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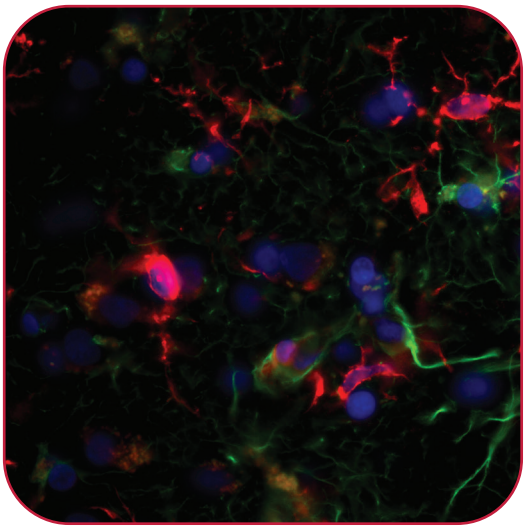
The photograph is a double immunofluorescence staining of a post-mortem human brain section for the astrocyte marker GFAP (green) and the microglia marker Iba-1 (red). Nuclei are stained with DAPI (blue).
Image is provided by Barbara Serafini, Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy.



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EDITORIAL
Newborn screening in Italy: a unique program of public health in Europe

Domenica Taruscio[1] and Andrea Piccioli[2]

[1]*Formerly Director, Centro Nazionale Malattie Rare, Istituto Superiore di Sanità, Rome, Italy*
[2]*General Director, Istituto Superiore di Sanità, Rome, Italy*

Newborn screening (NBS), also called neonatal screening, identifies shortly after birth pre-symptomatic conditions that can affect a child's long-term health or survival. Thus, early detection, diagnosis, and intervention can prevent death, disability or ameliorate the clinical manifestations of diseases, enabling children to reach their potential for health and well-being.

NBS was initiated in Europe during the 1960s for screening phenylketonuria (PKU) [1]. The introduction of tandem mass spectrometry (MS/MS) increased the technical possibility to screen more conditions using a single dried blood spot, therefore the panel of screened disorders ("conditions") gradually expanded [2].

Advances in molecular medicine contributed to enlarge the NBS panels. NBS is now performed in many countries worldwide. Where NBS is available, the number of disorders included in panels varies from one to over 40, including many inherited metabolic diseases, cystic fibrosis, severe combined immunodeficiency, and others [3]. Following the Wilson and Jungner criteria [4] several factors influence the selection of the diseases screened, including disease prevalence in the population, the availability of treatment, etc.

However, countries differ not only in the number of conditions screened, but also in the pre-screening information and support offered to parents, the time of sample collection, the accreditation status of the laboratories conducting screening and the governance, regulation and monitoring of the whole NBS pathway [5]. The major factor is represented by the health care system organization of each country.

Italy runs a universal public healthcare system since 1978, provided to all citizens; the system is organized under the Ministry of Health and administered on a regional basis.

Whereas during the past decades NBS pilot projects were available in several regions [6], in 1992 NBS became a mandatory provision of secondary prevention at national level by law [7] for the identification and early treatment of congenital hypothyroidism, phenylketonuria and cystic fibrosis.

In the years 2016-2017 a combined legislative framework established a nationwide NBS for more the 40 disorders [8-10], including genetic neuromuscular diseases, severe congenital immunodeficiencies and lysosomal storage diseases.

According to the legal framework, the NBS is funded and supported by the National Health System, and it included within the essential levels of assistance provided by the State. The framework defines the requirements in order to favor the maximum uniformity at national level of the regional implementation of NBS. Under this respect it establishes at the Istituto Superiore di Sanità (ISS) the NBS Coordination Center, coordinated by the Director of the ISS, which includes experts and representatives from central and regional institutions as well as three patient associations, Cometa Associazione Studio Malattie Metaboliche Ereditarie (Cometa A.S.M.M.E.), Associazione Italiana Sostegno Malattie Metaboliche Ereditarie (A.I.S.M.M.E.) and Associazione Immunodeficienze Primitive, on behalf of UNIAMO.

The screening program at regional level is a system articulated into four main functional structures, namely: screening laboratory, laboratory for confirmatory diagnosis, clinical centers, and regional coordination/supervision centre. The legislation also defined the panel of screening conditions, the timing for specimen collection, the screening methodology, the confirmatory tests and the clinical follow up. A periodic review of the list of conditions/diseases to be screened is set up by Ministry of Health, in collaboration with other government agencies and organizations. This task is carried out by *ad hoc* working group, co-ordinated by the Ministry of Health, including experts and representatives of institutions and patients associations, including UNIAMO. This working group has also the mission to elaborate an operational protocol including procedures for the management of positive NBS, positive diagnosis and accessing therapies [11].

The NBS Coordination Center performs the following tasks:
- promote and monitor the maximum uniformity in the implementation of newborn screenings at national level, also by identifying common standards;
- collaborate with the Regions for the dissemination of best practices;

Address for correspondence: Domenica Taruscio, Centro Nazionale Malattie Rare, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: domenica.taruscio@iss.it.

establish the procedures for collecting and delivering blood samples to screening centres;
- define the catchment area of each reference screening center for the Region, also in order to facilitate inter-regional collaboration (Italian Regions have quite different sizes);
- supply codified and standardized information to local services in order to inform newborns parents on the risks deriving from hereditary metabolic diseases, the benefits achievable through the screening activity, as well as the best available treatments for the specific disease;
- most important, set up a centralized archive on the results of neonatal screening in order to allow assessing the effectiveness, also in terms of cost-to-benefit, of the NBS program.

The NBS Coordination Center, with the scientific technical support of the National Centre for Rare Diseases of the ISS, works in collaboration with the Regions. Its activities are implemented through national surveys, working meetings, training courses, workshops, conferences and congresses. The results of such activities are published by the ISS as ISTISAN Reports [12-14]. The Reports also present a number of recommendations, aimed at improving the provision of information to citizens, the communication of diagnosis to parents, the consistent and uniform application of NBS on the national territory, as well as on specific technical steps (e.g., blood sampling).

Overall, the NBS is a public health action, building an organized and structured program for secondary prevention, funded by the National Health System.

The main keywords characterizing the features of the Italian NBS model are:

- Public health: NBS is an important public health action aimed at reducing the disease burden through secondary prevention; hence, public resources are devoted to its application.
- Equity: NBS is a measure of secondary prevention for all newborns of the national territory, regardless of region of birth, social status, gender, etc.
- Scientific evidence: planning, implementation and assessing the program are based on scientific evidence and exploit the consistent integration of multidisciplinary expertise.
- Consistency: in the Italian Health System, Regions have an extensive autonomy, also reflecting their different sizes and socio-demographic characteristics. Hence, the Regions, or also groups of Regions, can organize the screening services according to their requirements. Meanwhile, the legal NBS framework ensures a consistent provision of high-standard screenings throughout the whole territory, by facilitating the continuous interaction between the central and regional levels of governance and expertise.
- Assessment and review: data collection and the flow of data to the centralized archive are all important for assessing the outcomes of the program, in terms of efficacy, efficiency and consistency, and provide evidence for possible corrective actions and updates.

Last but certainly not least, participation, starting from the birth of the legal framework, that was also due to the proactive intervention by patient associations. This is reflected by the organization of the NBS. At central governance level, the patients have their place and they actively contribute, through their representatives. Indeed, the consistent and structured patient contribution, building-up from direct experience, is providing important inputs and rates among the most successful features of the Italian NBS.

In conclusion, the Italian NBS system is creating a positive exchange and a virtuous loop among Institutions, science and society, which should feed each other continuously. Assessment of uncertainties, an important aspect in the field of rare diseases [15], should facilitate and address the production of further scientific evidence. Institutions should maintain and strengthen their continuous efforts in supporting the system and maintaining and open dialogue with stakeholders; assessment of the current NBS status highlights the consistent implementation throughout the whole country as a point for attention.

The Institutions should further develop the current interchange with the European Union and EU Member States. This should be implemented at level of EU regulations, where rare diseases have already a significant place, as well as by fostering the Italian contribution to relevant technical-scientific networks and learned societies: under this respect, the European Reference Networks such as MetabERN and RITA [16] and EURORDIS [5] carry out activities of major relevance for the updating and further development of NBS, also in terms of achieving equity and innovation. On the other hand, disseminating and discussing the objectives and achievements of the Italian NBS can significantly contribute to the growth of neonatal screenings as a participated public health action at EU and international level. Society is essential in NBS implementation through advocacy and proactive participation including constructive criticisms. The involvement of patient representatives is essential for building and assessing NBS programs; in the meanwhile, beyond patient associations, citizens awareness of the value of neonatal screening is important for the NBS success.

Conflict of interest statement

The Authors declare that there are no conflicts of interest.

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Two years of COVID-19. Impacts on accessibility of a mental health service for immigrants and individuals in socio-economic difficulties

Massimiliano Aragona, Gianluca Nicolella, Giovanna Laurendi, Andrea Cavani, Gianfranco Costanzo and Concetta Mirisola

Istituto Nazionale per la Promozione della Salute delle Popolazioni Migranti e per il Contrasto delle Malattie della Povertà (INMP), Rome, Italy

Abstract

Objectives. Mental health services utilization decreased dramatically during the COVID-19 pandemic. For persons who are highly vulnerable and at risk of health and social care exclusion, restrictions negatively affected the accessibility to treatments and their mental conditions.

Methods. All psychiatric and psychological interviews carried out at National Institute for Health, Migration and Poverty (INMP) Italy from January 2018 to February 2022 were included in the study. To measure services use, an interrupted time-series analysis using March 2020 as the starting data of COVID-19 pandemic period was considered, and first visits *vs* follow-up session numbered.

Results. A significant decrease was observed in March 2020 due to the lockdown restrictive measures ($p<0.001$). Later on, the number of psychological interventions significantly increased ($p<0.05$), whereas the increment of the psychiatric interventions was not significant. By the end of February 2022 the number of visits returned to pre-COVID-19 levels, although recovery was slower than expected, especially for psychiatric visits.

Conclusions. After a dramatic drop during the lockdown, access to mental health outpatient clinics slowly returned to pre-pandemic levels in the next two years. Considering that mental health needs have increased during the pandemic, mental health services should improve their efforts to reduce barriers of access and to implement outreach referral.

Key words

- coronavirus
- mental health services
- vulnerable populations
- refugees
- homeless
- social care exclusion

INTRODUCTION

The first autochthonous case of SARS-CoV-2 infection was reported in Italy the 21st of February 2020. Some days later, the 8 March, in response to the growing pandemic of COVID-19 in the country, the Italian government imposed a national restriction of movements of the population except for buying food and other necessary goods (drugs, disinfectants, etc.), essential works, and health urgencies [1]. It was the first lockdown in Western countries; at that time the general belief was that after a necessary period of total restriction the crisis would have ended, and people could return to "normal" life. However, after two years the situation is still uncertain. Restrictions have been eased but the daily number of new cases is still high (about 90,000 at the time of the writing of this article).

Due to their pre-crisis vulnerability, it was expected that within the general population some groups could be particularly at risk in this situation. In particular, those in poor socioeconomic conditions [2, 3], homeless people [4], migrant workers [5, 6], asylum seekers and refugees [7, 8], and patients with mental disorders [9, 10].

Early evidence showed that migrants with mental problems were more likely to worsen during the lockdown if they were without VISA, unemployed or reporting food insecurity [11, 12], suggesting that part of the negative impact on mental health was mediated by social difficulties.

Another significant factor involved was the availability of mental health services in terms of accessibility and treatment adherence. Indeed, during the lockdown the accessibility of mental health outpatient services was generally reduced [13, 14], with a potential higher impact on asylum seekers and refugees [15]. Even for those mental health services that remained open and available during the lockdown, the general restrictions

Address for correspondence: Massimiliano Aragona, Istituto Nazionale per la Promozione della Salute delle Popolazioni Migranti e per il Contrasto delle Malattie della Povertà, Via di San Gallicano 25a, 00153 Rome, Italy. E-mail: massimiliano.aragona@inmp.it.

on mobility negatively impacted on the possibility of people in poor socioeconomic conditions to access the services, reducing both accessibility of new patients and follow-up visits of people already in treatment [16]. Moreover, discontinuation of the psychopharmacological treatment during that period resulted in significant worsening of mental conditions [11]. In general, the COVID-19 pandemic has exacerbated social and health inequities, particularly within refugee and migrant populations, challenging the way mental health care is usually delivered [17].

After two years, a few restrictions persist (e.g., the requirement of the so-called "green pass" to access workplace, restaurants, etc.), but, although the personal mobility to reach health services is guaranteed, accessibility of health services may remain difficult, e.g., services working with free-access models had to reshape their organization and visits must now be booked in advance by telephone, some services reduced their opening hours, etc.

The aim of this study was to evaluate the evolution of the impact of COVID-19 during these two years (from March 2020 to the end of February 2022), particularly on the accessibility of outpatient services specifically dedicated to the mental health of patients with a story of immigration and/or in socioeconomic difficulties. Provided that at the beginning of the coronavirus crisis the number of both first psychiatric interviews and follow-up visits decreased dramatically [16], is this problem recovering after two years? And, provided that psychological treatments were also significantly affected by the crisis [11], how did psychological treatment change in the same period?

METHODS

We conducted an interrupted time-series (ITS) analysis using data collected at the National Institute for Health, Migration and Poverty (INMP) in Rome, Italy. The study design, based on the retrospective analysis of routinely collected data, received ethical approval by the Projects & Research board of the Institute. We considered the number of monthly visits of the INMP Mental Health Unit between 1st January 2018 and 28th February 2022. This period was divided into two phases. A pre-COVID-19 era, until February 2020, and a COVID-19 pandemic period, from March 2020 to the end of February 2022. The INMP Mental Health Unit remained open and available for visits uninterruptedly during the COVID-19 pandemic.

For this purpose, we used the same procedure as proposed by Schuengel, *et al*. [18]. We first detrended data using Loess regression and smoothing [19]. Then we looked for a possible seasonality following the recommendations by Ollech [20]. If the presence of seasonal effects was identified, a seasonal adjustment was performed. At the end, we tested change in intercept and slope from the pre-COVID-19 period to the COVID-19 pandemic period using Poisson regression [21]. Furthermore, we performed the same ITS analysis on different subgroups in order to assess any differences between first and follow-up visits or between psychiatric and psychological visits.

All statistical analyses were performed using R (version 4.1.3). Statistical significance was defined as $p< 0.05$.

RESULTS

From January 2018 to February 2022 (N=50 months) a total of 16,841 visits and a mean of 336.82 visits per month (SD=66.26) were recorded. In the 26 months before COVID-19 restrictions a mean of 355.35 visits per month (SD=57.80) were reported, compared with 316.75 visits per month during the COVID-19 pandemic period (SD=70.10). *Figure 1* shows the total visits to the Mental Health Unit. After detrending the data, no seasonality was identified. Poisson regression detected a significant drop from pre-COVID-19 restriction levels to the start of the COVID-19 restriction phase (b=-0.41; SE=0.10; t=-3.97; p<0.001). The increase in mental health visits during COVID-19 period and pre-COVID-19 period was not significantly different (b=0.01; SE=0.01; t=1.54; p=0.13).

Subgroups analyses are shown in *Figure 2* (first and follow-up visits) and *Figure 3* (psychiatric and psychological visits).

Across the study period, 21.54% of all visits are represented by first visits. A total of 3,627 first visits and a mean of 72.54 first visits per month (SD=19.38) were recorded. After detrending the data no seasonality was identified. Poisson regression analysis shows a significant linear increase over the years (b=0.01; SE=0.01; t=2.21; p=0.03), a significant drop at the beginning of the COVID-19 restriction phase (b=-0.65; SE=0.14; t=-4.56; p<0.001), and a non-significant difference between the estimated slopes for the pre-COVID-19 and COVID-19 period (b=0.01; SE=0.01; t=1.51; p=0.14).

Follow ups were 13,214 (78.46% of all visits), with a mean of 264.28 visits per month (SD=53.29) and no seasonality was identified. Poisson regression analysis shows a significant drop at the beginning of the COVID-19 restriction phase (b=-0.34; SE=0.11; t=-3.16; p=0.002) and a no significant difference between the estimated slopes for the pre-COVID-19 and COVID-19 period (b=0.01; SE=0.01; t=1.33; p=0.19).

About a quarter of the total visits (4,250 interviews = 25.24%) were related to psychiatry, with a mean of 85 visits per month (SD=19.66). The other 12,591 visits regarded psychological treatments (74.76% of all visits), with a mean of 251.82 visits per month (SD=53.26). After detrending the data no seasonality was identified. Poisson regression analysis for psychiatric visits shows a significant increase over the years (b=0.01; SE=0.005; t=2.37; p=0.02) and a significant decrease at the start of the COVID-19 restriction phase (b=-0.39; SE=0.12; t=-3.20; p=0.002).

Same analysis for psychological treatments indicates a significant drop at the start of the COVID-19 restriction phase (b= 0.41; SE=0.11; t=-3.69; p<0.001) and that the slope for the COVID-19 period was significantly higher than for the pre-COVID-19 period (b=0.01; SE=0.01; t=2.02; p=0.049).

DISCUSSION

This study follows a previous one of our group showing that the lockdown had a strong effect on the availability

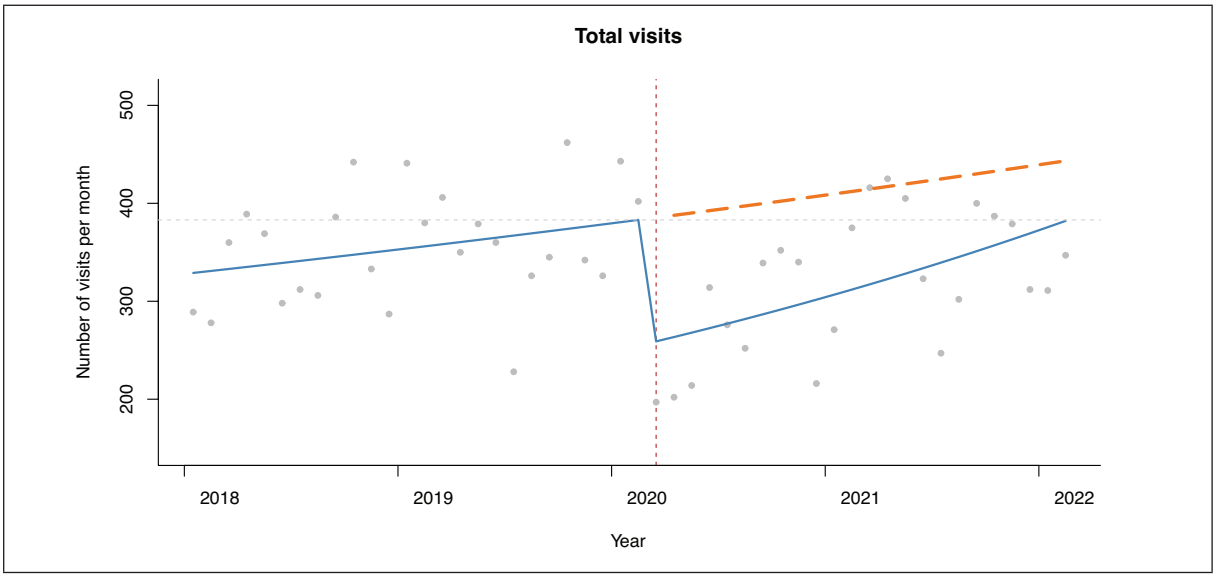

Figure 1
Number of monthly visits before and during the pandemic COVID-19. Vertical dashed line: introduction of restrictions. Continuous line: trend over the years. Dashed line: counterfactual scenario. Horizontal dashed line: pre-pandemic level.

of mental health facilities for immigrants and persons in poor socio-economic conditions, resulting in a significant reduction of the number of both first and follow-up psychiatric visits [16]. In the present study, previous indicators of treatment availability and adherence were extended temporarily to study their trend before and after the onset of the COVID-19 pandemic, from January 2018 to February 2022. Moreover, we considered the entire spectrum of mental health visits available in our service (psychiatric and psychological visits).

As expected, at the beginning of the crisis the rigid lockdown caused a dramatic drop of both first and follow-up visits, not only in psychiatry but also in psychological activities. However, after the initial lockdown the restrictions were gradually removed and then reshaped differently according to the different pandemic phases. As a result, people returned to be free to move to reach health facilities. Our data show that following this change, the total number of visits to mental health services increased progressively over time, and after two years they returned to pre-COVID-19 levels. A similar trend is shown when first visits are differentiated from follow-up visits. However, the present number of visits is still below the level expected if the pre-pandemic trend of visits had continued over time. Moreover, it is noteworthy that the return to pre-pandemic levels has been so slow and has taken so long, despite the restrictions of movements were largely removed already in June 2021. This is a serious concern, considering that during the pandemic it is frequently reported an increase in mental health needs, particularly in vulnerable populations like immigrants, patients with pre-existing mental health issues, and people in poor socio-economic condition (which are also increased in number due to the social and economic disaster produced by the pandemic) [22-25]. Accordingly, we would have expected a faster recovery in the utilization of mental health services after the lockdown, as well as a significant increase of total numbers. Although a "ceiling-effect" (see limitations of the study) can have influenced this result, the effect should be less on the slope than on rough numbers, so it is likely that barriers of access are also operating and proactive changes of the organization of the service are needed to reach unexpressed needs.

