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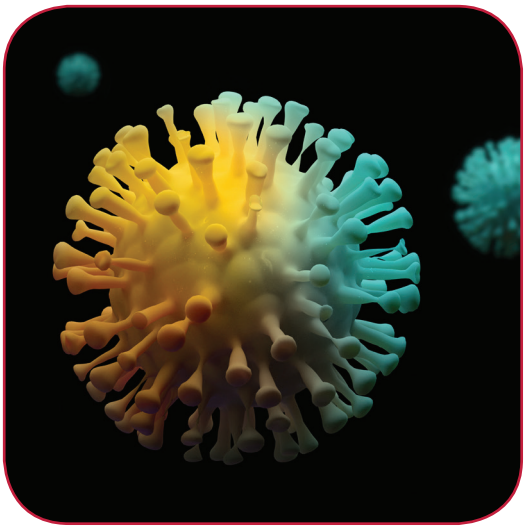

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Original articles and reviews

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Classification of the ibuprofen active pharmaceutical ingredients by chemical patterns combining HPLC, ^{1}H-NMR spectroscopy and chemometrics: traceability of legal medicines

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Abstract

Introduction. Ibuprofen is one of the widespread used non-steroidal anti-inflammatory drugs. Ibuprofen active ingredient is manufactured in many sites located all around the world. The aim of this paper was to classify the geographical source of ibuprofen active pharmaceutical ingredients (APIs) from the legal market, based on chemical characteristics and its impurity pattern and to define a geographical fingerprint.

Methods. To classify ibuprofen in different geographical groups, the chemometrics by principal component analysis (PCA) and Cluster analysis was applied to HPLC, ^{1}H-NMR data of twenty-four samples of APIs from approved manufacturers located in different European and Asian countries.

Results. The PCA showed clearly two different geographical groups, based on particular patterns of European or Indian samples; the cluster analysis showed the similarity of group.

Conclusion. The chemometric analysis is an important tool for tracking the geographical origin of APIs. This could be useful to supplement the quality control ensuring safety of the medicinal products in legal market and dealing with the evolving changes of the illegal market.

Key words

- active pharmaceutical ingredient (API)
- ibuprofen
- HPLC
- ^{1}H-NMR
- chemometrics

INTRODUCTION

Traceability of medicinal products is meant as the full knowledge of the medicine route from manufacturing of the active pharmaceutical ingredients (APIs) and excipients to commercial distribution.

APIs from the legal chain have similar high-quality standards as they are manufactured through validated processes in authorized facilities according to the stringent good manufacturing procedures (GMP) rules and are released in line with regulatory approved specifications [1, 2]. Accordingly, it is more challenging to discriminate the geographical origin of legal active ingredients which are a quite homogeneous "clean" whole. Even though medicinal products containing regular APIs should not represent a critical safety issue, awareness of the sources helps in the risk-based evaluation for regulatory inspections of manufacturing facilities and in promoting fast effective action in case of unforeseen adverse events. Examples of undue events in the legal chain of APIs are the worldwide case of heparin adulterated by oversulfated chondroitin sulfate (OSCS) [3-5]; Valsartan containing impurities as N-nitrosodimethylamines (NDMA) and N-nitrosodiethylamines (NDEA) and, lately, Ranitidin and Metformin also contaminated by NDMA [6-9].

It is acknowledged that the origin of active substances can be traced by comprehensive analysis of their physical and chemical properties and their impurity pattern.

The possible analytical approaches for impurity and chemical profiling and characterization of APIs are based on chromatographic and spectroscopic methods (NMR, NIR, IR), or on the isotopic nucleus ratio by IRMS (isotope ratio mass spectrometry) [10-15]. All these approaches proved effective in locating the origin of APIs, even though APIs are usually manufactured from reagents and starting materials that are sourced

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from different locations. In any case, advanced analytical techniques produce a huge amount of information that are difficult to evaluate for relevance if considered separately without a chemometric approach. Chemometrics consists in the application of multivariate analysis to handle chemical or process information as a whole, thus highlighting concealed patterns and relationships in the collected data [16-19].

The output of chemometrics can be used as a predictive tool of the properties of the investigated samples or for descriptive purposes to evidence hidden connections and similarities in a group of samples. Results are applicable to research, quality control, manufacturing in the pharmaceutical industry and to fighting falsification. Since 2016 the European Pharmacopeia (Ph. Eur. 9.5 Ed.) has dedicated a general text to the chemometric methods applied to analytical data outlining the importance of chemometrics in the processing of data sets to obtain maximal chemical information on the investigated samples (Ph. Eur. 5.21 Chemometric methods applied to analytical data).

Ibuprofen is the most widespread non-steroidal anti-inflammatory drug (NSAID). It had a calculated turnover of 169.7 million (euros) with reference to the Italian market for the year 2018 and it represented the second active ingredient for the expense of self-medication in Italy among self-medication drugs [20]. Ibuprofen, as an active substance, is sourced from suppliers located all over the world. On the illegal side, it is well known that ibuprofen popularity triggers counterfeiting which implies unknown location of the manufacturing site. Therefore, traceability of ibuprofen manufacturing location increases knowledge of the quality and the safety of this medicinal substance with a high impact on public health in the general population.

The analysed ibuprofen samples were all compliant to the Ph. Eur. Monograph ibuprofen and were within the validity period. Chemometrics, by principal component analysis (PCA) method with multivariate data compression and cluster analysis, were applied to the HPLC and ^{1}H-NMR data sets. The outcomes demonstrated that it was possible to classify such a homogeneous group of samples according to their geographical source based on chromatographic and spectroscopy profiles.

This study reports the results of a chemometric analysis on twenty-four ibuprofen APIs from legally approved manufacturers located in different countries. The evaluation was carried on with the purpose of defining the specific chemical characteristics of ibuprofen manufacturing among several countries and tracing the APIs origin.

MATERIALS AND METHODS

Collection of samples

Twenty-four different batches of ibuprofen active substance were collected during the Italian post-marketing surveillance program. The APIs were produced by seven different manufactures, placed in different European and Asian countries. All the samples were compliant to the specific monograph of the European Pharmacopoeia and were within the validity period.

Analytical methods

Two analytical methods were performed for the analysis of ibuprofen samples: HPLC and ^{1}H-NMR. In this phase the variability of the methods was not explored.

HPLC

A Waters Alliance HPLC 2695 (Waters Corporation, Millford, MA, USA) separation module equipped with an UV-DAD Waters 2996 was used. The analytical procedure was based on the method reported in Ph. Eur. 9.5 monograph of ibuprofen (0721).

The column used was a Symmetry C18-endcapped (150×4.6 mm - 5 µm particle size), whereas, the gradient elution is showed in *Table 1*: the Phase A was composed by water-acetonitrile-phosphoric acid (600:400:0.5, v/v/v), and the Phase B by acetonitrile.

The other parameters settings were: 2 ml/min for flow rate, 30 °C for column temperature, 20 µl for Injection volume, 20 mg/ml in mobile phase A for sample concentration, and 214 nm for detection (Wavelength prescribed in the Ph. Eur. Monograph).

It was investigated if additional peaks were present at different wavelengths than those ones indicated by the Ph. Eur. Monograph. The chromatograms were evaluated at wavelengths between 210 and 400 nm, but no further peaks were detected and the intensity of known secondary peaks was not higher than that measured at 214 nm. Therefore, the considered wavelength was confirmed at 214 nm. The run time was set at 70 minutes.

^{1}H-NMR

A Bruker Avance NMR spectrometer (Bruker BioSpin Gmbh, Billerica, Massachusetts, USA) operating at a frequency of 400 MHz for protons (9.4 Tesla), equipped with a 5 mm BBI Z-GRD- ^{1}H-^{13}C probe head was used for recording the experiments.

For each experiment, 10 mg of ibuprofen were dissolved in 0.7 ml of DMSO-d6 99.80% and TMS and the solution was stirred for two minutes to complete dissolution. ^{1}H-NMR 1D experiments were run at temperature of 298.0 K, 32 scans were acquired with a delay time of 20 s, pulse was set at 90°, spectral window was 20 ppm.

Chemometric analysis

Dataset description and pre-processing

HPLC – For the qualitative and quantitative analysis, the chromatograms obtained at wavelength of 214 nm were considered and processed using Empower software version 3 (Waters Corporation, Millford, MA, USA).

Table 1
Gradient elution of HPLC

Time (minutes)	Phase A (per cent V/V)	Phase B (per cent V/V)
0-25	100	0
25-55	100-15	0-85
55-70	15	85

V/V: volume/volume; HPLC: high performance liquid chromatography.

Only peaks not detected in the blank run were considered in the analysis, and the disregard limit was set to 90 ppm based on S/N ratio >10 for each sample. Quantity (area under the peak in %) of each peak, identified by the retention times (RTs), was obtained by normalising relevant area to the area of ibuprofen peak (RT of 13.96 min). The RTs of ibuprofen and Ph. Eur. impurities peaks were well-known; further/other peaks were taken into account only if they fulfilled the aforementioned requirements. The area % of each peak was evaluated by Empower software.

NMR - ^{1}H-NMR data were processed by TopSpin 3.4 software (Bruker BioSpin Gmbh, Billerica, Massachusetts, USA), applying 0.3 Hz line broadening and calibration to the TMS signal at 0 ppm. Spectra were overlapped and only signals with S/N ratio > 3 not belonging to ibuprofen structure were considered for qualitative data evaluation.

Two datasets were analysed: quantitative measurements represented by the area percent of the HPLC peaks, and qualitative measurements reported as presence or absence of signals at specific chemical shifts for ^{1}H-NMR. The presence of signals was indicated by number 1, and the absence by number 0. The samples were divided *a priori* in two classes by geographical area of manufacturing, according to the information reported in the certificates of analysis of the samples. The classification was class 1 for European countries and class 2 for Asian countries. Six signals for HPLC chromatograms and 16 signals of NMR spectra were selected. In total, twenty-two measurements were obtained in the overall dataset (*Table 2*).

Table 2
Characteristics of measurements analysed for ibuprofen samples

Analytical methods	Signals for datasets	Measurements
HPLC	RT 3.51 RT 5.2 RT 8.7 RT 13.2 RT 48.13 RT 66.41	Area of signal (%)
^{1}H-NMR	7.93 ppm 7.91 ppm 7.44 ppm 7.42 ppm 7.13 ppm 2.19 ppm 1.90 ppm 1.55 ppm 1.53 ppm 1.45 ppm 1.21 ppm 1.14 ppm 1.11 ppm 1.09 ppm 0.97 ppm 0.76 ppm	Presence/Absence of signal

HPLC: high performance liquid chromatography; ^{1}H-NMR: proton-nuclear magnetic resonance;
RT: retention time; ppm, parts per million; area of signal, area under chromatographic peak (%).

At first, the data of each analytical method were evaluated separately; subsequently, the effect of overall data (HPLC plus ^{1}H-NMR) was estimated. HPLC data were pre-treated with scaling method to analyse the HPLC and NMR measurements in a unified database.

Chemometric methods

Two unsupervised methods were applied: principal component analysis (PCA) and cluster analysis. The PCA reduced the number of measurements in the principal components (PCs), which represented highest variability of datasets and classified the ibuprofen APIs between the two classes of different geographical manufacturing area. The cluster analysis was carried out to evaluate similarities among samples by Euclidian distance to indicate the presence or absence of natural groupings between analysed samples.

Software

Data pre-treatment, PCA and cluster analysis were performed using Matlab R2018b software (The Mathworks, Natick, MA, USA). The algorithms for PCA and cluster analysis were part of the PCA_toolbox for Matlab-version 1.5 of Milano Chemometrics and QSAR Research Group [21].

RESULTS

The manufactures were localized for 54.2% in Europe (Italy, UK and Germany), 45.8% in Asia (India and China). They were grouped in two classes: Europe (class 1) and Asia (class 2).

PCA and HPLC

About 87.98% of variance were explained by two principal components (PC1 and PC2). The PC1 represented the highest variation of the data (70.31%) and the PC2 represented the 17.67% of variation of data. The PCA scores plot showed a separation between class 1 and class 2. Class 2 was characterised by negative PC1 and negative PC2. The PCA loadings showed that the associated measurements with class 2 (Asian samples) were RT 3.51 minutes and RT 13.2 minutes (*Figure 1S, available online as Supplementary Material*). The peaks eluted at these RTs could be typical of Asian ibuprofen manufacturing.

PCA and ^{1}H-NMR

Two principal components represented the 84.88% of variance for ^{1}H-NMR dataset: PC1 represented 63.32% and PC2 21.56% of variation of the data. The PCA scores plot demonstrated; separation between class1 (European samples) and class 2 (Asian samples). The class 2 was characterised by positive PC2. The chemical shifts associated with class 2 were: 1.09 ppm, 1.11 ppm, 7.42 ppm, 7.44 ppm, 7.91 ppm and 7.93 ppm (*Figure 2S, available online as Supplementary Material).* The signals in the spectra at these chemical shifts could be characteristic Asian ibuprofen manufacturing.

PCA & HPLC and ^{1}H-NMR

The overall dataset was composed by 22 measurements. Using the PCA, with two PCs, the variance was

82.51% (57.29% and 25.22%, respectively for PC1 and PC2).

The PCA scores plot confirmed the separation between class 1 and class 2 of ibuprofen samples. Class 2 was represented by positive PC2. Only score S13, corresponding to the only Chinese sample, was classified differently in respect to the Asian class with a negative PC2. The loadings plot showed that the Asian classification was determined by RT 3.51 minutes and by signals at chemical shifts 1.09 ppm, 1.11 ppm, 1.21 ppm, 7.42 ppm, 7.44 ppm, 7.91 ppm and 7.93 ppm which had a positive PC2 (*Figure 1*).

Cluster analysis

Four groups were defined on the basis of chromatography and spectroscopy similarities of samples, and of their distance (*Figure 2*). The first homogenous group of samples was represented by cluster A, which showed to be less related to the other clusters. Cluster A was composed of European samples. These results highlighted its difference from the other groups, in particular from cluster D, composed of Indian samples. Closer related cluster B, represented by sample S8 only, shared similar characteristics with cluster A. Cluster C showed one Asian sample (S13 from China) together with one European sample (S24 from UK). The presence of Asian and European samples within the same group C suggests that some basic characteristics of ibuprofen manufacturing (i.e., starting material, intermediate of synthesis, other materials) could be similar between the two geographical areas. Establishing the similarity of these samples was not possible due to the

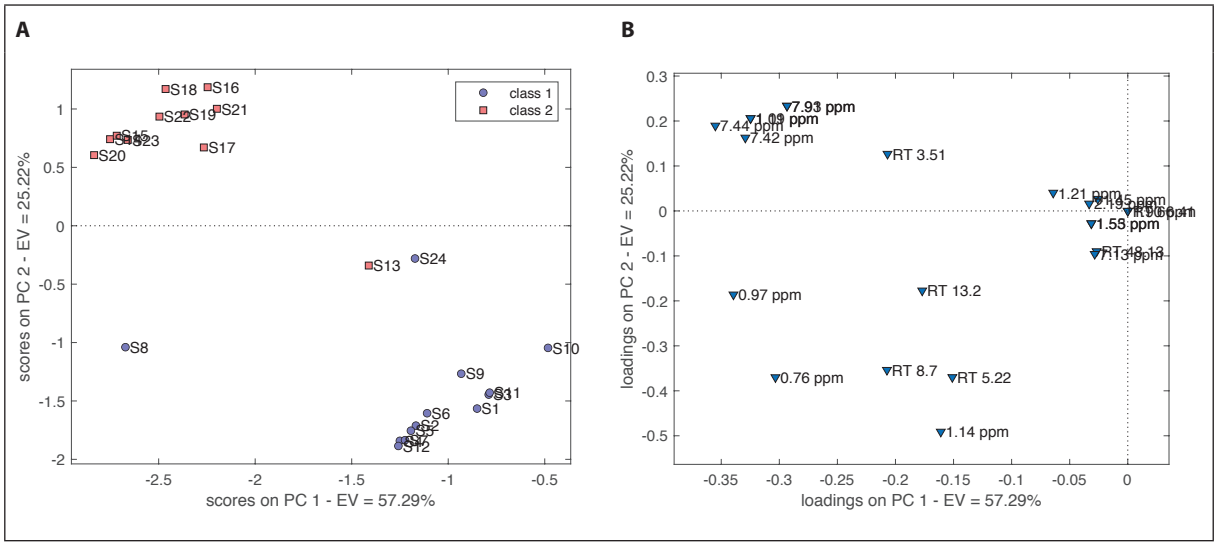

Figure 1
PCA scores plot (**A**) and loadings plot (**B**) of the overall dataset of the 22 combined HPLC and ^{1}H-NMR measurements.

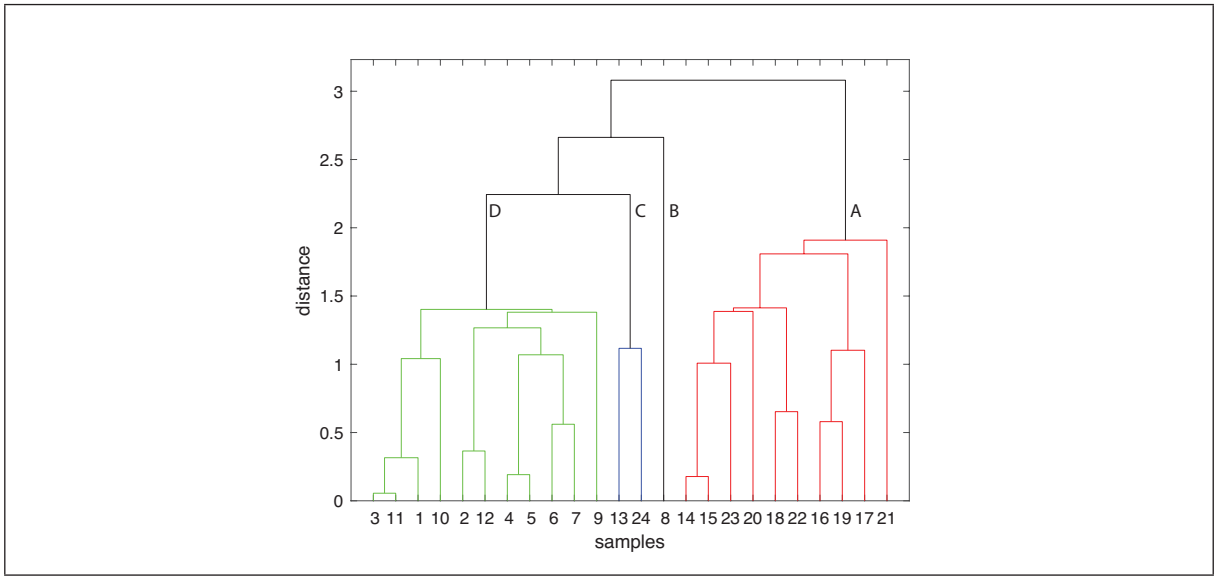

Figure 2
Dendrogram of cluster analysis for European and Asian samples.

low availability of samples for each country. Cluster D showed to be a homogenous group of samples, and it was represented by the Indian samples. These findings suggest that some characteristics of manufacturing of S13 (the only Chinese sample in Cluster C) could be different from the other Asian samples. For sample S24, the only ibuprofen sample manufactured in UK, the characteristics of manufacturing could be different from the other European countries.

DISCUSSION

This study demonstrated that marketed medicines containing active ingredient, such as ibuprofen, from different geographical origins, can be characterized by the active substance chemical and impurity profile. Indeed, the outcomes of this study demonstrated that by HPLC, ^{1}H-NMR and Chemometric tools, it is possible to classify APIs sharing the same chemical structure but being manufactured in different geographical areas. In fact, the first multivariate analysis, highlighted differences in legal ibuprofen APIs depending on manufacture geographical area. The PCA results, in fact, demonstrated that the European and Indian products were classified in two well distinct groups according to their different chromatography and ^{1}H-NMR spectroscopy fingerprints. The classification of samples improved when both HPLC and ^{1}H-NMR data were combined in the PCA analysis. Cluster analysis confirmed the classification in four clusters corresponding to the manufacturing region of ibuprofen. Hence, on the basis of the distance of the two relative clusters and of the chromatographic and spectroscopic profiles, the 24 samples were demonstrated to be not identical and that they could be classified in different homogenous groups.

Notwithstanding, the limited number of API samples, categorization between India and Europe was clearly noticeable.

Although the true root cause of the classification is unknown, some assumptions can be done. One hypothesis is that the purchase of the starting materials used for the API synthesis from manufacturers located in the same region of the world, would originate "similar" impurities pattern in the final API [22, 23]. Another possibility could be a different approach to GMP rules in the regulated countries. The possibility that the different patterns may be linked to different API manufacturing processes seems less convincing as the manufacturing processes to obtain ibuprofen are well known and established worldwide. However, this possibility cannot be completely ruled out as the choice of a manufacturing process can be based on the availability and cost of the starting materials which once again, may be linked to the region of origin.

CONCLUSION

The results of the present study confirmed chemometrics as a powerful predictive tool and stimulated further developments such as improving the geographical localization of the APIs source and to provide APIs fingerprints based on HPLC and ^{1}H-NMR. The chemometric analysis can be applied to different analytical method and can be useful to classify finished medicines or excipients. The new analytical methods provide huge amount of data which can be evaluated with chemometrics to better understand the characteristics of specific class of active substance.

The knowledge of the characteristics of the APIs manufactured in several areas of the world is also an important factor to oppose the illegal commerce of falsified medicines [16, 17]. Tracking geographical origin of APIs constitutes a major challenge to the OMCls either to assess the quality of legal medicines in post-marketing surveillance studies or to deal with the constantly evolving changes of the illegal market of falsified medicines. Furthermore, the knowledge of API fingerprint could be a very useful tool for Inspectorates to drive GMP inspections in particular geographical area.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

MR: conceptualization, methodology, data curation, software, roles/writing original draft. AB: conceptualization, methodology, formal analysis, roles/writing original draft. MB: formal analysis, roles/writing original draft. AM: resources. GG: conceptualization, methodology, formal analysis, roles/writing original draft.

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REFERENCES

1. European Medicines Agency (EMA). ICH Topic Q7 Good Manufacturing Practice for active pharmaceutical ingredients. CPMP/ICH/365/96 01/11/2000; 2000.
2. World Health Organization. WHO good manufacturing practices for active pharmaceutical ingredients. WHO Technical Report Series, No. 957; 2010.
3. Beyer T, Matz M, Brinz D, Rädler O, Wolf B, Norwig J, Baumann K, Alban S, Holzgrabe U. Composition of OSCS-contaminated heparin occurring in 2008 in batches on the German market. Eur J Pharm Sci. 2010;40:297-304. doi: 10.1016/j.ejps.2010.04.002
4. European Medicines Agency (EMA). CHMP Assessment Report for Medicinal Products Containing or Derived from Heparin. Under Article 5 (3) of Regulation (EC) No 726/2004.EMEA/CHMP/278280/2008; 2008.
5. Food and Drug Administration (FDA). Heparin for drug and medical device use: monitoring crude heparin for quality; 2013.
6. European Medicines Agency (EMA). Sartan medicines: companies to review manufacturing processes to avoid

presence of nitrosamine impurities. EMA/248364/2019 Rev 1; 2019.
7. World Health Organization. Update on Nitrosamine Impurities. EMP/RHT/Information Note_Nitrosamine impurities. WHO; 2019.
8. European Directorate for the Quality of Medicines and HealthCare (EDQM). The EDQM's response to nitrosamine contamination 2019; EDQM; 2019.
9. European Medicines Agency (EMA). EMA update on Metformin diabetes medicines. EMA/660975/2019; 2019.
10. Acevska J, Stefkov G, Cvetkovikj I, Petkovska R, Kulevanova S, Cho J, Dimitrovska A. Fingerprinting of Morphine using chromatographic purity profiling and multivariate data analysis. J Pharm Biomed Anal. 2015;109:8-27. doi: 10.1016/j.jpba.2015.02.016
11. Deconinck E, Cauwenbergh T, Bothy JL, Custers D, Courselle P, De Beer JO. Detection of sibutramine in adulterated dietary supplements using attenuated total reflectance-infrared spectroscopy. J Pharm Biomed Anal. 2014;100:279-83. doi: 10.1016/j.jpba.2014.08.009
12. Ruggenthaler M, Grass J, Schuh W, Huber CG, Reischl RJ. Levothyroxine sodium revisited: A wholistic structural elucidation approach of new impurities via HPLC-HRMS/MS, on-line H/D exchange, NMR spectroscopy and chemical synthesis. J Pharm Biomed Anal. 2017;135:140-52. doi: 10.1016/j.jpba.2016.12.002
13. Harms ZD, Shi Z, Kulkarni RA, Myers D. Characterization of near-infrared and Raman spectroscopy for in-line monitoring of a low-drug load formulation in a continuous manufacturing process. Anal Chem. 2019;91:8045-53. doi: 10.1021/acs.analchem.8b05002
14. Deconinck E, van Nederkassel AM, Stanimirova I, Daszykowski M, Bensaid F, Lees M, Martin GJ, Desmurs JR, Smeyers-Verbeke J, Vander Heyden Y. Isotopic ratios to detect infringements of patents or proprietary processes of pharmaceuticals: two case studies. J Pharm Biomed Anal. 2008;48:27-41. doi: 10.1016/j.jpba.2008.04.023
15. Gilevska T, Gehre M, Richnow HH. Multidimensional isotope analysis of carbon, hydrogen and oxygen as tool for identification of the origin of ibuprofen. J Pharm Biomed Anal. 2015;115:410-7. DOI: 10.1016/j.jpba.2015.07.030.
16. Krakowska B, Custers D, Deconinck E, Daszykowski M. Chemometrics and the identification of counterfeit medicines. A review. J Pharm Biomed Anal. 2016;127:112-22. doi: 10.1016/j.jpba.2016.04.016
17. Biancolillo A, Marini F. Chemometric methods for spectroscopy-based pharmaceutical analysis. Front Chem. 2018;6:576. doi: 10.3389/fchem.2018.00576
18. Monakhovaa YB, Holzgrabec U, Diehla BWK. Current role and future perspectives of multivariate (chemometric) methods in NMR spectroscopic analysis of pharmaceutical products. J Pharm Biomed Anal. 2018;147:580-9. doi: 10.1016/j.jpba.2017.05.034
19. Calvo NL, Maggio RM, Kaufman TS. Characterization of pharmaceutically relevant materials at the solid state employing chemometrics methods. J Pharm Biomed Anal. 2018;147:538-564. doi: 10.1016/j.jpba.2017.06.017
20. The Medicines Utilisation Monitoring Centre. National Report on Medicines use in Italy. Year 2018. Rome: Italian Medicines Agency, 2019.
21. Ballabio D. A MATLAB toolbox for principal component analysis and unsupervised exploration of data structure. Chemometrics and intelligent laboratory systems. 2015;149:Part B, 1-9. doi: 10.1016/j.chemolab.2015.10.003
22. Remaud GS, Bussy U, Lees M, Thomas F, Desmurs JR, Jamin E, Silvestre V, Akoka S. NMR spectrometry isotopic fingerprinting: a tool for the manufacturer for tracking active pharmaceutical ingredients from starting materials to final medicines. Eur J Pharm Sci. 2013;48:464-73. doi: 10.1016/j.ejps.2012.12.009
23. Gavin PF, Olsen BA, Wirth DD, Lorenz KT. A quality evaluation strategy for multi-sourced active pharmaceutical ingredient (API) starting materials. J Pharm Biomed Anal. 2006;41:1251-9. doi: 10.1016/j.jpba.2006.03.013

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An insight on drug resistance in *Plasmodium vivax*, a still neglected human malaria parasite

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Abstract
Plasmodium vivax has been considered for years as responsible for a mild form of malaria, due to the absence in the majority of its infections of the severe form of the disease, typical instead of the deadly human parasite *P. falciparum*. In the last decade, studies on *vivax* malaria have had a partial step ahead especially after the completion of the whole genome project, but there is still a gap of knowledge in the biology of this parasite. Moreover, the emergence of *P. vivax* antimalarial resistance in 1980s and its subsequent spread in the Southeast Asia have indicated new concerns about the possibility to control this parasite. *P. vivax* drug resistance poses a major threat to endemic countries and without important international efforts, we could assist in a near future to the paradox of seeing different malaria co-endemic countries, that have successfully controlled/eliminated *P. falciparum*, still fighting against *P. vivax*.

Key words
- malaria
- *Plasmodium vivax*
- drug resistance

In memoriam of ***Professor Giancarlo Majori, 1943-2020***

INTRODUCTION

The biology of *Plasmodium vivax* is characterized by the existence of hypnozoites, which are characteristic dormant liver stage forms able to cause relapses weeks or years later from the first exposure and clinical manifestation *(Figure 1)*. The discovery of these dormant forms occurred in 1982 [1]. Some of the sporozoites inoculated by the bite of an *Anopheles* mosquitoes, on arriving in liver tissue do not turn into merozoites, which are responsible for the primary malaria attack, but instead become hypnozoites responsible for relapse of this disease. Relapses can occur after different time intervals, based on the *P. vivax* strain and climatic zone: infection by the tropical strain named "Chesson" can result in relapse after just two weeks, whereas strains originating from temperate zones may cause relapses even years after the infection. [2]. Some other biological features make this parasite peculiar and, in some way, more difficult to control than *P. falciparum.* From the epidemiological point of view, an important aspect for *vivax* transmission is the fact that the gametocytes, the parasite forms able to infect the mosquito vectors, appear in the human blood circulation very early, making the patient potentially infectious to the mosquitoes very soon, i.e. at the appearance of the clinical symptoms. Finally, the ability of this parasite to complete its life cycle in the vector at temperature below the 20 °C (the life cycle of *P. vivax* does not occur below the 15 °C) explains the presence of this parasite outside tropical areas and we can assume that in cases of resurgence of malaria in these regions, *P. vivax* will be the responsible plasmodial species [3, 4].

In Europe, in the early 20th century, before the industrial era and before the development of a malaria-control program initiated just after the Second World War, *P. vivax* was the most common species of the four human plasmodia then known. At that time, the annual number of cases of malaria caused by *P. vivax* was much greater than that caused by *P. falciparum*. *P. vivax* was the dominant parasite in temperate areas and most tropical areas except in Africa.

Nowadays, *P. vivax* represents the geographically most widespread *Plasmodium* species, with more than 2.5 billion people at risk of infection and an estimated 15.8 million of clinical cases per years. [5, 6]. According

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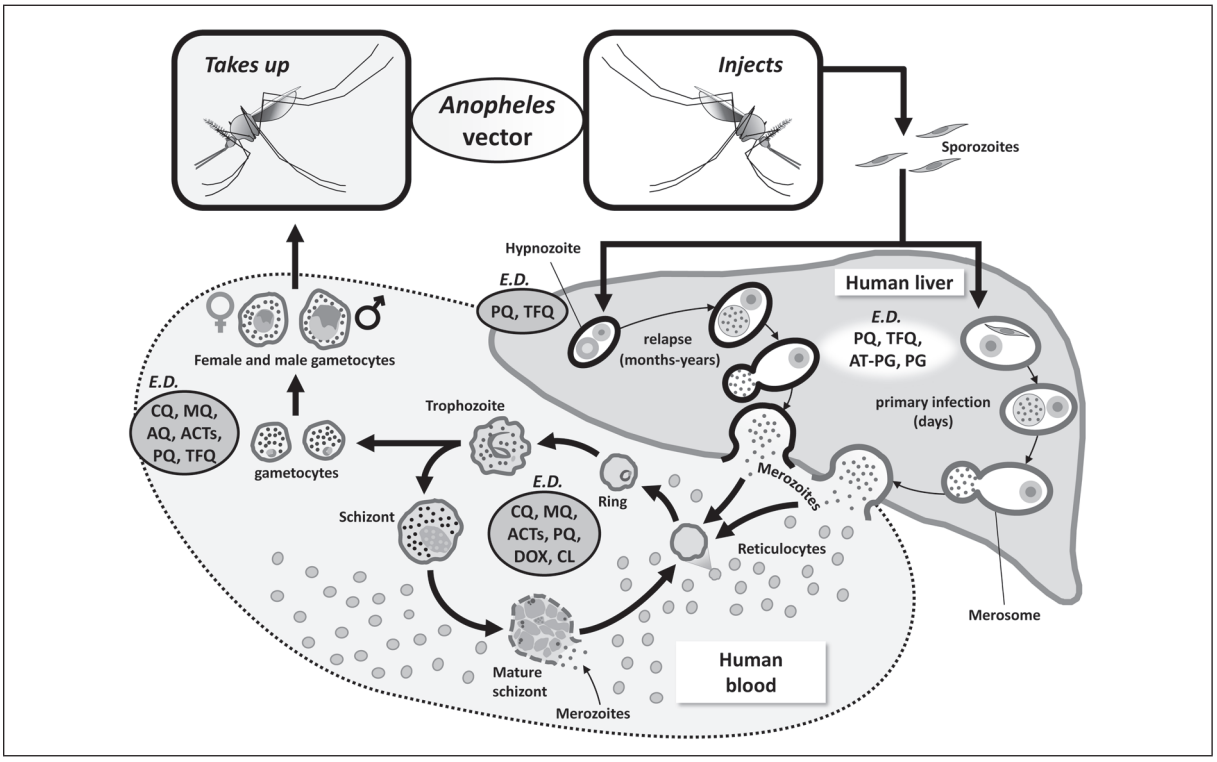

Figure 1

The complexity of the life cycle of *Plasmodium vivax* is illustrated by the fact that it is possible that three different parasitic populations can circulate in the blood of a person infected with *P. vivax*: a responsible population of primary attack, a resurgent population if the strain is resistant to drugs, and a population from a relapse of which hypnozoites are the origin. The diagnostic tools currently available are unable to distinguish these three populations. Specific antimalarial drugs for relevant parasite cell target are also indicated in this figure.

ED: effective drug; CQ: chloroquine; PQ: primaquine; AQ: amodiaquine; MQ: mefloquine; PG: proguanil; AT: atovaquone; TFQ: tafenoquine; DOX: doxycycline; CL: clindamycin; ACTs: artemisinin combination therapies.

to the latest Word Malaria Report, released by WHO in 2019, the highest incidence of *P. vivax* was reported in the region of America, with 75% of malaria cases in the 2018, and Southeast Asia; [7]. Until few years ago, *P. vivax* has been rarely studied in Africa, since the dominance of Duffy-negative blood group in blacks, as key determinant of natural resistance factor of *P. vivax* infection [8]. Nevertheless, several studies have recently shown evidence of *P. vivax* transmission across Africa in Duffy-negative populations [9-11].

In Europe malaria are currently reported as imported cases. Sporadic non-imported/indigenous cases of malaria caused by *P. vivax* were reported through the last years, as occurred in Italy in 1997 [12], in Corsica during the summer of 2006 [13] and in Spain in October 2010 [14]. Even if in 2016, the WHO European Region was declared malaria free [15], nevertheless an outbreak of *vivax* malaria occurred in Greece [16] in the district of Lakonia, Peloponnese between 2011 and 2012 and after an initial successful control of the situation in the two following years (0 local transmission in 2014) some indigenous cases have been still registered in the year 2015-2018 (10 cases in 2018). In France, Spain, and Italy, where malaria was endemic until the end of the sixties, some areas were monitored to assess the risk of reintroduction of malaria [17, 18]. These areas are the Camargue, the largest wetland in the south of France; the Ebro delta; and the Maremma, a great rural territory that stretches between Lazio and Tuscany, in central Italy. Particular eco-climatic conditions and a significant presence of mosquitoes potentially vectors of malaria (phenomenon known as "anophelism without malaria") make these zones prone to malaria reintroduction.

More recently, five events of local malaria transmission (non-imported cases) have been reported recently in the EU. Three of these events were associated with either mosquito-borne transmission from an imported case (introduced malaria) or an imported infected mosquito (airport malaria), in Greece and northern Cyprus (*P. vivax*), and in France (*P. falciparum*); and two of the cases were most likely associated with nosocomial mosquito-borne or iatrogenic transmission of *P. falciparum*, in Italy and Greece [19]. These cases demonstrate that the re-starting of malaria transmission in Europe, even if in the form of small epidemics, is a possibility and the magnitude of risk still under-evaluated.

For over 50 years, chloroquine has been used as the schizonticidal drug choice for the treatment of *vivax* malaria, administrated in combination with primaquine, a drug used to prevent relapses due to hypnozoites. Unfortunately, drug resistance to chloroquine started to emerge at the end of 1980s [20], and over the following years several reports described the extent of

Table 1
Therapeutic protocols currently in use for the treatment of uncomplicated *Plasmodium vivax* malaria

Drugs	Dose	Duration	Notes
Chloroquine (+ primaquine or tafenoquine as below)	600 mg initial 300 mg after 6-8 hrs 300 mg/single daily – day2 and day3	3 days	
Primaquine phosphate	30 mg/single daily *(0.75 mg/kg - single weekly × 8 weeks)*	14 days *(2 months)*	*Not to be used in pregnancy* *To be avoided in G6PD deficiency* *(Prevention of relapse in patients with mild-moderate G6PD deficiency)*
Tafenoquine	300 mg single dose (2 tablets of Krintafel®, 150 mg each)	1 day	*Not to be used in pregnancy* *To be avoided in G6PD deficiency*
Artemether-lumefantrine (20 mg/120 mg) (+ primaquine or tafenoquine as above)	4 tablets initial 4 tablets/single at 8, 24, 36, 48 and 60 hrs	3 days	
Dihydroartemisinin-piperaquine (320 mg/40 mg) (+ primaquine or tafenoquine as above)	3 tablets/single daily × 3 days (36-75 kg bw)	3 days	*>75 kg = 4 tablets/ single daily x 3 days*
Atovaquone-proguanil (+ primaquine or tafenoquine as above)	4 tablets/single daily × 3 days	3 days	

chloroquine resistance spread across most of *vivax*-endemic countries [21]. A summary of therapeutic protocols currently in use for the treatment of uncomplicated *Plasmodium vivax* malaria is reported in *Table 1*. In areas where chloroquine is high ineffective due to resistance, artemisinin combination therapy, co-administrated with primaquine, has been used as alternative drug strategy [22]. Recently, in July 2018, tafenoquine, commercially known as Krintafel® and Arakoda®, has been approved by FDA as a longer acting anti-hypnozoite drug for the radical cure of *vivax* malaria and as a possible substitute of primaquine. It is administrated in a single oral dose in combination along with a schizonticide, resulting in a better compliance linked to the dose regimen [23]. As for primaquine, this drug cannot be used in glucose-6-phosphate dehydrogenase (G6PD) deficiency and in pregnancy, due to the unclear risks of its use in these two circumstances.

Since years, scientific research on *P. vivax* has been fewer compared to *P. falciparum*. Moreover, in the recent decades, there have been relatively few programs and funds made available for targeted action against *P. vivax*, probably because malaria mortality is linked mainly to *P. falciparum* infections and occurs primarily in African children. Instead, *P. vivax* epidemiology shows several peculiarities that would suggest the importance of increasing international efforts to shed light on the biology of this parasite and to channel more resources to support endemic countries for collaborative international studies and to develop effective control programs for its detection, characterization and containment. Just to highlight the most important features: *P. vivax* is endemic in most of the world's malarial areas and exerts in all these areas a socio-economic burden that is anything but negligible; the transmission of this parasite is mostly driven by the reactivation of hypnozoites (relapses) and the observed increase of *vivax* infection after *falciparum* malaria treatment make mandatory the development and implementation of an effective radical cure for malaria in areas of coendemicity; the report of severe/fatal clinical forms and the proven transmission among the Duffy-negative people of sub-Saharan Africa deny two of the most believed dogmas related to *P. vivax* infection (*P. vivax* is a benign form of malaria; Duffy-negative people are resistant to *vivax* infection); the emergence and spread of chloroquine resistance, especially in southeast Asia [24].

Above all, drug resistance is considered one of the biggest challenges in the fight and control against malaria; it has contributed to the spread of malaria to non-endemic countries and the re-emergence of the disease in areas where it had been eradicated. The combination of these epidemiological and biological features of the *P. vivax*, including the resistance mechanisms, justify an updated picture of the situation. A comparison between what is known about *P. falciparum* and *P. vivax* in term of drug resistance is also presented in this concise review, in order to highlight the lack of biological knowledge about this important human plasmodial parasite.

DRUG RESISTANCE IN *P. VIVAX*

P. vivax resistance to chloroquine (*Pv*CQR) has been detected in the late 1980s in isolates from Papua New Guinea [20, 25] and Indonesia [26], about 30 years later than the emergence of chloroquine resistance in *P. falciparum*. Same situation has been described for other antimalarial drugs, like primaquine, mefloquine and pyrimethamine/sulfadoxine [27]. Mode of action of the pyrimethamine and sulfadoxine and the mechanism for resistance to these drugs are the only ones well documented in *P. vivax*. There are several antimalarials targeting the blood forms of *P. vivax*, such as chloroquine, amodiaquine, piperaquine, artesunate, artemether, lumefantrine, dihydroartemisinin, but chloroquine is still the first-line drug for the treatment of *P. vivax*.

From the discovery of the first isolate of chloroquine-

resistant (CQR) *P. vivax* in Papua New Guinea in 1989 [20-26], this resistance has spread throughout Southeast Asia and the world, even though its progress is slower than for *P. falciparum* resistance [21-28]. Due to the emergence and spread of *Pv*CQR, the treatment of malaria caused by *P. vivax* is expected to become more difficult in the coming years. Despite having been reported more than 30 years ago, mechanisms underlying *Pv*CQR have not been completely understood due to the difficulties to maintain a continuous *in vitro* culture system for this parasite. Antimalarial drug resistance is based on the accumulation of specific mutations occurring in genes encoding essential enzymes or proteins involved in the parasite biology. The development of resistance to chloroquine is a serious issue because this drug is the recommended one as first-line treatment for this parasite. In any case, *Pv*CQR is limited if considering the worldwide use of chloroquine. The radical cure of the parasite, the erythrocyte forms and liver forms responsible for relapse, is based on a combination of chloroquine with primaquine. This treatment, used for sixty years, sometimes failed in different malarious areas in Southeast Asia and primaquine itself has constraints and side effects that limit its use [22]. Unfortunately, as other important aspects of the biology of this parasite, antimalarial resistance mechanisms in *P. vivax* remain no deciphered yet.

Therapeutic protocol based on artemisinin-based combination therapies (ACTs) are currently the treatment of choice for uncomplicated malaria attacks caused by *P. falciparum*. Recent studies have shown evidence of a faster *P. vivax* parasites clearance of ACTs respect to chloroquine with a considerable reduction in recurrence [29, 30]. Notably, the combination of dihydroartemisinin and piperaquine (DHA-PPQ) results in a rapid reduction of parasitemia and in an optimal prophylaxis strategy against potential relapses. The efficacy of this therapeutic approach depends on the combination of a fast acting (DHA) and a longer acting drug (PPQ) highly effective in the prevention of recurrences up to 56 days [31]. A first study in Thailand in 2000 [32] showed that artesunate and artemether quickly eliminated parasites in the blood of patients compared with other antimalarials. More recent studies [33-34] have shown the efficacy of the combination artemether-lumefantrine (AL, Coartem® and Riamet®) in the treatment of *P. vivax* infections. The advantage of a common therapeutic approach, i.e. using ACT to treat all malaria cases, would be of paramount importance in areas where *P. vivax* and *P. falciparum* coexist (e.g. in Southeast Asia and South America). As described by Bassat *et al.* in 2014, the efficacy of AL with other ACTs whose partner drugs have a longer half-life (DHA-PPQ, artemether-mefloquine) were compared. Data showed that *vivax* relapses are only delayed and not eliminated [34]. Using ACT would also limit the use of chloroquine, preserving its effectiveness against *P. vivax*. However, AL combination is not active against hypnozoites, and does not protect against recurrence and exposes patients to the risk of new infections in a shorter period if it is not associated with treatment with primaquine [35]. As described by Commons *et al.*, 2019, in areas where *P. falciparum* and *P. vivax* coexist, there is a high risk of subsequent *P. vivax* parasitemia (by day 63 more than 15%) after treatment of *falciparum* malaria with an effective ACT [36]. The recurrence of *vivax* parasitemia depends on the reactivation of the dormant liver stages, demonstrating that ACTs result efficacy for the treatment of the erythrocytic stages of the two parasites, but not on the hypnozoites. Previous studies have been proved the ability of primaquine to act as a schizonticidal drug on the asexual blood stages of *P. vivax* [37], and as a gametocytocidal drug on *P. falciparum* [38]. For this reason, Commons *et al.* hypothesized that the administration of an artemisinin-combination regimen along with primaquine would decline the incidence of recurrent *P. falciparum* and *P. vivax* infections, and reduce the risk of transmission in patients with *falciparum* monoinfection [36]. Mixed infections of *falciparum*/*vivax* are thus an important issue for the treatment of malaria patients in co-endemic countries; and the study of potential interactions between the two species could provide more information about determinants of drug resistance *in vivo*. A unified treatment strategy for asexual forms of two infections (*falciparum* and *vivax*) offers significant advantages in areas where the two are co-endemic.

The unanswered question is: how long does one have to follow a patient to see if he has a *vivax* relapse? According to several studies, generally, the follow-up ranges between 4-6 weeks, as in the case of *P. falciparum* infections, up to 6 months. Nevertheless, this period is not enough to capture long-latency relapses, which can appear 8-9 months after the first exposure [39]. Finally, even in countries where chloroquine remains highly effective, the administration of an artemisinin-based regimen could remove more rapidly the *vivax* gametocytes biomass, reducing the risk of transmission [40].

THE CHALLENGE OF EVALUATING THE DRUG RESISTANCE IN *P. VIVAX*

The resistance of the malaria parasite is a complex phenomenon that involves many parameters and can be measured by different approach. In general, as for example is for *P. falciparum,* three approaches are used to identify and evaluate the level of drug resistance in plasmodial isolates: *in vivo* tests, *in vitro* tests and molecular markers analysis.

In vivo tests (efficacy therapeutic tests, ETTs) can be used in which clinical and parasitological symptoms in malaria patients treated with these drugs are followed long enough to see whether parasite re-appearance occurs. *In vivo* tests are the gold standard, because they provide clinical evidence of treatment outcome, but, unfortunately, *in vivo* tests are difficult to implement in the frame of control programs, since following cohorts of patients for about a month is rarely possible in endemic area.

In vitro tests (or phenotypic analysis) include putting a culture of the parasite in the presence of various concentrations of drugs to determine the effective dose and to define thresholds for each drug.

Finally, molecular markers analysis (in general, the assessment of polymorphisms present in plasmodial

genes involved in drug resistance) could represent a useful way to survey the emergence and spread of drug resistance surveillance in endemic areas [41].

In vivo tests (ETTs) for *P. vivax* are limited by the same parasite biology. Indeed, *P. vivax* has intra-hepatocyte forms (hypnozoites) that escape most drugs, which can cause re-appearance of the parasite several weeks or months after infection. It then becomes very difficult to know whether a patient was properly treated and relapsed, was infected with a resistant parasite that escaped drug treatment, or was infected by a parasite dormant form, which are protected from the drug. Actually, the outcome of a treatment is very often challenging in *vivax* endemic areas, since it is hard to known if a parasitemia re-appearance in a given patient might be due to a real treatment failure or to the hypnozoite reactivation or a new infection.

Highly standardized methods are available for *P. falciparum* continuous cultures since long time, making affordable a wide range of biological studies, as for example the evaluation of resistance of this parasite to the different antimalarials. In the case of *P. vivax*, the situation is different: we do not have a continuous culture of *P. vivax in vitro*, and therefore, *in vitro* tests is in general somewhat difficult. The main problem is probably that *P. vivax* favors young erythrocytes, i.e., reticulocytes, which represent only one percent of red blood cells in human blood. It also appears that, even if we add a constant amount of reticulocytes to the culture, the parasite has difficulty reproducing and multiplying *in vitro*.

The only way of maintaining *P. vivax* strains *in vitro* is to alternate cultures in flasks with the inoculation of the parasite in monkeys. *P. vivax* infects several species of non-human primates, including chimpanzees, gibbons, and "squirrel monkeys" (Saimiri sciureus), without problems [42], but the research groups able to breed and manipulate monkeys in the laboratory are very few in the world.

In 2007, a group of researchers from Thailand presented an *in vitro* method in which *P. vivax* survives for a few parasitic cycles [43], but the protocol turned to be complex and difficult to reproduce. Subsequently, in 2015, the same protocol has been improved allowing the *in vitro* cultivation for over 26 months, even if with a low parasite density [44]. More recently, different protocols of "short-term culture" for *P. vivax* isolates have been presented in the literature but the protocols are still limited in efficacy especially when compared to the high-standardized protocol for *P. falciparum* culturing [45, 46].

In summary, the situation about *P. vivax* culturing is frustrating after more than 100 years of attempts (the first protocol of *vivax* cultures was published in 1912 by Bass and Johns) and the lack of *in vitro* cultures of *P. vivax* makes it difficult to monitor the sensitivity of this parasite to drugs and constitutes the main gap in biology knowledge of this parasite [47].

The analysis of molecular marker polymorphisms in general supports *in vitro* and *in vivo* tests assays and could be a useful way to try to identify resistance of *plasmodium* parasites to drug treatments [41]. However, in *P. vivax,* the value of these markers depends on the ability to analyze them in a patient where treatment has failed, and the ability to distinguish whether a patient was properly treated and relapsed, was infected with a resistant parasite that escaped drug treatment, or a hepatocyte which was infected with a dormant form of the parasite, which was protected from the drug treatment. Currently there is not a truly reliable method to assess the sensitivity or resistance of *P. vivax* to treatment. Meanwhile, several observations of treatment failures raise concern about the development of resistance in *P. vivax*.

The difficulties in detecting and studying *P. vivax* resistance than *P. falciparum* depends on the basic biology and epidemiology of the two parasites. The major and latest differences obtained towards understanding the drug resistance in *P. falciparum* and *P. vivax* are shown in *Table 2*.

The search for the identification of reliable molecular markers in *P. vivax* has been focused in the recent years on the *P. vivax* putative transporter protein gene (*pvcrt-o, P. falciparum* orthologues CQR-genes), multidrug resistance gene (*pvmdr-1,* CQR) and dihydrofolate reductase and dihydropteroate synthase (*pvdhfr/pvdhps* genes, PYR/SUL-R)

In the case of *P. falciparum*, CQR is measured by a decrease in the action of the drug at the parasite's food vacuole. Mutations of genes *pfcrt* and in part, *pfmdr1* are involved in this process. Shortly after the identification of the gene *crt* in *P. falciparum* and demonstration of its involvement in CQR, the orthologous gene (*crt-o*) in

Table 2
Differences in drug resistance knowledge between *Plasmodium vivax* and *Plasmodium falciparum*

Plasmodium spp.	Antimalarial drugs with known resistance	Identified molecular marker genes for drug resistance
Plasmodium vivax	CQ, PQ, QN, PYR/SUL	*crt-o* = CQR? *mdr1* = CQR?
Plasmodium falciparum	CQ, QN, AQ, MQ, PPQ, PYR/SUL, PG, ARTder, ATQ	*dhfr/dhps* = PYR/SUL, PG *crt, mdr1, nhe,* = CQ, QN, MQ, AQ *pm2* = PPQ *cytb* = ATQ *k13* = ARTder

Drugs – CQ: chloroquine; PQ: primaquine; QN: quinine; AQ: amodiaquine; MQ: mefloquine; PPQ: piperaquine; PRG: proguanil; PYR/SUL: pyrimethamine/sulfadoxine; ATQ: atovaquone; ARTder: artemisinin derivatives (dihydroartemisinin, artesunate, artemether).
Genes – *mdr1:* multidrug resistance1; *crt:* chloroquine resistance transporter; *nhe*: Na^+/H^+ exchanger; *dhfr/dhps:* dihydrofolate reductase/dihydropoteroate synthase; *cytb*: cytochrome b; *pm2*: plasmepsin 2; *k13*: kelch-*on chromosome*-13.

P. vivax was identified [48]. Studies with several different strains of the parasite found in endemic areas have not shown an association between the polymorphism of this gene and resistance to CQ. A 2006 study in Brazil showed a decreased response to CQ for a *P. falciparum* strain transfected with the *crt P. vivax* (heterologous expression system), suggesting a possible involvement of this gene in CQR [49].

More recently, Sà *et al.*, investigated again the possible role of the *crt-o* in *P. vivax* CQR and described an upregulation of this gene through crossing different parasite population with different sensitivity to CQ [50]. Despite the studies above mentioned, the involvement of *crt-o* gene in *vivax* CQR remain to be deciphered yet.

The *mdr1* gene in *P. falciparum* plays an important role in modulating resistance against several drugs, most of which belong to the class of quinolines, but also to artemisinin derivatives [51]. The decrease in antimalarial sensitivity is related to the presence of polymorphic loci in the gene or an increase in the copy number of the gene in the genome of the parasite, a condition known as "multidrug resistance effect". For example, the Thailand-Myanmar, *P. falciparum* isolates have amplification of the gene *Pfmdr1* associated with decreased sensitivity to mefloquine, quinine, lumefantrine, halofantrine, and artemisinin derivatives [52]. In 2005, Brega *et al.* were the first to demonstrate the role of *P. vivax mdr1* gene in drug resistance [53]. While early studies have not shown a relationship between the presence of mutations of this gene and sensitivity of the parasite to quinoline [54, 55], studies conducted more recently in Southeast Asia (Thailand, Indonesia [56, 57] and Papua New Guinea [58]) have sought to clarify the role of *Pvmdr1* in antimalarial drug resistance, taking into consideration either the gene polymorphism or the number of copies and comparing the molecular results with the *in vitro* and *in vivo* sensitivity results. We can summarize the results of these studies as follows: chloroquine and mefloquine exert a competitive evolutionary pressure on *Pvmdr1*, identical to that observed with *P. falciparum*; the polymorphism at the codon 976 (Y976F) of the *Pvmdr1* gene can be used as an indicator to monitor *P. vivax* resistance to chloroquine; amplification of the gene *Pvmdr1* in multidrug-resistance shows an effect similar to that observed in *P. falciparum*. This appears to be limited to areas with endemic zones characterized by the circulation of these drugs. Amplification of copy number was observed in isolates from Thailand but not in isolates from Papua New Guinea, where mefloquine is not used [56]. Finally, in 2017 a study carried out in *P. vivax* field isolates from Mauritania, Sudan and Oman investigated single nucleotide polymorphisms in *Pvmdr1* and, for the first time, in *PvMCA1-cd* (spelling) gene, to look for a potential role of these two genes in *P. vivax* drug resistance [57].

Pyrimethamine and sulfadoxine (S/P) are inhibitors of dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS). Due to the quick rise of *P. falciparum* resistance and the occurrence of rare but severe adverse events, currently this drug combination is rarely used for malaria treatment. However, this association is still recommended by WHO in intermittent preventive treatment in pregnant women (ITPs) and it is also used in association with artemisinin derivatives. Resistance of *P. vivax* to S/P appeared very quickly and DHFR/DHPS inhibitors have not been used for the treatment of *P. vivax* malaria because preliminary tests showed low effectiveness against *P. vivax*. *P. vivax* was considered intrinsically resistant to these two drugs. But recent studies have shown a correlation between the point mutations in the *dhfr* gene of *P. vivax* isolates from different geographical areas and resistance to antifolate: Thailand, India, Madagascar, Comoros [58] and Papua New Guinea [59]. The mechanism of resistance to S/P is the only well-known mechanism for this parasite because the situation is similar to that of *P. falciparum:* mutations in the genes encoding the DHFR and DHPS enzymes are responsible for a change in the 3-D structure of these proteins and, therefore, these mutations lead to a decrease in the affinity of the mutated enzyme vis-a-vis antifolates. Pécoulas *et al.*, in 1998, isolated and cloned the DHFR-TS domain of the *dhfr* gene of *P. vivax* and different alleles of the gene have been identified [60, 61] and more recently, *P. vivax dhps* gene has been identified and characterized [62, 63]. Nevertheless, further studies on *dhfr/dhps* polymorphisms are needed to properly assess the extent of genetic variation in these *P. vivax* resistance markers.

After a long search to identify a specific locus implicated in artemisinin resistance, the kelch propeller domain of the *k13* gene on chromosome 13 was recently identified as a molecular marker of artemisinin resistance in *P. falciparum*: several mutations in the kelch propeller domain have now been associated with *in vitro* ring stage survival assays and delayed parasite clearance rates in patients treated with artemisinins [64]. A recent study identified the *Pfk13* ortholog for *P. vivax*, *Pvk12*, showing that non-synonymous mutations in this gene are already circulating at very low frequencies in Cambodia [65]. More recent studies conducted in Southeast Asia confirmed the limited polymorphism of *Pvk12* making the role of this gene in artemisinin resistance unclear [66, 67].

ARE HYPNOZOITES RESERVOIR OF RESISTANCE?

The presence of these dormant liver forms enormously complicates control of this parasite in endemic areas as well as in non-endemic areas for the management of *vivax* imported cases. Knowledge of the biology of hypnozoites is very limited and they escape most drugs. Several articles report that the introduction of artemisinin derivatives in Asia has caused a drop in cases of *P. falciparum* in different endemic areas without having the same efficacy on *P. vivax* [68]. The hystorical drug able of acting on hypnozoites and preventing relapse is primaquine (PQ). However, this drug has two weaknesses: hemolysis risk in the case of red blood cell of the patient is G6PD deficient, and a bad compliance due to its long course of treatment (15 days). *P. vivax* resistance to primaquine has been already reported also [69, 70].

Hypnozoites and their reactivation allow *P. vivax* to survive in temperate zones characterized by a marked seasonality of vector populations. Several questions on

the biology of hypnozoites are currently unanswered, which has direct implications for the control of this pest. What is the "signal alarm" for the hypnozoites? A Finnish study sought to provide an answer to this question, assuming that the saliva of *Anopheles* injected during the bite by the mosquito can be responsible for the triggering [71].

The ability to artificially induce the reactivation of hypnozoites is a fascinating perspective that might provide a new and effective control strategy for this elusive human parasite.

CONCLUSION

WHO recognized in its recent agenda that more attention has to be paid to *P. vivax* infections to move forward in the elimination efforts. Even though TQ does not overcome all shortcomings of PQ, the TQ single dose anti-relapse treatment for hypnozoites of *P. vivax* as well as of *P. ovale* markedly improves the patient compliance to the treatment regimen for these malaria species [72]. The changes in the WHO agenda might have given the final impulse to keep TQ in the development pipeline and finally bring it to the market. Currently no other drugs are available or in pipeline for anti-relapse therapy [73].

Considering that development from the early clinical phase to market approval takes ~10 years, most probably no alternative to 8-AQ for anti-relapse therapy will be available in the near future. It is ultimately conceivable that in the near future TQ will replace current treatment regimens with PQ and play a crucial role in the treatment protocol included in programs for the control of *P. vivax* malaria [72].

The European Commission and the major international stakeholders are now the only ones who can effectively support research programs of the magnitude needed to overcome the bottlenecks in knowledge in the fight against *P. vivax*. Yet, obtaining financing in these frameworks is very uncertain, and the money offered is rarely compatible with these objectives. In an international scenario in which funding made available for *falciparum* malaria control programs had an increase stop in 2017 and even a decrease in 2018 [7] and it is necessary to define priorities in the use of public or private funds, malaria control projects focused on *P. vivax* have little chance of winning.

In conclusion, despite the completion of the whole genome sequencing, several steps forward in the knowledge of the biology of this parasite and the availability of a new drug for radical treatment, *P. vivax* malaria is still to be considered among the neglected human diseases.

It is of pivotal importance to invest in international control programs targeting *P. vivax* otherwise in the near future we could assist to the paradox of seeing different malaria co-endemic countries, that have successfully controlled/eliminated *P. falciparum*, still fighting against *P. vivax*.

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CS, SP, MLE and EP wrote the manuscript. FS edited Tables and Figures. CS, SP, MLE, EP and FS reviewed the manuscript and approved the final version of the manuscript.

Conflict of interest statement

The Authors declare no conflicts of interest.

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REFERENCES

1. Krotoski WA, Garnham PC, Bray RS, Krotoski DM, Killick-Kendrick R, Draper CC, Targett GA, Guy MW. Observations on early and late post-sporozoite tissue stages in primate malaria. I. Discovery of a new latent form of *Plasmodium cynomolgi* (the hypnozoite), and failure to detect hepatic forms within the first 24 hours after infection. Am J Trop Med Hyg. 1982;31(1):24-35.
2. Cogswell FB. The hypnozoite and relapse in primate malaria. Clin Microbiol Rev. 1992;5(1):26-35. doi: 10.1128/cmr.5.1.26
3. Petersen E, Severini C, Picot S. *Plasmodium vivax* malaria: a re-emerging threat for temperate climate zones? Travel Med Infect Dis. 2013;11(1):51-9. doi: 10.1016/j.tmaid.2013.01.003
4. Piperaki ET, Daikos GL. Malaria in Europe: emerging threat or minor nuisance? Clin Microbiol Infect. 2016;22(6):487-93. doi: 10.1016/j.cmi.2016.04.023
5. Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE, Baird JK, Hay SI. Global Epidemiology of Plasmodium vivax. *Am J Trop Med Hyg*. 2016;95(6 Suppl):15-34. doi: 10.4269/ajtmh.16-0141
6. Hupalo DN, Luo Z, Melnikov A, Sutton PL, Rogov P, Escalante A, Vallejo AF, Herrera S, Arévalo-Herrera M, Fan Q, Wang Y, Cui L, Lucas CM, Durand S, Sanchez JF, Baldeviano GC, Lescano AG, Laman M, Barnadas C, Barry A, Mueller I, Kazura JW, Eapen A, Kanagaraj D, Valecha N, Ferreira MU, Roobsoong W, Nguitragool W, Sattabonkot J, Gamboa D, Kosek M, Vinetz JM, González-Cerón L, Birren BW, Neafsey DE, Carlton JM. Population genomics studies identify signatures of global dispersal and drug resistance in *Plasmodium vivax*. Nat Genet. 2016;48(8):953-8. doi: 10.1038/ng.3588
7. World Health Organization. World Malaria Report 2019. Geneva: WHO; 2019.
8. Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy. N Engl J Med. 1976;295(6):302-4. doi: 10.1056/NEJM197608052950602
9. Twohig KA, Pfeffer DA, Baird JK, Price RN, Zimmerman PA, Hay SI, Gething PW, Battle KE, Howes RE. Growing evidence of *Plasmodium vivax* across malaria-endemic Africa. PLoS Negl Trop Dis. 2019;13(6):e0007525. doi: 10.1371/journal.pntd.0007525

10. Gunalan K, Niangaly A, Thera MA, Doumbo OK, Miller LH. *Plasmodium vivax* infections of Duffy-negative erythrocytes. Historically undetected or a recent adaptation? Trends Parasitol. 2018;34(5):420-9. doi: 10.1016/j.pt.2018.02.006
11. Zimmerman PA. *Plasmodium vivax* infection in Duffy-negative people in Africa. Am J Trop Med Hyg. 2017;97(3):636-8. doi: 10.4269/ajtmh.17-0461
12. Baldari M, Tamburro A, Sabatinelli G, Romi R, Severini C, Cuccagna G, Fiorilli G, Allegri MP, Buriani C, Toti M. Malaria in Maremma, Italy. Lancet. 1998;351(9111):1246-7. doi: 10.1016/S0140-6736(97)10312-9
13. Armengaud A, Legros F, Quatresous I, Barre H, Valayer P, Fanton Y, D'Ortenzio E, Schaffner F. A case of autochthonous *Plasmodium vivax* malaria, Corsica, August 2006. Travel Med Infect Dis. 2008;6(1-2):36-40. doi: 10.1016/j.tmaid.2007.09.042
14. Santa-Olalla Peralta P, Vazquez-Torres MC, Latorre-Fandós E, Mairal-Claver P, Cortina-Solano P, Puy-Azón A, Adiego Sancho B, Leitmeyer K, Lucientes-Curdi J, Sierra-Moros MJ. First autochthonous malaria case due to *Plasmodium vivax* since eradication, Spain, October 2010. Euro Surveill. 2010;15(41):pii=19684.
15. Eurosurveillance editorial team. World Health Organization announces European Region malaria free. Euro Surveill. 2016;21(17):pii=30216. doi: 10.2807/1560-7917.ES.2016.21.17.30216
16. Andriopoulos P, Economopoulou A, Spanakos G, Assimakopoulos G. A local outbreak of autochthonous *Plasmodium vivax* malaria in Laconia, Greece – a re-emerging infection in the southern borders of Europe? Int J Infect Dis. 2013;17(2):e125-e128. doi: 10.1016/j.ijid.2012.09.009
17. Ponçon N, Tran A, Toty C. A quantitative risk assessment approach for mosquito-borne diseases: malaria re-emergence in southern France. Malar J. 2008;7:147. doi: 10.1186/1475-2875-7-147
18. Romi R, Boccolini D, Vallorani R, Severini F, Toma L, Cocchi M, Tamburro A, Messeri G, Crisci A, Angeli L, Costantini R, Raffaelli I, Pontuale G, Thiéry I, Landier A, Le Goff G, Fausto AM, Di Luca M. Assessment of the risk of malaria re-introduction in the Maremma plain (Central Italy) using a multi-factorial approach. Malar J. 2012;11:98. doi: 10.1186/1475-2875-11-98
19. European Centre for Disease Prevention and Control. Multiple reports of locally-acquired malaria infections in the EU – 20 September 2017. Stockholm: ECDC; 2017.
20. Rieckmann KH, Davis DR, Hutton DC. *Plasmodium vivax* resistance to chloroquine? Lancet. 1989;2(8673):1183-4. doi: 10.1016/s0140-6736(89)91792-3
21. Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant Plasmodium vivax: a systematic review and meta-analysis. Lancet Infect Dis. 2014;14(10):982-91. doi: 10.1016/S1473-3099(14)70855-2
22. World Health Organization. Guidelines for the Treatment of Malaria. 3rd edition. Annex 6, Treatment of *Plasmodium vivax, P. ovale, P. malariae* and *P. knowlesi* infections. Geneva: WHO; 2015. Available from: www.ncbi.nlm.nih.gov/books/NBK294430/.
23. Lacerda MVG, Llanos-Cuentas A, Krudsood S, Lon C, Saunders DL, Mohammed R, Yilma D, Batista Pereira D, Espino FEJ, Mia RZ, Chuquiyauri R, Val F, Casapía M, Monteiro WM, Brito MAM, Costa MRF, Buathong N, Noedl H, Diro E, Getie S, Wubie KM, Abdissa A, Zeynudin A, Abebe C, Tada MS, Brand F, Beck HP, Angus B, Duparc S, Kleim JP, Kellam LM, Rousell VM, Jones SW, Hardaker E, Mohamed K, Clover DD, Fletcher K, Breton JJ, Ugwuegbulam CO, Green JA, Koh GCKW. Single-dose tafenoquine to prevent relapse of *Plasmodium vivax* malaria. N Engl J Med. 2019;380(3):215-28. doi: 10.1056/NEJMoa1710775
24. Price RN, Commons RJ, Battle KE, Thriemer K, Mendis K. New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. Trends Parasitol. 2020; 6:560-70.
25. Whitby M, Wood G, Veenendaal JR, Rieckmann K. Chloroquine-resistant *Plasmodium vivax*. Lancet. 1989;2(8676):1395. doi: 10.1016/s0140-6736(89)92002-3
26. Baird JK, Basri H, Purnomo, Bangs MJ, Subianto B, Patchen LC, Hoffman SL. Resistance to chloroquine by *Plasmodium vivax* in Irian Jaya, Indonesia. Am J Trop Med Hyg. 1991;44:547-52. doi: 10.4269/ajtmh.1991.44.547
27. World Health Organization. Global report on antimalarial efficacy and drug resistance: 2000-2010. Geneva: WHO; 2010.
28. Gonçalves LA, Cravo P, Ferreira MU. Emerging *Plasmodium vivax* resistance to chloroquine in South America: an overview. Mem Inst Oswaldo Cruz. 2014;109(5):534-9. doi: 10.1590/0074-0276130579
29. Visser BJ, Wieten RW, Kroon D, Nagel IM, Bélard S, van Vugt M, Grobusch MP. Efficacy and safety of artemisinin combination therapy (ACT) for non-*falciparum* malaria: a systematic review. Malar J. 2014;13:463. doi: 10.1186/1475-2875-13-463
30. Tavul L, Hetzel MW, Teliki A, Walsh D, Kiniboro B, Rare L, Pulford J, Siba PM,Karl S, Makita L, Robinson L, Kattenberg JH, Laman M, Oswyn G, Mueller I. Efficacy of artemether-lumefantrine and dihydroartemisinin-piperaquine for the treatment of uncomplicated malaria in Papua New Guinea. Malar J. 2018;17(1):350. doi: 10.1186/s12936-018-2494-z
31. Sinclair D, Gogtay N, Brand F, Olliaro P. Artemisinin-based combination therapy for treating uncomplicated *Plasmodium vivax* malaria. Cochrane Database Syst Rev. 2011 Jul 6;(7):CD008492. doi: 10.1002/14651858.CD008492.pub2
32. Pukrittayakamee S, Chantra A, Simpson JA, Vanijanonta S, Clemens R, Looareesuwan S, White NJ. Therapeutic responses to different antimalarial drugs in *vivax* malaria. Antimicrob Agents Chemother. 2000;44(6):1680-5. doi: 10.1128/aac.44.6.1680-1685.2000
33. Eibach D, Ceron N, Krishnalall K, Carter K, Bonnot G, Bienvenu AL, Picot S. Therapeutic efficacy of artemether-lumefantrine for *Plasmodium vivax* infections in a prospective study in Guyana. Malar J. 2012;11:347. doi: 10.1186/1475-2875-11-347
34. Bassat Q. The use of artemether-lumefantrine for the treatment of uncomplicated *Plasmodium vivax* malaria. PLoS Negl Trop Dis. 2011;5(12):e1325. doi: 10.1371/journal.pntd.0001325
35. Senn N, Rarau P, Manong D, Salib M, Siba P, Reeder JC, Rogerson SJ, Genton B, Mueller I. Effectiveness of artemether/lumefantrine for the treatment of uncomplicated *Plasmodium vivax* and *P. falciparum* malaria in young children in Papua New Guinea. Clin Infect Dis. 2013;56(10):1413-20. doi:10.1093/cid/cit068.
36. Pukrittayakamee S, Vanijanonta S, Chantra A, Clemens R, White NJ. Blood stage antimalarial efficacy of primaquine in *Plasmodium vivax* malaria. J Infect Dis. 1994;169(4):932-5. doi: 10.1093/infdis/169.4.932
37. Smithuis F, Kyaw MK, Phe O, Win T, Aung PP, Oo AP, Naing AL, Nyo MY, Myint NZ, Imwong M, Ashley E, Lee SJ, White NJ. Effectiveness of five artemisinin com-

bination regimens with or without primaquine in uncomplicated *falciparum* malaria: an open-label randomised trial. Lancet Infect Dis. 2010;10(10):673-81. doi: 10.1016/S1473-3099(10)70187-0

38. Commons RJ, Simpson JA, Thriemer K, Hossain MS, Douglas NM, Humphreys GS, Sibley CH, Guerin PJ, Price RN. Risk of Plasmodium vivax parasitaemia after *Plasmodium falciparum* infection: a systematic review and meta-analysis. Lancet Infect Dis. 2019;19(1):91-101. doi: 10.1016/S1473-3099(18)30596-6
39. Rijal KR, Adhikari B, Ghimire P, Banjara MR, Das Thakur G, Hanboonkunupakarn B, Imwong M, Chotivanich K, Day NPJ, White NJ, Pukrittayakamee S. Efficacy of primaquine in preventing short- and long-latency *Plasmodium vivax* relapses in Nepal. J Infect Dis. 2019;220(3):448-56. doi: 10.1093/infdis/jiz126
40. Douglas NM, Anstey NM, Angus BJ, Nosten F, Price RN. Artemisinin combination therapy for *vivax* malaria. Lancet Infect Dis. 2010;10(6):405-16. doi: 10.1016/S1473-3099(10)70079-7
41. Baird JK. Resistance to therapies for infection by *Plasmodium vivax*. Clin Microbiol Rev. 2009;22(3):50834. doi: 10.1128/CMR.00008-09
42. Borlon C, Russell B, Sriprawat K, Suwanarusk R, Erhart A, Renia L, Nosten F, D'Alessandro U. Cryopreserved *Plasmodium vivax* and cord blood reticulocytes can be used for invasion and short term culture. Int J Parasitol. 2012;42(2):155-60. doi: 10.1016/j.ijpara.2011.10.011
43. Udomsangpetch R, Kaneko O, Chotivanich K, Sattabongkot J. Cultivation of *Plasmodium vivax*. Trends Parasitol. 2008;24(2):85-88. doi: 10.1016/j.pt.2007.09.010
44. Roobsoong W, Tharinjaroen CS, Rachaphaew N, Chobson P, Schofield L, Cui L, Adams JH, Sattabongkot J. Improvement of culture conditions for long-term *in vitro* culture of *Plasmodium vivax*. Malar J. 2015;14:297. doi: 10.1186/s12936-015-0815-z
45. Rangel GW, Clark MA, Kanjee U, Lim C, Shaw-Saliba K, Menezes MJ, Mascarenhas A, Chery L, Gomes E, Rathod PK, Ferreira MU, Duraisingh MT. Enhanced *ex vivo Plasmodium vivax* intraerythrocytic enrichment and maturation for rapid and sensitive parasite growth assays. Antimicrob Agents Chemother. 2018;62(4):e02519-17. doi: 10.1128/AAC.02519-17
46. Chaorattanakawee S, Lon C, Chann S, Thay KH, Kong N, You Y, Sundrakes S, Thamnurak C, Chattrakarn S, Praditpol C, Yingyuen K, Wojnarski M, Huy R, Spring MD, Walsh DS, Patel JC, Lin J, Juliano JJ, Lanteri CA, Saunders DL. Measuring *ex vivo* drug susceptibility in *Plasmodium vivax* isolates from Cambodia. Malar J. 2017;16(1):392. doi: 10.1186/s12936-017-2034-2
47. Noulin F, Borlon C, Van Den Abbeele J, D'Alessandro U, Erhart A. 1912-2012: a century of research on *Plasmodium vivax in vitro* culture. Trends Parasitol. 2013;29(6):286-94. doi: 10.1016/j.pt.2013.03.012
48. Nomura T, Carlton JM, Baird JK, del Portillo HA, Fryauff DJ, Rathore D, Fidock DA, Su X, Collins WE, McCutchan TF, Wootton JC, Wellems TE. Evidence for different mechanisms of chloroquine resistance in 2 Plasmodium species that cause human malaria. J Infect Dis. 2001;183(11):1653-61. doi: 10.1086/320707
49. Sá JM, Yamamoto MM, Fernandez-Becerra C, de Azevedo MF, Papakrivos J, Naudé B, Wellems TE, Del Portillo HA. Expression and function of pvcrt-o, a *Plasmodium vivax* ortholog of pfcrt, in *Plasmodium falciparum* and *Dictyostelium discoideum*. Mol Biochem Parasitol. 2006;150(2):219-28. doi: 10.1016/j.molbiopara
50. Sá JM, Kaslow SR, Moraes Barros RR, Brazeau NF, Parobek CM, Tao D, Salzman RE, Gibson TJ, Velmurugan S, Krause MA, Melendez-Muniz V, Kite WA, Han PK, Eastman RT, Kim A, Kessler EG, Abebe Y, James ER, Chakravarty S, Orr-Gonzalez S, Lambert LE, Engels T, Thomas ML, Fasinu PS, Serre D, Gwadz RW, Walker L, DeConti DK, Mu J, Bailey JA, Sim BKL, Hoffman SL, Fay MP, Dinglasan RR, Juliano JJ, Wellems TE. *Plasmodium vivax* chloroquine resistance links to pvcrt transcription in a genetic cross. Nat Commun. 2019;10(1):4300. doi: 10.1038/s41467-019-12256-9
51. Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, Patel R, Laing K, Looareesuwan S, White NJ, Nosten F, Krishna S. Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number. Lancet. 2004;364(9432):438-47. doi: 10.1016/S0140-6736(04)16767-6
52. Sidhu AB, Uhlemann AC, Valderramos SG, Valderramos JC, Krishna S, Fidock DA. Decreasing pfmdr1 copy number in *Plasmodium falciparum* malaria heightens susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin. J Infect Dis. 2006;194(4):528-35. doi:10.1086/507115
53. Brega S, Meslin B, de Monbrison F, Severini C, Gradoni L, Udomsangpetch R, Sutanto I, Peyron F, Picot S. Identification of the *Plasmodium vivax* mdr-like gene (*pv*mdr1) and analysis of single-nucleotide polymorphisms among isolates from different areas of endemicity. J Infect Dis. 2005;191(2):272-7. doi: 10.1086/426830
54. Sá JM, Nomura T, Neves Jd, Baird JK, Wellems TE, del Portillo HA. *Plasmodium vivax*: allele variants of the mdr1 gene do not associate with chloroquine resistance among isolates from Brazil, Papua, and monkey-adapted strains. Exp Parasitol. 2005;109(4):256-9. doi: 10.1016/j.exppara.2004.12.005
55. Picot S, Brega S, Gérôme P, et al. Absence of nucleotide polymorphism in a *Plasmodium vivax* multidrug resistance gene after failure of mefloquine prophylaxis in French Guyana. Trans R Soc Trop Med Hyg. 2005;99(3):234-7. doi:10.1016/j.trstmh.2004.09.007
56. Suwanarusk R, Russell B, Chavchich M, Chalfein F, Kenangalem E, Kosaisavee V, Prasetyorini B, Piera KA, Barends M, Brockman A, Lek-Uthai U, Anstey NM, Tjitra E, Nosten F, Cheng Q, Price RN. Chloroquine resistant *Plasmodium vivax: in vitro* characterisation and association with molecular polymorphisms. PLoS One. 2007;2(10):e1089. doi: 10.1371/journal.pone.0001089
57. Sow F, Bonnot G, Ahmed BR, Diagana SM, Kebe H, Koita M, Samba BM, Al-Mukhaini SK, Al-Zadjali M, Al-Abri SS, Ali OA, Samy AM, Hamid MM, Ali Albsheer MM, Simon B, Bienvenu AL, Petersen E, Picot S. Genetic diversity of *Plasmodium vivax* metacaspase 1 and *Plasmodium vivax* multi-drug resistance 1 genes of field isolates from Mauritania, Sudan and Oman. Malar J. 2017;16(1):61. doi: 10.1186/s12936-017-1687-1
58. Imwong M, Pukrittakayamee S, Looareesuwan S, Pasvol G, Poirreiz J, White NJ, Snounou G. Association of genetic mutations in *Plasmodium vivax* dhfr with resistance to sulfadoxine-pyrimethamine: geographical and clinical correlates. Antimicrob Agents Chemother. 2001;45(11):3122-7. doi: 10.1128/AAC.45.11.3122-3127.2001
59. Tjitra E, Baker J, Suprianto S, Cheng Q, Anstey NM. Therapeutic efficacies of artesunate-sulfadoxine-pyrimethamine and chloroquine-sulfadoxine-pyrimethamine in *vivax* malaria pilot studies: relationship to *Plasmodium vivax* dhfr mutations. Antimicrob Agents Chemother. 2002;46(12):3947-53. doi: 10.1128/aac.46.12.3947-3953.2002
60. de Pécoulas PE, Tahar R, Ouatas T, Mazabraud A, Basco

LK. Sequence variations in the *Plasmodium vivax* dihydrofolate reductase-thymidylate synthase gene and their relationship with pyrimethamine resistance. Mol Biochem Parasitol. 1998;92(2):265-73. doi: 10.1016/s0166-6851(97)00247-8

61. Marfurt J, de Monbrison F, Brega S, Barbollat L, Müller I, Sie A, Goroti M, Reeder JC, Beck HP, Picot S, Genton B. Molecular markers of *in vivo Plasmodium vivax* resistance to amodiaquine plus sulfadoxine-pyrimethamine: mutations in pvdhfr and pvmdr1. J Infect Dis. 2008;198(3):409-17. doi: 10.1086/589882
62. Menegon M, Majori G, Severini C. Genetic variations of the *Plasmodium vivax* dihydropteroate synthase gene. Acta Trop. 2006;98:196-9.
63. Imwong M, Pukrittayakamee S, Cheng Q, Moore C, Looareesuwan S, Snounou G, White NJ, Day NP. Limited polymorphism in the dihydropteroate synthetase gene (dhps) of *Plasmodium vivax* isolates from Thailand. Antimicrob Agents Chemother. 2005;49(10):4393-5. doi: 10.1128/AAC.49.10.4393-4395.2005
64. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, Kim S, Duru V, Bouchier C, Ma L, Lim P, Leang R, Duong S, Sreng S, Suon S, Chuor CM, Bout DM, Ménard S, Rogers WO, Genton B, Fandeur T, Miotto O, Ringwald P, Le Bras J, Berry A, Barale JC, Fairhurst RM, Benoit-Vical F, Mercereau-Puijalon O, Ménard D. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;505(7481):50-5. doi: 10.1038/nature12876
65. Popovici J, Kao S, Eal L, Bin S, Kim S, Ménard D. Reduced polymorphism in the Kelch propeller domain in *Plasmodium vivax* isolates from Cambodia. Antimicrob Agents Chemother. 2015;59(1):730-3. doi:10.1128/AAC.03908-14
66. Wang M, Siddiqui FA, Fan Q, Luo E, Cao Y, Cui L. Limited genetic diversity in the *Pv*K12 Kelch protein in *Plasmodium vivax* isolates from Southeast Asia. Malar J. 2016;15(1):537. doi: 10.1186/s12936-016-1583-0
67. Duanguppama J, Mathema VB, Tripura R, Day NPJ, Maxay M, Nguon C, von Seidlein L, Dhorda M, Peto TJ, Nosten F, White NJ, Dondorp AM, Imwong M. Polymorphisms in *Pv*kelch12 and gene amplification of *Pv*plasmepsin4 in *Plasmodium vivax* from Thailand, Lao PDR and Cambodia. Malar J. 2019;18(1):114. doi: 10.1186/s12936-019-2749-3
68. Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE, Baird JK, et al. Global epidemiology of *Plasmodium vivax*. Am J Trop Med Hyg. 2016;95:15-34
69. Fernando D, Rodrigo C, Rajapakse S. Primaquine in *vivax* malaria: an update and review on management issues. Malar J. 2011;10:351. doi:10.1186/1475-2875-10-351
70. Dijanic C, Nickerson J, Shakya S, Dijanic A, Fabbri M. Relapsing malaria: a case report of primaquine resistance. Case Rep Infect Dis. 2018;2018:9720823. doi: 10.1155/2018/9720823
71. Huldén L, Huldén L, Heliövaara K. Natural relapses in *vivax* malaria induced by *Anopheles mosquitoes*. Malar J. 2008;7:64. doi: 10.1186/1475-2875-7-64
72. Hounkpatin AB, Kreidenweiss A, Held J. Clinical utility of tafenoquine in the prevention of relapse of *Plasmodium vivax* malaria: a review on the mode of action and emerging trial data. Infect Drug Resist. 2019;12:553-70. doi:10.2147/IDR.S151031
73. Medicines for Malaria Venture. MMV Annual Report 2016. Medicines for Malaria Venture. Published June 14, 2018. Available from: www.mmv.org/newsroom/publications/mmv-annual-report-2016.

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Physical activity as a tool for health promotion: the evolution of international strategies and interventions

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Abstract
Physical activity (PA) has a great potential impact in the prevention and control of non-communicable diseases. However, epidemiologic data reporting a high percentage of inactive people, still indicate a scarce perception of PA benefits. Therefore, in the past decades, a number of documents has been produced by international organizations with the aim of changing policies and institutional actions towards the promotion of PA. Several actions have been put in place and an evolution process in international strategies for PA promotion is ongoing. Nevertheless, there is a need to continue updating these policies in light of new knowledge about evidence-based PA health effects. A stimulating discussion about effective PA promotion programs is useful for future planning of interventions. The aim of this work is to report the evolution of international strategies aimed to PA promotion, from early PA recommendations, to the recent WHO Global Action Plan on Physical Activity 2018-2030.

Key words
- physical activity
- health promotion
- non-communicable diseases
- prevention
- recommendations

INTRODUCTION

Physical activity (PA) is defined as "any bodily movement produced by skeletal muscles that require energy expenditure" [1], including that done during leisure time, for transport or work. The term "PA" should not be confused with "exercise", which is "a subcategory of PA that is planned, structured, repetitive, and aims to improve or maintain one or more components of physical fitness" [2, 3].

Physical inactivity is instead defined as "not meeting the applicable physical activity World Health Organization (WHO) recommendations" [2]. In recent years, a consistent body of epidemiological evidence proved that physical inactivity is a risk factor for the major non-communicable diseases (NCDs) responsible for premature death and disability in western countries [4, 5]. Moreover, sedentary behavior is defined as "any waking behavior characterized by an energy expenditure ≤1.5 metabolic equivalents" [6]. People's sedentary behavior has been described as the fourth leading risk factor for death in the world [7]. In recent years, especially among children and young people, PA has been replaced by more sedentary habits, and in adults and the elderly the opportunities to be active tend progressively to decrease [8].

On the contrary, it is widely recognized that PA is a main tool for the prevention of non-communicable diseases and the improvement of psycho-physical well-being and quality of life, for both sexes and at every age *(Table 1)*. Moreover, physically active populations tend to live longer than inactive ones. As a consequence of all these benefits, PA may reduce direct and indirect costs deriving from a sedentary lifestyle [9]. This is of particular relevance, if considering the population ageing occurring in developed countries, that is determining a growing burden of chronic diseases [10]. However, there is evidence that anyone who increases their level of physical activity, even after long periods of inactivity, can obtain health benefits irrespective of their age. Therefore, the WHO, the European Union (EU), and National Governments in recent years have directed their policies towards the promotion of an active lifestyle. Increasing the PA level in the population requires a whole of society and culturally relevant approach and therefore demands a collective effort across different sectors and disciplines [11]. Indeed, PA promotion is important not only at the individual level, but also for the entire society, and a multi-disciplinary and multi-sectorial approach is needed.

The best strategy is to act primarily on both individual and collective behaviors with legislative and regulatory interventions and/or health education. In this sense, the

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Table 1
Health effects related to physical activity as described by health-related organisations (Health Promotion and Disease Prevention Knowledge Gateway, at https://ec.europa.eu/jrc/en/health-knowledge-gateway/promotion-prevention/physical-activity)

Effect	Benefit
Cardiovascular health	Reduced risk of cardiovascular and coronary heart disease, and stroke Prevention of arterial hypertension and better control of arterial blood pressure in high blood pressure suffering individuals Reduction of LDL- and not HDL-cholesterol
Cardiorespiratory health	Good cardio-pulmonary function
Diabetes 2	Reduced risk of diabetes 2
Body weight and energy intake	Increased fat utilization, weight control and less risk of obesity Healthier body mass and composition
Mental health and cognitive performance	Maintenance of cognitive functions and lowered risk of depression and dementia Lower stress levels and associated improved sleep quality Improved self-esteem and optimism Reduction of anxiety disorders Decrease of feelings of fatigue
Musculoskeletal function	Improved bone mineralization. Prevention of bone fractures and osteoporosis Improvement in muscular strength, walking speed, muscular fitness and balance Better capacity in daily living activities. Lower risk of falling
Risk of cancer	Lowered risk of breast, prostate, colon, endometrial and bladder cancer
All cause mortality and premature death	All-cause mortality is delayed by regularly engaging in physical activity
Other health effects	Improved digestion and regulation of intestinal rhythm
General quality of life	Decreased absenteeism from work Delaying of chronic illnesses associated with ageing

concept of "prevention" is in line with that of "health promotion" [12].

Several actions have been put in place and an evolution process in international strategies for PA promotion is ongoing. Nevertheless, there is a need to continue updating these policies in light of new knowledge about evidence-based PA health effects.

In this review, we analyze the steps in the evolution of international strategies aimed to PA promotion, from early PA recommendations, to the recent WHO Global Action Plan on Physical Activity, retracing the history of strategies and programs, as well as the legislative evolution and intervention projects *(Figure 1)*.

A stimulating discussion about effective PA promotion programs can be useful for future planning of interventions.

PHYSICAL ACTIVITY AND HEALTH PROMOTION

In 1948, the WHO defined health as "(...) a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity" [13]. This definition introduces and places for the first time the emphasis on cultural and psychic factors, including them in the meaning of "health", and highlighting the role of social and political environment. In this way, health represents a global, social and political process, aimed at changing social, economic and environmental conditions, in such a way that the impact of risk factors on public and individual health is reduced. The subsequent Alma Ata Declaration on Primary Health Care [14] gave rise to a different and new perspective on public health, drawing attention to the importance of prevention in national and international health policies, as well as within the national health systems.

The first and most important document for the "Health Promotion" was the Ottawa Charter, elaborated in 1986 in the context of the First International Health Promotion Conference, that defined health promotion as "… the process of enabling people to increase control over, and to improve, their health" [12]. The same document also shows how health promotion is based on concrete and effective community actions to achieve a better health status and focuses on defining priorities, making decisions, planning strategies and implementing them.

A further step in this direction was made in 1997 by the 4th International Conference on Health Promotion of Jakarta, entitled "New players for a new era: leading health promotion into the 21st century". The Jakarta Declaration [15] highlighted the need of using a wider range of resources to address the issue of health determinants in the 21st century, as it is essential that health promotion evolves to cope with social changes.

An important part of the Jakarta Declaration was the "Call for Action", in which the participants to the Conference were invited to share its key messages with their governments, institutions and communities, and to put the proposed actions into practice. The "Health Promotion" concept developed by WHO aims to answer to two main questions: how is health created? How to keep yourself healthy for as long as possible? Therefore, it works to motivate people to choose a healthy lifestyle in a conscious and autonomous way. In this context, PA will be one of the tools chosen and promoted to achieve this result.

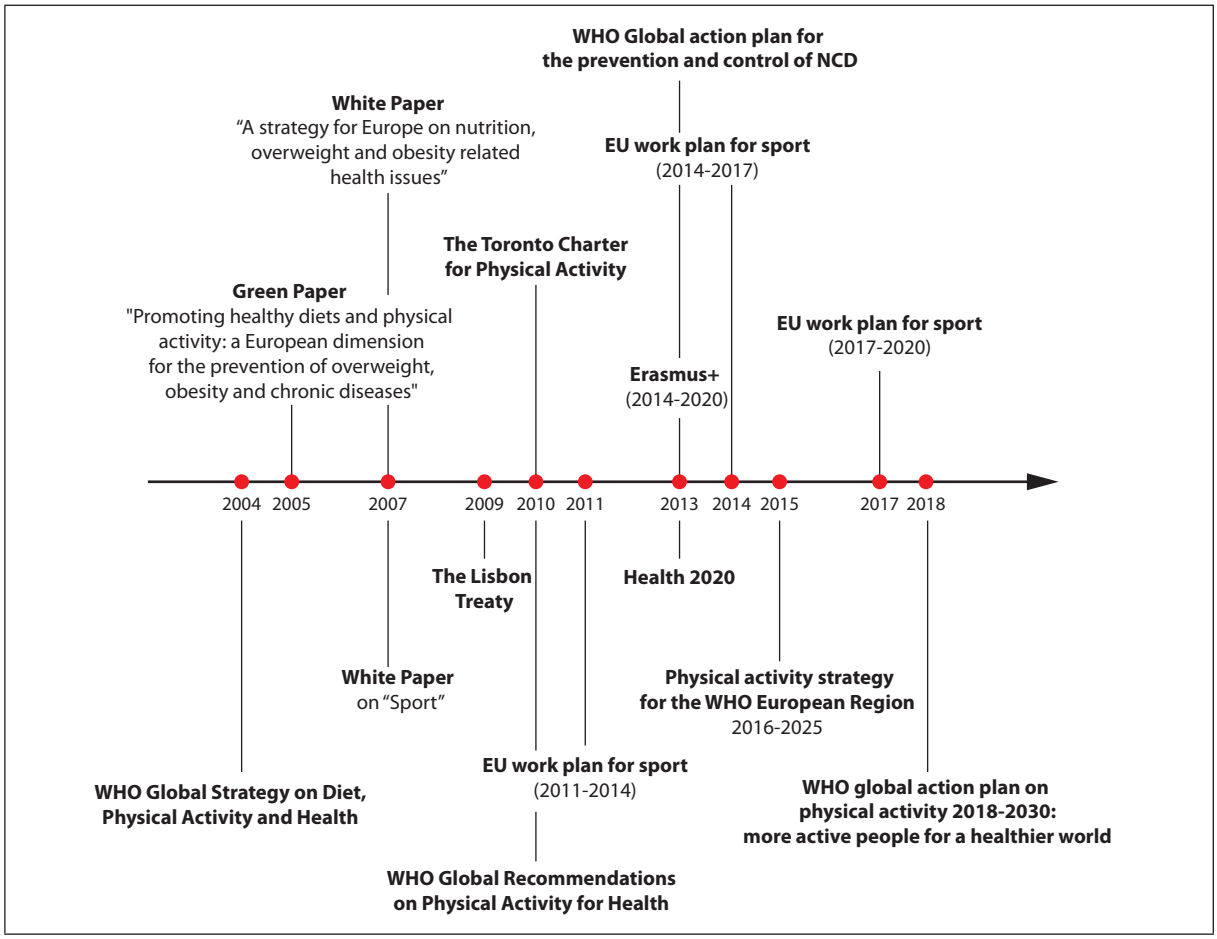

Figure 1
Evolution of international strategies and interventions for health promotion through physical activity.
WHO: World Health Organization; EU: European Union; NCD: non-communicable diseases.

The implementation of policies for health promotion through PA relies both on recommendations about type and dose of activity providing health benefits for people, and monitoring about PA levels in the population, as starting point for promoting initiatives.

Physical activity guidelines

The recognition of the functional risks of a sedentary lifestyle has led to the dissemination of numerous recommendations and guidelines about PA levels useful to improve public health.

The attention of scientific research on the relationship between exercise or PA and health began only in the second half of the 20th century and included two main relevant research fields: a) epidemiological studies, in which the relationship between PA and health outcome is assessed, and b) exercise training studies in a controlled laboratory environment, in which the potential of aerobic and/or strength exercise is the physiological variable taken into consideration [16].

The first systematic investigation on the health risks, with specific concern to coronary heart disease (CHD), associated with a sedentary lifestyle at work or during leisure time was conducted by Morris and coworkers [17]. Since that, hundreds of reports have been published in scientific peer-reviewed literature proving health risks determined by a physically inactive lifestyle, and advantages of PA practice [18, 19].

A limit of epidemiologic studies carried out until the mid-1980s was that data collected about PA levels were self-assessed, thus the inaccuracy linked to this type of data collection made difficult to measure the exact dose of exercise, in terms of type, quantity and intensity, associated with the observed health benefits. Subsequent studies used the cardiorespiratory fitness as more objective index to evaluate the relationship between exercise "dose" and health benefits [20-25]. Other investigators conducted controlled training studies providing quantification of the exercise dose needed to improve physical work capacity. This research flows provided the scientific basis for the recommendations: epidemiological research was used for the development of health-related activity guidelines, while exercise training research to quantify the frequency, intensity and duration of recommended PA. The American College of Sports Medicine (ACSM) and the American Heart Association (AHA) were the first to provide recommendations on specific exercises for clinical and rehabilitative medicine [26-33]. However, early guidelines and recommendations were based primarily on endurance exercise to enhance performance,

especially aerobic capacity. In subsequent years, results from large epidemiological studies showed benefits of moderate-intensity activities of daily living: quantity and quality of exercise needed to attain health related benefits may differ from that recommended for fitness benefits. Thus, a subsequent document from the Centers for Disease Control and Prevention (CDC)/ACSM [34] gave more specific indications, suggesting the practice of ≥30 minutes of moderate intensity PA each day. This report contains the most widely known evidence-based PA recommendations for public health, which were adopted by many other authorities worldwide, like the National Institutes of Health [35] and the WHO [36].

Since then, other specific recommendation of PA to control weight gain followed, like those by the IOM (Institute of Medicine) Committee on Dietary Reference Intakes [37] and the International Association for the Study of Obesity [38].

More recently, new recommendations based on updated scientific evidence have been published, such those from the US Physical Activity Guidelines Advisory Committee [39], the AHA/ACSM for adults and older adults [40], the US Department of Health and Human Services (US DHHS) [41], that contain specific guidelines for young people, people with disabilities, pregnant and postpartum women, and the WHO "Global Recommendations on Physical Activity for Health" [2], addressing three age groups: 5-17, 18-64 and 65 years old and above. All these documents introduced some differences respect to the first ACSM reports: recommendations are specific for target groups; some vigorous exercise and also muscle- and bone-strengthening activities are included; PA time is indicated as total weekly activity time (150 min per week).

Now, Physical Activity Guidelines have been published in several countries, as well as in the context of the WHO. The WHO's documents, that are based on the most recent scientific evidence, focus on PA as a tool for population-based primary prevention.

In Europe, the EU Physical Activity Guidelines were published in 2008 and several EU Member States have national PA Guidelines which help government agencies and private bodies to work together in order to promote PA [42].

At the moment, recommendations are oriented to suggest moderate-intensity activities, planned as total weekly PA, and targeted to the whole population and to specific target groups. Thus, providing evidence-based information about the relationship between PA and health, they represent the basis for strategies and policies of PA promotion at national and regional level [43].

Data collection and monitoring

The surveillance of population levels of PA using a standardized protocol is an important and necessary starting point in PA promotion policies. This kind of investigation is generally carried out through questionnaires, that are inexpensive and easy to administer, such as the Global Physical Activity Questionnaire (GPAQ, www.who.int/ncds/surveillance/steps/GPAQ_EN.pdf) developed by the WHO about a decade ago.

In Europe, the first manifestations of interest for studies and research on sports participation appeared in the '70s, when the sport began to be included in welfare policies. These early investigations examined the socio-economic and demographic characteristics of the participants, the modalities of participation, the reasons for sport practice or inactivity.

People participation in sport activities ("sport for all") reached a peak in the '80s and was monitored both at European and extra-European level. Subsequently, a new approach for measuring sports participation was designed, with the aim of harmonizing data collection between countries: the Compass project (Co-Ordinated Monitoring of Participation in Sports) [44, 45] and the subsequent Eurobarometer, a service through which the European Commission measures and analyzes the trends of public opinion in all Member States (MSs) in order to better prepare legislative proposals, to make decisions and to evaluate the EU work. The first Special Eurobarometer Physical Activity [46] evaluated PA in the Member States (MSs) using the "International Physical Activity Questionnaire" (IPAQ). The IPAQ analyzed frequency, duration and intensity level of respondents' PA over the last 7 days, as well as the context in which they were physically active and the perception of environmental and local opportunities that favor the practice of PA. Several Eurobarometers on sport and PA were produced in the following years [47-50].

However, data collected in recent years are not encouraging. The WHO estimated that, at a global level, 25% of adults is not sufficiently active and 80% of adolescents do not reach recommended levels of PA (www.who.int/news-room/fact-sheets/detail/physical-activity). Also, in the EU nearly half (46%) of Europeans never exercise or play sport, and that proportion has increased gradually since 2009. Only 7% exercise regularly (at least five times per week), and a high proportion of adults in Europe spend more than 5 h/day sitting [50]. Lack of time is the principal barrier for those with a sedentary behavior, but there are conflicting opinions among the states on the fact that local authorities do not enough provide their citizens with appreciable opportunities (39%). Finally, it should be noted that the survey observed a good perception of sport as a benefit to physical and mental health, reflecting citizen awareness of the role that PA plays in the prevention/treatment of several disease.

In Italy PA levels are monitored continuously through the following national surveillance systems, promoted by the Ministry of Health and led by the *Istituto Superiore di Sanità*: *Okkio alla salute* (Child Obesity Surveillance Initiative) collects data on children aged 8-9 years; The Health Behaviour in School-aged Children (HBSC) survey collects data on adolescents aged 11, 13 and 15 years; *Progressi delle Aziende Sanitarie per la Salute in Italia* (PASSI) collects data on adults aged 18-69 years. Additional data are collected by the National Institute for Statistics [51, Italy Physical Activity Factsheet]. According to these data, the estimated prevalence of sufficient PA levels is 82% for children 8-9 years old, 11% for adolescents 11-13 years old and 31% for people aged 18-69 years.

MAIN POLICIES AND INTERNATIONAL PROGRAMMES

During last years, WHO published several documents and gave a series of suggestions to guide policies towards PA promotion through an intersectoral approach. Moreover, to support member states, WHO set up partnerships with various organizations, the United Nations Organization for Education, Science and Culture (UNESCO) and the United Nations Office on Sport for Development and Peace (UNOSDP).

In 2004, in consideration of the dramatic increase of chronic degenerative diseases and obesity, WHO approved the Global Strategy on Diet, Physical Activity and Health [52], which aimed to design and suggest a worldwide interventional program to improve the situation. After this, all countries proposed political initiatives to control and promote interventions in line with WHO suggestions. In particular, in 2005 a Green Paper entitled "Promoting healthy diets and physical activity: a European dimension for the prevention of overweight, obesity and chronic diseases" has been published in Europe [53].The Green Paper gave rise to two documents in 2007: the White Paper on "A strategy for Europe on nutrition, overweight, and obesity related health issues" [54], and the White Paper on sport, including the "Pierre de Coubertin Action Plan" [55].

In 2008, the 61st session of the World Health Assembly approved the "Action plan for the global strategy for the prevention and control of NCDs", which is considered a strong worldwide initiative to emphasize the importance of PA as a prevention strategy [56]. In this document physical inactivity is listed as a risk factor for NCDs, together with tobacco consumption and unhealthy diet, and the goal of the global strategy was to reduce the level of exposure to these risk factors and to develop norms and guidelines for interventions to reduce the incidence of NCDs and improve health care. A few years later, the Global Action Plan for the Prevention and Control of NCDs 2013-2020 was also published [57].

These documents show how the promotion of PA is a topic of real interest at the global and at the European level. The WHO and the European Commission are currently involved in promoting and supporting policies that aim at an active lifestyle. One of the most recent results is the "Physical activity strategy for the WHO European Region 2016-2025" (see below) [58].

2005: The Green Paper on "Promoting healthy diets and physical activity: a European dimension for the prevention of overweight, obesity and chronic diseases"

The Green Paper presented by the European Commission in 2005 in Brussels set out various concepts for public debate on the promotion of a healthy lifestyle through proper nutrition and PA [53].

The document first illustrated the epidemiological situation at the European level in that period: incorrect nutrition and lack of PA emerged as main causes of preventable diseases and premature deaths in Europe; the growing prevalence of obesity across Europe was one of the major public health problems. The Council invited the Commission to contribute to the promotion of healthy lifestyles and to develop strategies to improve food habits in the EU, promoting healthy diets and PA. The Council stressed also the need to include issues concerning nutrition and PA in the relevant policies at European level.

Furthermore, a series of initiatives were launched in that period: the European Platform for Action on Diet, Physical Activity and Health, the Network on Nutrition and Physical Activity, and the Public Health Action Program, aimed to support projects to promote healthy eating habits and PA.

A very important part of the Green Paper is the Annex 2: "Relationship between diet, physical activity and health", containing scientific news to support the theme on which the book is based. The relationship between diet, PA and health has been established scientifically, in particular regarding the role of lifestyles as determinants of chronic NCDs and conditions such as obesity, heart disease, type 2 diabetes, hypertension, cancer and osteoporosis [59]. All these inputs, made known worldwide by the "Global Strategy on Diet, Physical Activity and Health" and at the European level by the Green Paper, will be the basis for practical proposals that will be contained in the subsequent White Papers [54, 55].

2007: The White Paper on "A strategy for Europe on nutrition, overweight and obesity related health issues"

The purpose of the first White Paper was to suggest an integrated European approach to improve population health status and decrease illness linked to poor nutrition, obesity and overweight [54]. It stressed the importance of individual responsibility for lifestyles improvement and emphasizes the important role of the public authorities in promoting the increase of PA level, developing a favorable physical and social environment.

Among the various topics addressed, the focus is on the "Encouraging physical activity" section. "The Commission believes that the MSs and the EU must take pro-active steps to reverse the decline in physical activity levels in recent decades (…). The individual's attempt to find ways to increase physical activity in daily life should be supported by the development of a physical and social environment" such as sustainable urban transport actions and walking and cycling projects.

Finally, the Commission ends this part by announcing that it will draw up a White Paper on Sport that will be "aimed at putting forward proposals to boost participation rates for sport in the EU and to extend the sport movement to include physical activity more generally."

2007: The White Paper on "Sport"

The White Paper on Sport was one of the main contributions to the theme of the role of sport in daily life of European population [55]. It contained several proposed actions, which were then brought together in the "Pierre de Coubertin Action Plan". The White Paper on Sport aimed to enhance the social role of PA to reach a social cohesion and inclusion of vulnerable groups; moreover, it aimed to enhance the role of PA in educating and training and to stress the concept of public

health through PA, to increase the rates of participation in sports and to set up a European network to promote sport as a health-enhancing method. The second part of the White Paper focused also on the economic dimension of sport, emphasizing the contribution given by PA as a growth and job creation in the European context. Then, the Commission monitored the progresses of all the initiatives with a structured dialogue in which the sport organizations were involved.

First of all, the indications concerning the minimum levels of PA recommended by the WHO were clarified: minimum 30 minutes of moderate PA per day for adults and 60 minutes for children. Public authorities and private organizations in the MSs should all contribute to achieving this goal, but generally, no progress worthy of note has been shown.

Therefore, the White Paper foresaw the following activities:

a) The Commission proposed to develop new guidelines on PA before the end of 2008. It recommended strengthening cooperation at ministerial level between the health, education and sport sectors in the MSs to define and implement consistent strategies to reduce overweight, obesity and other health risks. In this context, the Commission encouraged MSs to examine how to promote the concept of active life through national education and training systems, including teacher training.
b) The Commission committed itself to supporting a European network of PA for the benefit of health (so-called HEPA activity: Health-Enhancing Physical Activity).
c) The Commission had set itself the goal of making "physical activity for the benefit of health" a reference point for its sports-related activities and sought to better take into account this priority in the relevant financial instruments, which included: the 7th Framework Program for research and technological development lifestyle aspects of health; the public health program 2007-2013; the youth and citizenship programs (cooperation between sports organizations, schools, civil society, parents and other partners at local level); the lifelong learning program (teacher training and cooperation between schools).

2009: The Lisbon Treaty

The 2007 White Paper on Sport and the related "Pierre de Coubertin Action Plan" launched in 2008 were of fundamental importance for laying the foundations for an EU sport policy and also if the treaties do not provide for a specific legal competence in sport matters before 2009, with the Lisbon Treaty the EU has acquired a specific competence in this area [60].

The EU became responsible for developing policies based on concrete elements, as well as promoting cooperation and managing initiatives to support PA and sport in Europe. Article 6 (e) of the Treaty on the Functioning of the European Union (TFEU) stated that the EU has power to carry out actions to support or supplement the action of MSs in the field of sport, while article 165 contains the detailed aspects of sport policy: "The Union shall contribute to the promotion of European sporting issues, while taking account of the specific nature of sport, its structures based on voluntary activity and its social and educational function". Article 165 (2) aims to develop "the European dimension in sport, by promoting fairness and openness in sporting competitions and cooperation between bodies responsible for sports, and by protecting the physical and moral integrity of sportsmen and sportswomen, especially the youngest sportsmen and sportswomen".

Given its many benefits, the attention at European level towards the promotion of PA has grown more and more, and the Lisbon Treaty has provided the legal basis for the EU to require the development of European dimension in sport and the promotion of sports issues at European level.

2010: The Toronto Charter for Physical Activity

In 2010 another fundamental Paper has been published: the "Toronto Charter for Physical Activity: a global call for Action", which raised the awareness of the national, regional and local decision-makers in the importance of promoting PA [61]. The Toronto Charter was written in 2010 by the International Society for Physical Activity and Health (ISPAH), a group of experts from the Global Advocacy for Physical Activity (GAPA), and then integrated in 2011 by "The best investments for Physical Activity" document; it became the worldwide reference text for the promotion of PA and defined a set of individual and social spheres of action in which to invest to obtain a more active and therefore healthier life. From 2011, all the organizations and individuals interested in promoting PA used this Charter to raise awareness and bring together decision-makers at national, regional and local levels in achieving a shared goal. These organizations include the health, transport, environment, sport and leisure time, education and urban planning sectors, as well as public administration, civil society and the private sector.

The Toronto Charter has identified and proposed, based on the most up-to-date scientific evidence, four fundamental action areas:

a) implement a policy and an action plan at national level;

b) introduce policies that support PA (from urban transport to communication campaigns, through sport and education);

c) redirect services and funding to give priority to PA (workplaces, green spaces, health, etc.);

d) develop partnerships for action (intersectoral working groups, collaborations between profit and non-profit organizations, etc.).

The four actions are based on guiding principles, and invite all countries, regions and communities to urge greater political and social commitment to enhance the importance of PA and improve the health of all.

Recommendations and strategies in Italy

Italian strategies are in line with WHO Action plans and the EU policies. All initiatives take into account health determinants conditioning lifestyle and aim to realize effective actions of health promotion with an intersectoral and integrated approach.

Since 2007 the action plan *Guadagnare salute: rendere facili le scelte salutari* (Gaining health: encouraging healthy choices) has been put into practice [62]. The plan is an integral part of the chronic disease prevention and control strategies for the "gaining health", promoted by the WHO in 2006. The main objectives are to prevent and change unhealthy conducts which are the main risk factors for non-communicable diseases with the highest epidemiological relevance and strongest impact on public health: cardiovascular diseases, cancer, diabetes mellitus, chronic respiratory pathologies, disorders of the muscle-skeletal and gastro-intestinal system, mental health problems. The initiative relies on the promotion of healthy choices and correct lifestyles (stop smoking, follow a correct diet, limit the alcohol intake and exercise regularly). Moreover, this supports local sanitary authorities in the implementation of the National Prevention Program (*Piano Nazionale della Prevenzione* 2014-2018), which aims to increase the prevalence of physically active adults (+30%) and people aged >64 (+15%) (www.salute.gov.it/portale/temi/p2_6.jsp?id=456&area=stiliVita&menu=attivita).

INTERVENTION PROJECTS

The introduction of legislative initiatives on sport and PA confirms the increase in global interests about this topic, with reference to the possibility of achieving improvement in the quality of life through the practice of PA and sport. This process has created the ideal basis for establishing real intervention programs whose main purpose is the promotion of PA to obtain a better health status.

Among the interventions, we find both initiatives mainly addressed to the sport field and initiatives focused on "PA for health".

Erasmus+ (2014-2020)

Sport is an integral part of the Erasmus+ program, the EU program for education, training, youth and sport starting in 2014. In accordance with one of the new elements introduced in the Lisbon Treaty, Erasmus+ supports activities aiming at developing the European dimension in sport. The Programme promotes the creation and development of European networks, providing opportunities for cooperation among stakeholders and the exchange and transfer of knowledge and know-how in different areas relating to sport and PA. The Programme intends promoting mobility opportunities for students and staff at various levels of education and for youth workers, by giving financial support to people submitting project proposals. In the 2014-2020 period, and for the first time, a budget line specifically dedicated to support projects and networks in the sports sector within the Erasmus+ program was made available. Almost 2% of the annual budget of the Erasmus+ program was dedicated to sport related activities, with the aim of supporting collaborative partnerships and non-profit European sporting events.

A particular initiative within this project is the "European Sport Week". This was proposed in the 2012 Resolution of the European Parliament following the dramatic data emerged from the above mentioned Eurobarometer. Each year, the EU promotes the European Sport Week with initiatives at EU, national, regional and local level to raise public awareness of the benefits of physical exercise for health. In general, such projects must lead to increased levels of participation in sport and the Erasmus+ Sport program will help develop the European dimension of sport by improving cooperation between sport organizations, public administrations and other interested parties.

Moreover, the network of Erasmus students annually organizes the International Erasmus Games (IEG). Countries send teams of Erasmus+ students to participate in sports competitions. In local and national qualifying rounds, teams from different cities, made up of friends of different nationalities, compete for the honor of representing the host country.

EU work plan for sport (2017-2020)

The EU work plan for sport is the most important document of the European sport strategy. The first plan was adopted by the Council in 2011 and the second in 2014. The 2014-2017 program contained three priorities: the integrity of sport, its economic dimension and the relationship between sport and society. To address these priority issues, the MSs and the Commission have set up five expert groups in the following areas: match-fixing, good governance, the economic dimension of sport, healthy physical activity (HEPA) and human resource development in sport.

On 23 May 2017, the Education, Youth, Culture and Sport Council in the sport session adopted the new EU work plan for sport (2017-2020). Under this plan, the Commission organized a forum of experts who discussed the general objectives previously analysed in the 2014-2017 program:

- integrity of sport, in particular promoting good governance including the safeguarding of minors, the specificity of sport, combating corruption and match fixing, as well as fighting doping;
- the economic dimension of sport, in particular innovation in sport, and sport and the digital single market;
- sport and society, in particular social inclusion, the role of coaches, education in and through sport, sport and health, sport and environment and sport and media, as well as sport diplomacy.

Although this Plan focuses more on strictly sports issues, it is worth highlighting that there is always a part dedicated to the health aspects of the practice of sport exercise.

Health 2020

"Health 2020" is a European policy model developed by the WHO through a long two-year consultation process and was adopted by the 53 MSs of the Region during the Sixty-second session of the Regional Committee for Europe of the WHO in September 2012 [63].

It is an important reference tool for implementing national, regional and local policies that are in harmony and in synergy, taking into account the specificities of the territories and social contexts.

Health 2020 "significantly improve the health and well-being of populations, reduce health inequalities,

strengthen public health and ensure people-centered health systems that are universal, equitable, sustainable and of high quality".

The document focuses on the main health problems and identifies four priority areas of political action:1. Investing in health through a life-course approach and empowering people; 2. Tackling the Region's major health challenges of non-communicable and communicable diseases; 3. Strengthening people-centered health systems, public health capacity and emergency preparedness, surveillance and response; 4. Creating resilient communities and supportive environments.

As part of the primary prevention of diseases, efforts in PA promotion through targeted interventions instead of mass-media campaigns has been highlighted in this document. Environmental measures such as changes in the transport system and the wider environment can be put in place to promote PA: where there are public green spaces and forests, people use them to walk, play, and cycle, turning PA into an integral part of their daily lives.

Physical activity strategy for the WHO European Region 2016-2025

This PA strategy was developed following the global goals set by the WHO Global Action Plan for the Prevention and Control of NCDs 2013-2020 [57].

The strategy is based on PA as a driving factor for the health and wellbeing of the European Region, with particular attention to the incidence of NCDs associated with insufficient levels of PA and sedentary behavior [58]. It covers all forms of PA practicable in the course of life.

The PA strategy aims to encourage governments and stakeholders to work to increase the levels of PA practiced by all citizens of the European Region. Obtaining a relative 10% reduction in the prevalence of insufficient PA by 2025 is one of the nine global goals. Indeed, increasing PA levels is an important factor to obtain a relative 25% reduction in early mortality due to cardiovascular diseases, tumors, diabetes or chronic respiratory diseases, to obtain a relative 25% reduction in the prevalence of hypertension, and to stop the increase in diabetes and obesity.

The second part of the document is dedicated to the priority areas, objectives and intervention tools that "MSs should consider developing or expanding, according to national context, strategies and action plans to promote PA". A special reference is given to the need to work together: "MSs should promote alliances between government, the media, civil society organizations and other stakeholders, including, but not limited to, public health and sports organizations and others, in order to promote physical activity for health across the life course" [58].

WHO global action plan on physical activity 2018-2030: more active people for a healthier world

Recently, the "WHO global action plan on physical activity 2018-2030: more active people for a healthier world" has been published [3]. The aim of this document is to ensure that all people have access to diverse opportunities to be physically active in their daily lives in a safe and enabling environment, resulting in an improvement of individual and community health and contributing to the social, cultural and economic development of all nations. The main target is to reduce a 15% of physical inactivity relative amount by 2030, using a baseline of 2016, in both adults and adolescents.

The document deals with various topics, divided into four main objectives to be pursued:

- create active societies: enhancing the knowledge and the understanding of, and appreciation for, the multiple benefits of regular PA and structured exercise, with the aim to create positive social norms and attitudes in all of society;
- create active environment: having equitable access to safe places and spaces in cities and communities dedicated to regular PA, by policy action addressing the need to create supportive spaces and places that promote and safeguard the rights of all people;
- create active people: helping people of all ages and abilities – individuals, families and communities – to engage in regular PA, outlining the multiple settings to increase programs and opportunities;
- create active systems: increasing PA and reducing sedentary behavior through policy actions that outline the investments needed to strengthen the systems necessary to implement effective international, national and subnational action. These actions address governance, leadership, multisectoral partnerships, workforce capabilities, advocacy, information systems and financing mechanisms across all relevant sectors.

This document also describes 20 evidence-based policy actions, recommended to achieve these four objectives. For example, to increase knowledge and skills related to the roles of professionals, it has been proposed to strengthen pre- and in-service training, within and outside the health sector, creating inclusive, equitable opportunities for an active society regarding transport, urban planning, education, tourism and recreation, sports and fitness.

The priorities to create an active environment are aimed to improve the integration of urban and transport planning policies (walking and cycling network infrastructures), as well as the access to good-quality public and green open spaces, green networks, recreational spaces and sports amenities by all people.

The societies and environmental modification will lead to the creation of active people, by strengthen provision of good-quality physical education and more positive experiences and opportunities for active recreation and sports. Moreover, the goal of future society is the implementation of systems of patient assessment and counseling on increasing PA and reducing sedentary behavior, as well as the establishment of prescription of structured exercise in health care services for patients with a history of cancer, diabetes and cardiovascular diseases [64-67].

Finally, this document underlines the importance of policy frameworks, leadership and governance systems, at the national and subnational levels, to support implementation of actions aimed at increasing PA and reducing sedentary behaviors at a multisectoral level. In the Appendix 2, the recommended specific roles for the WHO Secretariat, WHO MSs and other stakeholders to support implementation are outlined for each action.

CONCLUSIONS

It is well known that PA is a powerful tool for the prevention of non-communicable diseases, through reduction of main risk factors, and the support and improvement of psycho-physical well-being and quality of life.

International recommendations about PA for health [2], taken up by the Action Plan for the Prevention and Control of Noncommunicable Diseases in the WHO European Region 2016-2025, are an important reference point for each MS. PA recommended levels can be easily obtained in everyday life (at school, at home, at recreational and working environments). However, in order to support change, increasing levels of physical activity in the population demands a multisectoral and multidisciplinary approach. This issue has been recently enphasized by the WHO Regional Office for Europe [68], which recognized the importance of multisectoral and intersectoral actions for improved health and well-being for all. This kind of collaboration should involve different figures, at national, regional and local levels, without forgetting international networks (i.e. WHO European Healthy Cities Network and the Regions for Health Network): policy-makers, civil society, the health sector, media, etc. Moreover, the WHO also gave practical indications, as town planning to ensure accessible and safe walking and cycling, active transport, intervention in workplaces and schools, providing advice or counsel in primary care, creating social networks to encourage PA [2].

Other supporting activities for PA promotion include regulations and strategies to modify living environments, to assign specific economic funding, to activate useful collaborations.

Such strategies and health policies not only respond to the need of reducing the burden of disease of non-communicable diseases, but also contribute to reducing sanitary costs. Indeed, an increase in people PA levels could remarkably reduce costs for the National Health Services.

It is therefore necessary to include prevention strategies based on the practice of PA in governmental interventions made by the global, European, state and local organizations. All these levels must operate in a concerted and cooperative manner: only in this way interventions can be made effective and have real benefits for people's health.

Thanks to strategies promoted and financed by these bodies, it is possible to render the population increasingly aware of the fact that an active lifestyle improves the quality of life and favours the achievement of a state of complete well-being. At the population level, some activities may help to make healthy choices easy choices, like educational of information initiatives to increase people consciousness of benefits deriving from PA and to motivate at a more active lifestyle.

In this context, in the last decade, new technologies received increasing attention as a tool for physical activity promotion, including the interactive exercise-based video games (or "exergames") that could promote physical activity providing enjoyable exercise opportunities for children and young adults [69]. The exergames have also been proposed to improve rehabilitation adherence in individuals with acute or chronic illness, or with physical or developmental impairment [70]. Moreover, in older people, technology-based exercise interventions revealed good adherence and several advantages over traditional exercise programs, offering a more enjoyable and stimulating exercise experience, and may provide a sustainable means of promoting physical activity and preventing falls [71].

In this way, people will be capable to acquire the ability to increase and improve control over their health.

Author's contribution statement

All Authors contributed to study conception and design. Mauro De Santi, Debora Contisciani, Giulia Baldelli and Giulia Amagliani co-wrote the paper. Giorgio Brandi and Giuditta Fiorella Schiavano provided critical revision of the article and final approval of the version to publish.

Conflict of interest statement

The Authors of the manuscript declare that they do not have any conflicts of interest.

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REFERENCES

1. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise and physical fitness: definitions and distinctions for health-related research. Public Health Rep. 1985;100:126-31.
2. World Health Organization. Global recommendations on physical activity for health. Geneva: WHO; 2010. Available from: www.who.int/dietphysicalactivity/global-PA-recs-2010.pdf.
3. World Health Organization. Global action plan on physical activity 2018-2030: more active people for a healthier world. Copenhagen: WHO; 2018. Available from: www.who.int/ncds/prevention/physical-activity/global-action-plan-2018-2030/en/.
4. Cochrane M, Watson PM, Timpson H, Haycox A, Collins B, Jones L, Martin A, Graves LEF. Systematic review of the methods used in economic evaluations of targeted physical activity and sedentary behaviour interventions. Soc Sci Med. 2019;232:156-67. doi: 10.1016/j.socscimed.2019.04.040
5. World Health Organization. Non-communicable diseases. WHO; 2018. Available from: www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases.
6. Tremblay M, Aubert S, Barnes JD, Saunders TJ, Carson V, Latimer-Cheung AE, Chastin SFM, Altenburg TM, Chinapaw MJM; SBRN Terminology Consensus Project Participants. Sedentary Behavior Research Network (SBRN)-Terminology consensus project process and outcome. Int J Behav Nutr Phys Act. 2017;14:75.
7. Lee I-M, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on ma-

jor non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. 2012;380:219-29.
8. Gallè F, Sabella EA, Da Molin G, Parisi EA, Liguori G, Montagna MT, De Giglio O, Tondini L, Orsi GB, Napoli C. Physical activity in older adults: an investigation in a metropolitan area of southern Italy. Int J Environ Res Public Health. 2020;6;17:1034. doi: 10.3390/ijerph1703103
9. Tremmel M, Gerdtham UG, Nilsson PM, Saha S. Economic burden of obesity: a systematic literature review. Int J Environ Res Public Health. 2017;14:435. doi: 10.3390/ijerph14040435
10. WHO, National Institute on Aging, National Institutes of Health, US Department of Health and Human Services. NIH Publication no. 11-7737. Global Health and Aging. 2011. Available from: www.who.int/ageing/publications/global_health.pdf.
11. World Health Organization. Health topics. Physical activity. WHO. Available from: www.who.int/health-topics/physical-activity.
12. WHO. Ottawa Charter for Health Promotion. First international conference on health promotion. Ottawa, 21 November 1986. Available from: www.who.int/healthpromotion/conferences/previous/ottawa/en/.
13. WHO. Constitution of the World Health Organization. 1948. Available from: www.who.int/governance/eb/who_constitution_en.pdf.
14. WHO. Alma Ata Declaration on primary health care. International Conference on Primary Health Care, 6-12 September 1978. Available from: www.who.int/publications/almaata_declaration_en.pdf.
15. WHO. Jakarta Declaration on Leading Health Promotion into the 21st Century. Jakarta, Indonesia, 21-25 July 1997. Available from: www.who.int/healthpromotion/conferences/previous/jakarta/declaration/en/.
16. Blair SN, LaMonte MJ, Nichaman MZ. The evolution of physical activity recommendations: how much is enough? Am J Clin Nutr. 2004;79:913S-20S.
17. Morris JN, Crawford MD. Coronary heart disease and physical activity of work: evidence of a national necropsy survey. BMJ. 1958;2:1485-96.
18. Warburton DER, Bredin SSD. Health benefits of physical activity: a systematic review of current systematic reviews. Curr Opin Cardiol. 2017;32:541-56. doi: 10.1097/HCO.0000000000000437
19. Tremblay MS, Colley RC, Saunders TJ, Healy GN, Owen N. Physiological and health implications of a sedentary lifestyle. Appl Physiol Nutr Me. 2010;35:725-40.
20. Blair SN, Kohl HW III, Paffenbarger RS Jr., Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality: a prospective study of healthy men and women. JAMA. 1989;262:2395-401.
21. Ekelund LG, Haskell WL, Johnson JL, Whaley FS, Criqui MH, Sheps DS. Physical fitness as a predictor of cardiovascular mortality in asymptomatic North American men: the Lipid Research Clinic's mortality follow-up study. N Engl J Med. 1988;319:1379-84.
22. Erikssen G, Liestol K, Bjornholt J, Thaulow E, Sandvik L, Erikssen J. Changes in physical fitness and changes in mortality. Lancet. 1998;352:759-62.
23. Kampert JB, Blair SN, Barlow CE, Kohl HW III. Physical activity, physical fitness, and all-cause and cancer mortality: a prospective study of men and women. Ann Epidemiol. 1996;6:452-7.
24. Peters RK, Cady LD Jr., Bischoff DP, Bernstein L, Pike MC. Physical fitness and subsequent myocardial infarction in healthy workers. JAMA. 1983;249:3052-6.
25. Slattery ML, Jacobs DR Jr. Physical fitness and cardiovascular disease mortality: the US railroad study. Am J Epidemiol. 1988;127:571-80.
26. American College of Sports Medicine. Guidelines for graded exercise testing and exercise prescription. Philadelphia: Lea & Febiger; 1975.
27. American College of Sports Medicine. Guidelines for graded exercise testing and exercise prescription. Philadelphia: Lea & Febiger; 1980.
28. American College of Sports Medicine. Guidelines for exercise testing and prescription. Philadelphia: Lea & Febiger; 1986.
29. American College of Sports Medicine. Guidelines for exercise testing and prescription. Malvern, PA: Lea & Febiger; 1991.
30. American College of Sports Medicine. ACSM's guidelines for exercise testing and prescription. Media, PA: Williams & Wilkins; 1995.
31. American College of Sports Medicine. ACSM's guidelines for exercise testing and prescription. Philadelphia, PA: Lippincott Williams & Wilkins; 2000.
32. American Heart Association. Committee on Exercise. Exercise testing and training of apparently healthy individuals: a handbook for physicians. 1972.
33. American Heart Association. Committee on Exercise. Exercise testing and training of individuals with heart disease or at high risk for its development:a handbook for physicians. 1975.
34. Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, Buchner D, Ettinger W, Heath GW, King AC. Physical activity and public health: a recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. JAMA. 1995;273:402-7.
35. National Institutes of Health. Consensus development panel on physical activity and cardiovascular health. Physical activity and cardiovascular health. JAMA. 1996;276:241-6.
36. Blair SN, Kohl HW III, Barlow CE, Paffenbarger RS Jr., Gibbons LW, Macera CA. Changes in physical fitness and all-cause mortality: a prospective study of healthy and unhealthy men. JAMA. 1995;273:1093-8.
37. Institute of Medicine of the National Academies of Science. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Whashington, DC: The National Academies Press; 2002. p. 1331.
38. Saris WH, Blair SN, van Baak MA, Eaton SB, Davies PS, Di Pietro L, Fogelholm M, Rissanen A, Schoeller D, Swinburn B, Tremblay A, Westerterp KR, Wyatt H. How much physical activity is enough to prevent unhealthy weight gain? Outcome of the IASO 1st Stock Conference and consensus statement. Obes Rev. 2003;4:101-14.
39. US Physical Activity Guidelines Advisory Committee. Physical Activity Guidelines Advisory Committee Report. Washington, DC: US Department of Health and Huistman Services; 2008. Available from: https://health.gov/paguidelines/2008/report/pdf/CommitteeReport.pdf.
40. Nelson ME, Rejeski WJ, Blair SN, Duncan PW, Judge JO, King AC, Macera CA, Castaneda-Sceppa C, American College of Sports Medicine, American Heart Association. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Circulation. 2007;28:1094-5.
41. US Department of Health and Human Services. Physical activity guidelines for Americans. Washington, DC:

US Department of Health and Human Services. 2008. Available from https://health.gov/paguidelines/2008/pdf/paguide.pdf.
42. European Union. EU Working Group "Sport & Health". EU Physical Activity Guidelines. Recommended policy actions in support of health-enhancing physical activity. Brussels, 10 October 2008. Available from https://ec.europa.eu/assets/eac/sport/library/policy_documents/eu-physical-activity-guidelines-2008_en.pdf.
43. Oja P, Titze S. Physical activity recommendations for Public Health: development and policy context. EPMA J. 2011;2:253-9.
44. UK Sport, Sport England and CONI. Sport participation in Europe: COMPASS 1999. London: UK Sport.
45. Gratton C, Rowe N, Veal AJ. International Comparison of sports participation in European countries: an update of the COMPASS Project. Eur J Sport Soc. 2011;8:99-116.
46. European Commission. Physical activity. Special Eurobarometer 183-6. Brussels: European Commission. 2003. Available from: https://ec.europa.eu/commfrontoffice/publicopinion/archives/ebs/ebs_183_6_en.pdf.
47. European Commission. The citizens of the European Union and sport: Special Eurobarometer 213. Brussels: European Commission. 2004. Available from: http://ec.europa.eu/public_opinion/archives/ebs/ebs_213_report_en.pdf.
48. European Commission. Physical activity and sport: Special Eurobarometer 334. Brussels: European Commission. 2009. Available from: https://data.europa.eu/euodp/en/data/dataset/S776_72_3_EBS334.
49. European Commission. Physical activity and sport: Special Eurobarometer 412. Brussels: European Commission. 2014. Available from: http://data.europa.eu/euodp/en/data/dataset/S1116_80_2_412.
50. European Commission. Physical activity and sport: Special Eurobarometer 472. Brussels: European Commission. 2018. Available from: http://data.europa.eu/euodp/en/data/dataset/S2164_88_4_472_ENG.
51. WHO. World Health Organization Physical Activity Factsheets for the 28 European Union Member states of the WHO European region. 2018. Available from: www.euro.who.int/_data/assets/pdf_file/0005/382334/28fs-physical-activity-euro-rep-eng.pdf.
52. WHO. Global strategy on diet, physical activity and health. 2004. Available from: www.who.int/dietphysicalactivity/strategy/eb11344/strategy_english_web.pdf.
53. Commission of the European Communities. Green Paper. Promoting healthy diets and physical activity: a European dimension for the prevention of overweight, obesity and chronic diseases. 2005. Available from: https://ec.europa.eu/health/ph_determinants/life_style/nutrition/documents/nutrition_gp_en.pdf.
54. Commission of the European Communities. White Paper on a strategy for Europe on nutrition, overweight and obesity related health issues. 2007. Available from: https://ec.europa.eu/health/ph_determinants/life_style/nutrition/documents/nutrition_wp_en.pdf.
55. Commission of the European Communities. White Paper on Sport. 2007. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52007DC0391&from=EN.
56. WHO. 2008-2013 Action plan for the global strategy for the prevention and control of noncommunicable diseases. 2008. Available from: www.who.int/nmh/publications/ncd_action_plan_en.pdf.
57. World Health Organization. Global action plan for the prevention and control of noncommunicable diseases 2013-2020. WHO; 2013. Available from: www.who.int/iris/bitstream/10665/94384/1/9789241506236_eng.pdf.
58. WHO. Physical activity strategy for the WHO European Region 2016-2025. WHO Regional Office for Europe. 2015. Available from: www.euro.who.int/__data/assets/pdf_file/0010/282961/65wd09e_PhysicalActivityStrategy_150474.pdf.
59. WHO/FAO Expert Consultation on diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO expert consultation. WHO technical report series 916. Geneva: 2003. Available from: https://apps.who.int/iris/bitstream/handle/10665/42665/WHO_TRS_916.pdf?sequence=1.
60. European Commission. Treaty of Lisbon. Official Journal of the European Union. 2007/C 306/01. 2007. Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2007:306:FULL:EN:PDF.
61. Bull FC, Gauvin L, Bauman A, Shilton T, Kohl HW 3rd, Salmon A. The Toronto Charter for physical activity: a global call for action. J Phys Act Health. 2010;7:421-2.
62. Italia. Decreto del Presidente del Consiglio dei Ministri del 4 maggio 2007. Documento programmatico "Guadagnare salute". G.U. Serie Generale, n. 117 del 22 maggio 2007.
63. WHO. Health 2020. A European policy framework and strategy for the 21st century. WHO Regional Office for Europe. 2013. Available from: www.euro.who.int/__data/assets/pdf_file/0011/199532/Health2020-Long.pdf.
64. Agostini D, Natalucci V, Baldelli G, De Santi M, Donati Zeppa S, Vallorani L, Annibalini G, Lucertini F, Federici A, Izzo R, Stocchi V, Barbieri E. New insights into the role of exercise in inhibiting mTOR signaling in triple-negative breast cancer. Oxid Med Cell Longev. 2018;30:5896786. doi: 10.1155/2018/5896786
65. Barbieri E, Falcieri E, De Santi M, Natalucci V, Vallorani L, Agostini D, Annibalini G, Stefani L, Szychlinska MA, Musumeci G. The "Journal of Functional Morphology and Kinesiology" Journal ClubSeries: highlights on recent papers in physical activity and sedentary behaviour. J Funct Morphol Kinesiol. 2018;3:23.
66. De Santi M. Physical activity promotion for cancer prevention. J Cancer Res Forecast. 2018;1:1008-9.
67. Kohl HW 3rd, Craig CL, Lambert EV, Inoue S, Alkandari JR, Leetongin G, Kahlmeier S, Lancet Physical Activity Series Working Group. The pandemic of physical inactivity: global action for public health. Lancet. 2012;21:294-305. doi: 10.1016/S0140-6736(12)60898-8
68. WHO Regional Office for Europe. Multisectoral and intersectoral action for improved health and well-being for all: mapping of the WHO European Region. Governance for a sustainable future: improving health and well-being for all. Final Report. Publications WHO Regional Office for Europe. Copenhagen: 2018. 100 p.
69. Klos L, Feil K, Eberhardt T, Jekauc D. Interventions to promote positive affect and physical activity in children, adolescents and young adults – a systematic review. Sports. 2020;8:26.
70. Staiano AE, Flynn R. Therapeutic uses of active videogames: a systematic review. Games for Health Journal. 2014;3:351-65.
71. Valenzuela T, Okubo Y, Woodbury A, Lord SR, Delbaere K. Adherence to technology-based exercise programs in older adults: a systematic review. J Geriatr Phys Ther. 2018;41:49-61.

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ORIGINAL ARTICLES AND REVIEWS

Gene flow and Bayesian phylogeography of serogroup C meningococci circulating in Italy

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Abstract

Introduction. Hyperinvasive strains of *Neisseria meningitidis* serogroup C have caused outbreaks of severe disease in Italy. Here, we report the analysis of the migration patterns of C:P1.5-1,10-8:F3-6:ST-11(cc11) meningococcal strains from different Italian regions collected between 2012 and 2017.

Methods. *N. meningitidis* genomes were sequenced through the whole genome sequencing (WGS) method and were analyzed using the BIGSdb Genome Comparator tool. The phylogeography was performed using BEAST. The gene flows in Italy were tested by using MacClade.

Results. The C:P1.5-1,10-8:F3-6:ST-11(cc11) hyperinvasive meningococcal strain, for the data available at the time of the analysis, from UK reached at first Emilia Romagna region, and then, in 2012, was detected in the outbreak occurred in the port of Livorno. The *"Tuscany-outbreak strain"* was likely introduced in Italy between 2013 and 2014. Most of the observed gene flow events occurred from the Center to Northern part of Italy.

Discussion. The phylogeographic analysis allowed to track the dissemination of C:P1.5-1,10-8:F3-6:ST-11(cc11) strains in the country.

Key words

- public health
- surveillance
- invasive meningococcal disease
- phylogeography
- outbreak investigation

INTRODUCTION

Neisseria meningitidis is still cause of epidemic and outbreak with a high case fatality rate [1]. Molecular epidemiology has provided clear observational evidence for an association between specific bacterial genotypes (hyperinvasive lineages) and invasive meningococcal disease (IMD) [2]. In particular, serogroup C strains have been a common cause of IMD outbreaks in Europe, some of them belonging to hypervirulent clones.

Rapid and early detection of outbreaks is critical to guide prompt and appropriate public health interventions to control the outbreaks and prevent additional cases. Molecular typing has played an important role in outbreak investigations, identifying outbreak strains by assessing their genetic relatedness, and providing evidence to understand the chain transmission and linkage.

The rapid turnaround time and availability of automated whole genome sequencing (WGS) analysis is, nowadays, in the routine use for outbreak investigation and surveillance of IMD [3-5].

In a previous paper [6] we identified the pattern of dispersal of hyperinvasive strains of serogroup C meningococci in a large geographical area and the routes leading to their introduction in our Country.

The present study was then planned to decipher the sequence information of the complete genome of circulating meningococci of serogroup C isolated in Italy from 2012 to 2017. In particular, an extensive comparative genomic analysis was carried out for identifying the unique features and for phylogeography on strains belonging to C:P1.5-1,10-8:F3-6:ST-11(cc11) through the Italian regions.

METHODS

Setting, whole genome sequencing and phylogenetic dataset

In this study, 63 genomes of meningococci of serogroup C collected at the National Reference Laboratory (NRL), in the frame of the National IMD Surveillance System at the Italian Institute of Public Health (Istituto Superiore di Sanità, ISS), from January 1st 2012 to December 31st 2017 were analyzed. Sixthy-one of them belonged to C:P1.5-1,10-8:F3-6:ST-11(cc11) and two to C:P1.5-1,10-8:F3-6:ST-2780(cc11). The whole

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genome sequences were previously performed with the Illumina MiSeq platform (kit v3, 600 cycles), according to the procedure previously described [7]. The phylogenetic dataset, ranging from 2007 to 2017, comprised the 63 *N. meningitidis* genomes from Italy (collection dates ranging from 2012 to 2017), together with 70 C:P1.5-1,10-8:F3-6:ST-11(cc11) genomes from other countries (acting as reinforcement isolates for Bayesian analysis), and were compared using the BIGSdb Genome Comparator [8], through the gene-by-gene analysis. The genomes were analyzed by the core genome MLST (cgMLST), in the PubMLST Neisseria website [9], and the core genome has been generated [8]. The sampling administrative regions of the sixty-three Italian isolates were: Tuscany (n= 29), Lombardy (n=13), Liguria (n=2),Veneto (n=1), Lazio (n=2), Trentino (n=1), Emilia-Romagna (n=6), Piedmont (n=3), Apulia (n=3), Sardinia (n=1), Marche (n=1) and Basilicata (n=1).

Phylogenetic analysis

Core Genome Recombination analysis was performed with BratNextGen (BNG) [10] in the aligned core genome of the *N. meningitidis* dataset to obtain recombination-free input sequences for further phylogenetic analyses, as previously described [11-13]. Single-nucleotide polymorphisms (SNPs) were based on the core genome shared by all the isolates. SNPs were exported as variable sites using MEGA, removing SNP sites with ambiguities, missing data and gaps [14]. The SNPs introduced by putative recombination events were removed. The evaluation of the best fitting model of nucleotide substitution was performed with the JModeltest [15, 16]. The phylogenetic signal was investigated by using Treepuzzle, with the likelihood mapping analysis using 10,000 random quartets [17] to obtain a comprehensive picture of the phylogenetic quality and to estimate the amount of phylogenetic information.

Evolutionary rate estimate and phylogeography

In order to investigate the phylogeography, including evolutionary rate and dates estimates of the *N. meningitidis* dataset, a Bayesian Markov Chain Monte Carlo (MCMC) method implemented in the BEAST software [18], with the GTR model, previously estimated, was used. Alternative clock models (strict and relaxed clock with an uncorrelated log normal rate distribution) and demographic models (constant population size, exponential growth, non-parametric smooth skyride plot Gaussian Markov random field, GMRF, and non-parametric Bayesian skyline plot, BSP) were tested, on the filtered core genome SNPs alignment, accounting for invariant sites. The Bayes factor (BF, using marginal likelihoods) tests were used to compare the models and to estimate the best fitting, as previously described [19]; only values of 2ln BF>6 were considered significant. Chains were conducted until convergence was reached and assessed by calculating the effective sampling size (ESS) for each parameter. Only parameter estimates with ESS's of >200 were accepted. Uncertainty in the estimates was indicated by 95% highest posterior density (95% HPD) intervals. The continuous-time Markov chain (CTMC) process over discrete sampling locations in the BEAST software [18], with Bayesian stochastic search variable selection (BSSVS) model was used for the phylogeographic inference. The maximum clade credibility (MCC) tree (the tree with the largest product of posterior clade probabilities) was summarized with Tree-Annotator, after a 10% burn-in. The final tree was manipulated in FigTree [20] for display purpose. Statistical support for specific clades was assessed by the posterior probability (pp>0.90). The analysis and visualization of the different aspects of the phylogeographic diffusion was performed with Spread [21]. The temporal dynamics of the spatial *N. meningitidis* diffusion were provided by snapshots-maps of the dispersal pattern by Google Earth [22].

Gene flow and migration analysis

The Mac Clade version 4 program (Sinauer Associates, Sunderland, MA) was used to test gene out/in flow in Italy, among *N. meningitidis* infected subjects, using a modified version of the Slatkin and Maddison test [23]. A maximum likelihood (ML) tree was built by using the Phyml [24] with the GTR model and used as starting tree for this analysis. A one-character data matrix was obtained from the dataset by assigning to each taxon in the tree a one-letter code indicating its own sampling location, according to three different geographic groups: A: Northern Italy including the following regions: Lombardy, Liguria, Piedmont, Veneto, Trentino Alto Adige; B: Central Italy including Emilia-Romagna, Lazio, Marche, Tuscany (also including the isolates from Livorno's port); C: Southern Italy including Basilicata, Apulia and Sardinia. The putative origin of each ancestral sequence (i.e., internal node) in the tree was inferred by finding the most parsimonious reconstruction (MPR) of the ancestral character. The final tree length, that is the number of observed gene flow events in the genealogy, can easily be computed and compared to the tree-length distribution of 10,000 trees obtained by random joining-splitting (null distribution). Observed genealogies significantly shorter than random trees indicate the presence of subdivided populations with restricted gene flow. The gene flow among the different geographic groups (character states) was traced with the State changes and stasis tool through the Mac Clade software [23], which counts the number of changes in a tree for each pair wise character state. When multiple MPRs were present, the algorithm calculated the average migration count over all possible MPRs for each pair.

RESULTS

Evolutionary rate and Bayesian phylogeography.

After removing the SNPs introduced by recombination, the phylogenetic signal was assessed by likelihood mapping analysis which indicated <30 % of "noise", meaning sufficient phylogenetic signal. The Bayes factor tests indicated that the relaxed clock fitted the data significantly better than the strict molecular clock (relaxed *vs* strict 2ln BF=226,754 in favor of the relaxed). The BF comparisons showed that, under the

relaxed clock, the demographic BSP model fitted the data significantly better than the other models (BSP *vs* exponential 2ln BF=38.326, BSP *vs* constant 2 ln BF=68.558, BSP *vs* GMRF 2ln BF=732.512). Under the selected relaxed BSP model, the estimated mean value of the evolutionary rate was 3.1×10^{-6} substitutions per site per year (95% HPD, lower value, 2.4×10^{-6}–95% HPD upper value, 3.8×10^{-6}).

The phylogeographic analysis (*Figure 1*), showed that the root of the tree dated back to the year 2005 (95% HPD: 2002-2007) and most probably originated in UK (sp=0.95). Six cluster (I, II, III, IV, V, VII) and a main clade (VI), all statistically supported, were identified (tMRCA and most probable location reported in *Table 1*). The information regarding the isolates included in this study is available as *Supplementary Material Table 1S*.

The main clade (VI) included the majority of isolates (*Figure 1*), dated back to 2007 (95% HPD: 2006-2010) and showed two distinct introductions, with strains segregating in sub-clade VI a and VI b. The sub-clade VIa, which was dated back to 2008 (95% HPD: 2007-2011), included two isolates from the UK, two from Emilia-Romagna, and four isolates from the Livorno's port out-

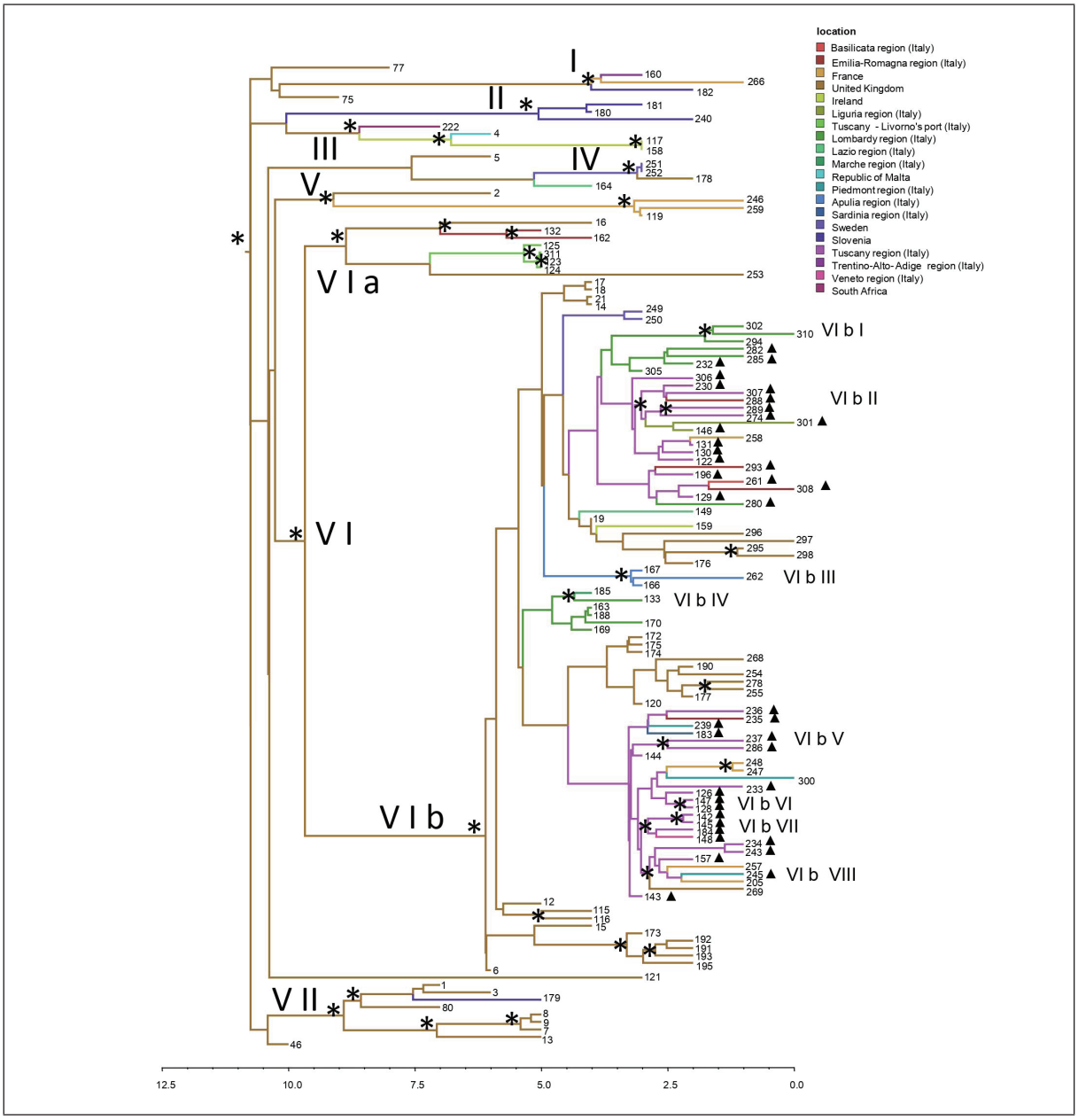

Figure 1

Bayesian phylogeographic tree of *N. meningitidis* strains belonging to the phylogenetic dataset. The scale axis below the tree showed the time (years) before the present. Main statistically supported clade and cluster were indicated. The * along the branch represents significant statistical support for the clade subtending that branch (posterior probability > 90%). Geographic locations were shown with different colors in the tree and reported as legend.

break. The four isolates from the port of Livorno ship outbreak occurring in 2012 were closely related to each other, as previously described [25], dated to 2011 (95% HPD: 2011-2012). The sub-clade VIb, dated to 2011 (95% HPD: 2009-2011), originated from UK (sp=0.99) and included European strains intermixed with Italian isolates from different regions (Tuscany, Piedmont, Veneto, Sardinia, Emilia-Romagna, Lombardy, Marche, Apulia, Lazio, Basilicata, Liguria) (*Figure 1*). Eight statistically supported internal clusters, dating from 2013 to 2015, were identified (VIb $_{I}$ - VIb $_{VIII}$, *Figure 1*, *Table 1*). All the isolates belonging to the "Tuscany-outbreak strain" were included inside VIb, (*Figure 1*, highlighted by a full triangle), some of them segregating in statistically supported clusters. The "Tuscany-outbreak strain" was likely introduced in Italy between 2013 and 2014. The two ST-2780 Italian meningococci (ID code 142 and 145) from Tuscany appeared closely related inside cluster VI b $_{VII}$, together with two Italian meningococci (ID code 184 and 148) collected respectively from Tuscany and Veneto regions in the same time period (year 2015).

Temporal dynamics of spatial diffusion

The spread of the hyperinvasive C:P1.5-1,10-8:F3-6:ST-11(cc11) meningococcal strains isolated in Italy over time, obtained from the location annotated MCC tree, indicated that starting from 2011 meningococci from UK reached at first Emilia Romagna region (*Supplementary Material: Figure 1S A, panel a*). By 2012, C:P1.5-1,10-8:F3-6:ST-11(cc11) meningococci from UK reached the port of Livorno causing a small outbreak, and subsequently, another migration occurred from UK to Lombardy (*Supplementary Material: Figure 1S A, panel b*). A spread from Lombardy to Marche and from UK to Lazio occurred by 2013 (*Supplementary Material: Figure 1S A*, *panel c*). Subsequently, waves of migration from UK to Tuscany (*Supplementary Material: Figure 1S A*, *panel d*), from Tuscany to Lombardy, and from UK to Apulia (*Supplementary Material: Figure 1S A*, *panel d*) were observed. By 2014 (*Supplementary Material: Figure 1S A*, *panel e*), flows from France to Trentino Alto Adige, from Tuscany to Liguria, and from Tuscany to Sardinia probably occurred. By the beginning of 2015, C:P1.5-1,10-8:F3-6:ST-11(cc11) meningococci probably continued to spread from Tuscany to Veneto, from Tuscany to Piedmont, from Tuscany to UK, from Tuscany to France (*Supplementary Material: Figure 1S A*, *panel f*). Between the end of 2015 and the beginning of 2016, a strain from Tuscany probably reached Basilicata. The most frequently invoked and supported links, involving Italian regions and foreign countries, were those between Slovenia and Sardinia, Slovenia and Marche, Slovenia and Piedmont, Slovenia and Basilicata, Sardinia and South Africa, Sardinia and Sweden, South Africa and Marche, Marche and Sweden, South Africa and Piedmont, South Africa and Basilicata, Piedmont and Sweden, Sweden and Basilicata (*Supplementary Material: Figure 1S B*). The most frequently invoked and supported links between Italian regions were, those between Apulia and Basilicata, Sardinia and Marche, Sardinia and Piedmont, Sardinia and Basilicata, Marche and Piedmont, Marche and Basilicata, Piedmont and Basilicata (*Supplementary Material: Figure 1S B*).

The gene flow analysis

The "null hypothesis" of panmixia (i.e., no population subdivision or complete intermixing of sequences from different geographic areas) was rejected by the

Table 1

Time to the most recent common ancestor for the internal nodes, 95% highest posterior density estimates, the most probable location and the state probability of the main clade and cluster

Clade and cluster	tMRCA (years)	95% HPD	Locality	State probability
I	2013	2008-2013	United Kingdom	0.51
II	2012	2011-2013	Slovenia	0.99
III	2008	2006-2009	United Kingdom	0.31
IV	2013	2012-2014	Sweden	0.54
V	2008	2006-2011	United Kingdom	0.95
VI	2007	2006-2010	United Kingdom	0.99
VI a	2008	2007-2011	United Kingdom	0.93
VI b	2011	2009-2011	United Kingdom	0.99
VI b I	2015	2014-2016	Italy: Lombardy	0.99
VI b II	2014	2013-2015	Italy: Tuscany	0.99
VI b III	2014	2013-2014	Italy: Apulia	0.99
VI b IV	2013	2012-2013	Italy: Lombardy	0.81
VI b V	2015	2014-2016	Italy: Tuscany	0.99
VI b VI	2015	2014-2015	Italy: Tuscany	0.99
VI b VII	2014	2014-2015	Italy: Tuscany	0.99
VI b VIII	2014	2014-2015	Italy: Tuscany	0.89
VII	2008	2006-2009	United Kingdom	0.99

tMRCA: time to the most recent common ancestor; HPD: highest posterior density.

randomization test, (P <0.0001) [23]. The gene flow analysis revealed that most of the observed gene flow events (54.5%) occurred from the Center to Northern Italy; a lower proportion of gene flow (18.2%) was identified from Northern to Center and from Center to Southern Italy (18.2%) (*Figure 2*). Only 9.1% of gene flow was observed from Northern to Southern Italy (*Figure 2*).

DISCUSSION

Neisseria meningitidis represents a public health issue globally [25-27] and a leading cause of morbidity and mortality in all age groups [1].

In Italy, where serogroup C is the second most common meningococcus serogroup [28], the hyperinvasive C:P1.5-1,10-8:F3-6:ST-11(cc11) strain caused several IMD sporadic cases, clusters, and small outbreaks in several regions, and a major outbreak in Tuscany in 2015-2017 [29]. In this study, a Bayesian MCMC approach was used to define the epidemiological history and phylogeographic analysis of C:P1.5-1,10-8:F3-6:ST-11(cc11) *N. meningitidis* isolates, circulating in different Italian regions, Moreover, the gene flow analysis was used to test the amount of gene out/in flow in Italy, among the different areas of the country.

The estimation of the rate of molecular evolution is critical for understanding a variety of evolutionary and epidemiological processes. In this study, we estimated a mean evolutionary rate of 3.1×10^{-6} substitutions per site per year (95% HPD: 2.4×10^{-6}–3.8×10^{-6}), which is comparable to those reported by Lamelas *et al.* 2014 [30] for *Neisseria meningitidis* serogroup A strains (3.1×10^{-6}; 95% HPD: 2.30×10^{-6}–3.85×10^{-6}). Values of the same order of magnitude were also reported in the literature [31] for *A. baumannii,* with a value of 3.15×10^{-6} (2.34×10^{-6} –4.44×10^{-6}), and for *Staphylococcus aureus,* which showed a mean rate of 2.43×10^{-6} (1.14×10^{-6}–3.98×10^{-6}) substitutions per site per year. The lowest estimates reported in the literature [31, 32] were those of *Klebsiella pneumoniae* and *Y. pestis*, with mean rates of 1.03×10^{-6} (95% HPD: 8.09×10^{-7} to 1.24×10^{-6}) and 1.57×10^{-8} (95% HPD: 1.03×10^{-8}–2.27×10^{-8}) substitutions per site per year, respectively.