Comparing trends in psychiatric and psychological activities, the psychiatric service utilization seems to have dropped less sharply during the national lockdown while the increase in psychiatric visits during the COVID-19 period appears slower. In contrast, after a relatively more dramatic drop, the increase in visits was faster for psychological activities, with a significantly higher slope for the COVID-19 period, surpassing the pre-pandemic levels. This result suggests that the persisting difficulties of access discussed above are mainly related to psychiatric treatments. Possible reasons for this difference may be related to the characteristics of treatments and to organizational factors. Overall, psychiatric visits were influenced less strongly by the initial lockdown, probably because some pharmacological treatments could not be stopped and in these cases the Italian lockdown restriction rules did not apply to patients directed to a hospital for essential treatments. On the contrary, psychotherapies were more likely to be considered non-essential treatments and were temporarily suspended with higher frequency. As regard to the post-lockdown phase, it is possible that after the removal of main restrictions, the more severe patients usually referred to the psychiatrist had more difficulties in reaching the service. This because in some cases other agencies (e.g., NGOs) providing outreach information and orientation to health services reduced their activity during these years, while in other cases the staff of reception centers for migrants was reduced in favor of smart working, decreasing their ability to detect early symptoms in resettled persons. As a consequence, this could have led to a reduction of the referral of, respectively, homeless people and asylum seekers. On the

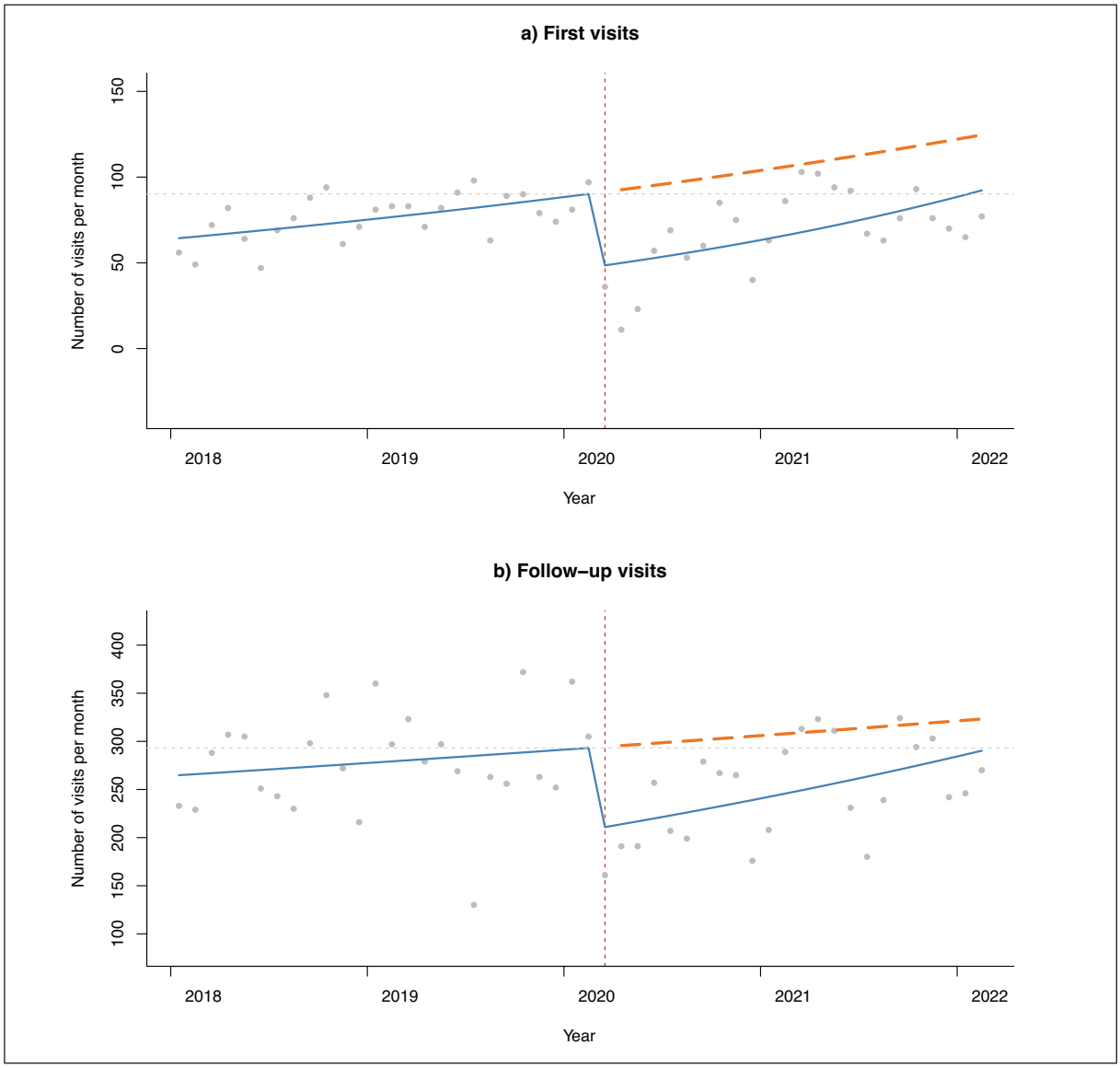

Figure 2
Monthly counts of first visits (a) and follow ups (b) before and during the pandemic COVID-19. Vertical dashed line: introduction of restrictions. Continuous line: trend over the years. Dashed line: counterfactual scenario. Horizontal dashed line: pre-pandemic level.

other side, psychological visits increased faster, probably because a part of these activities were influenced differently by contextual factors. For example, in our service a part of the psychological interviews was dedicated to the evaluation of asylum seekers for legal support in their request of an international protection VISA. While this activity was completely stopped during the lockdown, after the removal of main restrictions it restarted as usual.

A first limitation of this study is that it is a single center study in a mental health service specifically dedicated to migrants and people in difficult socioeconomic conditions, thus the findings cannot be directly generalized to the other mental health services of the National Health System. However, they can be representative of the situation in similar mental health facilities for migrants and low socioeconomic status, in Italy and abroad (e.g., those of charities or non-governmental organizations). Another limitation is in the counterfactual predictions. With the same number of psychiatrists and psychologists, it is possible that in the pre-pandemic period we were nearly the apex of our possibilities, so a sort of ceiling-effect shall be considered. In this case, it would be normal that the trend does not continue to increase even after total numbers are returning around the pre-pandemic levels. However, the slope of the curve in the period after the strict lockdown phase should had been more sharp, so the finding remains informative, suggesting an upturn which is slower than expected (especially for psychiatry).

In conclusion, our study shows that once the strict lockdown measures were eased, mental health patients in vulnerable conditions (migrants and persons in economic and social difficulty) returned to be healed at levels which are not very different from pre-pandemic ones. There is still relatively more difficulty for psychiatric treatments than for psychological interventions.

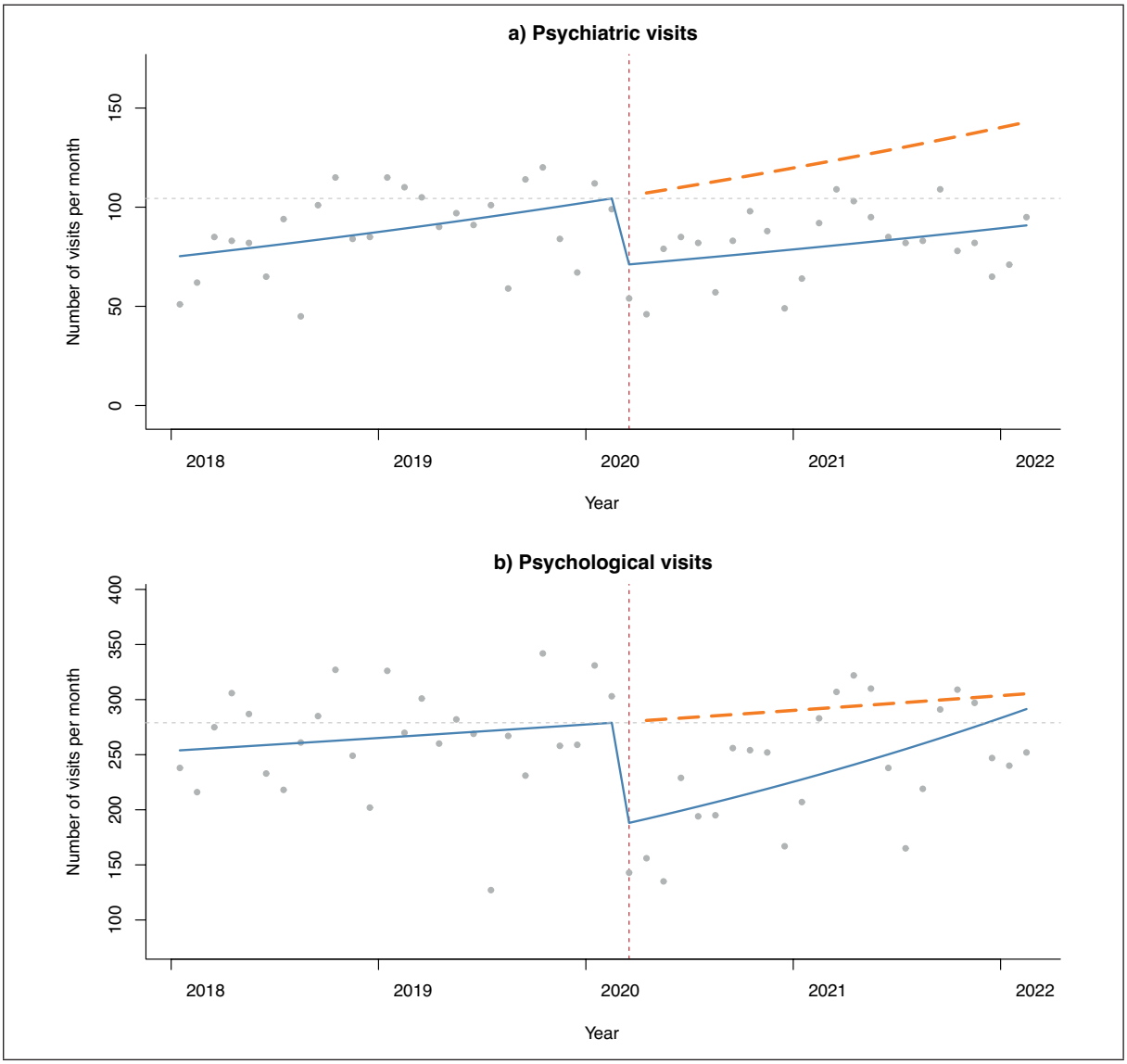

Figure 3
Monthly counts of psychiatric (a) and psychological visits (b) before and during the pandemic COVID-19. Vertical dashed line: introduction of restrictions. Continuous line: trend over the years. Dashed line: counterfactual scenario. Horizontal dashed line: pre-pandemic level.

This is probably due to a higher impact on psychiatric patients of persisting difficulties in the territory to reach the most vulnerable patients and to refer them to psychiatric services. The other relevant finding is that it took two years to return to the pre-pandemic levels. Considering that in the meanwhile mental health needs should have increased due to the economic and social consequences of the pandemic, mental health services should improve their efforts to reduce barriers of access and to implement outreach referral.

Ethical considerations

This study has been performed in accordance with the Helsinki declaration. Research design and ethicality of the study reviewed and approved by INMP Review Board.

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None.

Authors' contributions

MA: study design, data collection and data entry, writing of the manuscript. GN: methodology, data analysis, writing of the manuscript. GL: methodological audit, text review. AC: study design, text review, bibliographic analysis. GC: methodological revision, text review. CM: conceptualization, text review.

Conflict of interest statement

None.

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ORIGINAL ARTICLES AND REVIEWS

Determinants of COVID-19 vaccination acceptance or hesitancy in Italy: an overview of the current evidence

Chiara Primieri[1], Carla Bietta[2], Irene Giacchetta[1], Manuela Chiavarini[3] and Chiara De Waure[3]

[1]*Scuola di Specializzazione in Igiene e Medicina Preventiva, Università of Perugia, Perugia, Italy*
[2]*Azienza USL Umbria 1, Dipartimenti di Prevenzione, Servizio di Epidemiologia, Perugia, Italy*
[3]*Dipartimenti di Medicina e Chirurgia, Sezione di Sanità Pubblica, Università di Perugia, Perugia, Italy*

Abstract

Introduction. Vaccine hesitancy is a major public health issue and a challenge for the implementation of COVID-19 immunization campaigns. The objective of this study was to address the determinants of COVID-19 vaccination acceptance or hesitancy in the Italian population.

Materials and methods. We conducted a rapid systematic review by searching PubMed until May 3rd, 2022, according to Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines. Articles assessing determinants of Italians' attitudes towards COVID-19 vaccination in terms of hesitancy and/or acceptance were considered eligible. Quality and risk of bias assessment was performed through the Newcastle Ottawa Scale appraisal tool. Determinants were grouped in three categories: contextual, individual and group, and vaccine/vaccination specific influences.

Results. Out of 606 articles, 59 studies were included in the analysis. Included studies demonstrated that, in Italy, COVID-19 vaccination acceptance or hesitancy is mostly influenced by perceived safety, efficacy and usefulness of the vaccine.

Conclusion. These findings should be considered to plan tailored interventions for counteracting COVID-19 vaccination hesitancy in Italy.

Key words

- COVID-19
- vaccine
- vaccination
- adherence
- hesitancy
- Italy

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by SARS-CoV-2, a pathogen that primarily spreads through close contact from person to person and targets the human respiratory system [1]. On January 30th, 2020, the World Health Organization (WHO) declared COVID-19 a public health emergency of international concern. On March 11th, 2020, WHO characterized COVID-19 as a pandemic [2]. Up to July 29th, 2022, there were 572,239,451 confirmed cases and 6,390,401 confirmed deaths worldwide [3, 4].

The development of safe and effective COVID-19 vaccines was the first step toward a long-term solution to the pandemic. The first mass vaccination program started in December 2020. At the date of May 17th, 2022, Italy had one of the highest COVID-19 vaccination coverage in Europe, with only Portugal, Malta and Spain exceeding Italy in terms of percentage of population vaccinated with at least one dose [5, 6]. As of July 27th, 2022, 86.6% of Italian eligible subjects completed the primary vaccination cycle and 83.7% got the booster dose too, with slight differences among Italian regions [7].

Vaccination is recognized as one of the most cost-effective methods of avoiding diseases. The WHO estimated that it currently prevents 2-3 million deaths a year and a further 1.5 million could be avoided if global vaccination coverage improved [8]. A recent study confirmed that COVID-19 vaccination has changed the course of the pandemic, avoiding 14.4 million deaths in 185 countries between December 2020 and December 2021 [9]. However, vaccine hesitancy, defined as a "delay in acceptance or refusal of vaccination despite availability of vaccination services" [10, 11], is a phenomenon that has existed since the first vaccines were administered and has become much more difficult to face in the age of social media. Because it undermines the progress made in addressing vaccine-preventable diseases, vaccine hesitancy was recognized among the top 10 threats to global health by the WHO in 2019 [8].

COVID-19 vaccination campaign achieved overall high coverages in Italy; however, some pockets among

Address for correspondence: Irene Giacchetta, Scuola di Specializzazione in Igiene e Medicina Preventiva, University of Perugia, Piazzale Gambuli 2, 06132 Perugia, Italy. E-mail: irene.giacchetta@gmail.com.

population did not vaccinate at all or did not get the booster dose. This issue may be attributable to several reasons, including the dynamics of supply and service delivery in the Italian health system, but also people's beliefs, attitudes, and behaviors. Among the barriers to the uptake of COVID-19 vaccination, vaccine hesitancy has been documented by a big body of evidence [12-22] as a key modifiable factor that places critical challenges to the successfully implementation of the COVID-19 vaccination campaign. Vaccine hesitancy is a complex and context-specific issue, varying across time, place, and vaccines [23-34]. According to the SAGE Working Group's Vaccine Hesitancy Determinants Matrix, factors that can influence hesitancy could be grouped in three categories: contextual influences (due to historic, socio-cultural, environmental, health system/institutional, economic or political factors), individual and group influences (arising from personal perception of the vaccine or from the social/peer environment), and vaccine/vaccination-specific issues (directly related to vaccine or vaccination) [11, 23-30].

Uninterrupted efforts should be made to vaccinate everyone who is eligible in every country and an effective vaccination program cannot avoid considering the understanding of concerns and expectations of individuals and communities regarding vaccines and vaccination. In fact, this could help in reaching pockets of unvaccinated people and addressing hard-to-reach populations, through tailored interventions, even in contexts where vaccination coverage is high. The monitoring of vaccination coverage and of reasons for non-vaccination is a required activity to ensure population Essential Levels of Care (LEA) [31]. However, albeit also the Italian Society of Hygiene (Società Italiana di Igiene, Medicina Preventiva e Sanità Pubblica, SItI) underlined the need of monitoring these issues, a national monitoring system has not been implemented yet [35]. Furthermore, despite the increasing body of literature investigating COVID-19 vaccine hesitancy and its determinants in Italy, all available evidence has not been summarized to date. For this reason, the objective of this study was to carry out a systematic literature review of the Italian studies on the topic, in order to collect and summarize the evidence on factors associated with acceptance or hesitancy of COVID-19 vaccination in the Italian population. The synthesis of this evidence will be useful for better understanding the reasons for COVID-19 vaccine acceptance or hesitancy and, consequently, supporting evidence-informed interventions to increase COVID-19 vaccination coverage in Italy.

MATERIALS AND METHODS

A systematic review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews (PRISMA) [36].

Search strategy

PubMed was searched to retrieve potential eligible articles published from the inception until May 3rd, 2022. The PubMed search was pursued with a search string developed on the PICO model (P, population/patient; I, intervention/indicator; C, comparator/control; and O, outcome) and reported below:

((vaccin*[tiab] OR immuniz*[tiab] OR immunis*[tiab]) AND (covid*[tiab] OR sars-cov-2[tiab] OR coronavirus[tiab] OR 2019ncov[tiab])) AND ((adherence[tiab] OR uptake[tiab] OR accept*[tiab] OR intent*[tiab] OR willingness[tiab] OR facilitator*[tiab] OR confiden*[tiab] OR trust[tiab] OR hesita*[tiab] OR refus*[tiab] OR reject*[tiab] OR unwillingness[tiab] OR opposition[tiab] OR barrier*[tiab] OR mistrust[tiab] OR distrust[tiab] OR anti-vaccin*[tiab] OR antivaccin*[tiab] OR exemption*[tiab] OR behaviour[tiab] OR attitude*[tiab] OR determinant*[tiab] OR predict*[tiab])) AND (Ital*).

The reference lists of included articles were hand-searched to look for additional eligible studies.

Inclusion and exclusion criteria

The systematic review included observational analytical studies conducted on the Italian population that assessed acceptance or hesitancy towards COVID-19 vaccination as outcomes and any favorable or unfavorable factor associated to them.

We excluded systematic reviews, non-empirical studies, conference, editorials, commentaries, book reviews, and abstracts without a full text. In addition, studies whose full text could not be retrieved were excluded. International studies that did not analyze and report disaggregated data by countries were also excluded; if disaggregated data were reported, we extracted only separately reported Italian data.

Study selection

The study selection was conducted by one author and further cross-checked by another author for accuracy. Disagreements were iteratively discussed until agreement was reached. The selection of eligible articles was carried out by screening titles and abstracts first and then full texts. The study selection was performed from May 2022 to June 2022.

Data extraction and synthesis

The full text review and data extraction were conducted by one author and further cross-checked by another author for accuracy. Disagreements were iteratively discussed until agreement was reached. The data extraction was performed from June 2022 to July 2022.

A dedicated data extraction form developed on Excel was used to gather the following information for each eligible study:

1) Study identification (first author, title, journal, and publication year);
2) Study characteristics (region/city, period, design and study population);
3) Study population characteristics (sample size, age, gender, and socio-cultural-economic characteristics, presence of any special health conditions, vaccination status);
4) Study outcome(s) (outcomes of the study with relevant descriptive statistics, percentages; factors associated with the outcome).

Because of expected heterogeneity among studies, the synthesis of data was conducted only qualitatively and reported in summary tables.

Factors associated with acceptance or hesitancy towards COVID-19 vaccination were grouped according to the categories identified by the SAGE Working Group in the Vaccine Hesitancy Determinants Matrix [7], namely contextual, individual and group, and vaccine/vaccination-specific influences.

Quality assessment and risk of bias

The methodological quality and risk of bias of included articles were assessed through the Newcastle Ottawa Scale - NOS in its original version [37] and in a version adapted for the assessment of analytical cross-sectional studies [38]. The assessment was conducted by one author and further cross-checked by another one. Disagreements were resolved by discussion with a third researcher.

To summarize the results of the quality assessment and risk of bias, the articles were grouped into four categories: excellent (10-11 points), good (9-7 points), sufficient (6-5 points) and poor (4-0 points) quality. The risk of bias decreases as the quality increases.

RESULTS

Results of the search strategy

PubMed search returned 606 articles, of which, after the screening by title and abstract and by full text, 91 papers were retrieved for the assessment of final eligibility. Of these, 59 articles [39-97] met eligibility criteria and were included in the systematic review. The study selection process is reported in *Figure 1*.

Characteristics of the included studies

Among included articles, 27 studies (45.8%) addressed the whole Italian population [43, 44, 47, 48, 51, 59, 61, 63-66, 68, 70, 74, 76, 77, 79, 84, 86, 88-90, 93-97], whereas 12 studies (20.3%) [42, 45, 60, 62, 67, 71, 73, 75, 80-82, 92] were conducted in northern Italy, 5 (8.5%) [40, 41, 54, 83, 87] in central Italy and 12 (20.3%) [39, 46, 49, 50, 52, 53, 55, 56, 58, 69, 85, 91] in southern Italy.

The studies were conducted between February 2020 and January 2022; in particular, 18 [43, 47-50, 55, 59, 60, 63, 65, 73, 76, 78, 79, 88, 90, 95, 97] (30.5%) studies were conducted before the start of the vaccination campaign in Italy, 33 (55.9%) [39-42, 44-46, 51-54, 56-58, 61, 64, 69, 70, 74, 75, 77, 80-83, 85-87, 89, 92-94,

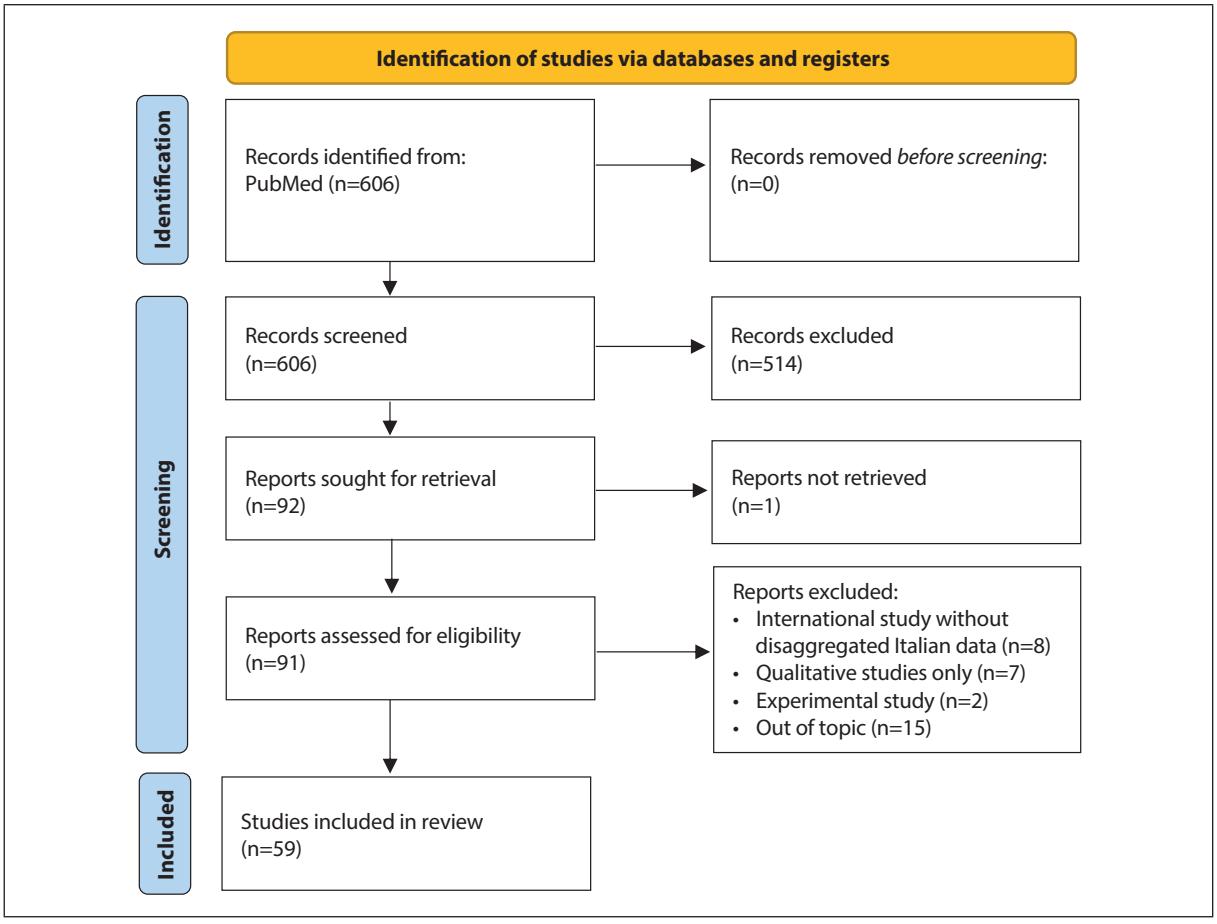

Figure 1

Preferred reporting items for systematic reviews (PRISMA) flowchart of the search strategy. *From*: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prismastatement.org/.