The phylogeographical analysis suggested that C:P1.5-1,10-8:F3-6:ST-11(cc11) *N. meningitidis* strains, after accumulating by 2005 (95% HPD: 2002-2007) in UK, probably spread a few years later to the locations identified through the phylogeographic tree and diffusion analysis [33]. C:P1.5-1,10-8:F3-6:ST-11(cc11) meningococci most probably reached Emilia Romagna in 2011 (95% HPD: 2011-2012), and by 2012 was responsible of an outbreak occurred in the port of Livorno. The spatial diffusion suggested an intensification of the migration pathway since 2013 and during the following years towards several Italian regions. This finding is consistent with epidemiological data reporting an increase in the number of Men C IMD cases in Italy [29, 34, 35]. In the same time period, linkages and migration events were also identified between other foreign countries and Italian regions, together with exchanges involving only Italian regions. As recently described [33] the outbreak occurred in Tuscany was probably due to the introduction of the C:P1.5-1,10-8:F3-6:ST-11(cc11) strain between 2013 and 2014 through a dispersal pattern originated from UK in 2011 (95% HPD: 2009-2011). The analysis of the diffusion rates allowed

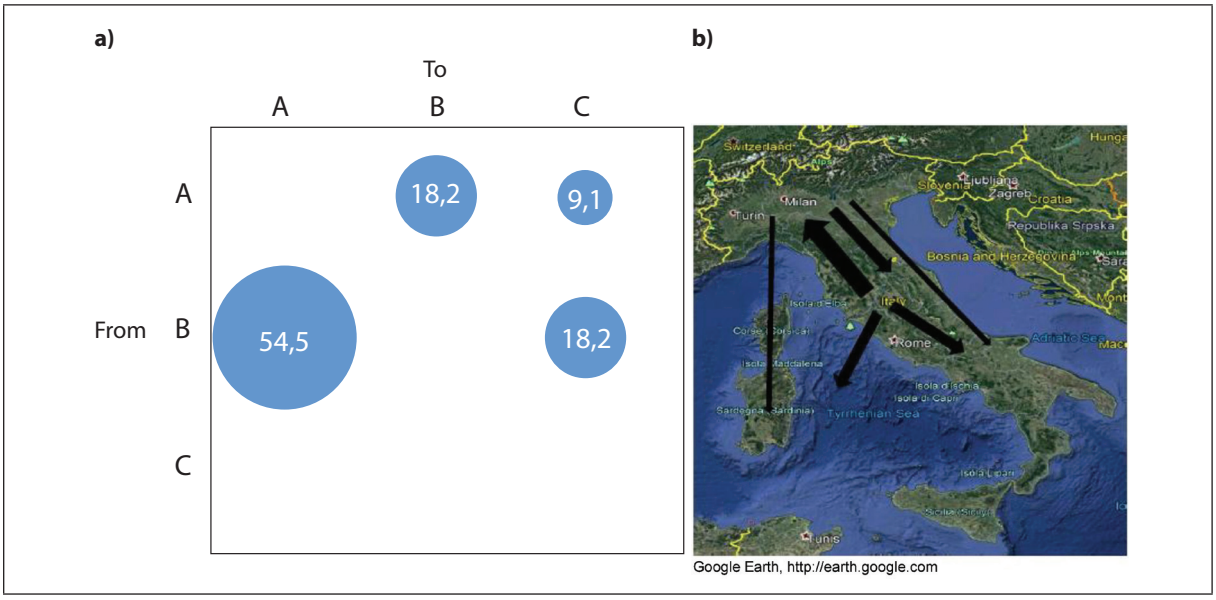

Figure 2

a) Maximum parsimony migration patterns of *N. meningitidis* strains to/from different areas of Italy. The bubblegram shows the frequency of gene flow (migrations) to/from different geographic areas, as the percentage of the total observed migrations estimated from the maximum likelihood tree. A, Northern Italy; B, Center- Italy; C, Southern Italy.

b) Projections and reconstructions of the *N. meningitidis* gene flows on a geographical map made available from Google Earth (http://earth.google.com; Google Earth Pro V.7.3.2.5491). The arrows indicate the gene flow to/from different areas of Italy (North, Center and South), the thickness of the arrows is proportional to the amount of gene flow (migrations obtained with Mac Clade software).

to highlight both frequently invoked rates acting as long distance connections and short distance linkages. Focusing only on the migrations identified by phylogeography and most probably occurred between Italian regions, most of the them regarded regions located in the Center and in the North of the country. Other migrations, probably involved the Center and the South of Italy. Few connections were identified between Northern and Southern regions. Linkages were also observed between regions located in the same geographic area. For example, Basilicata region resulted connected with Apulia located in Southern Italy, and with Sardinia. It is worth to mention how the phylogeographic analysis allowed to track the dissemination of the strains and to identify the highly connected locations. To this purpose, the migration patterns identified local and intercountries exchanges, probably explained by increased mobility of affected population, attendance to places of interconnection or crowded sites, close contacts with carriers or symptomatic individuals.

In this study, we also reported for the first time the gene flow analysis of C:P1.5-1,10-8:F3-6:ST-11(cc11) *N. meningitidis* isolates, which is able to test the migrations to/from different geographical areas. This analysis identified the Center of Italy as a major source of dissemination, with a 54.5% of gene flows to the North. Furthermore, as mentioned above, Central Italy showed a significant flow (18.2%) to the South, suggesting that C:P1.5-1,10-8:F3-6:ST-11(cc11) *N. meningitidis* mainly expanded from the Center to the North and, to a lesser extent to the South of the country. A low proportion of *N. meningitidis* gene flow from the North to the Center and to the South of Italy was also identified. Overall, these data, even though obtained by using a different methodology, confirm what previously observed using Bayesian phylogeography, which also showed a greater number of migrations between the Center and the North of Italy. Our data highlighting the Center of Italy as a major source of dissemination, can be probably explained by the increased number of meningococcal disease cases observed in Tuscany between 2015 and 2016 [30], together with an efficient surveillance system. Another possible assumption could be linked to the high prevalence of risk-behaviour that facilitate close contacts with infected individuals, promoting the spread of meningococci as previously described by Miglietta *et al*., 2018 [35]. Finally, our findings according to the dynamics of migration and phylogeography highlight the increase of IMD cases due to MenC in the Tuscany outbreak among adults [29, 34, 36] and the smaller increase of MenC incidence rates in other Italian regions in the same period, i.e. Emilia-Romagna, Lombardy, Trentino Alto Adige, Piedmont, Veneto [34, 36].

CONCLUSIONS

Invasive meningococcal disease due to hypervirulent serogroup C strain needs to be carefully monitored considering phylogeographic analysis of genomes in order to follow the spread in the country.

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Conflict of interest statement

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REFERENCES

1. Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. Lancet. 2007;369(9580):2196-210. doi: 10.1016/S0140-6736(07)61016-2
2. Caugant DA, Maiden MC. Meningococcal carriage and disease-population biology and evolution. Vaccine. 2009;24;27(2):B64-70. doi: 10.1016/j.vaccine.2009.04.061
3. Didelot X, Bowden R, Wilson DJ, Peto TE A, Crook DW. Transforming clinical microbiology with bacterial genome sequencing. Nat Rev Genet. 2012;13;601-12.
4. Loman N J, Pallen M J. Twenty years of bacterial genome sequencing. Nat Rev Microbiol. 2015;13:787-94.
5. Joseph S J, Read TD. Bacterial population genomics and infectious disease diagnostics. Trends Biotechnol. 2010;28:611-8.
6. Lo Presti A, Neri A, Fazio C, Vacca P, Ambrosio L, Grazian C, et al. Reconstruction of dispersal patterns of hypervirulent meningococcal strains of serogroup C:cc11 by phylogenomic time trees. J Clin Microbiol. 2019;23;58(1). pii: e01351-19. doi: 10.1128/JCM.01351-19
7. Stefanelli P, Fazio C, Neri A, Ciammaruconi A, Balocchini E, Anselmo A, et al. Genome-based study of a spatio-temporal cluster of invasive meningococcal disease due *to Neisseria meningitidis* serogroup C, clonal complex 11. J Infect. 2016;73(2):136-44. doi: 10.1016/j.jinf.2016.05.003
8. Jolley KA, Maiden M CJ. BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics. 2010;11:595. doi: 10.1186/1471-2105-11-595
9. PubMLST Neisseria data-base. Available from: http://pubmlst.org/neisseria/.
10. Marttinen P, Baldwin A, Hanage WP, Dowson C, Mahenthiralingam E, Corander J. Bayesian modeling of re-

combination events in bacterial populations. BMC Bioinformatics. 2008;7;9:421. doi: 10.1186/1471-2105-9-421
11. Croucher NJ, Harris SR, Fraser C, Quail MA, Burton J, van der Linden M, McGee L, et al. Rapid pneumococcal evolution in response to clinical interventions. Science. 2011;331:430-4. doi: 10.1126/science.1198545
12. Méric G, Miragaia M, de Been M, Yahara K, Pascoe B, Mageiros L, et al. Ecological overlap and horizontal gene transfer in *Staphylococcus aureus* and *Staphylococcus epidermidis*. Genome Biol Evol. 2015;16:1313-28. doi: 10.1093/gbe/evv066
13. Marttinen P, Hanage WP, Croucher NJ, Connor TR, Harris SR, Bentley SD, Corander J. et al. Detection of recombination events in bacterial genomes from large population segments. Nucleic Acids Research. 2011;40(1):e6. doi: 10.1093/nar/gkr928
14. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecul Biol Evol. 2013;30:2725-9. doi: 10.1093/molbev/mst197
15. Posada D. jModelTest: phylogenetic model averaging. Mol Biol Evol. 2008;25:1253-6. doi: 10.1093/molbev/msn083
16. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 2012;30:772. doi: 10.1038/nmeth.2109
17. Strimmer K, von Haeseler A. Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. Proc Natl Acad Sci USA. 1997;94:6815-9.
18. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol. 2007;7:214.
19. Kass RE, Raftery AE. Bayes Factors. J Am Statistic Assoc. 1995;90:773-95.
20. A. Rambaut. FigTree. Available from: http://tree.bio.ed.ac.uk/software/figtree/.
21. Bielejec F, Rambaut A, Suchard MA, Lemey P. SPREAD: Spatial phylogenetic reconstruction of evolutionary dynamics. Bioinformatics. 2011;27:2910-2. doi: 10.1093/bioinformatics/btr481
22. Google Earth Pro V.7.3.2.5491. Available from: http://earth.google.com.
23. Slatkin M, Maddison WP. A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics. 1989;123(3):603-13.
24. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies. Assessing the performance of PhyML 3.0. System Biol. 2010:59(3):307-21. doi: 10.1093/sysbio/syq010
25. Moore PS. Meningococcal meningitis in sub-Saharan Africa: a model for the epidemic process. Clin Infect Dis. 1992;14(2):515-25.
26. Rosenstein N. Commentary. Opportunities to decrease mortality and long-term sequellae associated with meningococcal disease in Africa. Int J Epidemiol. 2001;30(6):1447-8.
27. Schuchat A, Robinson K, Wenger JD, et al. Bacterial meningitis in the United States in 1995. Active Surveillance Team. N Engl J Med. 1997;337:970-6.
28. Istituto Superiore di Sanità, ISS (Italian National Institute of Health). [Surveillance data on invasive bacterial diseases updated on 23 April 2019]. Italian. Available from: http://old.iss.it/binary/mabi/cont/Interim_Report_2018_finale.pdf.
29. Stefanelli P, Miglietta A, Pezzotti P, Fazio C, Neri A, Vacca P, et al. Increased incidence of invasive meningococcal disease of serogroup C / clonal complex 11, Tuscany, Italy, 2015 to 2016. Euro Surveill. 2016;21:pii=30176.
30. Lamelas A, Harris SR, Röltgen K, Dangy JP, Hauser J, Kingsley RA, et al. Emergence of a new epidemic *Neisseria meningitidis* serogroup A Clone in the African meningitis belt: high-resolution picture of genomic changes that mediate immune evasion. MBio. 2014;21:e01974-14.
31. Duchêne S, Holt KE, Weill FX, Le Hello S, Hawkey J, Edwards DJ, et al. Genome-scale rates of evolutionary change in bacteria. Microb Genom. 2016;30;2(11):e000094. doi: 10.1099/mgen.0.000094
32. Bowers JR, Kitchel B, Driebe EM, MacCannell DR, Roe C, Lemmer D, et al. Genomic analysis of the emergence and rapid global dissemination of the Clonal Group 258 Klebsiella pneumonia Pandemic. PLoS One. 2015;21;10(7):e0133727. doi: 10.1371/journal.pone.0133727
33. EpiCentro. Available from: www.epicentro.iss.it/problemi/meningiti/report_mening_veneto.asp.
34. Ferro A, Menegon T. Emergenza meningite: l'operatività locale e regionale. Available from: https://salute.regione.veneto.it/c/document_library/get_file?uuid=b4861725-d3be-4c48-be8f-7333baf21223&groupId=73838.
35. Miglietta A, Fazio C, Neri A, Pezzotti P, Innocenti F, Azzari C, et al. Interconnected clusters of invasive meningococcal disease due to *Neisseria meningitidis* serogroup C ST-11 (cc11), involving bisexuals and men who have sex with men, with discos and gay-venues hotspots of transmission, Tuscany, Italy, 2015 to 2016. Euro Surveill. 2018;23. ES.2018.23.34.
36. Italia. Regione Toscana. Campagna contro il meningococco C. Misure di profilassi e prevenzione Available from: www.regione.toscana.it/-/campagna-contro-il-meningococco-c.

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Epidemiological data and treatment strategies in children with severe haemophilia in Italy

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Abstract

Objectives. In a period of important therapeutic changes in the field of haemophilia care, we provide updated statistics on children with severe haemophilia (0-12 years of age) in Italy.

Methods. Data presented are from the Italian National Registry of Congenital Coagulopathies (NRCC) – survey 2017.

Results. Children with severe Haemophilia A (HA) were 242, those with severe haemophilia B (HB) 48. Prophylaxis was adopted in 92.1% of individuals with severe HA and 88.6% with severe HB. Thirty-nine children (14.8%) were on treatment for inhibitors. FVIII prescribed to children with severe HA represented 11.1% of the total consumption, of which 4.6% was extended half-life (EHL). FIX given to children with HB accounted for 7.2% of the total FIX, of which 19.1% was EHL-FIX.

Conclusion. The paediatric population analysed is characterized by a great adherence to therapy, so this data may constitute a benchmark for use of new, alternative therapies in the coming years.

Key words

- paediatric individuals
- haemophilia A
- haemophilia B
- treatment strategies

INTRODUCTION

Haemophilias and von Willebrand's Disease are the most frequent congenital bleeding disorders. Haemophilia A (HA) and haemophilia B (HB) are characterized by the deficiency of one of the proteins involved in blood clotting: factor VIII (FVIII) in HA and factor IX (FIX) in HB. Clinical picture in haemophilia is classified in three main groups based on residual FVIII or FIX coagulant activity: severe (<1% of normal activity levels), moderate (<5%) and mild (5-40%) [1]. Patients with severe haemophilia often have spontaneous bleeds, above all haemarthrosis, chronic pain and impaired joint function [2], and are at risk of life-threatening bleedings, most commonly in young children [3]. Patients with moderate or mild haemophilia usually suffer from abnormal bleeding after trauma or surgery.

Rapid and reliable identification of these diseases is important to allow the adoption of appropriate replacement therapies based on plasma-derived or recombinant FVIII and FIX concentrates that can be administered for the treatment of a bleeding event (on demand) or on a regular basis to prevent bleeding episodes (prophylaxis). Although haemophilia is an inherited bleeding disorder, about a third of cases of mild and moderate HA and HB, almost half of cases of severe HB and more than half of cases of severe HA are sporadic, being the first occurrence in the family [4].

Children diagnosed with haemophilia need regular follow-up that, in Italy, is provided by the national health system through the 54 Haemophilia Treatment Centres (HTCs). In children with severe haemophilia, prophylaxis today is considered the first-choice therapy for reducing bleedings and preserving joint health [5-7]. Patients with haemophilia on prophylaxis require frequent intravenous injections owing to the short half-life of FVIII and FIX. In this light, over the last years a new generation of coagulation factors characterised by extended half life (EHL) has been developed for treatment of patients with haemophilia. By reducing the frequency of administrations, these products may, in the

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future, improve adherence to treatment and the quality of life, especially in young patients [8].

Nowadays, an important issue in haemophilia treatment remains the risk, especially in children, of the development of neutralizing antibodies, or inhibitors, to FVIII and FIX. In the literature, inhibitor development is reported in about 30% of previously untreated individuals with severe HA [9] and in up to 10% of those with severe HB [10].

Currently, the only known intervention to successfully eradicate inhibitors is immune tolerance induction (ITI) that consists in the infusion of high doses of FVIII, regularly administered for months or years [11, 12]. Patients with high-titre inhibitors also require treatment with bypassing agents – activated prothrombin complex concentrates (aPCC, FEIBA®) and recombinant activated factor VII (rFVIIa, NovoSeven®) – which bypass the role of the FVIIIa-FIXa complex within the clotting cascade. Both, rFVIIa and aPCCs, are effective for the treatment of acute bleeds in haemophilia with inhibitors [13]. Since the second half of 2018, another treatment for HA subjects with inhibitors, emicizumab, an antibody that bridges FIXa and FX mimicking the function of FVIII has become available in Italy [14].

The last annual survey of the Italian National Registry of Congenital Coagulopathies (NRCC), for the year 2017, indicates that children ≤12 years of age with severe haemophilia are about three-hundred. We have addressed our study to the analysis of this paediatric population because, in an age of important therapeutic changes, it represents the population that can benefit most from the recent innovations in haemophilia therapy and, considering the great adherence to therapy in this cohort of individuals, it can represent the benchmark for further management in the coming years.

In this paper, we provide information on this cohort of paediatric individuals with severe haemophilia, as it emerges from the Italian Annual Survey 2017. The following have been analysed: epidemiological data, treatment regimens, treatment-related complications, and concentrate consumption.

MATERIALS AND METHODS

The 54 Italian HTCs are coordinated by the Italian Association of Haemophilia Centres (AICE) that develops uniform therapeutic and diagnostic strategies and contributes to data monitoring and transmission to the NRCC.

The patients' demographic and clinical data are recorded in a password-secured web-based platform, managed by AICE and shared anonymously with the Italian National Institute of Health for elaboration by NRCC [15, 16]. Individual data are collected in accordance with the current EU standards on privacy and the subjects enrolled in the registry were asked to sign a consent form to allow data collection for epidemiological and clinical research purposes. A specific section of the registry is dedicated to drug prescriptions. Data are collected on the basis of the factor concentrate prescriptions by the HTCs, mandatory for patients with inherited bleeding disorders [16]. Data were analysed by age groups (0-3, 4-6, 7-9 and 10-12 years of age).

The total of FVIII and FIX concentrate prescriptions reflects the voluntary adhesion of each HTC to report on the prescriptions in the registry database. We have considered EHL recombinant products those that meet the classification proposed by Mahlangu [17, 18]. In 2017 novel EHL available in Italy were: recombinant FVIII and FIX fused to the Fc portion of human immunoglobulins (rFVIII-Fc, Elocta®, from the second half of 2016; rFIX-Fc, Alprolix®, from 2017), recombinant FIX fused to albumin (rFIX-FP, Idelvion®, from 2017) and rFVIII-SingleChain (Afstyla®, available from the second half of 2017).

Concentrates and bypassing agents utilized for inpatients were not included in the study because data on prescriptions are not available.

RESULTS

Epidemiology

The NRCC counted 542 paediatric individuals with haemophilia: 435 with HA and 107 with HB, accounting for 10.4% and 11.9% of the total number of registered patients with HA and HB, in 2017. The severe forms accounted for 290 individuals: 55.6% and 44.9% of the total number of registered children with HA and HB, respectively *(Table 1)*.

Since the treatment of these patients started after the introduction of recombinant products and the adoption of robust methods for viral inactivation and highly sensitive assays for testing plasma-derived products, none of the patients, as expected, resulted positive to hepatitis nor Human Immunodeficiency Virus (HIV).

Data on HA and HB child patients were provided from 48/54 HTCs; the six missing centres were non-paediatric HTCs.

Home treatment

The assessment of treatment regimens for home therapy was based on the HTC prescriptions to HA and HB children for home treatment. As shown in *Table 1*, prescriptions provided to the registry database during 2017 covered almost all the patients with the severe forms registered in this cohort, as high as 92.1%

Table 1
Children with haemophilia A and B, registered in the NRCC, as of 2017

Pathology	Children registered	Children with therapeutic prescriptions	Coverage (%)
Severe haemophilia A	**242**	**223**	***92.1***
Moderate haemophilia A	67	46	*68.7*
Mild haemophilia A	126	47	na
Severe haemophilia B	**48**	**40**	***83.3***
Moderate haemophilia B	22	16	*72.7*
Mild haemophilia B	37	13	na

na: not applicable; NRCC: National Registry of Congenital Coagulopathies.

Table 2
Children with severe haemophilia A and B registered in the NRCC, as of 2017

Age group	Children registered	Children with therapeutic prescriptions	Coverage (%)
		Severe HA	
0-3	59	58	*98.3*
4-6	48	44	*91.7*
7-9	65	59	*90.1*
10-12	70	62	*88.6*
Total	**242**	**223**	***92.1***
		Severe HB	
0-3	11	10	*90.1*
4-6	16	15	*93.7*
7-9	12	8	*66.7*
10-12	9	7	*77.8*
Total	**48**	**40**	***83.3***

in the severe cases, but lower in the moderate forms (68.7%). For HB the coverage was 83.3% for the severe and 72.7% for the moderate form. Patients with mild HA and HB rarely needed replacement therapy, especially those mild HA patients who can benefit for the desmopressin. For this reason, we did not deem it appropriate the analysis of replacement therapies in the population of mild HA *(Table 1)*. The analysis by age groups showed that therapeutic prescriptions covered more than 88% in all age groups of children, except in the two oldest cohorts (7-9 and 10-12 years of age) of children with severe HB *(Table 2)*. The coverage differences were not statistically significant (Chi-square test, p=0.2304).

Prophylaxis resulted the therapeutic regimen of choice for 92.1% of children with severe HA and for 88.6% with severe HB. Details on therapeutic regimens distinguished by age group are given in *Table 3*. Excluding the individuals with an inhibitor, the on demand treatment was used (25.5% of the patients) only in the 0-3 years of age group with severe HA, while prophylaxis was the most widely used therapeutic regimen in the other age groups of both severe HA and HB subjects (more than 97.1% in HA and more than 83.3% in HB). The mean annual amount of FVIII and FIX prescribed for children with severe haemophilia in prophylaxis, detailed by age group, is reported in *Table 4*. The proportion of children switched to prophylaxis with EHL-FVIII and -FIX was 10.3% and 29.0%, respectively *(Table 4)*. In severe HA children on prophylaxis, plasma-derived FVIII concentrates were employed only in 2.9% of the patients (5/174).

Treatment of inhibitor patients

During 2017, 39 individuals with severe haemophilia were under treatment for inhibitors: 15.2% (n=34) of children with severe HA and 12.5% (n=5) of those with severe HB. The age distribution of these individuals and the treatment regimens used are reported in *Table 3*. Among the 34 children with severe HA, ITI alone was used in 12 children (35.3%), and combined treatment with bypassing agents and ITI was used in 10 children (29.4%). The 5 individuals with severe HB and inhibitors were treated with rFVIIa alone.

Drugs prescribed for home treatment

The total amount of FVIII prescribed to children with severe HA, was about 46,000,000 international units (IU): 85.5% was conventional recombinant FVIII, 4.6% EHL-FVIII and the remaining 9.9% plasma-derived FVIII (*Table 5*). This value (46,000,000 IU) represents 11.1% of the FVIII prescribed to all individuals (adults and children alike) with severe HA [16].

As for FIX, the total amount prescribed to children with severe HB was about 3,400,000 IU, all in recombinant form: 80.9% conventional recombinant FIX and 19.1% EHL-FIX *(Table 5)*. The prescription in the pae-

Table 3
Therapeutic regimens used for children with severe haemophilia A and haemophilia B, as of 2017

	Age group	Children without inhibitor			Children with inhibitor		Total
Severe HA		**Prophylaxis**	**On demand**	**ITI**	**ITI + aPCC and/or rFVIIa**	**aPCC and/or rFVIIa**	
	0-3	38	13	2	3	2	**58**
	4-6	33	1	4	5	1	**44**
	7-9	50	-	5	1	3	**59**
	10-12	53	1	1	1	6	**62**
	Total	**174**	**15**	**12**	**10**	**12**	**223**
Severe HB		**Prophylaxis**	**On demand**	**ITI**	**ITI + rFVIIa**	**rFVIIa**	
	0-3	7	1	-	-	2	**10**
	4-6	10	2	-	-	3	**15**
	7-9	8	-	-	-	-	**8**
	10-12	6	1	-	-	-	**7**
	Total	**31**	**4**	**-**	**-**	**5**	**40**

aPCC: activated prothrombin complex concentrate; HA: haemophilia A; HB: haemophilia B; ITI: immune tolerance induction; rFVIIa: recombinant activated factor VII.

Table 4
Mean annual dose used by children with severe HA and HB in prophylaxis, as of 2017

		Conventional FVIII				EHL – FVIII			
	Age group	n	Mean in prophylaxis (IU per pt)	CI (95%)		n	Mean in prophylaxis (IU per pt)	CI (95%)	
Severe HA	0-3	32	133,500	86,300	180,800	6	64,300	21,000	107,600
	4-6	30	125,400	102,800	148,000	3	105,300	46,400	164,200
	7-9	46	182,100	150,800	213,500	4	164,000	70,600	257,400
	10-12	48	213,000	189,700	236,200	5	204,000	162,300	245,700
	Total	**156**	**170,800**	**154,400**	**187,300**	**18**	**132,100**	**94,000**	**170,300**
		Conventional FIX				**EHL – FIX**			
Severe HB	0-3	7	47,300	24,300	70,300	-	-	-	-
	4-6	8	110,700	83,100	138,300	2	53,000	51,000	55,000
	7-9	3	117,000	73,900	160,100	5	93,400	72,300	114,500
	10-12	4	168,000	84,700	251,400	2	100,000	92,160	107,800
	Total	**22**	**107,800**	**80,329**	**135,325**	**9**	**85,900**	**69,300**	**102,500**

CI: confidence interval; FVIII: factor VIII; FIX: factor IX; HA: haemophilia A; HB: haemophilia B; IU: international units; n: number; pt: patient.

diatric population accounts for 7.2% of the total FIX prescribed to severe HB subjects [16].

The amount of bypassing agents prescribed to children with inhibitors was ~3,500,000 IU of aPCC and ~7,700 milligrams of rFVIIa.

DISCUSSION

In this article, we have described the epidemiological data of the Italian children (≤12 years of age) with severe HA and HB and analysed the prescriptions of FVIII and FIX concentrates in this age group as well as the therapeutic regimens adopted.

A meta-analytic approach using national registries from Canada, France and the United Kingdom reported an overall prevalence of 6.0/100,000 males for severe HA and 1.1/100,000 males for severe HB [19], similar to that reported by the NRCC: severe HA=6.3/100,000 males and severe HB=1.1/100,000 males [16].

The prescriptions for home therapy in the 0-12 year cohort, covered about 90% of children with severe HA and severe HB registered in the demographic of the same registry. This percentage, higher than that observed in the total haemophilic population [16], indicates a special attention to disease management and an accurate involvement of parents for the management and monitoring of children with severe haemophilia.

The coverage of the treatment prescriptions for the moderate and mild cases was lower than that of severe cases: this reflects the reduced demand for replacement therapies in these paediatric subgroups as these individuals, particularly those with the mild forms, do not require frequent treatment, namely because most of those with mild HA respond to desmopressin [6].

Prophylaxis has gradually become the standard of care in the developed world and, as expected, is largely adopted in the children analysed in this study. Prophylaxis was less frequently adopted only in the 0-3 years age group; this is probably due to the difficulty of venous access or because bleeds are relatively infrequent, especially in the first year of age. The amount of FVIII

Table 5
Factor VIII and factor IX usage in severe HA and HB children, by age group, regimen and concentrates, as of 2017

	0-3	4-6	7-9	10-12	Total
FVIII for prophylaxis and on demand (IU)	4,728,250	4,041,750	9,299,250	11,405,000	29,474,250
FVIII for ITI (IU)	2,668,000	7,510,000	4,326,000	1,880,000	16,384,000
Total FVIII (IU)	**7,396,250**	**11,551,750**	**13,625,250**	**13,285,000**	**45,858,250**
Plasma-derived FVIII	*4.9%*	*17.4%*	*11.1%*	*4.8%*	*9.9%*
Recombinant FVIII	*89.8%*	*80.7%*	*84.1%*	*88.9%*	*85.5%*
Extended Half-Life FVIII	*5.3%*	*1.9%*	*4.8%*	*6.3%*	*4.6%*
Total FIX (IU)	**340,000**	**1,192,750**	**833,000**	**1,006,500**	**3,372,250**
Plasma-derived FIX	*0.0%*	*0.0%*	*0.0%*	*0.0%*	*0.0%*
Recombinant FIX	*100.0%*	*91.1%*	*47.5%*	*90.1%*	*80.9%*
Extended Half-Life FIX	*0.0%*	*8.9%*	*52.5%*	*9.9%*	*19.1%*

FVIII: factor VIII; FIX: factor IX; ITI: immune tolerance induction; IU: international units.

and FIX concentrates prescribed in children with severe haemophilia in prophylaxis, was similar to that reported in France, Germany, Spain, the United Kingdom [20] and Australia [21].

All Italian patients with severe haemophilia are regularly monitored for the occurrence of inhibitor development, as management of bleeding episodes in individuals with inhibitors is particularly difficult. Analysing the therapeutic prescriptions, 15.2% of children with severe HA and 12.5% of those with severe HB were under treatment for inhibitors during the year 2017 and the highest percentage of individuals treated for an inhibitor was in the 4-6 years of age group of severe HA patients (22.7%).

The therapies offered to inhibitor children were ITI and bypassing agents, according to the evidence provided by the medical literature that ITI is effective for the inhibitor eradication and the proven efficacy of recombinant factor VIIa and aPCC for the treatment of bleeding episodes in haemophilia patients with inhibitors [11-13].

The high prevalence of children with severe HA and inhibitors on ITI (64.7%) indicates the popularity of ITI in our country derived from the longstanding experience in this field and the overall success rate of 60-80% [22, 23]. Since the second half of 2018, the Italian national health system provides another treatment for HA patients with inhibitors, emicizumab, an antibody that mimics the function of FVIII [14]. This product has been shown to be safe and efficacious in reducing the incidence of bleeding episodes in patients with inhibitor to FVIII [24]. The benefits of inhibitor eradication include the possibility to restore prophylaxis and to reduce the costs of the long-term inhibitor therapies. This situation could be significantly affected by the availability of emicizumab. Children with HB complicated by an inhibitor respond less frequently to ITI and HB in itself is a poor prognostic indicator of ITI success [25]. As a consequence, no one child with severe HB and an inhibitor was treated with an ITI regimen during the year 2017.

Recombinant factors were the most prescribed products in children with haemophilia: 90% in patients with HA and 100% in those with HB. In over 20 years of clinical trials and worldwide experience with recombinant products, the transmission of infectious pathogens was virtually eliminated and no increase in the inhibitor occurrence in the treated haemophilia population was observed. Recently, the introduction of EHL concentrates that facilitate prophylaxis and improve the quality of life, namely in children, has represented a major breakthrough in haemophilia treatment [8]; especially the EHL-FIX products reduce the frequency of intravenous infusions and are very effective, both in the treatment and in prevention of bleeds [18]. For the first time in 2017, the prescription of the EHL products was reported in the NRCC as they were licenced in Italy at the end of 2016.

The European Medicine Agency has recently licensed emicizumab for severe HA patients without inhibitors and the drug is presently undergoing evaluation by the Italian Drug Agency (AIFA, Agenzia Italiana del Farmaco) to make it available through the national health system. Emicizumab requires dosing frequencies significantly reduced in comparison to EHL-FVIII and the advantage of the subcutaneous infusion makes the drug substantially easier usage, especially in children.

CONCLUSIONS

We have analysed the data reported to the NRCC with reference to the paediatric cohort of children with severe haemophilia (0-12 years of age). This paediatric population is characterized by a great adherence to therapy so it may represent a benchmark for use of new therapies, in the future.

In the time of important therapeutic changes in the field of haemophilia care, the NRCC can represent a powerful tool for the comparative evaluation of the efficacy of new drugs and for the assessment of their side effects.

Authors' contributions

FA, HJH, ES, RA, MB and AG made substantial contributions to conception, design, acquisition of data, analysis and interpretation of data. All Authors and co-authors have been involved in drafting the manuscript and revising it critically for important intellectual content. All Authors gave final approval of the version to be published.

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REFERENCES

1. Bolton-Maggs PH, Pasi KJ. Haemophilias A and B. Lancet. 2003;361:1801-9.
2. Mannucci PM, Tuddenham EG. The hemophilias – from royal genes to gene therapy. N Engl J Med. 2001;344:1773-9.
3. Chalmers EA, Alamelu J, Collins PW, Mathias M, Payne J, Richards M, et al. Intracranial haemorrhage in children with inherited bleeding disorders in the UK 2003-2015: A national cohort study. Haemophilia. 2018;24:641-7. doi: 10.1111/hae.13461
4. Kasper CK, Lin JC. Prevalence of sporadic and familial haemophilia. Haemophilia. 2007;13:90-7.
5. Iorio A, Marchesini E, Marcucci M, Stobart K, Chan AK. Clotting factor concentrates given to prevent bleeding and bleeding-related complications in people with hemophilia A or B. Cochrane Database Syst Rev. 2011;CD003429. doi: 10.1002/14651858.CD003429.pub4
6. Rocino A, Coppola A, Franchini M, Castaman G, Santoro C, Zanon E, et al. Principles of treatment and update of recommendations for the management of haemophilia and congenital bleeding disorders in Italy. Blood Transfus. 2014;12:575-98. doi: 10.2450/2014.0223-14
7. Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A, et al. Treatment Guidelines Working Group on Behalf of The World Federation of Hemophilia. Guidelines for the management of hemophilia. Haemophilia. 2013;19:e1-47. doi:10.1111/j.1365-2516.2012.02909.x
8. Thornburg CD, Duncan NA. Treatment adherence in hemophilia. Patient Prefer Adherence. 2017;11:1677-86. doi: 10.2147/PPA.S139851
9. Franchini M, Coppola A, Rocino A, Santagostino E, Tagliaferri A, Zanon E, et al. Systematic review of the role of FVIII concentrates in inhibitor development in previously untreated patients with severe hemophilia A: a 2013 update. Semin Thromb Hemost. 2013;39:752-66. doi: 10.1055/s-0033-1356715
10. Franchini M, Santoro C, Coppola A. Inhibitor incidence in previously untreated patients with severe haemophilia B: a systematic literature review. Thromb Haemost. 2016;116:201-3. doi: 10.1160/TH16-02-0116
11. Athale AH, Marcucci M, Iorio A. Immune tolerance induction for treating inhibitors in people with congenital haemophilia A or B. Cochrane Database Syst Rev. 2014;24:CD010561. doi: 10.1002/14651858.CD010561.pub2
12. Mariani G, Siragusa S, Kroner BL. Immune tolerance induction in hemophilia A: a review. Semin Thromb Hemost. 2003;29(1):69-76. doi:10.1055/s-2003-37941
13. Matino D, Makris M, Dwan K, D'Amico R, Iorio A. Recombinant factor VIIa concentrate versus plasma-derived concentrates for treating acute bleeding episodes in people with haemophilia and inhibitors. Cochrane Database Syst Rev. 2015;16:CD004449. doi: 10.1002/14651858.CD004449.pub4
14. Kitazawa T, Igawa T, Sampei Z, Muto A, Kojima T, Soeda T, et al. A bispecific antibody to factors IXa and X restores factor VIII hemostatic activity in a hemophilia A model. Nat Med. 2012;18:1570-4. doi: 10.1038/nm.2942
15. Iorio A, Oliovecchio E, Morfini M, Mannucci PM; Association of Italian Hemophilia Centres Directors. Italian Registry of Haemophilia and Allied Disorders. Objectives, methodology and data analysis. Haemophilia. 2008;14(3):444-53. Erratum in: Haemophilia. 2009;15:637. doi: 10.1111/j.1365-2516.2008.01679.x
16. Abbonizio F, Hassan HJ, Riccioni R, Santagostino E, Arcieri R, Giampaolo A; Italian Association of Haemophilia Centres. New data from the Italian National Register of Congenital Coagulopathies, 2016 annual survey. Blood Transf. 2020;18:58-66. doi 10.2450/2019.0211-18
17. Mahlangu J, Young G, Hermans C, Blanchette V, Berntorp E, Santagostino E. Defining extended half-life rFVIII-A critical review of the evidence. Haemophilia. 2018;24:348-58. doi: 10.1111/hae.13438
18. Mahlangu JN. Updates in clinical trial data of extended half-life recombinant factor IX products for the treatment of haemophilia B. Ther Adv Hematol. 2018;9:335-46. doi: 10.1177/2040620718802606
19. Iorio A, Stonebraker JS, Chambost H, Makris M, Coffin D, Herr C. Establishing the prevalence and prevalence at birth of hemophilia in males: A meta-analytic approach using National Registries. Ann Intern Med. 2019;171:540-6. doi: 10.7326/M19-1208
20. Berntorp E, Dolan G, Hay C, Linari S, Santagostino E, Tosetto A, et al. European retrospective study of real-life haemophilia treatment. Haemophilia. 2017;23:105-14. doi: 10.1111/hae.13111
21. Mason JA, Parikh S, Tran H, Rowell J, McRae S. Australian multicentre study of current real-world prophylaxis practice in severe and moderate haemophilia A and B. Haemophilia. 2018;24:253-60. doi: 10.1111/hae.13375
22. Coppola A, Di Minno MN, Santagostino E. Optimizing management of immune tolerance induction in patients with severe haemophilia A and inhibitors: towards evidence-based approaches. Br J Haematol. 2010;150:515-28. doi:10.1111/j.1365-141.2010.08263.x
23. Mariani G, Kroner B; Immune Tolerance Study Group (ITSG). Immune tolerance in hemophilia with factor VIII inhibitors: predictors of success. Haematologica. 2001;86:1186-93.
24. Oldenburg J, Mahlangu JN, Kim B, Schmitt C, Callaghan MU, Young G, et al. Emicizumab prophylaxis in hemophilia A with inhibitors. N Engl J Med. 2017;377:809-18. doi: 10.1056/NEJMoa1703068
25. Ljung R, Auerswald G, Benson G, Dolan G, Duffy A, Hermans C, et al. Inhibitors in haemophilia A and B: Management of bleeds, inhibitor eradication and strategies for difficult-to-treat patients. Eur J Haematol. 2019;102:111-22. doi: 10.1111/ejh.13193

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Dermopigmentation of the nipple-areola complex in a dedicated breast cancer centre, following the Treviso Hospital (Italy) LILT model

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Abstract

Background. Dermopigmentation, also known as medical tattooing, is a complementary technique in the reconstruction of the nipple-areola and an adjuvant procedure to improve colour mismatch. In 2009, tattooing of the nipple-areola complex (NAC) was introduced by Treviso Hospital through a project conducted in cooperation with the local section of the Italian Anti-Cancer League (LILT).

Methods. From 2010 to 2016, 169 patients treated for breast cancer underwent dermopigmentation treatments. Patients were selected by the hospital plastic and breast surgeons. Dermopigmentation was performed at the LILT (*Lega Italiana per la Lotta contro i Tumori,* Italian Cancer League) facility following a specific procedure to ensure safety. A sterile disposable surgical set was used.

Results. Of 169 patients treated in 309 treatment sessions, no serious complications were reported after tattooing, with only three cases seen of minor complications. Patients expressed a high level of satisfaction (90%) with the aesthetic results.

Conclusion. The study found that dermopigmentation of the NAC is a safe approach, providing benefits both to the patients and the hospital itself. Medical tattooing of the NAC is a simple and safe nonsurgical technique that reduces missed workdays and increases the time available for other commitments. This ultimately translates into savings for society and the healthcare system.

Key words
- tattooing
- nipple areola complex
- restore
- scar

INTRODUCTION

Breast cancer is the most common tumour found among women in Italy. In 2018, 52,800 new cases were estimated, accounting for 29% of affected women [1]. Surgery is usually the initial treatment and, in the early stages of the disease, conservative surgery associated with radiotherapy has replaced radical mastectomy, which is still the standard approach in more advanced stages of the disease. When the mammary neoplasm affects the tissue below the areola and/or invades the surrounding skin, excision of the whole nipple-areola complex (NAC) may be necessary [2], which seriously compromises the aesthetic results of any subsequent reconstruction. This may lead to a more severe psychological impact on the patient.

Considerable attention is now given to the likely psychological reaction to the reconstruction of affected areas. In order to improve outcomes, breast cancer in Italy today is tackled by a multidisciplinary group under the organizational structure of a dedicated breast

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centre. In this multidisciplinary specialist centre (called the "Breast Unit" locally), experts from different backgrounds work together to provide patients with customized treatment to successfully treat the disease and to guarantee a satisfactory quality of life.

Reconstruction of the NAC after mastectomy is a straightforward procedure from a technical point of view, but its importance from an aesthetic perspective [3] should not be underestimated. NAC reconstruction can be performed with all types of reconstructive procedures, and at any time following surgery. Studies have shown that recreation of the NAC has a high correlation with overall patient satisfaction and acceptance of body image [4].

The evolution of NAC reconstruction started with the initial description of the nipple-areola graft and labial graft [5, 6]. Following this, Millard proposed the nipple sharing concept, where the contralateral nipple tissue was used as a composite graft for the reconstructed nipple [7].

These techniques were, to some degree, successful in providing passable aesthetic results, but at the expense of significant donor site morbidity [8, 9].

Dermopigmentation (medical tattooing) can be used as a complement to reconstructive surgery [10, 11], and is generally used to cover skin conditions and restore the appearance of healthy skin.

Tattooing of the areola in post-mastectomy reconstruction, initially introduced by Rees in 1975 and Spear *et al*., is an important nonsurgical technique that serves as an adjunct to areola reconstruction [12-14].

Recently, 3D dermopigmentation has become a technique of choice in the NAC reconstruction process; it mimics the nipple-areola complex by means of defining the areolar contour and Montgomery's tubercles [15, 16].

As part of this approach, dermopigmentation is often used in NAC reconstruction as it is the easiest and safest way to restore much of the appearance of the original anatomy in women who have undergone mastectomy [17, 18].

This tattooing technique is a simple, quick and safe procedure which has achieved a high level of patient satisfaction and can provide an excellent areolar colour match with limited morbidity [19, 20]. As a result, the aesthetic improvements obtained can reduce the psychological impact of physical changes following surgery [21].

In the province of Treviso, about 800 new cases of breast cancer are reported each year. Since 2009, they have been managed following the advice of a multidisciplinary team based at the dedicated unit ("Breast Unit" of Treviso Hospital). In 2009, a project was launched to introduce tattooing in the reconstruction of the nipple-areola complex. This project resulted from the synergy created between the multidisciplinary team of the Operational Plastic Surgery Unit of the former Local Health and Social Care Services Unit number 9 and the Treviso section of the Italian Cancer League (LILT).

This dedicated breast unit was the first centre in Italy to apply this technique, in 2010, and was the first to officially recognize the role of "dermopigmentators" in its Diagnostic and Therapeutic Pathway for breast cancer. A dermopigmentator is defined as a professional who has been suitably trained to take part in a multidisciplinary team treating patients; he/she works by complying fully with a strict health and hygiene protocol specifying the use of appropriate materials and equipment in a suitable work environment.

Dermopigmentation is indicated for:

- tattooing of the nipple-areola complex, specifically for correcting dyschromia due to surgery and correction of the newly reconstructed areola;
- eyebrow and infracillary eyeliner, known as Permanent Make Up (PMU), which restores the effect of eyebrows and eyelashes in patients with alopecia due to chemotherapy;
- correction of scars due to demolitive surgery or the camouflaging of scars due to surgery.

Since 2013, the Treviso team has been collaborating with the *Istituto Superiore di Sanità* (National Institute of Health, ISS). The latter contributed to the evaluation and improvement of the medical tattooing procedure used, under an agreement with the Italian Ministry of Health that aimed to evaluate risks and benefits of this type of treatment compared with the traditional treatments. This cooperation started with the National Centre ONDICO and is currently continuing with the National Centre for Innovative Technologies in Public Health (TISP Centre), which has carried out various studies regarding the safety and the advantages of medical tattooing.

This paper reports on the dermopigmentation of the nipple-areola complex performed from 2010 to 2016 on cancer patients treated in the specialist unit of Treviso Hospital.

The aims of the project were to:

- improve breast aesthetics by creating, as far as possible, an areola and a nipple matching the contralateral breast or its original appearance before surgery, thus improving patients' self-confidence;
- design a specific procedure to ensure the complete safety of the patient;
- measure the levels of patient satisfaction with the results of the treatment received;
- assess the usefulness of dermopigmentation for patients;
- provide benefits both to the patients and the health care unit.

MATERIALS AND METHODS

According to the diagnostic and therapeutic pathway set down by Treviso Hospital's Breast Centre, tattooing is used after the completion of reconstructive surgery; the Breast Centre laid down a detailed procedure that establishes when and how to intervene. Patients selected for dermopigmentation are chosen by the plastic and breast surgeons. After careful assessment of the patient's medical history, they complete a form authorizing tattooing. The following factors are taken into consideration and assessed: original disease, type of surgery and adjuvant therapies undergone by the patient (chemotherapy, radiotherapy), general state of health, administration of medication, allergies, and

time lapse before tattooing can take place. In the case of Treviso Hospital, dermopigmentation is performed at least 6-12 months after the completion of breast reconstruction, which is enough time for the healing to complete and for the breast to stabilize.

If no contraindication is found, consent for treatment is given and a briefing with the professional in charge of performing the tattoo is held. The plastic surgeon presents the patient's case and precautions to be taken during the treatment. Before tattooing, the patient receives information about the treatment and the associated risks. The patient signs the informed consent form to declare that she has fully understood the information and suggestions received and commits to carefully and strictly following the aftercare guidance. The patient also reports any local or general diseases, anomalies in the wound healing process, allergies and intolerances, medical treatments in progress, and abnormal reactions following previous tattooing or PMU treatments. She also declares that she is not pregnant or breastfeeding. Photographs are taken before and after the treatments.

Dermopigmentation is performed at the LILT facility, in close cooperation with the Treviso multidisciplinary team.

The training and professional experience of the operator dealing with the medical tattoo are crucial. The tattooing needs to be performed swiftly and with full mastery of the dermograph, avoiding excessively stimulating or inflaming the tissue as this may jeopardise the patient's general health. In addition, the pigment should be injected at the right depth in the skin to avoid excessive loss of colour.

For patients affected by dermatological problems (e.g. a nickel allergy), a specific procedure is agreed with the dermatologist. According to the procedure laid down, the most suitable ink colours are selected and the patient is referred to the dermatological unit where the pigments' safety data sheets and composition are assessed to exclude the risk of specific reactions. If in doubt, an allergological test is carried out. Upon completion of the investigations, the treatment can start, if the medical opinion is favourable.

In the case of patients affected by psoriasis or conditions likely to induce reactive isomorphism, treatment modes are assessed on a case-by-case basis by the dermatologist.

NAC tattooing is performed on cancer patients who may present different degrees of immunodepression. Thus, strict sterility applies both to instruments and devices, and to the execution of the tattoo according to a detailed procedure.

For that purpose, a sterile disposable surgical set has been created to be used at the workstation. It is classified as a Medical Device and commonly referred to as a "sterile field" *(Figure 1)*, and includes:

- covering coupled sterile sheets (100x150 cm);
- cotton yarn 70 cm;
- camera protection cover 14x254 cm to cover the dermograph;
- 10 TNT bandages 10x10 cm with 4 layers;
- 2 chlorhexidine soaked wipes;
- 1 100 ml bowl;
- 1 coupled sterile sheet with adhesive holes (15 cm diameter) 150x100 cm;
- 1 dermographic marker;
- 2 needles 21gx10;
- Sterile transparent cover 50x60 cm for the dermograph console and transformer;
- 1 tray 24x14x2,5 cm;
- 1 gown and towel for the operator;
- double sided adhesive tape 5x15 cm.

The sterile field is essential to avoid complications.

Pigments comply with specific product and process requirements. In particular, pigments are:

- atoxic, hypoallergenic and compliant with the requirements of ResAP 2008/1;

Figure 1
Example of a workstation and practice (sterile field set and disposable equipment).

- disposable;
- manufactured in a sterile chamber with medical grade water;
- sterilised at the end of the manufacturing process using Gamma rays;
- free from metallic impurities likely to cause complications during certain diagnostic tests.

Following research and study, pigments were carefully chosen according to their specific characteristics to enable a selection of inks that could be well tolerated by patients, while simultaneously serving their purpose. The PMU colours commonly marketed are not specific to NAC tattoos as they last for too short a time, thus requiring retouching after a very short period (once a year or every two years). This would cause excessive stress to the skin that would in turn react with the formation of scar tissue. Similarly, colours used for artistic tattoos should not be used as their result is definitive and final. Often, after 5 or 6 years (the average time between retouches) the natural contralateral areola may change in size and colour due to weight or hormonal changes or other reasons. In such cases, retouching is needed to adjust the result. The ideal pigments should be sufficiently stable to enable intervention when deemed appropriate to adjust colour and shape without creating scar tissue.

Thus, inks have been selected keeping in mind that their composition and molecular weight must not interfere with diagnostic tests, as this could lead to false positives or produce vibrations likely to interfere with MRIs [22, 23]. Moreover, the inks used are made with pigments having the same light fastness code to avoid colour alterations following light exposure. Each batch undergoes chemical and microbiological checks.

Stocks of inks and devices must be managed and stored in sterile conditions during their shelf-life.

Special care is taken regarding the choice of the diluent used to create new shades. This diluent also needs to be sterile, disposable and compatible with the formulation of the pigment to be diluted.

Pigments are mixed in special sterile disposable ink cups immediately prior to use.

Before starting, the expert consultant in medical tattooing and the patient choose the colour that best matches the contralateral NAC or her complexion and carry out a test in front of the mirror to obtain a more precise idea of the final result.

To create the same light and shade effects, the expert in dermopigmentation takes pictures before treatment (one in macro mode) of the natural nipple-areola complex to be used as a reference during the execution of the tattoo. The work carried out is photographed and documented; a sterile silicon gauze soaked in vitamin E is applied; and the tattooed area is covered with sterile gauze and a cotton pad, both allowing transpiration.

The photograph, the adhesive labels for the inks, and anything else needed to trace the materials used are attached to the informed consent form which is put in the patient's records and filed for future use.

At the end of the treatment, the patient is taught the correct aftercare procedure and given written self-care instructions. The patient is instructed to ensure the tattoo is kept well hydrated. For daily hygiene, the patient is instructed to carefully wash the tattooed area with lukewarm water and mild detergent and to delicately dab dry. The patient is discouraged from visiting saunas or Turkish baths or coming into contact with sea or swimming pool water during the first 10 days, and is advised to avoid intense physical activity likely to cause excessive transpiration during the first week. Furthermore, during the first 15 days, the tattoo should not be exposed to direct sunlight or sun lamps without using full screen protection cream/lotion. Applying sunscreen is also advisable after the said period to prevent photosensitization and ensure the colour lasts longer.

The result is assessed after about two months and, on that occasion, a second treatment may be decided upon. A third treatment may be needed, particularly when the original tattoo is more superficial in order to avoid complications (i.e. if the skin is particularly sensitive and thin, or if scars are very thick, or if the patient is taking medication that slows down the ordinary healing process). In this case also, treatment will be performed a minimum of two months after the previous procedure.

Patients tattooed from 2010 to 2016 were asked to rate their level of satisfaction regarding their dermopigmentation treatment in phone interviews conducted in 2017 by LILT volunteers. During the interview, patients were also asked to rate the usefulness of the project that enabled them to have medical tattooing, delivered according to their needs, cost free, under the medical supervision of the specialist.

To rate their satisfaction regarding the results of treatment, the patients were asked to express their views by selecting one of the following options: Very satisfied, Satisfied, Not very satisfied, Dissatisfied.

To assess the usefulness of the project, the patients were also asked to rate it by selecting one of the following options: Very useful, Useful, Neither useful nor useless, and Useless.

RESULTS

From 2010 to 2016, Treviso Hospital operated on an average of 300 breast tumour cases annually, of which 40% were mastectomies (approx. 120 patients). Of the latter, the surgery involved the sacrifice of the NAC in approximately 50 cases per year (a total of 350 cases over the period).

In this period, 169 patients were treated using dermopigmentation. The number of sessions required to achieve a satisfactory result ranged from a minimum of 1 to a maximum of 3 per patient, amounting to 309 treatment sessions in total *(Table 1)*.

Treatments of various types were performed:

- tattooing of the NAC, with a surgically reconstructed nipple;
- 3D tattooing with missing NAC;
- restoration of symmetry between NACs and/or reduction in differences in terms of position, shape, dimension, or colour.

Cases and figures are shown in *Table 1*.

In addition to medical tattooing performed as part of breast reconstruction, the following treatments were

Table 1
Number of sessions and treatments performed from 2010 to 2016 compared with the number of patients per type of treatment

Number of treatment sessions required to achieve final result		
n. of patients treated	**n. sessions**	**n. of treatments performed**
42	1	42
114	2	228
13	3	39
169	**Total**	**309**
Type of treatment		
49	3D tattoo with missing NAC	
110	NAC tattoo following nipple reconstruction surgery	
6	Tattoo to restore NAC symmetry	
4	3D tattoo with missing NAC on one breast, plus tattoo on surgically reconstructed nipple on the other breast	
169	**Total**	

NAC: nipple-areola complex.

performed: 33 permanent makeup, scar camouflage, and tattooing of the vitiligo of the NAC.

It should be noted that out of 169 treated patients, two were affected by psoriasis, and 38 patients, one of whom was allergic to nickel, had previously undergone radiotherapy.

In one case, a patient who had to undergo tattooing presented erythema and tenderness. She was therefore referred to the dermatologist who diagnosed erysipelas. Tattooing was postponed for three months, accompanied by appropriate prophylaxis. The area tattooed subsequently healed without complications.

Complications

Of 169 treated patients, no serious complications or toxicity were reported after tattooing.

In three cases, there were minor complications:

- One patient reported altered sensitivity and localized soreness, which the dermatologist diagnosed as dysesthesia due to irritation of the scar. These symptoms completely resolved after 3 weeks of physiotherapy.
- One patient who received a tattoo on a labia majora skin graft displayed small areas of skin abrasion covered by tiny scabs that lasted for about 10 days (on average, small scabs remain for 5/6 days, so in this case the healing process was slower). The clinical picture resolved completely after topical treatment of tetracycline medicated cream;
- One patient reported an allergic reaction that was resolved with topical cortisone treatment.

No treated patients showed late sequelae.

Satisfaction level

At a later stage, the 169 patients who underwent dermopigmentation of the nipple-areola complex between 2010 and 2016 were contacted by phone to respond to a satisfaction survey on the treatment received.

Of these, 141 subjects answered the survey: 137 patients reported they were "Very satisfied" or "Satisfied", and only four were "Not very satisfied" or "Dissatisfied" *(Table 2, a)*. Reasons for dissatisfaction were: in one case, early fading of the colour compared with the average duration time initially communicated to the patient; in another case, dissatisfaction was due to a very light areola against which the tattooed NAC did not look very realistic. The other two cases of dissatisfaction were due to psychological factors implying rejection of their new personal image.

Project usefulness

As for the usefulness of the project, 137 patients rated the opportunity of having dermopigmentation treatment as "Very useful"/"Useful". The project was considered "Neither useful nor useless" or "Useless" by only four interviewees, those that reported low satisfaction with the results of the treatment. *(Table 2, b)*.

DISCUSSION AND CONCLUSION

Medical tattooing of the NAC complements the process of breast reconstruction and seeks to achieve a more natural and pleasing result. It contributes to patient recovery and psychological and physical integrity. Tattooing the NAC is a simple and safe technique that can accompany plastic reconstructive surgery, enhancing the final aesthetic result and, in some cases, replacing the need for reconstructive surgery.

The benefits can be numerous: the procedure does not require anesthesia; in addition, it does not require tissue transfer from other areas and does not leave any scars; quite the opposite, it can cover any existing scar tissue [15, 17, 24, 25].

The patient has an immediate economic advantage as she receives the treatment free of charge. It should also be noted that medical tattooing of the nipple-areola complex was initially considered a mere complement to surgical NAC reconstruction. However, it is increas-

Table 2
Patients' level of satisfaction regarding the treatment received compared with patients' opinion regarding the usefulness of the project

a) Level of satisfaction		**n. cases**	**%**
	Very satisfied	128	90.8
	Satisfied	9	6.4
	Not very satisfied	1	0.7
	Dissatisfied	3	2.1
	Total	**141**	**100%**
b) Usefulness of the project	Very useful	96	68.1
	Useful	41	29.1
	Neither useful nor useless	2	1.4
	Useless	2	1.4
	Total	**141**	**100%**

ingly becoming an effective alternative to grafts of pigmented skin taken from other areas of the body. And in the case of 3D tattooing, it could replace nipple reconstruction itself [16]. In these cases, the patient can take advantage of the fact that she does not need to undergo another surgical operation and be hospitalized. This reduces missed workdays and increases social interaction opportunities for patients.

Another benefit is the savings for hospitals opting for this service as an alternative to traditional surgery. It should be noted that cost analyses of different NAC reconstruction techniques are not available in Italy as dermopigmentation of the NAC has only recently been introduced. To our knowledge, this technique has been adopted in Treviso and a few hospitals in Friuli Venezia Giulia, Lazio and Liguria. In the literature, it would seem that only one study, carried out in the USA by Koumanis DJ *et al*. [26] has provided a cost analysis of NAC reconstruction under general versus local anesthesia.

As the dermopigmentation of the nipple-areola complex described in this study is an outpatient procedure, there are no variable costs included. If we consider costs such as operating room time (approximately 1 hour), medical and nursing staff, recovery room, pharmacy, medical supplies, and anesthesia fees, we could calculate a minimum cost of about 1,600 euros which could be saved. Koumanis *et al.,* showed that a local anesthesia procedure costs 3,157 US dollars and, therefore, we could assume that dermopigmentation of the NAC, as an alternative to surgery reconstruction, may allow saving a minimum of 1,600 euros to 3,157 US dollars [26], about 2,900 euros.

The nipple-areola complex tattoo is an important treatment for women who want to recover their sense of self and their femininity, after overcoming cancer. As such, it ought to be considered an integral part of the rehabilitation process that starts after total or partial breast removal [27].

As mentioned above, the dedicated breast unit is specialized in supporting women affected by breast cancer. Women treated in these centres show higher survival rates than those treated in non-specialist units. Above all, such women report a better quality of life, as they feel supported through all the various stages of their treatment [28]. These treatments enable them to accept their self-image more easily, along with their role within the family and society. Today, the Italian National Healthcare System (SSN), through its Essential Levels of Assistance (LEA), includes tattoos for pigmenting the nipple-areola complex among its specialist outpatient assistance services (code 86.02.3), provided that certain conditions are met [29].

Special care is needed when tattooing cancer patients, as they are particularly vulnerable. They have low immune defenses as well as skin alterations, depending on their specific disease, chemotherapy in progress, and any radiotherapy treatment given.

Such treatments and/or the administration of medicines to treat other diseases can make dermopigmentation more complicated and increase the risks involved. They can, for example increase skin sensitivity, trigger skin reactions [30, 31], hinder blood coagulation [32, 33] or slow down the scar healing process. Given the above, cooperation between the breast unit's medical team and the operator who carries out the NAC tattoo is crucial to ensure patient safety. In our experience, the dermopigmentator's role fits appropriately into the dedicated breast unit as he or she complements the multidisciplinary team.

The study described in this paper suggests that cooperation and synergy between a public healthcare facility (Treviso Hospital) and a volunteer patients' association (such as LILT) can overcome certain bureaucratic obstacles and, above all, provide patients with a safe and effective service. In fact, no severe complications were reported following this treatment in either the short or the long term - possible complications may include allergies to ink components, risk of infection, scars, or granuloma [34, 35]. Furthermore, only a small number of minor complications were reported by the patients, amounting to 1.78% (3/169).

It should be pointed out that the 169 patients evaluated in this study is a relatively low number compared with the number of cases involving the breasts that were indicated for medical tattooing. This is due to several bureaucratic reasons within the public health service which, with the help of LILT, were partially overcome by employing a part-time dermopigmentator. It was not possible to treat all the cases indicated as only one to two days per month of free treatment were available.

As for the level of satisfaction, it should be noted that one hundred percent satisfaction cannot be guaranteed and the degree of success depends on the patient's expectations, the type of skin (dryness, oiliness, sun damage, acidity), the presence of scars or traumatized tissues from surgery, the tattooing method used and the characteristics of the operator (skill, experience and professional preparation). Furthermore, the habitual use of alcohol, smoking, sun exposure and/or tanning lamps, sweating, frequent swimming in pools and/or Turkish baths, the habitual use of the hot tubs and/or saunas might affect the results [36]. The degree of satisfaction with the final aesthetic result found in our study was high: 97% (137/141) of patients said they were satisfied/very satisfied. The same level of satisfaction was expressed concerning the usefulness of the project.

These results were achieved thanks to a well-designed procedure, to a careful selection of patients, suitable facilities, careful selection of pigments, and the dermopigmentator's advanced training and extensive experience.

One limit of the study should be mentioned; the results obtained for patient satisfaction may be influenced somewhat by a methodological bias. Measurement through only a single evaluation represents a cognitive and interpretative limit. The protocol followed was focused on patient safety and did not include specific evaluation procedures; therefore, a phone survey was conducted after the period of study. Essentially, the parameters considered were based on subjective satisfaction and self-evaluation, taking inspiration from certain papers found in the literature [37, 38].

In any case, despite being an incomplete evaluation, it nevertheless provides clear indications of the high

level of patient satisfaction. Future studies ought to ensure more detailed evaluations, following specific criteria and evaluation parameters. The surgeon, the Breast Centre team, the dermopigmentator, and the patient should all be involved. All these subjects should give an opinion on: size and shape of the NAC, colour, three-dimensional effect, similarity with the contralateral NAC.

As already mentioned at the beginning of the paragraph, hospitals opting for this service as an alternative to traditional surgery make savings by avoiding the use of operating rooms, surgeons and medical staff who can therefore be used to treat new patients, thus reducing waiting lists. This ultimately translates into savings and increased productivity for both the hospital and the healthcare system.

Hypothetically, if this model were to be scaled up to a national level, it would lead to considerable savings for the National Healthcare System.

The NAC dermopigmentation described in our work was carried out over the period 2010-2016, and this was not subject to specific official regulation until 2018. Subsequently, the Ministry of Health issued an order on the 15th May 2019 that regulates tattooing of the NAC [39]. Now, the operator authorized to carry out this kind of tattooing must be a health professional, which means a specialist recognized by law no. 43 (2006), and such health professionals can only operate within accredited or authorized centres. This order does not define a new specific profession to carry out this kind of treatment, instead, it envisages specialist training within University Faculties of Medicine and Surgery, open to existing health professionals. While these courses are yet to be established and completed, it will be difficult to find certified operators capable of carrying out such treatments.

Therefore, there is concern that patients needing this corrective treatment of the NAC may turn to unsuitable operators in unauthorized centres if they cannot access this service under the National Health Service; clearly there are associated risks.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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REFERENCES

1. Associazione Italiana di Oncologia Medica, Associazione Italiana Registro Tumori, Fondazione AIOM e Progressi delle Aziende Sanitarie per la Salute in Italia. I numeri del cancro in Italia 2018. Available from: www.aiom.it/wpcontent/uploads/2018/10/2018_NumeriCancro-operatori.pdf.
2. Fanceschini G, Martin Sanchez A, Di Leone A, Magno S, Moschella F, Accetta C, Masetti R. New trends in breast cancer surgery: a therapeutic approach increasingly efficacy and respectful of the patient. G Chir. 2015;36:145-52.
3. Few JW, Marcus JR, Casas LA, et al. Long-term predictable nipple projection following reconstruction. Plast Reconstr Surg. 1999;104:1321-4.
4. Evans KK, Rasko Y, Lenert J, et al. The use of calcium hydroxylapatite for nipple projection after failed nippleareolar reconstruction: early results. Ann Plast Surg. 2005;55:25-9.
5. Berson MI. Construction of pseudoareola. Surgery. 1946;20:808.
6. Adams WM. Labial transplant for correction of loss of the nipple. Plast Reconstr Surg (1946). 1949;4:295-8.
7. Millard DR Jr. Nipple and areola reconstruction by splitskin graft from the normal side. Plast Reconstr Surg. 1972;50:350-3.
8. Brent B, Bostwick J. Nipple-areola reconstruction with auricular tissues. Plast Reconstr Surg. 1977;60:353-61.
9. Klatsky SA, Manson PN. Toe pulp free grafts in nipple reconstruction. Plast Reconstr Surg. 1981;68:245-8.
10. Boulart L, Mimoun M, Noel W, Malca N, Chaouat M, Boccara D. Dealing with tattoos in plastic surgery. Complications and medical use. Ann Chir Plast Esthet. 2017;62:e23-e29. doi: 10.1016/j.anplas.2016.09.004. Epub. 2016 Oct 12
11. Drost BH, van de Langenberg R, Manusama OR, Janssens AS, Sikorska K, Zuur CL, Klop WM, Lohuis PJ. Dermatography (medical tattooing) for scars and skin grafts in head and neck patients to improve appearance and quality of Life. JAMA. Facial Plast Surg. 2017;19:16-22. doi: 10.1001/jamafacial.2016.1084
12. Buchman HH, Larson DL, Huang TT, Lewis SR. Nipple and areola reconstruction in the burned breast. The "double bubble" technique. Plast Reconstr Surg. 1974;54:531-6.
13. Rees TD. Reconstruction of the breast areola by intradermal tattooing and transfer. Case report. Plast Reconstr Surg. 1975;55:620-1.
14. Spear SL, Convit R, Little JW. 3rd. Intradermal tattoo as an adjunct to nipple-areola reconstruction. Plast Reconstr Surg. 1989;83:907-11.
15. Halvorson EG, Cormican M, West ME, Myers V. Three-dimensional nipple-areola tattooing: A new technique with superior results. Plast Reconstr Surg. 2014;133:1073-5.
16. Uhlmann NR, Martins MM, Piato S. 3D areola dermopigmentation (nipple-areola complex). Breast J. 2019;25:1214-21. doi: https://doi.org/10.1111/tbj.13427
17. Bodin F, Bruant-Rodier C, Ruffenach L, Dissaux C. The reconstruction of the nipple-areolar. Ann Chir Plast Esthet. 2018;63:559-68. doi: 10.1016/j.anplas.2018.06.010. Epub 2018 Jul 7
18. Sisti A, Grimaldi L, Tassinari J, Cuomo R, Fortezza L, Bocchiotti M A, Roviello F, D'Aniello C, Nisi G. Nipple-areola complex reconstruction techniques: A literature review. EJSO 2016;42:441e465. doi: http://dx.doi.org/10.1016/j.ejso.2016.01.003
19. Goh SCJ, Martin NA, Pandya AN, Cutress RI. Patient satisfaction following nipple-areolar complex reconstruction and tattooing. J Plast Reconstr Aesthet Surg. 2011;64:360-3. doi: 10.1016/j.bjps.2010.05.010. Epub 2010 Jun 8
20. K El-Ali, M Dalal, CC Kat. Tattooing of the nipple-areola

complex: review of outcome in 40 patients. J Plast Reconstr Aesthet Surg. 2006;59:1052-7. Epub 2006 Jun 12
21. Franceschini G, Di Leone A, Masetti R. The Breast Unit Update on advantages and the open issues. Ann Ital Chir. 2014;85:407-12.
22. Ross JR, Matava MJ. Tattoo-induced skin "burn" during magnetic resonance imaging in a professional football player: a case report. Sports Health. 2011;3:431-4.
23. Callaghan MF, Negus C, Leff AP, Creasey M, Burns S, Glensman J, Bradbury D, Williams E, Weiskopf N. Safety of tattoos in persons undergoing MRI. N Engl J Med. 2019;380:495-6. doi: 10.1056/NEJMc1811197
24. White CP, Gdalevitch P, Strazar R, Murrill W, Guay NA. Surgical tips: Areolar tattoo prior to nipple reconstruction. J Plast Reconstr Aesthet Surg. 2011;64:1724-6. doi: 10.1016/j.bjps.2011.06.031. Epub 2011 Jul 21
25. Børsen-Koch M, Bille C, Thomsen JB. Promising results after single-stage reconstruction of the nipple and areola complex. Danish Medical Journal. 2013;60:A4674.
26. Koumanis DJ, Colque A, Eisemann ML, Smith J. Breast Surgery under local anesthesia: second-stage implant Exchange, nipple flap reconstruction, and breast augmentation. Clin Plast Surg. 2013;40:583-91.
27. Bykowski MR, Emelife PI, Emelife NN, Chen W, Panetta NJ, de la Cruz C. Nipple-areola complex reconstruction improves psychosocial and sexual well-being in women treated for breast cancer. J Plast Reconstr Aesthet Surg. 2017;70:209-14. doi:10.1016/j.bjps.2016.10.009. Epub 2016 Nov 9.
28. Kesson EM, Allardice GM, George WD, Burns HJG, Morrison DS. Effects of multidisciplinary team working on breast cancer survival: retrospective, comparative, interventional cohort study of 13 722 women. Br Med J. 2012;344:1-9.
29. Italia. Decreto del presidente del consiglio dei ministri 12 gennaio 2017. Definizione e aggiornamento dei livelli essenziali di assistenza, di cui all'articolo 1, comma 7, del decreto legislativo 30 dicembre 1992, n. 502. Gazzetta Ufficiale - Serie Generale n. 65, 18 marzo 2017.
30. Fabbrocini G, Cameli N, Romano MC, Mariano M, Panariello L, Bianca D, Monfrecola G. Chemotherapy and skin reactions. J Exp Clin Cancer Res. 2012;31:50. doi:10.1186/1756-9966-31-50
31. Cox JD, Stetz J, Pajak TF. Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC). Int J Radiat Oncol Biol Phys. 1995;31:1341-6.
32. Wu Y, Aravind S, Ranganathan G, Martin A, Nalysnyk L. Anemia and thrombocytopenia in patients undergoing chemotherapy for solid tumors: a descriptive study of a large outpatient oncology practice database, 2000-2007. Clin Ther. 2009;31 Pt 2:2416-32. doi:10.1016/j.clinthera.2009.11.020
33. Miarons M, Velasco M, Campins L, Fernández S, Gurrera T, Lopez-Viaplana L. Gradual thrombocytopenia induced by long-term trastuzumab exposure. J Clin Pharm Ther. 2016;41:563-5. doi:10.1111/jcpt.12416. Epub 2016 Jul 18
34. Islam PS, Chang C, Selmi C, Generali E, Huntley A, Teuber SS, Gershwin ME. Medical Complications of Tattoos: A Comprehensive Review. Clin Rev Allergy Immunol. 2016;50:273-86. doi:10.1007/s12016-016-8532-0
35. Bassi A, Campolmi P, Cannarozzo G, Conti R, Bruscino N, Gola M, Ermini S, Massi D, Moretti S. Tattoo-associated skin reaction: the importance of an early diagnosis and proper treatment. Biomed Res Int. 2014;2014:354608. doi:10.1155/2014/354608. Epub 2014 Jul 23
36. NHS Salisbury. Nipple Tattooing/Areola Complex. NHS Foundation Trust. Available from: www.salisbury.nhs.uk/wards-departments/departments/breast-reconstruction-surgery/nipple-tattooing-areola-complex/.
37. Smallman A, Crittenden T, Yip J M, Dean NR. Does nipple-areolar tattooing matter in breast reconstruction? A cohort study using the BREAST-Q. JPRAS. 2018;16:61. doi: 10.1016/j.jpra.2018.01.003.eCollection 2018 Jun
38. Heo JW, Park SO, Jin US. A nipple-areolar complex reconstruction in implant-based breast reconstruction using a local flap and full-thickness skin graft. Aesthetic Plast Surg. 2018;42:1478-84. doi:10.1007/s00266-018-1162-6
39. Italia. Circolare del Ministero della Salute prot. n. 14138 del 15/05/2019. Nota circolare sui tatuaggi con finalità medica; chiarimenti in merito alla pigmentazione dell'areola capezzolo. Available from: www.salute.gov.it/portale/news/p3_2_1_1_1.jsp?lingua=italiano&menu=notizie&p=dalministero&id=3744.

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ORIGINAL ARTICLES AND REVIEWS

Dioxins and PCBs contamination in mussels from Taranto (Ionian Sea, Southern Italy): a seven years spatio-temporal monitoring study

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Abstract

Introduction. Taranto is of particular Public Health relevance for the presence of industrial sources of dioxins and PCBs. The aim of this study was to monitor these pollutants in mussels produced from 2012 to 2018.

Materials and methods. Mussels were collected on a monthly basis with random sampling. Concentrations were determined through accredited methods.

Results. 622 samples were collected. Dioxins and PCBs showed higher median concentrations in Mar Piccolo 1st Inlet (p_s<0.0001; Dioxins: 1.43 pg WHO-TEQ/g ww; Dioxins+DL-PCBs: 5.98 pg WHO-TEQ/g ww; DL-PCBs: 4.57 pg WHO-TEQ/g ww; NDL-PCBs: 61.54 ng/g ww) and in III trimester for all basins (p_s<0.02). In Mar Piccolo 1st Inlet, there was a linear increase of dioxins and PCBs moving North (p_s<0.05).

Conclusions. These findings confirmed higher concentrations in Mar Piccolo 1st Inlet during the summer period and supported the validity of the Public Health measures adopted by the Department of Prevention of Taranto.

Key words
- dioxins
- PCBs
- environmental contamination
- Taranto
- mussel

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and polychlorinated biphenyls (PCBs) are classified by WHO as environmental pollutants with a global distribution and high resistance to degradation [1-6].

Long-term exposure to dioxins (PCDD/Fs) and some PCBs, referred to as dioxin-like PCBs (DL-PCBs) due to their similar toxicological properties, has been shown to cause a range of adverse effects on the nervous, immune and endocrine systems, to impair reproductive function and to cause cancer. Other PCBs referred to as non-dioxin-like PCBs (NDL-PCBs) have a different mechanism of toxicity, but they too can seriously damage human health [1, 3, 5-9].

More than 80% of total exposure is attributable to dietary intake, that represents the main route of PCDD/Fs and PCBs exposure for humans [1, 5, 7]. In particular, the consumption of animal origin foods, like milk, meat, eggs, leads to a greater risk of bioaccumulation due to the lipophilic properties of these pollutants [1, 5].

Taranto, a coastal city in the South of Italy (Ionian Sea, Puglia Region), is of particular relevance in this context due to the type of industrial settlements accounting for known potential sources of PCDD/Fs and PCBs (the most important steel plant in Europe, an oil refinery, a cement works, thermoelectric plants, waste incinerators, discharges and military harbours) and to the environmental contamination present in different matrices, including marine sediments - in particular in Mar Piccolo 1st Inlet [10-14]. In this regard, the city is surrounded by two basins, the Mar Grande (a wide roadstead with maximum depth of about 35 meters and a mean salinity of 37.8‰) and the Mar Piccolo (a inner basin with lagoon features, divided into two inlets with a maximum depth of 13 and 8 meters, respectively, and a mean salinity of 35.1‰), that are home to several mussel (*Mytilus galloprovincialis*, Lamarck 1819) culture areas which account for a significant production at national level [13, 14].

Mussels can represent a food at risk of contamination, because are filter-feeding organisms with high bio-

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accumulation and low biotransformation potential for both organic and inorganic contaminants [14-16].

Since 2011, the Department of Prevention of the Local Health Authority of Taranto has carried out a monitoring plan of in order to assess PCDD/Fs and PCBs contamination in *M. galloprovincialis* from Mar Piccolo and Mar Grande. The results of a study on mussel samples collected between March and December 2011 [14] as well as of the activities carried out in these years by the Department of Prevention of the Local Health Authority of Taranto, showed results of non-compliance with European legislation [16, 17] as regards the levels of PCDD/Fs and DL-PCBs in the 1st Inlet of the Mar Piccolo during the summer period. This led the Department of Prevention of the Local Health Authority of Taranto to propose to Puglia Region the adoption of the Regional Ordinance n. 188/2016 to block the removal and handling of commercial mussels in Mar Piccolo 1st Inlet, with the only possibility of moving juvenile mussels (<5 cm) from Mar Piccolo 1st Inlet to the other basins by March 31, after a sampling result in accordance with EU standards for dioxins and PCBs [16, 17]. In September 2018, the Puglia Region published an update of the Ordinance (n. 532/2018), anticipating the temporal limit to February 28, in order to account for an increase in dioxins/PCBs mussels contamination found starting from the II trimester.

The aim of this study was to update those evidences through the monitoring of the content and the spatio-temporal distribution of PCDD/Fs, DL-PCBs and NDL-PCBs in mussels produced from January 2012 to December 2018 in all the mussel farming plants of the Mar Grande and Mar Piccolo of Taranto, in order to guarantee the healthiness of the product placed on the market, identify marine areas and critical seasons for mussel contamination, and verify and develop effective public health strategies to protect the health of consumers together with the production chain of the territory.

MATERIALS AND METHODS

Sampling

We included in this study the mussel samples (*M. Galloprovincialis*) collected by the staff of the Department of Prevention of Local Health Authority of Taranto between January 2012 and December 2018 in Mar Piccolo and Mar Grande. For each sample, numerous aliquots of product (at least 1 kg total) were taken from various points between 0 and 4 meters deep, in order to obtain a representative sample of any different levels of contamination present in the mussels both in contact with sediments or in aquatic suspension. Mussels were collected on a monthly basis with random sampling using predefined square grid cells within the three basins. The geographic coordinates of the sampling areas were established from the georeferencing maps created by ISPRA (National Institute for Environmental Protection and Research), taking into account the level of contamination of the sediments. The samples were sent for chemical analysis to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (National Reference Laboratory for Halogenated POPs in food and feed).

Chemical analysis

Chemicals

Solvents such as n-hexane, dichloromethane, acetone, toluene and isooctane were organic residue analysis quality (Honeywell Burdick & Jackson, Seezle, Germany). Ultra-pure water was generated within the laboratory by means Purelab option-Q system (ELGA LabWater, High Wycombe, United Kingdom). Other reagents included anhydrous sodium sulphate, concentrated sulphuric acid and sodium chloride, all at reagent grade (Honeywell Burdick & Jackson, Seezle, Germany).

Prepacked multilayer silica, alumina, and carbon columns were obtained from Fluid Management Systems (Massachussetts, USA).

All standard solutions were supplied by Wellington Laboratories (Guelph, Ontario, Canada). Calibration solutions DF-CVS (CS1 through CS4), $^{13}C_{12}$-labeled internal standard DF-LCS-C200, and recovery standard DF-IS-J were used for PCDD/Fs analysis. Calibration solutions WP-CVS (CS1 through CS7), $^{13}C_{12}$-labeled internal standard WP-LCS, and recovery standard P48-RS-STK were prepared for DL-PCB analysis. Calibration solutions P48-M-CVS (CS1 through CS5), $^{13}C_{12}$-labeled internal standard P48-M-ES, and recovery standard P48-RS-STK were selected for NDL-PCB analysis.

Analytical methodology

The 17 PCDD/Fs, the 12 DL-PCBs and the 6 indicator NDL-PCBs [16, 17] were determined through accredited methods in accordance with ISO EN 17025, based on US EPA (1994) Method 1613 B for PCDD/Fs and US EPA (2008) Method 1668 B for PCBs. Both methods are based on isotopic dilution and high resolution mass spectrometry (HRMS) detection. In order to adapt the analytical procedures to the matrix under examination, variations have been made in the extraction and purification phases of the sample.

All the samples under examination were homogenized by a knife mill Grindomix GM-200 (Retsch, Dusseldorf, Germany) and a representative rate of approximately 5±2 g was taken from them. The samples were dried for at least 8 hours in an oven at a temperature of 40±5°C and, before extraction, were mixed with anhydrous sodium sulphate in a ratio of 1:3 (w/w) and fortified with a mixture of the internal standards containing: 17 PCDD/Fs $^{13}C_{12}$-labeled (0.2-0.4 ng); 12 DL-PCBs $^{13}C_{12}$-labeled (1.0 ng); 6 NDL-PCBs $^{13}C_{12}$-labeled (2.0 ng).

The samples were extracted by accelerated solvent extraction with n-hexane-acetone (80:20, v/v) using a Dionex ASE 350 (Thermo Fisher Scientific, Waltham, MA) at 1,500 psi and 125°C.

The extract, collected in a glass vial, was filtered on anhydrous sodium sulphate and collected in a volumetric flask, dried on a rotary evaporator with a water bath at 40±5°C.

After solvent evaporation, the extract was dissolved in hexane and subjected to liquid-liquid partitioning with concentrated sulfuric acid, 20% aqueous potassium hydroxide, and saturated aqueous sodium chloride.

Subsequently, the extract was purified, in sequence, on chromatographic columns of multilayer silica gel, alumina and carbon, using an automated clean-up process with a Power-Prep™ system (FMS, Massachusetts, USA).

The two eluates containing PCDD/Fs and PCBs, were concentrated by evaporation in nitrogen stream and dissolved in the corresponding recovery standards solutions ($^{13}C_{12}$-labeled PCDD/Fs and PCBs different from the previous ones).

The instrumental analysis was performed using high resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS), using GC Trace Series 2000 coupled to a MAT 95 XL (Thermo Fisher Scientific, USA) and a Trace Series 1310 GC, coupled to a DFS (Thermo Fisher Scientific, USA). The chromatographic separation of the 17 PCDD/Fs was carried out on a DB-5 MS capillary column 60m x 0.25mm x 0.10µm (J&W Scientific, California, USA). The chromatographic separation of DL-PCBs and NDL-PCBs was carried out on HT8-PCB capillary column 60m x 0.25mm x 0.25µm (SGE Analytical science, Melbourne, Australia). The acquisition of the masses was carried out in Single Ion Monitoring (SIM) mode at a resolution of 10,000, selecting the masses indicated by the method. As regards 17 PCDD/Fs and 12 DL-PCBs, TEQ concentrations were determined by multiplying the analytical result of each congener by the corresponding WHO-TEF, while for NDL-PCBs, the result was reported as the sum of the 6 indicator congeners. All values have been reported as upper bound concentrations, that is all values below the limit of quantification (LOQ) are supposed to be equal to the respective LOQ.

A laboratory blank and a control sample were analyzed for each batch of 10 and 20 samples, respectively. Recovery rates of labeled congeners ranged from 60% to 90%, and the analytical uncertainty was in the order of ±18% for WHO-TEQs and the sum of six NDL-PCBs. Method performance was in agreement with the requirements for method of analysis used in official control of the levels of PCDD/Fs and PCBs in foodstuff [16, 17] and has been successfully verified in many proficiency tests over 15 years.

Statistical analysis

Statistical analysis was performed using R version 3.6.2 (released on 2019-12-12). Statistical significance α was fixed to 0.05. Maps were created with Microsoft Excel version 2002 (Build 12527.20194).

In order to account for non-normality, evaluated through Shapiro-Wilk test, numerical variables (means of the measured values of the four pollutants concentrations) [16, 17] were reported as median and IQR and compared first through Kruskal Wallis rank sum test and then through pairwise Wilcoxon rank sum test with Benjamini & Hochberg correction for p values (False Discovery Rate). Comparisons were carried out between basins (on the overall sample) and between trimesters (separately for each of the three basin). For each basin and pollutant (values), a multivariable linear median regression model (linear quantile regression with τ=0.5) was then fitted in order to assess the effect of latitude (hundredths of °N) and longitude (hundredths of °E) on the median of each pollutant values. The algorithmic method used to compute the fit was the modified version of the Barrodale and Roberts algorithm. Standard errors were computed through a Huber sandwich estimate using a local estimate of the sparsity. β coefficients can be interpretable as the increase in pollutant values median (median difference) for an increase of one hundredth of °N or °E. In order to assess the pairwise correlation between the four pollutants (values) non-normally distributed, Spearman rank correlation coefficient ρ was calculated for each basin and combination. P-values were computed via the asymptotic t approximation.

Categorical variables (means of the measured values of the three pollutant concentration minus the associated expanded uncertainty that are above the established EU maximum level) [16, 17] were reported as absolute and relative frequencies and, in order to account for low expected frequencies (n<5), were compared first through Fisher Exact test (Fisher-Freeman-Halton Exact Test for contingency tables larger than 2x2) and then through pairwise Fisher Exact test with Benjamini & Hochberg correction for p values (False Discovery Rate). Comparisons were carried out between basins (on the overall sample) and between trimesters (separately for each of the three basin). For each basin and pollutant (values above EU maximum level) with sufficient frequencies (n>30), a multivariable binary logistic regression model was then fitted in order to assess the effect of latitude (hundredths of °N) and longitude (hundredths of °E) on the odds of pollutant value above the EU maximum level. Odds Ratio can be interpretable as the increase in the odds of pollutant value above the EU maximum level for an increase of one hundredth of °N or °E. In order to assess the pairwise correlation between pollutants values above the EU maximum level, Pearson correlation coefficient ϕ was calculated for each combination in Mar Piccolo 1st Inlet. P-values were computed via t distribution.

RESULTS

622 mussel samples were collected between 2012 and 2018, 208 in Mar Piccolo 1st Inlet, 207 in Mar Piccolo 2nd Inlet and 207 in Mar Grande.

Spatial distribution of the pollutants values between the three basins was shown in the left side of *Figure 1*. Results of overall pollutants values distribution and comparisons between basins were reported in *Table 1*. Kruskal Wallis rank sum test showed a significant difference between basins for all pollutants (p_s<0.0001). Pairwise Wilcoxon rank sum test showed: higher values in Mar Piccolo 1st Inlet compared both to Mar Piccolo 2nd Inlet and Mar Grande for all pollutants (p_s<0.0001); higher values in Mar Piccolo 2nd Inlet compared to Mar Grande for dioxins and NDL-PCBs (p_s<0.0001); lower values in Mar Piccolo 2nd Inlet compared to Mar Grande for DL-PCBs (p=0.0067).

Results of Mar Piccolo 1st Inlet pollutants values distribution and comparisons between trimesters were reported in *Table 2*. Kruskal Wallis rank sum test showed

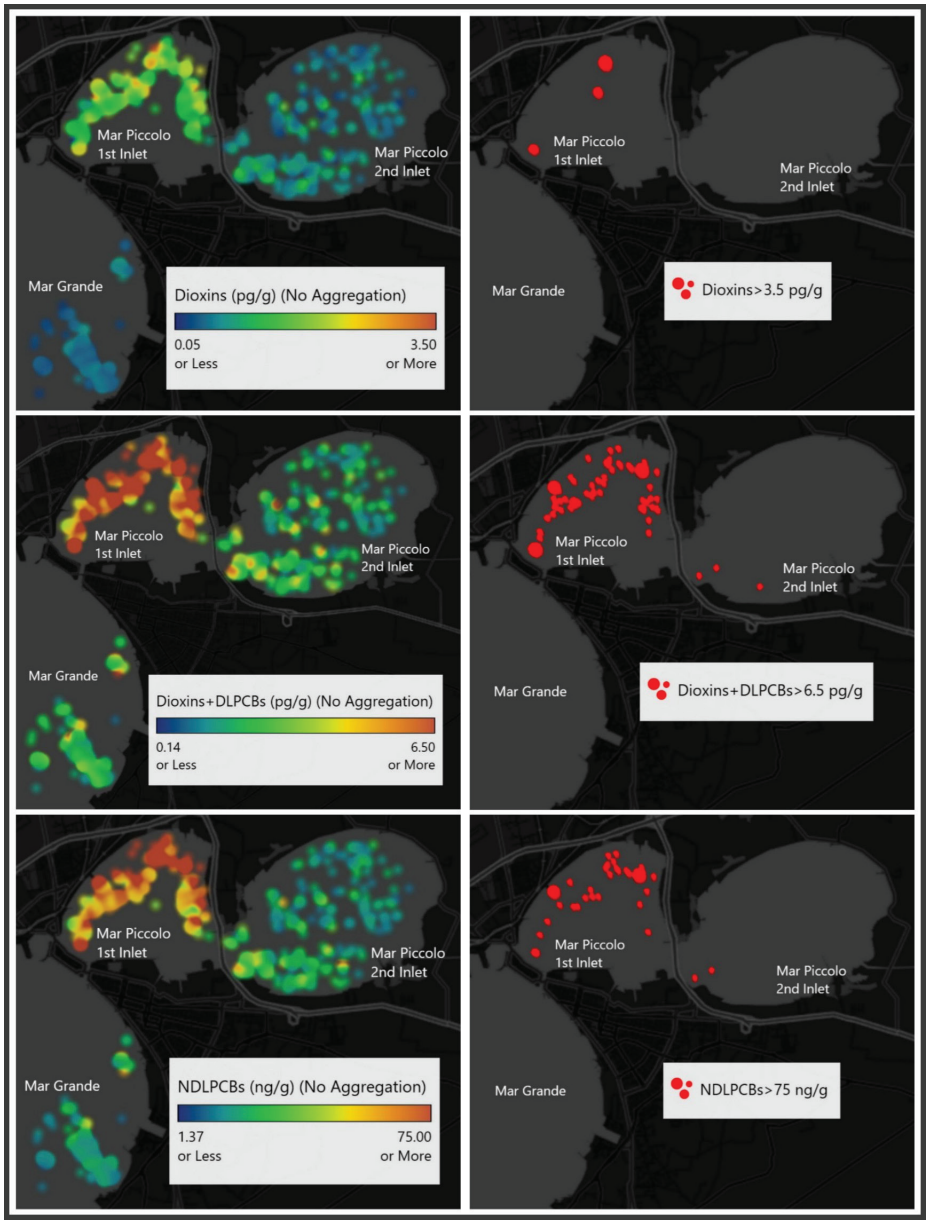

Figure 1
Spatial distribution of mussel pollutants (values and values above EU maximum level) between and within the three basins.
Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants on the left: mean of the measured values, wet weight; pollutants on the right: mean of the measured values minus the expanded uncertainty of the mean, wet weight [16, 17].

a significant difference between trimesters for all pollutants (p_s<0.0005). Pairwise Wilcoxon rank sum test showed: lower values of dioxins in both trimesters I and II compared both to III and IV (p_s<0.004); differences in Dioxins+DL-PCBs and DL-PCBs between all trimesters with higher values in trimester III (p_s<0.03); except for II-IV comparison (p=0.071), lower values of NDL-PCBs in both trimesters I and IV compared both to II and III (p_s<0.02).

Results of Mar Piccolo 2nd Inlet pollutants values distribution and comparisons between trimesters were reported in *Table 2*. Kruskal Wallis rank sum test showed a significant difference between trimesters for all pollutants (p_s<0.0001). Pairwise Wilcoxon rank sum test showed: lower values of dioxins and NDL-PCBs in trimesters I, II and IV compared to III (p_s<0.002); except for I-IV comparisons (p_s>0.05), differences in Dioxins+DL-PCBs and DL-PCBs between all trimesters with higher values in trimester III (p_s<0.003).

Results of Mar Grande pollutants values distribution and comparisons between trimesters were reported in *Table 2*. Kruskal Wallis rank sum test showed a significant difference between trimesters for all pollutants (p_s<0.0006). Pairwise Wilcoxon rank sum test showed:

Table 1
Mussel pollutants values distribution between basins (overall), Kruskal Wallis rank sum test and Pairwise Wilcoxon rank sum test (Benjamini-Hochberg correction)

2012-18	Dioxins (pg/g)		Dioxins+DLPCBs (pg/g)		DLPCBs (pg/g)		NDLPCBs (ng/g)	
Overall (n=622)	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**
Basin		**<.0001**		**<.0001**		**<.0001**		**<.0001**
Mar Piccolo 1st Inlet (n=208)	1.43 (0.98)		5.98 (4.20)		4.57 (3.78)		61.54 (35.06)	
Mar Piccolo 2nd Inlet (n=207)	0.63 (0.41)		2.00 (1.29)		1.34 (0.96)		20.05 (12.79)	
Mar Grande (n=207)	0.41 (0.28)		2.00 (1.74)		1.61 (1.67)		16.24 (9.67)	
Basin (pairwise)								
Mar Piccolo 1st Inlet - Mar Piccolo 2nd Inlet		**<.0001**		**<.0001**		**<.0001**		**<.0001**
Mar Piccolo 1st Inlet - Mar Grande		**<.0001**		**<.0001**		**<.0001**		**<.0001**
Mar Piccolo 2nd Inlet - Mar Grande		**<.0001**		.56		**.0067**		**<.0001**

Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); all pollutants: mean of the measured values, wet weight [16, 17].

Table 2
Mussel pollutants values distribution between trimesters (Mar Piccolo 1st Inlet, Mar Piccolo 2nd Inlet and Mar Grande), Kruskal Wallis rank sum test and Pairwise Wilcoxon rank sum test (Benjamini-Hochberg correction)

2012-18	Dioxins (pg/g)		Dioxins+DLPCBs (pg/g)		DLPCBs (pg/g)		NDLPCBs (ng/g)	
Mar Piccolo 1st Inlet (n=208)	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**
Trimester		**<.0001**		**<.0001**		**<.0001**		**.0004**
I (n=56)	1.13 (0.63)		4.05 (2.19)		2.98 (2.28)		50.35 (28.74)	
II (n=62)	1.15 (1.02)		7.40 (4.30)		5.98 (3.43)		70.33 (35.38)	
III (n=42)	1.97 (1.17)		9.63 (4.24)		7.92 (3.54)		74.21 (35.74)	
IV (n=48)	1.60 (0.81)		5.26 (2.76)		3.61 (2.06)		59.87 (24.73)	
Trimester (pairwise)								
I - II		.58		**<.0001**		**<.0001**		**.0060**
I - III		**<.0001**		**<.0001**		**<.0001**		**.0029**
I - IV		**.0001**		**.0031**		**.024**		.098
II - III		**.0001**		**.0006**		**.0043**		.44
II - IV		**.0038**		**.0040**		**.0001**		.071
III - IV		.060		**<.0001**		**<.0001**		**.010**
Mar Piccolo 2nd Inlet (n=207)	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**	**Median (IQR)**	**p**
Trimester		**<.0001**		**<.0001**		**<.0001**		**<.0001**
I (n=57)	0.55 (0.28)		1.50 (0.75)		0.96 (0.45)		17.57 (11.41)	
II (n=63)	0.60 (0.48)		2.23 (1.21)		1.58 (0.79)		20.94 (9.50)	
III (n=40)	0.91 (0.64)		3.81 (3.68)		2.88 (2.72)		27.14 (26.40)	
IV (n=47)	0.59 (0.22)		1.80 (0.90)		1.11 (0.64)		19.31 (9.82)	
Trimester (pairwise)								
I - II		.46		**<.0001**		**<.0001**		.059
I - III		**<.0001**		**<.0001**		**<.0001**		**<.0001**
I - IV		.21		.18		.34		.46
II - III		**<.0001**		**<.0001**		**<.0001**		**.0019**
II - IV		.78		**.0022**		**<.0001**		.23
III - IV		**<.0001**		**<.0001**		**<.0001**		**.0003**

Continues

Table 2
Continued

Mar Grande (n=207)	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p
Trimester		**<.0001**		**<.0001**		**<.0001**		**.0005**
I (n=58)	0.42 (0.19)		1.42 (0.57)		0.97 (0.50)		14.38 (6.04)	
II (n=59)	0.27 (0.13)		2.37 (1.32)		2.08 (1.30)		14.75 (7.13)	
III (n=45)	0.61 (0.29)		3.74 (1.66)		3.19 (1.68)		22.07 (9.63)	
IV (n=45)	0.45 (0.26)		1.74 (0.96)		1.26 (0.83)		16.24 (9.30)	
Trimester (pairwise)								
I - II		**<.0001**		**<.0001**		**<.0001**		.35
I - III		**<.0001**		**<.0001**		**<.0001**		**.0005**
I - IV		.18		**.0093**		**.0019**		.35
II - III		**<.0001**		**<.0001**		**<.0001**		**.0021**
II - IV		**<.0001**		**.0078**		**.0002**		.79
III - IV		**.0056**		**<.0001**		**<.0001**		**.015**

Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); all pollutants: mean of the measured values, wet weight [16, 17].

Table 3
Multiple linear median regression for each basin and mussel pollutant (values)

2012-18	Dioxins (pg/g)		Dioxins+DLPCBs (pg/g)		DLPCBs (pg/g)		NDLPCBs (ng/g)	
Mar Piccolo 1st Inlet (n=208)	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**
Latitude ($_{hundredth}$°N)	0.31 (0.01;0.61)	**.042**	1.60 (0.42;2.78)	**.0082**	1.43 (0.34;2.52)	**.011**	16.67 (5.89;27.46)	**.0026**
Longitude ($_{hundredth}$°E)	0.00 (-0.12;0.12)	.99	-0.34 (-0.84;0.16)	.19	-0.22 (-0.67;0.23)	.33	-3.01 (-7.36,1.34)	.17
Mar Piccolo 2nd Inlet (n=207)	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**
Latitude ($_{hundredth}$°N)	-0.04 (-0.12;0.04)	.34	-0.26 (-0.50;-0.02)	**.036**	-0.12 (-0.31;0.07)	.21	-1.40 (-3.43;0.63)	.17
Longitude ($_{hundredth}$°E)	-0.02 (-0.07;0.04)	.53	-0.10 (-0.27;0.08)	.28	-0.13 (-0.27;0.01)	.062	-2.40 (-3.91;-0.89)	**.0020**
Mar Grande (n=207)	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**	**β (95%CI)**	**p**
Latitude ($_{hundredth}$°N)	0.03 (-0.04;0.11)	.39	0.15 (-0.40;0.71)	.59	0.19 (-0.38;0.76)	.51	2.38 (-0.59;5.36)	.12
Longitude ($_{hundredth}$°E)	0.09 (0.03;0.15)	**.0042**	-0.22 (-0.67;0.22)	.32	-0.23 (-0.64;0.18)	.26	2.93 (1.09;4.77)	**.0019**

Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); all pollutants: mean of the measured values, wet weight [16, 17].

except for I-IV comparison (p=0.18), differences in dioxins between all trimesters with higher values in trimester III (p_s<0.006); differences in Dioxins+DL-PCBs and DL-PCBs between all trimesters with higher values in trimester III (p_s<0.01); lower values of NDL-PCBs in trimesters I, II and IV compared to III (p_s<0.02).

Spatial distribution of the pollutants values within the three basins was shown in the left side of *Figure 1*. Results of multiple linear median regression for each basin and pollutant (values) were reported in *Table 3*. In Mar Piccolo 1st Inlet, all pollutants showed significant differences as regards to latitude, with an increase moving North (p_s<0.05). In Mar Piccolo 2nd Inlet, dioxins+DL-PCBs showed significant differences as regards to latitude, with a decrease moving North (p=0.036), while NDL-PCBs showed significant differences as regards to longitude, with a decrease moving East (p=0.0020). In Mar Grande both dioxins and NDL-PCBs showed significant differences as regards to longitude, with an increase moving East (p_s<0.005).

Results of Spearman rank correlation ρ for each basin and combination of pollutant (values) were reported in *Table 4*. All pairwise combinations in all basins showed significant correlation (p_s<0.0001). Dioxins+DL-PCBs

Table 4
Spearman rank correlation ρ for each basin and mussel pollutant (values)

2012-18		Mar Piccolo 1st Inlet (n=208)		Mar Piccolo 2nd Inlet (n=207)		Mar Grande (n=207)	
Poll. 1	**Poll.2**	**ρ**	**p**	**ρ**	**p**	**ρ**	**p**
Dioxins (pg/g)	Dioxins+DLPCBs (pg/g)	0.72	**<.0001**	0.82	**<.0001**	0.50	**<.0001**
Dioxins (pg/g)	DLPCBs (pg/g)	0.61	**<.0001**	0.67	**<.0001**	0.35	**<.0001**
Dioxins (pg/g)	NDLPCBs (ng/g)	0.74	**<.0001**	0.65	**<.0001**	0.59	**<.0001**
Dioxins+DLPCBs (pg/g)	DLPCBs (pg/g)	0.98	**<.0001**	0.97	**<.0001**	0.98	**<.0001**
Dioxins+DLPCBs (pg/g)	NDLPCBs (ng/g)	0.83	**<.0001**	0.82	**<.0001**	0.75	**<.0001**
DLPCBs (pg/g)	NDLPCBs (ng/g)	0.80	**<.0001**	0.82	**<.0001**	0.70	**<.0001**

Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); all pollutants: mean of the measured values, wet weight [16, 17].

seemed to be more correlated to DL-PCBs (0.98>ρ>0.97) than to dioxins (0.82>ρ>0.50), in particular in Mar Grande (ρ=0.98 vs ρ=0.50). The lowest correlation was found between dioxins and DL-PCBs in Mar Grande (ρ=0.35).

Temporal trends of the pollutants values over the observed years for each basin were shown in the left side of *Figure 2*.

Spatial distribution of the pollutants values above the EU maximum level between the three basins was shown in the right side of *Figure 1*. Overall pollutants values above EU maximum level frequency distribution between basins (p from Fisher-Freeman-Halton exact test) were:

- 4 (1.9%) in Mar Piccolo 1st Inlet and 0 (0.0%) in Mar Piccolo 2nd Inlet and Mar Grande for Dioxins>3.5 pg WHO-TEQ/g ww (p=0.036);
- 73 (35.1%) in Mar Piccolo 1st Inlet, 3 (1.4%) in Mar Piccolo 2nd Inlet and 0 (0.0%) in Mar Grande for dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww (p<0.0001);
- 38 (18.3%) in Mar Piccolo 1st Inlet, 2 (1.0%) in Mar Piccolo 2nd Inlet and 0 (0.0%) in Mar Grande for NDL-PCBs>75 ng/g ww (p<0.0001).

Pairwise Fisher exact test showed higher frequencies in dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww and NDL-PCBs>75 ng/g ww in Mar Piccolo 1st Inlet compared both to Mar Piccolo 2nd Inlet and Mar Grande for all pollutants (p_s<0.0001).

Mar Piccolo 1st Inlet pollutants values above EU maximum level frequency distribution between trimesters (p from Fisher-Freeman-Halton exact test) were:

- 0 (0.0%) in trimesters I and II, 2 (4.8%) in III and 2 (4.2%) in IV for Dioxins>3.5 pg WHO-TEQ/g ww (p=0.077);
- 2 (3.6%) in trimester I, 33 (53.2%) in II, 33 (78.6%) in III and 5 (10.4%) in IV for dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww (p<0.0001);
- 4 (7.1%) in trimester I, 16 (25.8%) in II, 15 (35.7%) in III and 3 (6.2%) in IV for NDL-PCBs>75 ng/g ww (p=0.0001).

Pairwise Fisher exact test showed: except for I-IV comparison (p=0.24), differences between all trimesters in dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww with higher frequency in III (p_s<0.02); lower frequencies of NDL-PCBs>75 ng/g ww in both trimesters I and IV compared both to II and III (p_s<0.02).

Spatial distribution of the pollutants values above the EU maximum level within the three basins was shown in the right side of *Figure 1*. Results of multiple binary logistic regression for Mar Piccolo 1st Inlet and NDL-PCBs>75 ng/g ww showed significant differences as regards to latitude, with a frequency increase moving North (OR 6.43, 95%CI 2.66;17.00, p=0.0001), while no differences were found as regard to longitude. No significant differences were found for DL-PCBs>6.5 pg WHO-TEQ/g ww (p>0.05).

All pairwise combinations of pollutant (values above EU maximum level) in Mar Piccolo 1st Inlet showed significant Pearson correlation ϕ (p_s<0.006), with low correlation of dioxins>3.5 pg WHO-TEQ/g ww with dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww (ϕ=0.19) and with NDL-PCBs>75 ng/g ww (ϕ=0.21) and higher correlation of dioxins+DL-PCBs>6.5 pg WHO-TEQ/g ww with NDL-PCBs>75 ng/g ww (ϕ=0.54).

Temporal trends of the pollutants values above the EU maximum level over the observed years for each basin were shown in the right side of *Figure 2*.

DISCUSSION

As mussel farming historically represents an important cultural and economic heritage for the citizens of Taranto, the Department of Prevention of the Local Health Authority have long been engaged on the dual front of protecting consumers and safeguarding primary production.

The results of our study confirmed higher levels of dioxins and PCBs concentrations as well as values exceeding the EU maximum levels mainly in Mar Piccolo 1st Inlet. This finding supports the validity of the Regional Ordinance 188/2016 and the consequent Public Health measures carried out by the Department of Prevention of the Local Health Authority of Taranto. The prohibition of exploiting the Mar Piccolo 1st Inlet for mussel farming, except for juvenile mussels handling by February 28, intercepts almost all the exceedances observed with respect to the EU limits for dioxins and PCBs and plays a pivotal role in ensuring the healthiness of the product placed on the market.

Higher levels of dioxins and PCBs concentrations as

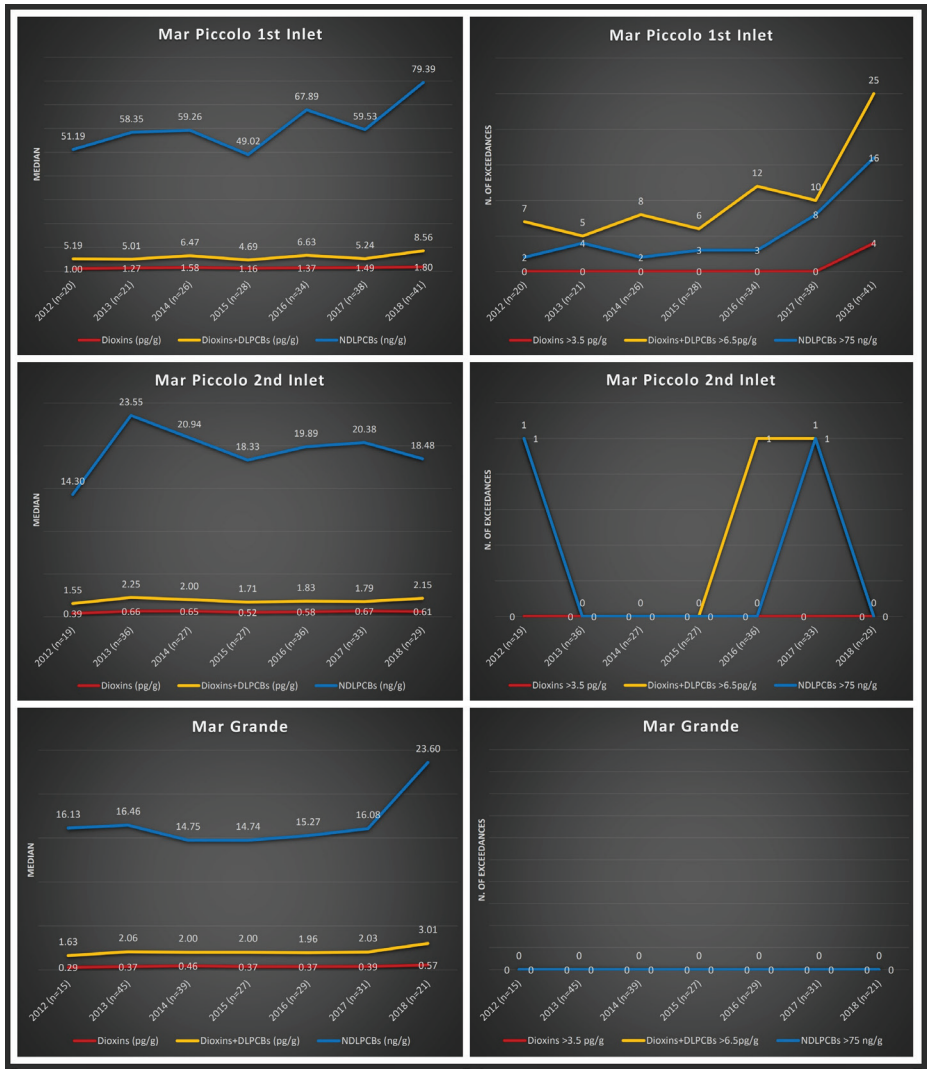

Figure 2
Temporal trends of mussel pollutants (values and values above EU maximum level) over the observed years.
Dioxins: sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs: sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs: sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs: sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants on the left: mean of the measured values, wet weight; pollutants on the right: mean of the measured values minus the expanded uncertainty of the mean, wet weight [16, 17].

well as values exceeding the EU maximum levels in Mar Piccolo 1st Inlet can be explained by the proximity of industrial settlements, which account for known potential sources of PCDD/Fs and PCBs, e.g. contaminating groundwater and freshwater in the northern area of the basin [14]. This is in line with the higher measured PCBs concentrations in marine sediments from Mar Piccolo 1st Inlet (Range 54 – 1684 µg/kg dw) compared to 2nd Inlet (Range 2 – 181 µg/kg dw) [13]. It is therefore not surprising that the mussels produced in Mar Piccolo 2nd Inlet and Mar Grande basins exhibited lower dioxins and PCBs concentrations which were basically always below the EU maximum levels.

In order to effectively deal with mussels contamination in Taranto, another key issue to consider is certainly the marked seasonality of measured dioxins and PCBs concentrations. As a matter of fact, summer is undoubtedly the most critical period, in accordance with a dependence of mussels filtration rate from temperature, as yet reported in literature [18-20]. The influence of temperature on filtration activity seems to be higher between 5°-15°C and 25°-30°C, lower from 15° to 25°C, while at 5° and 30°C, filtration drops to very low values [18]. The linear increase of filtration rate with temperature in mussels can be explained by increased biological activity as well as by decreased water viscosity [19, 20]. In Mar Piccolo, the scarce hydrodynamism and the low water exchange with Mar Grande determine, mainly in summer, a high water stratification and a significant increase in the average water temperature compared to the open sea [13].

Besides, the observed seasonal concentrations pattern could be linked to cyclical variations of mussels physiological state and lipid content during the year re-

lated to their reproductive cycle [14]. Infact, in the later stages of gametogenesis (March-May), the increase in mussels lipid content may lead to a greater risk of bioaccumulation due to the strong lipophilic properties of dioxins and PCBs [1, 5].

Our results showed also a differential fluctuation of dioxins and PCBs concentrations during the year, with higher dioxins concentrations during the 3rd trimester and higher PCBs concentrations during both 2nd and 3rd trimester. To explain this different temporal pattern, we could hypothesize a difference between dioxins and PCBs toxicokinetics in mussels that account for a delay in dioxins concentrations increase compared to PCBs' and, maybe, for higher PCBs measured concentrations. In fact, is of the utmost importance to underline that the majority of observed EU limits exceedances (96.7%) referred to PCBs rather than dioxins, suggesting that PCBs constitute the most abundant contaminant in marine sediments of Taranto [13]. This finding is of particular Public Health relevance considering that exposure to PCBs is associated with melanoma (sufficient evidence), Non-Hodgkin lymphoma and breast cancer (limited evidence) [21].

Moreover, early rise of PCBs concentrations starting from the 2nd trimester corroborates the effectiveness of the Regional Ordinance 532/2018, which anticipates the temporal limit for juvenile mussels handling to February 28, a choice that has also been considered in relation to the Southern Italy climate pattern of last years, characterized by a progressive increase in temperatures linked to climate change [22].

On the other hand, the possibility of moving juvenile mussels (<5 cm) from Mar Piccolo 1st Inlet to the other basins by February 28 is confirmed to be an option that guarantee the healthiness of the product, in light of the fact that 100% of the detected exceedances in Mar Piccolo 1st Inlet during the I trimester refers to commercial size mussels (≥5cm).

An interesting finding of this study is the linear relationship between some pollutants and geographical coordinates. In Mar Piccolo 1st Inlet, there was a linear increase of dioxins and PCBs moving North, in accordance with the marine sediments resuspension near the northern coast. In Mar Piccolo 2nd Inlet, we found a linear increase of some pollutants moving South or West, i.e. moving towards the Mar Piccolo 1st Inlet entrance channel. In Mar Grande, there was a linear increase of some pollutants moving East, in accordance with the marine sediments resuspension near the eastern coast. Marine sediments resuspension near the coast could be explained by several factors, e.g. low water depths, boats passage, water agitation by groundwater and freshwater flowing, human activities and, near the North-Western coast of Mar Piccolo 1st Inlet, a significant water outflow due to the presence of water-scooping machines that supply seawater to the steel plant cooling system. Finally, the correlation analysis showed stronger correlation between most of the pollutants in Mar Piccolo, leading to the hypothesis of related sources of contamination, while in Mar Grande the weaker correlation between dioxins and PCBs may suggest different unrelated pollution sources.

The possibility to identify a relationship between the levels of mussels contamination and the marine sediments resuspension stimulates an important reflection of public health. As a matter of fact, regardless the public health measures carried out by the Regional Ordinances as well as by the Local Health Authority have effectively remedied an emergency situation, nevertheless they cannot be considered definitive solutions, as the only way to deal with the problem of persistent organic pollutants contamination in Taranto basins is the remediation of contaminated marine sediments. In particular, in Mar Piccolo 1st Inlet, where we may suppose a common source sediments contamination (as suggested by the strong correlation between the pollutants), an environmental remediation plan would meet the needs of safeguarding the health of consumers as well as of ensuring the long-term survival of mussel farming.

In fact, the prohibition of exploiting the water body of the Mar Piccolo 1st Inlet for the mussel farming, on one hand forced mussel farmers to use almost exclusively the area of Mar Piccolo 2nd Inlet, leading to a significant overcrowding of installations, to the death of mussels and to the overall loss of most of the product legally raised in recent years, on the other hand to an increase of illegal production of unsafe mussels in Mar Piccolo 1st Inlet, which raises a serious public health concern.

As a partial solution, to deal with the contamination of the sediments in Mar Piccolo 1st Inlet as well as with the water warming and overcrowding in the Mar Piccolo 2nd Inlet, the further implementation of mussel farming in Mar Grande could favor the exploitation of many currently unused areas. However, given the peculiar environmental, geographical and cultural context of Taranto, the health protection cannot be separated from a rigorous policy action to reduce pollutant emissions, as well as from the environment restoration, which is also a key ethical issue. Moreover, the EU WFD (Water Framework Directive) requires Member States to achieve a good chemical status for all waterbodies in Europe as regard to dioxins and DL-PCBs, which are classified as priority substances in the field of water policy [23]. Anyhow, the sediment remediation plan should be carefully and appropriately applied in order to preserve the ecosystem of the area.

Finally, analyzing the problem from an historical perspective, the temporal trends of the pollutants over the observed years showed a concentrations increase in 2018 in Mar Piccolo 1st Inlet and Mar Grande. We do not know the reasons for this finding, but we can make some hypotheses: rising water temperatures, increase in human activities in the basins causing sediment resuspension, or, in Mar Piccolo 1st Inlet, overcrowding of installations leading to the death of the product and to the consequent release of accumulated pollutants in the water near the other filtering mussels.

In conclusion, a structured environmental plan for remediation of contaminated marine sediments, alongside with the reduction of pollutant emissions, appears to be the only effective, efficient and ethical long-term solution to protect the health of consumers, preserve the local production chain and restore the marine environment.

Conflict of interest statement
None.

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REFERENCES

1. EFSA. Update of the monitoring of levels of dioxins and PCBs in food and feed. EFSA J. 2012;10:2832. doi:10.2903/j.efsa.2012.2832
2. UN Environment. Stockholm Convention on Persistent Organic Pollutants (POPs). Text and annexes. Revised in 2017. Secretariat of the Stockholm Convention; 2018. Available from: http://chm.pops.int.
3. Fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and coplanar polychlorinated biphenyls. In: Safety evaluation of certain food additives and contaminants. WHO Food Additives Series No. 48. WHO; 2002. Available from: www.inchem.org/documents/jecfa/jecmono/v48je20.htm.
4. World Health Organization. Polychlorinated biphenyls: Human health aspects. Concise International Chemical Assessment Document 55. WHO; 2003. Available from: www.who.int/ipcs/publications/cicad/en/cicad55.pdf.
5. EFSA Panel on Contaminants in the Food Chain (CONTAM), et al. Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA J. 2018;16:5333. doi: 10.2903/j.efsa.2018.5333
6. Van den Berg M, Birnbaum L, Bosveld AT, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect. 1998;106:775-92. doi:10.1289/ehp.98106775
7. Eightieth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Non-dioxin-like polychlorinated biphenyls. Supplement 1 in: Safety evaluation of certain food additives and contaminants. WHO Food Additives Series: 71-S1. WHO; 2016. Available from: http://apps.who.int/iris/bitstream/10665/246225/1/9789241661713-eng.pdf.
8. EFSA Panel on Contaminants in the Food Chain (CONTAM). Opinion of the Scientific Panel on contaminants in the food chain related to the presence of non dioxin-like polychlorinated biphenyls (PCB) in feed and food. EFSA J. 2005;3:284. doi: 10.2903/j.efsa.2005.284
9. Van den Berg M, Birnbaum LS, Denison M, et al. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci. 2006;93:223-41. doi:10.1093/toxsci/kfl055
10. ARPA Puglia. Siti contaminati – Siti di interesse nazionale da bonificare. In: Relazione sullo stato dell'ambiente 2012. Regione Puglia; 2013. Available from: https://rsaonweb.weebly.com/uploads/9/6/2/6/9626584/2-sin.pdf.
11. Comba P, Pirastu R, Conti S, et al. Environment and health in Taranto, southern Italy: epidemiological studies and public health recommendations. Epidemiol Prev. 2012;36:305-20. Available from: www.epiprev.it/articolo_scientifico/ambiente-e-salute-taranto-studi-epidemiologici-e-indicazioni-di-sanità-pubblica.
12. Galise I, Serinelli M, Morabito A, et al. The integrated environmental health impact of emissions from a steel plant in Taranto and from a power plant in Brindisi, (Apulia Region, Southern Italy). Epidemiol Prev. 2019;43:329-37. doi:10.19191/EP19.5-6.P329.102
13. Cardellicchio N, Buccolieri A, Giandomenico S, Lopez L, Pizzulli F, Spada L. Organic pollutants (PAHs, PCBs) in sediments from the Mar Piccolo in Taranto (Ionian Sea, Southern Italy). Mar Pollut Bull. 2007;55:451-8. doi:10.1016/j.marpolbul.2007.09.007
14. Di Leo A, Annicchiarico C, Cardellicchio N, et al. Monitoring of PCDD/Fs and dioxin-like PCBs and seasonal variations in mussels from the Mar Grande and the Mar Piccolo of Taranto (Ionian Sea, Southern Italy). Environ Sci Pollut Res Int. 2014;21:13196-207. doi:10.1007/s11356-014-2495-6
15. Ortiz-Zarragoitia M, Cajaraville MP. Biomarkers of exposure and reproduction-related effects in mussels exposed to endocrine disruptors. Arch Environ Contam Toxicol. 2006;50:361-9. doi:10.1007/s00244-005-1082-8
16. EC. Consolidated text: Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). EU; 2018. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R1881-20180319.
17. EC. Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014 (Text with EEA relevance). EU; 2017. Available from: https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:32017R0644.
18. Schulte EH. Influence of algal concentration and temperature on the filtration rate of Mytilus edulis. Marine Biology.1975;30:331-41. doi:10.1007/BF00390638
19. Kittner C, Riisgård HU. Effect of temperature on filtration rate in the mussel Mytilus edulis: no evidence for temperature compensation. Marine Ecology. 2005;305:147-52. doi: 10.3354/meps305147
20. Riisgård HU, Larsen PS. Viscosity of seawater controls beat frequency of water-pumping cilia and filtration rate of mussels Mytilus edulis. Marine Ecology Progress Series. 2007;343:141-50. doi: 10.3354/meps06930
21. IARC. Polychlorinated biphenyls and polybrominated biphenyls. Monographs Volume 107. IARC; 2015. Available from: https://publications.iarc.fr/131/.
22. WHO, UN. Climate change and health country profile: Italy. WHO; 2018. Available from: https://apps.who.int/iris/handle/10665/260380.
23. EU. Consolidated text: Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. EU; 2014. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02000L0060-20141120.

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ORIGINAL ARTICLES AND REVIEWS

Turmeric (*Curcuma longa* L.) food supplements and hepatotoxicity: an integrated evaluation approach

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Abstract

Introduction. Turmeric is the common name for the rhizome of *Curcuma longa* L. In the recent years, food supplements containing turmeric have been marketed and widely used by an increasing number of consumers. Spontaneous reports of suspected adverse reactions to food supplements are collected within the Phytovigilance system.

Methods. An ad hoc multidisciplinary group investigated the suspected cases of hepatotoxicity reported to the Italian Phytovigilance system associated with the assumption of turmeric food supplements with the methodology specific to pharmacovigilance as well as for the evaluation of the quality and safety of food supplements.

Results. A cluster of 28 spontaneous reports of acute hepatitis, mostly with cholestasis, associated with turmeric products were sent to the Italian Phytovigilance system in the first six months of 2019.

In all cases, except one, the causality assessment was at least possible. The suspected products were collected and analysed for the presence of drugs, heavy metals, aflatoxins, pesticides, synthetic dyes and pyrrolizidine alkaloids.

Conclusion. On the basis of the results of all the activities performed by multidisciplinary group, regulatory intervention was taken. This study highlights the importance of developing an integrated evaluation approach for the evaluation of the adverse effects associated with the use of food supplements.

Key words

- *Curcuma longa*
- food supplements
- adverse reactions
- food safety
- chemical analyses

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INTRODUCTION

Turmeric is the common name for the rhizome of *Curcuma longa* L. (*C. domestica* Valeton, Fam. *Zingiberaceae*). Its main chemical components are curcuminoids (3-5%), including curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-dien-3,5-dione] (77%), demethoxycurcumin (17%) and bisdemethoxycurcumin (3%). These compounds are part of a mixture of yellow-colored substances chemically derived from diferuloylmethane. Turmeric also contains carbohydrates, proteins, lipids, minerals and essential oils (5.8%). Essential oils and curcuminoids are pharmacological markers for drug quality control; indeed, the dry rhizome must contain at least 2% curcuminoids, calculated as curcumin, and at least 25 ml/kg of essential oil [1].

The powder of rhizome is most widely used as a spice to color and flavor food such as mustard, cheese and butter, it is incorporated into tea and it is a base component in many culinary spice blends, such as curry. Curcumin, is also used as food dye (E 100), authorized for specific purposes in food and beverage production. The European Union legislation has established specific characterization and purity requirements for this substance in Reg. 231/2012 and subsequent amendments [2]. In this context, the European Food Safety Authority (EFSA) established an Acceptable Daily Intake (ADI) for curcumin of 3 mg/kg/day, equal to 210 mg/day for an adult of 70 kg of body weight [3].

Moreover, turmeric based products are present on the market as food supplements, containing *Curcuma longa* extracts, often standardized up to 95% curcuminoids, obtained after extraction with organic solvents like ethanol, methanol, or ethyl acetate.

Since the bioavailability of curcumin, almost insoluble in water, is very low (1%) [4], various methods have been devised to enhance the bioavailability, including complexation with piperine (an alkaloid extracted from *Piper nigrum*). Recently, also complexes of curcumin with phospholipids, polymeric micellar formulations and curcumin-loaded solid lipid nanoparticles have been used to increase the absorption of curcumin improving its oral bioavailability [5].

Commercial food supplements containing *Curcuma longa* extracts, alone or in combination with other plants, are used in rheumatologic diseases (such as arthritis) for the reputed anti-inflammatory activity and for reduction of body weight [6-8]. In addition, curcumin is under clinical evaluation as neuroprotective agent in the management of cognitive disorders even if the results appear contradictory [9, 10].

The European Union herbal monograph [11] on *Curcuma longa* supports its traditional use for relief of mild digestive problems, such as feelings of fullness, slow digestion and flatulence. This means that, although there is insufficient evidence from clinical trials, the efficacy of these herbal products is plausible, according to traditional use, confirmed that they have been used safely for those mild conditions for at least 30 years (including at least 15 years within the EU). As undesirable effects the monograph lists: mild symptoms of dry mouth, flatulence and gastric irritation. The monograph lists mild symptoms of dry mouth, flatulence and gastric irritation as *Curcuma longa* and reports that it is not recommended in case of obstruction of the bile duct, cholangitis, liver disease, gallstones and any other biliary diseases, due to possible stimulation of bile secretion. In experimental animal models, turmeric stimulated the contraction of the gall bladder and the bile secretion, these effects are specific to choleretic activity. The chemical components responsible for choleretic activity are curcuminoids and sesquiterpenes (α-turmerone and β-turmerone) [12]. Human studies report an increased contraction of the gallbladder with various dosages of curcumin (from 20 to 80 mg) [13-15]. Turmeric is used as powdered herbal substance (posology is 0.5-1 g 2-3 times daily), herbal tea (0.5-1.0 g as an infusion 2-3 times daily), tincture (the dilution is 1:10 or 1:5 and the posology is respectively 0.5-1 ml 3 times daily and 10 ml once daily or 5 ml 3 times daily), and dry extract [11].

In Italy, no drug containing turmeric has been registered for traditional use to date.

In recent years, different types of food supplements containing turmeric have been marketed. In Italy, sales data of these products indicated an increase of 25% between 2018 and 2019 (from almost 4 million of packages in 2018 to almost 5 million in 2019) [16].

A first signal of hepatotoxicity of turmeric-based products arose by a cluster of spontaneous reports of liver damage associated with turmeric products consumption that arrived to the Italian Phytovigilance system in the first six months of 2019. The frequency of new cases of liver damage, the homogeneity of the diagnoses and of the products taken by the subjects, were in line with a risk signal of hepatotoxicity associated with the use of food supplements containing turmeric. Immediately, preventive actions have been taken, in particular the EFSA Focal Points of other European countries were contacted exchanging information, and at the same time, samples of suspected products were collected to perform analytical assays and rule out the presence of any hazard of concern.

The work describes the activities carried out by an ad hoc multidisciplinary group to evaluate the suspected cases of hepatotoxicity reported to the Italian Phytovigilance system associated with the assumption of turmeric food supplements and drive any decision to reduce the associated risk. In order to ascertain the presence of harmful substances and to substantiate the risk, the study was performed according to: i) the methodology specific to pharmacovigilance; as well as ii) the evaluation of the quality and safety of food supplements.

MATERIALS AND METHODS

Phytovigilance methodology

The Phytovigilance system, coordinated by the Italian National Institute of Health – NIH (Istituto Superiore di Sanità), collects spontaneous reports of suspected adverse reactions to food supplements and galenic preparations containing plants. The surveillance system was activated in 2002 as a research project and became in 2012 a national system to support the Ministry of Health in monitoring safety of products of its regulatory competence.

The Phytovigilance system activities are conducted

separately from the Pharmacovigilance system, which is coordinated by the Italian Medicines Agency (Agenzia Italiana del Farmaco – AIFA).

Anybody observing a suspected adverse reaction associated with the above-mentioned products can report the reaction. Online report is possible through the website VigiErbe (www.vigierbe.it). Diagnoses are coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Hospital physicians provide follow-up for hospitalized patients.

A request, for "exchange of information" on the presence of turmeric supplements and any adverse reactions to these supplements in the different EU countries was sent through the EFSA Italian Focal Point. Causality assessment was performed for all cases using an evaluation scale adapted from the World Health Organization causality categories [17]. It was not possible to apply Roussel Uclaf Causality Assessment Method (RUCAM) because important information was missing in the reports.

Analytical chemical methodology

To identify the substances potentially responsible for the adverse reactions observed, the suspected products were collected and analysed.

On one side, for the safety issue, the analyses were focused on the search of voluntary added drugs, accidental contaminants, residues, and intentional synthetic adulterants, on the other side the analysis of the curcuminoids was also carried out to characterize the quality of the product. In particular, the following classes of substances were checked: non-steroidal anti-inflammatory drugs (e.g. nimesulide), narcotic or psychotropic substances, heavy metals (cadmium and lead), aflatoxins (B1, B2, G1, and G2), pesticides, pyrrolizidine alkaloids (26 alkaloides plus 20 N-oxides), synthetic dyes.

The analytes previously indicated have been selected for the following aspects: substances used to fraudulently connote turmeric-based products, contaminants from the environment and substances known for their potential hepatotoxic effect.

Indeed, turmeric is often subject to adulteration with potentially toxic compounds such as synthetic dyes that mimic the colour appearance of curcumin as well as the mixing with lower-cost botanical ingredients such as other *Curcuma* species. The replacement of natural curcumin with synthetic curcumin, which is intended to serve a lower-cost substitute, is a deliberate practice also [18]. Moreover, anti-inflammatory drugs (e.g. nimesulide) and narcotic or psychotropic substances could be added to enhance the effectiveness of the supplements.

The quali-quantitative analysis of the curcuminoids, the principal bioactive compounds of suspected products, including curcumin, demethoxycurcumin and bisdemethoxycurcumin and the presence of other Curcuma species such as *C. zedoaria* and *C. zanthorrhiza* was also carried out in order to evaluated and to investigate the content of curcumin and its natural origin.

The analytical techniques used for the determinations of the substances studied are listed below:

1) HPLC (High Performance Liquid Chromatography) with diode array detector (HPLC-DAD) used for the determination of synthetic dyes (2G yellow, fast yellow, naphthol yellow S, metanil yellow, oil orange S, orange 2, orange 6, sudan 1, yellow sudan, orange sudan G, red para). The chromatographic profile obtained by HPLC, was evaluated to investigate the content of curcumin and its natural origin;

2) HPLC (High Performance Liquid Chromatography) with fluorescence detector (HPLC-FLD) used for the determination of aflatoxins, namely aflatoxin (B1, B2, G1 and G2);

3) HPTLC (High-Performance Thin Layer Chromatography) was used to investigate the presence of other species of Curcuma (*C. zedoaria* and C. *zanthorrhiza*) as well as to compare the chromatographic profile of curcuminoids obtained by HPLC;

4) LC-MS/MS (Liquid Chromatography coupled with Tandem Mass Spectrometry) used for the determination of aflatoxins, confirmation of the presence of red dyes, narcotic or psychotropic substances, pyrrolizidine alkaloids, volatile pesticides, non-steroidal anti-inflammatory drugs and NSAIDs (e.g. nimesulide). In order to ensure the quality of the analytical data, the analyses performed by LC MS / MS were conducted, in collaboration between ISS and IZS, on the same samples;

5) GC/MS/MS (Gas Chromatography coupled with Tandem Mass Spectrometry) used for volatile pesticides;

6) ICP-MS (Inductively Coupled Plasma Mass Spectrometry) used for the determination of heavy metals (such as lead and cadmium).

Analyses were carried out by the NIH, the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZS LT) and the Istituto Zooprofilattico Sperimentale della Lombardia e della Emilia-Romagna (IZS LER).

RESULTS

Analysis of reports

From April 1st 2002 to July 15th 2019, 76 spontaneous reports of suspected adverse reactions to products, mainly food supplements, containing turmeric alone or in combinations with other ingredients were registered in the Italian Phytovigilance system. Thirty-nine of these reports (51%) indicated symptoms attributable to liver damage. We excluded eleven reports from the analysis because of lack of clinical documentation.

In the following, we refer to 28 reports associated with products containing turmeric, with date of onset between November 10th 2018 and June 17th 2019, reported to the Phytovigilance system between December and July (*see Table 1S in Supplementary Material available online*). In almost all cases, diagnosis, as described on the reporting form or derived from follow-up report, was acute hepatitis, mostly with cholestasis. For all included cases a diagnosis of viral hepatitis was ruled out. Negative tests for HAV, HBV, HCV, Epstein-Barr virus, cytomegalovirus were indicated.

The median age of patients was 55 years (range 27-71 years), mostly women (86%). Twenty-five patients (89%) were hospitalized. As for the products, 19 cases (67%) assumed food supplements containing high titre curcumin and various dosage of piperine; six cases assumed food supplements with turmeric and other ingre-

dients (2 with echinacea, 3 with boswellia and one with red yeast rice and berberine). One patient assumed a food supplement containing only *Curcuma longa* extract, one assumed a spice containing turmeric and ginger and one assumed two herbal teas containing turmeric, ginger and dandelion. In one case the assumed product was a galenic preparation containing curcumin and piperine. In one report, the name of the food supplement was not specified. Duration of use varied from 8 days to 8 months (median 2 months). As for the reason of using the food supplements, in 8 cases (29%) weight loss was indicated; in 4 pain associated to arthrosis, arthritis, joint pain and senile osteoporosis. In one report, the reason for use was "digestion". Other reasons for use were "detoxification", "as antioxidant" and hypercholesterolemia. In eight reports the reason for use was not indicated.

In 8 of the 28 reports, it was explicitly specified that no concomitant drugs were assumed. In ten cases (36%) concomitant use of drugs was reported, some of which with known or suspected hepatotoxicity. Two patients assumed in addition to drugs, other food supplements not containing turmeric. In 10 cases, the presence of concomitant drugs was not indicated: in these cases, it was not possible to determine if the information about concomitant drugs was missing or if there were not concomitant drugs.

The outcome was "complete resolution" in 15 cases, "persistent" in 2 cases and "resolution with sequelae" in one case, in 10 cases the information was not reported; dechallenge was positive in 17 cases, in one case negative and in 10 cases the information was not reported; in one case the rechallenge was positive.

In 17 cases (61%) the causality assessment was "probable", in 10 cases (36%) was "possible" and in one case it was not possible to evaluate the causal relation because the hospital did not provide complete data.

Twenty-three reports were sent from hospital physicians or specialists, 4 from Poison Control Centre, and one from a hospital pharmacist. Results are synthetized in *Table 1*.

The correspondence between the recommended dose, reported on the label, and the assumed dose (taken from the reporting forms, where available) was verified. The assumed dose was equal or even higher than the recommended dose.

For 18 reports, the amount of curcuminoids/curcumin taken by patients (ranging from 40 to 1425 mg/die) was calculated considering what was declared by the patients or at the dose indicated by the manufacturer. In 67% of cases, the curcumin daily intake was 1.6 to 7 times higher than the daily reference value set by EFSA [19].

Of particular interest were the answers of EFSA focal points of Spain and Greece obtained following the Italian request for "exchange of information". Spain does not have a list of plants allowed in supplements and allows mutual recognition for turmeric supplements from other European Union countries. At national level, Spain has appointed the scientific committee of the Spanish Food Safety and Nutrition Agency to determine the maximum levels of curcumin that are eli-

Table 1
Summary of the reports

Variable		
Age (years)		
Mean (range)	55	(27-71)
Duration of use (months)	**n**	**%**
Median (range)	2	(0.25-8)
Sex		
Males	4	14.3
Females	24	85.7
Hospitalization		
Yes	25	89.3
No	3	10.7
Product components		
Curcumin+piperine	19	67.9
Turmeric+other	6	21.4
Turmeric+echinacea	*2*	*7.1*
Turmeric+boswellia	*3*	*10.7*
Turmeric+red yeast+berberine	*1*	*3.6*
Curcuma longa extract	1	3.6
Spice (Turmeric+ginger)	1	3.6
Herbal tea (Turmeric+ginger+dandelion)	1	3.6
Reasons for use		
Weight loss	8	28.6
Pain (arthrosis, arthritis, joint, senile osteoporosis)	4	14.3
Digestion	1	3.6
Other (detoxification, antioxidant, hypercholesterolemia)	7	25.0
Not reported	8	28.6
Concomitant drugs		
No	8	28.6
Yes	10	35.7
Not reported	10	35.7
Outcome		
Complete resolution	15	53.6
Persistent reaction	2	7.1
Resolution with sequelae	1	3.6
Not reported	10	35.7
Dechallenge		
Positive	17	60.7
Negative	1	3.6
Not reported	10	35.7
Rechallenge		
Positive	1	3.6
Not reported	27	96.4
Causality		
Probable	17	60.7
Possible	10	35.7
Indeterminable	1	3.6
Report from		
Hospital physicians or specialists	23	82.1
Poison Control Centre	4	14.3
Hospital pharmacist	1	3.6

gible for use in supplements, with reference to the ADI established by EFSA. The Greek National Medicines Agency suggests not to exceed the daily dose of 210 mg of curcumin, based on the EFSA opinion [19].

Analyses of the products

From May to June 2019, 18 samples related to 16 reports, identified by the product name and production batch, were collected and analysed, of these, six were products assumed by hospitalized patients and shipped directly from hospitals (*Table 2*).

In 13 (about 50%) of the 28 reports associated with the products containing turmeric (*see Table 1S in Supplementary Material available online*) no data on the production batch of the suspected products were reported. Only for three of these products a sample with a different production batch was collected and consequently analysed.

All the analysed products were food supplements with the exception of one food marketed as spice, containing turmeric and ginger powders and one galenic preparation containing curcuma Meriva®, *Curcuma longa* dry extract (titrated 95% in curcuminoids) and *Piper nigrum* (titrated 95% in piperine). With regard to the composition of the analysed food supplements, in most cases the ingredients declared on the label consisted of *Curcuma longa* dry extract (titrated 95% in curcuminoids) in association with *Piper nigrum* dry extract (titrated 95% in piperine), in some cases the dry extracts of *C. longa* were complexed with phospholipids (curcuma Meriva® phytosome).

In none of the analysed samples the presence of drugs, NSAIDs, synthetic dyes and pyrrolizidine alkaloids was detected. The presence of heavy metals, aflatoxins, pesticides, where determined, were found to be close to limit of quantification.

As regard to the analysis performed to evaluate the quali-quantitative composition in curcuminoids of turmeric extract, used as ingredient in the food supplements, the obtained results showed that:

a) in 100% of analysed sample (11/18) other species of curcuma were not found (e.g. *C. zedoaria* and *C. zanthorrhiza*);
b) the concentration in curcuminoids of the analysed samples was in compliance with the declaration on the label;
c) in about 60% of samples the turmeric demethoxylated compounds were not detected.

In *Table 2* the results of the qualitative composition in curcuminoids of the analysed products are shown. The "patient ID" in *Table 1S in Supplementary Material available online* and in *Table 2* represents the same cases.

Table 2
Experimental data on composition in curcuminoids of the analysed products

		Curcuminoids	
Patient ID	**Product**	**Curcumin**	**Demethoxylated forms of curcumin**
1	Supplement	Detected	Detected
3	Supplement	Detected	Detected
8	Spice	Detected	Detected
9	Supplement	Detected	Not detected
9*	Supplement	Detected	Not detected
10	Supplement	Detected	Not detected
10*	Supplement	Detected	Not detected
11*	Galenic preparation	Detected	Detected
12	Supplement	Detected	Detected
13	Supplement	Detected	Not detected
15	Supplement	Detected	Not detected
16	Supplement	Detected	Not detected
17*	Supplement	Detected	Not detected
18*a	Supplement	Detected	Detected
18 b	Supplement	Detected	Detected
20	Supplement	Detected	Not detected
21*	Supplement	Detected	Not detected
22	Supplement	Detected	Not detected

*find (product assumed by hospitalized patients and shipped from hospital).
a, b: different products from the same report.

DISCUSSION

Based on the frequency of new cases of liver damage, the homogeneity of the diagnoses and of the products taken by the subjects, a risk signal associated with the use of food supplements containing turmeric was pointed out. The signal arose through a cluster of 28 spontaneous reports of suspected cholestatic hepatitis associated with turmeric containing food supplements sent to the Italian Phytovigilance system between December 2018 and June 2019. The seven reports from Tuscany Region were described in detail in a paper recently published [20].

This system, almost unique among European countries, has shown during the years its value for monitoring the safety profile of dietary supplements, as a guarantee for protection of citizens' health considering the wide use of these products in Italy (Italy is the first country for consumption of supplements in Europe) [21].

Considering that, for their nature, food supplements fall under the regulatory framework of food control, the signal was managed using both methodologies specific to pharmacovigilance and food safety control.

With regard to pharmacovigilance strategies and considerations applied in this context, it has to be underlined that spontaneous reports of suspected adverse reactions provide a qualitative signal, thus do not allow the quantification of a risk. The good reporting level of the Italian Phytovigilance system in comparison with other European countries and the quality of the reported data, with the possibility of follow-up in serious cases, should be underlined.

The evaluation of the possible causal association in individual cases (causality assessment) was the first step of signal validation, to which further investigations (disproportionality analysis, observed/expected analysis) should follow, when feasible and appropriate. Further-

more, an important consideration to be taken into account when trying to estimate the entity of the potential risks associated with food supplements is under-reporting of adverse reactions to the so-called "natural" products. This under-reporting is partly showed in our situation by the geographical variability of the number of reports: the reports were received mostly from Tuscany and Lombardy, where it is known that there is a greater sensitivity to pharmaco- and phyto-vigilance activities. Cannot be excluded that the increase in reports could also be due to the "notoriety bias" (increase in reports following the spread of a problem), indeed the spread of the news occurred between January and May 2019.

Another important element in the evaluation of risk signals in the pharmacovigilance field is the possible biological plausibility of the reaction observed with regard to the substance assumed. Regarding the mechanisms at the basis of suspected hepatotoxicity from turmeric, many hypotheses can be formulated.

The choleretic and cholecystokinetic action of curcumin may be also considered. Curcumin is excreted through the bile and excretion is mediated by ATP-dependent efflux pumps called Mrp2 (multidrug resistance-associated protein 2) [22]. These pumps are also physiologically responsible for the transport of bile acids from the liver to the bile canaliculi to form bile. At the level of these transporters a competition can be created between curcumin and bile acids which therefore accumulate in the liver inducing cholestasis. Therefore, the cholecystokinetic effect of curcumin, possibly enhanced by piperine, could be a risk factor for hepatotoxicity [23-25].

Some studies show that curcumin, as well as piperine, modulates the activity of different enzymes involved in the metabolism of some drugs. Curcumin has been shown to inhibit the activity of cytochrome P450, glutathione-S-transferase and UDP-glucuronosyl transferase. Inhibition of these enzymes in subjects taking curcumin can lead to an undesirable increase in blood concentrations of some drugs causing toxicity [26]. In this context, it was shown that CYP3A4 inhibitors could alter the metabolism of numerous drugs commonly used in the elderly, e.g. amiodarone and quinidine, increasing the risk of dangerous ventricular arrhythmias [27]. Piperine inhibits both the drug trasporter P-glycoprotein and the CYP3A4: these proteins are expressed in enterocytes and hepatocytes and contribute to a major extent to first-pass elimination of many dugs [28]. Therefore, the metabolic effect of curcumin, especially if associated with piperine, could be a risk factor for numerous drug interactions especially in patients undergoing polytherapy.

Many weaknesses and limitations emerged. In the first place, as already stated, spontaneous reports provide qualitative information and, in some cases, not all the necessary information were reported, such as the name of the product, batch, concomitant drugs or food supplements, follow-up of the patient. Thus, spontaneous report only can generate signals for further investigations.

During the qualitative and quantitative analysis phases, several aspects arose related, in particular, to the quantities of curcumin and other curcuminoids contained in the products. Many of the extracts analysed contained high concentrations of curcumin, but in about 60% of these, the other curcuminoids normally contained in the extracts of *Curcuma longa* were not present. For this reason, it was hypothesized that the different raw materials could not be totally of natural origin (and therefore derived from a normal extraction process) but consisted of synthetic substances added to enrich the finished product. However, this adulteration reduces turmeric quality, but should not impact its safety.

EFSA established the ADI of curcumin as safe considering the low bioavailability of this substance due to the negligible absorption in gastrointestinal tract [3]. However, this ADI is not referring to cases in which substances that increase the bioavailability of curcumin are added. Almost all the products indicated in our reports contained high titre curcumin associated with substances such as piperine to increase bioavailability. In these cases, the products no longer comply with the conditions examined by EFSA, therefore the benefit/risk ratio must necessarily be reassessed. All the aspects that emerged from the analyses need further investigation. In conclusion, the emerged problems can potentially be observed for any other product or substance available on the market, therefore it is appropriate to understand the need to plan interventions that may be reproducible for each situation. The detailed analysis of spontaneous reports represents the first step to face these problems and adopt appropriate intervention measures aimed at guaranteeing the safety of food supplements widely used by the population. During the first months of 2019 the Ministry of Health published a list of products implicated in the adverse reactions. The companies have autonomously withdrawn the products from the trade.

Spontaneous reports were the starting points for qualitative and quantitative analyses of the substances contained in some of the samples of the products. On the basis of the results of all the activities performed by the multidisciplinary group, regulatory intervention was taken, and further actions have been planned in the immediate future. The Ministry of Health has decided, to adopt a specific warning for the labelling of turmeric-based food supplements, aimed at discouraging their use for subjects with alterations of the hepato-biliary function. Moreover, the Ministry of Health invited to seek doctor's advice when using concomitant drugs.

CONCLUSION

Considering the results of the above investigations, it is concluded that the ADI established by EFSA (3mg/kg /day) for curcumin is a toxicological value that represents the quantity of the substance that can be taken daily for life without recognizable adverse effects. ADI was set by EFSA for curcumin as such and not in association with a molecule that increases its availability or biological effects. Therefore, this value cannot be used directly to assess the safety of the substance taken for limited periods. No illegal substances added voluntarily to the user have been detected by the analytical chemis-

try activity currently carried out. The levels of contaminants detected were always lower or close to the quantification limits. These supplements are formulated with associations of substances that increase the absorption of curcumin. Most of the supplements indicated on the label have a dosage which determines the exceeding of the ADI.

At the current state of knowledge and on the basis of the evidence collected so far, in the reported cases a cause-effect link of the dosage and formulation of the supplements in question with the hepatitis might not be excluded.

In order to investigate further interpretative scenarios of the phenomenon in the period under study, such as the increased exposure of the population to turmeric supplements and the presence of harmful substances, unidentified to date it is desirable to acquire further information on the various formulations present on the market over time for a better characterization of the supplement production chain (origin of raw materials, traceability, formulation, marketing).

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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REFERENCES

1. Council of Europe. Turmeric rhizome monograph. (10th Ed.). Ph Eur Monograph. (ref. 2543). Strasbourg, France; 2020.
2. EU Commission. Commission regulation (EU) no 231/2012. Laying down specifications for food additives listed in Annexes II and III to Regulation (EC) no 1333/2008 of the European Parliament and of the Council. Official Journal of the European Union 83 (pp. 270-1); 2012. Available from: http://data.europa.eu/eli/reg/2012/231/oj.
3. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) Scientific Opinion on the re-evaluation of curcumin (E 100) as a food additive. EFSA Journal. 2010;8(9):1679. Available from: www.efsa.europa.eu/en/efsajournal/pub/1679.
4. Lestari MLAD, Indrayanto G. Curcumin. Profiles of drug substances, excipients and related methodology. 2014;39:113-204.
5. Wang R, Han J, Jiang A, Huang R, Fu T, Wang L, Zheng Q, Li W, Li J. Involvement of metabolism-permeability in enhancing the oral bioavailability of curcumin in excipient-free solid dispersions co-formed with piperine. Intern J Pharmacol. 2019;561:9-18. doi: 10.1016/j.ijpharm.2019.02.027.
6. Panahi Y, Rahimnia AR, Sharafi M, Alishiri G, Saburi A, Sahebkar A. Curcuminoid treatment for knee osteoarthritis: A randomized double-blind placeboc trial. Phytother Res. 2014;28:1625-31. doi: 10.1002/ptr.5174
7. Panahi Y, Hosseini MS, Khalili N, Naimi E, Majeed M, Sahebkar A. Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with metabolic syndrome: A randomized controlled trial and an updated meta-analysis. Clin Nutrit. 2015;34:1101-8. doi: 10.1016/j.clnu.2014.12.019
8. Ganjali S, Sahebkar A, Mahdipour E, Jamialahmadi K, Torabi S, Akhlaghi S, Ferns G, Parizadeh SM, Ghayour-Mobarhan M. Investigation of the effects of curcumin on serum cytokines in obese individuals: A randomized controlled trial. Scient World J. 898361;2014. doi: 10.1155/2014/898361
9. Mazzanti G, Di Giacomo S. Curcumin and resveratrol in the management of cognitive disorders: What is the clinical evidence? Molecules. 2016;21:1243. doi: 10.3390/molecules21091243
10. Syarifah-Noratiqah SB, Naina-Mohamed I, Zulfarina MS, Qodriyah HMS. Natural polyphenols in the treatment of Alzheimer's disease. Curr Drug Targets. 2018;19:927-37. doi: 10.2174/1389450118666170328122527
11. Committee on Herbal Medicinal Products (HMPC). European Union herbal monograph on *Curcuma longa* L., rhizoma. EMA/HMPC/329755/2017, Final 25 September 2018. Available from: www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-curcuma-longa-l-rhizoma-revision-1en.pdf.
12. Wang L, Zhu X, Wang D, Li X. Choleretic activity of turmeric and its active ingredients. J Food Sci. 2016;81, H1800-H18006. doi: 10.1111/1750-3841.13348
13. Rasyid A, Lelo A. The effect of curcumin and placebo on human gall-bladder function: an ultrasound study. Aliment Pharmacol Therap. 1999;13:245-9. doi: 10.1046/j.1365-2036.1999.00464.x
14. Rasyid A, Rahman AR, Jaalam K, Lelo A. Effect of different curcumin dosages on human gall bladder. Asia Pac. J Clin Nutr. 2002;11:314-8. doi: 10.1046/j.1440-6047.2002.00296.x
15. Marciani L, Cox EF, Hoad CL, Totman JJ, Costigan C, Singh G, Shepherd V, Chalkley L, Robinson M, Ison R et al. Effects of various food ingredients on gall bladder emptying. Eur. J Clin Nutr. 2013;67:1182-7. doi: 10.1038/ejcn.2013.168
16. Federsalus. Integratori di curcuma: cosa c'è da sapere. 31 July 2019. Available from: www.federsalus.it/integratori-di-curcuma-cosa-sapere/.
17. Uppsala Monitoring Centre. The use of the WHO-UMC system for standardised case causality assessment; 2018. Available from: www.who.int/medicines/areas/quality_safety/safety_efficacy/WHOcausality_assessment.pdf.
18. Bejar E. Turmeric (*Curcuma longa*) root and rhizome, and root and rhizome extracts. Bot Adulterants Bull. 2018;May:1-11.
19. European Food Safety Authority. Refined exposure assessment for curcumin (E 100). EFSA J. 2014;12:3876. Available from: www.efsa.europa.eu/en/efsajournal/pub/3876.
20. Lombardi L, Crescioli G, Maggini V, Ippoliti I, Menniti Ippolito F, Gallo E, Brilli V, Lanzi C, Mannaioni G, Firenzuoli F, Vannacci A. Acute liver injury following turmeric use in Tuscany: an analysis of the Italian Phytovigilance database and systematic review of case reports. Br J Clin Pharmacol. 13 July 2020. doi: 10.1111/bcp.14460
21. Statistics & Facts Published by Statista Research Department. Health supplements in Italy; Jan 15, 2020. Available from: www.statista.com/topics/5149/health-supplements-market-in-italy.
22. Lee JH, Oh JH, Lee YJ. Biliary excretion of curcumin is mediated by multidrug resistance-associated protein 2. Biol Pharmaceut Bull. 2012;35:777-80. doi: 10.1248/

bpb.35.777
23. Bi, X., Yuan, Z., Qu, B., Zhou, H., Liu, Z., Xie, Y. Piperine enhances the bioavailability of silybin via inhibition of efflux trasporters BCRP and MRP2. Phytomedicine. 2019;54:98-108. doi: 10.1016/j.phymed.2018.09.217
24. Chand, S., Hair, C., Beswick, L. A rare case of turmeric-induced hepatotoxicity. Intern Med J. 2020;50:258-9. doi: 10.1111/imj.14727
25. Suhail FK, Masood U, Sharma A, John S, Dhamoon A. Turmeric supplement induced hepatotoxicity: A rare complication of a poorly regulated substance. Clin Toxicol. (Phila); 2020;58:216-7. doi: 10.1080/15563650.2019.1632882
26. Burgos-Moron E, Calderon-Montano JM, Salvador J, Robles A, Lopez-Lazaro M. The dark side of curcumin. Intern J Cancer. 2010;126:1771-5. doi: 10.1002/ijc.24967
27. Mancuso C, Barone E. Curcumin in clinical practice: myth or reality? Trends Pharmacol Sci. 2009;30:333-4. doi: 10.1016/j.tips.2009.04.004
28. Bhardwaj RK, Glaeser H, Becquemont L, Klotz U, Gupta SK.,Fromm, MF. Piperine, a major constituent of Black Pepper, inhibits human P-glycoprotein and CYP3A4. J Pharmacol Experim Therap. 2002;302:645-50. doi: 10.1124/jpet.102.034728

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Taking care of minor migrants' health: the professionals' perception and training needs

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Abstract

Introduction. In Italy, minor migrants represent 21.8% of the non-EU citizens. The care of minor migrants might be challenging as this population is characterized by higher vulnerability and special needs. The study aim was to describe the perceptions on the provision of care, the bio-psycho-social needs of migrant children and the professional training needs.

Methods. The study is qualitative descriptive. In May 2019 three focus group, involving health and social professionals, cultural mediators and NGOs operators, were organized.

Results. The study explored different areas of the provision of care to minor migrants including bio-psycho-social needs, care provision, barriers to care and professionals' training needs.

Discussion and conclusions. The provision of care should consider the specific migration journey and narrative. In some cases healthcare is fragmented, generating obstacles to access especially in minors with lower levels of health literacy. Training plays a key role in the development of cultural competence.

Key words

- bio-psycho-social needs
- minor migrants
- training needs
- health literacy
- cultural competence

INTRODUCTION

In 2017, 2.4 million immigrants entered the European Union (EU) from non-EU countries [1]. Out of these, around 240,000 immigrated to Italy [1]. According to the Unicef [2], one in four of the 82,000 refugees and migrants arriving in Europe through Mediterranean migration routes (i.e., 19,800) is a child. Despite a significant reduction compared to 2017, the number of unaccompanied minors arriving in the EU remained high in 2018 [3].

In Italy, minor migrants represent 21.8% of the non-EU citizens [4]. The provision of care to minor migrants might be challenging for health and social professionals as this population group is characterized by high vulnerability and special needs.

The interaction of health professionals with persons with different migration backgrounds requires effective communication strategies (e.g. mediation and interpreting) as well as the knowledge of the migratory process and cultural background [5]. Health professionals recognize the need for special knowledge and skills when taking care of migrants, and that their training is often scarce or non-specific, making them inadequate in providing appropriate care [6]. Specific training in the field of cultural competence allows a greater understanding of the psychosocial context in the care of migrants [7]. Cultural competence means "a multidimensional learning process that integrates transcultural skills into cognitive, practical and affective dimensions that aim to achieve culturally congruent care" [8]. A study conducted with General Practitioners has shown that those who have received specific training for the development of intercultural competences seem to provide higher quality standards of care [9]. This cross-cultural knowledge and skills might also result from the continuous exposure to migrant patients [10]. There is

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evidence that training on cultural competence improves the knowledge, attitudes and skills of health professionals and positively affects patients' satisfaction [11]. Training in cultural competences has also proved effective for the professionals of rehabilitation facilities [12].

A controlled clinical trial, conducted to assess how a 3-day training affects the cultural skills of pediatric nurses, has shown improvements in cultural knowledge, skills, and desire to learn more about the topic of "culturally competent health services" [13]. When taking care of migrant populations, health professionals face several challenges: different expectations, difficulties in communication, and creating a trustful relationship, as well as a lack of specific training and inadequate organizational and management systems of care [14].

To our knowledge, there are no studies in the Italian context that have assessed the training needs of health and social professionals who are involved, at different levels, in the provision of care to migrant children.

Therefore, the objective of the present study was to describe the perceptions of health and social professionals on the provision of care, the bio-psycho-social needs of migrant children and the training needs and methodologies.

METHODS

The study design is qualitative descriptive. A series of focus group (FG) was organized within the European Union-Vocational Education and Training (EU-VET) care Project "Strengthening capacities for better health care to refugee and migrant children" [15], whose goal is to improve the training of professionals who are involved in the care of migrant/refugee children. The FGs aimed to explore the perception of key stakeholders regarding the care system addressing migrant/refugee children's health and wellbeing. Participants were recruited through a purposeful, theoretical built sampling and included physicians, psychologists, social workers, cultural mediators, child protection legal experts and administrative collaborators. All participants were contacted by telephone by an EU-VET project manager through the project stakeholders' network. The call has then been followed by an email invitation. All participants were actively involved in the care of migrant children in different settings at the moment of the study. The FGs were facilitated by experienced researchers of the Italian National Institute of Health in Rome, following a semi-structured set of questions. This questions guide was built by the contribution of all researchers, shared with EU-VET project managers, then tested during the first focus group, not requiring changes during data collection (*Table 1*). Each discussion lasted around 90 minutes. All the FGs were audio-recorded and transcribed, with the informed consent of the participants. Socio-demographic data were also collected in an anonymous form. The transcripts were independently read and coded by two authors who then discussed the categories for defining the tree-nodes. In case of disagreement, a third researcher was involved. Most categories were defined in advance according

Table 1
Aims and questions

Aim 1. Barriers and activators related to the care provision for migrant/refugee children
1a) In your opinion, what are the *main obstacles* in the provision of care for migrant/refugee children? Why? Can you give some examples? • What makes your job difficult? • Are you able to help them? If not why? • Does this age group have characteristics that make it difficult to help? • Are there skills/knowledge that would allow you to be more effective in helping? • What are these skills/knowledges? 1b) In your opinion, *what supports* the provision of care for migrant/refugee children? Why? Can you give some examples? • What would help your job? • What skills/knowledge would facilitate your work with these children?
Aim 2. Perceptions and attitudes about professional training on the provision of care for migrant/refugee children
2a) In your opinion, *how important is professional training* related to the provision of care for migrant/refugee children and why? *What impact* does professional training have on your care provision for migrant/refugee children in your practice? And what impact does the professional training have on the *interventions' outcomes* (health, biopsychosocial well-being)? 2b) What is your educational experience related to migrant/refugee children care? (search for knowledge, gaps and training needs of the participants) • Did you ever attend specific training on migrant/refugee children care? • If not, did you attend specific training on assistance to migrants/refugees in general? • How would you describe these learning experiences? Effective, engaging or not? Pros and cons and why. • What would you change in these training? Why? • What additional training do you consider necessary? • What has made you/would make you feel satisfied in this training? What makes training on these issues effective? • What instead makes it unsatisfactory?
Aim 3. Proposals to improve training on provision of care for migrant/refugee children
3a) Do you think that further training is needed for staff dealing with migrant/refugee children? 3b) Would you find further training useful for you? 3c) What content should training include, in order to cover needs and overcome the obstacles you have listed include? Why? • Who should be trained? • Should training be differentiated by profession or not? How would you structure it? • What content would you include related to the health and care provision of migrant/refugee children? • Which training methodologies? (e.g. case studies, role plays, other?) • What other issues should be included to promote motivation, involvement and active participation? (e.g. duration, certification, etc.)

to the main research questions (deductive approach) while additional categories were defined as emerging during the coding process (inductive approach). The software used for the qualitative analysis was NVivo 12 Plus. Data saturation was reached when no new information emerged. The tree-nodes has been applied to the whole of the transcripts and the most meaningful verbatim were identified.