96] after the start of the vaccination campaign and 8 (13.6%) [62, 66-68, 71, 72, 84, 91] straddling the two periods. Twenty-five (41.7%) [41, 42, 47, 51, 56, 59-61, 63, 65, 66, 72, 74, 76, 79, 80-82, 84, 86, 88, 89, 95-97] studies investigated the attitudes of general adult population towards COVID-19 vaccination, and two [43, 58] (3.3%) the attitude of the elderly. Ten (17%) [44, 45, 52, 54, 64, 68, 69, 75, 87, 91], focused on potentially more fragile and/or at-risk population groups (patients with chronic diseases, persons previously tested positive for SARS-CoV-2, prisoners, migrants). Eight studies (13.3%) [46, 48, 50, 62, 70, 77, 90, 93] investigated the attitudes towards vaccination of healthcare workers. Eight (13.3%) [39, 40, 49, 57, 67, 73, 78, 85] involved students and/or university staff. Seven studies (11.7%) [53, 55, 69, 71, 83, 92, 94] investigated parents' attitudes towards COVID-19 vaccination of their children, one [55] of which also assessed parents' propensity to vaccinate for themselves.

In 25 studies (42.4%), the study population was balanced between females and males, 28 (47.4%) study populations were predominantly formed by females (>60% of the sample) while only two (3.4%) [52, 62] were predominantly formed by males; eventually four articles (6.8%) [46, 66, 76, 84] did not report gender distribution of the study population.

Two studies (3.4%) [39, 56] evaluated populations that had already undergone a full cycle of vaccination.

A full description of the characteristics of the included studies is given in *Table 1*.

Only 3 studies (5.1%) [41, 54, 82] assessed actual vaccine uptake as an outcome, while the others investigated attitudes towards vaccination considering the intention to vaccinate.

Table 1
Description of the characteristics of the studies included in the systematic review, about COVID-19 vaccination acceptance or hesitancy in Italy

Author, year	Region/ city	Period	Study population	Sample size (N)	Sex (female %)	Age	Study outcomes and results				Quality score
							Hesitancy	%	Acceptance	%	
Aliberti 2022 [39]	Salerno	May-August 2021	University lecturers undergoing full cycle of Vaxzevria	500	59.20	range: 26-66	Vaccine hesitancy (Vaxzevria)	32.70			7
Baccolini 2021 [40]	Roma	March-June 2021	University students unvaccinated	5,369	61.50	mean (SD): 23.5 (4.5)	Vaccine hesitancy	26.00			5
Barello 2022 [51]	Italia	March 2021	Adult population	866	50.80	range: 18-70	Delay in vaccination while waiting for a 'better' vaccine	46.00			9
Belingheri 2021 [62]	Monza-Brianza	December 2020-January 2021	Health workers	421	28.50	≥25			Intention to vaccinate	82.20	7
Belingheri 2021 [73]	Lombardia	December 2020	Healthcare students	422	82.90	median (IQR): 21 (20-22)			Intention to vaccinate	80.80	5
Bucchi 2022 [84]	Italia	October 2020	Adult population	991	NA	>15			Intention to vaccinate (as soon as possible)	36.02	7
		January 2021		987						59.90	
		May 2021		977						83.80	
Buonsenso 2022 [94]	Italia	November 2021-January 2022	Parents of children/ adolescents with a previous diagnosis of COVID-19	121	81.20	median (IQR): 42 (38-47)			Intention to vaccinate one's children	56.20	7
Caserotti 2021 [95]	Italia	February-June 2020	Adult population	2,267	69.90	mean (SD): 38.1 (14.0)			Intention to vaccinate	40.10	6
Caserotti 2022 [96]	Italia	January-February 2021	Adult population	5,006	50.00	range: 18-70			Intention to vaccinate	88.00	9
Caserotti 2022 [97]	Italia	May-June 2020	Adult population	448	70.80	mean (SD): 33.8 (13.9)			Intention to vaccinate	NA	5
Cesaroni 2022 41]	Lazio	December 2021	Adult population	3,186,728	54.00	mean (SD): 58.9 (14.3)	Non-vaccination	10.30			11
Cocchio 2022 [42]	Veneto	January 2021	Adult population	4,467	51.10	mean (SD): 46.8 (16.0), median (IQR): 48 (34-59)	Vaccine hesitancy	15.70			6

Continues

Table 1
Continued

Author, year	Region/ city	Period	Study population	Sample size (N)	Sex (female %)	Age	Study outcomes and results				Quality score
							Hesitancy	%	Acceptance	%	
Contoli 2021 [43]	Italia	August-December 2020	Elderly population	1,876	53.60	≥65	Vaccine hesitancy	45.00			10
							Vaccine refusal	16.00			
Costantino 2021 [44]	Italia	February 2021	Patients suffering from inflammatory bowel disease	1,252	58.20	median (IQR): 48 (37-58)	Vaccine hesitancy	18.10			7
							Vaccine refusal	2.70			
Costantino 2021 [45]	Milano	February 2021	Patients suffering from coeliac disease	103	78.60	range: 18-77	Vaccine hesitancy	25.20			7
							Vaccine refusal	4.80			
Costantino 2022 [46]	Palermo	January-March 2021, October 2021	Health workers	1,450; 1,391	64.70	mean (SD): 46.3 (15.7);			Intention to vaccinate	64.00	9
Del Riccio 2021 [47]	Italia	December 2020	Adult population	7,605	65.50	median (IQR): 47 (34-58)			Intention to vaccinate	81.90	5
Di Gennaro 2021 [48]	Italia	October 2020	Health workers	1,723	57.70	mean (SD): 35.5 (11.8)	Vaccine hesitancy	33.00			5
Di Giuseppe 2021 [49]	Caserta-Napoli	September-November 2020	University staff	1,501	60.80	mean (SD): 36 (14.2); range: 18-73			Intention to vaccinate	84.10	9
Di Giuseppe 2021 [50]	Caserta-Napoli	September-November 2020	Health workers	738	42.30	mean (SD): 40.4 (12.8); range: 19-70			Intention to vaccinate	80.70	9
Di Giuseppe 2022 [52]	Campania	March-April 2021	Prisoners	865	0.00	mean (SD): 42.4 (11.9); range: 18-78			Intention to vaccinate	63.90	8
Di Giuseppe 2022 [53]	Napoli	April-May 2021	Parents of children/ adolescents	607	82.40	mean (SD): 42.3 (6.5); range: 22-63			Intention to vaccinate one's children	68.50	10
Di Noia 2021 [54]	Roma	March 2021	Patients suffering from oncological diseases	914	61.00	range: 21-97			Vaccinated	88.80	6
Di Valerio 2021 [93]	Italia	1 January-16 February 2021	Healthcare professional members of a Facebook private group	10,898	77.90	≥18	Vaccine hesitancy	1.10			4
Fedele 2021 [55]	Napoli	November 2020	Parents of children/ adolescents	640	73.90	NA	Vaccine hesitancy regarding the vaccination of one's children	82.80			5
							Vaccine refusal regarding the vaccination of one's children	34.50			
							Vaccine hesitancy	73.40			
							Vaccine refusal	23.40			
Folcarelli 2022 [56]	Napoli	November-December 2021	Adult population vaccinated with full cycle	615	57.40	mean (SD): 32.1 (15.9); range: 19-76	Vaccine hesitancy on booster dose administration	24.70	Intention to vaccinate (booster dose)	85.70	10
Gallè 2021 [57]	Bari, Napoli, Roma	February-April 2021	University students	3,226	56.00	mean (SD): 23.3 (3.9); median (IQR): 22 (21-25); range: 18-45			Vaccinated or Intention to vaccinate	92.90	8
Gallè 2021 [58]	Apulia	June-August 2021	Elderly population	1,041	58.30	mean (SD): 76.6 (6.5)			Vaccinated or Intention to vaccinate	92.70	8

Continues

Table 1
Continued

Author, year	Region/ city	Period	Study population	Sample size (N)	Sex (female %)	Age	Study outcomes and results				Quality score
							Hesitancy	%	Acceptance	%	
Genovese 2022 [59]	Italia	February-July 2020	Adult population	4,116	64.10	mean (SD): 33 (13)			Intention to vaccinate	76.00	8
Gerussi 2021 [60]	Udine	September-November 2020	Adult population with a previous diagnosis of COVID-19	599	53.40	mean (SD): 53 (15.8); range: 19-76	Vaccine hesitancy	59.10			5
							Vaccine refusal	24.90			
Giuliani 2021 [61]	Italia	January-February 2021	Adult population	1,074	67.50	range: 18-88			Intention to vaccinate	85.40	5
Graffigna 2020 [63]	Italia	May 2020	Adult population	1,004	50.90	mean (SD): 44 (14); range: 18-70			Intention to vaccinate	58.60	8
Guaraldi 2021 [64]	Italia	January 2021	Patients sufferingfrom diabetes	1,176	73.10	>18	Vaccine hesitancy	15.70			5
Heyerdahl 2022 [65]	Italia	December 2020	Adult population	1,000	50.40	range: 18-65			Vaccination acceptance	66.00	5
Lindholt 2021 [66]	Italia	September 2020-February 2021	Adult population	2,411	NA	>18			Vaccination acceptance	60.00	9
Lo Moro 2022 [67]	Torino	November 2020-February 2021	Health students	902	63.50	median (IQR): 24 (23-26)	Vaccine hesitancy	6.70			6
							Vaccine refusal	0.50			
Magon 2021 [68]	Italia	June-August 2020, October 2020-March 2021	Patients undergoing anticoagulant therapy	288	50.50	mean (SD): 58 (20)	Vaccine hesitancy	35.60			7
Miraglia del Giudice 2022 [69]	Napoli	December 2021-January 2022	Parents of children/ adolescents with chronic diseases	430	86.50	mean (SD): 40.5 (6.1); range: 25-57	Vaccine hesitancy regarding the vaccination of one's children	26.30	Intention to vaccinate one's children	38.80	10
Monami 2021 [70]	Italia	January 2021	Health workers	7,881	76.30	NA	Vaccine hesitancy	2.40			5
Montalti 2021 [71]	Bologna	December 2020-January 2021	Parents of children/ adolescents	4,993	76.60	NA	Vaccine hesitancy regarding the vaccination of one's children	39.50			5
							Vaccine refusal regarding the vaccination of one's children	9.90			
Montalti 2021 [72]	Bologna, Palermo	December 2020-February 2021	Adult population	443	56.40	>18			Intention to vaccinate	75.60	5
Moscardino 2022 [74]	Italia	June 2021	Adult population	1,200	49.40	mean (SD): 29.8 (6.5); range: 18-40	Vaccine hesitancy	25.10			9
							Vaccine refusal	7.50			
Page 2022 [75]	Milano	February-May 2021	Migrants	126	67.20	median (IQR): 41 (20)			Vaccination request	52.00	8
Palamenghi 2020 [76]	Italia	May 2020	Adult population	1,004	NA	NA			Intention to vaccinate	59.00	6
Papini 2021 [77]	Italia	February-April 2021	Health workers	2,137	71.70	NA	Vaccine hesitancy	6.70			7
Pastorino 2021 [78]	Milano, Brescia, Piacenza, Cremona, Roma	June-July 2020	University students	436	70.40	median (IQR): 23.1 (21.3-24.7)			Intention to vaccinate	94.70	6
Prati 2020 [79]	Italia	April 2020	Adult population	624	54.00	mean (SD): 32.3 (12.7); range: 18-72			Intention to vaccinate	75.80	6
Reno 2021 [80]	Emilia-Romagna	January 2021	Adult population	1,011	55.20	mean (SD): 46.9 (11.5); range: 19-70			Intention to vaccinate	68.90	8

Continues

Table 1
Continued

Author, year	Region/ city	Period	Study population	Sample size (N)	Sex (female %)	Age	Study outcomes and results				Quality score
							Hesitancy	%	Acceptance	%	
Reno 2021 [81]	Emilia-Romagna	January 2021	Adult population	1,011	55.20	mean (SD): 46.9 (11.5); range: 19-70			Intention to vaccinate	68.90	8
Russo AG 2021 [82]	Milano-Lodi	September 2021	Adult population	2,981,997	52.10	>18			Vaccinated	84.40	11
Russo L 2021 [83]	Roma	July-August 2021	Parents of children/ adolescents	1,696	81.60	median (IQR): 42 (37-47)			Vaccinated or Intention to vaccinate one's children	32.20	6
Salerno 2021 [85]	Palermo	May 2021	University students unvaccinated	2,667	68.10	mean (SD): 22.74 (3.81)	Vaccine hesitancy (mRNA vaccine)	8.20			5
							Vaccine refusal (mRNA vaccine)	1.00			
							Vaccine hesitancy (viral vector vaccine)	42.60			
							Vaccine refusal (viral vector vaccine)	12.20			
Santirocchi 2022 [86]	Italia	March-May 2021	Adult population	971	57.60	>18			Intention to vaccinate	78.50	7
Scoccimarro 2021 [87]	Firenze	January-April 2021	Patients suffering from diabetes	502	60.20	>18	Vaccine hesitancy	18.30			7
Simione 2021 [88]	Italia	April 2020	Adult population	350	8.00	mean (SD): 40.8 (10.8)			Intention to vaccinate	NA	7
Steinert 2022 [89]	Italia	June 2021	Adult population	1,087	51.20	>18	Vaccine hesitancy	15.00			8
Trabucco Aurilio 2021 [90]	Italia	December 2020	Health workers	531	73.40	NA			Intention to vaccinate	91.50	6
Viola 2021 [91]	Messina	October 2020-June 2021	Patients suffering from inflammatory bowel disease	470	43.60	mean (SD): 48 (18)			Vaccination acceptance (vaccinated or vaccine booking)	85.00	7
Zona 2021 [92]	Modena	July-August 2021	Parents of children/ adolescents	1,799	76.40	mean (SD): 45 (5.8)			Intention to vaccinate one's children	26.50	7

Vaccination hesitancy: refers to delay in acceptance or refusal of vaccination despite availability of vaccination services.
Vaccination acceptance: refers to vaccinated subject, subject who has already booked to vaccinate and intention to receive vaccination.
SD: standard deviation; IQR: interquartile range.

The majority of the articles referred to COVID-19 vaccination in general, except for three studies (5.1%) which referred to Vaxzevria, [39], to mRNA [85] and viral vector [56] vaccine type; moreover, one study (1.7%) [56] specifically assessed the attitude towards the administration of the booster dose.

Among studies investigating COVID 19 acceptance and /or hesitancy, there is a considerable variability in definition of outcomes, in study population type and in periods assessed (*Table 1*). Vaccination hesitancy showed the highest values in a study conducted in November 2020 on a population of parents, who stated that they were not positively inclined to vaccinate themselves in 73.4% of cases or to vaccinate their children in 82.8% [55]. Regarding hesitancy about vaccinating children, a lower percentage (26.3%) was found among parents of children with chronic diseases between December 2021 and January 2022 [69]. The lowest percentage of vaccination hesitancy (2.4%) was recorded among healthcare professionals [70]. The vaccination acceptance ranged from 94.7% in a study conducted among students of the Catholic University of the Sacred Heart in July 2020 [78] to 36.2% in a study performed on the general population in October 2020 [84]; this study found an increase in acceptance rate up to 83.8% in May 2021 too [84].

Results of the quality assessment and risk of bias

The details of the quality assessment are shown in detail in the *Supplementary Material* available online

whereas the overall scores are reported in *Table 1*. The quality scores ranged from 4 to 11 (median: 7; mean: 7.05). The quality was evaluated as "very good" for 6 studies (10.2%) [41, 43, 53, 56, 69, 82], "good" for 29 studies (49.2%) [39, 44-46, 49-52, 57-59, 62, 63, 66, 68, 74, 75, 77, 80, 81, 84, 86-89, 91, 92, 94, 96] and "sufficient" for 23 studies (39.0%) [40, 42, 47, 48, 54, 55, 60, 61, 64, 65, 67, 70-73, 76, 78, 79, 83, 85, 90, 95, 97], while for only one study (1.7%) [93] was evaluated as "low". With regard to risk of bias, thirteen studies [40, 47, 48, 55, 61, 64, 70-73, 85, 93, 97] could be considered at high risk of selection bias as they were scored zero in three out of four items considered, namely *representativeness of the sample, sample size and non-respondent*. Three studies [54, 60, 65] have a zero score in the item of comparability, while no article has a zero score in the domain referred to outcome assessment. Special attention should be paid to the article of Di Valerio, 2021 [93], which totalized a NOS score of 4, so it is reasonable to assume that it is at high risk of bias. Nevertheless, the evidence on factors associated with acceptance or hesitancy of COVID-19 vaccination, that are hereafter summarized, came from many studies, thus minimizing the hazard of making conclusions based only on studies at high risk of bias.

Factors associated with COVID-19 vaccine acceptance or hesitancy

The complete matrix of factors associated with COVID-19 vaccination acceptance or hesitancy is reported in *Table 2* and, hereafter, summarized according to the groups of influences.

Contextual influences. Among the contextual influences, socio-demographic and cultural factors have been the most investigated. Age was associated with adherence to vaccination, with a greater propensity to be vaccinated among older subjects than younger ones [41, 42, 52, 55, 56, 60, 64, 66, 71, 72, 74, 76, 79, 82, 84, 86, 88, 89, 91, 92, 96]. Similarly, a significant association was found between the higher age of children/adolescents and the propensity of parents to vaccinate them [53, 69, 71, 94]. Only few studies have come to opposite conclusions. In all except than two studies [40, 49] female gender was found to be associated with hesitancy [41-44, 47, 50, 55, 60, 61, 66, 71-73, 77, 80, 82, 84, 86, 88-90, 92, 96]. A medium/higher level of education was overall associated with a greater propensity to vaccination [40, 44, 55, 58, 61, 64-66, 69, 73, 84, 86, 88, 89, 96, 97], while a low educational level was associated with hesitancy [41-43, 71, 72, 74, 80, 81]. The evidence about health workers showed that they are more predisposed to accept vaccination [40, 48, 50, 61, 77, 96]. With regard to the source of information, there is a clear relation between the consultation of scientific/institutional information and the acceptance of vaccination [50, 55, 56, 81, 96], while the collection of information from mass media is associated to hesitancy [48, 58, 66, 71, 75, 81, 86, 96]. In the political sphere, both trust in government and institutions [47, 61, 74, 79, 86, 97] and support for health policies [66, 71, 96] are predictors of vaccination acceptance.

Individual and group influences. Beliefs, attitudes, and knowledge/awareness were the factors mostly addressed among individual and group influences. In particular, the attitude to preventive behaviours (such as use of masks, adherence to therapies, adherence to the flu vaccination campaign and cancer screening) was significantly associated with COVID-19 vaccination acceptance in half of the studies [40, 42, 43, 45-48, 51, 53, 57, 59, 60, 62, 64, 66, 67, 70, 72, 73, 76, 78, 82, 83, 86, 90, 91, 95, 97]. Twenty-five articles [39, 44-47, 57, 59, 61, 63, 66, 69, 71, 74, 76, 80, 81, 83, 84, 86, 88, 89, 92, 95-97] investigated the relationship between vaccination and confidence in science, medicine, health institutions and healthcare professionals, as well as confidence in vaccines in general; in contrast, propensity to alternative medicine [44] and previous experience of adverse events linked to vaccinations [67, 70, 93] were related to hesitancy. A positive association with acceptance was also found in relation to health literacy and health engagement [63, 68]. With regards to the perception of risk of disease, some studies showed a significant association between the perception of risks of COVID-19 and vaccination acceptance [40, 43, 44, 46, 49, 50, 52, 53, 61, 63, 66, 69, 77, 78, 80, 81, 83, 84, 86, 89, 95-97]. The perception of the safety [40, 47, 49, 50, 83, 85], efficacy [40, 83, 85, 90] and usefulness [46, 47, 53, 85] of the vaccine, as well as the experience of negative consequences of the disease among family members, friends and acquaintances [43, 56, 94, 96] were associated with vaccination acceptance. Vaccination hesitancy was associated with the perception of insufficient information about the vaccine [39, 56, 69]. Eventually, other factors associated with vaccination acceptance were the concern about emergency [40, 43, 78, 79] and economic situation [66, 96].

Vaccine- and vaccination-specific influences: among these influences short time needed to develop COVID-19 vaccines was reported as a cause of concern and therefore for vaccination hesitancy [85].

DISCUSSION

It has been estimated that in Italy, from January 2021 to January 2022, about 8 million cases, over 500,000 hospitalizations, over 55,000 hospitalizations in intensive care units and about 150,000 deaths were directly prevented by COVID-19 vaccination [98]. However, the phenomenon of vaccine hesitancy, both against COVID-19 vaccines and vaccination in general, skyrocketed since the beginning of the pandemic, with differences related to several aspects [99]. For this reason, every effort to understand the phenomenon is of great value to guide counteracting actions.

Our review addressed the determinants of COVID-19 vaccination acceptance and hesitancy in the Italian population, being the first one, to the best of our knowledge, to provide a broad and overall overview of the topic. The findings of our review showed that, as expected, the major reasons behind COVID-19 vaccination hesitancy were individual and group factors, such as perceived safety, efficacy and usefulness of the vaccine. In addition, the lack of awareness and information was often reported to negatively impact on vaccination attitudes too.

The reasons for COVID-19 vaccination acceptance or hesitancy have been investigated worldwide by a huge

amount of literature, addressing not only the overall population but also specific groups, such as healthcare professionals and students [100-103], or subgroups with expected lower vaccine uptake, such as pregnant women [104, 105], ethnic minority [106-108], adolescents/young adults [109] and parents in respect to their children [110, 111]. Also, all this evidence highlighted that the main reasons for vaccine hesitancy belonged to individual and group influences, including lack of information or misinformation [100, 102, 104, 108],

Table 2
Matrix of factors associated with COVID-19 vaccination acceptance or hesitancy, with bibliographical references

Macroareas of factors	Factors associated with:	Hesitancy [references]	Acceptance [references]
Contextual influences	**Socio-demographic factors, religion, culture, gender**		
	Age		
	Young	[71, 72, 79, 80]	[48, 59]
	Intermediate	[42, 76, 81, 95]	[93]
	Advanced	[74, 82, 88, 89]	[41, 52, 55, 56, 60, 64, 66, 84, 86, 91, 92, 96]
	Higher in children		[53, 69, 71, 94]
	Gender (female)	[41-44, 47, 50, 55, 60, 61, 66, 71-73, 77, 80, 82, 84, 86, 88-90, 92, 96]	[40, 49]
	Citizenship/birth abroad	[41, 82]	
	Marital status (married)	[86]	[49]
	High household size		[53]
	Educational level		
	Low	[41-43, 71, 72, 74, 80, 81]	
	Medium-high	[51]	[73]
	High		[40, 44, 55, 58, 61, 64-66, 69, 84, 86, 88, 89, 96, 97]
	Low income	[74, 80, 81, 89]	
	Occupation		
	Unemployed	[65, 69, 74]	[47, 92]
	In contact with the public	[42]	[60]
	Entrepreneurs	[97]	
	Administrative staff	[49]	
	Health workers and in particular doctors compared to other health professionals		[40, 48, 50, 61, 77, 96]
	Deprivation (high)	[41, 82]	
	Residence		
	North	[70, 74]	
	Central	[43]	
	South		
	High population density areas	[43]	
	Religious affiliation	[88]	
	Information		
	Media	[48, 71, 75, 81, 96]	[58]
	Institutional and scientific information sources		[50, 55, 56, 81, 96]
	Belief in misinformation		[66, 86]
	Policy		
	Political ideology	[40, 66]	
	Trust in government and institutions		[47, 61, 74, 79, 86, 97]
	Support for public health policies (e.g., compulsory vaccination)		[66, 71, 96]
	Lockdown phase, during the emergency		[95]

Continues

Table 2
Continued

Macroareas of factors	Factors associated with:	Hesitancy [references]	Acceptance [references]
Individual and group influences	**Knowledge, beliefs, attitudes, experiences about health and prevention**		
	Confidence in science, medicine, health institutions, health professionals		[39, 61, 66, 69, 71, 76, 84, 86, 88, 96, 97]
	Positive attitude to alternative medicine	[44]	
	Attitude towards for preventive behaviour (e.g., use of masks, flu vaccination, screening, adherence to possible therapies)		[40, 42, 43, 45-48, 51, 53, 57, 59, 60, 62, 64, 66, 67, 70, 72, 73, 76, 78, 82, 83, 86, 90, 91, 95, 97]
	Confidence vaccines (in general)		[44-47, 57, 59, 63, 74, 80, 81, 83, 89, 92, 95-97]
	Health literacy (highlevel)		[68]
	Health engagement		[63, 68]
	Underlying chronic diseases	[39, 70, 87]	[41, 43, 54, 80-82, 96]
	Perceived health status (good)	[39]	[55, 61]
	Living with fragile subjects	[70, 85] (viral vector vaccines)	[48, 85] (mRNA vaccines)
	Previous reactions after vaccination	[67, 70, 93]	
	Vaccine and disease perception		
	Vaccine perception		
	Safety		[40, 47, 49, 50, 83, 85]
	Efficacy		[40, 83, 85, 90]
	Usefulness/Utility		[46, 47, 53, 85]
	Insufficient information	[39, 56, 69]	
	Desire to choose the type of vaccine	[85]	
	Disease perception		
	Risks related to COVID-19 (due to severity of illness, high exposure, susceptibility to infection)	[51]	[40, 43, 44, 46, 49, 50, 52, 53, 61, 63, 66, 69, 77, 78, 80, 81, 83, 84, 86, 89, 95-97]
	Previous infection (confirmed or presumed)	[50, 62, 69, 70, 74]	[82, 85]
	Experience of the disease and its consequences (e.g., hospitalisation, death) among relatives/friends/ acquaintances		[43, 56, 94, 96]
	Human-psychological factors		
	Negative affective state		[96]
	External health locus of control	[52, 61]	
	Conspiracy mentality	[51, 66, 74, 79, 85, 88, 96]	
	Calculation	[51]	
	Low perception of social support from family and friends	[74]	
	Desire to protect		[48, 67, 96]
	Desire to return to normality		[78]
	Economic concerns		[66, 96]
	Concern about the emergency situation		[40, 43, 78, 79]
	Attachment to the home country		[74]
	Social life (extremely active or very inactive)	[42]	
	Relatives/friends opposed to the vaccine	[67]	
Vaccine and vaccination-specific influences	**New vaccines**		
	Speed of new vaccine development	[85]	
	Role of health professionals		
	Recommendation by the doctor		[69, 71]

together with concerns about vaccine safety [100, 102-104, 106], efficacy [102-103, 106], and adverse events [100-102, 104]. Social and institution trust/mistrust was also identified as a relevant determinant [102, 103, 108, 109]. These factors were found to be significant determinants of COVID-19 vaccine acceptance or hesitancy in our review as well as in other reviews addressing the same topic at worldwide level [112-115] or in respect to other pandemics [116].

According to our review, contextual influences were the most studied factors after individual and group influences. In particular, socio-demographic factors, such as female gender, younger age, low income, and low educational level were found to be associated with COVID-19 vaccine hesitancy in Italy. These factors were found to be relevant determinants of COVID-19 vaccine hesitancy also by other reviews addressing the worldwide population [114, 115, 117, 118]. It is worthwhile to observe that influences of this kind are particularly relevant also in respect to children vaccination, according to our review as well as other ones [110]. Prevalent women's role as children's caregivers should particularly call for tailored programs addressing their concerns about vaccines to increase their compliance with vaccination for themselves and their children too.

Further studies should surely better disentangle the interrelationship between determinants of vaccine hesitancy and vaccination uptake and assess the effectiveness of context-specific interventions to counteract vaccine hesitancy. However, the available huge body of evidence on the topic suggests that interventions to counteract COVID-19 vaccine hesitancy should address information and health literacy to offer people the possibility of making evidence-based choices. Furthermore, these interventions should be primarily targeted to some population groups that are shown to be more hesitant, namely women, young people, and with low income.

As the Italian population mostly identifies the health scientific community as a reliable source of information [119], it is essential to seize the enormous opportunity offered by this position to counter vaccine hesitancy, both with structured continuous intervention programs and with targeted interventions aimed at specific population subgroups. On the other hand, especially to reach also those pockets among population that do not rely on science and on scientific community, innovative real effective communication strategies are needed to be applied; indeed, the point is not only giving more detailed information, but rather offering it in a more effective and reliable way. To reach this goal, healthcare professionals are especially called to face their main competitor as source of information, namely social media. Vaccine hesitancy seems to be strictly related to erosion of public trust on scientific and social institutions that is strongly amplified by misinformation widely spread and sustained on social media. In contrast to traditional media, social media are characterized by its potential to rapidly spread a huge amount of information in a disintermediate environment and easily produce infodemics.

The intersection between social media-supported infodemics and epidemics certainly represents one of the most critical areas for future studies and interventions. Indeed, as social media radically changed the mechanism of accessing information and forming opinions, we need to better understand how individuals do acquire or avoid information and how their decisions can affect their behaviour. Including the complexity of human behaviour in the management of an epidemic is of critical importance to address its many facets through a scientifically based approach, in order to support the design of effective communication strategies and develop tools to correctly manage both the infodemics and the epidemics. To achieve this goal and capture the overall dimensions of epidemic/infodemic management, health professionals cannot work alone relying on medical competences only, but a multidisciplinary approach is essentially needed [120]. As recognized and underlined also in the National Prevention Plan 2020-2025 [121], a such effort should not be limited to the pandemic context alone, but should be transformed into a structured and continuous program targeted to the population, and in particular to the new generations, to improve health literacy increase and provide people with the necessary tools to make conscious choices for their own health.

This review has some limitations that should be considered when interpreting results. One of the major limitations is the PubMed search approach. However, our objective was to conduct a rapid synthesis of the evidence on factors associated to COVID-19 vaccine hesitancy in Italy and PubMed is a standalone, reliable platform to effectively retrieve most relevant publications. Evidence summarized from PubMed-based articles could indeed provide an initial but yet informative guidance for informing interventions to reach out hesitant people. Another limitation is that the protocol of this systematic review was not registered and that a potential bias in the selection of studies cannot be completely ruled out, even though selection was performed independently by two researchers. Eventually, the heterogeneity of studies' methodology prevented us making a quantitative analysis and issuing more conclusive finding. In this respect, it should be said that the whole literature on vaccine hesitancy and its determinants is still undermined by the lack of standardization of definitions (i.e., confidence, acceptance and uptake are generally used interchangeably), data collection, and analysis. Nonetheless, to the best of our knowledge, this is the first systematic review giving an overview of determinants of COVID-19 vaccine hesitancy in the Italian population. Furthermore, as further strengths, most of the included studies were judged of moderate to good quality and the Vaccine Hesitancy Determinants Matrix was used to summarize the evidence.

CONCLUSION

Vaccine hesitancy represents a challenge for the successful implementation of COVID-19 vaccination in Italy. Our review demonstrated that various factors, particularly belonging to individual and group influences such as misinformation and perceived vaccine safety, efficacy, and usefulness, influence acceptance or

hesitancy towards COVID-19 vaccination. Real effective interventions to increase vaccine uptake in Italy are needed and should rely on a multidisciplinary approach to address individuals' concerns over vaccines, vaccine-related misinformation, social media-related infodemic dynamics and health literacy in order to support individuals in making conscious choices for individual and collective 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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The new Italian National Immunization Technical Advisory Group (NITAG) and its commitment to endorse a new efficient National Immunization Plan in COVID-19 times

Andrea Silenzi[1], Andrea Siddu[1], Anna Carole D'Amelio[2], Sarah Cataldi[2], Camilla Fasano[2], Francesco Maraglino[1], Giovanni Rezza[1] and Carlo Signorelli[2]

[1]*Direzione Generale della Prevenzione Sanitaria, Ministero della Salute, Rome, Italy*
[2]*Università Vita-Salute San Raffaele, Milan, Italy*

Abstract
Among the objectives of the WHO Global Vaccination Action Plan 2020-2025, there is the establishment, in all countries, of a National Immunization Technical Advisory Group (NITAG), an independent body with the aim of supporting and harmonising vaccination policies. Italy firstly established a NITAG in 2017; it contributed to the nation's immunization policies but fell short of its goal of becoming a true reference group. The newly appointed NITAG, made up of 28 independent experts, has the ambitious goal to promote the new National Immunization Prevention Plan (PNPV), to harmonise the current vaccination schedule with the anti-COVID-19 campaign, and to recover the vaccination coverage decline that occurred during the pandemic. The contact with the ECDC EU/EEA, the WHO Global NITAG networks, and all the national stakeholders needs to be reinforced in order to accomplish these aims. This paper describes the structure, organisation, and strategy of the new Italian NITAG.

Key words
- NITAG
- vaccine preventable diseases
- vaccination policies
- national immunization plan
- COVID-19

INTRODUCTION

The rapid onset and harshness of COVID-19 pandemic has emphasised the importance of ensuring reliable and visible public health leadership, the need to improve people's trust in science, and to prevent misinformation [1]. Never in history has the vaccination been so much the focus of public attention. In particular, the increase of vaccine hesitancy represents an important issue to figure out [2].

Evidence-based decision making has constantly proven to be a successful means for countries to hold immunization programmes [3]. Currently, communication is crucial to increase awareness and coherence among countries throughout the world. To this end, special bodies called National immunization Technical Advisory Groups (NITAGs) are set up, with the aim of promoting and developing new national immunization policies [4, 5]. The Global Vaccine Action Plan called for all countries to establish or have access to such a NITAG by 2020 [6].

Vaccination represents an important part of primary health care and an undeniable human right [7], and contributes to individual health, community welfare and economic benefits [8]. Therefore, the World Health Organization (WHO) suggests the establishment and strengthening of NITAGs in all countries, as multidisciplinary groups of national experts whose role is to provide evidence-based recommendations to the Ministry of Health (MoH), supporting the decision making process on immunization policies and programmes [9, 10]. They have primarily a technical and advisory role with the aim of improving scientific rigour and credibility to the complex process of establishing immunization policies, without political, industrial, or personal interests [11, 12]. NITAGs systematically collect, review, and evaluate available evidence, for developing recommendations, even according to the social and epidemiological contexts [13].

WHO has defined six main criteria for characterising functional NITAGs, to ensure the systematic and comparable trend monitoring: i) a formal written terms of reference; ii) a legislative or administrative basis; iii) a

Address for correspondence: Andrea Silenzi, Direzione Generale della Prevenzione Sanitaria, Ministero della Salute, Viale Giorgio Ribotta 5, 00144 Rome, Italy. E-mail: a.silenzi@sanita.it.

minimum of five different areas of expertise in the core membership; iv) at least one meeting per year; v) circulation of the agenda and background materials at least one week prior to meetings; vi) a declaration of interests policy for all members [14].

NITAGs had to cope with the pandemic's fast changing COVID-19 knowledge, a dearth of data on vaccination product characteristics, and health misinformation. In this context, one of the most practical and effective ways for sharing and updating recommendations between countries was represented by the use of online platforms and the scheduling of updated webinars [13, 15]. Moreover, the decline in routine vaccination occurring during the pandemic exposed people at a higher risk of VPD [16].

Some NITAGs around the world serve as a model for the creation of a long-term operational work plan due to their long history of effective engagement and advocacy. The Australian Technical Advisory Group on Immunization (ATAGI), the German Standing Committee on Vaccination (STIKO), and the Canadian National Advisory Committee on Immunization (NACI) stand out for their experience. These ones have succeeded in adapting over time, gaining expertise through the production of evaluation reports, statements based on the most recent, accurate scientific knowledge, programs and campaigns aimed at improving health and forecasting the future issues the committee is likely to face.

HISTORY OF NATIONAL IMMUNIZATION TECHNICAL ADVISORY GROUPS IN ITALY

The first Italian NITAG was established in 2017 by the Minister of Health (Mrs Beatrice Lorenzin, the longest-serving health Minister of the Italian Republic). That year, when an innovative national vaccination plan came into force in Italy, is remembered for major awareness-raising and advocacy actions on the importance of vaccination due to the introduction of the Compulsory Vaccination Act with the aim of rapidly raising vaccination coverage after an increase in measles cases in the population [17-19].

In 2018, NITAG was revoked when all the technical bodies of the Italian MoH were renamed after the change of government. Despite the best of intentions, neither in the first nor in the second case did the Italian NITAG reach the target of becoming a reference point for Italian vaccination policies: the meetings were infrequent, and the number of documents produced was small. After a hiatus, due to the centralisation of vaccination decisions and policies related to the COVID-19 campaign to the institutional bodies of the MoH (i.e., National Health Council) and the Presidency of the Council of Ministers (i.e. National Technical and Scientific Committee on the COVID-19 pandemic), the new NITAG was appointed by the MoH in September 2021 and settled in early 2022 [20]. The main goals are linked to the achievement of the objectives of all the NITAGs set by the WHO, to the approval process of the new National Vaccine Prevention Plan (2022-2025) and to the recommendations of actions for the recovery of vaccinations not administered during the COVID-19 pandemic.

STRUCTURE AND MEMBERS OF THE ITALIAN NITAG

In Italy, NITAG composition is established by MoH Decree based on the proposal of the Director General of Health Prevention. Experts are chosen on their own merits, not as representatives of institutions, groups, or associations to which they belong. Hence, as soon as they are appointed, NITAG members are required to file an initial statement of conflicts of interest.

The overall composition of the NITAG includes core and non-core members. Core members are reputable professionals who do not represent a particular group or stakeholder. Non-core members are further divided into ex-officio members from governmental departments and liaison members, who represent immunization professional societies or organisations [21].

Currently, the new Italian NITAG includes different core members as follows: 10 public health physicians, 1 epidemiologist, 1 immunologist, 1 infectious diseases specialist, 1 communication expert, 1 psychologist and behavioural science expert, 1 forensic medicine expert, 1 expert in ethics, 3 paediatricians, 2 public health nurses and 1 general practitioner. Among non-core members, we can distinguish 3 ex-officio members and 2 liaison members.

The Italian NITAG has a chairperson and an executive secretariat. The three-year term of office is further prolonged if required. Furthermore, the nomination of one or more members may be withdrawn at any moment by the MoH or the Director General of Health Prevention. According to the WHO guidelines [13], the wide range of experience among NITAG members enables the committee to address several issues (vaccine recommendations, scheduling and prioritisation) that call for both scientific competence on vaccines and proficiency on public health policy matters. Currently, the Group consists of 28 members whose 23 are core members.

ITALIAN NITAG TASKS AND GOALS

According to the Decree, NITAG is expected to: 1) support the MoH in the monitoring of immunization programmes and assessment of their impact via annual reports; 2) provide information about epidemiological trends of diseases and about immunization coverage; 3) support elaboration of effective strategies detecting further data and useful information; 4) develop recommendations for immunization programmes, organisational models and control strategies for vaccine-preventable diseases, considering local epidemiology, vaccine efficacy and security, Health Technology Assessment (HTA), vaccine possible impact and social context; 5) provide recommendations for strategies, research, new vaccine development and technologies for the future; 6) to support regions in establishing relationships with Regional Vaccine Committees [20].

The main goals of the new Italian NITAG are summarized in *Table 1*.

Reinforcement of credibility and legitimacy of vaccination

Although vaccination is acknowledged as one of the most successful medical achievements of modern civi-

Table 1
Goals of the Italian National Immunization Technical Advisory Group (NITAG)

The Italian NITAG goals
Reinforcement of credibility and legitimacy of vaccination
Good governance
Proper management of Conflict of Interest
Reinforcement of institutional integration to promote sustainability and credibility
Increasing networking and regional collaboration: the global NITAG network

lization, an increasing number of people believe it to be unnecessary and life-threatening [22]. In fact, vaccine hesitancy poses the biggest threat to vaccination campaigns [23]. Research proved that trust in government is strongly correlated to vaccine acceptance and can promote public compliance with recommended actions [24]. Currently, enhancing the transparency of NITAG's decision-making process and supporting the development of best practices among the NITAGs is crucial to ensure people trust in recommended vaccination programmes.

To achieve this objective, NITAG must define terms of reference that are consistent with current NITAG practice. Moreover, the decision framework must adhere to a standardized approach, such as GRADE, and be evidence-based, structured, transparent, reproducible, and reliable [1]. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) is an unofficial group of people who cooperate to deal with the shortcomings of grading systems in health care. Therefore, the methodology based on quality of evidence, which is applied by the GRADE working group, has been suggested as the operational mode. This operational mode is widely applicable in various contexts and helps in evaluating the strengthening of recommendations in healthcare [9, 25, 26].

Another step towards this goal is represented by the establishment of transparency in public communication with, agenda, decisions, and technical reports made available to those who request them. Meetings should ideally be accessible to everyone who desires to attend. Lastly, economic concerns (cost-effectiveness analysis and budget impact estimate) should be included in the framework analysis at NITAG level, while price and reimbursement should be handled by other bodies [27].

Good governance

To improve competent policymaking and eliminate vaccine hesitancy, good governance is essential. Notably, accountability, integrity, openness, accessibility, and proportionality are essential components of good governance. These ones are employed to evaluate NITAG operations and foundational tools, according to different criteria such as composition, member integrity, remit and values, organisation independence, practice procedures, evidence and policy, performance evaluation [28].

Proper management of conflict of interest

Once they are appointed, NITAG members should formally declare their interests in order to avoid conflict of interest (CoI). Identification of current, past, and potential future interests is necessary; besides this, regular updates on any new interest should be provided in order not to influence future recommendations. After declaration, interests are evaluated by the NITAG chair, possibly in consultation with the secretariat. The strength of each interest can be assessed on the basis of the amount of benefit considered, the frequency of the relationship with this source of interest, and the age and length of interest. Typically, the NITAG member has three outcomes: participation in the debate and decision-making, participation in the debate but not the vote, or exclusion from both the debate and the vote. [29, 30].

Reinforcement of institutional integration to promote sustainability and credibility

Raising awareness on NITAG's role as an advisory committee is one of the main and essential prerogatives to strengthen the relationship with the MoH and its technical scientific bodies (e.g., National Health Council). In fact, national authorities' misconception about NITAG role could represent an obstacle to the achievement of NITAG goals. These concerns could consist in NITAG's independence being seen as a threat which could undermine MoH's authority and could cast doubt on his prerogatives. Nevertheless, concerns could be allayed by improving awareness of their individual duties since MoH nominates NITAG members, designates the secretariat, takes part in the agenda and is the final decision-maker [14].

Increasing networking and regional collaboration: the global NITAG network

Executive secretariats should develop and maintain relationships with other NITAGs to constantly improve and compare outcomes [14]. That's why the Global NITAG Network (GNN) has been created. The rising initiative of this platform started in 2016 and officially established in Berlin in 2017. The GNN steering committee functions as an executive decision-making body. It is composed by two members of the NITAG secretariat and six NITAG members, who each represent one of the six WHO regions. The group's makeup represents the wide diversity of socioeconomic backgrounds, organizational experiences, and maturity levels present in the NITAG community. Fifty-three countries were part of the network in 2020. Based on priorities, meetings are organised by the network on a yearly basis in different locations and rotating WHO regions. GNN serves as a common ground in which experiences and practices are shared among NITAGs. Its mission is to improve efficiency in the development of evidence-informed recommendations on immunizations through global cooperation with input from regional networks [31]. The usage of webinars is a significant support for this endeavour as they enable more regular and timely information and guidance dissemination along with simpler participation of highly qualified worldwide experts [15].