The main themes addressed during the FGs were:

- the characteristics of care provision to migrant children (probe: barriers and activators of the care provision for migrant/refugee children);
- the bio-psycho-social needs and the ability, at system and at individual level, to address these needs;
- the training needs and methodologies.

RESULTS

In May 2019, 3 FGs were conducted at the Italian National Institute of Health (Istituto Superiore di Sanità) in Rome involving a total of 15 participants (93,3% response rate). *Table 2* summarizes the participants' characteristics. Among the physicians, three were pediatricians; the organizations were both public and private, with a representation of NGOs of national and international relevance (Medecins du monde, Save the Children, International Organization for Migration, InterSOS, Medecins sans Frontieres, Caritas, Red Cross, National Institute for Health, Migration and Poverty) and operators of the Reception Centers (RC). The majority of the involved physicians and psychologists worked in the Roman area within the Health System, in international institutions and reception centers.

Table 2
FGs participants' characteristics (N = 15)

Data of participants	N (%)
Country of origin	
Italy	15 (100)
Mean age in years	46.3
Women	12 (80)
Men	3 (20)
Education	
Bachelor's degree or higher	14 (93.3)
Some technical school	1 (6.7)
Professions	
Physician	6 (40)
Pediatricians (3)	
Psychologist	3 (20)
Cultural mediator	2 (13.3)
Child protection expert	2 (13.3)
Social operator	1 (6.7)
Administrative assistant	1 (6.7)
Mean working time in years	
Adult or any age	9.7
Migrant minor	7.7
Specific training	
Any migrant	8 (53.3)
Minor migrant	8 (53.3)
Development of training course	
Any migrant	10 (66.7)
Minor migrant	9 (60)

The average working experience with migrants was 10 years with regard to adults or any age and 8 years with regard to minors, respectively. In most cases, they did not have specific training on both migrants in general and on migrant children, but most of them had participated in the development of training courses related to social and health care.

Bio-psycho-social needs

One of the main emerged themes was the identification of minor migrants' health needs, framed in a bio-psycho-social holistic perspective (*Figure 1*). The achievement of unaccompanied migrant minors' (UMM) primary needs, like "give a supportive space... a shower, a family space", is part of the activities implemented and to be implemented, although often accompanied by linguistic and cross-cultural barriers and by unfamiliar care settings.

Migration has been described as a "fragmented" event that breaks down the child's needs on several levels of care. In this context, the professionals and the operators are requested to connect "these pieces" and, therefore, the minor's identity. This fragmentation causes a need for reassurance and trust, which is especially expressed on arrival and reflects the fear of "being sent away or not being accepted". Often their migration project does not comply with "our migration stereotype which often does not coincide with their history". Therefore, including their migration goal in the provision of care means responding to their need to achieve it. Although many experiences, in particular the "journey", require migrant minors to assume responsibility as adults, professionals recognize their need and right to be recognized and treated as children especially when they "have to manage their own health and the health of their parents".

Sometimes, the need for caring relationships in reception overcomes the health needs because it allows to "enhance all aspects beyond the more technical ones". About specific health needs, one of the main themes is the reduced vaccination coverage or the difficulty in reconstructing the child/adolescent's vaccination history, and the consequent lack of access to school. Another issue is the higher risk of obesity "because diets change". In addition, the implementation of an inclusion and integration process requires a guarantee for working autonomy. Thus, adolescents need appropriate training and/or gradual job placement.

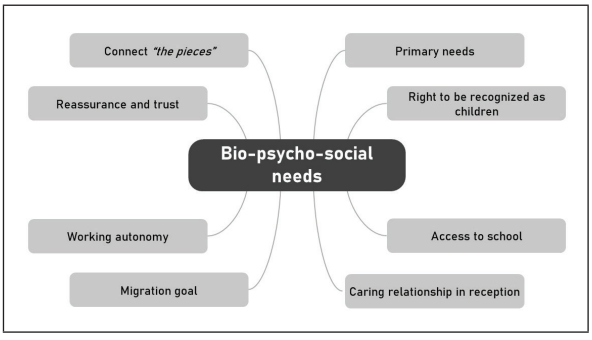

Figure 1
Bio-psycho-social needs.

Care provision

Professionals report an "infantilization" of minor migrants and the consequent tendency to not recognize their "edge over" that allows reaching a greater degree of autonomy.

Volunteer tutors are an important resource because they help to build a therapeutic relationship that is not only conveyed "by pieces of paper". The excess of bureaucracy is also due by the recognition of the minor age, which is not always univocal, generating difficulties and slowdowns. The recognition of minor frailties is challenging as it does not always occur in a multidisciplinary and holistic way. In the case of a psychic vulnerability, especially in adolescents and UMMs, "little work is being done towards prevention", but rather at an emergency level. Provision of care in the first 1000 days and childhood emerged as being complicated by several issues emerge. In Western Countries, pregnancy is considered as a potential risk condition, while migrant women deem it as a physiological process. Breastfeeding for 2 years and beyond is a well-received practice by migrant women who unwillingly accept early weaning (around 4-5 months), "they would like to start weaning later".

Regarding the UMMs, the organization should know, at various levels, their narratives, especially for those who have a greater vulnerability. Taking care of adolescents mainly concerned the psychic area, such as boredom and "suspended time". To cope with this discomfort, it is the care facilities' task to engage the adolescent in "a calendar of activities". The family custody of these adolescents seems to be a long and tiring journey, and this prevents the adolescents from completing their migration project, whether it is to reach the "country of hope" or to work in the host country. Furthermore, there seems to be a "difficulty of the system to take care of girls", especially in the case of victims of trafficking for whom "the offer of individual psychological support is not always the most suitable solution" and should be accompanied by support groups. These girls had experienced "missed adolescence and childhood" that could be aggravated during the stay in the reception centers: "... arrives the maman and they became victims three times, because they could no longer even talk to us". The UMMs, especially adolescents, are identified in the streets by the community services, within their "informal network".

With regard to accompanied migrant minors, the difficulties are bureaucratic, mainly related to the lack of access to services due to residence problems (e.g. the registration with the family pediatrician) and family problems "because the fragility of the family always affects the child". The new adults and minors approaching 18 years of age are particularly vulnerable "because they simply do not exist", as they become adults without the possibility of inclusion in institutional reception systems. This greater vulnerability leads to psychological problems without structured care pathways.

In the care relationship, the participants described two main types of minors: the translator or interpreter of the family unit and "adult minor". It has been a common opinion that children, by fulfilling these functions, are more fragile because they are invested with a "responsibility that they should absolutely not have". In some cases the figure of "translator" is a direct consequence of a system that does not provide the cultural mediator as a support in the provision of care to the migrant population. On the other hand, "adultization" allows to reach a level of autonomy that differentiates these boys/girls from the adolescents of the host country.

The participants provided indications to better respond to minors needs. Among these, briefing, mutual help, discussion and sharing within a multi-professional and multidisciplinary team emerged, "to always have focused everything on how to proceed". This would help to understand and overcome the critical points, and to improve care. Clinical protocols would allow all the actors involved in migrant children's care to work in a synergistic, clear and defined way. According to some of the participants, the wide reception centres (RCs) could represent a solution that allows them to welcome migrants in a multidisciplinary way in order to "feel part of this world". On the other hand, others have expressed favourable opinions for the widespread small centres, linked by networks with local services, because they could facilitate a real opportunity of integration and a "truly intercultural world".

The opportunistic provision of services, compared to a pro-active offer, leads to inappropriate access to services by migrants (minors and their families), such as greater use of emergency services. Moreover, cultural mediators, differentiated by gender, especially for girls, and ethnicity can facilitate the comprehension and the response to bio-psycho-social needs.

The participants also addressed the topic of the vicarious trauma experienced by operators in situations of emotional involvement, when coming into "contact with dramatic stories". The participants highlighted the need of listening, sharing and support services for operators, to guarantee them the possibility of unburdening any problems they may be facing before emotional distress occurs. There is also a need for "external supervision, both technical and psychological", to overcome the operators' feeling of loneliness and not recognition. Moreover, the participants raised the attention on the emergence of stereotypes, that could lead to attribute labels to the migrant person, and this "is very dangerous, because once it is given, all the other information is read in this key".

Barriers to care

The three FGs revealed obstacles to accessing health services (*Figure 2*). The scarce availability of orientation information and the reduced health literacy of migrants make it difficult to access and use health services properly. While the prevention services are underused, there is excessive and inappropriate use of emergency services. Health literacy is a key point for the adequate access to services, as well as other aspects like food safety (especially for the preservation of food) and the conscious use of drugs (e.g. over/misuse of antibiotics or vitamins in children).

The barriers to the provision of care resulted to be

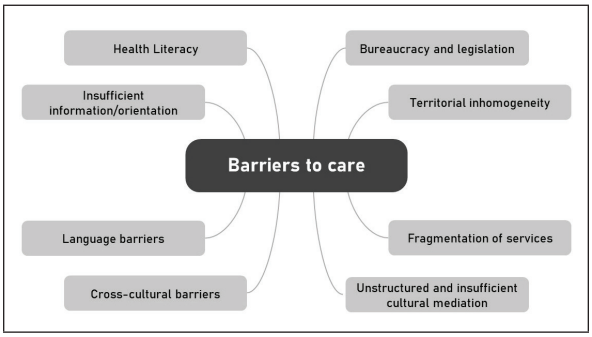

Figure 2
Barriers to care.

also bureaucratic and legislative, e.g. the difficulty in obtaining residence. A "bureaucratic delay" was found, and it appears to be opposed to the common representation that migrants have of "a structured, fast and efficient European world". The cultural mediator, however, is able to explain, especially to children, the rationale behind bureaucracy and the necessary administrative procedures, making them more comprehensible and acceptable. One of the main difficulties is the registration with the National or Regional Health Service, that is essential for the universal, free of charge care system.

The national repressive migration policies have generated insecurity and distrust among migrants, as the offer of the reception system has been downsized. Moreover, the territorial and national inhomogeneity contributes to create obstacles because "a guideline is missing, protocols are lacking, in short, more standardized directions are missing". This generates a fragmentation of the services that often do not communicate with each other, causing a "fragmentation" of the minor's needs and identity, and undermining continuity of care. In spite of this, "some realities have successfully developed a virtuous pathway" "…modelling their interventions taking into account some cultural peculiarities".

Language barriers represent one of the greater obstacles to the provision of care, from the perspective of migrants, professionals and administrative personnel (e.g. information desk). This adds up to the lack of cultural mediators to accompany the migrant throughout his/her reception pathway. This figure is often not recognized by the reception system, it is not structurally integrated within the Social-Health System and it is not part of the programming of the services. This leads to communication problems, reinforcing the idea that cultural mediation, beyond translation, implies a transcultural dialogue between the migrant person and the care system.

The cross-cultural barriers between operators and migrant minors were perceived as an obstacle that affects both the migrant and the operators. As an example, male circumcision, practiced during childhood in some cultures/religions, could be "made possible in public structures, hospitals, Local Health Authority" contributing to eliminate the undeclared and dangerous practice. Often the operators don't know the culturally different ways of caring for infants, because "we don't have the same cultural reference on caring". Among the barriers that emerged for migrants, there is poor adherence and compliance to long-term drug therapies, especially by minor migrants; the non-acceptance of diagnostic techniques (e.g. blood sampling); psychological disorders culturally interpreted in a different way by the caregiver. The gender difference also seems to be an obstacle, "in many cities the ability to take care of girls is very lacking", e.g. human trafficking and sexual victims.

Training

The target should include health professionals, educators, mediators, reception managers, technicians, administrators, and, "in general, all those who will take care of the child on a daily basis". Training should be planned according to the different professional specificities, with a special focus on those who interact with children. The tutors and families to whom the minors could possibly be entrusted were also identified as a target of the training. It should be a specific training, aimed to describe the process, the timing and the dynamics of welcoming and entrusting.

Training is considered as "a very individual thing" that requires different timing for everyone, because "starting from the same conditions, there are those who develop immediately [knowledge and abilities]… and others who do not" and help to be aware of one's attitudes. At the same time, it is evident that professionals attend these courses according to their individual predisposition, causing a selection bias.

The training contents include the development of counselling skills, the identification of the risk profile of the minor migrant, and the social health determinants. The latter, according to a "global health approach, therefore an approach that takes into account the effect of the social determinants of health on people's lives. We are talking about inequalities in health". This will support early identification of the situations of greatest vulnerability. Another content to address is the processing of sensitive data. Moreover, "training should be centred on how to make these skills talk to each other".

The training on the first 1000 days should involve infant care, in its cultural declinations, breastfeeding, complementary feeding, infant massage and job placement of mothers. In addition, it is useful to give directions to the professionals on culturally sensitive good practices related to child feeding, in order to not responding to a typical vision of our western societies but to the mothers' desire and cultural background. It is the case of weaning, which is often advised before 6 months and according to pre-set Italian dietary schemes. The need to have a cross-cultural food pyramid emerged. Another topic was infant feeding in emergencies and, therefore, training "on the importance of breastfeeding in war and emergency areas".

The training methodologies considered most effective were those that provide for a higher level of active participation of the learners (role-playing, discussion of cases, etc…). In this type of training, the young migrants can play the role of trainers, because the testimony of their experience would allow people to get in touch with each other ("Making stories speak"). The Community of Practice could be an adequate tool, to

promote collaboration among "police headquarters, prefecture, immigration office, Local Health Authority and reception centers". It should be "a long-term solution, which requires strong hierarchical coordination both at the micro-level in the management of the single center and at the macro-level in the management and taking care of the minor".

In participants' experiences, the strengths of effective training were: multidisciplinarity and multi-professionalism; field training; a combination of theoretical and practical training methodologies. The weaknesses were, instead, pre-established training modules that do not allow the active involvement of participants in the construction of training objectives and needs and obsolete training programs that do not raise awareness on reception.

The proposals to improve training included the involvement of different professions, a training needs analysis and the activation of the participants on their own needs. Moreover, a follow up is required to monitor the results achieved and whether these are preserved over time. Pre-service and in-service training should be included both in university curricula and in continuous training, through Continuing Medical Education (CME) accreditation.

DISCUSSION

In our study, we explored the health needs of minor migrants and the barriers to care as a means to understand and improve the professionals' training and the provision of care. During our FG, the participants recursively addressed and showed greater interest in the theme of minor migrants' frailties and barriers to care. Among the other aspects, the FGs brought to light the professionals' need for listening and discussion as they experience a sense of isolation. As for the study results, although the scientific literature is not extensive, we found some consistency with other published studies.

The UMMs' emerging needs are often the expression of inadequate living conditions due to the migration route from the country of origin to the host country. Migrant minors in general, but in particular the UMMs, face risks related to the social and political situations of their countries of origin, and the modality, distance and duration of the journey, and the arrival in the host country; all elements that negatively impact on the psycho-physical health. These findings are consistent with the ISSOP position statement on minor migrant health, reporting that these children show high rates of depression and post-traumatic stress disorder (PTSD) in the first years after resettlement [16].

In our results, these aspects cause difficulties in taking care of minors. Language and cross-cultural barriers undermine communication between service providers and people, produce insecurity in migrants, make more difficult to build a therapeutic relationship based on mutual trust and this can also negatively affect the provision of primary care, the quality, and continuity of care, as showed also by Dauvrin *et al.* [17]. Moreover, the care practices can be misinterpreted and consequently not accepted by the migrant population. A metasynthesis [18] analysed migrants' views on obstacles to accessing services. They included linguistic and cross-cultural barriers related to belonging to an ethnic group, religion, or country of origin that produce distrust towards organizations and health professionals. As emerged from our focuses, a strategy that migrants put in place to deal with communications barriers is the use, within the family, of children as translators. This leads to problems related to greater fragility and responsibility of those children who are invested in carrying out this function. Additional problems that emerge from the literature concern the impairment of the parents' authority who rely on their child for interpretation; the lack of school attendance by children who must accompany a family member to a health visit; parents and children can experience emotional trauma, fear, embarrassment and shame [19]. Sometimes, the figure of the "minor-translator" is needed to overcome the lack of a system that does not include the structural integration of cultural mediation and interpretation. In scientific literature, the difference between cultural mediators and interpreters is not always clear. According to the Regional Office for Europe of the World Health Organization (WHO), mediators encourage and improve the use of services by explaining the health care provided and promoting the trust of migrants in the service and the staff [19]. Interpreters, on the other hand, translate spoken information from one language to another [19]. Our participants strongly support for active provision of cultural mediation in the context of standard health/social care, in a way that is efficient and sensitive to gender and ethnicity. The shortage of cultural/linguistic mediation is consistent with other authors' findings [20], causing the child to be used as interpreter and to become an "adultized child". "Adultization" also occurs in other circumstances, e.g. when migrant children try to improve the economic conditions of their families [21].

At the same time, there is often a tendency to infantilize children by not recognizing their degree of autonomy. Kanics *et al.* describe how this negatively impact on the ability to exploit integration opportunities [22]. According to our findings, an intercultural approach is necessary to address the difficulties to adapt to a new culture and country while maintaining the native culture, as described also by Nakeyar C. *et al.* [23].

Failure to recognize the minors' psycho-physical frailties, the lack of multidisciplinary and multi-professional management, and no specific cross-cultural skills, represents an obstacle to treatment. In our results, among the frailties, emerged an increased risk of obesity and overweight due to changes in eating habits. These data are also confirmed by the Italian surveillance system, which shows how the family context can influence the lifestyles [24]. With regard to mental health, migrant children are at high risk of mental and psychosocial problems, including depression and PTSD [16]. These frailties are reported in our study and result in difficulties of integration, provision of holistic care, and access to services that can harm the migrants' health and increase vulnerability. The inhomogeneity and, in some cases, the lack of integration of health services, not always oriented to continuous and tailored care, reduces

the possibility of adequate use of services, in particular those relating to ordinary care. This leads to greater use of emergency services. Several studies showed that the probability of using emergency services is higher among migrants than the local population [25] [26]. Another reason that could explain this increased access is the use, in the countries of origin, of emergency services as first-line access to healthcare; this is especially evident in countries where primary and community health care is not widespread. Many migrant children may not have access to adequate community preventive care and, therefore, may not have adequate vaccination coverage resulting in limited access to school. The poor vaccination coverage is mainly due to poor implementation of primary prevention programs in the countries of origin [26], but, as emerged from our study, it may also be due to a difficulty in tracking and certifying the vaccination pathways.

Our study highlights some areas that need to be considered when planning training aimed at operators involved in the provision of care for minor migrants. These areas include context analysis and the capacity to identify and address the main children's needs and barriers to care, comprising specific frailties and conditions related to the migration process (e.g. PTSD, depression). Another area to be addressed is the professionals' cultural competence and appropriate communication and counselling skills. The professionals should also improve their capacity to work according to a trans-disciplinary, trans-sectoral, and trans-cultural approach. Therefore, the training should promote a transformative (trans-formative) process and the co-construction of a new/different way of provision of care, both on individual and organizational level. Some specific topics regard self-medication and therapeutic appropriateness, abuse screening and prevention, sexual health, health promotion, lifestyles and health literacy; maternal and child health should be treated in the training according to the WHO/UNICEF recommendations. The training should also be aimed to improve awareness on bias and prejudices of health professionals, that can contribute to the vulnerability and impact health outcomes. On the other hand, the operators are more likely to develop a vicarious trauma, which exposes them to a greater risk of "short circuit". Aside from peer professional support, specific training can help in developing awareness and coping strategies, in order to prevent, recognize and seek help in case of vicarious trauma and burnout.

A trans-cultural training on minor migrant health is intended for a broad target. Indeed, there is a "selection bias" that leads professionals with higher motivation to be more frequently involved in social and healthcare pathways for minor migrants, compared to those who are less interested in the migration issue. For this reason, a transcultural approach falls within the core competencies addressed by pre-service and in-service (CME) education for all the health, educational, and social personnel.

Our participants expressed a preference for training methodologies that allow greater involvement and active participation of learners. Among these, role-playing, case-studies, and the activation of a Community of Practice were mentioned. Berlin *et al.* (2010) described the effectiveness of an educational method that combines theory, practice, seminars, activities, and discussion [13]. Previous studies on cultural competence among health professionals have used various methods, such as didactic presentations, discussions, role-playing, videos, and exercises [27]. A 2017 study, which used and assessed the participatory approach of the Community of Practice, showed how this could be able to improve knowledge, attitudes, perceived skills, and involvement of members belonging to the Community, in order to optimize resources and skills by generating new knowledge [28].

Continuous discussion and comparison through audit and feedback strategies would help to make a critical analysis of successful and unsuccessful strategies and find shared solutions to improve the provision of care. In general, clinical experiences have shown how these moments of sharing and discussion between professionals lead to small but important changes in professional practice [29].

This study has some limitations: the purpose sampling and its small size may not allow considering all the possible experiences in this field. Further research involving the UMMs would provide a broader comprehension of the phenomena from their own perspective.

CONCLUSIONS

In our study, multiple organizational and individual challenges have been reported in both care provision to minor migrants and professionals' training needs. In light of these results, a guidance is given to trainers, lecturers, health/social/educational professionals, reception systems, associations, NGOs, decision and policy makers, and other stakeholders to orient training planning, content and methodologies. A broad, a trans-cultural approach is needed to promote the core competencies during pre-service and in-service education. Further studies are needed to develop and evaluate the effectiveness of training programs in improving the cultural competence of the operators and the consequent improvement in migrants' health and care.

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REFERENCES

1. Statistics Explained. (2020, May 17). Available from: https://ec.europa.eu/eurostat/statistics-explained/index.php/Main_Page.
2. United Nations Children's Fund – UNICEF. Refugee and Migrant Response in Europe. 2019.
3. European Migration Network. Annual Report on Migration and Asylum 2018.
4. Istituto Nazionale di Statistica – Istat. Cittadini non comunitari in Italia. Anni 2018-2019.
5. Alpern JD, Davey CS, Song J. Perceived barriers to success for resident physicians interested in immigrant and refugee health. BMC Med Educ. 2016;16(1):1-6. doi:10.1186/s12909-016-0696-z
6. Mengesha ZB, Perz J, Dune T, Ussher J. Preparedness of health care professionals for delivering sexual and reproductive health care to refugee and migrant women: A mixed methods study. Int J Environ Res Public Health. 2018;15(1):174. doi: 10.3390/ijerph15010174
7. Hudelson P, Perron NJ, Perneger TV. Measuring physicians' and medical students' attitudes toward caring for immigrant patients. Eval Heal Prof. 2010;33(4):452-72. doi: 10.1177/0163278710370157
8. Jeffreys MR. Teaching cultural competence in nursing and health care. New York: Springer Publishing Company; 2016.
9. Papic O, Malak Z, Rosenberg E. Survey of family physicians' perspectives on management of immigrant patients: Attitudes, barriers, strategies, and training needs. Patient Educ Couns. 2012;86(2):205-9. doi: 10.1016/j.pec.2011.05.015
10. Watt K, Abbott P, Reath J. Cultural competency training of GP Registrars-exploring the views of GP Supervisors. Int J Equity Health. 2015;14(1):1-10. doi: 10.1186/s12939-015-0226-3
11. Beach MC, Price EG, Gary TL, Robinson KA, Gozu A, Palacio A, Smarth C, Jenckes MW, Feuerstein C, Bass EB, Powe NR, Cooper LA. Cultural competency: A systematic review of health care provider educational interventions. Med Care. 2005;43(4):356-73.
12. Chipps JA, Simpson B, Brysiewicz P. The effectiveness of cultural-competence training for health professionals in community-based rehabilitation: A systematic review of literature. Worldviews Evidence-Based Nurs. 2008;5(2):85-94. doi: 10.1111/j.1741-6787.2008.00117.x
13. Berlin A, Nilsson G, Törnkvist L. Cultural competence among Swedish child health nurses after specific training: A randomized trial. Nurs Heal Sci. 2010;12(3):381-91. doi: 10.1111/j.1442-2018.2010.00542.x
14. Kalengayi FKN, Hurtig AK, Nordstrand A, Ahlm C, Ahlberg BM. "It is a dilemma": Perspectives of nurse practitioners on health screening of newly arrived migrants. Glob Health Action. 2015;8(1):1-11. doi: 10.3402/gha.v8.27903
15. EU-VET CARE - Strengthening capacities for better healthcare to refugee & migrant children. 2020. Available from: https://www.euvetcare.eu/
16. International Society for Social Pediatrics and Child Health Migrationa Working Group. ISSOP position statement on migrant child health. Child Care Health Dev. 2018; 44(1):161-70. doi: 10.1111/cch.12485
17. Dauvrin M, Lorant V, Sandhu S, Devillé W, Dia H, Dias S, Gaddini A, Ioannidis E, Jensen NK, Kluge U, Mertaniemi R, Puigpinos i Riera R, Sarvary A, Strabmayr C, Stankunas M, Soares JJF, Welbel M, Piebe S. Health care for irregular migrants: Pragmatism across Europe. A qualitative study. BMC Res Notes. 2012;5:1-9. doi: 10.1186/1756-0500-5-99
18. Agudelo-Suárez AA, Gil-gonzález D, Vives-Cases C, Love JG, Wimpenny P, Ronda-Pérez E. A metasynthesis of qualitative studies regarding opinions and perceptions about barriers and determinants of health services' accessibility in economic migrants. BMC Health Serv Res. 2012;12:1-13. doi: 10.1186/1472-6963-12-461
19. McGarry O, Hannigan A, Manuela De Almeida M, Severoni S, Puthoopparambil SJ, MacFarlane A. What strategies to address communication barriers for refugees and migrants in health care settings have been implemented and evaluated across the WHO European Region? Copenhagen: WHO Regional Office for Europe; 2018 (Health Evidence Network (HEN) synthesis report 62)
20. Straßner C, Gewalt S, von Rose P, Lorenzen D, Szecsenyi J, Bozorgmehr K. Quality circles to identify barriers, facilitating factors, and solutions for highquality primary care for asylum seekers. BJGP Open. 2017;1(3):1-4. doi: 10.3399/bjgpopen17X101133
21. Accorinti M. Unaccompanied foreign minors in Italy: A political and social analysis. Soc Sci Learn Educ. 2016;1(9):27-41. doi: 10.15520/sslej.v1i09.15
22. Kanics J, Senovilla Hernandez D, Touzenis K. Migrating alone: Unaccompanied and separated children's migration to Europe. France: UNESCO Publishing; 2010
23. Nakeyar C, Esses V, Reid GJ. The psychosocial needs of refugee children and youth and best practices for filling these needs: A systematic review. Clin Child Psychol Psychiatry. 2017;23(2):186-208. doi: 10.1177/1359104517742188
24. Nardone P, Spinelli A, Buoncristiano M, Lauria L, Pierannunzio D, Galeone D. Il Sistema di sorveglianza OKkio alla SALUTE: risultati 2016. Roma: Istituto Superiore di Sanità; 2018.
25. Credé SH, Such E, Mason S. International migrants' use of emergency departments in Europe compared with non-migrants' use: A systematic review. Eur J Public Health. 2018;28(1):61-73. doi:10.1093/eurpub/ckx057
26. Ismail SA, Abbara A, Collin SM, Orcutt M, Coutts AP, Maziak W, Sahloul Z, Dar O, Corrah T, Fouad FM. Communicable disease surveillance and control in the context of conflict and mass displacement in Syria. Int J Infect Dis. 2016;47:15-22. doi: 10.1016/j.ijid.2016.05.011
27. Thom DH, Tirado MD, Woon TL, McBride MR. Development and evaluation of a cultural competency training curriculum. BMC Med Educ. 2006;6:1-9. doi: 10.1186/1472-6920-6-38
28. Giusti A, Perra A, Lombardo F. The experience of a nationwide Community of Practice to set up Regional Prevention Plans in Italy. Heal Res Policy Syst. 2017;15(1):1-10. doi: 10.1186/s12961-017-0226-4
29. Ivers N, Jamtvedt G, Flottorp S, Young JM, Odgaard-Jensen J, French SD, O'Brien MA, Johansen M, Grimshaw J, Oxman AD. Audit and feedback: Effects on professional practice and healthcare outcomes. Cochrane Database Syst Rev. 2012;13(6). doi: 10.1002/14651858.CD000259.pub3

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ORIGINAL ARTICLES AND REVIEWS

Early mortality from malignant mesothelioma in Italy as a proxy of environmental exposure to asbestos in children

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Abstract
Malignant mesothelioma (MM) is a rare neoplasm caused by asbestos. Mortality from MM in ≤50 years old people, considering the long latency, is likely related to asbestos exposure in childhood. Mortality from MM (C45, ICD10 code) is described among ≤50 years (ys) old people in Italy, in 2003-2016. National and regional Standardized Rates (SRs) were computed by age-class. The North-South trend of regional SRs, increasing in >50ys age-class, showed a flat cline in ≤50ys old people. Municipal Standardized Mortality Ratios (SMRs) were computed, with respect to regional figures, for ≤50 ys old population. In Italy, 487 people ≤50 ys old died from MM, in 2003-2016 (2.5% of all MM deaths), corresponding to 35/year. The highest SMRs were observed in Northern Regions, the most industrialized areas. Exceeding SMRs were found in 10 municipalities where former asbestos-cement plants, shipyards, and a quarry contaminated by fluoro-edenite fibres were present. Early mortality from MM, proxy of childhood environmental asbestos exposure, deserves particular concern.

Key words
- epidemiology
- mesothelioma
- mortality
- asbestos
- young adults

INTRODUCTION

Malignant mesothelioma (MM) is a rare neoplasm, originating from mesothelial cells of serous cavities (pleura, peritoneum, pericardium, and vaginal tunic of the testicle). Pleural MM represent about 80% of all MM cases. MM is highly lethal and characterised by a long period of latency (about 40 years and over) [1]. More than 80% of MM cases are attributable to asbestos exposure. All asbestos types are ascertained carcinogenic to human (Group 1), causing with sufficient evidence mesothelioma and lung, larynx and ovary cancers. A positive association with cancers of pharynx, stomach and colon-rectum was also reported [2]. Erionite, a naturally occurring fibrous mineral, was also confirmed to be an ascertained carcinogenic to human, causing mesothelioma [2]. In addition, in 2017 IARC defined fluoro-edenite, a previously unknown asbestos-like fibrous mineral, as carcinogenic to humans (Group 1), on the basis of the ascertained causal link with MM [3].

The number of deaths from MM is currently used to estimate the population burden of this neoplasm, in light of its high lethality. Incidence data, as a matter of fact, are not always available. Odgerel and colleagues estimated the global burden of MM in the range from 36,300 to 38,400 deaths per year, in a 20 year-period (1994-2014), considering 230 countries [4].

Asbestos is one of the most widespread occupational carcinogens: the World Health Organization (WHO) has estimated that around 125 million people worldwide are currently exposed to asbestos at workplace (www.who.int/ipcs/assessment/public_health/asbestos/

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en/). The Global Burden of Diseases (GBD) study estimated about 63% of occupational cancers attributable to asbestos in 2017 at global level, including 27,000 cases of mesothelioma [5].

Estimating the burden of MM cases due to non-occupational exposure is particularly difficult, though the risk of pleural mesothelioma caused by the residence near asbestos fibres sources (quarries, asbestos-cement plants) is known [1, 6]. Some estimates show that about 20% of MM cases at global level could be caused by non-occupational exposure to asbestos [6].

Italy was one of the main producers and importers of raw asbestos until the ban in 1992 [7]. In Italy, in 2003-2014 period, 16,086 persons died from MM, corresponding to 1,340 per year. Temporal trends show an increase of mortality from MM, in particular from pleural MM among men, in the last years [8]. The most recent report of the Italian Registry of Mesothelioma (ReNaM) identified 27,356 incident cases of mesothelioma from 1993 to 2015, corresponding to about 1,600 incident cases per year in the last period [9]. Information on the exposure context was available for 21,387 cases (78.2%): among these, 70.0% experienced occupational exposure (certain, probable, possible), 4.9% domestic and 4.4% environmental exposure, meanwhile for 1.5% of them the asbestos exposure was related to leisure or hobby activity. For 20% of cases, asbestos exposure was unlikely or unknown [9]. On the basis of exposure ways reported in ReNaM database, clusters of MM cases due to environmental exposure were mainly related to the presence of asbestos-cement plants, shipbuilding and repair activities and soil contamination [1]. Regarding the last asbestos exposure source, local investigations reported an exceeding risk of MM in some Italian areas with naturally asbestos fibres presence [10, 11]. MM cases were reported in excess in an area of Pollino Mount (Basilicata Region, Sothern Italy), where naturally serpentine and metabasite outcrops, containing asbestos fibres (namely, tremolite, actinolite and chrysotile), occurred [10]. In early 2000s, pleural and peritoneal malignancies were found in excess in some municipalities of Upper Susa Valley (Piedmont Region, Northern Italy), where tremolite asbestos in rocks surfaced by natural ground erosion or originating from construction activities [11]. MM cases environmentally exposed were reported in areas close to chrysotile and fluoro-edenite fibres containing quarries [12, 13]. An investigation focused on female MM cases found that non-occupational case-list of ReNaM is characterized by the prominence of women [14].

The improvement of early diagnosis of MM and the establishment of registries recording people exposed to asbestos are among the public health actions recommended by WHO (www.who.int/ipcs/assessment/public_health/asbestos/en/). Moreover, during the Sixth Ministerial Conference on Environment and Health, the 53 countries of WHO European Region committed to develop national programs to eliminate asbestos-related diseases, in agreement with the objectives of 2030 UN Agenda for Sustainable Development [15].

The issue of health risks originating from asbestos exposure in children is rarely addressed, mainly due to the low number of young mesothelioma cases and to the difficulties to detect a causal role of past exposure occurred during childhood. A systematic review on the age at first exposure to asbestos and the risk of asbestos-related diseases reported the studies published up to July 2012 [16]. The object of the review was highlighting the difference in MM risk, on the basis of the age at first asbestos exposure. Six studies reporting risk of MM mortality by age-class, childhood included, among occupational and environmental asbestos exposed subjects [17-22] were considered. Four studies found the highest risk in subjects aged 15 years (ys) or less at first exposure [17-20], meanwhile the other one reported a lower risk in subjects exposed at young age (less than 20 ys) than in adulthood [21]. The subjects exposed at birth showed the highest risk in the only study that included birth as age at first exposure [22]. Regarding the highest risk age at first asbestos exposure, the results of all studies, also restricting to only residential exposure investigations, were not consistent. The Authors concluded that because of the low number of the studies and their limitations the results are inconclusive and further studies are needed [16].

More recently, some cohort studies analysed the risk of MM among populations residentially exposed to asbestos in childhood. Dalsgaard and colleagues performed a cohort study on the incidence of MM in former children attending four schools located near an asbestos-cement plant in Aalborg [23]. The median age at diagnosis (similar in school and reference cohort) was 61 years (34-74 ys). MM was diagnosed more than 30 years after the school attendance in the majority of cases. Higher risk (Hazard Ratio: HR) in school cohort, adjusted for occupational and familial occupational exposure, was found and the results were confirmed also restricting the analysis to the subjects without occupational or familial occupational exposure. Similar HRs were shown in men and women, with M/F ratio in the school cohort equal to 1.2:1, as assumed for environmental exposure. The results suggested that childhood environmental exposure is an important risk factor for MM in late life [23]. Increased risk (OR = 3.3; 95% CI: 1.4-7.7) in subjects attending grammar school in Casale Monferrato, where the largest Italian asbestos-cement plant operated, was also reported in a case-control study on pleural MM incidence [24].

The risk of asbestos-related diseases in the population living near the Wittenoom crocidolite mine in Australia was investigated in several studies [21, 25, 26]. In the cohort analysis after 30 years and more of follow-up, increased incidence and mortality risk from several cancers, including mesothelioma, were found among adults living at Wittenoom in childhood (aged <15 ys) [25]. However, in a subsequent analysis of MM incidence, difference in risk between the subjects exposed in childhood (<15 ys) and adulthood was not observed [26], confirming the previous findings [21].

In 2017 a study on the association between residential exposure to Libby amphibole asbestos (LAA) prior to age 18 and respiratory symptoms in late life (median age: 25 ys) was published [27]. Pleural or interstitial changes

on chest x-ray or HRCT were not found, but several respiratory symptoms, including self-reported pleural chest pain, among young adults were associated to childhood environmental exposure to LAA and were highlighted as a possible indicator of future respiratory diseases [27].

The Surveillance, Epidemiology and End Results (SEER) database reported a lower M/F rates ratio for MM cases aged under 65, with respect to those aged 65 and over [28].

Few studies on MM risk have been performed in Latin-American countries. A recent mesothelioma case-series in the municipality of Sibaté, where a major Colombian asbestos-cement facility is located was published. Some of the characteristics of the observed MM cases are represented by the early age at diagnosis, the sex-ratio approaching one and the absence of occupational exposure to asbestos, thus suggesting a major role of environmental exposure [29].

In Italy, the mortality from pleural mesothelioma by age-class was reported for 2003-2009 period [30]. In 0-39 age-class, 8 male and 7 female deaths were observed, corresponding to 0.01 (95% CI: 0.005-0.02)/100,000 in men and 0.01 (95% CI: 0.004-0.02)/100,000 in women. In the same period the corresponding rate in the overall population was 2.8 (95% CI: 2.7-2.9) and 0.8 (95% CI: 0.8- 0.9) /100,000, in men and women, respectively. The M/F ratio in 0-39 year age-class was equal to 1, meanwhile in the older age-classes male death rate was about 3-fold that of females rate: M/F = 3.4 in subjects aged 40-75 and M/F = 3.3 in 76-99 age-class [30].

The incidence of MM cases in Italy, in different age-classes by modality of exposure, on the basis of ReNaM database, was reported in Marinaccio, *et al.* 2015 [1]. MM cases aged less than 45 ys at diagnosis were rare, accounting for 2.4% of all cases recorded in 1993-2008 period (15,845 MM cases). Significantly, lower mean age at diagnosis was observed in non-occupationally exposed, in particular in those with an environmental exposure, compared to the cases exposed in occupational settings (67.2 and 66.1, respectively, *vs* 68.1). The mean age at first exposure was significantly lower in subjects environmentally exposed than in those occupationally exposed (17 *vs* 22.5 years, p value <0.001) [1].

Considering the long period of latency, the high mortality rate and the high attributable fraction to asbestos exposure, early deaths from MM could represent a *proxy* of exposure in childhood. Studying the early occurrence of mesothelioma has relevant public health and ethical implications in terms of health protection by unintentional exposure to environmental hazards in children, also considering the hypothesis of a highest vulnerability to environmental risk of this age-class population [31, 32]. Temporal and spatial distributions of early MM deaths could contribute in estimating the health impact of non-occupational exposure to asbestos in childhood. This is the case, for example, of the children living in areas contaminated by asbestos fibres or indirectly exposed to asbestos in domestic context, because of occupational activities of the parents.

In Italy, a preliminary analysis of early malignant pleural neoplasms (MNP) mortality, showed 1,594 early deaths (≤50 ys) from MNP in 1980-2010 period (55 per year on average, annual standardized rate = 0.2/100,000) and identified 147 municipalities where early mortality from MNP was significantly higher than the expected (mainly located in Regions mostly affected by activities involving asbestos exposure) [33].

The present study describes mortality from MM in Italy in people younger than 50 years (≤50 ys) and its geographical distribution, as a possible marker for environmental exposure to asbestos in children.

MATERIALS AND METHODS

This study is based on data of mortality from malignant mesothelioma (MM) at municipal level that are included in the cause-specific mortality database managed by the Statistical Service of the National Institute for Health, and provided by the Italian National Institute of Statistic (Istat).

Mortality from MM was analysed in a 14 year-period (2003-2016), the most recent years available at the beginning of the study, from ICD10 revision application. The MM deaths included in the study were all those recorded in the specific diagnostic category of malignant mesothelioma C45 (ICD-10).

National and regional standardized mortality rates (SR, direct method, 2013 European population as reference: https://ec.europa.eu/eurostat/web/products-manuals-and-guidelines/-/KS-RA-13-028) and their 90% Confidence Intervals (90% CI), in the population ≤50 and >50 ys old, were computed by gender.

Standardized Mortality Ratios (with 90% CI) in the subpopulation ≤50 ys old were computed for each of the 21 Italian Regions and Autonomous Provinces and for the 8,047 Italian municipalities, using national and regional age-class and gender specific rates as references, respectively. 90% CIs were estimated based on Poisson's distribution, if the observed cases were less than 100, otherwise on Byar method. Considering the low number of cases, SMRs were computed for the overall population, including both men and women, to reach a less broad CI.

RESULTS

In Italy, during 2003-2016 period, 487 persons ≤50 ys old died from MM (34.8 cases/year, on average), corresponding to 0.096 cases/100,000 inhabitants. These cases represent 2.5% of all deaths from MM in the same period (19,315 cases). *Table 1* shows the number of all MM cases and the corresponding standardized rates (SR), by age-class (≤50 and >50 ys) and gender.

The number of deaths from MM, by site, age-class and gender is reported in *Table 2*. The percentage of peritoneal MM, with respect to all MM deaths, is higher in ≤50 ys old people than in >50 ys old, among both sex (12.5% *vs* 3.8% in men and 16.7% *vs* 5.7% in women). Among young adults, the percentage of pleural MM, with respect to all MM, in men is higher than in women (73.5% *vs* 66.7%); in the latters, the percentage of peritoneal and other MM, equal to 16.7%, is higher than in male population.

The ratio of male to female standardized rates (SRm/SRf) is equal to 1.8 in the young sub-population (≤50

Table 1
Mortality from malignant mesothelioma: cases and age-standardized death rate, by age-class and gender. Reference: 2013 European population. Period: 2003-2016

Men		
Age (years)	**Cases**	**ASR (90% CI)**
≤50	313	0.16 (0.14-0.18)
>50	13,511	9.34 (9.21-9.18)
Women		
≤50	174	0.088 (0.074-0.105)
>50	5,317	2.73 (2.67-2.80)
Overall		
≤50	487	0.12 (0.11-0.14)
>50	18,828	5.57 (5.50-5.63)

ASR: Age Standardized death Rate (n. deaths/100,000 inhabitants); 90% CI: Confidence Interval.

Table 2
Mortality from malignant mesothelioma (MM), by site, sex and age-class: number of cases and percentage with respect to all MM deaths. Period: 2003-2016

Cause of death	ICD	Men cases (%)	Women cases (%)	Overall cases (%)
Age ≤50				
Mesothelioma of pleura	C45.0	230 (73.5)	116 (66.7)	346 (71)
Mesothelioma of peritoneum	C45.1	39 (12.5)	29 (16.7)	68 (14)
Other mesothelioma	C45.2-C45.9	44 (14.1)	29 (16.7)	73 (15)
Mesothelioma (all)	C45	313 (100)	174 (100)	487 (100)
Age >50				
Mesothelioma of pleura	C45.0	11080 (82)	4246 (79.9)	15326 (81.4)
Mesothelioma of peritoneum	C45.1	514 (3.8)	303 (5.7)	817 (4.3)
Other mesothelioma	C45.2-C45.9	1917 (14.2)	768 (14.4)	2685 (14.3)
Mesothelioma (all)	C45	13511 (100)	5317 (100)	18828 (100)

ys) and 3.4 in older population (>50 ys), in overall period 2003-2016. *Figure 1* shows the national M/F ratio by year.

The geographical trend of regional MM mortality standardized rates, by Istat macroarea (North-West, North-East, Centre, South and Islands) and age-class is shown in *Figure 2*. In ≤50 ys old population the cline is flat, meanwhile a decreasing North-Southern trend is observed in overall and >50 ys old people *(Figure 2)*.

Regional SMRs from MM in young population (≤50 old) are showed in *Table 3* and *Figure 3* reports the geographical distribution.

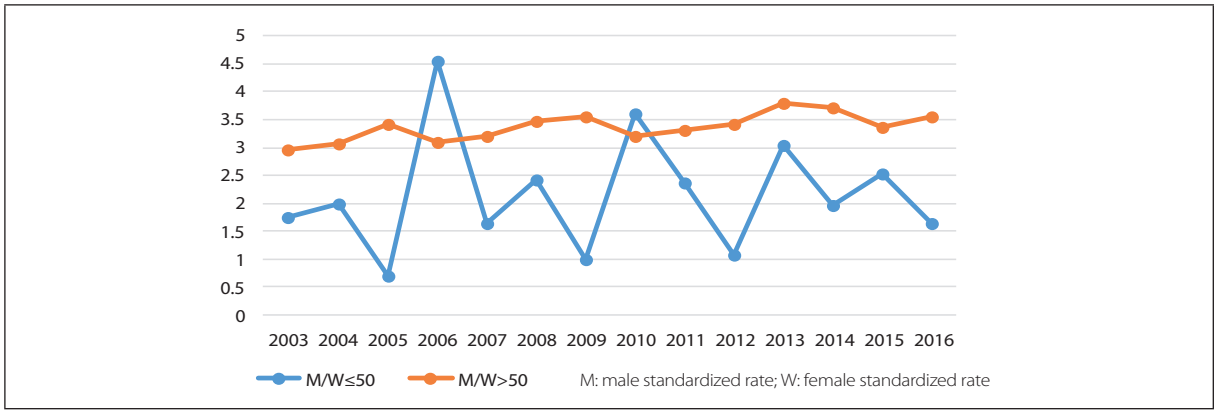

Figure 1
Mortality from malignant mesothelioma. Annual trend of sex ratio (male standardized rate/female standardized rate), by age-class. Period: 2003-2016.

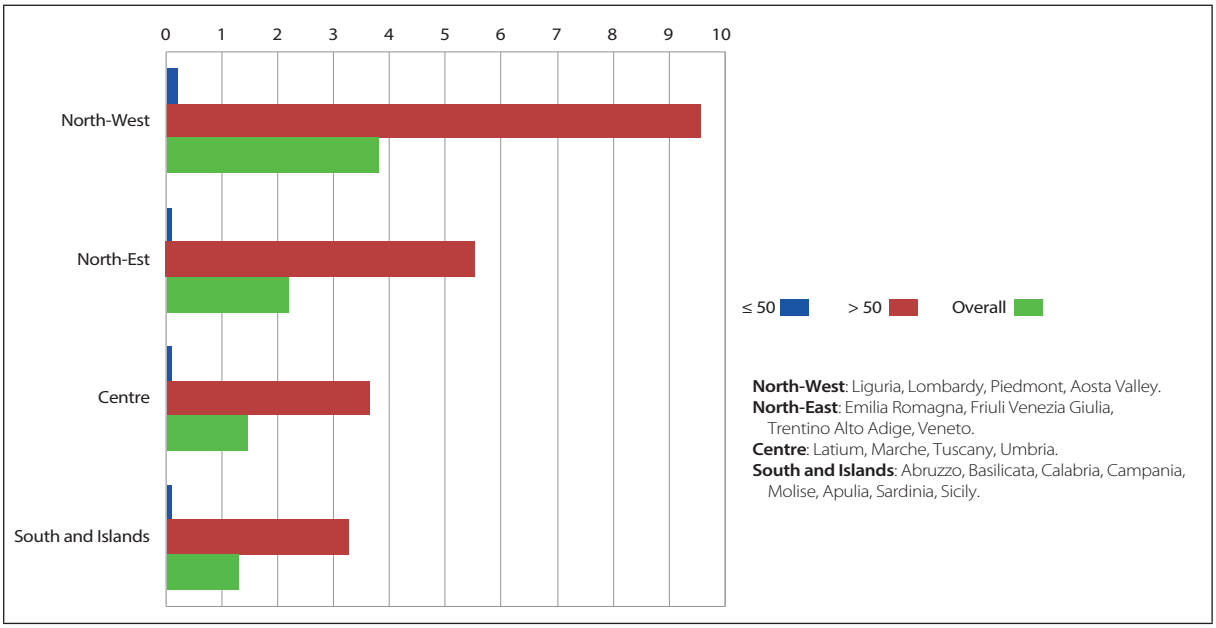

Figure 2
Mortality from malignant mesothelioma. Regional standardized rates (/100,000), by Istat macroarea (North-West, North-East, Centre, South and Islands) and age-class. 90% Confidence Intervals. Period 2003-2016.

Table 3
Mortality from malignant mesothelioma, in ≤50 years old population. Standardized Mortality Ratio by Region. Reference: National Rate. Period: 2003-2016

Region	OBS	SMR (90% CI)
Piedmont	73	204.0 (168.4-247.3)
Aosta Valley	1	93.2 (20.8-417.7)
Lombardy	115	141.1 (121.1-164.5)
Bolzano	5	120.3 (58.6-247.1)
Trento	1	23.1 (5.16-103.8)
Veneto	25	60.9 (43.9-84.4)
Friuli-Venezia Giulia	5	49.7 (24.2-102)
Liguria	18	141 (95.9-207.2)
Emilia-Romagna	35	97.7 (74.1-129)
Tuscany	28	93.7 (68.8-127.7)
Umbria	5	71.8 (34.9-147.5)
Marche	10	81.3 (48.6-136)
Latium	34	72.6 (54.8-96.2)
Abruzzo	4	37.9 (17.0-84.5)
Molise	2	79.7 (26.4-241)
Campania	46	99.7 (78.3-126.6)
Apulia	26	81.1 (58.8-111.9)
Basilicata	2	42.9 (14.2-129.6)
Calabria	12	77.5 (48.4-124)
Sicily	35	89.4 (67.8-118)
Sardinia	5	35.8 (17.4-73.5)

OBS: Observed cases; SMR: Standardized Mortality Ration; CI: Confidence Interval.

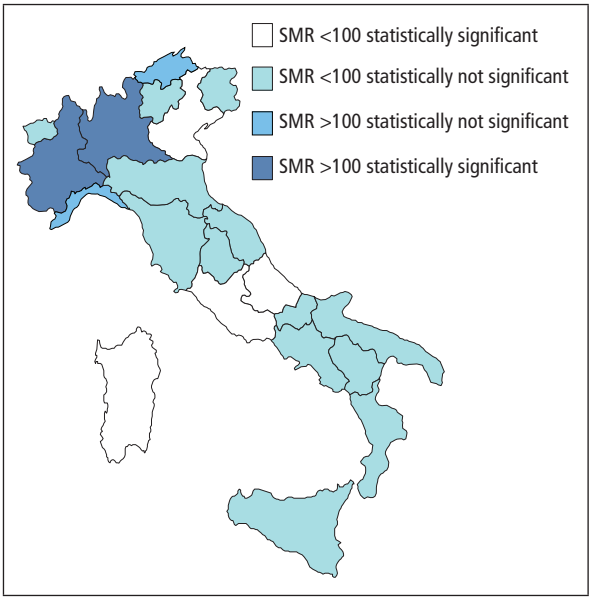

Figure 3
Mortality from malignant mesothelioma, in ≤50 years old population. Geographical distribution of Standardized Mortality Ratio (SMR), by Region. Period: 2003-2016.

The early mortality (≤50 ys) from MM at regional level as compared to national rates, show higher risks in some Northern Italian Regions: Piedmont and Lombardy display SMRs significantly higher than 100, Liguria and Bolzano Province have rates higher than the National one, even if the CI lower limit is <100.

In the analysis at municipal level, 357 out of the 8,078 Italian municipalities showed at least one MM death ≤50 years old, in the study period. In ten municipalities

Municipality	OBS	SMR (90% CI)
La Spezia	3	396.4 (158.4-991.8)
Stradella	3	3132.2 (1251.8-7837.4)
Quattro Castella	3	2728 (1090.2-6825.8)
Torre Annunziata	3	927.01 (370.5-2319.6)
Alessandria	4	538.2 (241.6-1198.8)
Biancavilla	4	2324.2 (1043.5-5177.1)
Monza	4	393.7 (176.8-877)
Broni	6	8095.0 (4186.0-15654.2)
Genova	11	229.9 (140.7-375.7)
Casale Monferrato	17	5805.9 (3906.1-8629.7)

OBS: Observed Cases; SMR: Standardized Mortality Ratio; CI: Confidence Interval.

Figure 4
Early mortality from malignant mesothelioma (≤50 years old). Municipalities with SMR and 90% Confidence Interval Lower Limit >100, based on at least 3 observed cases. Period: 2003-2016.

a statistically significant excess risk (based on at least 3 cases) was observed *(Figure 4)*.

DISCUSSION

In Italy, 2.5% of all deaths from MM occurred in ≤50 years old people (487 cases in 14 years, 2003-2016), corresponding to around 35 deaths per year, on average. Considering the long period of latency of the disease and the high attributable fraction to asbestos exposure, these deaths could be probably due to asbestos exposure occurred in childhood.

Several previous investigations, reporting excesses of early mortality from MM in people exposed to asbestos in childhood [23-26], corroborate this hypothesis. For this reason, localized excesses of MM mortality in ≤50 ys old people, could also be used as a sentinel event of past, or current, contexts of environmental exposure to asbestos.

Considering the causal link between MM and fibres [2, 3], we highlight that no information on the presence of erionite outcrops either erionite exposure contexts for the population in Italy has been reported. Contexts of exposure to fluoro-edenitic fibres were detected in a specific area that will be considered in the comments of the present findings.

The lower MM mortality ratio of male to female in young age-class, compared to that in >50 ys old population (1.8 *vs* 3.4), corroborates the hypothesis of a non-occupational asbestos exposure in early MM cases. This hypothesis is confirmed by data from ReNaM, that showed a significantly higher F/M gender ratio in environmentally exposed cases than in overall and occupationally exposed cases (1.19 *vs* 0.38 and 0.14, respectively) [14]. MM Female/Male rate ratio about 1:1 in under 65 people was reported also in US population [28] and among MM cases residentially exposed to asbestos in childhood, in Aalborg [23].

In addition, the percentage of incident cases reported in ReNaM database with a history of occupational exposure is lower among subjects ≤50 years than in >50ys old (37% *vs* 56%); the proportion of MM incident cases with environmental/familial exposure is around two-fold significantly higher in the young (≤50 ys) with respect to older cases (16% *vs* 8%) [9]. Furthermore, ReNaM findings provide evidence of a percentage of unknown or unlikely modalities of exposure higher in young MM patients (≤50 ys old at diagnosis) than in older cases (24.6% and 15.6%, respectively) [9].

The analysis of mortality by MM site, age-class and gender *(Table 2)* highlighted a higher percentage of peritoneal MM in young adult (≤50 old) than in >50 old people, among both gender (12.5% *vs* 3.8% in men and 16.7% *vs* 5.7% in women). In addition, our evaluation showed that in both age-classes the percentage of peritoneal MM, with respect to all MM deaths, is higher in women than in men. In a previous investigation on peritoneal mesothelioma risk in Italy, based on multiple-causes mortality and ReNaM incidence database, the 0-44 years age group had a higher proportion of incident peritoneal MM cases (6.2%) than of pleural MM (2.4%). In the same age group, 34.7% of deaths and 44.4% of incident cases for peritoneal MM occurred among women [39]. The issue of misclassification of ovarian cancers in peritoneal mesothelioma, as

well as of other abdomen contiguous tumours, has been discussed in recent papers [39-42].

The analysis of regional SMR distribution showed the highest mortality risk from MM among young adults in some Northern Regions. The same geographical pattern was observed in the general population mortality from MM, following the industrial geographical distribution in the country, with the highest industrialization rate in Northern Italy [8, 30, 38]. In particular, the former Italian largest asbestos-cement plants were located in Broni and Casale Monferrato, Lombardy and Piedmont Region (North-West) respectively. In Liguria Region (North-West Italy), several shipyards and naval industries are still operating. The high SMR found in Bolzano Province has never showed before, and deserves further in-depth analysis.

The presence of these activities could explain the geographical North-South trend observed in overall and >50 ys old people, with the highest SRs in North-West macroarea. The flat cline observed in young adults (≤50 ys old) corroborates the hypothesis of a less contribute of occupational exposures in early MM mortality.

At municipal level, significant exceeding SMRs (based on 3 or more observed cases) in young adults (≤50 ys old) were observed in municipalities with known asbestos sources: asbestos-cement plants (Broni and Stradella, Casale Monferrato), shipbuilding and repair activities (Torre Annunziata, La Spezia and Genova) and a quarry contaminated by fluoro-edenite fibres (Biancavilla), characterized by a high mortality from MM in the general population also [8]. The presence of the same asbestos sources was related to clusters of non-occupationally exposed MM incident cases [1].

The case of Biancavilla municipality (Sicily Region, South Italy) represents an example of appropriateness of using early mortality as a marker of residential asbestos exposure in children. A high risk of mesothelioma in the population living in this municipality related to environmental exposure to fluoro-edenitic fibres contained in the stone quarry located in the municipality has been highlighted, since the late Nineties [34]. The epidemiological investigations performed in Biancavilla, corroborated by animal studies, contributed to the evaluation by IARC of the carcinogenicity of fluoro-edenite [3]. In 1980-2010 period, 6 deaths from MNP *vs* 0.6 expected were observed in this municipality (SMR = 1,003, CI 90%: 437-1,980) among people aged 50 ys or less. Extending the analysis to municipalities located wit:hin a radius of 10 km from Biancavilla, the number of MNP deaths rose to 11 (SMR = 367, CI 90%: 206-608) [35]. Same results were found by the analysis of MM incident cases living at the diagnosis in Biancavilla, based on Regional Operating Centre of ReNaM database. A higher value of Standardized Incidence Ratio (SIR), with respect to regional figures, was shown in people aged less than 50 years (5 cases, SIR = 2,134, 95% CI: 693-5,000) than among older people (19 cases, SIR = 474, 95% CI: 285-739), even if based on a low number of cases [13].

Regarding Alessandria, Monza and Quattro Castella, the other municipalities reported in *Figure 4*, no specific etiological hypotheses have so far been raised. An excess of mortality from pleural mesothelioma, in the male population, was previously observed in Quattro Castella [36]. In these contexts, *ad hoc* in-depth studies appear to be warranted.

In spite of the uncertainty of the computed estimates, due to the low number of observed and expected cases, these results deserve specific concerns, in view of the rarity of the phenomenon and the ethical implications, considering the possibility of a childhood exposure to environmental risks.

Some limitations of the present study need to be discussed.

A limitation is related to the use of mortality data in the detection of mesothelioma cases. A possible underestimate of MM cases in asbestos occupational cohort studies using mortality data, with respect to incidence data, was debated [37]. Misclassification caused by the use of death certificates was discussed also in previous papers on the surveillance of mesothelioma mortality in Italy [30, 38]. The use of the 10th revision ICD code, available in Italy at national level since 2003, including the specific morphological code of malignant mesothelioma reduces the possible misclassification. In addition, the high mortality rate of the disease mitigates the possible bias, but a remaining effect could not be ruled out, and some prudence in the interpretation of the data is appropriated.

Moreover, a limitation could be represented by the use of the residence at death as a *proxy* of the childhood residence. The geographical analysis, performed in order to identify the areas with possible asbestos sources, was carried out on the basis of the residence transcribed in death certificate (as Istat database) while, taking into account the long period of the latency, the place of residence during childhood, where exposure to asbestos probably occurred, might have been different.

In addition, regional and municipal SMRs have been computed for men and women combined to improve the precision of the estimates, considering the ratio F/M close to one in early MM mortality. Studies analysing the distribution of early MM deaths by site and gender, with suitable methods, appear appropriated.

Further investigations, based on the integration of MM mortality and incidence data, the latter from ReNaM database, could reduce parts of these limitations and furnish a useful focus on this issue. The analysis of early MM occurrence, by site of MM, sex and exposure modality, based on individual database, appears of particular concern, taking into account also the specific F/M ratio among young adults.

CONCLUSION

The analysis of early mortality from MM showed that in Italy, in 2003-2016 period, 487 people ≤50 ys old died from MM, corresponding to 2.5% of all MM deaths, due to a likely non-occupational asbestos exposure in childhood.

Geographical distribution highlighted regions and, particularly, municipalities with the highest risks of MM mortality for this specific population age group.

These signals, though characterised by uncertainty, require to implement specific public health and environmental remediation actions, and further in-depth in-

vestigations, in the light of their ability to identify past, or still on-going, environmental sources of exposure that could impact on childhood population.

The findings of the present study provide evidence of the usefulness to use early MM mortality data as a *proxy* of asbestos exposure in people affected in young age, particularly where individual assessment of exposure is not available. This possibility might be common in low- and middle-income countries where environmental exposure to asbestos in children is a critical issue [43] and where suitable experiences of surveillance systems of mesothelioma incident cases, including the individual evaluation of the modalities of exposure, still lack.

The use of the adopted methods, based on mortality data, to replicate the study in other countries could give an important information on the environmental exposure to asbestos, at global level.

Author's contributions statement

Conception: II, LF, PC, SC, GM, CB, AZ; Acquisition and analyses of data: GM, MDS; Interpretation of data: AB, AM, AZ, CB, CM, II, LF, PC, SC; Writing-Original draft: LF, II; Writing: Revising and final approval: LF, AB, AM, AZ, CB, CM, GM, MDS, PC, SC, II.

Conflict of interest statement

The Authors declare that neither they nor their Institutions have financial or personal relationship with other people or organizations that could inappropriately bias conduct and findings of the study.

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REFERENCES

1. Marinaccio A, Binazzi A, Bonafede M, Corfiati M, Di Marzio D, Scarselli A, et al. and ReNaM Working Group. Malignant mesothelioma due to non-occupational asbestos exposure from Italian national surveillance system (ReNaM): epidemiology and public health issues. Occup Environ Med. 2015;72:648-55. doi: 10.1136/oemed-2014-102297
2. International Agency for Research on Cancer. Arsenic, metals, fibres and dusts. IARC monographs on the evaluation of carcinogenic risks to humans; Vol. 100C. Lyon: IARC; 2012.
3. International Agency for Research on Cancer. Fluoro-edenite. In: IARC. Some nanomaterials and some fibres. IARC monographs on the evaluation of carcinogenic risks to human; Vol. 111. Lyon: IARC; 2017. p. 215-42.
4. Odgerel CO, Takahashi K, Sorahan T, Driscoll T, Fitzmaurice C, Yoko-o M, Sawanyawisuth K, Furuya S, Tanaka F, Horie S, van Zandwijk N, Takala J. Estimation of the global burden of mesothelioma deaths from incomplete national mortality data. Occup Environ Med. 2017;74:851-8. doi: 10.1136/oemed-2017-104298
5. GBD 2017 Risk Factor Collaborators. Global, regional and national comparative risk assessment of 84 behavioural, environmental, and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Diseases Study 2017. Lancet. 2018;392:1923-94.
6. Goldberg M, Luce D. The health impact of non-occupational exposure to asbestos: what do we do? Eur J Cancer Prev. 2009;18:489-503. doi: 10.1097/CEJ.0b013e32832f9bee
7. Italia. Legge n. 257, 27 Marzo 1992. Norme relative alla cessazione dell'impiego dell'amianto, Gazzetta Ufficiale - Serie Generale n.87, Suppl. Ordinario n. 64, 13 aprile 1992.
8. Fazzo L, Minelli G, De Santis M, Bruno C, Zona A, Conti S, Comba P. Epidemiological surveillance of mesothelioma mortality in Italy. Cancer Epidemiol. 2018;55:184-91. doi: 10.1016/j.canep.2018.06.010
9. Marinaccio A, Binazzi A, Bonafede M, et al. (Eds.). Il Registro Nazionale dei Mesoteliomi (ReNaM). Sesto Rapporto. Monografia INAIL. INAIL; 2018. Available from: www.inail.it/cs/internet/docs/alg-pubbl-registro-nazionale-mesoteliomi-6-rapporto.pdf [in Italian].
10. Caputo A, De Santis M, Manno V, Cauzillo G, Bruno BM, Palumbo L, Conti S, Comba P. Health impact of asbestos fibres naturally occurring in Mount Pollino area (Basilicata Region, Southern Italy). Epidemiol Prev. 2018;42:142-50. doi: 10.19191/E18.2.P142.04311
11. Mirabelli D, Cadum E. Mortality among patients with pleural and peritoneal tumors in Alta Valle di Susa. Epidemiol Prev. 2002;26:284-6.
12. Mirabelli D, Calisti R, Barone-Adesi F, Fornero E, Merletti F, Magnani C. Excess of mesotheliomas after exposure to chrysotile in Balangero, Italy. Occup Environ Med. 2008;65:815-9. doi: 10.1136/oem.2007.037689
13. Bruno C, Tumino R, Fazzo L, Cascone G, Cernigliaro A, De Santis M, Giurdanella MC, et al. Incidence of pleural mesothelioma in a community exposed to fibres with fluoro-edenitic composition in Biancavilla (Sicily, Italy). Ann Ist Super Sanità. 2014;50:111-8. doi: 10.4415/ANN_14_02_02
14. Marinaccio A, Corfiati M, Binazzi A, Di Marzio D, Scarselli A, Ferrante P, Bonafede M. et al.; ReNaM Working Group. The epidemiology of malignant mesothelioma in women: gender differences and modalities of asbestos exposure. Occup Environ Med. 2018;75:254-62. doi:10.1136/oemed-2016-104119
15. World Health Organization. Declaration of the sixth ministerial conference on environment and health. WHO; 2017. EURO/Ostrava2017/6. Available from: www.euro.who.int/data/assets/pdf_file/0007/341944/OstravaDeclaration_SIGNED.pdf.
16. Kang D, Myung MS, Kim YK, Kim JE. Systematic review of the effects of asbestos exposure on the risk of cancer between children and adults. Ann Occup Environ Med. 2013;25:10. doi:10.1186/2052-4374-25-10
17. Chen M, Tse LA, Au RK, Yu IT, Wang XR, Lao XQ. Mesothelioma and lung cancer mortality: a historical cohort study among asbestosis workers in Hong Kong. Lung Cancer 2012;76:165-70. doi: 10.1016/j.lungcan.2011.11.003
18. Lacourt A, Leffondré K, Gramond C, Ducamp S, Rolland P, GilgSoittllg A, Houot M, Imbernon E, Févotte J, Goldberg M, Brochiard P. Temporal patterns of occupational asbestos exposure and risk of pleural mesothelioma. Eur Respir J. 2012;39:1304-12. doi: 10.1183/09031936.00005111

19. Rake C, Gilham C, Hatch J, Damton A, Hodgson J. Peto J. Occupational, domestic and environmental mesothelioma risks in British population: a case-control study. Br J Cancer. 2009;100:1175-83. doi: 10.1038/sj.bjc.6604879
20. Pira E, Pelucchi C, Piolatto PG, Negri E, Discalzi G, La Vecchia C. First and subsequent asbestos exposures in relation to mesothelioma and lung cancer mortality. Br J Cancer. 2007;97:1300-4. doi: 10.1038/sj.bjc.6603998
21. Reid A, Berry G, de Klerk N, Hansen J, Heyworth J, Ambrosini G. Age and sex differences in malignant mesothelioma after residential exposure to blue asbestos (crocidolite). Chest. 2007;131:376-82. doi: 10.1378/chest.06-1690
22. Luce D, Bugel I, Goldberg P, Goldberg M, Salomon C, Billon-Galland MA. Environmental exposure to tremolite and respiratory cancer in new Caledonia: a case-control study. Am J Epidemiol. 2000;151:259-65. doi: 10.1093/oxfordjournals.aje.a010201
23. Dalsgaard SB, Würtz ET, Hansen J, Røe OD, Omland Ø. Environmental asbestos exposure in childhood and risk of mesothelioma later in life: a long term follow-up register-based cohort study. Occup Environ Med. 2019;76:407-13. doi:10.1136/oemed-2018-105392
24. Magnani C, Dalmasso P, Biggeri A, Ivaldi C, Mirabelli D, Terracini B. Increased risk of malignant mesothelioma of the pleura after residential or domestic exposure to asbestos: a case-control study in Casale Monferrato, Italy. Environ Health Perspect. 2001;109:915-9. doi: 10.1289/ehp.01109915
25. Reid A, Franklin P, Olsen N, Sleith J, Samuel L, Aboagye-Sarfo P, de Klerk N, Musk AW. All-cause mortality and cancer incidence among adults exposed to blue asbestos during childhood. Am J Ind Med. 2013;56:133-45. doi: 10.1002/ajim.22103
26. Reid A, Franklin P, Berry G, Peters S, Sodhi-Berry N, Brims F, Musk AW, de Klerk NH. Are children more vulnerable to mesothelioma than adults? A comparison of mesothelioma risk among children and adults exposed non-occupationally to blue asbestos at Wittenoom. Occup Environ Med. 2018;75:898-903. doi:10.1136/oemed-2018-105108
27. Ryan PH, Rice CH, Lockey JE, Black B, Burkle J, Hilbert TJ, Levin L, Brokamp C, McKay R, Wolfe C, LeMasters GK. Childhood exposure to Libby amphibole asbestos and respiratory health in young adults. Environ Research. 2017;158:470-9. doi: 10.1016/j.envres.2017.07.013
28. Howlader N, Noone A.M, Krapcho M, Miller D, Brest A, Yu M, Ruhl, J, Tatalovich, Z, Mariotto A, Lewis DR, et al. SEER Cancer Statistics Review, 1975-2014. Bethesda, MD: National Cancer Institute; 2017. Available from: https://seer.cancer.gov/csr/1975_2014/
29. Ramos-Bonilla JP, Cely-Garcia MF, Giraldo M, et al. An asbestos contaminated town in the vicinity of an asbestos-cement facility: the case study of Sibaté, Colombia. Environ Res. 2019;176:108464. doi: 10.1016/j.envres.2019.04.031
30. Fazzo L, Minelli G, De Santis M, Bruno C, Zona A, Marinaccio A, Conti S, Pirastu R, Comba P. Mesothelioma mortality surveillance and asbestos exposure tracking in Italy. Ann Ist Super Sanità. 2012;48:300-10. doi: 10.4415/ANN_12_03_11
31. Anderson LM, Diwan BA, Fear NT, Roman E. Critical windows of exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental animal models. Environ Health Persp. 2000;108:573-94.
32. Christopher PW, Jos K. Children and increased susceptibility to environmental carcinogens: evidence or empathy? Cancer Epidemiol Biomarkers Prev. 2003;12:1389-94.
33. Iavarone I, Conti S, De Santis, M, Pirastu R, Comba P. Early Mortality from malignant neoplasms of pleura, a proxy for environmental exposure to asbestos in childhood. Annual ISEE conference, 24-28 August 2014, Seattle, Washington. ISEE Congress, 2014. Abstract n. 2368. Available from: https://ehp.niehs.nih.gov/doi/10.1289/isee.2014.P3-716.
34. Bruno C, Bruni BM, Comba P (Eds.). Health impact of fibres with fluoro-edenitic composition: the case of Biancavilla (Italy). Ann Ist Super Sanità. 2014;50:108-38.
35. Conti S, Minelli G, Manno V, Iavarone I, Comba P, Scondotto S, Cernigliaro A. Health impact of exposure to fibres with fluoro-edenitic composition on the residents in Biancavilla (Sicily, Italy): mortality and hospitalization from current data. Ann Ist Super Sanità. 2014;50:127-32. doi: 10.4415/ANN_14_02_04
36. Comba P, Fazzo L (Ed.). Pleural mesothelioma mortality in Italy, 2003-2014. Roma: Istituto Superiore di Sanità; 2017. (Rapporti ISTISAN 17/37). [in Italian]
37. Ferrante D, Chellini E, Merler E, Pavone V, Silvestri S, Miligi L, et al. Italian pool of asbestos workers cohorts: mortality trends of asbestos-related neoplasms after long time since first exposure. Occup Environ Med. 2017;74:887-98. doi: 10.1136/oemed-2016-104100
38. Fazzo L, De Santis M, Minelli G, Bruno C, Zona A, Marinaccio A, Conti S, Comba P. Pleural mesothelioma mortality and asbestos exposure mapping in Italy. Am J Ind Med. 2012;55:11-24. doi: 10.1002/ajim.21015
39. Conti S, Minelli G, Ascoli V, Marinaccio A, Bonafede M, Manno V, Crialesi R, Straif K. Peritoneal mesothelioma in Italy: trends and geography of mortality and incidence. Am J Ind Med. 2015;58:1050-8. doi: 10.1002/ajim.22491
40. Zona A, Fazzo L, Minelli G, De Santis M, Bruno C, Conti S, Comba P. Peritoneal mesothelioma mortality in Italy: spatial analysis and search for asbestos exposure sources. Cancer Epidemiol. 2019;60:162-7. doi: 10.1016/j.canep.2019.04.001
41. Krasuki P, Pontecka A, Gai E. The diagnostic challenge of peritoneal mesothelioma. Arch Gynecol Obstet. 2002;266:130-2. doi: 10.1007/s004040100189
42. Clement PB. Selected miscellaneous ovarian lesions: small cell carcinomas, mesothelial lesions, mesenchymal and mixed neoplasms, and non-neoplastic lesions. Mod Pathol. 2005;18(Suppl. 2):S113-29. doi: 10.1038/modpathol.3800313
43. Laborde A, Tomasina F, Bianchi F, Bruné MN, Buka I, Comba P, et al. Children's health in Latin America. The influence of environmental exposures. Environ Health Perspect. 2015;123:201-9. doi: 10.1289/ehp.1408292

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A few ethical issues in translational research for medicinal products discovery and development

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Abstract

The results obtained with basic research showing significant therapeutic promise are often not translated into clinical applications. The purpose of translational research is to favour the transition of basic research to application at the patient's bedside, and from here to routine clinical practice (without excluding the opposite pathway, in which the evidence generated by clinical practice helps to guide research). Although translational research can provide patients with valuable therapeutic resources, it is not risk-free. The most significant ethical issues in translational research on medicinal products derive from the risk of the intention to shorten the timeframes for the application of the results of the research making the scientific methods adopted and the regulatory requisites to be satisfied along the long path from the bench to the patient's bedside less rigorous. This is also relevant during pandemics when shortening the timeline from basic research to bedside is even more crucial. It is therefore necessary to establish defined and agreed requisites in order to guarantee the ethicality of translational research, by promoting the good of individuals and minimising the risks.

Key words

- ethics
- human experimentation
- risk
- translational research

WHAT IS TRANSLATIONAL RESEARCH?

Translational research has been defined in many different ways. According to the American Physiological Society, translational research promotes "the transfer of knowledge gained from basic research to new and improved methods of preventing, diagnosing, or treating disease, as well as the transfer of clinical insights into hypotheses that can be tested and validated in the basic research laboratory" [1]. Indeed, many scientific discoveries never go beyond the laboratory bench and are failing to be translated efficiently into tangible human benefit. Translational research aims to overcome this situation.

The objectives of translational research are: i) to translate scientific knowledge from laboratory and preclinical research to clinical research on human subjects; and ii) to transfer the knowledge generated in biomedical research into clinical practice [2].

The "translational research" debate has ranged for a long time and has been particularly lively since the 1970s [3]. A number of definitions of "translational research" have been put forward [4]. Generally speaking, the most frequently used expression and that summarises the various different definitions is "from bench to bedside". Indeed, "a translational researcher is someone who takes something from basic research to a patient and measures an endpoint in a patient" [5].

The European Society for Translational Medicine (EUSTM) defines translational medicine as an "interdisciplinary branch of the biomedical field supported by three main pillars: benchside, bedside and community. The goal is to combine disciplines, resources, expertise, and techniques within these pillars to promote enhancements in prevention, diagnosis, and therapies" [6].

With a broader outlook, translational research is "a dynamic and iterative process that includes synthesis, dissemination, exchange and ethically sound application of knowledge to improve the health (…), provide more effective health services and products and strengthen the health care system" [7].

The definition given by the American Physiological Society [1] also stresses the fact that translational research must not exclude the opposite pathway, on the basis of which the experience obtained through clinical practice can guide the developments of research [8].

Favouring the transition from laboratory data to practical application is necessary in all areas of biomedical research, but it is particularly important in the case of

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medicinal products, including vaccines, discovery and development. As is well known, there is a particularly long interval between basic research and the registration of a new medicinal product: this can be to the detriment of patients, especially when there are no alternative treatment options available or during epidemics as in the current case of SARS-CoV-2.

The case of vaccines, however, has some peculiarities that differentiate it from clinical trials of medicinal products. In particular, the spread of SARS-CoV-2 has reopened a heated debate on the possibility of authorizing and carrying out "challenge trials". Human challenge studies involve the deliberate infection of healthy volunteers. Such studies can be faster to conduct than vaccine field trials, in part because far fewer participants need to be exposed to experimental vaccines in order to provide preliminary estimates of efficacy and safety. The World Health Organization [9] has provided conditional approval to this extraordinarily contentious practice of 'challenge studies' for Covid-19 vaccines. However, challenge studies are difficult to admit due to serious ethical issues.

The path of translational research starts from laboratory research and reaches clinical application. Hence, it also includes animal experimentation. This contribution, however, focuses on human experimentation, from the "first in man" phase to the commercial availability of a new drug: it is in human experimentation, in fact, that the most relevant ethical problems arise.

TYPES AND PHASES OF RESEARCH

The pathway that research takes from the lab through to clinical application can be split into a number of stages. A number of different classifications have been proposed on the basis of different criteria.

One reprocessed approach [10] based on the classification put forward by Stokes [11] sees two orthogonal axes *(Figure 1)*. The horizontal axis represents relevance to immediate application. The vertical axis represents relevance to the advancing of knowledge. The area between the two axes has been split into four quadrants. The quadrant that is least relevant with regard to both immediate application and the advancing of knowledge constitutes the "waste quadrant", and is home to those research projects that do not make significant contributions to either the advancing of knowledge, or immediate application. The quadrant corresponding to high relevance to immediate application but little relevance with regard to the advancing of scientific knowledge is known as the "Doll quadrant", because of Richard Doll's work with Bradford Hill to identify smoking as a cause of lung cancer. The quadrant corresponding to the area of high relevance to advancing of knowledge, but poor relevance to immediate application, is known as the Curie quadrant, referring to the importance of the research Marie Curie conducted on radiation. The quadrant referring to the area of high relevance for both immediate application and the advancing of knowledge is known as the "Pasteur quadrant", as the scientific knowledge generated by Louis Pasteur had an enormous impact in reducing the morbidity, mortality and economic costs of infections. Translational research falls in this quadrant.