THE NEW NATIONAL IMMUNIZATION PREVENTION PLAN

One of the first activities of the new NITAG has been the evaluation and promotion of the new National Immunization Prevention Plan (PNPV). Traditionally, the National Vaccine Plan (PNV), represented a multi-year planning tool to which the vaccination schedule, created with the contribution of both ministerial committees and the main scientific societies, was also linked [17]. Since 2012 it has been referred to as the National Vaccine Prevention Plan, equivalent to the worldwide National Immunization Programmes (NIPs). This time, the first draft, prepared by an *ad hoc* commission of the MoH, provided for some general four-year objectives along with an immunization schedule that may be updated annually. The NITAG, with its own skills, will have to integrate the proposal and favour rapid approval. The main objectives of the PNPV will be: I) to maintain polio-free status; II) to achieve measles-free and rubella-free status; III) to strengthen prevention of cervical cancer and other HPV-related diseases; IV) to reach and maintain target coverage through a fair and free vaccination offer among age groups and populations at risk; V) to counteract inequalities by promoting vaccination interventions in at-risk population for adverse socioeconomic status, occupational exposure or marginalization; VI) to complete the digitalization of a nationwide vaccination register that can be consulted from each region; VII) to improve the surveillance of vaccine-preventable diseases; VIII) to increase adherence to vaccinations; IX) to promote vaccinations among healthcare workers to achieve the target coverage [17, 31-33]. The COVID-19 pandemic's effects on vaccination coverage and the organizational lessons learned during the nationwide anti-SARS-CoV2 vaccination campaign are both considered in the latest version of the PNPV. Additionally, a patient-centered vaccination strategy is being emphasized, strengthening the role of the local healthcare network for the inclusion of vulnerable patients and their care.

RECOVERY OF VACCINATION COVERAGE DECREASED DURING THE PANDEMIC

The health emergency caused by COVID-19 has had a significant impact on the world population. National vaccination rates (about year 2020) have undoubtedly been impacted by the pandemic, which disrupted pediatric routine immunizations and caused parents to reschedule visits out of fear of infection or due to "stay-at-home" safety measures. The national immunization coverage levels for mandatory vaccinations slightly decreased during 2020 compared with the previous year, with only one exception that regards chicken pox, which underwent a significant increase (+2.2%). Almost all the recommended vaccinations showed a moderate decrease (range -1.4% to -2.7%). Particularly, anti-Men C revealed a remarkable drop (-8.5%) unlike vaccine coverage rates for anti-Men ACWY, anti-rotavirus and anti-HPV, that raised (+4.8%, +9.4% and +1.8%, respectively) [34].

One of NITAG's main goals is to propose the new PNPV (expired in 2019), to harmonise the current vaccination schedule with the anti-COVID-19 campaign and to recover the concerning vaccination drops that occurred during the pandemic. Interaction between the WHO Global NITAG networks and the ECDC EU/EEA networks can help to more effectively accomplish all of these objectives.

OTHER PERSPECTIVES OF THE ITALIAN NITAG

The new NITAG's mission is to advance and maintain the strategic goals established by the PNPV 2017-2019, with an emphasis on revising the current immunization schedule and formulating recommendations for coverage levels that are sustainable and attainable [35]. According to NITAG, these actions need to be continuously assessed using a system of indicators (to be developed) that can measure vaccination coverage and the accomplishment of preventative goals.

The introduction of new practices, such as the creation of a standard protocol for the introduction of vaccinations on the immunization schedule and a revision of the current essential levels of care (*Livelli essenziali di assistenza, LEA*), will be a key topic of debate [32].

There is still work to be done on two other crucial issues: the regional vaccine prevention gaps in Italy as well as the focus on at-risk groups, to whom specific vaccinations, free of charge, are offered (e.g., pregnant women, healthcare workers and subjects suffering from chronic diseases).

The new Italian NITAG holds another ultimate responsibility which is to complete the evaluation of the current law on mandatory vaccinations and to raise public confidence in the role of the latter during these trying times, via communication campaigns: after all, good communication could contribute to an increase in vaccination rates in Italy, which is essential to realizing the opportunity for everyone to live a healthy life [36].

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Conflict of interest statement

The Authors do not have any conflict of interest or financial interest.

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Diagnosis of Respiratory Syncytial Virus (RSV) infection in children by Respiratory Panel utilized during the COVID-19 pandemic

Paola Alessandra Petrocelli[1], Veronica Cunsolo[1], Marianna Melito[1], Giovanni Scuderi[1], Rebecca Testa[1], Stefano Messina[1], Francesca Tucci[1], Lorenzo Sardone[1], Daria Colligiani[1], Maria Nardone[1], Stefano Rapi[1] and Elisabetta Stenner[2]

[1]UOC Laboratorio Analisi Chimico-Cliniche, Ospedale San Luca, USL Toscana Nord Ovest, Lucca, Italy
[2]UOC Laboratorio Analisi Chimico-Cliniche, Ospedali Riuniti, USL Toscana Nord Ovest, Livorno, Italy

Abstract

Background. In the months of October and November 2021, there was throughout Italy and in our specific case in the area of Lucca and Versilia, a disturbing increase of SARS-CoV-2 infections and cases of Respiratory Syncytial Virus (RSV) in new-borns. The aim of this paper is to compare the cases of RSV infection diagnosed in recent years to the cases recorded during the SARS-CoV-2 pandemic to November 2022.

Materials and methods. The study consisted of evaluating the results of requests for RSV diagnosis from 2015 to November 2022, using molecular biology techniques.

Results. The data obtained show that the number of cases of RSV infection in children during the winter season had a constant trend from 2015 to 2019. From November 2020 to February 2021 there were no cases of RSV respiratory infections. Starting from September 2021, on the other hand, there was a resumption of cases of RSV infections in conjunction with an increase in the number of children affected by COVID-19. From January 2022, after a peak in cases of SARS-CoV-2 infection, there has been a decrease in RSV infections. From September 2022 to November 2022, there was no increase of cases of RSV infections in new-borns but on the contrary, there was a trend in respiratory infections comparable to the pre-pandemic period.

Conclusion. The data that emerged from the study conducted, show the onset of an outbreak of RSV in new-borns. This incidence is linked to the implementation of rigorous non-pharmacological public health interventions in 2020, aimed at combating COVID-19 infection. The use of the molecular panel made it possible to identifying the responsible agent and highlighting the most suitable clinical and therapeutic path.

Key words

- RSV
- respiratory infections
- SARS-CoV-2
- pandemic

INTRODUCTION

Clinically relevant respiratory infections, caused by a variety of viral and bacterial pathogens, commonly occur in both adults and children. Respiratory infections have the highest incidence at paediatric ages, particularly in the first years of life. In 80% of cases, the etiological agents of Recurrent Respiratory Infections (RRIs) are the Respiratory Syncytial Virus (RSV), the Influenza and Parainfluenza Viruses, the Adenovirus, the Rhinovirus and the Bocavirus [1]. The Respiratory Syncytial Virus is an RNA virus, classified as Pneumovirus, of which two subgroups have been identified, namely A and B. RSV is one of the respiratory viruses that circulates the most in the world and is the most common cause of bronchiolitis, an inflammation of the small airways of the lungs [2, 3]. Human Respiratory Syncytial Virus is present in the bronchi, bronchioles and pulmonary alveoli and can cause necrosis of epithelial cells and interstitial infiltrates of inflammatory mononuclear cells. The Respiratory Syncytial Virus affects all ages, but more severely infants in the first months of life and the elderly with multiple pathologies. There are people who are more at risk of contracting respiratory infections, such as children with heart disease, severe prematurity, or neurodevelopmental problems [4, 5]. In elderly people, with comorbidities, RSV is a dangerous virus like SARS-CoV-2, which can cause the premature death of the patient. Most children get infected at least

Address for correspondence: Veronica Cunsolo, Ospedale San Luca, Via Guglielmo Lippi Francesconi n. 556, Loc. San Filippo, 55100 Lucca, Italy.
E-mail: veronica.cunsolo@uslnordovest.toscana.it.

once in their lifetime, usually in the first two years, but they do not always develop severe manifestations. Children can also be reinfected by the virus, because a first RSV infection does not make them completely immune; however, subsequent infections are milder than the first [6]. Infants or children in the first few months of life are at increased risk of developing the most severe form of the disease, with respiratory complications and pneumonia and consequent dangers to the baby's life. In infants, especially when premature, the first symptoms are apnoea or those typical of respiratory diseases such as colds, coughs, sore throats, and fever [7]. In the following days they may begin to manifest more serious symptoms including respiratory distress, a condition in which the use of oxygen is necessary [8, 9]. Respiratory Syncytial Virus usually has a seasonal peak, causing respiratory infections starting from mid-November and then lasting until the end of. The seasonality of the virus allows, therefore, in the case of premature babies, to program a pharmacological prophylaxis in order to prevent the onset of the most serious symptoms [10, 11]. Prophylaxes based on the use of monoclonal antibodies, which have a high cost and are reserved only for some special cases indicated by the guidelines, do not give immunity but confer protection from respiratory infections during the winter season. During the months of October and November 2021, throughout Italy and in our specific case in the areas of Lucca and Versilia, we observed a disturbing increase in cases of new-borns with SARS-CoV-2 infections and cases of Respiratory Syncytial Virus. This epidemic of RSV broke out earlier than in previous years. Numerous RSV outbreaks, with hospital admissions of paediatric patients, were recorded as early as the end of October 2021 when temperatures were not yet typically of winter. In the first weeks of December 2021, the ratio in terms of the number of hospitalizations of children for respiratory syncytial virus and those of COVID-19 was about 10 to 1. There were therefore 10 times more children who ended up in hospital for RSV than those who needed hospitalization for coronavirus SARS-CoV-2 infection [12, 13]. Since January 2022 a constant decrease in cases of RSV infection in young patients was observed until no cases were recorded during summer period. From October 2022 to November 2022 an increase of infections caused by RSV in children has been observed, but the number of cases reflecting the seasonality of respiratory infections [14, 15]. The introduction from September 2021 of the One-step RT Real-time PCR multiplex test for the screening of infectious agents, has made it possible to process a greater number of samples routinely and quickly for a correct differential diagnosis of RSV from other pathogens and guide the hospital pathways for the management of young patients. Using rapid diagnostic techniques also means reducing hospitalization times, hospital costs and often avoids the administration of inappropriate antibiotic therapy, thus preventing the phenomena of drug resistance [16, 17].

SUBJECTS, MATERIALS AND METHODS

Subjects

The study done at the Clinical Chemical Analysis Laboratory of the San Luca Hospital in Lucca was carried out by considering the results of the requests for RSV diagnosis from 2015 until November 2022. A population of 500 patients, which included new-borns and children up to 11-12 years old with respiratory diseases was analysed. In past years, the search for Respiratory Syncytial Virus was performed using the RSV Card of Beta Diagnostici, subsequently, diagnostic platforms based on molecular biology techniques were used: Cepheid Xpert® Flu / RSV from Seegene Inc, FilmArray® multiplex PCR from BioFire Diagnostics Inc. and finally, the Seegene Allplex™ Respiratory Full Panel Assay kit.

Immunochromatographic method

The RSV Card is a qualitative lateral flow assay for the diagnosis of RSV antigen in nasopharyngeal specimens. The RSV antigens, if present in the sample, react with anti-RSV antibodies immobilized in the strip and combined with particles are capable of generating a colorimetric reaction. The time for the analysis and reporting is 15 minutes.

Molecular Biology Methods

A diagnostic platform used for RSV analysis is the FilmArray®, designed by BioFire Diagnostics, which uses a nested multiplex PCR (nmPCR) protocol for the identification of 16 viral and 4 bacterial targets (at gender and/or species), in a biological sample. The integrated FilmArray® platform is a closed system, which is based on the use of a disposable and compact pouch inside which the reactions of extraction, purification, and amplification of the nucleic acid of the eventual pathogen, present in a single sample, take place biologically. The execution time of the analysis is approximately one hour. The second platform in use is the Cepheid Xpert® Flu / RSV system, a diagnostic test in molecular biology, which allows one to identify the A, B and RSV Influenza Virus in just 20 minutes. Recently, an analytical platform for the diagnosis of symptomatic airway infections has been introduced, which permits examination for the presence of a wider variety of viruses and bacteria, from a single nasopharyngeal swab. The Seegene Allplex™ Respiratory Full Panel Assay kit is a multiplex One-step RT Real-time PCR assay, which in 22 samples simultaneously detects and identifies, 16 viruses, 7 bacteria and 3 subtypes of Flu A, agents of respiratory diseases. Analysis time is approximately four hours (*Table 1*).

RESULTS

From the analysis of the positive cases of RSV diagnosis over time, in particular before the pandemic period (end of February 2020), during the pandemic (from the lockdown until March 2022), and after the pandemic period (from April 2022 to November 2022), it clearly emerges that the Respiratory Syncytial Virus is a pathogen that causes seasonal winter epidemics (as shown in *Figure 1*). The data obtained from our study, starting from 2015, showed an increase in the cases recorded for RSV during the winter season, starting from November, with a peak in the month of January and then decreasing, with fewer cases of infections during the spring/

Table 1
The Seegene Allplex™ Respiratory Full Panel Assay that is composed of 4 different panels and is a multiplex One-step RT Real-time PCR assay to detect and identify, 16 viruses, 7 bacteria and 3 subtypes of Flu A

Allplex™ Respiratory Full Panel Assay			
Panel 1	**Panel 2**	**Panel 3**	**Panel 4**
Influenza A virus	Adenovirus	Bocavirus	Mycoplasma pneumoniae
Influenza B virus	Enterovirus	Rhinovirus	Chlamydophila pneumoniae
Respiratory syncytial virus A	Parainfluenza virus 1	Coronavirus NL63	Legionella pneumophila
Respiratory syncytial virus B	Parainfluenza virus 2	Coronavirus 229E	Haemophilus influenzae
Flu A-H1	Parainfluenza virus 3	Coronavirus OC43	Streptococcus pneumoniae
Flu A-H1pdm09	Parainfluenza virus 4	Internal Control	Bordetella pertussis
Flu A-H3	Metapneumovirus		Bordetella parapertussis
Internal Control	Internal Control		Internal Control

summer. The seasonal trend of RSV cases appears to be constant in the pre-pandemic years. On the other hand, no cases were recorded from November 2020 to February 2021, and this is related to the implementation of rigorous non-pharmacological public health interventions to combat COVID-19. From September 2021, on the other hand, there has been an increase in RSV cases and at the same time also of children, aged between a few months from birth up to 11 years, with SARS-CoV-2 infections. From the beginning of December 2021 to March 2022 there was another peak of SARS-CoV-2 infection involving a heterogeneous age group. At the same time, there was a steady decrease, starting from January 2022, in the number of respiratory infections caused by RSV in children. In September 2022 there was no anomalous increase in RSV cases as in 2021, but on the contrary, an increase of the cases RSV infection was detected up to November 2022 which correlates with a pathogen that causes seasonal winter epidemics (as shown in *Figure 2*).

DISCUSSION

The data that emerged from the study conducted in our laboratory describe a situation that corresponds to what has also happened in other countries in different continents of the Northern Hemisphere and the Southern Hemisphere [18-22]. In Italy, Finland, Belgium, the UK, and the USA the implementation of restrictions, starting from March 2020, coincided with an apparent sudden and earlier end of the RSV epidemic season, compared to previous years and almost no cases detected in the following months. In the Southern Hemisphere, SARS-CoV-2 restrictive measures were implemented just before winter and were maintained for different periods according to SARS-CoV-2 diffusion [23-25]. Starting from September 2021, in Italy and

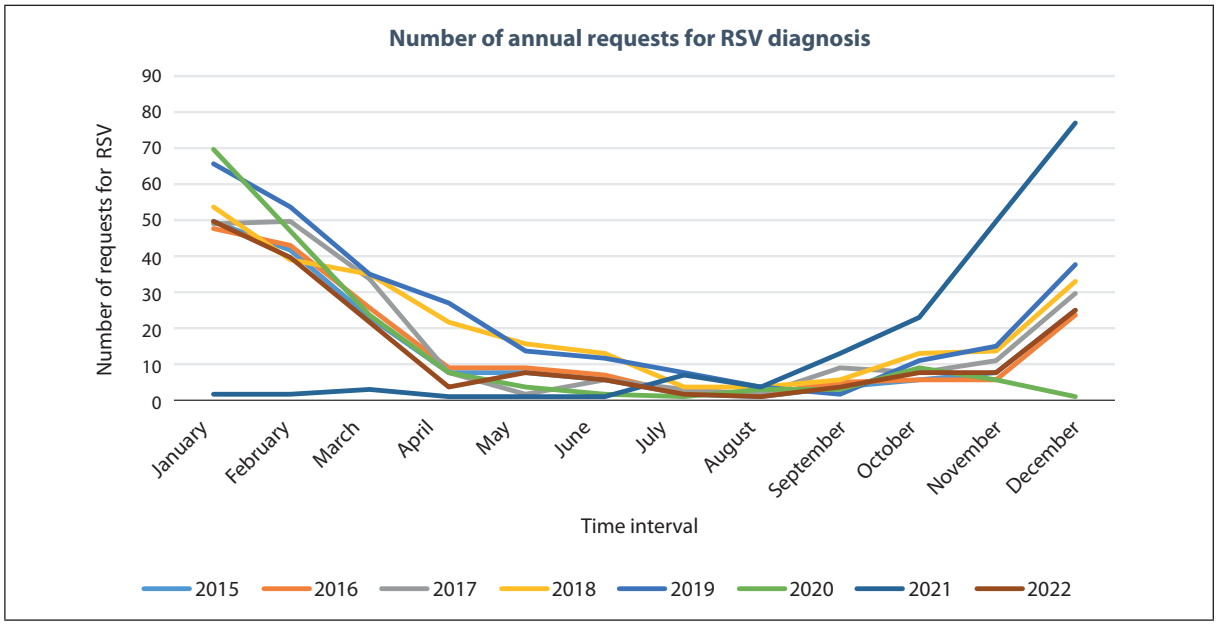

Figure 1
Number of annual requests for research of the Respiratory syncytial virus (RSV) genome using molecular biology techniques.

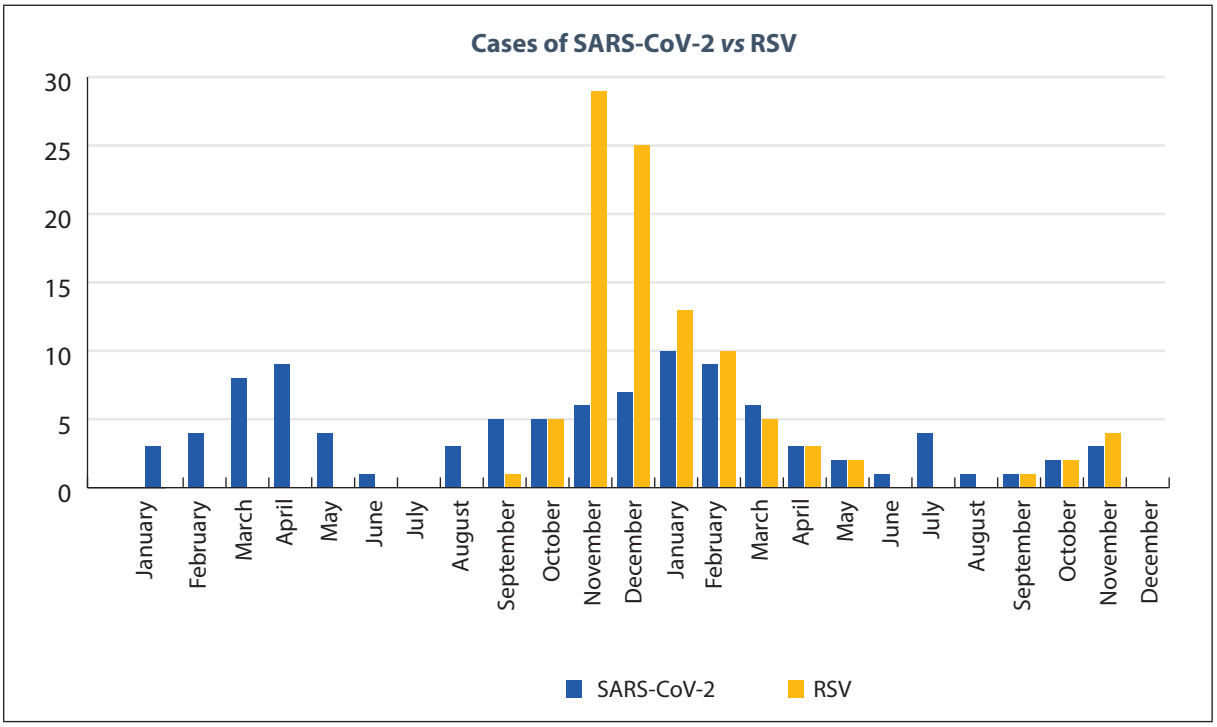

Figure 2
Comparison of the number of Respiratory syncytial virus (RSV) cases compared to SARS-CoV-2 infections in children, aged between a few months from birth up to 11 years old, during the pandemic and post-pandemic period up to November 2022.

in our specific case in the areas of Lucca and Versilia, another epidemiological phenomenon has also been observed, a disturbing increase in cases of new-borns with respiratory infection of Respiratory Syncytial Virus. This epidemic broke out earlier than in previous years. In other countries of the northern hemisphere like in France, RSV arrived late in February 2021 and expanded during the spring with an outbreak of a duration comparable to the previous season, although of a lesser magnitude [26, 27]. In the USA, the RSV epidemic started at the end of March 2021 covering the spring–summer months, extending into the autumn in some states [28]. This epidemiological shift was previously observed in the southern hemisphere. In Australia and New Zealand, after a 2020 winter season with RSV virtually absent, an unusual reappearance of the virus was observed during the summer, with an even larger outbreak than previous epidemic seasons [29]. The data conducted in our laboratory has been highlighted that the association of SARS-CoV-2 infection and an increase in cases of children with RSV respiratory infections it was correlated to the fact that children were more vulnerable than usual to respiratory viruses and seasonal infections because they had been underexposed to germs during the measures decreed at the beginning of the pandemic (social distancing, the use of Personal Protective Equipment). Respiratory RSV infections did not spread during the COVID-19 lockdown and consequently pregnant women developed a reduced number of antibodies to be transmitted to their children, not having been exposed to the related infectious agents. With the easing of anti-Covid measures, the Respiratory Syncytial Virus has presented itself earlier and more aggressively, than would be expected in a normal winter season. In post-lockdown Italy there were several hospitalizations of children due to respiratory infections, a condition that supports the theory of immunity debt, a concept used to describe the poor protective immunity resulting from long periods of low exposure to a given pathogen, accumulated thanks to the measures adopted in times of pandemics. What we saw in that period was due to the boomerang effect of COVID-19. We assisted to a change in the immune system, particularly in younger children, as a result of major social and health changes related to the pandemic. Fortunately, the risk of death, unlike in developing countries, is exceptionally low in Italy, but the virus can leave permanent damage, starting with the development of asthma in adulthood which occurs in 50% of cases. Without seasonal exposure, therefore, immunity decreases and susceptibility to potentially more serious future infection increases (https://www.cdc.gov/flu/symptoms/flu-vs-covid19.htm). After an epidemic of RSV at the end of 2021 and a new peak of COVID-19, starting from the beginning of January 2022 there was a constant decrease over time in the number of cases of respiratory infections of RSV in children (https://www.cdc.gov/coronavirus/2019-ncov/long-term-effects/index.html.). This would seem to be associated with the easing of restriction measures to counter the spread of SARS-CoV-2 and the gradual return to pre-pandemic habits favouring, therefore, a lower susceptibility to seasonal respiratory viruses (https://www.cdc.gov/flu/season/faq-flu-season-2021-2022.htm.). The epidemio-

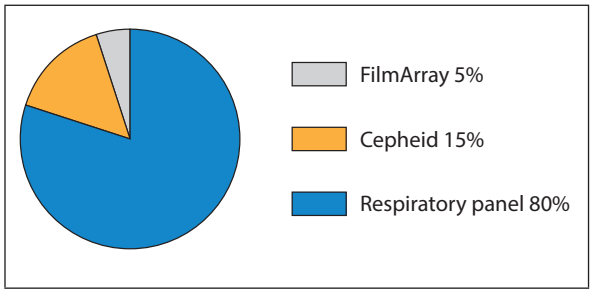

Figure 3
Percentage of diagnostic platforms use based on molecular biology techniques.

logical phenomenon that has been observed in these years, has highlighted the important role that the analytical laboratory in providing results quickly and thus help the clinician for the diagnosis. In order to optimise the number of samples to be processed, quickly and at moderate costs, it was decided to routinely introduce, in the Molecular Biology sector, a diagnostic platform based on the use of an analytical panel. Urgent requests for RSV diagnosis, on the other hand, are treated by analysing the nasopharyngeal swab with the Cepheid Xpert® Flu / RSV system or with the FilmArray® system from BioFire Diagnostics, faster but more expensive analysis techniques, permitting the analysis of a few samples simultaneously (as shown in *Figure 3*). With this type of panel, it was possible to perform the differential diagnosis of the two subgroups of the RSV, A and B. The pathology caused by subgroup A appears to be more serious and, in most cases, requires the hospitalization of the child.