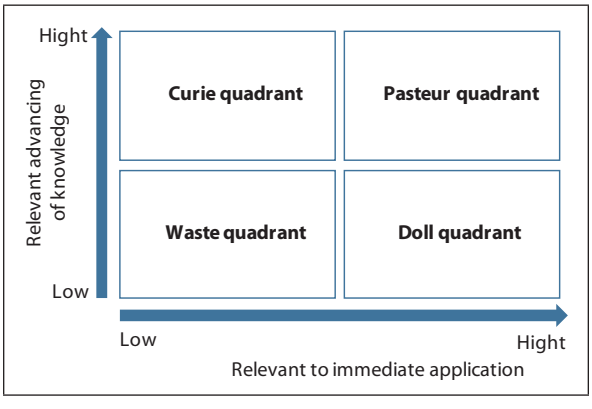

Figure 1
Quadrant model of scientific research.

The different interpretations of the development of research also translate into different interpretations of the role of translational research. Three major "families" of interpretations can be identified [12]:

1. the "gap" model, which has adopted, above all, by the "Clinical Research Roundtable" (CRR) working group [13]. According to this model, translational research bridges the gap between basic knowledge and clinical research in order to translate the knowledge generated from basic research into benefits for patients and / or the general population;

2. the "continuum" model corresponds with the interpretation given, for example, by Khoury *et al.* [14]. According to this model, basic research and clinical research are part of a same process in which phases are relatively continuous. In this perspective, the advancing of knowledge is translated through a "continuous research spectrum and phases in this continuum are labelled by common setting or research methods" [12];

3. the "mixed" model interprets the initial phases (basic research) in the same way as the "gap model" and the later stages of applied research in the same way as the "continuum" model [15].

ETHICAL ASPECTS

In the debate on translational research, significant space is dedicated to research organisation and management, for example regarding funding and staff training. For example, according to the abovementioned "Clinical Research Roundtable" (CRR) working group, the shortcomings in the transfer of the knowledge obtained with basic research to application are due to "2 major obstacles, or translational blocks: impeding the translation of basic science discoveries into clinical studies and of clinical studies into medical practice and health decision making in systems of care" [13]. According to the CCR, these two obstacles are associated with four "central challenges facing clinical research at present", namely "public participation, information systems, workforce training, and funding" [13].

Although the organisational and managerial aspects are relevant, in this paper we intend to focus in par-

ticular on the aspects that are most important from an ethical standpoint. It goes without say that the organisational and managerial aspects are closely intertwined with the ethical aspects [14]. For example, analyses of translational research promotion policies often reveal a need for funding: it would be important to adopt a national investment plan for research. However, without playing down the importance of adequate investments, the efficacy of translational research depends above all on the capacity to draw new lines and innovate, and this has a significant ethical relevance. This is particularly important in the case of research for new medicinal product discovery and development, where, despite disposing of significant funding from large companies, some of the many research projects that prove to be fruitless could, in actual fact, lead to useful applications if developed appropriately.

At the same time to make available new drugs and vaccines in an equitable way requires considering the so called "innovation science": to be successful in a system innovation requires precise reflections, planning and implementation policies.

Innovation Science encompasses a broad space [15]. It can contribute to the improvement of translational research by moving from a "hardware" approach (by restructuring organisation charts, upgrading procedures, etc.) to a "software" one (which reflects the complexity of the system and respect its resilient features, which often appear unexpectedly, arising from interaction of smaller or simpler entities). In this perspective, we might adopt new models that appreciate the complexity of the systems ad understand that changes is always unpredictable and needs to be tailored to the setting [16].

In other terms, the traditional conception of medical knowledge as a linear pipeline moving from evidence created in the laboratory through clinical trials and finally, via new tests, drugs or vaccines into clinical practice, is no longer sustainable. Complexity science forces us to consider the dynamic properties of the systems and the varying characteristics: the health system is probabilistic and stochastic rather than strictly deterministic, and often the characteristics of the components are secondary to the relationships of the components themselves [17].

Without playing down the ethical and non-ethical relevance of general policy, the most significant ethical issues in translational research on medicinal products derive from the risk of the intention to shorten the timeframes for the application of the results of research making the scientific methods adopted and the regulatory requisites to be satisfied along the long path from the bench to the patient's bedside less rigorous [18]. This could expose the subjects taking part in the research to undue risks. This is a crucial topic, with many different facets [19]:

1. *The safety and welfare of the subjects taking part in the study*. The researchers are under obligation to put patient welfare before any interest regarding the advancing of knowledge, society or profits;

2. *Direct and indirect benefits*. As recognised in the World Medical Association's Declaration of Helsinki (art. 8), "While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects" [20]. However, the precedence of the research subject's welfare over the interests of science or society does not, in any way, preclude the possibility of the participants obtaining direct benefits. In these circumstances, the need to analyse and restrict the potential risks is particularly important.

3. *Proportionality between risks and benefits*. All research involves risks. One crucial step, in which due caution is required, is the transition from laboratory research and/or research using animal models to trials on humans. It goes without say that the risk level is different in different studies and risks and benefits have to be balanced correctly. In special situations (for instance serious diseases for which there are no efficacious therapies available and epidemic situations), risk levels that would be unacceptable in other circumstances are permitted. Striking this balance is made difficult by the unpredictability that characterises all research. In this context, it is dutiful to guarantee special protection for individuals in conditions of particular vulnerability.

4. *Doctor-patient relationship*. In the case of translational research, in which the objective of the practical application of the research takes precedence, special attention must be dedicated to the relationship between doctor and patient, when providing the patient with information and the procedures required to obtain consent. Although the requisites set forth in the 3 previous points refer in particular to the ethical principles of beneficence and non-maleficence, the doctor-patient relationship, information and consent refer, in particular, to the ethical principle of autonomy [21].

5. *Equality of access*. When promoting the applicability of the results of the research, it is necessary to adequately consider access to the applications and avoid discrimination. This corresponds to the ethical principle of justice [21].

6. *Integrity of the research*. Translational research must always comply with the principles of research integrity. Research Integrity may be defined as adherence to the ethical principles, rules and professional standards essential for the responsible conduct of research [22]. Research Integrity is about getting to the scientific knowledge using the highest scientific, professional and ethical standards. Ethical practice, honesty, trustworthiness, and high regard for the scientific methods are essential attributes of any scientist with an interest in conducting research that benefits mankind. For research institutions, integrity is about ensuring commitment to creating an environment that promotes responsible conduct by embracing standards of excellence, trustworthiness, and lawfulness in the conduct of research by staff and all members affiliated to the institution. The term "Responsible Conduct of Research" (RCR) is often used by institutions to refer to a wide range of areas of research compliance [23].

7. *Conflict of interest*. Due to its nature, and especially for the potential commercial repercussions, translational research is particularly subject to the risk of conflicts of interest. A conflict of interest occurs when professional judgement regarding a primary interest (the

health of a patient, the validity of a study or a product, the truthfulness of the results of a study, etc.) is potentially influenced by a secondary interest, such as economic gain or personal advantage. Conflict of interest is therefore a condition (and not necessarily a behaviour) that could compromise the autonomy of a researcher and the impartiality of his/her professional actions.

8. *Need for monitoring*. In translational research, it is particularly important not only to assess protocols thoroughly, but also to provide constant monitoring, by qualified staff, throughout the conduct of the study. Special vigilance is required in this sense as the intention to obtain useful applications could lead some to be less rigorous on a methodological level or even to falsify data.

CONCLUSIONS

The ethical assessment of the risks and benefits of translational research must be performed on a case-by-case basis, considering the circumstances and the added value that it is expected the new medicinal product will bring (or, in general, a new therapeutic resource) over what is already available [24].

Translational research is not a set of methods and skills, but a strategy that requires experience and coordination. It is not a sequential activity, but a constant interaction.

Translational research requires cooperation between the academic and industrial worlds and needs a favourable administrative and political framework, whilst avoiding undue conflicts of interest: the primary objective must be the patient's welfare.

Therefore, in translational research, particularly stringent ethical requirements must be adopted and ethics committees can play a crucial role in guaranteeing the welfare and rights of the individuals taking part in the research.

The CoViD-19 pandemic has made it even more evident the importance of developing and making new medicines rapidly available. Moments of crisis are rarely good moments to develop ethics. However, it is necessary to question oneself on criteria and procedures, without derogating from the safety requirements for patients and rigor in the scientific method. An international conference could be important to define shared criteria, also for the purpose of any regulatory adjustments.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings.

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REFERENCES

1. Hall JE. The promise in translational physiology. Am J Physiol Endocrinol Metab. 2002;283:E193-E194.
2. Strauss SE, Tetroe J, Graham ID. Knowledge translation in healthcare. Moving from evidence to practice, 2nd ed. Oxford: Wiley-Blackwell - BMJ Books; 2013.
3. Wolf S. The real gap between bench and bedside. N Eng J Med. 1974;290:802-3.
4. Petrini C. From bench to bedside and to health policies: ethics in translational research. La Clin Ter. 2011;162:51-9.
5. Birmingham K. What is translational research. Nat Med. 2002;8:647.
6. Cohrs R, Martin T, Ghahramani P, Bidaut L, Higgins PJ, Shahzad A. Translational Medicine definition by the European Society for Translational Medicine. New Horizons in Translational Medicine. 2015;2:86-8.
7. Canadian Institutes of Health Research. Knowledge translation: definition. 2018. Available from: http://www.cihr-irsc.gc.ca/e/29529.html.
8. Petrini C. From bench to bedside and to health policies (and back): ethics in translational research. Ann Ist Super Sanità. 2014;50:62-6.
9. World Health Organization. Key criteria for the ethical acceptability of COVID-19 human challenge studies. 2020. Available from: www.who.int/ethics/publications/key-criteria-ethical-acceptability-of-covid-19-human-challenge/en/.
10. Chalmers I, Bracken MB, Djulbegovic B, Garattini S, Grant J, Gülmezoglu AM, Howells DW, Ioannidis JPA, Sandy O. Research: increasing value, reducing waste 1. How to increase value and reduce waste when research priorities are set. Lancet. 2014;383:156-65.
11. Stokes D. Pasteur's quadrant – basic science and technological innovation. Washington, DC: Brookings Institution Press; 1997.
12. Fort DG, Herr TM, Shaw PL, Gutzman KE, Starren Justin B. Mapping the evolving definitions of translational research. J Clin Translat Sci. 2017:1:60-6.
13. Sung NS, Crowley WF, Salber P, Sandy L, Sherwood LM, Johnson SB, Catanese V, Tilson H, Getz K, Larson EL, Scheinberg D, Reece EA, Slavkin H, Dobs A, Grebb J, Martinez RA, Korn A, Rimoin D. Central challenges facing the National Clinical Research Enterprise. JAMA. 2003;289:1278-87.
14. Khoury MJ, Gwinn M, Yoon PW, Dowling N, Moore CA, Bradley L. The continuum of translation research in genomic medicine: how can we accelerate the appropriate integration of human genome discoveries into health care and disease prevention? Genet Med. 2007;9:665-74.
15. Greenhalgh T, Robert G, Macfarlane F, Bate P, Kyriakidou O. Diffusion of innovations in service organizations: systematic review and recommendations. Milbank Q. 2004;82:581-629.
16. Braithwaite J. Changing how we think about healthcare improvement. BMJ. 2018;361:k2014.
17. Braithwaite J, Churruca K, Long JC, Ellis LA, Herkes J. When complexity science meets implementation science: a theoretical and empirical analysis of systems change. BMC Medicine. 2018;16:63.
18. Woolf SH. The meaning of translational research and why it matters. JAMA. 2008;299:211-3.
19. Petrini C. Ethical issues in translational research. Perspect Biol Med. 2010;53:517-33.
20. World Medical Association. Declaration of Helsinki – Ethical principles for medical research involving human

subjects (adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended). 2013. Available from: www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/.
21. Beauchamp TL, Childress JF. Principles of biomedical ethics. 7th ed. New York: Oxford University Press; 2012.
22. Institute of Medicine and National Research Council. Integrity in scientific research: Creating an environment that promotes responsible conduct. Washington, DC: The National Academies Press; 2002. Available from: https://doi.org/10.17226/10430.
23. Ndbele P. Integrity: Research. In: Ten Have H. (Ed.). Encyclopedia of global bioethics. Switzerland: Springer International Publishing; 2016.
24. Kimmelman J, London AJ. Predicting harms and benefits in translational trials: ethics, evidence, and uncertainty. PLoS Med. 2011;8:e1001010.

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BRIEF NOTE

Need for a sustainable use of medicinal products: environmental impacts of ivermectin

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Abstract
It is worldwide recognized that the use of pharmaceuticals for human and veterinary purposes could lead to unsustainable effects on the environment. A strategy to reduce the impact of pharmaceuticals on the environment has been recently established at European level, where guidelines to evaluate the impacts of veterinary drugs used to treat animal diseases are in place. The aim of this article is to focus on the worldwide used antiparasitic drug ivermectin (IVM) and its potential impact on the environment. A specific section is related to the IVM resistance that the massive use of this drug could generate enhancing the risk scenarios also for human health. The application of stringent measures for the veterinary use of this substance, in line with the recommendations provided by International frameworks such as One Health and EcoHealth, is recommended.

Key words
- environment
- ecotoxicity
- resistance
- antiparasitic drug
- ivermectin

INTRODUCTION

The treatment of many human and animal diseases relies on access to effective pharmaceuticals. At the same time, the environmental pollution caused by some pharmaceuticals is an emerging problem because of their residues discharged and released in the ecosystems during the manufacture, use and disposal. The evidence of risks for the environment is well-documented [1].

The communication about the pharmaceutical strategy on the environment supports the aim of the European Commission (EC) (COM/2019/128 final) to deliver a sustainable Europe by 2030, according to the United Nations (UN) Sustainable Development Goals (SDGs). The strategy has been established in the context of the EU Water Framework Directive that has the aim to achieve a good chemical and ecological status for all water resources. Furthermore, this strategy is a component of the Union's One Health Action Plan against Antimicrobial Resistance.

The largest source of pharmaceuticals entering the environment is represented by their use. Moreover, the general pressure of pharmaceuticals will increase due to demographic developments (e.g. increasing population density and age) and due to climate changes, an insufficient constant dilution of pharmaceutical run offs and effluents can be expected in many areas. The route of exposure likely differs depending on whether human or veterinary use is involved. However, in some cases, up to 90% of the active ingredient is excreted (or washed off) in its original form due to the stability and the adsorption of the chemical substance. The release of veterinary drugs in the environment tends to come from untreated diffuse sources such as the spreading of manure. In this respect, the evaluation of the environmental risk of veterinary medicinal products within marketing authorisation procedures has been discussed in European Union (EU) since the mid-1990. Moreover, a first guidance document defining how to perform the environmental risk assessment (ERA) was prepared by the European Medicines Agency (EMEA) in 1997. For the EU, additional guidance in support of the guidelines was provided by EMEA in 2008 [2] and further revisions have been provided in 2019. In this guidance, predicted environmental concentrations (PECs) are estimated based on the dose and frequency of the product applied. If the PEC exceeds the trigger value of 100 µg/kg dry weight in the soil for intensively reared and pasture animals, studies on environmental fate and effects on selected non-target species have to be performed during the phase II. In parallel if a PEC in water compartments exceeds 100 ng/L it triggers additional risk assessment steps. In the same phase, the environmental risk is deterministically characterized by

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comparing the PECs with the predicted no-effect concentrations (PNECs) in several environmental compartments.

Inivermectin (IVM) belongs to the avermectins and these are widespread used antiparasitic medical products representing a serious potential threat for the environment [3]. They have been used for several purposes for example in agriculture and horticulture for the protection of fruit, cotton, vegetables, and ornamental, thanks to their effectiveness against a wide range of nematodes, mites, and insects. Among the avermectins, IVM is mainly used for controlling livestock parasites. With over 5 billion doses worldwide sold since its market introduction in the early 1980s, this veterinary medical product has become the most widely used antiparasitic drug. It is used regularly as a pesticide for cattle, pigs, sheep, horses, and dogs. Its use is effective against internal parasites, such as gastrointestinal and pulmonary nematodes, and external parasites, such as mange mites and blood lice. IVM can be administrated orally, topically or intramuscularly. Oral applications tend to result in sharp excretion peaks, with most of the dose excreted over a few days.

In connection with the excretion pathways, IVM and its metabolites were excreted mainly in faeces (90%) and only 1% in urine. Other authors who tried to determine IVM in urine did not find the parent drug nor its metabolites. Bile is the main route of excretion.

Peak elimination of injectable or topical formulations usually occurs within 2 to 7 d post-treatment, followed by a long tail that may sustain for more than 4 to 6 weeks, whereas peak elimination levels of sustained-release formulations may occur over several weeks post-treatment.

Already a decade ago, Liebig et al. [4] have demonstrated that, with regard to its environmental aspects, IVM is a substance of high concern. The environmental risk assessment of IVM was mainly performed according to the mentioned international and European guidelines, using a large number of new data on its fate and effects, and additional results from 2-species, multispecies, semifield, and field studies. This case study has clearly demonstrated unacceptable risks for living organisms (e.g., for daphnids and dung invertebrates), and the authors suggested the necessity of reassessing IVM containing veterinary medicinal products. Moreover, when it is used in crop protection, there are evidence of ecotoxicity for non-target species, with insects generally and bees in particular being at the highest risk [5]. For human use ivermectin (IVM) has also been shown to be effective in *in vitro* tests against a broad range of viruses, including HIV, Dengue, Influenza, and Zika. Recently, a collaborative study led by the Monash Australian University and published in the journal *Antiviral Research* has showed that a single dose of IVM could stop the COVID-19 virus growing in cell cultures. The next step of this research will aim to evaluate the correct dosage [6].

IVERMECTIN EFFECTS ON AQUATIC AND TERRESTRIAL ORGANISMS

Aquatic invertebrates

Notable effects of IVM have been reported on various aquatic invertebrate species such as *Daphnia magna* and *Gammarus pulex*. A 2007 study [7] observed the extreme sensitivity of *D. magna* to this drug. 10 acute tests were carried out and a 48 h LC_{50} of 5.7 ng/L *(Table 1)* was found, while chronic tests revealed high toxicity on reproduction and growth rate even at lower concentrations. The nominal LOEC obtained was 0.001 ng/L and the NOEC 0.0003 ng/L. These values are analogue to those of extremely toxic compounds that are classified as priority hazardous substances by the Water Framework Directive. Among the Amphipods, a wide order of crustaceans, high sensitivity to IVM has been reported in particular for *Gammarus pulex* and *Gammarus fossarum* [8]. A 2018 study [9] observed a 100% mortality in young American lobsters (*Homarus americanus*) exposed to ivermectin-treated sediment (61.0 and 300.0 ng/g sediment) for a 25 days period, while a prolonged exposure to lower concentrations (0-3.0, 6.4 and 14.7 ng/g sediment) induced sublethal effects IVM shows a very high acute and chronic toxicity to crustaceans in the 5.7 ng/L and 0.3 pg/L range and this should be considered in future risk management measures. At these levels no robust analytical controls can be expected. Moreover, such a very high toxicity is indicating the need of reducing any aquatic exposure of IVM to very low levels, in order to protect a wide range of aquatic invertebrates.

Table 1
Comparison between the effect concentrations and Initial PEC values in water and dung [4]

Compartment	Species	Effect concentration	PEC (best/worst case)
Water	*Daphnia magna*	EC_{50} 5.7 ng/L PNEC 0.0057 ng/L(acute) PNEC 0.00003 ng/L (chronic)	0.1/7.2 (IR) 0.2/2.5 (P) 83/523 (P) ng/L
	Oncorhynchus mykiss	LC_{50} 3.0 µg/L PNEC 3.0 ng/L	0.1/7.2 (IR) 0.2/2.5 (P) 83/523 (P) ng/L
Dung	*Musca autumnalis*	$EC_{50emerg.rate}$ 4.65µg/kg dung fresh wt	4.8/12.7 (P) mg/kg dung fresh wt
	Aphodius constans	LC_{50} 176 µg/kg dung fresh wt	4.8/12.7 (P) mg/kg dung fresh wt

PEC: predicted environmental concentrations; IR: intensively reared animals; P: pasture animals.

Fishes

Many studies over the years focused on the consequences of the IVM use on seawater fishes: this drug is indeed commonly used to treat sea lice infestations in aquaculture, mainly in Atlantic salmon (*Salmo salar*) farms. Sea lice (*Lepeoptherius sp.* and *Caligus sp.*) are marine ectoparasitic copepods able to induce serious health issues to fishes and consequently significant economic losses. In a 2012 [10] study, Ucán-Marín *et al.* observed several toxicological effects in salmons exposed to IVM through diet at similar concentrations to those currently used in aquaculture. The lethal concentration found was 0.174 mg/kg, only three times higher than those used in operational context, and with concentrations slightly higher than those commonly used. Effects such as lethargy, dark skin and reduced feeding behavior were reported. Negative consequences derived from the exposure to IVM were investigated also in freshwater fish. In *Oncorhynchus mykiss* a LC_{50} of 3.0 µg/L has been detected [4]. In *Danio rerio*, one of the most commonly used organisms for ecotoxicological assays Domingues *et al.* [11] observed the effects produced by a chronic exposure to concentrations between 0.25 and 25 µg/L in adult Danios. While in the acute test a 96 hours LC_{50} was calculated with higher values, the behavioural patterns of the fish changed even at the lowest concentration of 0.25 µg/L, as they tended to spend more time in the lowest part of the tank. This behavior altered their feeding ability causing a weight loss during the exposure time, more emphasized in males. At the highest concentration (25 µg/L) the effects were lethargy, reduced activity, dark coloration and mild curvature of the spine.

Algae and plants

The stability and persistence of IVM in sediment and water, especially in wetlands, can cause constant exposure for algae and plants [12]. The toxicity effects of IVM was studied since 1989 by Halley *et al.* Phytotoxicity of IVM on algae and plants is reported in several new studies. In a study aimed to compare the possible effects of IVM on both *D. magna* and *Pseudokirchneriella subcapitata* [13] the algae showed a considerably lower sensitivity: only at the highest concentrations adopted (1250 and 4000 µg/L) a significant effect on algal growth was reported. Eichberg *et al.* [14] reported inhibition of germination in three grassland plant species (*Centaurea jacea*, *Galium verum*, *Plantago lanceolata*) from moxidectin, an antihelmintic drug similar to IVM. Another study, on *Sinapis alba,* was carried out by Vokřál *et al.* in 2019 [15] using a "seed germination and early roots growth" test with two different concentrations, 50 nM (0.044 µg mL^{-1}) and 500 nM (0.44 µg/mL). The results of this study showed phytotoxicity for both concentrations, with a 20% growth inhibition for the lowest concentration and 24% for the highest. It is to be underlined that the exposure to a concentration of 44 µg/L can be realistically found in the environment where IVM-treated cattle excrements are present.

Terrestrial organisms and dung insects

The large use of IVM as an anti-parasitic drug for livestock implies its constant release in the environment. In all the possible administration routes, the IVM is in fact largely excreted via faeces, where it can remain for a long period of time. For this reason, many insect species that live in close proximity with dung can be affected by this chemical. In particular, dung beetles tend to excavate dung in order to create brood balls in which they lay their eggs; this kind of behavior has significant positive effects on the environment, as it prevents the accumulation of dung and promotes the fertilization of the soil [16]. The toxicity of IVM on dung beetles has already been observed in several papers over the years [4]. Pecenka *et al.* [17] highlights the negative correlation of ivermectin quantity in cattle pats with dung beetle abundance and diversity. In particular, Martinez et al. [18] exposed the dung beetle *Euoniticellus intermedius* to IVM (3.16, 10.0, 31.6, 63.2, 100, and 316 µg/kg fresh dung) in order to determine the toxicity on adults and larvae. After 10 days of exposure, adult females showed a decrease in fecundity. Ishikawa and Iwasa [19] exposed four different species of dung beetle, *C. acutidens*, *O. bivertex*, *O. lenzii* and *P. auratus* to IVM-treated dung; the IVM was applied on cattle by pour-on formulation with the dose of 500 µg/kg of body weight and dung was collected 1, 3, 7, 14 and 21 days post-treatment. In *C. acutidens* an high mortality was observed in adults exposed to dung collected 3- or 7-days post-treatment compared to the control (exposed to non-treated dung). In the same dung, few or no brood balls were reported and in dung from 7- or 14-days post-treatment the emergence rate was significantly lower compared to the control. IVM shows a high toxicity for dung insects in the 3,16-316 µg/kg range of fresh dung, therefore, any terrestrial exposure should be minimized if possible.

Environmental fate and behaviour

When considering the impact upon ecosystems, the environmental fate of a drug like IVM should be taken into account. The log K_{ow} of IVM of 3.2 [4] indicates bioaccumulation potential in organisms. Moreover, IVM has the potential to bioaccumulate in *L. variegatus* (biota-sediment accumulation factors ranged from 2.1 to 16.6). Several studies have shown a high persistence of this substance in mesocosms, wetland and terrestrial environment [20]. Dissipation half-lives (DT_{50}) in soil can be rather variable depending on soil type, sorption capacity, temperature, and oxygen availability, ranging from 16 to 1520 days [4]. IVM can be also very persistent in mixtures of soil and manure or faeces (7-217 days). Log K_{oc} have ranges of 3.6-4.2 [4]. As a widely used drug in animal husbandry and farming, IVM frequently occurs in cattle faeces. Movement of IVM from dung to the underlying soil and to the nearby plants has been observed. Concentrations of IVM have been also detected in the roots of macrophytes living in wetlands subjected to different cattle uses in roots and leaves of aquatic plants in a simulated aquaculture ecosystem [21]. Moreover, IVM accumulates in dung, sediment, and water of wetlands, representing a serious threat for such ecosystems. A similar fate of IVM has been assessed in water and sediment of simulated aquaculture [21]. Overall, IVM shows

bioaccumulation and persistence indicating a need of a specific environmental risk management.

DRUG RESISTANCE IN PARASITIC PROTOZOA

The IVM binding with ligand-gated ion channels, particularly glutamate-gated chloride channels in nematode and insects, cause an influx of negatively charged chloride ions, resulting in hyperpolarisation of synapses and paralysis of organisms. Further, recent studies in other biological systems suggest that IVM can affect additional pathways too [22]. Due to its widespread, use the protozoans developed resistance and it appeared at first time in small ruminants, then in cattle parasites and free-living *Caenorhabditis elegans*, The results of several studies suggested that both target mutation and transport alteration can lead to ivermectin resistance in particular through the changes in mRNA transcription of the ABC transport proteins and the glutathione-related genes [23, 24]. The IVM resistance is a serious problem for parasite control in livestock and there is a concern about resistance development and spread in nematode parasites of humans. Since 2000s, the World Health Organization included parasites in the list of antimicrobial resistant microorganisms together with bacteria, fungi and viruses (www.who.int/health-topics/antimicrobial-resistance). Studying and understanding drug resistance mechanisms of these organisms is important in terms of human and animal health.

CONCLUSIONS

The data summarized in this brief note demonstrate that the veterinary use of IVM can lead to an unacceptable and unsustainable risk for both aquatic and terrestrial ecosystems. IVM shows a very high acute and chronic toxicity to crustaceans. In fish, IVM can cause lethargy, dark skin and reduced feeding behavior. Moreover, growth inhibition for algae and high toxicity for terrestrial organisms and dung insects were underlined. The use of this veterinary medical product, applying the ERA methodology, can cause a high risk. Specifically, for daphnids and dung organisms with a very low PNEC value, indicating a high toxicity level analogue to chemical substances classified as priority hazardous by the European Union. Based on these findings, we suggest a reduction (or elimination) in the use of this substance as veterinary drug considering the related high risk in particular for aquatic ecosystems. If the use will be authorised more stringent measures should be applied in order to reduce and/or mitigate the impact of IVM on the environment and its organisms. For this reason, IVM should be considered in the context of the EU Water Framework Directive monitoring programmes due to the lack of monitoring data in rivers and aquatic ecosystems. The monitoring data can also improve the knowledge on the role of the environment in the development of resistance in parasitic organism described in the brief note. In some cases, due to the IVM toxicity at very low levels, the use of effect-based methods can be also recommended in areas where the presence of IVM can be predicted in order to detect the real effects on the ecosystems. In conclusion, IVM is a pharmaceutical used worldwide for its properties for the treatment of several diseases in humans and animals, an example is the recent study mentioned in the introduction about the possible use against COVID-19; but for the achievement of SDGs the potential environmental effects of IVM described in this article cannot be neglected. In particular, IVM as a very toxic, persistent and resistance building medical product with high use is maybe one of the most suitable candidates to be assessed in a sustainability strategy. These considerations are also in line with the One Health and EcoHealth approaches that are recommended by several European and international institutions (FAO, WHO, EFSA). In these international frameworks, the environment is strictly linked to human and animal health and every medicinal use authorised, should be sustainable for the environment. Moreover, the main holistic target of this approach should be the overall protection of environmental, animal, and human health.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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REFERENCES

1. Mudgal S, De Toni A, Lockwood S, Salès K, Backhaus T, Sorensen BH. Study on the environmental risks of medicinal product. Final report. Executive Agency for Health and Consumers; 2013.
2. European Medicines Agency. Revised guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38. London, UK: Committee for Medicinal Products for Veterinary Use (CVMP), EMEA; 2008.
3. Omura S. Ivermectin: 25 years and still going strong. Int J Antimicrob Agric. 2008;31:91-8.
4. Liebig M, Fernandez ÁA, Blübaum-Gronau E, Boxall A, Brinke M, Carbonell G, Egeler H, Fenner K, Fernandez C, Fink G, Garric J, Halling-Sørensen B, Knacker T, Krogh KA, Küster A, Löffler D, Cots M, Ángel P, Pope L, Prasse C, Römbke J, Rönnefahrt I, Schneider MK, Schweitzer N, Tarazona JV, Ternes TA, Traunspurger W, Wehrhan A, Duisy K. Environmental risk assessment of ivermectin: a case study. Integr Environ Assess Manag. 2010;6:567-87.
5. Bai SH, Ogbourne SM. Eco-toxicological effects of the avermectin family with a focus on abamectin and ivermectin. Chemosphere. 2016;154:204-14.
6. Caly L, Druce DJ, Catton GM, Jans AD, Wagstaff MK. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 *in vitro*. Antiviral Research. 2020;178:104787.
7. Garric J, Vollat B, Duis K, Pe´ry A, Junker T, Ramil M,

Fink G, Ternes TA. Effects of the parasiticide ivermectin on the cladoceran Daphnia magna and the green alga Pseudokirchneriella subcapitata. Chemosphere. 2007;69:903-10.
8. Alonso Á, De Lange HJ, Peeters ET. Contrasting sensitivities to toxicants of the freshwater amphipods Gammarus pulex and G. fossarum. Ecotoxicology. 2010;19:133-40.
9. Daoud D, McCarthy A, Dubetz C, Barker DE. The effects of emamectin benzoate or ivermectin spiked sediment on juvenile American lobsters (Homarus americanus). Ecotoxicology and Environmental Safety. 2018;163:636-45.
10. Ucán-Marin F, Ernst W, O'Dor RK, Sherry J. Effects of food borne ivermectin on juvenile Atlantic salmon (Salmo salar L.): survival, growth, behavior, and physiology. Aquaculture. 2012;334-337:169-75.
11. Domingues I, Oliveira R, Soares AMVM, Amorim MJB. Effects of ivermectin on Danio rerio: a multiple endpoint approach: behaviour, weight and subcellular markers. Ecotoxicology. 2016;25:491-9.
12. Mesa L, Gutiérrez MF, Montalto L, Perez V, Lifschitz A. Concentration and environmental fate of ivermectin in floodplain wetlands: An ecosystem approach. Sci Total Environ. 2020;706:135692.
13. Garric J, Vollat B, Duis K, Pe´ry A, Junker T, Ramil M, Fink G, Ternes TA. Effects of the parasiticide ivermectin on the cladoceran Daphnia magna and the green alga Pseudokirchneriella subcapitata. Chemosphere. 2007;69:903-10.
14. Eichberg C, Wohde M, Muller K, Rausch A, Scherrmann C, Scheuren T, During RA, Donath TW. The anthelmintic ingredient moxidectin negatively affects seed germination of three temperate grassland species. Plos One. 2016;11:e0166366.
15. Vokřál I, Michaela Š, Radka P, Jiří L, Lukáš P, Dominika S, Kateřina L, Barbora S, Lenka S. Ivermectin environmental impact: Excretion profile in sheep and phytotoxic effect in Sinapis alba. Ecotoxicol Environ Saf. 2019;169:944-9.
16. Beynon SA, Wainwright WA, Christie M. The application of an ecosystem services framework to estimate the economic value of dung beetles to the UK cattle industry. Ecological Entomology. 2015;40:124-35.
17. Pecenka JR, Lundgren JG. Effects of herd management and the use of ivermectin on dung arthropod communities in grasslands. Basic and Applied Ecology. 2019;40:19-29.
18. Martínez MI, Lumaret JP, Ortiz Zayas R, Kadiri N. The effects of sublethal and lethal doses of ivermectin on the reproductive physiology and larval development of the dung beetle Euoniticellus intermedius (Coleoptera: Scarabaeidae). Can Entomol. 2017;149:461-72.
19. Ishikawa I, Iwasa M. Toxicological effect of ivermectin on the survival, reproduction, and feeding activity of four species of dung beetles (Coleoptera: Scarabaeidae and Geotrupidae) in Japan. Bullet Entomol Res. 2020;110:106-14.
20. Sanderson H, Laird B, Pope L, Brain R, Wilson C, Johnson D, Bryning G, Peregrine AS, Boxall A, Solomon K. Assessment of the environmental fate and effects of ivermectin in aquatic mesocosms. Aquatic Toxicology. 2007;85:229-40.
21. Wang D, Han B, Li S, Due X, Cao Y, Lu T. Assessment of the fate and effect of ivermectin in a simulated aquaculture ecosystem. Aquaculture Research. 2020;51:535-41.
22. Dent JA, Smith MM, Vassilatis DK, Avery L. The genetics of ivermectin resistance in Caenorhabditis elegans. Proc Natl Acad Sci USA. 2000;14;97:2674-9.
23. Laing R, Gilan V, Devaney E. Invermectin – Old drug, new tricks? Trends in Parasitol. 2017;33:463-72.
24. Ouellett ME. Biochemical and molecular mechanisms of drug resistance in parasites. Trop Med Int Health. 2001;6:874-82.

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BRIEF NOTE

Unequal effects of the national lockdown on mental and social health in Italy

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Abstract

With the exception of a few countries that chose a different approach, the worldwide reaction to the COVID-19 pandemic was a (longer or shorter) period of national lockdown. While the economic consequences of shutting down national economies were immediately evident, the sociopsychiatric implications of the social confinement of the entire population remain hidden and not fully understood. Italy has been the first European country to be severely impacted by the COVID-19 pandemic, to which it responded through strict lockdown measurements. The results of a timely survey on mental and social health, carried out by students and teachers of a middle school in Rome, might help identify the most vulnerable groups of the population. This evidence could be crucial in conceiving and enacting targeted public health policies to mitigate the consequences of the pandemic on mental health and to prevent intolerance to containment measures in some population segments, which could hamper worldwide efforts in the fight against COVID-19.

Key words

- mental health
- health policy
- adolescents and women health
- COVID-19 epidemic

On March 5, all the schools in Italy closed as a reaction to the exponential growth in the number of COVID-19 cases, in anticipation for a nation-wide lockdown four days later. Nonetheless, all the teaching activities continued, sustained by a commendable effort by teachers to engage students from their homes through the screen of an electronic device. Teachers from the middle school Michelangelo Buonarroti in Rome, Italy, took a further step in this new era of online learning, by guiding and motivating their students to create an online survey to study the emotional response of the population to the national lockdown.

Similar to bird watchers who annotate the species they identify [1] or volunteers who tag images of polluted body of water on a computer screen [2], teachers and students took on themselves to create a comprehensive picture of the social and mental health consequences of the lockdown in Italy. Reaching beyond the barriers of structured, in-classroom education, this informal science endeavor constitutes an instance of citizen science spurred by the emergency posed by COVID-19. As any authentic citizen science project [3], this effort helped the scientific community through original data collection, and it benefitted volunteers, students and teachers, who increased their scientific literacy on the topic and acquired first-hand knowledge of the impact of the virus on our lives.

This commendable initiative was recognized by the

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World Health Organization – European Region in April 2020 as an example of adolescence active involvement in fighting the psychological effects of the pandemic. The survey was filled by thousands of individuals of all ages starting April 17, offering a unique snapshot of how society first responded to this life-changing event. Such a snapshot could help identify the traits of the individuals who were most affected by the lockdown, thereby informing effective public health approaches in the short and long term fight against COVID-19.

The experimental protocol was approved by the institutional review board (IRB) at Istituto Comprensivo Regina Elena, Scuola Media Michelangelo Buonarroti, Ministry of Education (protocol number 737.I.8). The survey was designed in Google modules to include questions on demographics (age, gender, and city of residence), on economic status (possible loss of job, and variations in the economic condition), and on feelings and activities when the lockdown was announced. The complete surveys, with their answers, can be found at https://osf.io/buahv/?view_only=2dfd654cf3944be49985db765b898226; excerpted questions underlying the variables used in the analysis, along with the possible answers, are presented below:

1. Gender
   Male / Female
2. Age
   10-14 / 15-19 / 20-30 / 31-50 / 51-70 / over 70
3. What did you feel when the quarantine was announced?
   Fear / Joy / Calm / Confusion / Anxiety / Indifference
4. What are your feelings now, about a month since the beginning of the quarantine? (3 answers maximum)
   Fear / Happiness / Serenity / Anxiety / Boredom / Nostalgia / Melancholy / Indifference.

The survey shares similarities to established tools, which were utilized by professional researchers to study the unfolding of the COVID-19 pandemic on mental health in the United States [4] and the effect of social distancing measures on productivity and workload of scientists across the globe [5].

Data until June 12 were exported in .csv files, which were then imported in Matlab for being analyzed. Participants were recruited by sharing the link to the survey through social media (WhatsApp, Instagram, and Facebook) and sending them emails with the following text: "Hello everybody! We are eighth grade students of the middle school Michelangelo Buonarroti in Rome, Italy, and we did a survey about how we are living during the quarantine and we imagine the future. Thank you for responding to this school project. (All answers are subject to privacy and will be used for educational work)".

Out of the 3990 individuals who filled the survey, we selected the 3562 respondents who selected Italian as the survey language. The demographic distribution of this sample is reported in *Figure 1*. Our analysis focused on identifying the differences in emotional response to the pandemic in relation to gender and age. We associated binary values with the gender (0 female, 1 male), and with each of the feelings in questions 3 and 4 (0 not felt, 1 felt). Then, we computed the Phi coefficient and the corresponding p-values. The results of this analysis are reported in *Tables 1* and *2*, where p-values lower than 0.01 (cells in green) indicate an association between feelings and age or gender. The Matlab code can be downloaded at https://osf.io/buahv/?view_only=2dfd654cf3944be49985db765b898226.

Here, we discuss two key results of this survey that we believe could contribute towards an improved understanding of the segments of the population that are most vulnerable to the social and mental health consequences of this pandemic. *Figure 2* shows that female and male respondents dramatically differed in their emotional response to the announcement of the lockdown and in their enduring reaction to it. Female respondents tended to experience anxiety, fear, melancholy, confusion, and nostalgia more than male respondents, thereby pointing at a higher level of distress associated with the lockdown.

These results are in line with the so-called gender paradox: the combination of biological and environmental factors produces a health inequality condition in which women, although having a higher life expectancy,

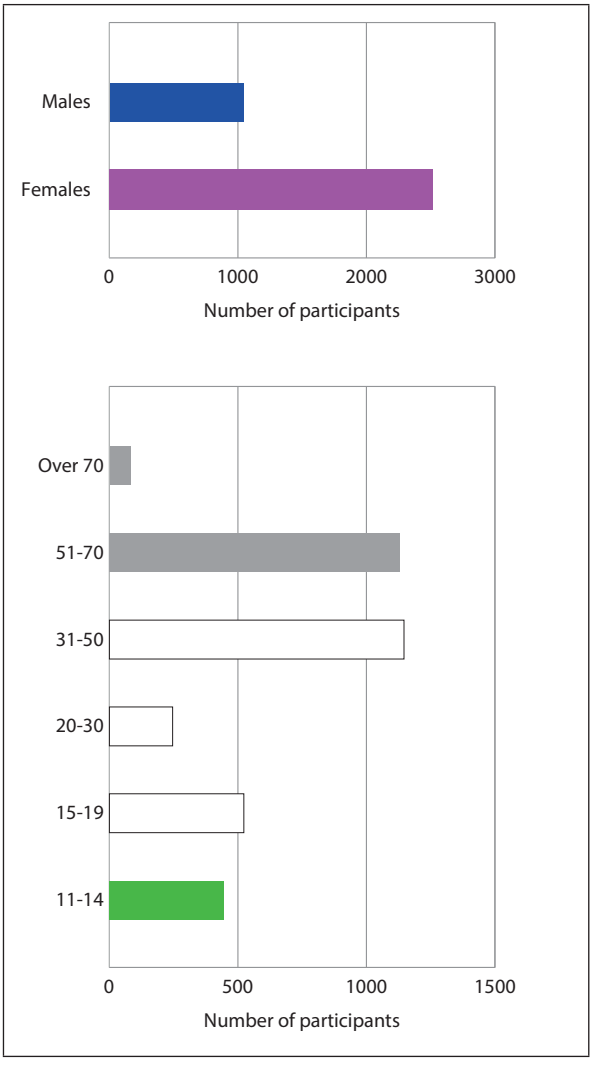

Figure 1
Gender (top panel) and age (bottom panel) distribution of the selected sample of participants.

Table 1
Phi coefficient for the association between age or gender and reaction to lockdown. When age is considered, we only focus on two categories of respondents, that is, *younger* (below 15 years of age) and *elder* (beyond 50 years of age), respectively. Cells in green correspond to p-values lower than 0.01

	Feelings when lockdown was announced					
	Fear	**Joy**	**Calm**	**Confusion**	**Anxiety**	**Indifference**
Age	0.02	-0.36	0.20	-0.04	0.17	-0.19
Gender	-0.10	0.00	0.14	-0.08	-0.11	0.18

Table 2
Phi coefficient for the association of age or gender to the feelings during lockdown. When age is considered, we only focus on two categories of respondents, that is, *younger* (below 15 years of age) and *elder* (beyond 50 years of age), respectively. Cells in green correspond to p-values lower than 0.01

	Feelings during lockdown							
	Fear	**Happiness**	**Serenity**	**Anxiety**	**Boredom**	**Nostalgia**	**Melancholy**	**Indifference**
Age	-0.01	-0.19	0.00	0.15	-0.33	-0.24	0.09	-0.10
Gender	-0.09	0.02	0.09	-0.11	0.16	-0.07	-0.12	0.12

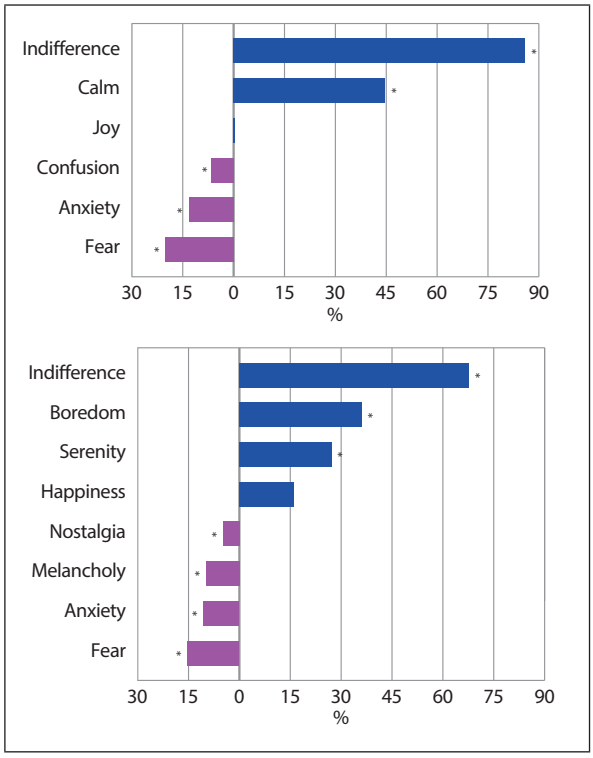

Figure 2
Feelings experienced when lockdown was announced (top panel) and during the lockdown (bottom panel). The magenta horizontal bars identify the feelings that were experienced more among the female rather than the male population, and their width corresponds to the increment (in percentage) compared to the entire population. Similarly, the blue bars identify the feelings that were experienced more among the male population. An asterisk identifies the feelings that were significantly correlated with gender (Phi coefficient, p-value less than 0.01).

end up living less healthy lives [6]. In the case of COVID-19, the paradox materializes in women and girls paying a higher price to the pandemic [5], although the mortality among them is lower than men and boys.

The higher impact of the pandemic on women and girls has also been recognized by the United Nations [7]. Key environmental factors explaining the vulnerability of women and girls, advocated by the United Nations, include their higher economic fragility, increased risk of gender-based violence, and greater involvement in unpaid care work towards children and elderly that grew during the pandemic. In its simplicity, the survey conceived by middle school students and teachers of Michelangelo Buonarroti provides sociopsychiatric backing to the observations made by the United Nations, by identifying differences in the emotional response of women and girls.

In addition to environmental factors, differences in emotional responses could be linked to gender-specific biological traits. The gender differences in the psychological and biological reactions to distress and in the way emotions are expressed are well-assessed, as well as the higher incidence of depression and post-traumatic stress syndrome in females [8]. The same stressful events can impact women and girls more, who may feel less in control and more prone to choose emotional *versus* problem-focused coping styles [9]. Every brain is a unique patchwork of traits, but quantitative analyses show that some traits are more prominent in one gender or another [10]. The different reactions to the lockdown could be then partially related to the fact that the masculine brain is structured to favor the perception-action connection, while the feminine brain is organized to facilitate the connection between analytic information processing and the intuitive analysis [11].

Overall, gender differences in the response to the lockdown should be sought in environmental and biological variations between male and female respondents. Neither of them should be considered the unique mediator for the observed differences in emotional response. Irrespective of their causes, we call to identify and enact suitable policies to protect the health of women and girls. It is of greatest urgency to provide and strengthen

community-based health and social services to compensate for gender inequalities, especially as women are taking a crucial role in the healthcare workforce [12].

The second key finding from the survey is reported in *Figure 3*, which highlights differences in the emotional experience of adults and young adolescents when the lockdown was announced and when it was in effect. A higher prevalence of joy and happiness among teenagers can be related to the indirect effects of the pandemic, with the schools closing three months earlier than usual. These results are in line with the social information processing network [13], which posits an earlier development of cerebral areas connected to emotions than areas connected to cognition. The higher incidence of joy and happiness in youth points toward a sense of immediate gratification, which is not mediated by the regulatory activity of the cognitive hub of the brain that could tune the emotional response to the seriousness of the situation [14].

However, the national lockdown not only implied early school closure, but also forced teenagers to stay home, abruptly interrupting their daily activities, including sports, meeting with their partners, socializing with friends, etc. This explains why, together with joy and happiness, teenagers also reported a higher prevalence of feelings of boredom and nostalgia.

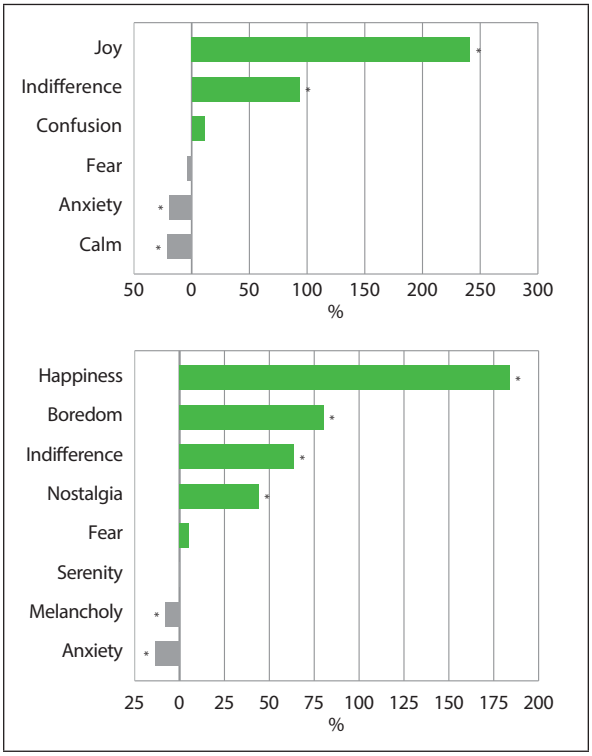

Figure 3
Feelings experienced when lockdown was announced (top panel) and during the lockdown (bottom panel). The green horizontal bars identify the feelings that were experienced more among the younger (less than 15 years of age) rather than the elder (more than 50 years of age) population, and their width corresponds to the increment (in percentage) compared to the population composed of youth and elderly. Similarly, the grey bars identify the feelings that were experienced more among the elder population. An asterisk identifies the feelings that were significantly correlated with age (Phi coefficient, p-value less than 0.01).

We believe that teenagers' feelings during the lockdown are an indicator of a stronger drive to return to the behaviors that were not allowed. Their emotional experience during lockdown might induce the violation of social distancing rules – which are crucial in containing the pandemic and preventing second or third waves – and might reinforce the tendency, typical of their age, to take imprudent behaviors that could increase morbidity [15].

Indeed, the prefrontal cortex, one of the main hubs of the cerebral circuits involved in human decisional processes, is subject to substantial changes during adolescence. Since decisional processes are inherently related to emotions and decision-making skills are only partially developed in teenagers, the additional emotional baggage associated with the lockdown could push teenagers toward riskier behaviors [16], with a less cogent judgement of the consequences of their decisions and more limited control and monitoring of their actions [17].

Teenagers could represent a vulnerable segment of the population that needs targeted measures for ensuring compliance with containment measures that are needed to halt the diffusion of the virus and prevent future waves, especially considering that the average age of infected individuals is decreasing in several countries [18, 19].

Although the pandemic is far from being defeated, from a sociopsychiatric perspective, there is an understandable desire to declare its social end, forgetting the fear and recovering the habits and behaviors of the pre-COVID-19 era [20]. How dramatic will be the second wave as teenagers are returning to school? Will the distancing measurements and guidelines that governments are setting in place worldwide be sufficient? We believe that these rules alone cannot be enough, and that public health campaigns targeted to teenagers will be crucial to proactively involve them in containing the pandemic, so that the social end of the pandemic will be as close as possible to its clinical end.

Failing to improve the risk perception and to promote prudent social behaviors and compliance of rules might lead to a further decrease in the age of infected individuals, with the youngest becoming the main carriers of the infection. The involvement of the target audience in the process of self-reflection has been proposed to be key for meaningful and sustainable changes in cognition and behavior [21]. During this pandemic, education is paramount to encourage students to become advocates for disease prevention and control in their homes, schools, and local community [22]. The survey makes a critical step in this direction by increasing students' awareness about the complexity of the moment we are living and making them active citizen scientists in the study of the COVID-19.

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Conflict of interest statement

The Authors declare no competing interests.

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REFERENCES

1. Sullivan BL, Wood CL, Iliff MJ, Bonney RE, Fink D, Kelling S. eBird: A citizen-based bird observation network in the biological sciences. Biological Conservation. 2009;142:2282-92. doi: 10.1016/j.biocon.2009.05.006
2. Laut J, Henry E, Nov O, Porfiri M. Development of a mechatronics-based citizen science platform for aquatic environmental monitoring. IEEE/ASME Transactions on Mechatronics. 2014;19:1541-51. doi: 10.1109/TMECH.2013.2287705
3. Bonney R, Shirk JL, Phillips TB, Wiggins A, Ballard HL, Miller-Rushing AJ, Parrish JK. Next steps for citizen science. Science. 2014;343:1436-7. doi: 10.1126/science.1251554
4. Holman EA, Thompson RR, Garfin DR, Silver RC. The unfolding COVID-19 pandemic: A probability-based, nationally representative study of mental health in the United States. Science Advances. 2020;6:eabd5390. doi: 10.1126/sciadv.abd5390
5. Myers KR, et al. Unequal effects of the COVID-19 pandemic on scientists. Nature Human Behaviour. 2020;4:880-3. doi: 10.1038/s41562-020-0921-y
6. World Economic Forum. The global gender gap report 2016. Switzerland: World Economic Forum; 2016. Available from: www3.weforum.org/docs/GGGR16/WEF_Global_Gender_Gap_Report_2016.pdf.
7. UN Women. UN Secretary-General's policy brief: The impact of COVID-19 on women. UN Women. 2020. Available from: www.unwomen.org/en/digital-library/publications/2020/04/policy-brief-the-impact-of-covid-19-on-women.
8. Verma R, Balhara YPS, Gupta CS. Gender differences in stress response: Role of developmental and biological determinants. Industrial Psychiatry Journal. 2001;20:4-10, doi: 10.4103/0972-6748.98407
9. Matud MP. Gender differences in stress and coping styles. Personality and Individual Differences. 2004;37:1401-15. doi: 10.1016/j.paid.2004.01.010
10. Joel D, Berman Z, Tavor I, Wexler N, Gaber O, Stein Y, Margulies DS. Sex beyond the genitalia: The human brain mosaic. Proceedings of the National Academy of Sciences of the United States of America. 2015;112:15468-73. doi: 10.1073/pnas.1509654112
11. Ingalhalikar M, et al. Sex differences in the structural connectome of the human brain. Proceedings of the National Academy of Sciences of the United States of America. 2014;111:823-8. doi: 10.1073/pnas.1316909110
12. Langer A, et al. Women and health: the key for sustainable development. The Lancet. 2015;386:1165-210. doi: 10.1016/S0140-6736(15)60497-4
13. Nelson EE, Leibenluft E, McClure EB, Pine DS. The social re-orientation of adolescence: a neuroscience perspective on the process and its relation to psychopathology. Psychological Medicine. 2005;35:163-74. doi: 10.1017/S0033291704003915
14. Yurgelun-Todd DA. Emotional and cognitive changes during adolescence. Current Opinion in Neurobiology. 2007;17:251-7. doi: 10.1016/j.conb.2007.03.009
15. Steinberg L. Risk taking in adolescence: What changes, and why? Annals of the New York Academy of Sciences. 2004;1021:51-8. doi: 10.1196/annals.1308.005
16. Powell K. How does teenage brain work? Science. 2006;442:865-7. doi: 10.1038/442865a
17. Eshel N, Nelson E, Blair RJ, Pine DS, Ernst M. Neural substrates of choice selection in adults and adolescents: development of ventrolateral prefrontal and anterior cingulate cortices. Neuropsychologia. 2007;45:1270-9. doi: 10.1016/j.neuropsychologia.2006.10.004
18. Epicentro. COVID-19 integrated surveillance: key national data. Istituto Superiore di Sanità; 2020. Available from: https://www.epicentro.iss.it/en/coronavirus/sars-cov-2-integrated-surveillance-data.
19. CNBC. Dr. Anthony Fauci says the average age of US coronavirus patients has dropped by 15 years as Sun Belt states gets hit. CNBC; 2020. Available from: www.cnbc.com/2020/07/06/dr-anthony-fauci-says-the-average-age-of-us-coronavirus-patients-has-dropped-by-15-years-as-sun-belt-states-gets-hit.html.
20. Murray SL. Fear and the front line. New England Journal of Medicine. 2020;382:1784-5. doi: 10.1056/NEJMp2003868
21. Greene K. The theory of active involvement: Processes underlying interventions that engage adolescents in message planning and/or production. Health Communication. 2013;28:644-56. doi: 10.1080/10410236.2012.762824
22. Unicef, World Health Organization. Key messages and actions for COVID-19 prevention and control in schools. UNICEF; 2020. Available from: www.who.int/docs/default-source/coronaviruse/key-messages-and-actions-for-covid-19-prevention-and-control-in-schools-march-2020.pdf?sfvrsn=baf81d52_4.

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YOUNG PEOPLE'S HEALTH IN ITALY: DATA FROM THE HEALTH BEHAVIOUR IN SCHOOL-AGED CHILDREN (HBSC) SURVEY 2018 AND SUGGESTIONS FOR ACTION

Edited by Paola Nardone, Silvia Ciardullo and Angela Spinelli

Preface

Paola Nardone, Silvia Ciardullo and Angela Spinelli

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Adolescence is often considered the healthiest time of life. It is a time when many aspects of good health are at their peak and adolescents appear to have fewer needs of health services than children or the elderly [1]. At the same time, a fast physical, emotional, cognitive, and social development can be observed during this stage of life. In particular, behaviours established during adolescence, affecting issues such as mental health, tobacco use, diet, physical activity levels, social media disorders and alcohol use, can persist in adult life. The wellbeing of young people is shaped by the complex interaction between a range of individual, behavioural, social, cultural, environmental and organizational factors changing over time. Understanding how these factors interact one with another is an important step to promote young people's health and wellbeing in adulthood [2, 3].

The Health Behaviour in School-aged Children (HBSC), a World Health Organization (WHO) collaborative cross-national study, is an international research study that aims to increase understanding of adolescent health behaviours and their social determinants, particularly the settings of family, peers and school, across different countries. HBSC was launched in the early 1980s and now involves 50 countries and regions across Europe and North America [4]. To ensure the cross-national comparability, all participant member countries adhere to a common international standard protocol, developed and systematically updated by the entire HBSC network through topic-based groups. Data from each wave of the Italian HBSC study were shared with an International Coordination Group and analyzed together with the results from the other countries of the HBSC network [5, 6].

Italy joined the HBSC international network in the year 2000 and, to date, five data collections (2002, 2006, 2010, 2014, 2018) have been performed; since 2010 each survey was promoted and funded by the Ministry of Health, coordinated with the Universities of Torino, Padova and Siena and supported by the Ministry of Education [7, 8]. In 2010, the responsibility for the coordination of Italian HBSC survey was extended to the Italian National Institute of Health (Istituto Superiore di Sanità). From then, the HBSC became the first Italian population-based survey on adolescent behaviours, representative at national and regional level and involving more than 65,000 students (aged 11, 13 and 15 years) every 4 years [9, 10].

Since 2017, an Italian Legislative Decree recognized HBSC as the only national surveillance of adolescence health; the responsibility for its coordination was given to the Italian National Institute of Health [11]. Hundreds of health workers and school staff are involved in the local organization and collection of data.

The monograph presented in this number of the *Annali dell'Istituto Superiore di Sanità* consists of four articles presenting the major results of the Italian HBSC 2018 survey on adolescents' eating habits, sexual behaviours, alcohol use and social media disorders. Each article also includes a comparison between Italian data and those of other countries participating in HBSC study. Furthermore, the details of the methodological protocol of the HBSC study are reported in Nardone *et al.*, *Appendix 1*.

The four articles are summarized below:

Nardone *et al.* describe dietary habits (i.e. consumption of breakfast, fruit, vegetables, legumes and carbonated-sugary drinks) and their possible association with geographical and socio-demographic characteristics among adolescents aged 11, 13 and 15 years in Italy.

Marino *et al.* focus on the prevalence of problematic social media use across Italian regions by age groups (11, 13 and 15 years) and gender and evaluate whether this increases the levels of health complaints (psychological and somatic symptoms).

Borraccino *et al.* investigate, among 15-year-olds, the role of different forms of social support in early sexual intercourse and contraceptive use.

In the same age groups, Charrier *et al.* draw a com-

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prehensive picture of the behavioural, social and psychological patterns of alcohol use and abuse.

In conclusion, each author enriched the knowledge of adolescents' health and emphasized the importance of this surveillance system to link knowledge and action to promote healthy behaviours in young people.

REFERENCES

1. Patton GC, Sawyer SM, Santelli JS, et al. Our future: a *Lancet* commission on adolescent health and wellbeing. Lancet. 2016;387(10036):2423-78.
2. Gore FM, Bloem PJ, Patton GC, et al. Global burden of disease in young people aged 10-24 years: a systematic analysis [published correction appears in Lancet. 2011 Aug 6;378(9790):486]. Lancet. 2011;377(9783):2093-102.
3. World Health Organization. Health for the World's Adolescents: a second chance in the second decade. WHO; 2014. Available from: www.who.int/maternal_child_adolescent/documents/second decade/en/.
4. Health Behaviour In School-Aged Children. Available from: /www.hbsc.org/.
5. Inchley J et al. (Eds). Growing up unequal: gender and socioeconomic differences in young people's health and well-being. Health Behaviour in School-aged Children (HBSC) study: international report from the 2013/2014 survey. Copenhagen; WHO Regional Office for Europe: 2016 (Health Policy for Children and Adolescents, No. 7).
6. Inchley J, Currie D, Budisavljevic S, Torsheim T, Jåstad A, Cosma A et al. (Eds). Spotlight on adolescent health and well-being. Findings from the 2017/2018 Health Behaviour in School-aged Children (HBSC) survey in Europe and Canada. International report. Volume 1. Key findings. Copenhagen: WHO Regional Office for Europe; 2020.
7. Stili di vita e salute dei giovani italiani 11-15 anni. Rapporto sui dati italiani dello studio internazionale HBSC 2001-2002. Available from: www.epicentro.iss.it/hbsc/pdf/Stili%20di%20vita%20e%20salute%20nei%20giovani%20italian%20-%202002.pdf.
8. Stili di vita e salute nei giovani italiani tra 11 e 15 anni. II rapporto sui dati italiani dello studio internazionale HBSC 2006. Available from: www.epicentro.iss.it/hbsc/pdf/Stili%20di%20vita%20e%20salute%20nei%20giovani%20Italian%20-%202006.pdf.
9. Cavallo F, Giacchi M, Vieno A, Galeone D, Tomba A, Lamberti A, Nardone P, Andreozzi S (Ed.). Studio HBSC-Italia (Health Behaviour in School-aged Children): rapporto sui dati 2010. Roma: Istituto Superiore di Sanità; 2013. (Rapporti ISTISAN 13/5).
10. Cavallo F, Lemma P, Dalmasso P, Vieno A, Lazzeri G, Galeone D. Report nazionale dati HBSC Italia 2014. Available from: www.epicentro.iss.it/hbsc/pdf/Report%20nazionale%20dati%20HBSC%20Italia%202014.pdf.
11. Italia. Decreto del Presidente del Consiglio dei Ministri, 3 marzo 2017. Identificazione dei sistemi di sorveglianza e dei registri di mortalità, di tumori e di altre patologie. Gazzetta Ufficiale – Serie Generale n. 109, 12 maggio 2017.

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Dietary habits among Italian adolescents and their relation to socio-demographic characteristics

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Abstract

Objective. The aim is to describe dietary habits and their association with socio-demographic characteristics in a large nationally representative sample of Italian adolescents aged 11, 13 and 15 years.

Materials and methods. Data from the 2018 Italian Health Behaviour in School-aged Children (HBSC) survey on 58,976 adolescents were analysed to determine eating habits. Logistic regression was used to investigate the association between incorrect dietary habits and potential predictors.

Results. 38.3% of boys and 48.1% of girls skipped breakfast and 54.1% did not consume fruit and/or vegetables daily. 15.9% of boys and 11.3% of girls drank carbonated-sugary beverages at least once a day. Incorrect dietary habits were more common among boys, adolescents with lower socio-economic conditions, residents in Southern Italy and those spending more time watching TV. Italian adolescents were more likely to have incorrect dietary habits compared with those from most other countries involved in 2018 HBSC.

Conclusions. Action is needed to improve dietary habits among adolescents.

Key words

- eating behaviour
- beverage
- vegetables
- fruit
- breakfast

INTRODUCTION

Adolescence is a critical time for adopting lifelong health behaviours [1, 2]; different habits, either positive or negative, are established during this period of life and health-related behaviours adopted by young people may persist throughout adulthood [3]. Unhealthy eating habits, insufficient physical activity, smoking, alcohol and substance abuse are some of the negative behaviours that may be observed among adolescents.

In particular, incorrect dietary habits, reduction of physical activity and time spent playing outside may lead to a higher prevalence of overweight and obesity in children and adolescents [4-6]. In addition, screen media exposure is another factor that might contribute to increase the risk of incorrect eating habits characterized by a high consumption of energy-dense foods and sweetened drinks during the viewing. Furthermore, low-nutrient food and beverage marketing can influence adolescent food preferences, purchases and consumption [5, 7, 8].

Diet plays an important role in the quality of life not only during adolescence but also through adult life. Unhealthy eating habits, in addition to other several environmental and genetic factors, might contribute to the development of overweight, obesity and diet-related non-communicable diseases [9-11]. In order to encourage healthy dietary choices by young people, the knowledge of their dietary habits is essential to promote innovative policies and focused actions. This knowledge can be obtained by surveillance and monitoring initiatives [12, 13].

The World Health Organization (WHO) recommends for adolescents a daily consumption of five portions (400 grams) of fruit and vegetables [5], but adolescent diets are often low in fruit and vegetables. A meta-analysis conducted between 2003 and 2011 found that in low/middle-income countries 74% of adolescents aged 12 to 15 years consumed fruit and vegetables less than 5 times per day [14]. The recent international report on 2018 Health Behaviours in School-aged Children (HBSC) survey stated that the daily consumption of fruit and vegetables among European adolescents

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aged 11, 13 and 15 years was for all age groups 40% and 38%, respectively [15].

Starting the day with breakfast is a basic dietary recommendation especially for children and adolescents [16]. Breakfast is considered the "most important meal of the day" and recent scientific reviews addressed many aspects related to its essential role in people diet and its possible impact on body weight control, as well as on other physiological, social and cultural aspects [17]. Breakfast consumption is positively associated with an adequate nutrient intake in children and adolescents [18]. In contrast, skipping breakfast in young people is linked with higher adiposity measures as found in both cross-sectional and longitudinal studies [19, 20]. Findings from the international 2018 HBSC report showed a significant difference between gender prevalence of breakfast consumption on every school day among 11-15 year old European adolescents (61% boys and 55% girls) [15].

Adolescent diet often involves high intakes of energy-dense and nutrient-poor foods, including sweet and salty snacks, sugar-sweetened beverages (SSB) and fast foods [21]. A recent review of sugar consumption in Europe and North America highlighted that the adolescent diet had a higher amount of added sugars than that of any other age-group [22]. As reported by the 2018 HBSC investigation, one in four (25%) European adolescents aged 11, 13 and 15 years consume sweet chocolates daily. Additionally, 16% of adolescents, between ages 11 and 15, participating in the HBSC international survey, claimed to consume sugared soft drinks daily [15].

A wide variety of social and economic factors is associated with eating patterns and behaviours of adolescents. These include peer influence, parental modelling, food availability, food preferences, cost, convenience, personal and cultural beliefs, mass media, body image, socio-economic status, family structure, parents' education and occupation. These factors might contribute to unhealthy diets and poor nutrition especially for the most vulnerable groups [23-27]. In order to adopt and implement appropriate policies and measures to promote healthy food choices as well as healthier lifestyles among young people, it is essential to understand how these factors can influence their eating habits [12, 13].

The aim of this study was to describe dietary habits (i.e. consumption of breakfast, fruit, vegetables, legumes and carbonated-sugary drinks) and their possible association with geographic and socio-demographic characteristics (i.e. Family Affluence Scale – FAS, family meals, parents' educational level) in a large nationally representative sample of Italian adolescents aged 11, 13 and 15 years. Furthermore, in the discussion these Italian data were compared with those from other European and North American countries involved in the international 2018 HBSC survey [15].

MATERIALS AND METHODS

In 2018, a survey was conducted in all Italian Regions on students (11, 13 and 15 year-old) in the framework of the international Health Behaviour in School-aged Children study. Target classes were the first and third grade of middle school and the second grade of high school. To all subjects attending the sampled classes a questionnaire was administered to collect information on their dietary habits, physical activity, risk behavior and well-being, their relationship with the school, parents and peers as well as general information concerning their health and social background. A stratified cluster sample design, with class as the primary sampling unit, was used (see *Appendix 1*).

In the self-completed anonymous questionnaire, students were asked to indicate parents' educational level and country of birth. For these analyses the highest educational level between the two parents was considered and three educational levels were taken into account: "low" (both parents with less than high school), "medium" (at least one of the parents with high school) and "high" (one of the parents with university degree or higher); a fourth category, regarding the answer "don't know", was also considered.

Country of birth was categorized into "both Italians", "at least one foreign parent" and "both foreigners".

The socio-economic position of the students' families was measured according to the FAS described in *Appendix 1*.

The numbers of hours spent using TV/Tablet/PC were calculated by adding up hours spent watching television (including videos and DVDs) on weekdays and on the weekend (possible responses were "never" to "about 7 or more hours a day") and hours spent playing on electronic devices (possible responses were "never" to "about 7 or more hours a day"). This sum was recoded into "≤2 hours a day" *vs* ">2 hours a day".

For nutritional habits, the frequency of family meals (possible responses were every day/most days/ about once a week/less than once a week/never) was dichotomized into "every day" *vs* "less than every day". Students were asked to indicate how many times they had breakfast (defined as having more than a glass of milk or fruit juice) during schooldays (possible responses were "never" to "five days") and during weekend (possible responses were "never" to "both days").

In the analysis, only breakfast consumption during school days was considered because it was assumed that during weekend days this would be different. Breakfast consumption on weekdays was dichotomized into "daily breakfast" (five days a week) and "no daily breakfast" (less than five days a week).

Students also reported how many times per week they consumed fruit, vegetables, legumes (i.e. peas, chickpeas, beans) and carbonated-sugary drinks (possible responses: never/less than once a week/once a week/ two to four times a week/five to six times a week/once a day/more than once a day). Consumption of fruit and vegetables was dichotomized into "at least once a day" *vs* "less than once a day"; in the logistic regression models the consumption of fruit and vegetable were combined and dichotomized into "fruit or vegetables at least once a day" *vs* "neither fruit nor vegetables at least once a day". Legume consumption was categorized as "at least twice a week" or "less than twice a week" and carbonated-sugary drinks as "less than once a day" *vs* "at least once a day".

Using the above 5 dietary indicators a global score was created by counting the number of correct habits, ranging from 0 to 5: daily breakfast (yes = 1, no = 0), consumption at least once a day of fruit (yes =1, no = 0) consumption at least once a day of vegetables (yes = 1, no = 0), consumption at least twice a week of legumes (yes = 1, no = 0) and consumption less than once a day of carbonated-sugary drinks (yes = 1, no = 0); this global score was dichotomized into "less than 3" *vs* "at least 3" correct habits.

Logistic regression models for dietary indicators were fitted to assess the association with socio-demographic and behavioural characteristics. The likelihood of unhealthy eating habits was described by odds ratios (OR) with a 95% confidence interval (CI). Stata software version 16.1 was used for all statistical analyses. Missing data were excluded from the analysis.

RESULTS

The Italian HBSC 2018 survey included 64,929 students from 4,183 selected classes. After data cleaning and applying the inclusion criteria, 58,976 students' data were eligible for analysis. *Table 1* shows the main characteristics of the sample. For each age group, the male and female ratio is 1:1 (male: 29,820 *vs* female: 29,156). Almost half of the sample were resident in the Northern regions (boys: 45.0%; girls: 46.2%), a third in the Southern Regions (boys: 37.3%; girls: 36.4%) and the remaining in Central Italy (boys: 17.7%; girls: 17.4%).

The majority of adolescents (boys: 86.7%; girls: 87.0%) reported to have both Italian parents, followed by students who had both foreign parents (boys: 7.8%; girls: 7.5%) and 5.5% of boys and girls with one foreign parent. More than one third of teenagers (boys: 35.8%;

Table 1
Socio-demographic and behavioural characteristics of the sample by age and gender. Italy, 2018

Sample characteristics*	11 years old N = 19,504		13 years old N = 20,554		15 years old N = 18,918		All age group N = 58,976	
	Boys (%)	Girls (%)	Boys (%)	Girls (%)	Boys (%)	Girls (%)	Boys (%)	Girls (%)
Residence area								
North	48.5	49.2	46.5	46.2	37.9	42.5	45.0	46.2
Centre	17.2	17.8	17.1	17.6	19.4	16.7	17.7	17.4
South	34.3	33.0	36.4	36.2	42.7	40.8	37.3	36.4
Missing	-	-	-	-	-	-	-	-
Parents' country of birth								
Both Italians	85.9	86.0	85.8	86.7	88.9	88.7	86.7	87.0
One foreign parent	5.4	5.1	5.8	5.7	5.1	5.8	5.5	5.5
Both foreigners	8.7	8.9	8.4	7.6	6.0	5.5	7.8	7.5
Missing	4.9	4.9	4.2	3.5	3.1	2.2	4.2	3.7
Parents' educational level**								
Low level of education	7.0	6.1	11.2	13.1	14.1	14.2	10.4	10.9
Medium level of education	21.7	21.9	42.1	44.3	46.2	47.5	35.8	37.3
High level of education	26.9	28.1	30.4	28.5	29.3	30.4	28.8	28.9
Don't know	44.4	43.9	16.3	14.1	10.4	7.9	25.0	22.9
Missing	5.6	5.3	3.0	2.1	4.8	2.7	4.4	3.4
Family Affluence Scale (FAS)								
Low	28.0	29.7	27.5	30.1	29.8	31.3	28.3	30.3
Medium	47.0	47.8	46.8	47.1	47.9	47.9	47.2	47.6
High	25.0	22.5	25.7	22.8	22.3	20.8	24.5	22.1
Missing	3.5	2.7	3.6	1.9	2.8	1.6	3.3	2.1
Hours "screen time" daily								
<= 2 hours	45.5	54.3	33.2	42.4	31.6	40.7	37.3	46.2
> 2 hours	54.5	45.7	66.8	57.6	68.4	59.3	62.7	53.8
Missing	3.5	2.5	3.3	1.5	2.8	1.7	3.2	1.9
Family meal every day								
Yes	55.9	55.9	59.1	57.0	56.9	54.3	57.3	55.8
No	44.1	44.1	40.9	43.0	43.1	45.7	42.7	44.2
Missing	0.9	0.9	0.6	0.3	0.5	0.3	0.7	0.5

*For each variable, percentages are calculated on the total of subjects excluding those with missing information. Percentage of those missings are calculated on the total.
**The highest educational level between the two parents.

girls: 37.3%) reported at least one parent with a medium level of education, one third (boys: 28.8%; girls: 28.9%) at least one parent with high level of education and the remaining (boys: 10.4%; girls: 10.9%) both parents with low educational level. About a quarter of the respondents did not know the educational level of their parents; this aspect was highest for 11-year-old and followed a decreasing trend with age. The majority of adolescents had a medium FAS level (boys: 47.2%; girls: 47.6%) and about one-third a low FAS level.

There were some differences between the adolescents' use of TV or other devices: more than one half (boys: 62.7%; girls: 53.8%) spent more than two hours per day watching television or other devices. This habit is more common among boys than girls for all age groups. More than one half of adolescents (boys: 57.3%; girls: 55.8%) said to consume at least one family meal every day.

Data quality in terms of completeness was good; missing values were less than 6% for each of the considered variables.

Dietary habits

The prevalence rates of dietary indicators by age and gender are presented in *Table 2*. Overall, more than one half of the adolescents (boys: 61.7%; girls: 51.9%) reported to consume breakfast every school day, while 38.3% of boys and 48.1% of girls skipped breakfast, a bad habit that increased with age. More than a half of the sample (boys: 67.5%; girls: 61.1%) reported not to eat fruit every day and this habit was more common among those who are 15-years old compared to the younger age groups.

77.9% of boys and 67.5% of girls did not eat vegetables daily and this was constant among age groups and the values were higher among boys. Overall, the percentage of adolescents that reported no daily consumption of fruit and/or vegetables was 54.1%.

About one-half of the adolescents (boys: 48.3%; girls: 51.1%) consumed legumes at least twice a week; this slightly increased with age and was more common among girls for all age groups.

15.9% of boys and 11.3% of girls drank carbonated-sugary beverages at least once a day, with lower percentages among girls for all age groups.

Considering these 5 dietary indicators, the adolescents who had less than 3 correct dietary habits were 40.3% at eleven years, 42.8% at thirteen years and 43.8% at fifteen years; the proportions were slightly higher among boys than girls.

Multivariate analyses

The results of the logistic regression model applied to the dietary habits are reported in *Table 3*. The risk of not consuming breakfast daily on school days significantly increased with age (13 years: OR = 1.36, 95% CI = 1.24-1.48; 15 years: OR = 1.49, 95% CI = 1.36-1.62). In addition, this risk is higher for girls (OR = 1.54, 95% CI = 1.44-1.64) and for those who live in the Central (OR = 1.12, 95% CI = 1.03-1.22) and Southern regions (OR = 1.70, 95% CI = 1.57-1.85). Also, the risk was found to be higher for those with both parents foreign (OR = 1.23, 95% CI = 1.09-1.40) and for adolescents who spend more than 2 hours a day watching TV or other devices (OR = 1.24, 95% CI = 1.16-1.32).

Geographical area of residence was associated with the consumption of fruit and/or vegetables. In detail, the results showed that students from Central and Southern regions were more likely to not consume fruit or vegetables every day than those from the North (Centre: OR = 1.10, 95% CI = 1.02-1.19; South: OR = 1.43, 95% CI = 1.33-1.55). A higher risk was also observed among adolescents that spend more than 2 hours a day watching TV or other devices (OR = 1.37, 95% CI = 1.29-1.45) and among students aged 13-15 years (13 years: OR = 1.12, 95% CI = 1.03-1.22; 15 years: OR = 1.17, 95% CI = 1.07-1.27).

The risk of daily consumption of carbonated-sugary drinks is higher among adolescents that live in the Southern regions (OR = 1.48, 95% CI = 1.31-1.67) compared with those from the Central and Northern ones and among adolescents who spend more than 2 hours a day watching TV or other devices (OR = 1.69, 95% CI = 1.53-1.86).

The consumption of legumes at least twice a week significantly increased with age and parents' educational level.

In general medium-high parents' educational level, in addition to medium-high family FAS and family meal consumption, were positively associated with some correct habits: daily consumption of fruit/vegetables and breakfast; weekly consumption of legumes; low intake of carbonated-sugary drinks.

The model of the score of correct dietary habits shows that adolescents who are more likely to have a bad diet (less than 3 correct dietary habits) spent more than 2 hours a day watching TV or other devices (OR = 1.55, 95% CI = 1.45-1.65) and are residents in Southern Italy (OR = 1.24, 95% CI = 1.14-1.34). On the contrary, adolescents who were more likely to have a better diet were characterized by higher educated parents (high educational level: OR = 0.51, 95% CI = 0.45-0.57) and a major FAS (high FAS: OR = 0.75, 95% CI = 0.69-0.82). Additionally, eating a meal with the family every day is positively associated with having a better diet (OR = 0.83, 95% CI = 0.78-0.88).

DISCUSSION

The results show that dietary habits in some Italian adolescents are not in line with the Dietary Guidelines [5, 28]. In detail, 4 out of 10 adolescents skipped breakfast, more girls than boys; 7 out of 10 adolescents did not consume vegetables daily and 1 in 2 did not consume both fruit and vegetables daily. Approximately 1 in 7 consumed carbonated-sugary beverages at least once a day and a lower percentage was found among girls for all age groups. Considering the total number of correct dietary habits, adolescents who had less than 3 correct dietary habits were 40.3% at eleven years, 42.8% at thirteen years and 43.8% at fifteen years; boys had higher prevalence of incorrect dietary habits than girls.

Comparing these findings to other research, it can be observed that most of the studies reported that at least 10-30% of children and adolescents never eat breakfast [29]. Additionally, an increasing prevalence was noticed

Table 2
Dietary indicators by age and gender. Italy, 2018

Dietary habits*	11 years old N = 19,504		13 years old N = 20,554		15 years old N = 18,918		All age group N = 58,976	
	Boys (%)	Girls (%)	Boys (%)	Girls (%)	Boys (%)	Girls (%)	Boys (%)	Girls (%)
Breakfast daily								
Yes	64.8	60.5	61.6	48.1	57.5	45.8	61.7	51.9
No	35.2	39.5	38.4	51.9	42.5	54.2	38.3	48.1
Missing	3.8	2.6	2.2	1.7	1.6	1.2	2.6	1.9
Fruit at least once a day								
Yes	35.6	41.0	31.8	38.2	29.2	37.1	32.5	38.9
No	64.4	59.0	68.2	61.8	70.8	62.9	67.5	61.1
Missing	0.5	0.4	0.4	0.2	0.2	0.1	0.4	0.2
Vegetables at least once a day								
Yes	23.1	31.1	22.2	33.0	20.7	33.7	22.1	32.5
No	76.9	68.9	77.8	67.0	79.3	66.3	77.9	67.5
Missing	0.6	0.4	0.6	0.2	0.3	0.1	0.5	0.3
Fruit/Vegetables at least once a day								
Yes	44.0	52.4	40.5	50.5	37.1	50.0	40.9	51.1
No	56.0	47.6	59.5	49.5	62.9	50.0	59.1	48.9
Missing	0.7	0.5	0.6	0.2	0.3	0.1	0.6	0.3
Legumes at least twice a week								
Yes	43.9	47.2	49.0	52.3	53.2	54.6	48.3	51.1
No	56.1	52.8	51.0	47.7	46.8	45.4	51.7	48.9
Missing	1.1	1.0	1.2	0.8	0.7	0.4	1.4	0.7
Carbonated-sugary drinks less than once a day								
Yes	83.8	87.5	84.1	88.4	84.3	90.4	84.1	88.7
No	16.2	12.5	15.9	11.6	15.7	9.6	15.9	11.3
Missing	0.6	0.4	0.4	0.1	0.3	0.1	0.5	0.2
Correct dietary habits								
Less than 3	42.8	37.7	43.2	42.3	45.5	42.3	43.7	40.7
At least 3	57.2	62.3	56.8	57.7	54.5	57.7	56.3	59.3
Missing	5.5	3.5	3.5	2.6	2.2	1.7	3.9	2.7
Number of correct dietary habits								
0	1.9	1.5	2.2	1.9	2.0	1.7	2.0	1.7
1	13.2	11.5	11.9	12.4	12.3	10.9	12.5	11.7
2	27.7	24.8	29.2	28.0	31.2	29.7	29.2	27.3
3	31.7	31.0	32.4	28.1	32.5	28.0	32.2	29.0
4	17.6	20.2	17.3	19.7	14.9	19.2	16.7	19.8
5	7.9	11.0	7.0	9.9	7.1	10.5	7.4	10.5
Missing	4.5	3.5	3.5	2.6	2.2	1.7	3.9	2.7

*For each variable, percentages are calculated on the total of subjects excluding those with missing information. Percentage of those missings are calculated on the total.

in adolescents, mainly in girls [29, 30]. Furthermore, since 2014, a significant decline in daily breakfast consumption was observed in most countries, including Italy [15, 31].

The determinants of fruit and vegetable intake among adolescents are numerous: gender, age, parental vegetable and fruit consumption, and the availability/accessibility of these foods at home. In addition, girls tend to have a higher or more frequent intake of fruit and vegetables than boys [32, 33]. In general our findings suggest that the daily frequency of fruit and vegetables consumption was very low among Italian young people.

Carbonated-sugary beverages are the leading source

Table 3
Logistic regression models for dietary indicators. Italy, 2018

Indipendent variables	No daily breakfast N = 57,874			Fruit/Vegetables less than once a day N = 58,708			Legumes less than twice a week N = 58,355			Carbonated-sugary drinks at least once a day N = 58,746			Less than 3 correct dietary habits N = 57,308		
	Prev	OR*	95% CI	Prev	OR*	95% CI	Prev	OR*	95% CI	Prev	OR*	95% CI	Prev	OR*	95% CI
Age															
11 years	37.3	1		51.9	1		54.5	1		14.4	1		40.3	1	
13 years	45.0	1.36	(1.24-1.48)	54.6	1.12	(1.03-1.22)	49.3	0.83	(0.76-0.91)	13.8	0.94	(0.83-1.07)	42.8	1.09	(1.00-1.20)
15 years	48.4	1.49	(1.36-1.62)	56.4	1.17	(1.07-1.27)	46.1	0.76	(0.69-0.83)	12.6	0.86	(0.75-0.98)	43.8	1.12	(1.03-1.23)
Gender															
Males	38.3	1		59.1	1		51.7	1		15.9	1		43.7	1	
Females	48.1	1.54	(1.44-1.64)	48.9	0.67	(0.62-0.71)	48.9	0.91	(0.86-0.97)	11.3	0.67	(0.61-0.74)	40.7	0.91	(0.85-0.97)
Residence area															
North	37.8	1		49.8	1		57.8	1		11.8	1		39.7	1	
Centre	40.9	1.12	(1.03-1.22)	52.6	1.10	(1.02-1.19)	51.4	0.76	(0.69-0.82)	12.1	1.03	(0.91-1.17)	39.7	0.97	(0.89-1.05)
South	51.0	1.70	(1.57-1.85)	60.2	1.43	(1.33-1.55)	40.5	0.47	(0.43-0.51)	16.7	1.48	(1.31-1.67)	46.5	1.24	(1.14-1.34)
Parents' country of birth															
Both Italians	42.9	1		54.5	1		50.5	1		13.2	1		42.2	1	
One foreign parent	42.0	1.03	(0.91-1.17)	52.9	0.94	(0.83-1.08)	51.0	0.97	(0.85-1.11)	13.2	1.10	(0.91-1.32)	40.8	0.97	(0.85-1.11)
Both foreigns	45.7	1.23	(1.09-1.40)	49.7	0.76	(0.67-0.87)	48.3	0.69	(0.61-0.78)	18.2	1.38	(1.17-1.62)	42.0	0.86	(0.76-0.98)
Parent's educational level**															
Low level of education	52.0	1		64.6	1		47.7	1		22.2	1		53.5	1	
Medium level of education	44.9	0.87	(0.79-0.97)	56.0	0.77	(0.69-0.86)	49.0	0.88	(0.79-0.98)	12.8	0.60	(0.52-0.68)	43.1	0.72	(0.65-0.80)
High level of education	39.1	0.75	(0.67-0.84)	45.3	0.54	(0.48-0.61)	47.5	0.78	(0.69-0.87)	8.5	0.40	(0.34-0.47)	33.1	0.51	(0.45-0.57)
Don't know	41.7	0.92	(0.82-1.03)	56.4	0.87	(0.77-0.99)	56.0	1.03	(0.91-1.16)	17.3	0.82	(0.70-0.96)	46.4	0.89	(0.79-1.00)
Family Affluence Scale (FAS)															
Low	48.2	1		60.1	1		48.2	1		17.0	1		48.2	1	
Medium	42.3	0.89	(0.83-0.96)	54.3	0.89	(0.82-0.96)	51.1	1.04	(0.96-1.13)	12.4	0.85	(0.77-0.95)	41.6	0.86	(0.80-0.93)
High	38.9	0.84	(0.76-0.92)	46.3	0.70	(0.64-0.77)	50.8	1.01	(0.93-1.10)	11.6	0.92	(0.80-1.06)	35.6	0.75	(0.69-0.82)
Hours "screen time" daily															
≤ 2 hours	39.4	1		48.6	1		47.7	1		10.0	1		35.3	1	
> 2 hours	45.8	1.24	(1.16-1.32)	58.0	1.37	(1.29-1.45)	51.9	1.25	(1.17-1.33)	16.0	1.69	(1.53-1.86)	46.9	1.55	(1.45-1.65)
Family meals															
Less than every days	44.9	1		57.2	1		52.0	1		12.0	1		44.2	1	
Every days	41.9	0.83	(0.78-0.89)	51.6	0.75	(0.71-0.79)	49.0	0.94	(0.89-1.00)	14.8	1.26	(1.15-1.37)	40.5	0.83	(0.78-0.88)

*Adjusted Odds Ratio for all variables list in the Table 3.
**The highest educational level between the two parents.

of added sugars in adolescents' diet; sugar-containing beverages and free sugars may increase the risk for overweight, obesity and dental caries as well as result in poor nutrient supply and decrease dietary diversity. For these reasons, it is especially important to avoid or limit free sugars in infants and obese/overweight children/adolescents [34-36]. In Italy, a "sugar tax" should be applied to carbonated-sugary beverages from 1st October 2020.

In accordance with other studies [37-40], our results underlined that high consumption of carbonated-sugary beverages as well as low intake of fruit and vegetables were associated with use of TV or other devices for more than 2 hours per day.

High Family Affluence and parents' high educational level were linked to better eating habits. These results are confirmed by several studies; in particular, socio-economically disadvantaged people showed more difficulties to change unhealthy behaviours since their environments offer fewer opportunities and a diet consisting of healthy foods is generally more expensive [41, 42].

On the contrary, family support as well as the habit to consume meal with family every day have been report-

ed to be positively associated with a better diet among adolescents and these behaviours are also evident for Italian young people [43].

The risk of having a less than 3 correct dietary habits was higher among the adolescents living in the Southern Italy in comparison to that found for the Northern and Central regions. This geographic gradient is also observed for other health indicators in Italian adolescents and children [4].

Eating habits: Italian HBSC results 2018 vs *International HBSC results 2018*

Dietary habit data obtained from Italian HBSC 2018 study were compared to those from International HBSC 2018 results, which involved 45 countries (including Italy).

Overall International HBSC 2018 data report that the consumption of breakfast every school day was more prevalent among boys than girls and younger adolescents. Concerning to the percentage of adolescents who did not consume breakfast every school day, Italy is in an intermediate position in the ranking of the 45 countries participating to the International HBSC 2018 study (29th place, 21st place and 19th place respectively for 11, 13 and 15 years old).

The results on fruit and vegetable daily consumption showed a lower intake among Italian adolescents compared to the other 45 countries: Italy ranks respectively at 45th, 41st and 36th for the consumption of vegetables among 11, 13 and 15 years old and respectively at 42th, 32th and 16th for 11, 13 and 15 years old for the consumption of fruits.

In comparison with the same international framework, rankings for a meal with family every day for Italian adolescents were: 21st, 17th and 10th for 11, 13 and 15 years, respectively. The International HBSC average showed a decrease of having meal with family every day among older adolescents.

The overall findings indicate that actions to improve healthy dietary habits among young people should be undertaken. In particular, the consumption of fresh fruit and vegetables as well as breakfast every day, in addition to decrease of routine consumption of nutrient-poor foods high in sugars, should be encouraged among both Italian and European adolescents. As reported by WHO, the taxation of sugar-sweetened beverages might be an important action to reduce sugar consumption among young people [44]. The importance of dietary factors to gain health and well-being for every age group of the population has been indicated by the WHO. In the "European Food and Nutrition Action Plan 2015-2020" report, the WHO suggested possible actions that governments should implement to improve the quality of life, such as the creation of healthy food and drink environments, promotion of a balanced and healthy diet by a life course approach taking into account population differences as well as the vulnerability of some groups [13]. In addition, schools can play an important role to encourage healthy food choice by promoting education and restricting the availability of unhealthy foods in school context [45].

STRENGTHS AND LIMITATIONS

The main strength of this study was the use of a large and representative Italian sample to investigate the association between dietary habits of adolescents and social-demographic characteristics with a low percentage of missing values.

However, the questionnaire used in HBSC study does not permit to characterize food consumption patterns by adolescents and portion sizes as well.

Moreover, the parental country of birth and the educational level was reported by the adolescents who, sometimes, did not know the answer to these questions. The HBSC methodology strengths and limitations were described in *Appendix 1*.

CONCLUSIONS

A healthy diet plays a crucial role in the quality of life during adolescence and also into the adult life. Data of this study underline the need to encourage healthy food consumption and the implementation of policies at national and local level. The policy-makers and stakeholders should use the survey's data to promote a cultural change to a healthy diet among all population groups, particularly children and adolescents.

APPENDIX 1. THE HBSC METHODOLOGY

Background

Data were collected as part of the 2018 Health Behaviour in School-aged Children (HBSC) study. HBSC is a World Health Organisation (WHO) Collaborative Cross-National Survey of school students, collecting data every four years on well-being, social environments and health behaviours in early adolescence (aged 11, 13, and 15 years). HBSC 2018 survey includes data from 45 countries across Europe and North America, all adhering to a detailed international study protocol [1, 2].

Italy joined HBSC international network in 2000 and has carried out five data collections (2002, 2006, 2010, 2014, 2018), promoted and funded by the Ministry of Health, and coordinated by the Universities of Torino, Padova and Siena, with the support of Ministry of Education and the Italian regions [3]. Since 2017, the Prime Minister's Decree on "registers and surveillance" has included the Surveillance of behavioural risks at 11-17 years of age among the Surveillance Systems of national and regional relevance, coordinated by the Italian National Institute of Health, with the collaboration of Universities of Torino, Padova and Siena [4].

Methods

Sampling procedures

The sampling procedure adopted in Italy has followed the rules agreed internationally. Class is the primary sampling unit, drawn by systematic cluster sampling of all public schools throughout the Italian regions on the behalf of the Ministry of Education, allowing a national and regional representative sample of youths aged 11, 13, and 15 years. In 2018, the Italian HBSC survey included around 4,100 classes and 85,000 students: the response rates were 86% of all sampled classes and 97% of students.

Data collection

Data were collected using the international questionnaire, including information on health indicators, health-related behaviours and socio-demographic characteristics. Socioeconomic status was assessed according to the Family Affluence Scale (FAS), a reliable indicator of family wealth [5]. The scale consists of six questions including family car ownership, whether adolescents have their own bedroom, number of holidays trips taken in the last year, number of computers owned by the family, dishwasher ownership, and number of bathrooms in the home. The obtained score (0-13) was recoded in a 3-point ordinal scale according to low (0-6), medium (7-9), and high (≥10) family affluence. The geographic area of residence was derived from the Region of residence and classified into North, Central and South Italy according to the Italian National Institute of Statistics (ISTAT) classification.

The questionnaires, distributed in schools, were self-filled and anonymous.

The responses to the questionnaires were acquired through optical reading. The construction of the database, the cleaning of the records and the subsequent analysis of the data were carried out centrally by the coordination group.

Ethics and privacy

Students' parents received an information note with the description of the purpose of the survey before the day of the data collection. Families could refuse the participation by filling in the note that was returned to the teachers of the involved class. In respect of anonymity and privacy, respondents can never be identified.

In 2018, the Italian HBSC study protocol and questionnaire were formally approved by the Ethics Committee of the Italian National Institute of Health (Ref. PROT-PRE876/17, 20 November 2017)

Strengths and limitations

Limitations of the HBSC are the cross-sectional design, which does not allow to draw conclusions about causation, and the self-reported information.

The main strengths are standardized and validated data collection procedures, based on the international HBSC study, and the representativeness of the findings, both at national and regional level, with the largest sample size available in these developmental ages.

References

1. Health behaviour in school-aged children. World Health Organization collaborative cross-national survey. Available from: www.hbsc.org.
2. Inchley J, Currie D, Budisavljevic S, Torsheim T, Jåstad A, Cosma A, Kelly C, Arnarsson AM. Spotlight on adolescent health and well-being. Findings from the 2017/2018 Health Behaviour in School-aged
3. Cavallo F, Lemma P, Dalmasso P, Vieno A, Lazzeri G, Galeone D (Eds). Report nazionale dati HBSC Italia 2014. Torino: Strampatre s.r.l; 2016.
4. https://www.epicentro.iss.it/hbsc/
5. Torsheim T, Cavallo F, Levin KA, et al. Psychometric Validation of the Revised Family Affluence Scale: a Latent Variable Approach. Child Ind Res. 2016;9:771-84.

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Conflict of interest statement

All authors declare that they have no conflict of interest.

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Ethical approval

The Italian HBSC study protocol and questionnaire were formally approved by the Ethics Committee of the Italian National Institute of Health (PROT-PRE876/17, 20 November 2017).

Authors' contributions

PN, SC, AS, DP conceptualized and designed the study. DP analysed the data. PN, SC wrote the first draft. AS contributed to the interpretation of data and reviewed the manuscript. GL and NC critically reviewed the manuscript and approved the final version.

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REFERENCES

1. Banspach S, Zaza S, Dittus P, Michael S, Brindis CD, Thorpe P. CDC Grand Rounds: Adolescence – Preparing for lifelong health and wellness. 2016;65(30):759-62 Available from: www.cdc.gov/mmwr/volumes/65/wr/mm6530a2.htm.
2. Patton GC, Viner R. Pubertal Transitions in Health. Lancet. 2007;369(9567):1130-9.
3. Patton GC, Sawyer SM, Santelli JS et al. Our future: a *Lancet* commission on adolescent health and wellbeing. Lancet. 2016;387(10036):2423-78.
4. Lauria L, Spinelli A, Cairella G, et al. Dietary habits among children aged 8-9 years in Italy. Ann Ist Super Sanita. 2015;51(4):371-81.
5. Inchley J, Currie D, Jewell J, Breda J, Barnekow V. Adolescent obesity and related behaviours: trends and inequalities in the WHO European Region, 2002-2014. Copenhagen: WHO Regional Office for Europe; 2017.
6. González K, Fuentes J, Márquez JL. Physical inactivity, sedentary behavior and chronic diseases. Korean J Fam Med. 2017;38(3):111-5.
7. Robinson TN, Banda JA, Hale L, et al. Screen media exposure and obesity in children and adolescents. Pediatrics. 2017;140(Suppl 2):S97-S101.
8. Borraccino A, Lemma P, Berchialla P, et al. Unhealthy food consumption in adolescence: role of sedentary behaviours and modifiers in 11-, 13- and 15-year-old Italians. Eur J Publ Health. 2016;26(4):650-6.
9. Clément K, Vaisse C, Lahlou N, et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature. 1998;392(6674):398-401.
10. Rankinen T, Pérusse L, Weisnagel SJ, Snyder EE, Chagnon YC, Bouchard C. The human obesity gene map: the 2001 update. Obes Res. 2002;10(3):196-243.
11. World Health Organization. Better food and nutrition in Europe: a progress report monitoring policy implementationin the WHO European Region. Copenhagen; WHO: 2018.
12. World Health Organization Regional Office for Europe. Regional Committee for Europe 64th Session. Copenhagen, Denmark, 15-18 September 2014.
13. World Health Organization. European Food and Nutrition Action Plan 2015-2020. Copenhagen: WHO; 2015.
14. Caleyachetty R, Echouffo-Tcheugui JB, Tait CA, Schilsky S, Forrester T, Kengne AP. Prevalence of behavioural risk factors for cardiovascular disease in adolescents in low-income and middle-income countries: an individual participant data meta-analysis. Lancet Diabetes Endocrinol. 2015;3(7):535-44.
15. Inchley J, Currie D,Budisavljevic S, Torsheim T, Jåstad A,Cosma A, Kelly C, Arnarsson AM. Spotlight on adolescent health and well-being. Findings from the 2017/2018 Health Behaviour in School-aged Children (HBSC) survey in Europe and Canada. International report. Copenhagen: WHO; 2020.
16. Wadolowska L, Hamulka J, Kowalkowska J, et al. Skipping breakfast and a meal at school: Its correlates in adiposity context. Report from the ABC of Healthy Eating Study of Polish Teenagers. Nutrients. 2019;11(7):1563.
17. Betts JA, Chowdhury EA, Gonzalez JT, Richardson JD, Tsintzas K, Thompson D. Is breakfast the most important meal of the day? ProcNutr Soc. 2016;75(4):464-74.
18. Barr SI, DiFrancesco L, Fulgoni VL. Breakfast consumption is positively associated with nutrient adequacy in Canadian children and adolescents – Corrigendum. Br J Nutr. 2015;113(1):190.
19. Hallström L, Labayen I, Ruiz JR, et al. Breakfast consumption and CVD risk factors in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr. 2013;16(7):1296-305.
20. Lazzeri G, Ahluwalia N, Niclasen B, et al. Trends from 2002 to 2010 in daily breakfast consumption and its socio-demographic correlates in adolescents across 31 Countries participating in the HBSC Study. PLoS One. 2016;11(3):e0151052.
21. Akseer N, Al-Gashm S, Mehta S, Mokdad A, Bhutta ZA. Global and regional trends in the nutritional status of young people: a critical and neglected age group [published correction appears in Ann N Y Acad Sci. 2017;1396(1):236]. Ann NY Acad Sci. 2017;1393(1):3-20.
22. Newens KJ, Walton J. A review of sugar consumption from nationally representative dietary surveys across the world. J Hum Nutr Diet. 2016;29(2):225-40.
23. Das JK, Salam RA, Thornburg KL, et al. Nutrition in adolescents: physiology, metabolism, and nutritional needs. Ann NY Acad Sci. 2017;1393(1):21-33.
24. Franko DL, Thompson D, Bauserman R, Affenito SG, Striegel-Moore RH and National Heart, Lung, and Blood Institute Growth and Health Study (NGHS). What's love got to do with it? Family cohesion and healthy eating behaviors in adolescent girls. Int J Eat Disord. 2008;41(4):360-7.
25. Marques A, Naia A, Branquinho C, Matos MG. Adolescents' eating behaviors and its relationship with family meals, body mass index and body weight perception. [Comportamiento alimentario de los adolescentes y su relación con las comidas familiares, el índice de masacorporal y la percepción del peso corporal]. Nutr Hosp. 2018;35(3):550-6.
26. Chzhen Y, Moor I, Pickett W, Toczydlowska E, Stevens GWJM. International trends in "bottom-end" inequality in adolescent physical activity and nutrition: HBSC study 2002-2014. Eur J Public Health. 2018;28(4):624-30.
27. Voráčová J, Sigmund E, Sigmundová D, Kalman M. Family affluence and the eating habits of 11- to 15-year-old Czech adolescents: HBSC 2002 and 2014. Int J Environ

Res Public Health. 2016;13(10):1034.
28. Linee Guida per una sana alimentazione. Centro di Ricerca Alimenti e nutrizione, 2008. Available from: www.crea.gov.it/documents/59764/0/LINEE-GUIDA+DEFINITIVO.pdf/28670db4-154c-0ecc-d187-1ee9db3b1c65?t=1576850671654.
29. Monzani A, Ricotti R, Caputo M, et al. A systematic review of the association of skipping breakfast with weight and cardiometabolic risk factors in children and adolescents. What should we better investigate in the future? Nutrients. 2019;11(2):387.
30. Smith KJ, Breslin MC, McNaughton SA, Gall SL, Blizzard L, Venn AJ. Skipping reakfast among Australian children and adolescents; findings from the 2011-2012 National Nutrition and Physical Activity Survey. Aust NZJ Publ Health. 2017;41:572-8.
31. Istituto Superiore di Sanità – EpiCentro. Indagine HBSC 2018: i dati nazionali. Available from: www.epicentro.iss.it/hbsc/pdf/sovrappeso%20e%20obesit%C3%A0.pdf.
32. Rasmussen M, Krolner R, Klepp KI, Lytle L, Brug J, Bere E, Due P. Determinants of fruit and vegetable consumption among children and adolescents: a review of the literature. Part I: Quantitative studies. Int J BehavNutrPhys Act. 2006;3:22.
33. Pedersen TP, Meilstrup C, Holstein BE, Rasmussen M. Fruit and vegetable intake is associated with frequency of breakfast, lunch and evening meal: cross-sectional study of 11-, 13-, and 15-year-olds. Int J Behav Nutr Phys Act. 2012;9:9.
34. Fidler Mis N, Braegger C, Bronsky J, et al. Sugar in infants, children and adolescents: A position paper of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2017;65(6):681-96.
35. World Health Organization. Guideline: Sugars intake for adults and children. Geneva: WHO; 2015.
36. Ebbeling CB, Feldman HA, Chomitz VR, et al. A randomized trial of sugar-sweetened beverages and adolescent body weight. N Engl J Med. 2012;367(15):1407-16.
37. Olafsdottir S, Berg C, Eiben G, et al. Young children's screen activities, sweet drink consumption and anthropometry: results from a prospective European study. Eur J Clin Nutr. 2014;68(2):223-8.
38. Hicks K, Pitts SJ, Lazorick S, Fang X, Rafferty A. Examining the association between screen time, beverage and snack consumption, and weight status among Eastern North Carolina youth. N C Med J. 2019;80(2):69-75.
39. Kelishadi R, Mozafarian N, Qorbani M, et al. Association between screen time and snack consumption in children and adolescents: The CASPIAN-IV study. J Pediatr Endocrinol Metab. 2017;30(2):211-9.
40. Fletcher EA, McNaughton SA, Crawford D, et al. Associations between sedentary behaviours and dietary intakes among adolescents. Public Health Nutr. 2018;21(6):1115-22.
41. Schalkwijk AAH, van der Zwaard BC, Nijpels G, Elders PJM, Platt L. The impact of greenspace and condition of the neighbourhood on child overweight. Eur J Pub Health. 2018;28(1):88-94.
42. Drewnowski A, Darmon N. Food choices and diet costs: an economic analysis. J Nutr. 2005;135(4):900-4.
43. Walton K, Horton NJ, Rifas-Shiman SL, et al. Exploring the role of family functioning in the association between frequency of family dinners and dietary intake among adolescents and young adults. JAMA Netw Open. 2018;1(7):e185217.
44. World Health Organization. Taxes on sugary drinks: Why do it? Geneva: WHO; 2017.
45. Kovacs VA, Messing S, Sandu P, Nardone P, Pizzi E, Hassapidou M, Brukalo K, Tecklenburg E, Abu-Omar K, Improving the food environment in kindergartens and schools: An overview of policies and policy opportunities in Europe. Food Policy. 2020;96 Available from: www.sciencedirect.com/science/article/abs/pii/S0306919220300324.

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MONOGRAPHIC SECTION

Problematic social media use: associations with health complaints among adolescents

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Abstract

Objective. Problematic Social Media Use (PSMU) has an addictive potential for young users. The aim of this study was to show the prevalence of PSMU across Italian regions and its association with health complaints.

Materials and methods. Data are gathered from the Italian 2018 Health Behaviour in School-aged Children survey using a representative sample of Italian adolescents aged 11, 13 and 15 years (50.6% males). Participants completed self-administered questionnaires assessing PSMU and health complaints.

Results. PSMU affects 8.9% adolescents in Italy and the prevalence is quite consistent across regions. 13-year-olds girls showed the highest percentage of PSMU (13%). Problematic users of social media are more likely to report multiple somatic (OR = 1.84 [95% CI 1.82-1.85]) and psychological (OR = 2.60 [95% CI 2.58-2.63]) symptoms.

Conclusions. PSMU represents a widespread problem in Italy. National prevention interventions are needed in order to promote a positive use of social media.

Key words

- social media
- problematic use
- adolescence
- psychosomatic symptoms

INTRODUCTION

Among the many activities available on Internet, the use of social media (e.g., Facebook, WhatsApp, Instagram, etc.) has been growing and now involves more than one third of the world population [1]. This impressive statistic has led to increasing interest in this phenomenon and its positive and negative impact on adolescents' health and well-being [2]. When conscious and moderate, the use of social media tends to be beneficial for adolescents' social relationships and adjustment, for example in terms of contact with peers, entertainment, and civic engagement [3, 4]. Nevertheless, many researchers have recently begun to focus on "Problematic Social Media Use" (PSMU) in youths' lives as it can be a potential behavioural addiction and it has been found to be associated with a number of negative outcomes, such as somatic symptoms [5], academic performance [6], and psychological distress [7]. It has recently been shown that the prevalence of PSMU in adolescence varied significantly across European countries with Italy showing one of the highest percentages of adolescents with PSMU [8]. The aim of the current study is to show a clear picture of the prevalence of PSMU across Italian regions and its association with somatic and psychological complaints among adolescents involved in the latest HBSC survey (2018).

PSMU has been conceptualized as the presence of "addiction-like" symptoms due to social media use: (i) preoccupation (i.e. constantly thinking about social media), (ii) tolerance (i.e. desiring to spend more time on social media), (iii) withdrawal (i.e. feeling bad when offline), (iv) persistence (i.e. failing to reduce time spent on social media), (v) escape (i.e. using social media to escape from negative feelings), (vi) problems (i.e. having arguments with others because of social media); and other specific features borrowed from the latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria for Internet Gaming Disorder [9]: (vii) deception (i.e. lying to family and friends about the time spent on social media), (viii) displacement (i.e.

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neglecting other activities because of social media use), and (ix) conflict (i.e. having serious conflicts with family due to social media use) [10]. Although the DSM-5 and the 11th Revision of the International Classification of Diseases (ICD-11) [11] have not recognized PSMU as a mental disorder yet, there is a growing body of evidence sustaining the addictive potential of social media and its association with physical/somatic and psychological problems [7, 12].

A positive association between Facebook addiction and somatic symptoms in adolescence was reported by Koc and colleagues [13] and Hanprathet and colleagues [14] who demonstrated a negative impact of problematic Facebook use on general health including somatic symptoms, anxiety, insomnia, depression, and social dysfunction. Cerutti and colleagues [15] showed that adolescent problematic Internet users (engaged in social media use and other online activities) have higher levels of somatic symptoms (e.g., stomach pain, headache, sore muscle, low energy) compared with non-problematic peers. Moreover, it has been suggested that problematic Internet use might put adolescents at greater risk of somatization both directly and indirectly via sleep disturbance [16]. It follows that PSMU might be related to poor sleep which, in turn, may negatively impact perceived physical health and school performance [6, 17].

Meta-analytic studies and systematic reviews [5, 7, 18-20] have been highlighting the association between PSMU, well-being, and psychological problems. Problematic social media users tend to report low levels of well-being (including self-worth, social adjustment, quality of life, and happiness) and low levels of satisfaction with life and with social relationships [8, 21, 22]. Moreover, problematic users also report frequent psychological complaints sustaining the idea that PSMU might contribute to increasing anxiety, depression, loneliness and mood swings, especially in children and young people [20, 23, 24].

It has been pointed out that (early) adolescence might be the crucial and vulnerable developmental stage in which young users might not be able to avoid the negative consequences of social media use [19]. Indeed, in the last decades, the prevalence of adolescent psychological distress has been growing in European countries [19] and younger users appear to be at greater risk to develop PSMU and mental health problems because of the addictive potential of social media [25]. To our knowledge, no studies have investigated the prevalence of PSMU and its association with adolescents' psychosomatic health in a representative sample of adolescents in Italy.

The aim of this study is twofold: (i) to estimate the prevalence of problematic social media use across Italian regions by age groups (11, 13 and 15 years) and gender; and (ii) to evaluate whether PSMU is associated with health complaints (psychological and somatic symptoms) in a representative sample of adolescents in Italy, in line with previous international studies [19].

MATERIALS AND METHODS

Setting and sampling

Data were gathered from the 2018 Italian Health Behaviour in School-aged Children (HBSC) survey. HBSC is a cross-national survey undertaken every four years in 50 countries in Europe and Canada with the collaboration of World Health Organization Regional Office for Europe (for details see [26]). This study involved a representative sample of youths aged 11, 13 and 15 years corresponding to the 6th, 8th and 10th grade (1st and 3rd grade of Italian middle school, and 2nd grade of Italian secondary school). For further methodological details see Appendix 1 in the paper by Nardone *et al*., published in this same issue of *Annali dell'Istituto Superiore di Sanità*.

Measures

Problematic Social Media Use. PSMU was assessed through the Social Media Disorder Scale [10] that was translated into Italian following the indications of the international protocol [8]. The scale includes 9 items covering the criteria for PSMU described by van den Eijnden and colleagues [10]: preoccupation (".. have you regularly found that you can't think of anything else but the moment that you will be able to use social media again?"), tolerance ("…regularly felt dissatisfied because you wanted to spend more time on social media?"), withdrawal ("…often felt bad when you could not use social media?"), persistence ("… tried to spend less time on social media, but failed?"), displacement ("… regularly neglected other activities (e.g. hobbies, sport) because you wanted to use social media?"), problem ("… regularly had arguments with others because of your social media use?"), deception ("… regularly lied to your parents or friends about the amount of time you spend on social media?"), escape ("… often used social media to escape from negative feelings?"), conflict ("… had serious conflict with your parents, brother(s) or sister(s) because of your social media use?"). Participants were asked to think about the last year and answer "yes" or "no" to each item. In order to be classified as "problematic users" (1), participants had to answer "yes" to six or more items; participants answering positively up to five items were classified as "non-problematic users" (0).

Health complaints. The HBSC Symptom Checklist [27] was used to assess health complaints. The scale comprises two subscales: (i) somatic symptoms (4 items: headache, stomachache, backache, feeling dizzy); and (ii) psychological symptoms (4 items: feeling low, irritable or in a bad mood, nervous and having difficulties falling asleep). Participants were asked to rate the frequency of symptoms over the last 6 months on a 5-point scale (from 1= "about every day" to 5= "rarely or never"). The two subscales were dichotomized: participants reporting 2 or more somatic/psychological symptoms more than once a week were considered to experience "multiple somatic symptoms" and "multiple psychological symptoms" [28].

Control variables

Age, gender and family affluence were used as control variables. The Family Affluence Scale (FAS) [29] was used as a proxy of socio-economic status and is described in Appendix 1.

Statistical analysis

Cronbach's alphas were calculated for the Social Media Disorder Scale and the two dimensions of the HBSC Symptom Checklist in order to assess the reliability of the scales in this study.

In order to show the prevalence of problematic social media users in Italy, percentages across regions, age groups and gender were calculated.

In order to test for the possible contribution of PSMU to somatic and psychological symptoms, six multivariable logistic regression analyses were run, controlling for the effect of children's age, FAS and, in some models, gender. Multiple somatic and psychological symptoms were the dependent variables and PSMU was the independent variable. Analyses were run on the total sample and for males and females separately. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated.

IBM SPSS Statistics 25 was used to perform the analyses.

RESULTS

Descriptive statistics

Overall, a total of 58,976 youths completed the questionnaire (mean age = 13.5 years, Standard Deviation (SD) = 1.6 years; 50.6% males), distributed across Italy's 21 regions (1281-5570 youth per region). For the purpose of this study, only youths with complete data in the variables of interest (i.e. answering the 100% of the items related to PSMU and health complaints) were included in the final analyses.

The Cronbach's alpha for the Social Media Disorder Scale was 0.72. The Cronbach's alpha was 0.69 for the somatic symptoms dimension and 0.77 for the psychological symptoms dimension of the HBSC Symptom Checklist.

Table 1 shows that PSMU affects 8.9% adolescents in Italy and that the prevalence of PMSU is quite consistent across regions, slightly varying from 6.6% (Valle d'Aosta and Friuli Venezia Giulia) to 10.8%-10.9% (Campania and Puglia). With regard to differences across age groups, 13-year-olds reported the highest percentages of PSMU (10.2%) followed by 11-year-olds (8.6%) and 15-year-olds (7.8%). Whereas boys and girls appear to be at similar risk of PSMU among 11-year-old adolescents (8.6% and 8.7% respectively), PSMU is higher among 13- and 15-year-old girls as compared to their male peers (13.0% *vs* 7.5%; 10.0% *vs* 5.5%, respectively). Specifically, at age 13 girls reported the highest percentages of PSMU in Italy (i.e. 17.0% in Puglia, 16.1% in Campania, and about 15.0% in Lombardia, Umbria and Abruzzo) whereas the lowest percentage

Table 1
Prevalence of problematic social media use by region, age group, and gender (n = 53292)

	Total %	11-year-olds (n = 17,160) %			13-year-olds (n = 19,292) %			15-year-olds (n = 16,840) %		
		Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total
Piemonte	8.1	8.3	8.9	8.6	5.6	13.0	9.2	4.3	8.4	6.2
Valle d'Aosta/Vallée d'Aoste	6.6	4.5	5.2	4.9	6.8	10.9	8.9	3.9	7.9	5.9
Lombardia	10.3	9.5	10.5	10.0	9.8	15.4	12.5	5.9	9.8	8.1
Bolzano/Bozen	9.1	9.3	7.4	8.4	6.9	12.5	9.9	8.9	8.7	8.8
Trento	8.2	10.7	8.3	9.4	9.0	8.8	8.9	5.5	7.3	6.5
Veneto	9.1	8.8	7.6	8.2	8.6	12.9	10.8	6.0	10.1	8.0
Friuli Venezia Giulia	6.6	8.1	5.6	6.8	4.0	9.7	6.9	4.5	7.5	6.1
Liguria	8.9	8.6	7.6	8.1	8.6	12.4	10.3	5.8	10.8	8.2
Emilia Romagna	8.3	5.7	7.5	6.6	8.5	10.2	9.4	5.2	11.2	8.7
Toscana	7.5	5.8	7.5	6.7	4.8	11.9	8.4	4.6	10.0	7.2
Umbria	8.5	7.1	5.8	6.4	9.5	15.1	12.2	6.7	6.6	6.7
Marche	8.6	9.6	11.9	10.7	4.7	12.4	8.4	4.6	8.6	6.8
Lazio	8.3	6.9	7.8	7.3	6.4	11.7	9.1	4.2	13.6	8.4
Abruzzo	9.3	9.7	9.1	9.4	6.6	15.5	10.7	4.3	10.7	7.8
Molise	9.5	10.4	10.2	10.3	7.0	13.9	10.1	6.6	9.0	7.9
Campania	10.8	11.1	13.6	12.2	8.9	16.1	12.5	4.6	11.0	7.8
Puglia	10.9	8.4	9.2	8.8	8.9	17.0	12.7	7.7	13.8	10.9
Basilicata	9.2	8.0	10.4	9.1	8.5	12.6	10.7	6.0	8.6	7.1
Calabria	10.0	9.1	10.3	9.6	7.5	14.5	11.1	6.9	11.4	9.3
Sicilia	10.0	9.4	10.4	9.9	10.4	14.7	12.6	4.6	11.2	7.7
Sardegna	9.2	9.3	9.6	9.5	6.5	12.9	9.5	5.1	10.5	8.0
Italy	**8.9**	**8.6**	**8.7**	**8.6**	**7.5**	**13.0**	**10.2**	**5.5**	**10.0**	**7.8**

(4.0%) was observed among boys in Friuli Venezia Giulia. At age 15, boys in Valle d'Aosta showed the lower percentage of PSMU (3.9%) and the biggest difference between girls (13.6%) and boys (4.2%) was observed in Lazio. Overall, the regions in which PSMU was spread the most were Campania among 11-year-olds (12.2%) and Puglia among 13- and 15-year-olds (12.7% and 10.9% respectively).

Findings of the logistic regression analyses

Results from the logistic regression *(Table 2)* shows that being problematic users of social media increased the likelihood of reporting multiple somatic symptoms [2 or more symptoms claimed more than once a week; OR = 1.84 (95% CI: 1.82-1.85)], controlling for age, gender, and FAS. The strongest associations (above OR = 2.24) between PSMU and somatic symptoms were observed in Sardegna, Calabria, Umbria and Valle d'Aosta, with boys from Umbria and Sardegna and girls from Valle d'Aosta and Calabria showing the highest impact of PMSU on multiple somatic symptoms. This effect was non-significant among girls in Abruzzo and among boys in eight regions (i.e. Piemonte, Valle d'Aosta, Trento, Bolzano, Toscana, Marche, Molise, Basilicata).

Table 3 shows that problematic users of social media are also more likely to report multiple psychological symptoms [2 or more symptoms reported more than once a week; OR = 2.60 (95% CI: 2.58-2.63)], adjusting for confounders. Across regions, the highest effects (above OR = 3.00) of PSMU on psychological symptoms were observed in Piemonte, Valle d'Aosta, Toscana, Lazio, Molise, and Sardegna. Among girls, the strongest associations were observed in Valle d'Aosta and Sardegna. However, male problematic users seem to experience more psychological symptoms in Umbria and Abruzzo. This effect was non-significant only among boys in Bolzano.

DISCUSSION

This study aimed to investigate the prevalence of PSMU and analysed its association with health complaints (psychological and somatic symptoms) in a representative sample of Italian adolescents. On average, almost one out of ten adolescent reported problematic social media use, which was associated with both somatic and psychological symptoms.

Our findings showed that PSMU is a widespread problem in Italy, with almost one out of ten adolescents (8.9%) reporting a problematic use of social media; the

Table 2
OR (95% CI) for multiple somatic symptoms (2 or more symptoms more than once a week); independent variable: Problematic Social Media Use (0 = no; 1 = yes)

	Boys[a] (n = 25,917)	Girls[a] (n = 26,225)	Total[b] (n = 52,142)
Piemonte	1.49(.82-2.72)	1.52(1.04-2.22)*	1.53(1.11-2.11)**
Valle d'Aosta / Vallée d'Aoste	1.45(.59-3.58)	2.69(1.55-4.68)***	2.24(1.42-3.54)***
Lombardia	2.05(1.31-3.20)**	1.78(1.28-2.47)***	1.87(1.43-2.44)***
Bolzano/Bozen	1.21(0.53-2.77)	1.79(1.12-2.88)*	1.60(1.07-2.41)*
Trento	1.68(0.96-2.92)	2.21(1.48-3.30)***	2.02(1.47-2.79)***
Veneto	1.99(1.35-2.93)***	1.55(1.17-2.06)**	1.71(1.36-2.15)***
Friuli Venezia Giulia	2.16(1.19-3.93)*	1.87(1.25-2.79)**	1.99(1.42-2.78)***
Liguria	2.02(1.21-3.37)**	1.57(1.06-2.32)*	1.74(1.17-2.37)***
Emilia-Romagna	1.79(1.04-3.10)*	1.95(1.37-2.77)***	1.91(1.42-2.57)***
Toscana	1.18(0.59-2.36)	1.82(1.25-2.65)**	1.65(1.19-2.27)**
Umbria	3.68(1.83-7.41)***	1.82(1.02-3.24)*	2.33(1.49-3.64)***
Marche	1.63(0.92-2.88)	1.82(1.28-2.58)***	1.77(1.31-2.38)***
Lazio	2.36(1.41-3.95)***	1.71(1.19-2.45)**	1.93(1.43-2.60)***
Abruzzo	2.00(1.18-3.40)**	1.37(0.95-1.98)	1.56(1.16-2.12)**
Molise	1.42(0.78-2.60)	2.09(1.41-3.10)***	1.86(1.34-2.57)***
Campania	1.71(1.07-2.75)*	1.61(1.14-2.26)***	1.66(1.26-2.19)***
Puglia	1.64(1.02-2.65)*	1.84(1.33-2.54)***	1.79(1.37-2.34)***
Basilicata	1.71(.90-3.25)	2.15(1.32-3.50)**	1.97(1.34-2.89)***
Calabria	1.89(1.17-3.07)**	2.70(1.94-3.77)***	2.43(1.86-3.18)***
Sicilia	2.71(1.74-4.22)***	1.52(1.07-2.17)*	1.94(1.46-2.57)***
Sardegna	3.12(1.61-6.07)***	2.17(1.30-3.45)**	2.44(1.64-3.63)***
Italy[c]	**2.05(2.01-2.08)*** **	**1.71(1.69-1.73)*** **	**1.84(1.82-1.85)*** **

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; [a]: control variables: age and family affluence – effects not reported for clarity seeking; [b]: control variables: age, gender and family affluence; [c]: weighed for region.

Table 3
OR (95% CI) for multiple psychological symptoms (2 or more symptoms more than once a week); independent variable: Problematic Social Media Use (0 = no; 1 = yes)

	Boys[a] (n = 25,898)	Girls[a] (n = 26,209)	Total[b] (n = 52,107)
Piemonte	2.15(1.58-3.79)***	3.64(2.38-5.57)***	3.06(2.26-4.13)***
Valle d'Aosta / Vallée d'Aoste	3.33(1.70-6.55)***	5.38(2.72-10.67)***	4.29(2.68-6.86)***
Lombardia	2.35(1.63-3.40)***	2.80(1.99-3.94)***	2.60(2.03-3.33)***
Bolzano / Bozen	1.61(0.90-2.87)	2.57(1.68-3.92)***	2.17(1.55-3.03)***
Trento	2.25(1.48-3.40)***	3.55(2.30-5.48)***	2.86(2.13-3.84)***
Veneto	1.98(1.45-2.71)***	2.80(2.08-3.77)***	2.41(1.95-2.98)***
Friuli Venezia Giulia	2.76(1.71-4.47)***	2.87(1.85-4.43)***	2.91(2.11-4.02)***
Liguria	2.25(1.50-3.39)***	3.39(2.14-5.36)***	2.75(2.04-3.70)***
Emilia Romagna	2.22(1.43-3.46)***	3.37(2.26-5.03)***	2.83(2.11-3.78)***
Toscana	2.83(1.74-4.60)***	3.07(2.06-4.57)***	3.00(2.20-4.07)***
Umbria	3.87(2.03-7.38)***	2.16(1.17-3.97)*	2.92(1.86-4.59)***
Marche	1.85(1.19-2.86)**	3.25(2.22-4.75)***	2.58(1.95-3.41)***
Lazio	2.62(1.66-4.12)***	3.70(2.41-5.67)***	3.24(2.39-4.40)***
Abruzzo	3.01(1.95-4.66)***	2.44(1.68-3.55)***	2.73(2.05-3.64)***
Molise	2.97(1.93-4.57)***	3.28(2.14-5.03)***	3.09(2.29-4.18)***
Campania	1.94(1.29-2.81)**	2.55(1.77-3.68)***	2.29(1.75-3.00)***
Puglia	2.32(1.58-3.41)***	2.63(1.84-3.74)***	2.51(1.94-3.25)***
Basilicata	1.98(1.15-3.43)*	2.42(1.44-4.06)***	2.19(1.51-3.18)***
Calabria	1.52(1.01-2.29)*	2.79(1.94-4.01)***	2.16(1.66-2.82)***
Sicilia	1.79(1.19-2.68)**	2.65(1.79-3.91)***	2.27(1.72-2.99)***
Sardegna	2.03(1.11-3.72)*	4.57(2.45-8.54)***	3.13(2.06-4.76)***
Italy[c]	**2.23(2.20-2.27)*** **	**2.89(2.85-2.93)*** **	**2.60(2.58-2.63)*** **

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; [a]: control variables: age and family affluence - effects not reported for clarity seeking; [b]: control variables: age, gender, and family affluence; [c]: weighed for region.

prevalence appears quite consistent across the country, although some variation was found in some regions. Even though it was not possible to identify a clear trend across geographic areas, it is worth noting that the two regions with the lowest prevalence of PSMU were in the North (Valle d'Aosta and Friuli Venezia Giulia), while two regions from the South (Campania and Puglia) showed the highest prevalence. These findings point out that PSMU characterizes a notable portion of adolescents everywhere in the country, with some regions reflecting the North-South inequalities observed for other health indicators, for example the prevalence of youth gambling [30].

A wider variation in the prevalence of PSMU was observed in relation to age groups. More specifically, 13-year-old adolescents showed the highest percentages of PSMU (10.2%), compared to 11- and 15-year-olds. It should be noted that, in Italy, 13 years old is the minimum age required to create personal accounts on social media. Therefore, it is possible that in this developmental stage, after a short period of familiarization with social media, adolescents have learned most of the social media tools but might not have the abilities to use them in a constructive way yet. Interestingly, 15-year-old adolescents appeared as the less problematic age group (7.8%), suggesting a decrease of PSMU from 13- to 15-years. It could be that social media become less pronounced (and then problematic) as adolescents get used to them until social media use become a way of being rather than a problematic behaviour. However, more studies are needed in order to identify the developmental stages more exposed to the risk of developing PSMU and explore the factors responsible for the highest prevalence in specific age groups.

Variation in the prevalence of PSMU was also observed across gender: with the exception of 11-year-old boys and girls, having a similar risk of developing PSMU, 13- and 15-year-old girls reported to use social media in a problematic way almost twice as much as boys. This is consistent with the literature sustaining that girls are more vulnerable to the negative consequences of the online environment as they are more frequently engaged in social media use than boys and they tend to prefer social online interactions, thus incurring in more addiction-like symptoms [7].

PSMU: Italian HBSC results 2018 vs International HBSC results 2018

The recent HBSC international report showed that overall 7.0% of adolescents in Europe and Canada can

be classified as problematic social media users [31]. Overall, Italy gets the 4° place among countries showing the highest percentages of adolescent problematic social media users as compared to other countries [8]. At age 11, Italian boys and girls appeared at major risk of PSMU as compared to the HBSC average prevalence across countries. Specifically, they are only less problematic than 11-year-old in Azerbaijan, Malta, and Romania [32]. Italy ranks at 8° and 17° for PSMU among 13- and 15-year-olds respectively. In line with international findings, PSMU in Italy increased from 11- to 13-year-olds and was more common among girls. However, overall in Europe and Canada, 15-year-old girls had the highest percentage of PSMU. In contrast, our findings highlighted that Italian 13-year-old girls were the most problematic group in Italy along with girls of 13 years from Malta, Wales, Romania, Ireland, and Greece. Interestingly, at age 15, Italian adolescents showed a modest decrease in PSMU (especially among boys) that, instead, remained overall stable in other countries.

PSMU and health complaints

Moreover, as expected, PSMU was associated with health complaints, with greater effects on psychological symptoms as compared to somatic ones. Regarding the association between PMSU and somatic symptoms, results of the present study appear in line with the literature, highlighting the negative effect of problematic use of new technologies on somatic and physical symptoms [5, 33]. As social media have become an integral part of adolescent lives and adolescents predominantly use social media on their smartphones [25], it could be argued that the negative impact of PSMU on somatic symptoms, such as headache and feeling dizzy, might be at least partially explained by the effect of smartphone use (or the exposure to other device screen) on physical health [34, 35]. At the same time, given the correlational nature of the data, it is also possible that adolescents experiencing somatic symptoms such as backache or stomachache are more likely to prefer sedentary online activities, thus having a higher risk of developing a problematic use of these technologies.

It has been suggested that the mechanism linking PSMU and psychological problems is not straightforward, in that problematic use and psychological symptoms might co-occur and influence each other [7]. On the one hand, being characterized by mood modification, cognitive preoccupation, and compulsive online behaviours, PSMU might result in negative consequences in terms of emotional, social, and school impairments [19, 36]. On the other hand, psychologically vulnerable users (for example, experiencing any distress due to poor social support or with low levels of psychological well-being) might use social media as a coping strategy to regulate their emotional and social difficulties putting themselves at major risk to develop a maladaptive use of social media [22, 37, 38]. Although establishing the final direction of the association between PSMU and psychological distress was not the aim of this study, PSMU did appear to worsen the levels of adolescents' psycho-somatic health in Italy.

The associations between PSMU and adolescent health were stronger for psychological than physical symptoms. This might be due to the fact that the somatic effects of problematic social media may mostly derive from the physical consequences of spending time in front screen devices, and to a lesser extent to the social media specific uses. The opposite might be true for psychological consequences of social media use, mostly arising not from the time spent online per se, but rather from the problematic aspects of PSMU, such as loss of control and relational problems with family and friends.

STRENGTHS AND LIMITATIONS

Using a representative sample of adolescents in Italy represents the main strength of this study in that it allowed to reliably estimate the association between PSMU and health complaints. Nevertheless, the present study comes with several limitations that have to be acknowledged. The cross-sectional design of the study precludes any causality statement. A comprehensive description of the strengths and limitations of HBSC methodology is provided in Appendix 1.

Moreover, the scale used to assess PSMU is relatively short and does not capture the preference for online social interactions over face-to-face communication, which is commonly considered a key factor of the problematic use of Internet and social media among youths [39]. Lastly, data were collected via self-report measures in line with the vast majority of the studies in the field. However, there is a need to combine self-reported information with objective data related to the "real" use of social media in order to understand how and what type of social media activity put adolescents at greater risk to develop PSMU.

CONCLUSIONS

This is the first study showing the prevalence of PSMU in adolescents across Italian regions. By showing a consistent association between the problematic use of social media and adolescent psychosomatic health, our findings underline the urgency of implementing prevention intervention in order to promote a positive use of social media and minimize the drawbacks [40]. Moreover, the prevalence of PSMU across the country (also as compared to the international prevalence) shows that in no regions the problem can be considered negligible, thus underlying the need of a national strategy to implement intervention educating adolescents to a healthy use of social media.

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Ethical approval

The Italian HBSC study protocol and questionnaire were formally approved by the Ethics Committee of the Italian National Institute of Health (PROT-PRE876/17, 20 November 2017).

Authors' contribution

CM, ML, NC, and AV conceptualized and designed the study. CM and AV analyzed the data. CM wrote up the first draft. ML and NC wrote sections of the article and contribute to the interpretation of data. DP and PD supervised the statistical analysis. All Authors critically reviewed the manuscript and approved the final version.

Conflict of interest statement

None of the Authors declares competing financial interests.

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REFERENCES

1. Statista. Number of social network users worldwide from 2010 to 2023. 2020. Available from: www.statista.com/statistics/278414/number-of-worldwide-social-network-users/.
2. Bell V, Bishop DV, Przybylski AK. The debate over digital technology and young people. BMJ. 2015;351:3064. doi:10.1136/bmj.h3064
3. Kuss DJ, Griffiths MD. Online social networking and addiction – a review of the psychological literature. Int J Environ Res PU. 2011;8:3528-52. doi: 10.3390/ijerph8093528
4. Lenzi M, Vieno A, Altoé G, Scacchi L, Perkins DD, Zukauskiene R, Santinello, M. Can Facebook informational use foster adolescent civic engagement? Am J Commun Psychol. 2015;55:444-54. doi: 10.1007/s10464-015-9723-1
5. Andreassen CS. Online social network site addiction: A comprehensive review. Curr Addict Rep. 2015;2:175-84. doi: 10.1007/s40429-015-0056-9
6. Van den Eijnden RJJM, Koning IM, Doornwaard S, et al. The impact of heavy and disordered use of games and social media on adolescents' psychological, social, and school functioning. J Behav Addict. 2018;7:697-706. doi:10.1556/2006.7.2018.65
7. Marino C, Gini G, Vieno A, Spada MM. The associations between problematic Facebook use, psychological distress and well-being among adolescents and young adults: A systematic review and meta-analysis. J Affect Disorders. 2018;226:274-81. doi:10.1016/j.jad.2017.10.007
8. Boer M, van den Eijnden RJ, Boniel-Nissim M, Wong SL, Inchley JC, Badura P, et al. Adolescents' intense and problematic social media use and their well-Being in 29 countries. J Adolescent Health. 2020:66:S89-S99. doi:10.1016/j.jadohealth.2020.02.014
9. American Psychiatric Association. Diagnostic and statistical manual of mental disorders Text revision 5th ed. Washington, DC: American Psychiatric Association; 2013.
10. Van den Eijnden RJJM, Lemmens J, Valkenburg PM. The social media disorder scale: Validity and psychometric properties. Comput Hum Behav. 2016;61:478-87. doi:10.1016/j.chb.2016.03.038
11. World Health Organization. Gaming disorder (6C51). Available from: https://icd.who.int/en.
12. Sarmiento IG, Olson C, Yeo G, Chen YA, Toma CL, Brown BB, et al. How does social media use relate to adolescents' internalizing symptoms? Conclusions from a systematic narrative review. Adolescent Res Rev. 2018;1-24:0123456789. doi:10.1007/s40894-018-0095-2
13. Koc M, Gulyagci S. Facebook addiction among Turkish

college students: The role of psychological health, demographic, and usage characteristics. Cyber psych Beh Soc N. 2013;16:279-84. doi: 10.1089/cyber.2012.0249
14. Hanprathet N, Manwong M, Khumsri JMS, Yingyeun R, Phanasathit M. Facebook addiction and its relationship with mental health among Thai high school students. J Med Assoc Thai. 2015;98:S81-S90.
15. Cerruti R, Spensieri V, Presaghi F, Valastro C, Fontana, A., Guidetti V. An exploratory study on Internet addiction, somatic symptoms and emotional and behavioral functioning in school-aged adolescents. Clin Neuro psychiatry. 2017;14:374-83.
16. Cerutti R, Spensieri V, Amendola S, Presaghi F, Fontana A, Faedda N, Guidetti V. Sleep disturbances partially mediate the association between problematic internet use and somatic symptomatology in adolescence. Curr Psychol. 2019;1-9. doi:10.1007/s12144-019-00414-7
17. Andreassen SC, Pallesen S. Social network site addiction-an overview. Curr Pharm Design. 2014;20:4053-61.
18. Best P, Manktelow R, Taylor B. Online communication, social media and adolescent wellbeing: A systematic narrative review. Child Youth Serv Rev. 2014;41:27-36. doi:10.1016/j.childyouth.2014.03.001
19. Keles B, McCraeN, Grealish A. A systematic review: the influence of social media on depression, anxiety and psychological distress in adolescents. Int J Adolesc Youth. 2020;25:79-93. doi:10.1080/02673843.2019.1590851
20. McCrae N, Gettings S, Purssell E. Social media and depressive symptoms in childhood and adolescence: A systematic review. Adolescent Res Rev. 2017;2:315-30. doi:10.1007/s40894-017-0053-4
21. Marino C, Hirst CM, Murray C, Vieno A, Spada MM. Positive mental health as a predictor of Problematic Internet and Facebook use in adolescents and young adults. J Happiness Stud. 2018;19:2009-22. doi:10.1007/s10902-017-9908-4
22. Satici SA, Uysal R. Well-being and problematic Facebook use. Comput Hum Behav. 2015;49:185-90. doi:10.1016/j.chb.2015.03.005
23. Richards D, Caldwell PH, Go H. Impact of social media on the health of children and young people. J Paediatr Child H. 2015;51:1152-7. doi:10.1111/jpc.13023
24. Shensa A, Escobar-Viera CG, Sidani JE, Bowman ND, Marshal MP, Primack BA. Problematic social media use and depressive symptoms among US young adults: A nationally-representative study. Soc Sci Med. 2017;182:150-7. doi:10.1016/j.socscimed.2017.03.061
25. Griffiths MD, Kuss D. Adolescent social media addiction (revisited). Educ Health. 2017;35:49-52.
26. Health Behavior in School-aged Children (HBSC). Available from: http://www.hbsc.org/
27. Haugland S, Wold B. Subjective health complaints in adolescence – reliability and validity of survey methods. J Adolescence. 2001;24:611-24. doi:10.1006/jado.2000.0393
28. Vieno A, Gini G, Lenzi M, Pozzoli T, Canale N, Santinello M. Cybervictimization and somatic and psychological symptoms among Italian middle school students. Eur J Public Health. 2015;25:433-7. doi:10.1093/eurpub/cku191
29. Torsheim T, Cavallo F, Levin KA, et al. Psychometric validation of the revised family affluence scale: a latent variable approach. Child Ind Res. 2016;9:771-84. doi:10.1007/s12187-015-9339-x
30. Canale N, Vieno A, Lenzi M, et al. Income inequality and adolescent gambling severity: Findings from a large-scale Italian representative survey. Front Psychol. 2017;8:1318. doi:10.3389/fpsyg.2017.01318
31. Inchley J, Currie D, Budisavljevic S, Torsheim T, Jåstad A, Cosma A, et al., (Eds.). Spotlight on adolescent health and well-being. Findings from the 2017/2018 Health Behaviour in School-aged Children (HBSC) survey in Europe and Canada. International report. Volume 1. Key findings. Copenhagen: WHO Regional Office for Europe; 2020.
32. Inchley J, Currie D, Budisavljevic S, Torsheim T, Jåstad A, Cosma A, et al., (Eds.). Spotlight on adolescent health and well-being. Findings from the 2017/2018 Health Behaviour in School-aged Children (HBSC) survey in Europe and Canada. International report. Volume 2. Key data. Copenhagen: WHO Regional Office for Europe; 2020.
33. Panova T, Carbonell X. Is smartphone addiction really an addiction? J Behav Addict. 2018;7:252-9. doi:10.1556/2006.7.2018.49
34. Fernandez-Guerrero IM. WhatsAppitis. Lancet. 2014;383:1040. doi:10.1016/S0140-6736(14)60519-5
35. Park J, Kim J, Kim J, et al. The effects of heavy smartphone use on the cervical angle, pain threshold of neck muscles and depression. Adv Sci Technol Lett. 2015;91:12-7. doi:10.14257/astl.2015.91.0.3
36. Marino C, Gini G, Angelini F, Vieno A, Spada MM. Social norms and e-motions in problematic social media use among adolescents. Addict Behav Rep. 2020;11:100250. doi:10.1016/j.abrep.2020.100250
37. Hong FY, Huang DH, Lin HY, Chiu SL. Analysis of the psychological traits, Facebook usage, and Facebook addiction model of Taiwanese university students. Telemat Inform. 2014;31:597-606. doi:10.1016/j.tele.2014.01.001
38. Oberst U, Wegmann E, Stodt B, Brand M, Chamarro A. Negative consequences from heavy social networking in adolescents: The mediating role of fear of missing out. J Adolescence. 2017;55:51-60. doi:10.1016/j.adolescence.2016.12.008
39. Marino C, Finos L, Vieno A, Lenzi M, Spada MM. Objective Facebook behaviour: Differences between problematic and non-problematic users. Comput Hum Behav. 2017;73:541-46. doi:10.1016/j.chb.2017.04.015
40. Primack BA, Escobar-Viera CG. Social media as it interfaces with psychosocial development and mental illness in transitional age youth. Child Adol Psych Cl. 2017;26:217-33. doi:10.1016/j.chc.2016.12.007

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Sexual behaviour in 15-year-old adolescents: insights into the role of family, peer, teacher, and classmate support

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Abstract

Objective. The aim was to investigate the role of different forms of social support in early sexual intercourse and contraceptive use.

Materials and methods. The study sample included 18,918 15-year-olds who took part in the 2018 Italian Health Behaviour in School-aged Children study. Multivariable multilevel logistic regressions were performed taking into account geographical region and socioeconomic status.

Results. 21.7% of adolescents reported early sexual intercourse. 71.9% used a condom at last sexual intercourse, 12.2% oral contraceptives 41.2% other contraceptive methods (multiple response question). High support from family and from teachers were associated with a lower likelihood of early sexual intercourse in both genders, while peer support was shown to increase this likelihood among boys. Adolescents with high social support were more likely to use condoms.

Conclusions. Social support can have a positive influence on adolescents' sexual behaviour. Sexual education programmes should aim to engage peers and those significant adults who can influence adolescents' lives, in and out of school.

Key words

- adolescent health
- sexual health
- sexual behaviour
- social environment

INTRODUCTION

Adolescence is an important life stage during which physical, emotional, social, and biological development influence both physiology and the behavioural transition into adulthood [1]. This period should be considered a moment of a great vulnerability, as, in addition to physiological changes, adolescents experience contrasting emotions, personal conflicts, and social interactions. All of these factors, combined with their exposure to an increasingly demanding environment, increase young people's chances of facing health risks [2]. It is now widely known that the impact of experiences during childhood and adolescence is not limited to these stages of life; it extends to future opportunities and prospects. Health decisions made at this age may also have important effects later in life [3].

Adolescence is also a period during which sexual maturation and reproductive function become visible. At the 1994 Cairo International Conference, the global community established a policy framework to promote the well-being of adolescents. The World Health Organisation (WHO) claims that positive sexual health is essential to achieving a sustainable development and the realisation of global health and human rights. Age-appropriate sexuality education, both in schools and at the community level, is part of a series of measures recommended by the WHO to promote healthy relationships in adolescents [4]. The theme of adolescent sexuality

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has also been the focus of numerous studies, with particular attention paid to its biomedical aspects, e.g., sexual initiation [5, 6], contraception [7], and reproductive and sexual health risks [8]. Indeed, sexual initiation represents a public health issue for several reasons among which being more likely engaging in more sexual partners, different sexual practices and repertoires, together with a higher likelihood of teen pregnancy, and in some situations, be more likely to ever have a sexual transmitted infection, appear to be the most important [9].

Sexual activity in itself is not a risky behaviour, but it is widely recognised as a threat to well-being when it occurs at an early age and without proper knowledge and competences [10]. Early sexual debut is generally defined according to the distribution of age at first sexual intercourse within the community. As such, its definition shifts over time and across countries according to community cultural norms [11]. Early sexual debut can also be defined according to physical and/or emotional immaturity, as a precondition that increases the risk of sexually transmitted infections, unintended pregnancy, and abortion, and affects the social and the psychological sphere [7, 9, 12]. Based on both of these definitions [13], early sexual debut in the US was identified as first sexual intercourse before the age of 16 years; the same definition has also been used in European studies [6, 14-16].

More recently, social support, both formal and informal, has been shown to have a positive connection with the psychophysical health of adolescents. Such support ensures a greater sense of stability and security, and it acts as a buffer against stressful events and risky behaviours, including those related to sexual health, such as early sexual debut [17]. For instance, good relationships with family and teachers have been reported to lead to better mental health, better subjective well-being, and reduced substance use, while relationships with peers have been associated both positively and negatively with the same health issues [18, 19]. To the authors' knowledge, research on the association of social support with early sexual intercourse and condom use in adolescents is still in need of expansion, and particularly among Italian adolescents [20]. Therefore, the aim of this study was to investigate the role of the support of family, peers, teachers and classmates on early sexual intercourse and contraceptive use among 15-year-olds in Italy.

MATERIALS AND METHODS

Study population and design

This paper is based on data from the 2018 Italian Health Behaviour in School-aged Children (HBSC) study. Questions on sexual behaviour were only submitted to 15-year-old students, limiting the analyses on this outcome to a subgroup of the all sampled population (18,918 adolescents). For more information on the study and its methods see *Appendix 1* of the paper by Nardone *et al.*, published in this issue of the *Annali dell'Istituto Superiore di Sanità*.

Geographical region

Italy has 20 regions of different sizes (Trentino Alto Adige is represented with two autonomous provinces), with varying geographical characteristics, economic development, civic culture, and institutional performance [21]. The study methods were organized to allow a representative sample of adolescents for each region across the country. In these analyses, regions were grouped into three main areas according to the Italian National Institute of Statistics classification [22]: Northern, Central, and Southern Italy.

Family structure

Family structure was determined by asking adolescents to identify the people who live, most of the time, in the same house in which they live [23]. Adolescents were then classified as "living with both parents" (including stepfather or stepmother) or "not living with both parents" [24] very early sexual initiation (<14 years.

Social support. Family and peer support

Family and peer support were both measured using a multidimensional scale consisting of four items. For family support, the items were: i) "My family really tries to help me"; ii) "I get the emotional help and support I need from my family"; iii) "I can talk about my problems to my family"; and iv) "My family is willing to help me make decisions" [25, 26]. For peer support, the items were: i) "My friends really try to help me"; ii) "I can count on my friends when things go wrong"; iii) "I have friends with whom I can share my joys and sorrows"; and iv) "I can talk about my problems with my friends" [26]. Response options for family and peer support ranged from "very strongly disagree" (1) to "very strongly agree" (7); a sum-score was then calculated for each scale (range 4-28) and divided by four. Missing data for one or more items were coded as missing data for that domain [27]. According to the most recent HBSC International Report, both scores were then dichotomised into low (<5.5) or high (≥5.5) support [26, 28].

Social support. Teacher and classmate support

Teacher support was measured by three items: i) "I feel that my teachers accept me as I am"; ii) "I feel that my teachers care about me as a person"; and iii) "I feel a lot of trust in my teachers" [29]. Similarly, classmate support was measured by three items: i) "The students in my class enjoy being together"; ii) "Most of the students in my class are kind and helpful"; and iii) "Other students accept me as I am" [29]. Response options for teacher and classmate support ranged from "strongly agree" (1) to "strongly disagree" (5). Original codes were reversed: strongly disagree (0) to strongly agree (4), and a sum-score was generated for each scale (range 0-12) and then divided by three. Missing data for one or more items were encoded as missing data for that domain. Both of the final scores were classified as low (<2.5) or high (≥2.5) support [27, 29].

Sexual intercourse

Fifteen-year-olds were asked "Have you ever had sexual intercourse (sometimes this is called making love, having sex, or going all the way)?" [26, 30]. Response options were "yes" and "no". Students who replied yes were categorised as having had early sexual intercourse.

Contraceptive methods

Those who reported early sexual intercourse were also asked about the contraceptive methods used at last sexual intercourse: "The last time you had sexual intercourse, did you or your partner use": "a condom", "birth control pill", "withdrawal" and "other methods" [30]. Response options for each contraceptive method were "yes", "no", and "not sure". The latter two options were grouped.

Statistical analysis

Descriptive analyses and comparisons between genders by geographical region, socioeconomic status, and social support were performed by the corrected weighted Pearson Chi square statistic. Due to the hierarchical structure of the data, a set of multilevel logistic regression analyses was performed, using school as the level. These analyses were performed to study the associations between social support and early sexual intercourse, condom use, and oral contraceptive use separately for boys and girls. All models were adjusted for geographical region and socioeconomic status. Results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). All analyses were performed using STATA software 14.1 (Stata Corp LP, College Station, TX, USA) and a two-tailed p value <0.05 was considered significant. Missing values (7.3% in sexual intercourse and 2.2% in condom use) were excluded by pairwise deletion in descriptive analyses and by list wise deletion in regressions.

RESULTS

Girls were approximately half (9,506; 50.2%) of the study sample, and 46.4% of 15-year-olds were from Northern Italy, 18.4% from Central and 35.2% from Southern Italy. Nearly the half of them reported a medium socioeconomic status (49.9%), and nearly the 80% lived with both parents. The majority of 15-year-olds reported high family support (67.5%), high peer support (64.5%), and high classmate support (69.9%), while 47.3% reported high teacher support (*Table 1*). Boys reported higher family, teacher, and classmate support, while girls reported higher peer support when compared to boys (p<0.001 for all these relationships).

The 21.7% of adolescents reported early sexual intercourse, among these the 8.4% had declared their initiation before the age of 15. Comparative analyses showed overlapping results with those who reported having had their first intercourse before and at 15 years of age (data not shown).

Among adolescents who reported sexual intercourses, the contraceptive method most commonly used in their last sexual intercourse was condom (71.9%), followed by other methods (41.2%, of which 38.6% declared to have used withdrawal and 2.6% other methods) and oral contraceptives (12.2%), finally the 4.7% reported not having used any contraceptive. Boys were significantly more likely to use condom than girls (74.3% *vs* 68.9%) and girls to use other contraceptive methods (36.0% *vs* 47.7%).

Boys from Central and Southern Italy showed higher odds ratios of sexual intercourse (OR 1.55, 95% CI 1.26-1.90; OR 2.35, 95% CI 2.00-2.78, respectively), see *Table 2*. The same was observed for boys with high socioeconomic status (OR 1.56, 95% CI 1.33-1.83). Living with both parents showed a lower probability of sexual intercourse in both genders (OR 0.79, 95% CI 0.69-0.90 in boys; OR 0.65, 95% CI 0.57-0.74 in girls), as did high family support (OR 0.84, 95% CI 0.75-0.95 for boys; OR 0.55, 95% CI 0.49-0.62 for girls) and high teacher support (OR 0.74, 95% CI 0.66-0.82 in boys; OR 0.64, 95% CI 0.57-0.72 in girls). High peer support was positively associated with higher odds of sexual intercourse only in boys (OR 1.47, 95% CI 1.31-1.65). Classmate support was inversely associated with sexual intercourse, with significant results observed only in girls (OR 0.78, 95% CI 0.69-0.88)

Table 3 reports the Odds of condom use at last sexual intercourse among boys and girls: the use of condom was associated with geographical region; adolescents from Central and Southern Italy were less likely to use condoms when compared to their counterparts from Northern Italy, with significant results for boys from Southern Italy [OR 0.70 and 0.76, respectively, for girls and boys in Central Italy; 0.97 for girls in Southern Italy, not-significant (95% CI 0.72-1.31); 0.64, 95% CI 0.49-0.84 for boys in Southern Italy]. Higher socioeconomic status was also associated with condom use in both girls and boys, with statistically significant results for medium and high socioeconomic status among boys (OR 1.44, 95% CI 1.11-1.86 and OR 1.51, 95% CI 1.12-2.04, respectively) and high socioeconomic status in girls (OR 1.50, 95% CI 1.07-2.10).

Adolescents who lived with both parents were also significantly more likely to have used a condom at last sexual intercourse (OR 1.30, 95% CI 1.02-1.68 in boys; OR 1.43, 95% CI 1.10-1.86 in girls). The same was observed for social support: high support was associated with higher odds of condom use, with significant results for high family support in girls (OR 1.45, 95% CI 1.13-1.82), and for high teacher support (OR 1.29, 95% CI 1.03-1.60), high peer support (OR 1.30, 95% CI 1.03-1.63), and high classmate support (OR 1.29, 95% CI 1.01-1.67) in boys.

Condom use was less frequent among adolescents using other contraceptive methods. Oral contraceptive use was negatively associated with condom use, with significant results, in particular for girls (OR 0.20, 95% CI 0.22-0.44). The same was observed for other contraceptive methods, which showed a negative association with condom use in both boys and in girls (OR 0.27, 95% CI 0.22-0.34 and OR 0.23, 95% CI 0.18-0.31, respectively). Further analyses not reported in the tables showed that oral contraceptive use was significantly less common in girls from Southern Italy (OR 0.50, 95% CI 0.33-0.81) and more frequent in girls who reported high family support (OR 1.43, 95% CI 1.11-1.96). The choice of other contraceptive methods was significantly more frequent in adolescents from Central and Southern Italy (OR 1.13, 95% CI 0.83-1.53 and OR 1.58, 95% CI 1.24-2.02, respectively, for boys; and OR 1.53, 95% CI 1.15-2.04 and OR 1.59, 95% CI 1.24-2.04, respectively, for girls); while a negative association was shown for girls living with both parents (OR 0.73, 95% CI 0.57-0.92).

Table 1
Descriptive analyses of study variables by gender and in the overall sample, the Health Behaviours in School-aged Children study, Italy, 2018

	Girls (n = 9,506)		Boys (n = 9,412)		Total (n = 18,918)	
	N	%	N	%	N	%
Geographical region						
Northern Italy	4,448	46.79	4,331	46.02	8,779	46.41
Central Italy	1,723	18.13	1,751	18.60	3,474	18.36
Southern Italy	3,335	35.08	3,330	35.38	6,665	35.23
Socioeconomic status[a]						
Low	2,536	27.25	2,257	24.89	4,793	26.08
Medium	4,620	49.64	4,558	50.26	9,178	49.95
High	2,151	23.11	2,253	24.85	4,404	23.97
Family structure						
Living with both parents	7,613	80.09	7,422	78.86	15,035	79.47
Not living with both parents	1,893	19.91	1,990	21.14	3,883	20.53
Family support[b]						
Low	3,330	35.45	2,714	29.48	6,044	32.49
High	6,063	64.55	6,493	70.52	12,556	67.51
Peer support[b]						
Low	2,903	30.86	3,714	40.29	6,617	35.53
High	6,503	69.14	5,504	59.71	12,007	64.47
Teacher support[b]						
Low	5,147	54.81	4,683	50.58	9,830	52.71
High	4,243	45.19	4,575	49.42	8,818	47.29
Classmate support[b]						
Low	3,379	35.78	2,266	24.38	5,645	30.13
High	6,064	64.22	7,027	75.62	13,091	69.87
Early SI[b]						
No	7,434	81.72	6,298	74.70	13,732	78.34
Yes	1,663	18.28	2,133	25.30	3,796	21.66
Condom use at last SI[bc]						
No	506	31.08	536	25.73	1,042	28.08
Yes	1,122	68.92	1,547	74.27	2,669	71.92
Oral contraceptive use at last SI[c]						
No	1,377	87.04	1,768	88.36	3,145	87.78
Yes	205	12.96	233	11.64	438	12.22
Other contraceptive methods at last SI[bc]						
No	820	52.30	1,280	63.97	2,100	58.84
Yes	748	47.70	721	36.03	1,469	41.16

[a]As measured by the Family Affluence Scale.
[b]chi-squared test significance for a value of p<0.05.
[c]percentages for participants who reported early SI.
SI: sexual intercourse.

DISCUSSION

Adolescence represents a time of great change, particularly with regard to sexual maturation, during which adolescents who may be more physically and/or emotionally immature may be at risk for sexually transmitted infections and unintended pregnancy [7, 9, 10, 12]. This risk seems more common in those with early sexual intercourse, commonly defined as first sexual intercourse before the age of 16 years [6, 14-16]. Furthermore, early sexual debut has been shown to be more

Table 2
Odds of early sexual intercourse in boys and girls in relation to geographical region, socioeconomic status, family structure, and social support. The Health Behaviours in School-aged Children study, Italy, 2018

	Girls		Boys	
	adjOR	**95% CI**	**adjOR**	**95% CI**
Geographical region				
Northern Italy	1		1	
Central Italy	1.21	1.00-1.47	**1.55**	**1.26-1.90**
Southern Italy	0.98	0.83-1.16	**2.35**	**2.00-2.78**
Socioeconomic status*				
Low	1		1	
Medium	0.88	0.76-1.00	1.10	0.96-1.26
High	1.10	0.93-1.29	**1.56**	**1.33-1.83**
Family structure				
Living with both parents	**0.65**	**0.57-0.74**	**0.79**	**0.69-0.90**
Social support				
High family support	**0.55**	**0.49-0.62**	**0.84**	**0.75-0.95**
High peer support	0.90	0.79-1.01	**1.47**	**1.31-1.65**
High teacher support	**0.64**	**0.57-0.72**	**0.74**	**0.66-0.82**
High classmate support	**0.78**	**0.69-0.88**	0.96	0.84-1.09

Analyses were adjusted for socioeconomic status and geographical region and expressed as adjusted odds ratios (adjORs) and 95% confidence intervals (CIs); in all dichotomous variables reference value considered is "not having"; Missing values in the outcome variable: n=981 (10.4%) in boys and 409 (4.3%) in girls. Statistically significant results are in bold.
*As measured by the Family Affluence Scale.