CONCLUSION

We assisted a serious epidemic of Respiratory Syncytial Virus in new-borns, starting from September 2021. This incidence has been correlated to the implementation of rigorous non-pharmacological public health interventions in 2020, aimed at combating COVID-19 infection. The use of the molecular panel made it possible to draw attention to respiratory infections in children with similar clinical symptoms, thus identifying the responsible agent and highlighting the most suitable clinical and therapeutic path.

Conflict of interest statement

The Authors declare no conflicts of interest.

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Should I give kids money? The role of pocket money on at-risk behaviors in Italian adolescents

Edoardo Lozza[1], Carlotta M Jarach[2], Giulia Sesini[1], Elena Marta[1], Alessandra Lugo[2], Eugenio Santoro[3], Silvano Gallus[2] and HBSC Lombardy Committee 2018*

[1]*Dipartimento di Psicologia, Università Cattolica del Sacro Cuore, Milan, Italy*
[2]*Dipartimento Ambiente e Salute, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy*
[3]*Dipartimento di Salute Pubblica, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy*
**The Members of the HBSC Lombardy Committee 2018 are listed before the References*

Abstract

Background. Discussion on the impact of pocket money on positive behaviors is still debated.

Objective. To investigate the effect of diverse money allowance schemes on risky behaviors (smoking, alcohol, binge drinking, drug use, gambling) during adolescence.

Method. 989 students aged 15 from Lombardy (Italy) reported information on money availability in the 2018 wave of the Health Behaviour in School-aged Children study. To analyze the relationship between money availability and risky behaviors we computed odds ratios and 95% confidence intervals through unconditional multiple logistic regression models.

Results. Spending more than 10€ weekly was associated with higher likelihood to smoke, binge drink or gamble. Receiving pocket money (rather than receiving money upon request) was related to higher likelihood to engage in risky behaviors.

Conclusions. Pocket money may have a negative impact on adolescents, particularly with a substantial amount of money. More research is needed to understand why providing money only if needed may serve as a protective factor against risky behaviors.

Key words

- pocket money
- smoking
- alcohol drinking
- gambling
- adolescents

INTRODUCTION

Pocket money (i.e., providing a fixed amount of money recurrently) is a common way to allow for intentional financial socialization during childhood and adolescence, but whether it represents an effective factor in endorsing positive behaviors and lifestyles is a controversial issue. Indeed, previous studies report both positive and negative effects, as well as no impact at all, of having a fixed amount of money available for children and adolescents.

On the one hand, scholars defend money allowances to children as a tool to endorse critical financial capabilities and responsibilities [1-4], to incentivize good behaviors as a mechanism of reward [5], and to equip children with resources to be used for their own consumption [6, 7]. Furthermore, past research suggests that pocket money may function as an intra-household resource allocation device, key to educating children [8] and associated to higher financial literacy levels [9]. In addition, it was also found that adolescents with pocket money are more likely to practice physical activity with higher frequency than their peers with no allowances [10].

On the other hand, another equally substantial body of studies claims that allowances alone do not improve competencies such as carrying a credit card balance, having a bank account or saving [11], suggesting instead a connection between allowances and financial dependence rather than capability [12]. Looking at research in the health sector, evidence confirms the role of pocket money in cigarette smoking in children and adolescents, with a higher amount of pocket money associated with a higher prevalence [13-19]. It was also found that pocket money represents a risk factor in boosting unhealthy eating habits, like consumption of soft drinks and out-of-home eating [20-23]. Furthermore, increasing levels of money availability are associated with higher risk of substance use [24] and higher likelihood of students' gambling [25].

As concerns the Italian context, evidence is quite limited, suggesting a connection between adolescents' pocket money availability with alcohol consumption and smoking habits [26-29].

This paper contributes to the existing literature by

Address for correspondence: Giulia Sesini, Dipartimento di Psicologia, Università Cattolica del Sacro Cuore, Largo Gemelli 1, 20123 Milan, Italy. E-mail: giulia.sesini1@unicatt.it.

providing insights on the effects of different money allowance schemes over five types of risky behaviors. Indeed, the research takes into account the differential effect of terms and conditions under which parents provide an allowance, rather than just the pocket money itself. Specifically, the analyses mostly concerned the impact of the amount of weekly expenses, the frequency of pocket money (regular *vs* irregular) and the amount of money available over five different risky practices (smoking, consuming alcohol, binge drinking, drug use, gambling). To the best of our knowledge, this is the first study investigating the effect of diverse money allowance schemes on risky behaviors during adolescence. Indeed, previous studies present some limitations. The majority of them only asked for the amount of money available to children and adolescents, with no differentiation in terms of the sources of such allowance [1, 10, 13, 15, 16, 18-20, 22, 23, 25, 27]. Furthermore, some studies focused only on regular pocket money [11, 26, 29], or they tested the effect of paid chores, earned income and allowances over risky behaviors [12, 24], rather than investigating the frequency of pocket money. Last, we can recall few other studies that treated all money sources at children's disposal as a unique variable, without distinguishing where the money comes from [2, 14, 17].

MATERIALS AND METHODS

We used Lombardy (Italy) regional data from the 2018 wave of the Health Behaviour in School-aged Children (HBSC) study, a cross-national investigation conducted on school children aged 11-15 in over 51 countries and regions across Europe and North America in collaboration with the World Health Organization (WHO) Regional Office for Europe [30].

The sampling procedure followed international guidelines. Classes were selected according to a systematic sampling method from the complete list of schools provided by the Italian Ministry of Education, University and Research. Participants were chosen via cluster sampling, with school classrooms serving as the primary sample unit. In the Italian HBSC, schools from Lombardy were oversampled to ensure sufficient statistical power to obtain robust frequency estimates at a regional level [31]. Details about the survey's sample methodology and data collection may be found elsewhere [31, 32]. The protocol for the study was approved by the National Institute of Health's Institutional Ethical Board (General protocol: PRE-876/17).

Outcome measures

Five at-risk behaviors were assessed: i) smoking and ii) drinking behavior, using the questions "How many days have you smoked at least one cigarette in the last 30 days" and "How many days have you consumed alcohol (if you ever had) in the previous 30 days", respectively. We then classified all those who answered with "at least one day" as smokers or drinkers; iii) binge drinking (yes/no) was assessed with the question "Have you ever drunk five or more glasses of alcoholic beverages on a single occasion in the last 12 months?"; and iv) cannabis use was assessed with the question "Have you ever smoked cannabis in your life?". Lastly, v) gambling was assessed with the question "Have you ever bet and/or gambled money in your life?", where gambling was defined as betting on the outcome of a contest or game – including those organized by charities – in which money can be won or lost.

Other measures

In Lombardy Region only, adolescents aged around 15 were asked about money availability for their personal use, the average amount of money per week spent without parental supervision (open question, in €) and the following sources of money: weekly pocket money, monthly pocket money, money asked when needed. For the present analysis, 989 students aged 15 from 67 classes who provided information on self-reported money availability were included in the study.

Statistical analysis

We evaluated the odds ratios (OR) and corresponding 95% confidence intervals (CI) of i) smoking behavior during the previous month, ii) alcohol usage within the last month, iii) lifetime binge drinking, iv) lifetime cannabis use, and v) gambling using unconditional multiple logistic regression models. We also evaluated the ORs for at least four of the above-mentioned at-risk behaviors (i.e., smoking in the last 30 days, drinking in the last 30 days, binge drinking, cannabis use and gambling). All the models were adjusted for sex, age (in continuous), and the highest level of education of the parents. SAS version 9.4 (Cary, North Carolina, USA) was used to perform all statistical analyses. Materials and analysis code for this study are available by emailing the corresponding Author.

RESULTS

Table 1 shows the ORs for selected at-risk behaviors (namely smoking in the last 30 days, drinking in the last 30 days, binge drinking, cannabis use and gambling) according to money availability in adolescents in their third year of high school. Adolescents spending more than 10€ per week were more frequently smokers (compared to less than 10€, OR=1.60; 95% CI: 1.07-2.37 for 10-20€, and OR=1.66; 95% CI: 1.14-2.41 for more than 20€ per week). Those receiving pocket money were more frequently smokers (OR=1.69; 95% CI: 1.25-2.27) while no significant relation was observed with receiving money only upon request. Moreover, adolescents receiving less than 10€ on request per week had a lower likelihood to smoke (OR=0.53; 95% CI: 0.34-0.84), binge-drink (OR=0.65; 95% CI: 0.45-0.95), and gamble (OR=0.63; 95% CI: 0.42-0.94). Compared to <10€ (independently on the source), the OR of binge drinking for adolescents having more than 20€ available for their weekly expenses was 1.44 (95% CI: 1.04-1.99) and the OR of gambling was 1.43 (95% CI: 1.02-2.02). *Supplementary Table 1* and *Supplementary Table 2* available online show the ORs for the same at-risk behaviors in males and females, respectively.

Table 2 shows the ORs for having at least four out of the five aforementioned at-risk behaviors. Compared to adolescents having less than 10€ per week, the OR

Table 1
Distribution of adolescents in their third year of high school by selected at-risk behaviors, according to money availability. Corresponding odds ratios (OR) and 95% confidence intervals (CI). HBSC Lombardy 2017-2018

	Smoking in the last month^		Alcohol in the last month^		Binge drinking¥		Cannabis use¥		Gambling¥	
Determinants	**%**	**OR* (95% CI)**	**%**	**OR* (95% CI)**	**%**	**OR* (95% CI)**	**%**	**OR* (95% CI)**	**%**	**OR* (95% CI)**
Total	25.3		53.1		38.1		22.6		37.9	
Weekly expenses§										
<10€	19.6	1.00°	50.0	1.00°	33.2	1.00°	18.4	1.00°	34.0	1.00°
10-20€	27.8	**1.60 (1.07-2.37)**	55.3	1.25 (0.89-1.75)	39.7	1.34 (0.95-1.89)	24.9	1.47 (0.98-2.21)	39.2	1.27 (0.88-1.83)
≥20€	29.0	**1.66 (1.14-2.41)**	55.1	1.21 (0.88-1.65)	42.0	**1.44 (1.04-1.99)**	24.9	1.44 (0.98-2.12)	41.8	**1.43 (1.02-2.02)**
P for trend		**0.012**		0.276		**0.032**		0.083		**0.042**
Pocket money§										
No	21.7	1.00°	51.8	1.00°	36.1	1.00°	21.1	1.00°	35.7	1.00°
Yes	31.8	**1.69 (1.25-2.27)**	55.2	1.14 (0.87-1.49)	41.7	1.26 (0.96-1.64)	25.5	1.24 (0.91-1.69)	41.5	1.24 (0.93-1.65)
Pocket money§										
No	21.7	1.00°	51.8	1.00°	36.1	1.00°	21.1	1.00°	35.7	1.00°
Yes										
<10€	28.8	1.41 (0.84-2.39)	51.3	0.97 (0.60-1.55)	35.4	0.94 (0.58-1.53)	15.9	0.70 (0.37-1.31)	35.4	1.01 (0.61-1.69)
10-20€	34.0	**1.93 (1.23-3.05)**	54.4	1.12 (0.73-1.71)	45.7	1.51 (0.99-2.30)	30.8	1.60 (1.00-2.54)	43.3	1.26 (0.80-1.97)
≥20€	31.9	**1.69 (1.15-2.47)**	57.7	1.26 (0.88-1.79)	42.3	1.28 (0.90-1.83)	27.0	1.33 (0.89-1.99)	43.6	1.35 (0.93-1.96)
Money if needed§										
No	27.3	1.00°	52.2	1.00°	40.9	1.00°	23.6	1.00°	40.8	1.00°
Yes	24.2	0.82 (0.60-1.12)	53.6	1.03 (0.79-1.35)	36.7	0.82 (0.62-1.08)	22.1	0.92 (0.66-1.26)	36.5	0.87 (0.65-1.16)
Money if needed§										
No	27.3	1.00°	52.2	1.00°	40.9	1.00°	23.6	1.00°	40.8	1.00°
Yes										
<10€	17.4	**0.53 (0.34-0.84)**	50.3	0.90 (0.62-1.29)	31.8	**0.65 (0.45-0.95)**	19.5	0.77 (0.50-1.21)	29.9	**0.63 (0.42-0.94)**
10-20€	23.9	0.81 (0.54-1.22)	54.4	1.08 (0.75-1.55)	34.2	0.74 (0.51-1.08)	20.7	0.86 (0.56-1.33)	35.9	0.87 (0.59-1.28)
≥20€	29.5	1.08 (0.75-1.55)	55.6	1.10 (0.79-1.53)	42.4	1.03 (0.74-1.44)	25.2	1.07 (0.73-1.57)	42.0	1.09 (0.77-1.56)

*ORs were estimated by unconditional multiple logistic regression models, after adjustment for sex, parental highest level of education and age of the pupil. Estimates in bold are those statistically significant at 0.05 level.
°Reference category.
§Whether adolescents did not indicate having money, the money availability's variables (i.e., weekly expenses, pocket money and money if needed) were categorized as "no" or as "less than 10 euros".
^Smoking use and alcohol use were asked within the last 30 days. ORs for smoking were based on 974 individuals, and for alcohol on 973.
¥Binge drinking was assessed with the question "Have you ever drunk five or more glasses of alcoholic beverages on a single occasion in the last 12 months?". Cannabis use was assessed with the question "Have you ever smoked cannabis in your life?". Gambling was assessed with the question "Have you ever bet and/or gambled money in your life?". ORs for binge drinking were based on 988, for cannabis use on 985, for gambling on 984.
HBSC: Health Behaviour in School-aged Children.

for those having 10-20€ was 1.76 (95% CI: 1.10-2.82) and the OR for those having more than 20€ per week was 1.69 (95% CI: 1.08-2.64). Those receiving pocket money were more likely to engage in at least four at-risk behaviors (OR=1.48; 95% CI: 1.05-2.10), while no significant relationship was found for those receiving money only upon request. Last, adolescents having less than 10€ on request were less likely to engage in at least four at-risk behaviors (OR=0.49; 95% CI: 0.28-0.83).

DISCUSSION

In light of the controversial evidence concerning the effect of pocket money on unsound activities, we examined the impact of different allowance schemes over five types of risky behaviors. Based on multiple logistic regression models on a sample of Italian adolescents, the analyses suggest that higher financial means are more likely to induce adolescents to adopt a risky lifestyle, characterized by excessive behaviors such as alcohol consumption and smoking as well as gambling. Such results echo and complement previous studies in the health sector, confirming the active role of pocket money and economic availability in cigarette smoking [17, 18], alcohol consumption [26] and gambling [25]. The novelty of the present study is to reveal the differential effect of diverse allowance payment schemes in inducing risky behaviors.

Indeed, the present research showed that differential money schemes have divergent results on the adoption of risky behaviors. On the one side, pocket money (i.e., providing a fixed amount of money recurrently) negatively impacted adolescents' lifestyles, above all those

Table 2
Distribution of 962 adolescents in their third year of high school who provided information on all the five selected at-risk behaviors, by at-risk behaviors according to money availability. Corresponding odds ratios (OR) and 95% confidence intervals (CI). HBSC Lombardy 2017-2018

	At least four at-risk behaviors		
Determinants	**N^**	**%**	**OR* (95% CI)**
Total	962	17.0	
Weekly expenses§			
<10€	275	12.4	1.00°
10-20€	283	19.8	**1.76 (1.10-2.82)**
≥20€	369	19.5	**1.69 (1.08-2.64)**
P for trend			**0.035**
Pocket money§			
No	613	14.8	1.00°
Yes	342	21.1	**1.48 (1.05-2.10)**
Pocket money§			
No	613	14.8	1.00°
Yes			
<10€	80	13.8	0.90 (0.46-1.79)
10-20€	99	23.2	1.69 (1.00-2.85)
≥20€	163	23.3	**1.68 (1.09-2.58)**
Money if needed§			
No	316	19.9	1.00°
Yes	642	15.7	0.74 (0.52-1.06)
Money if needed§			
No	316	19.9	1.00°
Yes			
<10€	190	11.1	**0.49 (0.28-0.83)**
10-20€	191	16.2	0.80 (0.49-1.29)
≥20€	261	18.8	0.91 (0.60-1.38)

^For each determinant the sum does not add to the total because of few missing values.
*ORs were estimated by unconditional multiple logistic regression models, after adjustment for sex, parental highest level of education and age of the pupil. Estimates in bold are those statistically significant at 0.05 level.
°Reference category.
§Whether adolescents did not indicate having money, the money availability's variables (i.e., weekly expenses, pocket money and money if needed) were categorized as "no" or as "less than 10 euros".
HBSC: Health Behaviour in School-aged Children.

related to smoking habits. Specifically, 10€ represents the weekly threshold above which the negative effect of allowance is registered. On the other side, granting money on request results as a protective factor over risky behaviors, in particular when limited to a restricted amount of money (i.e., less than 10€ per week). In fact, giving children limited amount of money on demand decreased the likelihood to smoke, incur in binge drinking and gambling patterns.

Different hypotheses might be formulated to interpret such result. First, requesting money from parents often implies to clarifying the reasons behind the request; this in turn could result in a sort of pre-commitment undertaken by children who might feel more likely to respect the commitment with parents. Second, we can speculate that the absence of regular money provision might induce adolescents to feel higher responsibility for money requested, felt not as a right whereas as a way of being part of family household management and balance. Indeed, as Lee and Mortimer [12] suggested, regular allowance payments might induce children to excessively rely upon parents promoting financial dependence rather than capability. Other strategies to provide money to children might obtain better effects, such as contingent upon chores [11, 33] and money on request. Such strategies allow to engage in discussions and negotiations about money which are key in the financial socialization process, while pocket money does not require regular conversations [34]. Further research is needed to assess such hypotheses and to shed light on the reasons why irregular money provision rather than recurrent pocket money might function as a protective factor towards risky behaviors.

In terms of implications for policies and public health, the findings suggest the importance to inform parents and caregivers of the potential negative impact of money availability (in particular when fixed) over risk behaviors. Additionally, they might benefit from education programs discussing the use of different money schemes and the impact of diverse allowance amounts. While pocket money and high amounts might represent a risk factor in inducing unhealthy activities, providing restricted amount of money on request might be a useful strategy to limit such behaviors during adolescence.

Our study has some limitations. First, the study was based on cross-sectional data, so causality between variables cannot be established. Second, data were collected on a relatively limited sample size. Therefore, data should be generalized with caution. Furthermore, future research should take into consideration additional variables which were not included in the present dataset, and which might provide further insight on the relationship between money allowance schemes and risky behaviors (e.g., parental monitoring and parental risky habits). Last, since data were self-reported by respondents, misreporting of risky behaviors might occur.

CONCLUSIONS

To conclude, the present study reports novel insights on the effect of different money allowance schemes and economic availability over a broad range of risky behaviors, being the first to consider both the impact of the amount of weekly expenses and the frequency of pocket money. Results show that pocket money alone and high economic availability might have a counteractive impact on adolescents and children, being related to risky behaviors, while money on request might function as a protective factor especially with restricted amount of money.

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Authors' contributions

EL: conceptualization, writing (original draft, writing) review & editing, project administration, supervision, data interpretation. CMJ: data curation, formal analysis, writing (original draft, writing) review & editing, data interpretation. GS: writing (original draft, writing) review & editing, data interpretation. EM: writing (review & editing, data interpretation). AL: writing (review & editing, supervision, data interpretation). ES: writing (review & editing, data interpretation). SG: conceptualization, data curation, formal analysis, writing (original draft, writing) review & editing, project administration, supervision, data interpretation. HBSC: Lombardy Committee data curation.

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Data sharing

Materials and analysis code for this study are available by emailing the corresponding Author.

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.

The members of the HBSC Lombardy Committee 2018

Regional coordination of HBSC Lombardy Study (DGWelfare of Lombardy Region): Corrado Celata, Lucia Crottogini, Claudia Lobascio, Liliana Coppola; Regional research group: Giusi Gelmi, Chiara Scuffi, Veronica Velasco; MIUR-Regional school office for Lombardy Region: Mariacira Veneruso; Representatives of health protection agencies: Giuliana Rocca, Paola Ghidini, Ornella Perego, Raffaele Pacchetti, Corrado Celata, Maria Stefania Bellesi, Silvia Maggi, Elena Nichetti; Referents of territorial school offices: Antonella Giannellini, Federica Di Cosimo, Mariacira Veneruso, Davide Montani, Marina Ghislanzoni, Carla Torri, Elena Scarpanti, Laura Stampini, Cosimo Scaglione, Angela Sacchi, Marcella Linda Casalini; HBSC Lombardy scientific supporting group: Franco Cavallo (University of Turin); Antonella Delle Fave (University of Milan); Elisabetta Nigris, Luca Vecchio, Marco Terraneo, Mara Tognetti (University of Milan-Bicocca); Lavinia Barone (University of Pavia); Silvia Salvatore (University of Insubria); Stefano Capolongo (Polytechnic University of Milan); Elena Marta, Edoardo Lozza (Catholic University of Milan); Aleksandra Torbica (Bocconi University); Vincenzo Russo (IULM University); Silvano Gallus, Eugenio Santoro (Mario Negri Institute).

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Compassionate drug uses in Italy. Analysis of regional and local diffusion

Daniela Pilunni[1] and Pierluigi Navarra[2]

[1]Farmacia Prodotti Sperimentali, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[2]Dipartimento di Sicurezza e Bioetica, Cattedra di Farmacologia, Facoltà di Medicina e Chirurgia, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

Abstract

Aims. Compassionate drugs are provided to patients with a specific disease and no further treatment option, most frequently via Early (or Expanded) Access Programs. In Italy, it often occurs that compassionate uses concern medicines whose price has not been negotiated yet (and therefore unavailable on the market), although their use has been approved in Europe. Thus, compassionate drug uses turn out to be a way to expedite the access to new innovative drugs with demonstrated efficacy. This study aims to investigate how widespread is the use of compassionate drugs throughout the Country.

Methods. We analyzed data from 20 early access programs implemented by 2 pharmaceutical companies in the last few years. Data were analyzed by the number of patients and centers in each Region and province, and a correlation was established between patients and centers in each Region and the resident population. A further analysis was carried out with the same criteria on the subpopulation of oncology patients, including more than 80% of total study population.

Results. In our sample, 7529 patients received compassionate drug treatments in 348 centers throughout Italy. A significant correlation exists between the resident population in each Region and the number of requesting centers (r^2=0.877) and patients treated (r^2=0.844) in the Region. Taking the value of the linear regression slope as the expected one, certain Regions show a better "performance", in terms of more patients treated than expected, namely Umbria, Emilia-Romagna, Lazio, Lombardy, Tuscany, Liguria and Friuli Venezia-Giulia.

Conclusions. In this study we showed that the use of compassionate drugs in Italy is diffused in a manner closely related to the population of each Region. A number of Regions – mostly but not exclusively from the South and Island areas – show a performance below the expectations, in terms of patients treated.

Key words

- compassionate drug use
- compassionate drug program
- Italy

INTRODUCTION

Compassionate Drug Use (CDU) is one of the ways through which patients with a specific disease and no further treatment option can access unauthorized treatments. Compassionate drugs can be given for individual use; more often, the patients are included into specific Compassionate Use Programs (CUPs) [1]. In either case, the Company producing the drug approves the request and covers the cost of treatment [1]. Moreover, in Italy local Ethics Committees are in charge to evaluate and approve CDU requests, provided that the Company has declared the availability to supply the treatment free of charge [2, 3].

Although Italy adhered to the European Community (EC) Regulation n. 726/2004 by issuing a specific act in 2017 [2, 3], the term "unauthorized treatment" is currently interpreted in Italy in a peculiar way, i.e., as a treatment whose price has not been negotiated yet by the Italian Drug Agency (Agenzia Italiana del Farmaco, AIFA). Thus, a medicine can be considered as "unauthorized" even though it was approved by the European Medicine Agency (EMA). Proof of this is the fact that many drugs classified as Cnn (C *non negoziato*: hence, virtually available on the Italian market at a free price, waiting for price negotiation) are accessed by patients through CUPs.

We have recently published a study describing CDUs at the Fondazione Policlinico Gemelli in Rome, the largest academic hospital in Italy, in the period 2018-2021 [4]. We found that only 20 out of 463 requests of CDU received in the period under scrutiny were concerning drugs that had no indication approved by EMA/EC at the cutoff date of June 30, 2021 [4]. This finding suggests that the vast majority of CDU requests deals with

Address for correspondence: Pierluigi Navarra, Dipartimento di Sicurezza e Bioetica, Cattedra di Farmacologia, Facoltà di Medicina e Chirurgia, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy. E-mail: pierluigi.navarra@unicatt.it.

medicines with at least one indication already approved in Europe; thus, CDUs and CUPs can be envisioned as a powerful tool to expedite the access to innovative treatments with demonstrated efficacy, while the complex (and often time-consuming) marketing procedures are still under way.

In such a scenario, it was interesting to investigate how diffuse is the CDU practice in Italy and verify whether the access to innovative treatments granted by CUPs is widespread throughout Italy or it is limited to specific areas. To this end, we have analyzed the databases provided by two pharmaceutical companies which implemented a large number of CUPs in Italy in the last few years.

MATERIALS AND METHODS

The databases were provided by Bristol Myers Squibb (BMS) Italia and Roche Italia S.p.A. Data from BMS included two products, nivolumab and luspatercept, which accounted for five and two CUPs respectively. Data from Roche included ten products, which were provided as compassionate drugs through three (atezolizumab), or two (pralsertinib, risdiplam) or a single CUP (alectinib, emicizumab, entrectinib, glofitamab, ocrelizumab, polatuzumab vedotin and trastuzumab emtansine) respectively, to a total of 14 CUPs. Emicizumab was not included in our analysis since only one patient was treated within the CUP. All the CUPs were closed at the time the Companies provided the data, except for praseltinib CUPs, which are planned to close on October 22, 2022, and glofitamab CUP, which was activated on March 3, 2022, and is currently ongoing. Overall, twenty CUPs involving twelve products were included in our analysis.

The two databases had similar structure; BMS data were based on the physician requesting the drug, and each string of information included: 1) the name of the physician, 2) the clinical Center and 3) the number of patients treated in that Center. Roche data were based on the Center requesting the drug, and included: 1) the clinical Center, 2) the Region where the Center is located and 3) the number of patients treated in that Center. Our analysis did not include the physicians.

For each CUP, data were analyzed per number of patients and number of Centers in each Region. Twenty-one Regions were considered, according to the approach used by AIFA, which takes separate the "autonomous provinces" of Bolzano and Trento (actually belonging to the same Region, Trentino-Alto Adige) (https://www.istat.it). Since the same Center could be involved in more than one CUP, data were re-analyzed considering any requesting Center regardless of the number of CUPs involving that Center. This second wave of analysis also included the provinces (districts) to which Centers belong. Moreover, data were also analyzed per therapeutic areas, with a special focus on oncology, which accounted for more than 80% of treated patients.

Having defined the number of patients and Centers from each Region, we calculated the correlation between the percent of Italian population resident in each Region, as estimated by the Italian Institute of Statistics (Istituto Nazionale di Statistica, ISTAT) on January 1, 2022 [5], and the regional percentage of patients and Centers over the total in Italy. A sort of "efficiency index" was also obtained for each Region by calculating the ratio between the number of patients and the number of Centers. All statistics used in this study are descriptive, except the correlations between regional populations and the number of Centers and patients in each Region, which required a linear regression analysis carried out with a PrismTM v.6 computer program (GraphPad, San Diego CA, USA).

RESULTS

The drugs and indications included in this analysis are reported in *Table 1*. The products are listed per number of patients enrolled. Out of twenty programs, sixteen involved indications in oncology/onco-hematology (6080 patients, 80.75% of total), three involved neurological/neuromuscular disorders (1246 patients, 16.55% of total) and one involved an indication in non-malignant hematology (203 patients, 2.7% of total). Half of the CUPs had a relatively low number of patients, i.e. less than 200, either because they were concerning rare diseases (e.g., spinal muscular atrophy) or oncology patients with low-incidence diseases or rare mutations, or else because of a recent start of the program.

For each CUP, we analyzed the number of patients (*Table 2)* and Centers (*Table 2S available online as Supplementary Material*) involved in each of the twenty-one Regions. The CUPs are listed in order of magnitude, from the highest number of patients or Centers onward. The tables also report the absolute numbers and the percentages of patients and Centers in the four Italian macro-areas, as defined by AIFA, i.e., North, Center, South and Islands [5].

As explained in Materials and Methods, the above analysis overestimates the number of Centers, since any single Center may be involved in more than one CUPs. At variance, *Table 3* shows the analysis whereby each requesting Center is considered once, regardless of the number of requests coming from that Center. In this analysis, the Centers are reported per Region and per province (district), in order to gain more insight into the diffusion of CDUs throughout the Country.

Table 4 reports the values and their relative percent over the total of the patients and the Centers, as resulting from the analysis shown in *Table 2* and *3*, respectively. Per each Region these data are reported along with the regional population [5] and the relative percent over the total of the country. The ratio between the patients and the Centers per each Region is also reported. These data served to estimate the correlation between the regional population and patients receiving compassionate drugs in that Region (*Figure 1A*) or between the regional population and the requesting Centers in the Region (*Figure 1B*).

There is a good correlation between regional populations and the number of patients treated in each Region (r^2=0.844; Y=1.031X – 0.145); even closer correlation exists between regional populations and the number of requesting Centers (r^2=0.877; Y=0.875X + 0.593). *Figure 1* also shows that some dispersion exists around the

Table 1
Drugs included in the analysis and indications for which a Compassionate Use Program (CUP) was activated

Company	Medicine	Indication	Centers	Patients
BMS	Nivolumab 1	Non-squamous Non-Small Cell Lung Cancer (NSCLC)	187	2186
Roche	Trastuzumab emtansine	HER 2 positive early-stage Breast Cancer (eBC)	206	1248
Roche	Ocrelizumab	Primary Progressive Multiple Sclerosis (PPMS)	85	1045
BMS	Nivolumab 2	Squamous Non-Small Cell Lung Cancer (NSCLC)	113	530
BMS	Nivolumab 3	Renal cell carcinoma (RCC)	108	519
BMS	Nivolumab 4	Unresectable malignant pleural mesothelioma	64	246
Roche	Alectinib	Advanced ALK positive Non-Small Cell Lung Cancer (NSCLC) pretreated with crizotinib	67	226
Roche	Atezolizumab 1	Bladder cancer	68	222
BMS	Luspatercept 1	Transfusion-dependent anaemia due to very low, low and intermediate-risk myelodysplastic syndromes (MDS)	64	215
BMS	Luspatercept 2	Transfusion-dependent anaemia associated with beta-thalassaemia	36	203
BMS	Nivolumab 5	Classical Hodgkin lymphoma (cHL)	68	195
Roche	Polatuzumab vedotin	Patients with relapsed/ refractory diffuse large B cell Lymphoma (r/r DLBCL) ineligible for haematopoietic stem cell transplantation and who have received prior therapy	70	167
Roche	Risdiplam 1	Spinal Muscolar Atrophy 2 (SMA2)	32	152
Roche	Atezolizumab 2	Metastatic non squamous Non-Small Cell Lung Cancer (NSCLC)	47	127
Roche	Atezolizumab 3	Locally advanced or metastatic Triple Negative Breast Cancer (TNBC)	50	94
Roche	Pralsetinib1	Adult patients with unresectable or metastatic RET gene fusion positive Non-Small Cell Lung Cancer (NSCLC) who are not candidates for treatment with approved therapeutic alternatives	30	65
Roche	Risdiplam 2	Spinal Muscolar Atrophy 1 (SMA1)	18	49
Roche	Pralsetinib 2	Adult patients with advanced or metastatic RET mutant medullary thyroid carcinoma (MTC) or RET gene fusion positive thyroid carcinoma requiring systemic therapy who are not candidates for treatment with approved therapeutic alternatives	8	15
Roche	Entrectinib	Metastatic or locally advanced solid tumor with NTRK translocation or Non-Small Cell Lung Cancer with ROS-1 rearrangement	10	14
Roche	Glofitamab	Patients with relapsed/ refractory diffuse large B-cell lymphoma (r/r DLBCL) or relapsed/ refractory high-grade lymphoma (r/r HGL) or relapsed/ refractory transformed follicular lymphoma (r/r trFL) or relapsed/ refractory primary mediastinal lymphoma (r/r PMBCL)	9	11
			1340	7529

theoretical slope, with certain Regions performing "better that expected" (i.e., the observed value is above the theoretical slope) concerning the number of patients treated or Centers or both, and other Regions vice versa (i.e., with the observed value being below the theoretical slope). *Figure 2A* and *B* show the variability over the percent population in each Region.

Figure 3 shows the patients/Centers ratio in each Region. The average ratio in the Country is 21.64 patients per Center. Nine Regions out of twenty-one present ratios higher than the mean for Italy, with Umbria, Emilia-Romagna, Lazio and Campania having more than 30 patients per Center on average.

The analysis on the subpopulation of oncology patients shows a similar trend compared to the general population, with an important difference: the gap between better- and worst-performing Regions is increased, with some Regions performing even better, namely Emilia-Romagna, Tuscany, Umbria and Friuli-Venezia Giulia, and the other way round for Piedmont, Campania, Puglia and Sicily (*Table 5*). Such increased dispersion around the theoretical slope translates into a weaker correlation between the regional populations and the percent of patients treated (r^2=0.783), although the goodness-of-fit remains statistically significant.

DISCUSSION

This investigation included twenty CUPs from two major pharmaceutical companies, and involved twelve new drugs, 348 prescribing Centers and 7529 patients throughout Italy. In its repository on CUPs, AIFA has registered 69 CUPs (including 36 closed programs, 2 temporarily closed programs and 31 ongoing programs) at the cutoff date of July 21, 2022 [6]. In AIFA repository, the number of patients enrolled in the CUPs is not

Table 2
Patients enrolled in Compassionate Use Programs (CUPs) in each Italian Region

DRUGS	North									Center					South					Islands		Total
	PI	VdA	LO	BZ	TN	VE	FVG	LI	ER	TU	UM	MA	LA	AB	MO	CAM	PU	BA	CAL	SI	SA	
Nivolumab 1	60	3	381	11	-	76	56	86	305	265	106	31	359	33	7	90	93	4	22	149	49	2186
Trastuzumab emtansine	95	1	244	19	22	86	17	57	121	92	15	26	148	19	-	93	59	13	18	88	15	1248
Ocrelizumab	72	-	137	6	2	62	10	42	54	19	10	19	183	43	63	120	75	9	11	108	-	1045
Nivolumab 2	12	1	106	-	-	12	14	14	79	62	27	6	56	6	2	52	33	1	3	31	13	530
Nivolumab 3	13	-	83	5	1	31	26	14	83	68	14	15	58	5	-	54	11	-	8	20	10	519
Nivolumab 4	35	-	55	3	-	20	5	17	27	39	5	1	9	-	-	13	7	2	1	2	8	246
Alectinib	13	1	28	-	-	18	12	2	18	28	10	5	54	3	-	14	12	1	-	2	5	226
Atezolizumab 1	15	-	32	3	-	13	11	3	29	55	-	4	18	11	-	11	6	-	2	4	5	222
Luspatercept 1	31	-	64	-	-	4	3	5	15	11	2	9	10	12	-	14	12	-	2	9	9	215
Luspatercept 2	27	-	34	-	-	9	-	1	9	10	1	-	4	-	-	-	28	-	6	29	45	203
Nivolumab 5	9	-	46	-	-	9	1	9	28	10	3	2	16	6	-	23	14	1	5	10	3	195
Polatuzumab vedotin	12	-	19	1	-	11	10	13	19	17	4	2	17	6	-	6	14	1	3	8	4	167
Risdiplam 1	13	-	38	2	4	6	10	9	10	10	1	4	22	-	-	5	11	-	1	4	2	152
Atezolizumab 2	3	-	21	3	-	20	8	1	14	19	1	5	20	3	-	5	1	-	2	1	-	127
Atezolizumab 3	6	-	15	2	3	12	1	6	4	3	-	2	15	-	-	10	4	-	-	11	-	94
Pralsetinib 1	6	-	13	-	-	3	-	1	13	4	5	-	16	-	-	-	1	-	-	-	3	65
Risdiplam 2	5	-	12	-	-	2	2	1	8	-	-	2	8	-	-	7	1	-	-	1	-	49
Pralsetinib 2	-	-	5	-	-	2	-	-	4	-	1	-	3	-	-	-	-	-	-	-	-	15
Entrectinib	4	-	5	-	-	-	-	-	3	1	-	-	-	-	-	-	-	-	1	-	-	14
Glofitamab	1	-	2	-	-	2	1	1	1	-	-	-	-	-	-	-	2	-	-	1	-	11
TOTAL	432	6	1340	55	32	398	187	282	844	713	205	133	1016	147	72	517	384	32	85	478	171	7529
	3576 (47.5%)									2214 (29.4%)					1090 (14.5%)					649 (8.6%)		

PI = Piedmont, VdA = Val d'Aosta, LO = Lombardy, BZ = Autonomous Province of Bolzano, TN = Autonomous Province of Trento, VE = Veneto, FVG = Friuli-Venezia Giulia, LI = Liguria, ER = Emilia-Romagna, TU = Tuscany, UM = Umbria, MA = Marche, LA = Lazio, AB = Abruzzo, MO = Molise, CAM = Campania, PU = Puglia, BA = Basilicata, CAL = Calabria, SI = Sicily, SA = Sardinia.

recorded [6]. Since we analyzed 17 closed programs, our sample represents about 50% of all closed CUPs, which is highly representative of the overall scenario.

After the analysis per Region of the patients and Centers involved in CUPs, we verified the hypothesis that CDUs are evenly diffused in the Country, by correlating the regional data of patients and Centers with the population in each Region. There is indeed a significant relationship between inhabitants on the one hand, and patients and Centers on the other hand (*Figure 1A* and *B*). Assuming the value corresponding to the theoretical slope as the expected value in each Region, we observed some variability, with: 1) Tuscany, Liguria, Friuli Venezia-Giulia, Umbria and Molise showing more patients and more centers than expected, 2) Lombardy, Lazio and Emilia-Romagna showing more patients and less Centers than expected, 3) Veneto, Piedmont, Marche, Abruzzo, province of Bolzano and Val d'Aosta showing less patients and more Centers than expected, and 4) Campania, Puglia, Sardinia, Sicily, Calabria, Basilicata and province of Trento showing less patients and less Centers than expected (*Figure 2*). In such ranking, those Regions having more patients than expected should be considered as having a good "performance" regardless of the lower number of Centers involved. In fact, enrolling patients within a low number of Centers not necessarily is to be envisioned as a negative paradigm.

Another analysis was carried out looking at the ratio between patients and Centers in each Region (*Figure 3*). Concerning the gap between Regions with better and worst performance, this analysis provided almost overlapping results with the previous analysis, with the only exception of Campania, which presented a high patient/Center ratio in front of a relatively low number of patients enrolled. Taken collectively, the results of regression analysis and the patients/Centers ratios show a trend toward a worst performance in the areas of South and Islands. However, such trend is counterbalanced by negative parameters in such northern Regions as Piemonte, Val d'Aosta, Veneto, and Trentino-Alto Adige. Thus, a clearcut conclusion that Regions of the Center/North Italy are better served than Regions of the South/Island cannot be drawn at this time.

A highly important factor for the diffusion of CDUs in the Country is the presence of Centers of clinical excellence at local level. We haven't carried out a quantitative analysis of this parameter. Here we provide only a paradigmatic example of the relevance of excellence Centers for CDUs: a single Center in Molise (i.e., "Neuromed" based in Pozzilli, district of Isernia) recruited

Table 3
Centers involved in Compassionate Use Programs (CUPs) in each Italian Region and district

Region	Districts and number of requesting Centers												Total
Lombardy	Milan 18	Brescia 7	Bergamo 6	Varese 6	Como 4	Monza 3	Pavia 3	Cremona 2	Sondrio 2	Lecco 1	Lodi 1	Mantova 1	54
Lazio	Rome 27	Latina 3	Frosinone 2	Viterbo 1									33
Piedmont	Turin 15	Cuneo 6	Alessandria 3	Verbania 3	Biella 2	Novara 2	Asti 1	Vercelli 1					33
Tuscany	Florence 9	Siena 7	Pisa 4	Livorno 2	Lucca 2	Prato 2	Arezzo 2	Pistoia 1	Grosseto 1	Massa Carrara 1			31
Veneto	Verona 7	Padova 6	Vicenza 5	Treviso 4	Venice 4	Belluno 2	Rovigo 1						29
Sicily	Catania 8	Messina 5	Palermo 5	Caltanissetta 3	Ragusa 2	Agrigento 1	Siracusa 1	Trapani 1					26
Emilia-Romagna	Modena 5	Bologna 4	Reggio Emilia 3	Rimini 3	Ferrara 2	Parma 2	Ravenna 2	Forlì – Cesena 1	Piacenza 1				23
Puglia	Bari 6	Lecce 5	Taranto 4	Brindisi 3	Foggia 2	BAT 1							21
Campania	Naples 11	Salerno 3	Avellino 1	Benevento 1	Caserta 1								17
Marche	Ancona 4	Pesaro Urbino 3	Ascoli Piceno 2	Fermo 2	Macerata 2								13
Abruzzo	Chieti 5	L'aquila 3	Teramo 2	Pescara 1									11
Liguria	Genova 4	Imperia 3	Savona 3	La Spezia 1									11
Calabria	Catanzaro 3	Cosenza 3	Reggio Calabria 2	Crotone 1									9
Sardinia	Cagliari 5	Sassari 3	Nuoro 1										9
Friuli VG	Udine 3	Pordenone 2	Trieste 2	Gorizia 1									8
Umbria	Perugia 5	Terni 1											6
Trentino (Bolzano)	Bolzano 5												5
Basilicata	Potenza 2	Matera 1											3
Molise	Isernia 2	Campobasso 1											3
Trentino (Trento)	Trento 2												2
Val d'Aosta	Aosta 1												1
													348

Table 4
Absolute values and relative percentages of regional populations, Centers and patients included in Compassionate Use Programs (CUPs) in each Region, with the relevant Patient/Center ratio, in Italy

Region	Population (January 1st, 2022)	% over Italian population	Centers	Centers % of the total in Italy	Patients	Patients % of the total in Italy	Patients/ Centers ratio
Lombardy	9,965,046	16.89	54	15.5	1337	17.8	24.76
Lazio	5,715,190	9.69	33	9.48	1018	13.5	30.85
Campania	5,590,681	9.48	17	4.89	517	6.87	30.41
Veneto	4,854,633	8.23	29	8.33	401	5.29	13.83
Sicily	4,801,468	8.14	26	7.47	478	6.35	18.38
Emilia-Romagna	4,431,816	7.51	23	6.61	844	11.2	32.46
Piedmont	4,252,279	7.21	33	9.48	432	5.74	13.09
Puglia	3,912,166	6.63	21	6.03	384	5.10	18.29

Continues

Table 4
Continued

Region	Population (January 1st, 2022)	% over Italian population	Centers	Centers % of the total in Italy	Patients	Patients % of the total in Italy	Patients/ Centers ratio
Tuscany	3,676,285	6.23	31	8.91	713	9.47	23.00
Calabria	1,844,586	3.13	9	2.59	85	1.13	9.44
Sardinia	1,579,181	2.68	9	2.59	171	2.27	19.00
Liguria	1,507,438	2.56	11	3.16	282	3.75	25.63
Marche	1,489,789	2.53	13	3.74	133	1.77	10.23
Abruzzo	1,273,660	2.16	11	3.16	145	1.95	13.18
Friuli Venezia Giulia	1,197,295	2.03	8	2.30	187	2.48	23.37
Umbria	859,572	1.46	6	1.72	205	2.72	34.16
Trentino (Trento)	542,158	0.92	2	0.57	32	0.43	16.00
Basilicata	539,999	0.92	3	0.86	32	0.43	10.66
Trentino (Bolzano)	535,774	0.91	5	1.44	55	0.73	11.00
Molise	290,769	0.49	3	0.86	72	0.96	24
Valle d'Aosta	123,337	0.21	1	0.29	6	0.08	6
TOTAL	58,983,122		348		7529		

63 patients within the CUP for Ocrelizumab, out of 72 patients in total from Molise, in three CUPs. Thus, the activity of a single center in a single CUP placed Molise among the Regions with better performance in Italy. Another aspect somewhat related to the presence and activity of Centers of excellence is the phenomenon of "health tourism", a condition which often sees the involvement of patients needing CDUs because of the lack of therapeutic options. Unfortunately, the available data do not allow to analyze this phenomenon, and its impact on the diffusion of CDUs in the Country.