Table 3
Odds of condom use at last SI among boys and girls with early SIs by geographical region, socioeconomic status, family structure, social support, and use of oral contraceptives or other contraceptive method. Health Behaviours in School-aged Children study, Italy, 2018

	Girls		Boys	
	adjOR	**95% CI**	**adjOR**	**95% CI**
Geographical region				
Northern Italy	1		1	
Central Italy	0.70	0.50-1.00	0.76	0.54-1.07
Southern Italy	0.97	0.72-1.31	**0.64**	**0.49-0.84**
Socioeconomic status*				
Low	1		1	
Medium	1.00	0.76-1.31	**1.44**	**1.11-1.86**
High	**1.50**	**1.07-2.10**	**1.51**	**1.12-2.04**
Family structure				
Living with both parents	**1.43**	**1.10-1.86**	**1.30**	**1.02-1.68**
Social support				
High family support	**1.45**	**1.13-1.82**	1.21	0.96-1.54
High peer support	1.04	0.81-1.34	**1.30**	**1.03-1.63**
High teacher support	1.28	1.00-1.65	**1.29**	**1.03-1.60**
High classmate support	1.10	0.87-1.40	**1.29**	**1.01-1.67**
Contraceptive method usedat last SI				
Oral contraceptives	**0.20**	**0.22-0.44**	0.92	0.64-1.30
Other contraceptive methods	**0.23**	**0.18-0.31**	**0.27**	**0.21-0.34**

Analyses were adjusted for socioeconomic status and geographical region and expressed as adjusted odds ratios (adjORs) and 95% confidence intervals (CIs); in all dichotomous variables reference value considered is "not having". Statistically significant results are in bold.
Missing values in the outcome variable: n=50 (2.3%) in boys and 35 (2.1%) in girls.
*As measured by the Family Affluence Scale.
SI: sexual intercourse.

frequent in adolescents with poor perceived social support [2]. The present study explored whether social support was associated with early sexual intercourse and lack of condom use, and whether some forms of social support could act as protective factors.

The proportion of Italian students reporting early sexual intercourse in this study was nearly the same as that observed in the 2010 HBSC wave (18% for females and 26% for males) [31] and slightly lower than that observed in that of 2014 (21% for females and 28% for males) [32]. Moreover, results are also similar to those reported in a study on adolescent health and sexuality among 14- and 15-years old students in Italy [33].

Sexual behaviour: Italian HBSC results 2018 vs *International HBSC results 2018*

Sexual habits data obtained from the Italian HBSC 2018 study were compared to those from International HBSC 2018 results, which for sexual habits involved 43 countries (including Italy).

Among all countries participating in the international study, an average of 14% of girls and 24% of boys reported early sexual intercourse, compared to 18% of girls and 25.3% of boys in our sample. However, the proportions we observed are still far from the highest percentages observed in Greenland (46% for girls) and Albania and Georgia (45% for boys), and far from the lowest reported in Kazakhstan and Armenia (1% for girls) and Kazakhstan and Russian Federation (14% for boys) [34].

Out of all 43 countries participating in the 2018 HBSC Italian boys and girls who reported sexual intercourse ranked at 10th and 11th place, respectively.

As for condom use there was a higher proportion of use at last sexual intercourse in boys than in girls (74.3% *vs* 68.9%), which is similar to that reported in 2014 Italian HBSC (75.3% and 69.9%) [32]. In a wider context, Italy was above the average of HBSC countries concerning condom use, but far from the highest proportion, which was reported for Spanish girls (78%) and for Hungarian boys (79%), and far from the lowest proportion observed in Wales (44%) for boys and in Albania (30%) for girls. On the overall all out of the 43 countries participating in the 2018 international study, boys and girls ranked 5th and 21st, respectively, for condom use [34].

Sexual behaviour and social support

Confirming the results of previous research, our study highlighted that living with both parents may reduce the likelihood of early sexual intercourse [5, 6, 35], even if this result did not reach statistical significance in other studies [24]. The same studies showed that students with lower socioeconomic status were at higher risk of early sexual intercourse [6, 24], which is different to what observed in Italy.

Previous studies have reported that adolescents' view of sexual readiness is influenced by gendered behaviours shaped by peer and social context, and family and significant adults have been revealed to have an important role [10, 36]. Accordingly, our results showed that high family support reduced the likelihood of early sexual intercourse in both genders, and also led to higher use of condoms or oral contraceptives, particularly in girls. This result has been confirmed by other studies that highlighted how adolescents who perceived that they had a supportive family and who had a strong relationship with their parents were more likely to delay sexual activity [10]. Parents can play an important role in socialisation and sexuality education through effective communication about sex. This helps adolescents have a greater sense of stability and security, which is needed to favour adequate sexual health practices and to facilitate their growth into sexually healthy adults [17, 37]. De Looze *et al*. also found that parent support and dialogue about protection and contraception reduced the likelihood of early adolescent sexual initiation [37]. Notably in our research, high family support was associated with the use of condoms or oral contraceptives in girls.

Templeton *et al.* suggested that the messages received from parents and other adults are perceived as very important by adolescents, who are torn between what adults communicate and the behaviours that are expected by peers [36]. Coherently, high teacher support in our results reduced the chances of early sexual intercourse in both genders. Moreover, the relationship between school and sexual debut, which has been investigated mainly in terms of aspiration and academic performance, showed that adolescents with high school aspirations and those who appreciate education were less likely to engage in early sexual behaviours [5]. In addition, sexuality education in school has been reported to be an important protective factor against risky sexual behaviour [38]. Interestingly, modes of delivery of sexuality education other than teachers have been associated with better sexual health outcomes (remaining sexually inactive, later age at first sexual intercourse, and condom use) [39]. Therefore, adolescents' beliefs and choices seem to be conditioned by adults; however, these relationships are worthy of further analysis, especially the one with teachers, which has been less explored.

In contrast to family and teacher support, in our study peer support favoured an early sexual initiation among boys. An analysis of the literature showed that this effect of peer support has also been demonstrated in other studies [24], and that this might be related to the social pressure that peers exert on adolescents [5, 36]. It has been found that early sexual intercourse is not an unplanned experience for many teens, and decisions about first sexual intercourse are greatly related to social environment, with peers playing an essential part in building a sense of normative behaviour [40]. Indeed, adolescents tend to modify their conduct when they feel that their peers are starting a new behaviour, suggesting that public health action plans must face this issue by targeting peer communities instead of focusing on individuals [40]. Interestingly, and by contrast, Burke *et al*. reported that having a wider network of peers might protect against early sexual intercourse, as it would allow a more accurate assessment of both the associated risks and the normative behaviour [10]. Moreover, our study showed a significant positive relationship between

peer and classmate support in favouring condom use. Indeed, it has been shown that communication regarding contraception is an important predictive factor for contraceptive use among adolescents [24].

STRENGTHS AND LIMITATIONS

The HBSC study has some overall strengths and limitation that were discussed in the common section (see *Appendix 1* in the paper by Nardone *et al.* published in this issue of *Annali dell'Istituto Superiore di Sanità*). This particular analysis was limited in exploring sexual intercourse and condom use. Given the need to better address adolescents' sexual health, it would be helpful to enlarge the questionnaire. Moreover, the dichotomisation of some of the variables could have caused a loss of information.

CONCLUSIONS

Promoting healthy sexual maturation among adolescents requires educators to adopt a holistic approach. Adolescents who have access to comprehensive sexuality and relationship education, confidential reproductive health services, and appropriate contraceptive methods have better sexual health [4]. The support of the family, peers, teachers and schoolmates is important in these ages, even if pushing in different directions. When planning and implementing actions, it is advisable that they are not limited to the individual level, but that involve families and teachers as significant adults [10, 36]. Communication about sex between parents and adolescents has been positively associated with adolescents' use of contraceptives, and of condoms in particular [41]. Moreover, it is advisable to deliver interventions that include peers and classmates, as these groups have been shown to have a beneficial impact on the process of building a sense of positive normative behaviour [40]. Given that in many European countries, including Italy, there is no evidence of a comprehensive policy on sexual health education in schools and communities [42], it is highly advisable to invest in age-appropriate, comprehensive sexuality education curricula. Educational guidance and standardised content on this subject should be created and made available for students from preschool to university, and programmes should aim to engage peers and the adults who influence adolescents' lives both in and out of school [4].

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Conflict of interest statement

All authors declare that they have no conflict of interest.

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Ethical approval

The Italian HBSC study protocol and questionnaire were formally approved by the Ethics Committee of the Italian National Institute of Health (PROT-PRE876/17, 20 November 2017).

Author's contribution statement

AB, GLM and PL edited the draft and completed the manuscript; PD supervised the process of analyses; GLM and LC conducted the statistical analyses and contributed to the writing; PN, SD, PB, ML, AS contributed to the final manuscript editing. All authors participated in designing the study and data collection as members of the HBSC Italian team. All authors have critically revised the manuscript and approved its final version.

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REFERENCES

1. Holder MK, Blaustein JD. Puberty and adolescence as a time of vulnerability to stressors that alter neurobehavioral processes. Front Neuroendocrinol. 2014;35(1):89-110. doi: 10.1016/j.yfrne.2013.10.004
2. Department of Human Services, Department of Planning and Community Development, Department of Education and Early Childhood Development. Vulnerable youth framework discussion paper. Development of a policy framework for Victoria's vulnerable young people. Melbourne: Victoria Government Department of Human Services; 2008.
3. Peter Adamson. Child well-being in rich countries: A comparative overview. Innocenti Report Card 11. UNICEF; 2013.
4. World Health Organization. Sexual health and its linkages to reproductive health. An operational approach. Human Repr. Geneva: WHO; 2017.
5. Lee RLT, Yuen Loke A, Hung TTM, Sobel H. A systematic review on identifying risk factors associated with early sexual debut and coerced sex among adolescents and young people in communities. J Clin Nurs. 2018;27:478-501. doi: 10.1111/jocn.13933
6. Hawes ZC, Wellings K, Stephenson J. First heterosexual intercourse in the United Kingdom: A review of the literature. J Sex Res. 2010;47:137-52. doi: 10.1080/00224490903509399
7. Steyn PS, Goldstuck ND. Contraceptive needs of the adolescent. Best Pract Res Clin Obstet Gynaecol. 2014;28:891-901. doi: 10.1016/j.bpobgyn.2014.04.012
8. McCracken KA, Loveless M. Teen pregnancy: An update. Curr Opin Obstet Gynecol. 2014;26:355-9. doi: 10.1097/GCO.0000000000000102
9. Heywood W, Patrick K, Smith AMA, Pitts MK. Associations between early first sexual intercourse and later sexual and reproductive outcomes: a systematic review of population-based data. Arch Sex Behav. 2015;44:531-69. doi: 10.1007/s10508-014-0374-3
10. Burke L, Gabhainn SN, Kelly C. Socio-demographic, health and lifestyle factors influencing age of sexual initiation among adolescents. Int J Environ Res Public Health. 2018;15(9):1851. doi: 10.3390/ijerph15091851
11. Bingham CR, Crockett LJ. Longitudinal adjustment patterns of boys and girls experiencing early, middle, and late sexual intercourse. Dev Psychol. 1996;32:647-58. doi: 10.1037/0012-1649.32.4.647
12. Nic Gabhainn S, Baban A, Boyce W, Godeau E, HBSC Sexual Health Focus Group the. How well protected are sexually active 15-year olds? Cross-national patterns in condom and contraceptive pill use 2002-2006. Int J Public Health. 2009;54(Suppl 2):209. doi: 10.1007/s00038-009-5412-x.
13. O'Donnell L, O'Donnell CR, Stueve A. Early sexual initiation and subsequent sex-related risks among urban minority youth. The reach for health study. Fam Plann Perspect. 2001:268-75.
14. Godeau E, Vignes. C, Duclos M, Navarro F, Cayla F, Grandjean H. Factors associated with early sexual initiation in girls: French data from the international survey health behaviour in school-aged children (HBSC)/WHO. Gynecol Obstet Fertil. 2008;36:176-82.
15. Magnusson BM, Masho SW, Lapane KL. Early age at first intercourse and subsequent gaps in contraceptive use. J Womens Health. (Larchmt) 2012;21:73-9. doi: 10.1089/jwh.2011.2893
16. Lavikainen HM, Lintonen T, Kosunen E. Sexual behavior and drinking style among teenagers: a population-based study in Finland. Health Promot Int. 2009;24:108-19.
17. Foster CE, Horwitz A, Thomas A, Opperman K, Gipson P, Burnside A, et al. Connectedness to family, school, peers, and community in socially vulnerable adolescents. Child Youth Serv Rev. 2017;81:321-31.
18. Moore GF, Cox R, Evans RE, Hall.ingberg B, Hawkins J, Littlecott HJ, et al. School, peer and family relationships and adolescent substance use, subjective wellbeing and mental health symptoms in Wales: a cross sectional study. Child Indic Res. 2018;11:1951-65. doi: 10.1007/s12187-017-9524-1
19. Lemma P, Borraccino A, Berchialla P, Dalmasso P, Charrier L, Vieno A, et al. Well-being in 15-year-old adolescents. A matter of relationship with school. J Public Health (Oxf). 2015;37:573-80. doi:10.1093/pubmed/fdu095
20. Marino C, Vieno A, Lenzi M, Santinello M. Time trends in adolescent sexual behaviour in Italy. Sex Health. 2014;11:379-80. doi: 10.1071/SH14094
21. Toth F. How health care regionalisation in Italy is widening the North-South gap. Heal Econ Policy Law. 2014;9:231-49. doi: 10.1017/S1744133114000012
22. Istituto Nazionale di Statistica – ISTAT. Annuario Statistico Italiano 2019. Roma: ISTAT; 2019.
23. Levin KA, Dallago L, Currie C. The association between adolescent life satisfaction, Family structure, family affluence and gender differences in parent-child communication. Soc Indic Res. 2012;106:287-305. doi: 10.1007/s11205-011-9804-y
24. Young H, Burke L, Gabhainn SN. Sexual intercourse, age of initiation and school-aged children (HBSC) Ireland study. BMC Public Health. 2018;18:362. doi: 10.1186/s12889-018-5217-z
25. Zimet GD, Dahlem NW, Zimet SG, Farley GK. The multidimensional scale of perceived social support. J Pers Assess. 1988;52:30-41.
26. Inchley J, Currie D (Dorothy B, Young T, Samdal O, Torsheim T, Augustson L, et al. Growing up unequal : gender and socioeconomic differences in young people's health and well-being: health behaviour in school-aged children (HBSC) study: international report from the 2013/2014 survey. Denmark: WHO Regional Office for Europe, 2016.
27. Dalmasso P, Borraccino A, Lazzeri G, Charrier L, Berchialla P, Cavallo F, et al. Being a young migrant in Italy. The effect of perceived social support in adolescence. J Immigr Minor Health. 2018;20:1044. doi: 10.1007/s10903-017-0671-8
28. Canty-Mitchell J, Zimet GD. Psychometric properties of the Multidimensional Scale of Perceived Social Support in urban adolescents. Am J Community Psychol. 2000;28:391-400.
29. Torsheim T, Wold B, Samdal O. The teacher and classmate support scale factor structure, test-retest reliability

and validity in samples of 13-and 15-year-old adolescents. Sch Psychol Int. 2000;21:195-212.
30. Young H, Költo A, Reis M, Saewyc EM, Moreau N, Burke L, et al. Sexual health questions included in the health behaviour in school-aged children (HBSC) study: An international methodological pilot investigation. BMC Med Res Methodol. 2016;16. doi: 10.1186/s12874-016-0270-8
31. Cavallo F, Giacchi M, Vieno A, Galeone D, Tomba A, Lamberti A, et al. Studio HBSC-Italia (health behaviour in school-aged children): rapporto sui dati 2010. 2013.
32. Cavallo F, Lemma P, Dalmasso P, Vieno A, Lazzeri G, Galeone D (Eds). Report Nazionale dati HBSC Italia 2014: 4° Rapporto sui dati HBSC Italia 2014. Torino: Stampatre s.r.l.; 2016.
33. Donati S, Andreozzi S, Medda E, Grandolfo ME. Salute riproduttiva tra gli adolescenti: conoscenze, attitudini e comportamenti. Rapp ISTISAN 00/7.
34. Inchley J, Currie D, Budisavljevic S, Torsheim T, Jåstad A, Cosma A, et al. (Eds). Spotlight on adolescent health and well-being. Findings from the 2017/2018 Health Behaviour in School-aged Children (HBSC) survey in Europe and Canada. International Report Volume 2. Key Data. Copenhagen, Denmark: WHO Regional Office for Europe; 2020.
35. Madkour AS, De Looze M, Ma P, Halpern CT, Farhat T, Ter Bogt TFM, et al. Macro-level age norms for the timing of sexual initiation and adolescents' early sexual initiation in 17 European countries. J Adolesc Health. 2014;55:114-121. doi: 10.1016/j.jadohealth.2013.12.008
36. Templeton M, Lohan M, Kelly C, Lundy L. A systematic review and qualitative synthesis of adolescents' views of sexual readiness. J Adv Nurs. 2017;73:1288-301. doi: 10.1111/jan.13207
37. De Looze M, Constantine NA, Jerman P, Vermeulen-Smit E, Ter Bogt T. Parent-adolescent sexual communication and its association with adolescent sexual behaviors: A nationally representative analysis in the Netherlands. J Sex Res. 2015;52:257-68. doi: 10.1080/00224499.2013.858307
38. Ramiro L, Reis M, De Matos MG, Diniz JA. Sex education among Portuguese adolescent students. Proc Soc Behav Sci. 2011;29:493-502. doi: 10.1016/j.sbspro.2011.11.268
39. Young H, Long SJ, Hallingberg B, Fletcher A, Hewitt G, Murphy S, et al. School practices important for students' sexual health: Analysis of the school health research network survey in Wales. Eur J Public Health. 2017;28:309-14. doi: 10.1093/eurpub/ckx203
40. Kinsman SB, Romer D, Furstenberg FF, Schwarz DF. Early sexual initiation: The role of peer norms. Pediatrics. 1998;102:1185-92. doi: 10.1542/peds.102.5.1185
41. Widman L, Choukas-Bradley S, Noar SM, Nesi J, Garrett K. Parent-adolescent sexual communication and adolescent safer sex behavior: A meta-analysis. JAMA Pediatr. 2016;170:52-61.
42. Beaumont Karolina, Maguire Marcia. Directorate General for Internal Policies. Policy department c: citizens' rights and constitutional affairs. Gender equality. Policies for sexuality education in the European Union. Note. Brussels; 2013. doi: 10.2861/11317

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Alcohol use and misuse: a profile of adolescents from 2018 Italian HBSC data

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Abstract

Objective. Alcohol remains the most commonly substance used by adolescents.The aim of the study was to draw a comprehensive picture of the behavioural patterns that characterize alcohol use and abuse among 15-year-olds.

Materials and methods. The study sample included 18,918 15-year-olds participating in the 2018 Italian Health Behaviour in School-aged Children (HBSC) survey. A Bayesian approach was adopted for selecting the manifest variables associated with alcohol consumption; a latent class regression model was employed to identify health-related risk patterns associated with alcohol use.

Results. 21% of our sample represented the cluster of heavy drinkers who shared other risk behaviours: heavy smoking habits (29%), cannabis (68%), gambling (52%) and drunkenness (76%) experience, binge drinking (96%) and sexual intercourse (51%).

Conclusions. Our results confirm multiple risk-taking behaviours among adolescents tending to cluster in behavioural patterns. This has implications for public health policies and must be considered when planning interventions and prevention strategies.

Key words

- adolescent health
- alcohol
- drunkenness
- co-substances use
- risk behaviour

INTRODUCTION

Despite a trend of declining in alcohol consumption simultaneously to its abstinence increase among adolescents highlighted by different authors [1-3] and confirmed by data from the two most recent waves of HBSC surveillance (2014 and 2018), alcohol remains the most commonly substance used by 15-year-olds: overall almost three in five have drunk alcohol in their lifetime, compared with one in four for smoking and around one in seven that use cannabis [4, 5]. For decades two main models of alcohol consumption among adolescents have been described in Europe: the "dry culture" model in Northern Europe, characterized by sporadic consumption, mainly concentrated in the weekend, outside of mealtimes with the primary aim to get drunk; the "wet culture", specific to the Mediterranean countries, characterized by more regular alcohol consumption, with greater overall quantities, but associated with meals and rituals. In this framework, Italy had a long-standing "wet culture" that has always been associated with high levels of alcohol consumption, especially wine [6-8]. However, recent reports indicate also for Italy and other Mediterranean countries an increase of the phenomenon of drinking "out of meal" and the growing tendency to approximate a Northern European style in the use of alcohol, that is, binge drinking (having experienced binge drinking – five or more drinks on one occasion – in the last 12 months was the question asked in the national HBSC questionnaire) [9, 10].

Data from the last international HBSC survey show that the overall prevalence of lifetime drunkenness remained relatively stable since 2014, with Italian data in

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line with the HBSC averages (about 20% of 15-year-old adolescents had been drunk twice or more in their lifetime).

Alcohol abuse in adolescence may have a variety of adverse social, physical, psychological consequences for young people including missing school, school failure, having unprotected sex (with unintended pregnancy and sexually transmitted diseases as consequences), destructive behaviour, increase in injury likelihood, violence and even deaths [11-20].

The behavioural pattern of adolescents who consume alcohol can become even more complex. Findings from many studies confirm the co-occurrence of health risk behaviours, the idea that young adolescents exhibit multiple health risk behaviours that tend to cluster together [21-27] and also the evidence of strong similarities between countries in the clustering of adolescent risk behaviours [2].

On the other hand, proximal contexts such as family environment and school may shape the behavioural pattern of adolescents. Previous studies suggest that living in an intact family structure, having good relations with parents and parental control are protective factors for alcohol use and abuse as well as adolescents who spend a lot of time doing homework, enjoy school and perceive their school climate as positive, have lower prevalence rates of all alcohol outcomes [28, 29].

Although previous studies have found these relationships separately, less it is known about the psychosocial pattern of alcohol in adolescents.

The aim of the present study was to draw a comprehensive picture of the behavioural social and psychological patterns that characterize alcohol use and abuse among 15-year-old adolescents in Italy.

MATERIALS AND METHODS

Study population and design

A summary of the methodology, the main areas and questions included in the Italian HBSC questionnaire can be found in a previous paper [30] and in the Appendix 1 of the paper by Nardone *et al*., published in this issue of *Annali dell'Istituto Superiore di Sanità*.

Participants

Because most risk behaviours showed more robust estimates of prevalence among 15-year-old students and some behaviours (i.e. gambling, cannabis use, binge drinking and sexual intercourse) were only assessed in this age-class, we limited our analyses to 15-year-old adolescents included in the Italian HBSC survey conducted in 2018.

Statistical analyses

Details about the statistical analysis plan can be found in a previous paper [26].

A logistic regression model (LRM) was run on the dependent variable "frequent alcohol use" (response "weekly" or "every day" at the question "How often do you drink alcohol at present?"), and a spike-and-slab prior approach was used for selecting variables associated to alcohol use among: gender, Family Affluence Scale (FAS), which measures the socioeconomic status of adolescents' family (see Appendix 1 in the paper by Nardone *et al*., published in this same issue of *Annali dell'Istituto Superiore di Sanità*), parents's country of birth, family structure, easy to talk to father and mother, drunkenness, binge drinking, friends that drink alcohol, friends drunk at least weekly, having smoked every day in the last 30 days, cannabis and gambling lifetime experience, ever had sexual intercourse, self-rated health, life satisfaction, health complaints (like headache, stomach aches, feeling low, irritable or bad tempered, and having difficulty getting to sleep), liking school, been bullied, bullied others, fight, Body Mass Index (BMI, Cole's classification), body image, consumption of fruit and vegetables, consumption of sweets, use of soft-drinks, breakfast during schooldays and meals with family.

Variables with a posterior probability of a non-zero coefficient greater than 5% were entered as manifest variables in a latent class regression (LCR) model to identify clusters of adolescents sharing similar drinking habits [31]. Since the LCR model requires the specification of the number of clusters (latent classes), a series of models were fitted for two to five clusters. The choice of the best model was based upon the lowest value of the Bayesian information criterion [32].

Statistical analyses were performed using R version 4.0.0 [33]. R package PoLCA [34] was used to carry out the LCR analysis and Boom Spike Slab for the spike-and-slab regression.

RESULTS

Analyses were performed on 18,918 questionnaires of 15-year-old students (9,506 females and 9,412 males).

Table 1 reports the characteristics of the study sample stratified for self-reported alcohol consumption among 15-year-old students. These data show high rates of other risk behaviours like heavy smoking habits, cannabis and gambling lifetime experience, drunkenness and binge drinking, fights, having friends who drink alcohol and experience frequent alcohol abuse and having had sexual intercourse, among adolescents that reported to drink at least weekly or every day. In addition, these adolescents tend to show lower life satisfaction, a lower rate of their health and more health complains than their peers who don't drink or drink rarely; they also seem to eat fruit and vegetables less frequently, and to consume sweets and soft-drinks at least once a day. High prevalence of alcohol consumption turned out to be associated with a family structure different from living with both parents and with more difficult relationships with adolescents' mother.

On the basis of the self-reported alcohol consumption (the possible responses to the question "How often do you drink alcohol at present?" ranged from never to every day – never; rarely; every month; every week; every day), the LCR model identified three latent classes for the second-grade HS students *(Figure 1)*, which separate the sample (cluster 1 *vs* cluster 2, $p < 0.001$; cluster 3 *vs* cluster 1, $p < 0.001$).

The first cluster (red line) consisted mostly of adolescents who drink alcohol every day; the second cluster (green line) was composed of mostly "more moderate"

Table 1
Characteristics of the study sample stratified for alcohol consumption (responses from "Never" to "Everyday" to the question "How often do you drink alcohol at present?")

	How often do you drink alcohol at present?					
	Never	**Rarely**	**Monthly**	**Weekly**	**Every day**	**p-value**
n	*3827*	*6376*	*3412*	*4093*	*718*	
Gender = female (%)	2025 (52.9)	3494 (54.8)	1860 (54.5)	1710 (41.8)	193 (26.9)	<0.001
FAS (%):						<0.001
Low	806 (21.6)	1208 (19.3)	528 (15.8)	666 (16.7)	137 (19.9)	
Medium	2024 (54.2)	3436 (54.8)	1741 (52.1)	2031 (50.9)	372 (54.1)	
High	903 (24.2)	1624 (25.9)	1075 (32.1)	1297 (32.5)	178 (25.9)	
Parents born (%):						<0.001
both in Italy	3000 (80.2)	5496 (87.3)	2960 (88.2)	3574 (88.4)	591 (84.7)	
1 abroad	267 (7.1)	440 (7.0)	225 (6.7)	281 (7.0)	46 (6.6)	
both abroad	473 (12.6)	361 (5.7)	172 (5.1)	186 (4.6)	61 (8.7)	
Family structure = living with both parents (%)	3083 (82.4)	5168 (82.2)	2686 (79.5)	3202 (79.5)	529 (77.2)	<0.001
Talk father = easy or very easy (%)	2117 (56.4)	3263 (51.8)	1599 (47.3)	2021 (49.9)	418 (60.0)	<0.001
Talk mother = easy or very easy (%)	2882 (76.7)	4675 (74.4)	2342 (69.5)	2738 (67.8)	490 (70.4)	<0.001
Drunkenness = lifetime, at least twice (%)	24 (0.6)	467 (7.3)	872 (25.7)	1784 (43.9)	366 (52.1)	<0.001
Binge drinking = yes in the past 12 months (%)	166 (4.4)	1752 (27.6)	2092 (61.5)	3162 (77.7)	563 (78.9)	<0.001
Friends that drink alcohol = lots-all (%)	1812 (47.3)	4047 (63.5)	2814 (82.5)	3587 (87.6)	558 (77.7)	<0.001
Friends drunk at least weekly = lots-all (%)	548 (14.3)	1038 (16.3)	657 (19.3)	1303 (31.8)	314 (43.7)	<0.001
Smoking = every day in the last 30 days (%)	46 (1.2)	252 (4.0)	246 (7.3)	692 (17.2)	230 (32.5)	<0.001
Cannabis = lifetime, yes (%)	181 (5.1)	846 (13.9)	970 (29.3)	1696 (42.9)	327 (50.3)	<0.001
Gambling = lifetime, yes (%)	888 (25.1)	2134 (35.2)	1437 (43.7)	2046 (51.8)	394 (60.9)	<0.001
Ever had sexual intercourse = yes (%)	300 (8.7)	936 (15.8)	750 (23.2)	1361 (35.1)	331 (52.5)	<0.001
Self-rated health = good/excellent (%)	3348 (88.0)	5600 (88.1)	2892 (85.0)	3467 (85.0)	562 (78.9)	<0.001
Life satisfaction ≥6 (%)	3267 (86.2)	5503 (86.9)	2836 (83.4)	3410 (83.8)	559 (79.1)	<0.001
Health complaints = at least 2 more than once a week (%)	2734 (72.2)	4927 (77.8)	2750 (80.9)	3387 (83.2)	590 (83.6)	<0.001
Body Mass Index (%):						<0.001
underweight	101 (3.0)	116 (2.0)	68 (2.2)	45 (1.2)	13 (2.1)	
proper weight	2719 (79.9)	4724 (80.7)	2571 (81.7)	3011 (79.4)	466 (74.4)	
overweight	494 (14.5)	845 (14.4)	430 (13.7)	614 (16.2)	116 (18.5)	
obese	87 (2.6)	167 (2.9)	76 (2.4)	123 (3.2)	31 (5.0)	
Body image = perceiving to be too fat (%)	1005 (26.7)	1893 (30.0)	1104 (32.6)	1329 (32.9)	200 (28.6)	<0.001
Liking school = a bit or a lot (%)	2633 (69.1)	4181 (65.8)	2013 (59.2)	2068 (50.7)	281 (39.7)	<0.001
Been bullied = never (%)	3414 (90.0)	5769 (91.2)	3107 (91.4)	3698 (91.0)	616 (87.4)	0.004
Bullied others = never (%)	3481 (91.8)	5695 (90.0)	2985 (87.8)	3392 (83.5)	517 (73.0)	<0.001
Fight = never in the last 12 months (%)	3112 (81.9)	4737 (74.8)	2283 (67.0)	2267 (55.8)	296 (41.9)	<0.001
Fruit = at least once a day everyday (%)	1396 (36.5)	2225 (34.9)	1185 (34.8)	1302 (31.9)	244 (34.2)	<0.001
Vegetables = at least once a day everyday (%)	1202 (31.5)	1949 (30.6)	1107 (32.5)	1134 (27.8)	200 (28.1)	<0.001
Sweets = less than once a day (%)	2853 (74.5)	4774 (74.8)	2560 (75.0)	2909 (71.0)	432 (60.2)	<0.001
Soft-drinks = less than once a day (%)	3480 (90.9)	5753 (90.2)	3103 (0.9)	3533 (86.3)	488 (68.0)	<0.001
Breakfast during schooldays = 5 days (%)	2224 (59.0)	3494 (55.5)	1742 (51.5)	1996 (49.3)	330 (46.9)	<0.001
Meals with family = every day (%)	2196 (57.4)	3525 (55.3)	1667 (48.9)	1988 (48.6)	385 (53.6)	<0.001

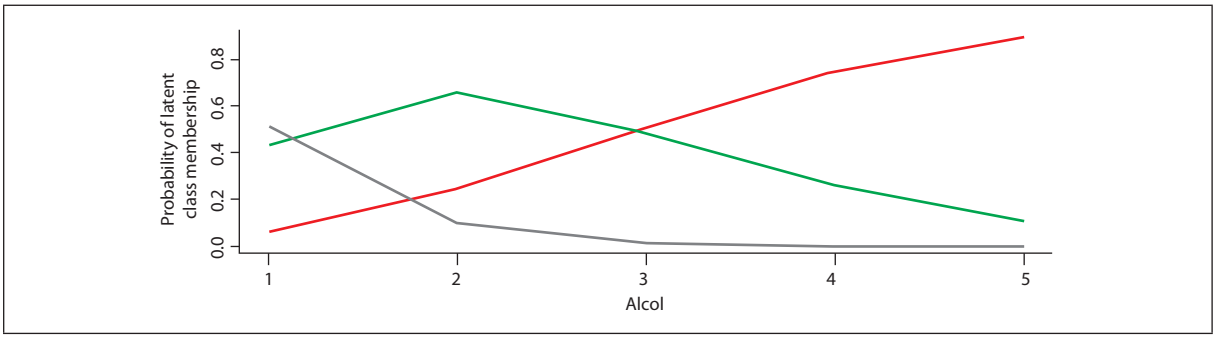

Figure 1
Alcohol consumption clusters of 15-year-old adolescents. Predicted prior probabilities of latent class membership based on the self-reported alcohol use of Italian adolescents involved in HBSC (with answer ranging from 1 -never to 5 -every day).

drinkers (students who refer to drink rarely or monthly); finally, the third cluster (grey line) comprised adolescents who do not drink or rarely drink alcohol.

According to the class-conditional probabilities estimated by the LRC model, the first cluster of heavy drinkers corresponds to the 21% of the sample. Adolescents who reported to drink everyday have a membership probability of about 89%; for those who reported to drink weekly but not every day it was 74% and for occasionally (monthly) drinkers 51%. The membership probability of those who reported to not drink was only 5%, while it was 25% for those who reported to drink rarely.

The second cluster (moderate) comprised adolescents who reported to drink rarely (66% of membership probability to the cluster) or monthly (48% of membership probability to the cluster) as well as non-drinkers (43% of membership probability to the cluster), and represents about the 41% of the sample.

Finally, the third cluster grouped mainly non-drinkers (50% of membership probability) and occasionally drinkers (9% of membership probability), who are about the 38% of the sample.

An indicator of the goodness of fit of the LCR models is the congruence between the estimated class population shares and the model predicted class membership. Estimated class population shares are 21%, 41% and 38% for cluster 1, cluster 2 and cluster 3 respectively, whereas model predicted class memberships are 20%, 40% and 40%, for cluster 1, cluster 2 and cluster 3, respectively.

Based on the assumption that adolescents with similar responses tend to cluster within the same risk profile, the class-conditional response probabilities of other self-reported risk behaviours were examined to obtain a risk profile of alcohol drinkers.

Figure 2 shows the profile of 15-year-old adolescents according to their propensity to consume alcohol.

As analyses showed similar profiles for boys and girls, the results are presented without stratifying by gender.

Adolescents who report heavy alcohol consumption (alcohol abuse profile) show higher prevalence of lifetime drunkenness (76%) compared to peer that drink less frequently (8%); they tend to have friends that drink alcohol as well, and experience weekly drunkenness (41% *vs* 12% among adolescents that do not drink).

Among moderate drinker adolescents (moderate profile), 83% reported to have friends who drink and 51% reported to have experienced binge drinking in the past 12 months. Similarly to adolescents who abuse alcohol, 47% of them experienced gambling (against 52% among teens who abuse alcohol). However, differently from adolescents who abuse alcohol, only 19% experienced cannabis use (against 68%) and a higher percentage reported to like school (61% against 45%).

Finally, among teens who do not drink or drink rarely alcohol (no Alcohol profile), only 52% reported to have friends who make use of alcohol, and 74% noticed to like school. None of them experienced drunkenness nor smoking every day in the last 30 days, and only 2% make binge drinking and cannabis use. Other risk behaviours are considerably lower: 14% reported fights and only 6% had sexual intercourse against 51% among heavy alcohol consumers for both items.

DISCUSSION

Adolescence is a transitional phase of life particularly susceptible to transgressions and risk-taking impulses, like smoking, cannabis use and harmful drinking [9, 35].Moreover, the evidence about the co-occurrence of risk behaviours among adolescents may have important implications for the design of intervention programs and the most promising approaches for reducing risk behaviours are those that simultaneously address several domains of risk and protective factors [23, 24].

To depict these aspects of adolescent health, we searched for the behavioural patterns that characterize different levels of alcohol consumption and drew up a profile of Italian teens who use and abuse alcohol.

Confirming previous findings that adolescents exhibit multiple health risk behaviours that tend to cluster in behavioural patterns [21-27], we have identified a cluster of about 21% of 15-year-old adolescents who abuse alcohol and show higher rates of other risk behaviours such as cannabis use, gambling experience, fights with peers, heavy smoking habits in addition to a more negative school approach with respect to peers with a more moderate alcohol use or adolescents who do not drink or drink rarely.

Other aspects that the literature recognized as protective for alcohol use and abuse [28, 29], like living in

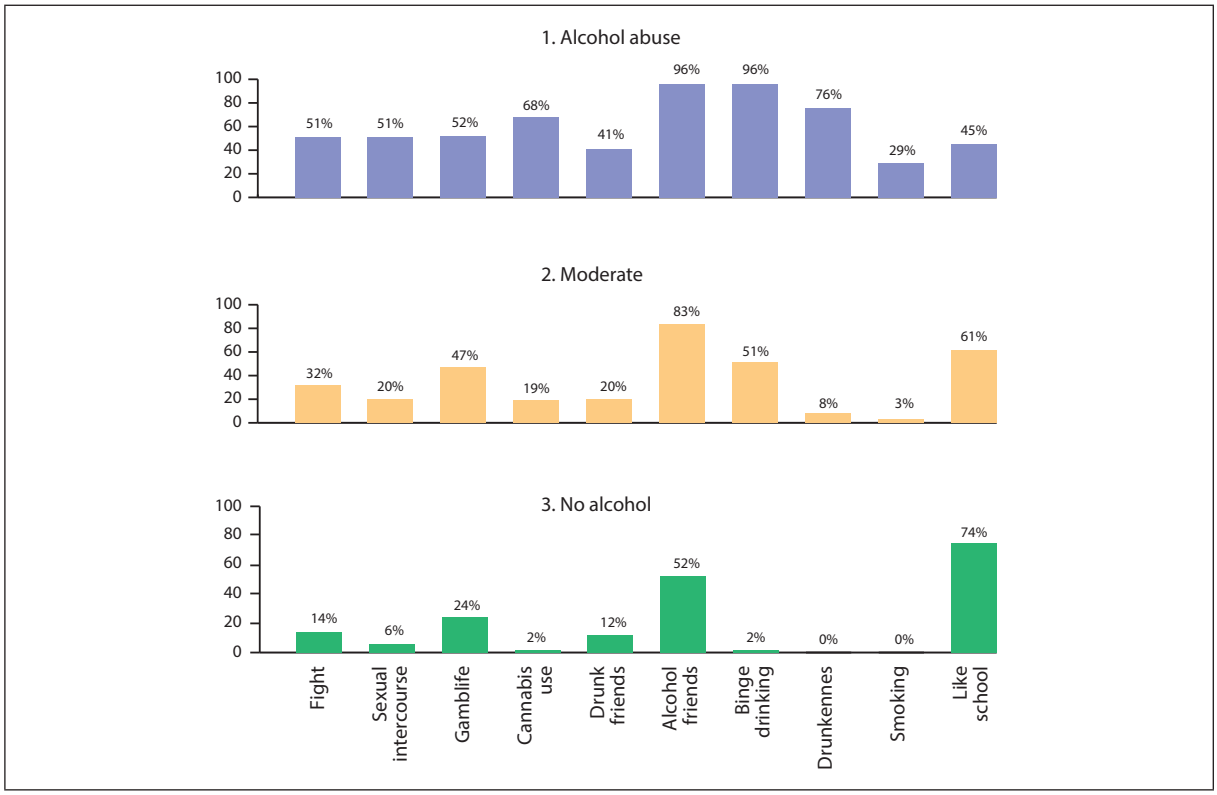

Figure 2
Profiles of 15-year-old adolescents who abuse alcohol, who are moderate drinkers and adolescents who do not drink or rarely drink alcohol.

intact families and having good relations with parents, did not turn out to be distinctive features of the behavioural pattern of teens in Italy. Conversely, in line with literature results, the role of peers showed a significant association with alcohol abuse, confirming that in this phase of life the influence of friends increases and becomes greater than that of parents [28, 36].

Alcohol use and abuse: Italian HBSC results 2018 vs International HBSC results 2018

Data about alcohol consumption obtained from Italian HBSC 2018 study were compared to those from the International HBSC 2018, which involved 45 countries (including Italy).

Data from the last international survey [5] confirm alcohol as the most commonly used substance by 15-year-olds, despite the decline of lifetime use since 2014: almost 3 in 5 have drunk alcohol in their lifetime, with a higher prevalence among boys, especially among younger adolescents. 1 in 5 15-year-olds had been drunk twice or more in their lifetime and almost 1 in 7 (15%) had been drunk in the last 30 days; drunkenness remains higher among boys than girls in all age groups and in almost all HBSC countries [5], even though an increase in alcohol use among adolescent girls was noticed in some countries [1], confirming the gender convergence of the last decade highlighted in previous studies [9].

Italy ranked among the HBSC countries with the highest prevalence of regular alcohol consumption (7° and 15° place for boys and girls, respectively, who reported having ever drunk alcohol in their lifetime; 4° and 13° place for having drunk alcohol in the last 30 days). Conversely, our country was in the second half of the international ranking for drunkenness for both genders (27° place for 15-year-olds who declare having been drunk at least twice in their lifetime and 26° place for having been drunk in the last 30 days).

Further research is needed to better understand the long-term impact of interventions with proven positive short-term effects and to improve the evidence of effectiveness of interventions addressing multiple risk-taking behaviours. In a public health perspective, the evidence of strong similarities between countries in the clustering of adolescent risk behaviours suggests to European and North American countries to collaborate in developing and implementing programs aimed at preventing or reducing adolescent risk behaviours [2]. Furthermore, interventions and policies focused on adolescent health should consider the presence of vulnerability in some young people evaluating differences and possible inequalities among them.

STRENGHTS AND LIMITATIONS

The main strength of this study was the use of a large and representative Italian sample that allowed to draw a profile of teens who drink and abuse alcohol.

To do that, we used a Bayesian approach to select variables for clustering adolescents into risk profiles,

which has proven advantageous in the analysis of highly correlated information.

Unfortunately, as details are lacking about some risk behaviours among younger students, we had to limit our analyses to 15-year-olds.

The HBSC methodology strengths and limitations were described in Appendix 1 of the paper by Nardone *et al.*, published in this issue of *Annali dell'Istituto Superiore di Sanità*.

CONCLUSIONS

Our findings highlighted that 1 in 5 Italian adolescents aged 15 years are heavy alcohol consumers and confirmed previous findings that risk-taking behaviours tend to cluster in behavioural patterns. In fact, the same adolescents that showed alcohol abuse, experienced other risk behaviours such as cannabis use, binge drinking, drunkenness, heavy smoking, gambling, sexual intercourse and fighting more frequently that their peers with a more moderate alcohol consumption.

This should be considered when designing and implementing programs to reduce risk behaviours among adolescents.

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Ethical approval

The Italian HBSC study protocol and questionnaire were formally approved by the Ethics Committee of the Italian National Institute of Health (PROT-PRE876/17, 20 November 2017).

Authors' contribution

LC, PB and PD participated in designing the study. LC, PB, AV, NC, SC, PD, PL AB and VS drafted the manuscript. PB conceived the analyses plan; PB and LC performed the statistical analysis. All authors participated in data collection as members of the Italian HBSC team.

Conflict of interest statements

All Authors declare that they have no conflict of interest.

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REFERENCES

1. Inchley J, Currie D, Vieno A, et al. Adolescent alcohol-related behaviours: trends and inequalities in the WHO European Region, 2002-2014. WHO Regional Office for Europe; 2018. Available from: /www.euro.who.int/en/publications/abstracts/adolescent-alcohol-related-behaviours-trends-and-inequalities-in-the-who-european-region,-20022014-2018.
2. Looze MD, Raaijmakers Q, Bogt TT, et al. Decreases in adolescent weekly alcohol use in Europe and North America: evidence from 28 countries from 2002 to 2010. Eur J Public Health. 2015;25:69-72.
3. Vieno A, Altoè G, Kuntsche E, Elgar FJ. Do public ex-

penditures on health and families relate to alcohol abstaining in adolescents? Multilevel study of adolescents in 24 countries: International trend in alcohol abstaining in adolescence. Drug Alcohol Rev. 2018;37:S120-S128.
4. Inchley J, Currie D, Cosma A, Samdal O. Health Behaviour in School-Aged Children (HBSC) study protocol: Background, methodology and mandatory items for the 2017/18 Survey. St Andrews: CAHRU; 2018.
5. Inchley J, Currie D, Budisavljevic S, Torsheim T, Jastad A, Cosma A. Spotlight on adolescent health and well-being. Findings from the 2017/2018 Health Behaviour in School-aged Children (HBSC) survey in Europe and Canada. International report. Volume 1. Key findings. Copenhagen: WHO Regional Office for Europe; 2020.
6. Room R. Intoxication and bad behaviour: understanding cultural differences in the link. Soc Sci Med. 2001;53:189-98.
7. Room R, Bullock S. Can alcohol expectancies and attributions explain western europe's north-south gradient in alcohol's role in violence? Contemp Drug Probl. 2002;29:619-48.
8. Collicelli C. Italian lifestyle and drinking cultures. In: Prina F, Tempesta E (Eds.). Salute e società. Youth and alcohol: Consumption, abuse and policies, an interdisciplinary critical review. Franco Angeli; 2010.
9. Vieno A, Lenzi M, Santinello M, Cavallo F. Gender convergence in adolescent drunkenness in different Italian regions. Int J Public Health. 2013;58:785-90.
10. Aresi G, Cleveland MJ, Vieno A, Beccaria F, Turrisi R, Marta E. A mixed methods cross-cultural study to compare youth drinking cultures in Italy and the USA. Appl Psychol Health Well-Being. 2020;12:231-55.
11. Perkins HW. Surveying the damage: a review of research on consequences of alcohol misuse in college populations. J Stud Alcohol Suppl. 2002;91-100.
12. Ellickson PL, Tucker JS, Klein DJ. Ten-year prospective study of public health problems associated with early drinking. Pediatrics. 2003;111:949-55.
13. Tomlinson KL, Brown SA, Abrantes A. Psychiatric comorbidity and substance use treatment outcomes of adolescents. Psychol Addict Behav. 2004;18:160-9.
14. Currie D, Small G, Currie C. Prevalence and profiles of substance and multi-substance use by adolescents: UK and international perspectives. London: Advisory Council on the Misuse of Drugs; 2005.
15. Crosnoe R. The connection between academic failure and adolescent drinking in secondary school. Sociol Educ. 2006;79:44-60.
16. Shope J, Bingham C. Teen drivingmotor-vehicle crashes and factors that contribute. Am J Prev Med. 2008;35:S261-S271.
17. Alex Mason W, Hitch JE, Kosterman R, McCarty CA, Herrenkohl TI, David Hawkins J. Growth in adolescent delinquency and alcohol use in relation to young adult crime, alcohol use disorders, and risky sex: a comparison of youth from low- versus middle-income backgrounds: Adolescent delinquency and alcohol use. J Child Psychol Psychiatry. 2010;51:1377-85.
18. Boden JM, Fergusson DM. The short- and long-term consequences of adolescent alcohol use. In: Saunders JB, Rey JM (Eds.). Young people and alcohol. Chichester, UK: John Wiley & Sons, Ltd; 2014.
19. Feldstein Ewing SW, Sakhardande A, Blakemore S-J. The effect of alcohol consumption on the adolescent brain: A systematic review of MRI and fMRI studies of alcohol-using youth. NeuroImage Clin. 2014;5:420-37.
20. Marshall EJ. Adolescent alcohol use: Risks and consequences. Alcohol Alcohol. 2014;49:160-4.
21. DuRant RH, Smith JA, Kreiter SR, Krowchuk DP. The relationship between early age of onset of initial substance use and engaging in multiple health risk behaviors among young adolescents. Arch Pediatr Adolesc Med. 1999;153:286-91.
22. French S, Rosenberg M, Knuiman M. The clustering of health risk behaviours in a Western Australian adult population. Health Promot J Austr. 2008;19:203-9.
23. Jackson C, Sweeting H, Haw S. Clustering of substance use and sexual risk behaviour in adolescence: analysis of two cohort studies. BMJ Open. 2012;2:e000661.
24. Spring B, Moller AC, Coons MJ. Multiple health behaviours: overview and implications. J Public Health. 2012;34:i3-i10.
25. Molinaro S, Benedetti E, Scalese M, et al. Prevalence of youth gambling and potential influence of substance use and other risk factors throughout 33 European countries: first results from the 2015 ESPAD study: Underage gambling in Europe. Addiction. 2018;113:1862-73.
26. Charrier L, Berchialla P, Dalmasso P, Borraccino A, Lemma P, Cavallo F. Cigarette smoking and multiple health risk behaviors: A latent class regression model to identify a profile of young adolescents. Risk Anal. 2019;39:1771-82.
27. Walsh SD, Sela T, De Looze M, et al. Clusters of contemporary risk and their relationship to mental well-being among 15-year-old adolescents across 37 countries. J Adolesc Health. 2020;66:S40-S49.
28. Steketee M, Jonkmanh H, Berten H, Vettenburg N. Alcohol use among adolescents in Europe: environmental reasearch and preventive actions. The Netherlands: the Verwey-Jonker Institute; 2013. Available from: http://hdl.handle.net/1854/LU-3257735.
29. Tomčíková Z, Dankulincová Veselská Z, Madarasová Gecková A, van Dijk JP, Reijneveld SA. Adolescents' drinking and drunkenness more likely in one-parent families and due to poor communication with mother. Cent Eur J Public Health. 2015;23:54-8.
30. Lazzeri G, Giacchi MV, Dalmasso P. The methodology of the Italian HBSC 2010 study (Health Behaviour in School-aged Children). Ann Ig Med Prev E Comunità. 2013;25:225-33.
31. Laska MN, Pasch KE, Lust K, Story M, Ehlinger E. Latent class analysis of lifestyle characteristics and health risk behaviors among college youth. Prev Sci. 2009;10:376-86.
32. Lin TH, Dayton CM. Model selection information criteria for non-nested latent class models. J Educ Behav Stat. 1997;22:249-64.
33. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2019.
34. Linzer DA, Lewis JB. poLCA : An R package for polytomous variable latent class analysis. J Stat Softw. 2011;42. doi: 10.18637/jss.v042.i10
35. Simons-Morton BG, Farhat T, Ter Bogt TFM, Hublet A, Kuntsche E, Saoirse Nic Gabhainn SN, Godeau E, Kokkevi A, and the HBSC Risk Behaviour Focus Group. Gender specific trends in alcohol use: cross-cultural comparisons from 1998 to 2006 in 24 countries and regions. Int J Public Health. 2009;54:199-208.
36. Currie C, Gabhainn S, Godeau E, et al. Inequalities in young people's health: HBSC international report from the 2005/06 Survey. Health Policy for Children and Adolescents, No. 5, Copenhagen, Denmark: WHO Regional Office for Europe; 2008.

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Book Reviews, Notes and Comments

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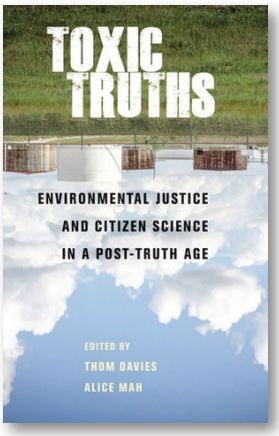

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We are used to the term "post-truth" when it comes to politics where the debate is often disconnected from facts and largely affected by appeals to emotion. In this book we understand how much the political culture of "alternative facts" and "fake news" has contaminated the nature of science, particularly the field of environmental science where science and expertise are increasingly under attack. Environmental justice (EJ), born in the 1980s, is one of the answers to this cultural trend. EJ is meant to guarantee to everyone "the same degree of protection from environmental and health hazards, and equal access to the decision-making process to have a healthy environment in which to live, learn, and work" (https://www.epa.gov/environmentaljustice). From the reading of this book we learn that indeed EJ themes were evoked from hundreds of years prior. The Editors provide as an example the yellow fever epidemic of the late nineteenth century that killed thousands of people in Philadelphia. While White people fled the city en masse, the Black people were organized to keep the city functioning. Fewer Black people than White people were dying because Black people had a greater immunity to the disease but people misunderstood how the disease was transmitted and spread rumors that Black people caused the epidemic. In 1973 Absalom Jones and Richard Allen – leaders of the Free African Society that was founded only six years earlier – wrote a document responding to the charges: this is considered the first document of EJ.

The chapters of this book address the three versions of EJ: *distributive*, *procedural*, and *capabilities*. The first type, distributive, is concerned with the geographic distribution of goods and/or burdens among groups of individuals of environmental hazards in relation to marginalized communities. The second type, *procedural*, is centered around the "meaningful involvement of all people regardless of race, colour, national origin or income with respect to the development, implementation and enforcement of environmental laws, regulations and policies." (Bullard and Johnson, 2000, *Journal of Social Issues*, 56(3), 555-578). The third version of justice is concentrated on *capabilities*, i.e. an approach that is meant to ensure the well-being of a population, where "justice is not about achieving an appropriate distribution of things between people, but rather about people being able to live lives that they consider worthwhile" (Edwards et al. 2016, *Progress in Human Geography*, 40(6), 754-769). The topics of citizen science with its pros and cons is widely analysed in this book that interrogates several ways that local communities, residents, and activists engage within EJ struggles.

The book is structured in four interconnected sections.

Part I, "Environmental Justice and Participatory Citizen Science", presents case studies of participatory EJ research. Among these I would like to mention the one concerning contamination by per- and polyfluorinated compounds (PFAS) by Phil Brown and coworkers (Chapter 1). PFAS contamination of drinking-water has been a relevant environmental and health problem in United States as well as in Italy in the Veneto Region. The Authors explore the important interconnections between scientific discovery, environmental justice activism, and the political, social, and economic components. They present as an example of success the Superfund Research Program Center established in 2017 at the University of Rhode Island that brings together scientists from various universities with communities on Cape Cod in a multi-project center with a strong community engagement core. The main goal of this Program is to learn more about the human and environmental impact of PFAS contamination, pushing for political action and disseminate lessons learned to help avoiding similar contamination problems in the future. Overall, this chapter as well as the others contained in Part I emphasize that, by remaining the need for a science-based approach, citizen science can contribute to change.

Part II, "Sensing and Witnessing Injustice", show how you can contribute making pollution visible without the aid of scientific knowledge and devices. Alternative tools of understanding pollution are presented. Examples are the Ecaudorian "toxic tours" (Chapter 5) where the observation of contaminated soil cores using an auger is a means of "sensing" injustice and the representation of pollution by e-waste in Ghana (Chapter 6) through participatory photography.

Part III, "Political Strategies for Seeking Environmental Justice", provides examples of citizen science projects and environmental inequalities that by mobilizing and politicizing communities can achieve EJ. Cases are presented that span from the tactics of "soft confrontation" against industrial pollution in China (Chapter 10) to utilizing top-down national data in Italy to

achieve EJ (Chapter 9). In particular, Chapter 9 is the contribution by Roberto Pasetto and Ivano Iavarone from the Department of Environment and Health of our Institute. With a focus on polluted sites in Italy they use an epidemiological surveillance approach to show how communities that are overburdened by the health impacts of environmentally hazardous industry are often also socially deprived.

Finally, part IV, "Expanding Citizen Science," explores the possibilities as well as limitations of citizen science for achieving EJ. To this regard I would like to mention the last chapter of this book by Nicolas Shapiro and coworkers (Chapter 14) who suggest that citizen scientists should look beyond the creation of exposure/toxicity data to combat pollution and concentrate on what they call "extra-numerical evidentiary projects" that are more centered on social and political change. In this last part we go back to the important question raised by Barbara Allen at the beginning of the book (Chapter 2) "What kind of science can serve as 'change-agent' knowledge – what are the ingredients that can facilitate action?". If you want to know the opinion of excellent scientists on this matter I encourage you to read this book.

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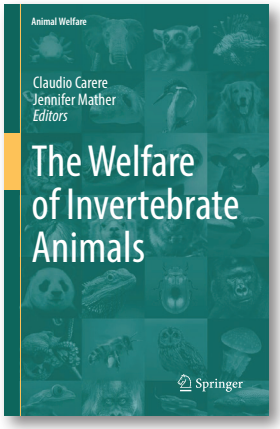

THE WELFARE OF INVERTEBRATE ANIMALS

Claudio Carere, Jennifer Mather (Eds)
Springer International Publishing (Animal Welfare, 18), 2019.
248 p. € 176,79
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Animal welfare issues are approached very differently among scientists. In particular, the composite universe of biomedical scientists, dealing more or less directly with animal behaviour, enlists very different professionals; often, e.g., neurophysiologists are much less sensitive to welfare than their colleagues more focused on subtle behavioural variables, including ethologists and zoologists carrying out extensive field work. Therefore, the very diverse research topics reflect as much diverse human and humane attitudes. This also includes the "inevitable bond", which enchains single scientists to their animal subjects [1]. It may also be a question of age. In fact, since a couple of decades, we are facing a progressively increasing level of awareness and susceptibility in the new generations (veterinary medicine seems especially attractive for undergraduates). For contemporary students, the psychophysical welfare of their animal subjects is becoming more and more a delicate issue, requiring novel skills and in many cases affecting experimental designs and settings. In parallel, and correlated to this, there is an ongoing change in the way the individual scientist empathizes with animal subjects. Some more "egoistic" component may play a role as well. In fact, "good welfare" is often reflected in the collection of better and sounder results, easy to replicate, since of course stressed or unhealthy subjects do not provide state-of-the-art behavioural and physiological results. As a consequence, welfare assessment is an important factor concerning data quality.

"Higher" vertebrates definitely include the most empathic animal species: dogs, cats and non-human primates which have a long and sometimes intricate history of "special protection", with specific guidelines. By contrast, "lower level" taxonomic groups have been somehow relegated to a level of non-problematic experimental concern. A kind of new era arose about thirty years ago. The European legislation on animal experimentation followed a precise trajectory. For many years (since 1993), the United Kingdom regulation put the octopus (*Octopus vulgaris*, belonging to Cephalopods, an evolutionarily very peculiar marine invertebrate taxon including also cuttlefish and squids) at about the same level of the vertebrate classes, the latter including since 1986 a strict general European legislation. The Canadian Council of Animal Care already mentioned Cephalopods in 1991, New Zealand in 1999, the Australian new regulation in 2004 [2].

In the last legislative revision [3] a major regulatory step occurred: all Cephalopod Molluscs became *ope legis* similar to vertebrates being eventually recognized as sentient species (similarly, the term "sentient" found a philosophical definition in St. Augustine's writings *Confessions*, a masterpiece of theological and epistemological thoughts) reviewing the matter of animal suffering in comparison with human suffering, an issue dating from Plato at least. Therefore, not only octopuses, but also cuttlefish and squids were assimilated to protected vertebrates and this prompted novel studies such as the recent curiosity to examine individual variability in stereotyped predatory behaviour of cuttlefish (*Sepia officinalis*) under laboratory conditions [4].

But what about all other invertebrates? In the Editors' words: "Invertebrates, like all the other animals, are an essential part of our lives. We eat them, we study them, and some of us keep them as "pets," for example, tarantulas and other spiders". In other words, not only they constitute about 99% of animal species, but they are also tremendously (and increasingly) exploited by humans, including research.

The present book focuses specifically on invertebrate psychophysical welfare. The starting question is "Why invertebrate welfare?" (chapter 1, signed by the co-editors themselves), summarizing the texture and the rationale of the book, its possibly hidden zoo-anthropological vo-

cation, while outlining its logical structure, contents and primary and secondary goals. This, in our view, is a true novelty for animal welfare science and community.

The Italian National Institute of Health (Istituto Superiore di Sanità, ISS) scientist Augusto Vitale and philosopher Simone Pollo (Rome University "Sapienza") contributed to the writing of the second chapter, entitled "Invertebrates and humans: science, ethics and policy". It provides a vivid reflection on the moral status of invertebrates, their rather peculiar "sentience" and the philosophical weapons to critically argue whether adequate protection to these animals is actually deserved.

The formulation of the next insect welfare chapter is entrusted to entomologists Michael Bopprè and Richard I. Vane-Wright. In particular, in chapter 3, they attempt to solve the dilemmas due to the increasingly widespread practice to keep insects in captivity, considering the huge variety of species and the still poor knowledge about their commercial breeding. Moreover, in the last years the proposal to exploit protein food of insect origin gained global attention due to the exploding increase of human population and associated food needs. The following chapter "Welfare of managed honey bees", by Claudia Garrido and Antonio Nanetti, is focused on the management of honey bee colonies with particular regard to their ecological "superpowers" and the relationship between honeybees and agriculture.

Chapters 5 and 6, "Spider welfare" and "Coral and cnidarian welfare in a changing sea", cover very original, relevant and timely issues, representing vivid and creative points for discussion for animal behaviourists. Economic and marketing stakeholders may well represent counterparts endowed by divergent feelings. Importantly, the former chapter bridges animal welfare with conservation and global-change biology, a link that is not yet fully realised by both students of animal welfare and biodiversity management and conservation, despite being a real need for the environmental challenges of the new millennium.

In chapter 7 Robert W. Elwood's contribution is structured in a detailed analysis regarding the physiological and behavioural responses to pain in crustaceans, nicely updating with the most recent results an issue that was previously brought to attention in this same journal *Annali dell'Istituto Superiore di Sanità* [5]. The mass of scientific evidence supports the active discussion about the need for some kind of special protection for crustacean Decapods since the last European Directive. This was, we believe, mostly due to the raising public awareness in relation to the use of lobsters, for example, as food. For a more strictly scientific point of view, it is a chapter full of comparative references among vertebrates, including humans. In fact, most of the emerging trends, i.e. the enhanced sensitivity of the general public, are also reflected in the way scientists performing animal experiments are evolving from the use of a variety of rodent models towards zebrafish or invertebrate models.

The chapters 8 and 9 are signed by both volume co-editor Jennifer Mather (Canada), a pioneer in Cephalopod behaviour and welfare, and a team partly belonging to the Stazione Zoologica "Anton Dohrn" in Naples, Italy, where most of the extraordinary behavioural and neural complexities of Cephalopods have been described even in past times. Specifically, the authors debated regarding the consciousness of Cephalopods, emphasizing their cognitive abilities and the regulatory aspects around issues linked to their welfare.

The authors conclude the book by expressing in the last chapter the need to focus attention on the individual personality for the provisions of animal welfare, which might appear weird for a butterfly or an earthworm. Yet, recent evidence shows that even ants may possess individual personalities, i.e. consistent clusters of behavioural traits shaped by genetic and epigenetic factors. This previously overlooked inter-individual variation is a topic at the frontiers of behavioural biology and stress physiology, including implications in translational medicine [6] because different personalities might react differently to experimental treatments and/or captive conditions and handling-even in an invertebrate. Therefore, tailoring welfare care and actions to the individual, rather than to the species-specific needs only, is a crucial refinement.

In some readers this book will provoke some skepticism, while triggering variable doses of cultural antibodies, e.g. raising the criticism that any ameliorative effort in the maintenance of experimental animals will inevitably result in higher costs, therefore making even invertebrate research less affordable. Nevertheless, still far from representing an exhaustive manual, this precisely-focussed compilation of essays represents the first useful and accurate outline on why and how the welfare and care management of invertebrates should be taken into consideration.

It is worth mentioning, since the present book review is published in this institutional journal, that the ISS played a major and pivotal role in governing the problem of animal experimentation in Italy. This Institute was historically committed in both promoting the 3R culture and in providing, since 1992, expert opinions to approve single scientific and/or industrial projects exploiting animal subjects. ISS veterinarian and animal experimentation expert Rodolfo Lorenzini, a lifelong supervisor of Italian activities carried out on vertebrates, reports in his accurate review [7] the history of the implementation of the European regulations at the national level, with the progressive steps in which ISS always played a pivotal role. Even the translation into Italian of the 1986 European Directive [8] was sketched by one of us (EA), despite its rather delayed implementation, six years later.

References

1. Visalberghi E, Alleva E. Book review of "The inevitable bond: Examining scientist-animal interactions". In: Davis D, Balfour D (Eds). Cambridge University Press, Cambridge (UK) (1992). Quarterly J Exp Psychol;1993;46B: 222-4.
2. Berry A, Vitale A, Carere C, Alleva E. EU guidelines for the care and welfare of an "exceptional invertebrate class" in scientific research. Ann Ist Super Sanità. 2015;51:267-9.
3. Council of Europe. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union L 276, 2010 Oct 20.
4. Zoratto F, Cordeschi G, Grignani G, Bonanni R, Alleva

E, Nascetti G, Mather JA, Carere C. Variability in the "stereotyped" prey capture sequence of male cuttlefish (*Sepia officinalis*) could relate to personality differences. Anim Cogn. 2018;21:773-85.
5. Gherardi F. Behavioural indicators of pain in crustacean decapods. Ann Ist Super Sanità. 2009;45:432-8.
6. Carere C, Alleva E. Taking into account animal and human personalities: relevance for health and disease. Ann Ist Super Sanità. 2011;47:331-2.
7. Lorenzini R. Il concetto di benessere degli animali per la ricerca scientifica negli ultimi quaranta anni: il ruolo dell'ISS. In: Ricciardi W, Alleva E, De Castro P, Giuliano F, Salinetti S (Eds). 1978-2018: quaranta anni di scienza e sanità pubblica. Roma: Istituto Superiore di Sanità; 2015.
8. Italia. Decreto Legislativo 27 gennaio, n. 116. Attuazione della direttiva n. 86/609/CEE in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici. Gazzetta Ufficiale, Serie Generale n. 40, 18 febbraio 1992 - Suppl. Ordinario n. 33.

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Publications from International Organizations on Public Health

Edited by
Annarita Barbaro

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)

The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets. Rome: Food and Agriculture Organization of the United Nations. 2020; 320 p. ISBN 9789251329016. The number of people affected by hunger globally has been rising since 2014. This report complements the usual assessment of food security and nutrition with projections of what the world may look like in 2030, if trends of the last decade continue. Projections show that the world is not on track to achieve Zero Hunger by 2030 and, despite some progress, most indicators are also not on track to meet global nutrition targets. The food security and nutritional status of the most vulnerable population groups is likely to deteriorate further due to the health and socio-economic impacts of the COVID-19 pandemic. This report also introduces new analysis of the cost and affordability of healthy diets around the world, by region and in different development contexts. It presents valuations of the health and climate-change costs associated with current food consumption patterns, as well as the potential cost savings if food consumption patterns were to shift towards healthy diets that include sustainability considerations. This report then concludes with a discussion of the policies and strategies to transform food systems to ensure affordable healthy diets, as part of the required efforts to end both hunger and all forms of malnutrition.

OECD-FAO Agricultural Outlook 2020-2029. Rome: Food and Agriculture Organization of the United Nations. 2020; 330 p. ISBN 9789251325391. The OECD-FAO Agricultural Outlook presents a consistent baseline scenario for the evolution of agricultural and fish commodity markets at national, regional and global levels over the coming decade (2020-2029). This Outlook thus focuses on the medium term, complementing both short-term market monitoring, outlook publications, and long-term projections. This current edition of the Outlook was being finalised under the unique circumstances generated by the COVID-19 pandemic. As the full impact of the pandemic on agricultural and fish markets remain uncertain, at least in quantitative terms, they were not incorporated into the baseline projections. The Outlook projections are inevitably uncertain because they extend ten years into the future and are based on assumptions regarding economic and policy conditions. These uncertainties are discussed in detail at the end of each of the commodity chapters.

Global Forest Resources Assessment (FRA) 2020. Main report. Rome: Food and Agriculture Organization of the United Nations. 2020; 184 p. ISBN 9789251329740. Since the first assessment in 1948, the report has evolved into a comprehensive evaluation of forest resources and their condition, management and uses, covering all the thematic elements of sustainable forest management. This report examines the status of, and trends in, more than 60 forest-related variables in 236 countries and territories in the period 1990-2020. The information provided by FRA presents a comprehensive view of the world's forests and the ways in which the resource is changing. Such a clear global picture supports the development of sound policies, practices and investments affecting forests and forestry. One of the key findings in this edition is that while deforestation continues, it has been reduced in the last five years.

Exposure of humans or animals to SARS-CoV-2 from wild, livestock, companion and aquatic animals. Qualitative exposure assessment. FAO animal production and health papers series. Rome: Food and Agriculture Organization of the United Nations. 2020; 81 p. ISBN 9789251330081 This assessment looks into risks, current knowledge gaps, evidence for susceptibility of different animal species to SARS-CoV-2, species prioritization for further research, and recommendations for targeted One Health investigations. Understanding the risk of exposure of humans or animals to SARS-CoV-2 from animals and their products is essential for containing virus spread, prioritizing research, protecting food systems, and informing national One Health investigations and mitigation measures. Results can inform country-level risk assessment and provide the evidence base for targeted SARS-CoV-2 investigations in animals and mitigation options. In detail, this publication provides an assessment of the risk of human or animal exposure to SARS-CoV-2 through contact with, handling or consumption of wild, domestic and aquatic animal species or their products; the identification of current knowledge gaps regarding the zoonotic origin or animal-human spill over of SARS-CoV-2 and recommendations on priority studies; a summary of available evidence for SARS-CoV-2 susceptibility of different animal species; evidence-based recommendations on how to prioritize animal species for targeted field investigations or research studies; and recommendations for targeted One Health investigations and epidemiological, laboratory, anthropological or seasonality studies to fill critical knowledge gaps evidenced by this exposure assessment.

ISC (INTERNATIONAL SCIENCE COUNCIL)

Open Science for the 21st Century. Draft ISC Working Paper. Paris: International Science Council (ISC). 2020; 30 p. This document is a response to the UNESCO global consultation on open science. It brings together strands of work that have developed within the International Science Council's (ISC) community to create a draft working paper that will be further developed through consultation with the Council's members as a formal ISC position paper on open science. The paper describes the rationale for and the origins of the modern open science movement, its dimensions and its applications. It makes recommendations to scientists, to universities, to UNESCO and to other science systems stakeholders about changes that are necessary for the effective operation of open science. The paper includes, at the end of relevant sections, an indication of ISC projects and programmes that are designed to support aspects of open science. The appendix includes answers to specific questions posed by UNESCO for which the detailed arguments are presented in the main text.

Hazard definition & classification review. Technical Report. Paris: International Science Council (ISC). 2020; 88 p. This report is the result of an international collaborative effort between the UN Office for Disaster Risk Reduction (UNDRR) and the International Science Council (ISC) to identify the full scope of hazards relevant to the Sendai Framework as a basis for countries to review and strengthen their risk reduction policies and operational risk management practices. The Sendai Framework for Disaster Risk Reduction 2015-2030 ('the Sendai Framework') was one of three landmark agreements adopted by the United Nations in 2015. The other two being the Sustainable Development Goals of Agenda 2030 and the Paris Agreement on Climate Change. This Technical Report supports all three by providing a common set of hazard definitions for monitoring and reviewing implementation which calls for "a data revolution, rigorous accountability mechanisms and renewed global partnerships".

EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA). **Safety of Schizochytrium sp. oil as a novel food pursuant to Regulation (EU) 2015/2283** EFSA Journal. 2020;18(10):e06242 doi: 10.2903/j.efsa.2020.6242. Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the safety of Schizochytrium sp. oil as a novel food (NF) pursuant to Regulation (EU) 2015/2283. Schizochytrium sp. is a single-cell microalga. The strain WZU477, used by the applicant (Progress Biotech bv), was found to belong to the species Schizochytrium limacinum and was obtained in a marine environment from rotted mangrove forest leaves. The NF, an oil rich in docosahexaenoic acid (DHA), is isolated from the microalgae by mechanical extraction. The applicant proposed to use the NF in infant formulae (IF) and follow-on formulae (FOF). The use level defined by the applicant was derived from Regulation (EU) 2016/127, which states the mandatory addition of DHA to IF and FOF at the level of 20-50 mg/100 kcal. The intake of DHA resulting from the use of the NF in IF and FOF is not expected to pose safety concerns. The composition of the NF indicates the absence of marine biotoxins in the NF. Furthermore, Schizochytrium limacinum was attributed the qualified presumption of safety (QPS) status with the qualification 'for production purposes only'. Based on the information provided, the microalga is not expected to survive the manufacturing process. Toxicological tests conducted with the NF were not performed. However, based on the available toxicological data on various forms of oils derived from Schizochytrium sp., the QPS status of the source of the NF, the production process and the composition of the NF, the Panel considers there are no concerns with regard to toxicity of the NF. The Panel concludes that the NF is safe under the proposed conditions of use.

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA). **Safety of chia seeds (Salvia hispanica L.) subject to thermal processing in relation to the formation of process contaminants as a novel food for extended uses** EFSA Journal. 2020;18(9):e06243 doi: 10.2903/j.efsa.2020.6243. Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the safety of chia seeds in foods subject to thermal processing which may result in the formation of process contaminants. The safety assessment of this novel food (NF) is based on previous assessments of chia seeds by the EFSA NDA Panel, information received from a public call for data by EFSA and information retrieved from an extensive literature search performed by EFSA. In 2019, during the overall safety assessment of chia seeds, the NDA panel retrieved one reference which, among others, investigated the formation of process contaminants, i.e. acrylamide, hydroxymethylfurfural and furfural, in wheat flour-based biscuits with added chia seeds flour. Based on this study, the Panel considers that there is a potential for substantial acrylamide formation in biscuits with 10-20% added chia seeds flour with low residual moisture contents (≤2%). The Panel is not aware of further scientific evidence corroborating these findings. The extensive new literature searches performed by EFSA did not show any relevant articles regarding either asparagine content or formation of process contaminants in chia seeds and products thereof. Information received from the call for data were either limited or inconclusive. The available evidence does not provide a basis to conclude whether or not the addition of chia seeds to foods undergoing heat treatment (at temperatures above 120°C) results in increased formation of acrylamide as compared to these

foods without chia seeds. Reported concentrations of hydroxymethylfurfural and furfural in heat-treated chia seeds do not pose a safety concern. No information on other process contaminants in chia seeds was found.

UNEP (UNITED NATIONS ENVIRONMENTAL PROGRAMME)

Frontiers 2018/19. Emerging issues of environmental concern. Nairobi: United Nations Environmental Programme. 2019; 80 p. This report series identifies and provides an insight into a broad range of emerging environmental issues that require attention and action from governments, stakeholders, decision makers as well as the public as large. Frontiers 2018/19, launched on 4 March 2019 prior to the fourth UN Environment Assembly in Nairobi, Kenya, covers five key emerging issues: the latest developments in synthetic biology, the critical advantages of landscape connectivity, the complex interactions and vulnerability of permafrost peatlands, the challenges of widespread nitrogen pollution, and the hazards of maladaptation in a world of climate change.

WORLD HEALTH ORGANIZATION (WHO)

The double burden of malnutrition: priority actions on ending childhood obesity. Geneva: World Health Organization. 2020; 115 p. ISBN: 978-92-9022-789-2. Despite the rising prevalence of overweight and obesity in children, responses in addressing the problem range are inadequate in many countries in WHO South -East Asia Region where undernutrition is common. In these settings, prevention of childhood obesity is not a priority despite the clear links between undernutrition and overweight and obesity and an ever increasing burden of noncommunicable disease. This technical report provides regional and country data on the double burden of malnutrition, and particularly on childhood overweight and obesity, highlights the need for an integrated approach to address overweight and obesity through integration into existing programmes while supporting the improvement of food environments. This report highlights the value of applying WHO-recommended double-duty actions. In line with the double-duty approach, countries should develop common programme packages for obesity and overweight that can be integrated into programmes that currently focus on undernutrition and micronutrient deficiencies. Special efforts should be made to include overweight and obesity considerations in the provision of quality maternal and antenatal care and diets; support breastfeeding and ensure optimum complementary feeding for young children; and promote healthy diets in older children and adolescents, along with adequate physical activity across all age groups.

Managing the COVID-19 infodemic. A call for action. Geneva: World Health Organization. 2020; 43 p. ISBN: 9789240010314. This report highlights the public impact of the "infodemic" or overabundance of information – some accurate, some not – that is spreading alongside the COVID-19 pandemic. Around the world, this overabundance of information confuses people about what actions to take to protect themselves and those around them from disease outbreak. This Report arises from a workshop hold in April, in which the WHO convened with over 1300 field experts to develop guidance to help individuals, community leaders, governments and private sector understand what 50 key actions they can take to manage the COVID-19 infodemic.

Global tuberculosis report 2020. Geneva: World Health Organization. 2020; 232 p. ISBN 978-92-4-001313-1 (electronic version) ISBN 978-92-4-001314-8 (print version). The World Health Organization (WHO) has published a global tuberculosis (TB) report every year since 1997 with the purpose of providing a comprehensive and up-to-date assessment of the TB epidemic in order to progress in the response to the epidemic, at global, regional, and country levels. The 2020 global TB report showcases the progress made towards ending the TB epidemic, and puts in stark perspective the current and potential impact of the COVID-19 pandemic, in eroding the hard-won gains of recent years. In recognition of the enormous health, social and economic impacts of the COVID-19 pandemic, the report includes a provisional assessment of how the pandemic will affect the TB epidemic, people with TB and progress towards global TB targets. In 2020, data were reported by 198 countries and territories that accounted for more than 99% of the world's population and estimated number of TB cases. Globally, the annual number of people reported to have accessed TB treatment has grown from about 6 million in 2015, to 7 million in 2018 and 7.1 million in 2019. Access to TB preventive treatment has also increased, from 1 million in 2015, to 2.2 million in 2018 and 4.1 million in 2019. More positively, the WHO European Region has almost reached the 2020 milestone, with a reduction of 19% in the TB incidence rate between 2015 and 2019, and the African Region has made good progress, with a reduction of 16%.

Referees

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Articles in journal

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Books and chapters in a book

Godlee F, Jefferson T. Peer review in health sciences. London: BMJ Books; 1999.

Van Weely S, Leufkens HGM. Background paper: orphan diseases. In: Kaplan W, Laing R (Eds). Priority medicines for Europe and the world – a public health approach to innovation. Geneva: World Health Organization; 2004.

Proceedings

Fadda A, Giacomozzi C, Macellari V. Comparative measurements to validate a new telemetric pressure insoles system. In: 2. International Symposium on measurement, analysis and modelling of human functions. 1. Mediterranean Conference on measurement. Workshop on evaluation check of traceability. Proceedings. Genova: June 14-16, 2004. p. 425-7.

Technical reports

Della Seta M, Di Benedetto C, Leone L, Pizzarelli S, Siegmund U. ETHICSWEB technical guides. Manual for the creation of standards and guidelines for sharing information about knowledge organization systems on ethics and science. Roma: Istituto Superiore di Sanità; 2011. (Rapporti ISTISAN, 11/32).

Legislation

Italia. Decreto legislativo 29 ottobre, n. 419. Riordinamento del sistema degli enti pubblici nazionali, a norma degli articoli 11 e 14 della legge 15 marzo 1997, n. 59. Gazzetta Ufficiale – Serie Generale n. 268, 15 ottobre 1999.

US Social Security Administration. Evidentiary requirements for making findings about medical equivalence. Final rules. Fed Reg. 2006 Mar 1;71(40):10419-33.

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