The analysis per therapeutic areas focused on oncology, which accounted for more than 80% of the whole sample of population. Two important differences emerged compared to the overall sample: 1) there is one less "good performing" Region, i.e., Molise, which recruited 87.5% of the patients in a non-oncologic area; 2) the gap between "good performing" and "bad-performing" Region is increased, as shown in *Table 5*.

In conclusion, this study shows that thousands of patients with limited therapeutic options could take advantage from CDUs in the last few years in Italy. Diffusion of CDUs throughout the Country is widespread, in close correlation to the density of population in each Region. Regions with a larger number of patients treated also show a higher number of requesting Centers, and/or a higher patients/Centers ratio. Based on these parameters, certain Regions can be envisioned as "better performing", namely Umbria, Emilia-Romagna, Lazio, Lombardy, Tuscany, Liguria and Friuli Venezia-Giulia. The role of the Centers of clinical excellence in such performances has been briefly discussed.

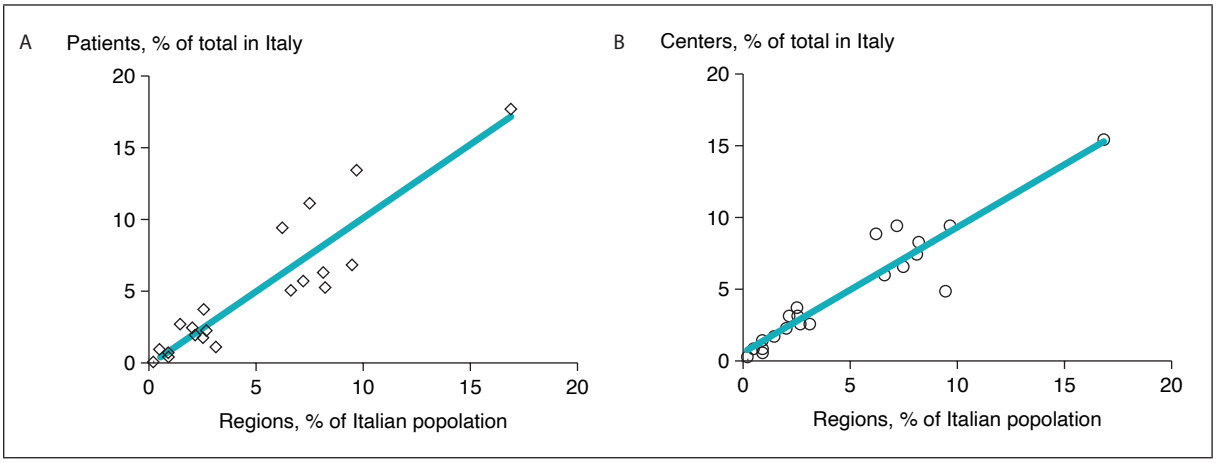

Figure 1
Panel A: The number of patients treated with compassionate drugs in each Region (on the Y axis) is directly related to the number of inhabitants in the Region (on the X axis). Panel B: The number of Centers recruiting each Region (on the Y axis) is directly related to the number of inhabitants in that region (on the X axis). Data are expressed as the percent of total population in Italy.

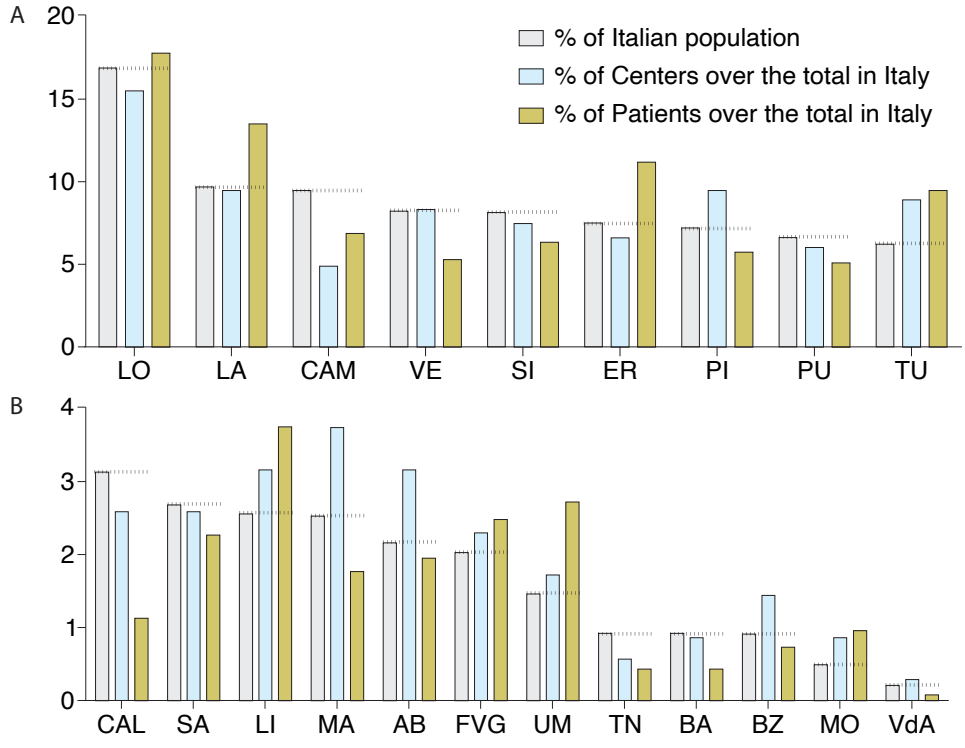

Figure 2
The figure shows the number of inhabitants, of recruiting Centers, and of patients treated with compassionate drugs in each Region. All data are expressed as percentages over the total in Italy. Panel A: Regions with more than 3.5-million inhabitants. Panel B: Regions with less than 2-million inhabitants.

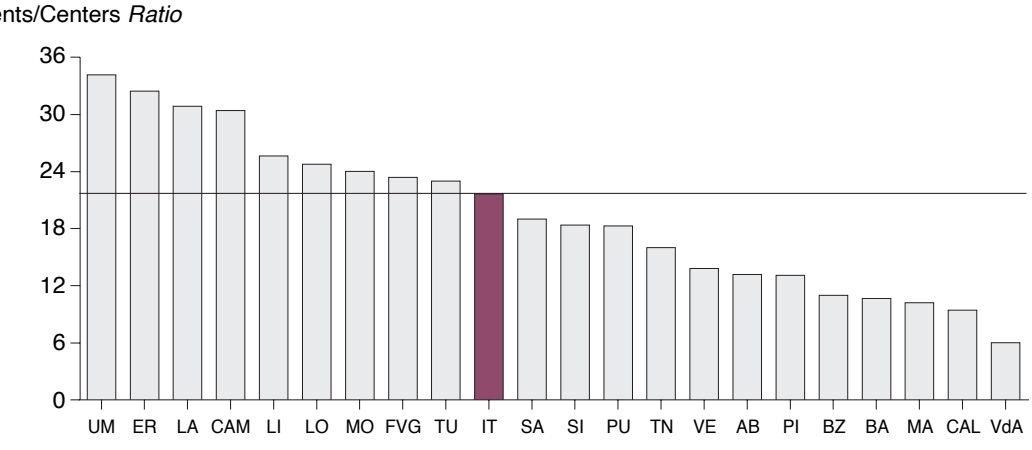

Figure 3
The figure shows the ratio between the number of patients treated and the number of centers in each Region. The value for Italy is reported in red.

Table 5
Analysis of the subpopulation of oncology patients in Italy

Region	Population (January 1st, 2022)	% over Italian population	Patients	Patients % of the total in Italy		Oncology patients	Oncology patients % of the total in Italy	
Lombardy	9,965,046	16.89	1337	17.8	+ 0.91%	1119	18.04	+ 1.15%
Lazio	5,715,190	9.69	1018	13.5	+ 3.81%	799	13.14	+ 3.45%
Campania	5,590,681	9.48	517	6.87	- 2.61%	385	6.33	- 3.15%
Veneto	4,854,633	8.23	401	5.29	- 2.94%	319	5.24	- 2.99%
Sicily	4,801,468	8.14	478	6.35	- 1.79%	336	5.52	- 2.62%
Emilia-Romagna	4,431,816	7.51	844	11.2	+ 3.99%	763	12.5	+ 4.99%
Piedmont	4,252,279	7.21	432	5.74	- 1.47%	315	5.18	-2.03%
Puglia	3,912,166	6.63	384	5.10	- 1.53%	269	4.42	- 2.21%
Tuscany	3,676,285	6.23	713	9.47	+3.24%	674	11.1	+ 4.80%
Calabria	1,844,586	3.13	85	1.13	- 2.00%	67	1.1	- 2.03%
Sardinia	1,579,181	2.68	171	2.27	- 0.41%	124	2.04	- 0.64%
Liguria	1,507,438	2.56	282	3.75	+ 1.19%	229	3.76	+ 1.20%
Marche	1,489,789	2.53	133	1.77	- 0.76%	108	1.77	- 0.76%
Abruzzo	1,273,660	2.16	145	1.95	- 0.21%	104	1.71	- 0.45%
Friuli Venezia Giulia	1,197,295	2.03	187	2.48	+ 0.45%	165	2.71	+ 0.68%
Umbria	859,572	1.46	205	2.72	+ 1.26%	193	3.17	+ 1.71%
Trentino (Trento)	542,158	0.92	32	0.43	- 0.49%	26	0.42	- 0.50%
Basilicata	539,999	0.92	32	0.43	- 0.49%	23	0.38	- 0.54%
Trentino (Bolzano)	535,774	0.91	55	0.73	- 0.18%	47	0.77	- 0.14%
Molise	290,769	0.49	72	0.96	+ 0.47%	9	0.15	- 0.34%
Valle d'Aosta	123,337	0.21	6	0.08	- 0.13%	6	0.1	- 0.11%
TOTAL	58,983,122		7529			6080		

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Authors' contributions

DP analyzed the data and critically reviewed the manuscript. PN conceived the study, analyzed the data and drafted the manuscript.

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The role of ecotoxicology in the health impact assessment: an innovative ecosystem approach for the protection of human health in Italy

Ines Lacchetti[1], Mario Carere[1], Walter Cristiano[1] and Laura Mancini[1]

Dipartimento di Ambiente e Salute, Istituto Superiore di Sanità, Rome, Italy

Abstract

Background. The Health Impact Assessment (HIA) is a procedure with the aim to protect the populations exposed to the impacts deriving from the establishment or upgrading of large industrial enterprises, i.e. large combustion plants (>300 MWth). In Italy a guideline for the HIA procedure has been published in compliance with the 2014/52/EU Directive on the Environmental Impact Assessment (EIA) requirements.

Italian HIA procedure. An ecotoxicological approach has been included for the first time in the HIA procedure with the aim to detect toxic effects caused by unknown not-monitored contaminants or mixtures in the ecosystem components affected by the potential emissions, discharges and releases of large industrial enterprises. Ecotoxicology plays an important bridge role between environment and human health in the scoping and monitoring step of the HIA procedure with a key function of early warning system and screening. The aim of this paper is to present the Italian experience in the first three years of the application of the new approach, proposing recommendations on specific case studies.

Conclusion and future perspective. 80% of enterprises, that applied HIA, have delivered a robust, integrated and detailed documentation in relation to the ecotoxicological assessment, this positive feedback will generate environmental and human health benefits to the areas where the plants are established.

Key words

- Health Impact Assessment
- ecotoxicology
- mixtures
- gas-fired power plant
- early warning system

BACKGROUND

Over the past decade the focus on biodiversity protection, sustainable development, climate changes and ecosystems health, has become the main key driver in European and international policy making.

Human health depends ultimately upon ecosystem products and services (such as availability of fresh water, food, pharmaceuticals, pollination) and significant direct and indirect human health impacts can occur if ecosystem services are no longer adequate to meet social needs, this concept is also embedded in the new European chemical strategy for sustainability that has been launched by the European Commission in October 2020 [1].

It is now well known that multiple substances occur simultaneously in the environment: a substance-by substance risk assessment therefore can underestimate the total chemical risk, as it does not take into account the fact that several substances present at the same time may have the same effects or modes of action and act additively or synergistically. The European chemical strategy recommends that "the effect of chemical mixtures needs to be taken into account and integrated more generally into chemical risk assessments" and plans to "introduce or reinforce provisions to take account of the combination effects in other relevant legislation, such as legislation on water, air, soil". The EU Action Plan "Towards zero pollution for air, water and soil" published in 2021 [2] sets out an integrated vision for 2050 to reduce environmental pollution to levels that are no longer harmful to human health and natural ecosystems. This means reducing the risk not only from single chemicals, but also the risk arising from their combined presence in the environment.

In this context every public and private project should therefore consider and limit the impact on environment of chemical and physical stressors including erosion, compaction and sealing. Specifically, the European Directive 2014/52/EU on the environmental impact assessment [3] aims to assure a high level of protection of

Address for correspondence: Ines Lacchetti, Dipartimento di Ambiente e Salute, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: ines.lacchetti@iss.it.

the environment and it highlights the importance of the protection of human health, through the establishment of minimum requirements for the assessment of the environmental impact of public and private projects. The Italian Legislative Decree 104/2017 has implemented the European Directive. The National Decree prescribes for new plants belonging to a specific category (e.g., large combustion plants, refineries) to carry out a Health Impact Assessment (HIA) procedure to protect the populations from the potential impacts caused by these plants, considering the economic development opportunities. In particular, it requires carrying out a HIA for new plants belonging to the categories of large combustion plants (>300 MWth), crude oil refineries, re-gasification and liquefaction plants or for plants that can have strong impacts on the land in relation to their production activity. Plants such as gas-fired power stations [4], steel industries and refineries can release in air, soil and water several chemical contaminants in low quantities, but their interaction can generate unpredictable effects for ecosystems and human health, for this reason tools and procedures are needed to detect and evaluate these effects earlier. In relation to air emissions a relevant pathway is also the atmospheric deposition on soil and surface waterbodies located around the plants. In this context, ecotoxicology [5] represents a valid and recognized instrument at European level which is fundamental for the management and understanding of the potential adverse effects resulting from multiple exposure to contaminants including those not covered by the legislation (emerging) and the mixtures. The aim of this paper is to present the Italian experience in the first three years in the application of the ecotoxicological approach in the context of HIA procedure and to propose recommendations.

THE ITALIAN HIA PROCEDURE

As reported by European Directive 2014/52/EU, in order to ensure a high level of protection of the environment and human health, screening procedures and environmental impact assessments should be considered the impact of the whole project, including, where relevant, its subsurface and underground, during the construction, transport and, where relevant, during the demolition phases. The Italian HIA guideline published in compliance with the law requirements [6] recommends that the proposers should compile a technical dossier with all information included in relation to several aspects relevant to the human health protection: description of the pressures and geographical area, exposure assessment, epidemiological and toxicological aspects. The dossier must be subsequently evaluated by the Competent Authority responsible for issuing the authorizations.

The procedure includes 5 phases among which the ecotoxicological assessment should be applied in the scoping and monitoring phase:

- *screening,* at this phase, it is assessed whether a HIA needs to be applied for the project subjected to EIA determining the potential health implication impact, through the identification of the exposed population, health profile of the exposed population, evaluation of the overlap of existing impacts with new ones determined by the work;
- *scoping,* health determinants are identified and addressed by the HIA such as the characterization of the area of interest, the exposed population, assessment of the state of health of the exposed population before the construction, definition of the socio-economic profiles of populations and communities and ecotoxicological evaluation;
- *assessment and appraisal,* the health effects determined by the realization of the project must be quantified and therefore a risk assessment (including an extensive exposure assessment) will be carried out. The conclusion of this stage determines the acceptability and feasibility of the project on the territory, the identification of the actions / technologies to be adopted to reduce the exposure of population;
- *monitoring,* definition of the health monitoring plan in relation to the environment to verify the assessments (toxicological, epidemiological, ecotoxicological and other health determinant evaluation) that have been carried out;
- *reporting,* drafting of the detailed report of the activities carried out: from bibliographic research to criteria for selecting the scientific literature consulted, models, environmental data health care utilized, the evaluation procedures adopted, the uncertainty levels of the estimates, the monitoring and control plan prepared.

THE ROLE OF ECOTOXICOLOGY IN HIA PROCEDURE

The ecotoxicological assessment has the aim to detect and assess before and after the building/upgrading of the plant effects caused by mixtures or pollutants not directly monitored. In a recent European technical document [7] drawn up in the context of the common implementation strategy of the EU Water Framework Directive 2000/60/EC [8] the use of effect-based methods (EBMs) (bioassays *in vivo* and *in vitro* and biomarkers) for ecosystems monitoring is recommended by a group of European experts in particular for their role as early warning systems and screening. Effects caused by chemical substances detected in ecosystems (e.g. genotoxic, embryotoxic, neurotoxic, endocrine disrupting effects) can indeed occur also in humans [9-11]. The HIA is an interdisciplinary procedure that is based on toxicological, epidemiological and ecotoxicological assessment. Ecotoxicology is connected to the exposure assessment of the HIA, for example the data related to the quality status of waterbodies (surface and ground) and soils are useful information to select the EBM that should be applied. If effects are detected in the ecosystems, this information will be considered also in the context of the other key evaluations (toxicological, epidemiological) foreseen by the HIA.

The importance of ecotoxicological assessment in environmental monitoring to support human health protection it is a requirement for the enterprises in two steps of the HIA procedure: scoping and monitoring *(Figure 1)*. In the scoping phase, when the pre-existing anthropogenic pressures on the territory are known the

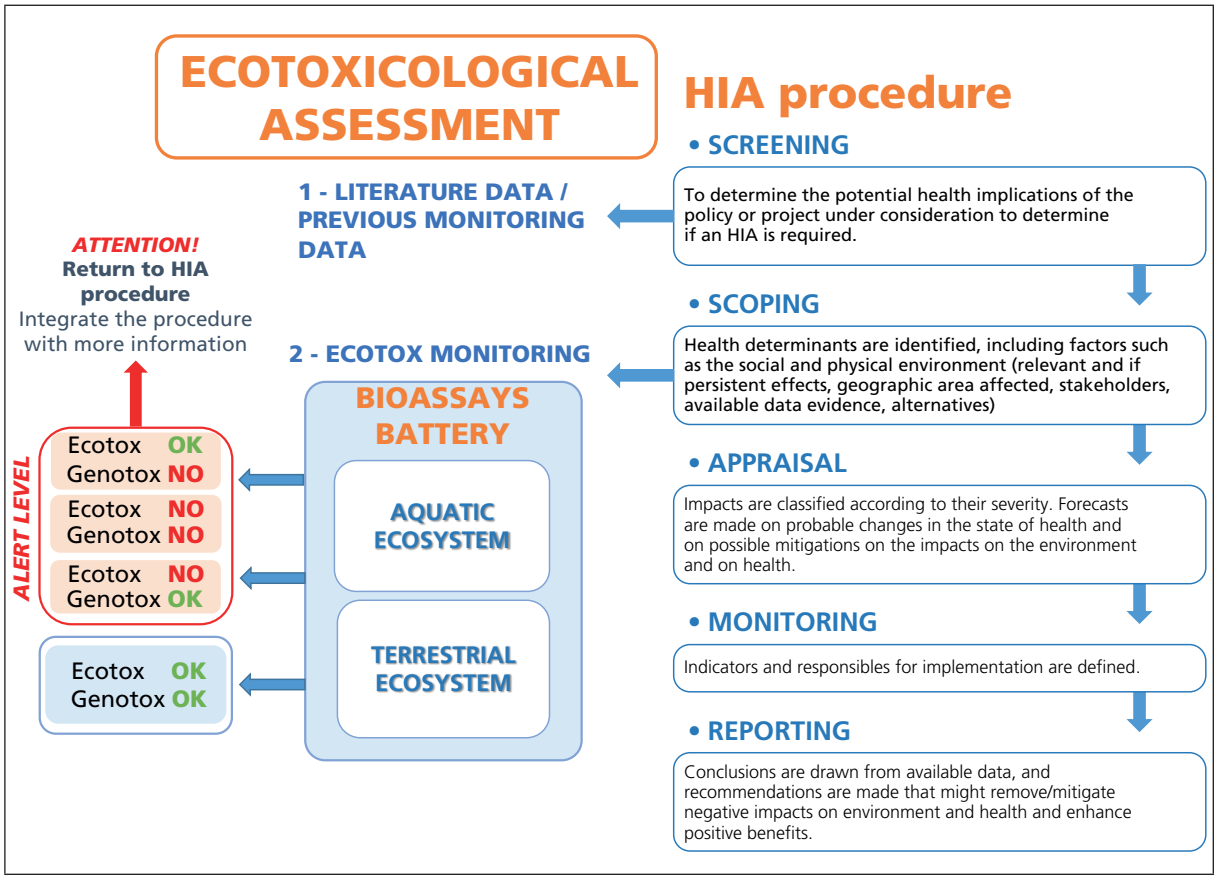

Figure 1
Ecotoxicological assessment flow scheme in the HIA procedure (modified from Dogliotti *et al.* [6]).

possibility of carrying out ecotoxicological investigations will be evaluated *ante-operam*; this is important in order to evaluate the trend of the effects after the construction/upgrading of the plant. During the monitoring phase a correct frequency and site selection strategy of application of effect-based methods should be performed.

The ecotoxicological investigation should be based on an appropriate choice of a site-specific bioassay battery (at least on three trophic levels) including acute, chronic ecotoxicity tests and eco-genotoxic tests since the mutagenic/genotoxic effects are closely related to human health [12, 13]; *in vitro* tests are recommended also because they are in line with the 3R (replacement, reduction, refinement) principle for animal welfare and also because they can detect very low levels of chemical contaminants. The bioassays should be selected following essential elements: the ecosystem in which the plant will be built (natural environment, urban environment, type of water bodies, aquatic or terrestrial ecosystems), type of industrial cycle and potential pollutants discharged and the routes of exposures for the population (drinking water, irrigation, agricultural and zootechnical activity). Testing should be conducted by the enterprises according to the main national, international guidelines (e.g. OECD) or validated protocols. On the basis of the results obtained through the ecotoxicological assessment, different scenarios may occur: 1) if no presence of ecotoxicity and eco-genotoxicity is detected, no warning for HIA should be reported; 2) if ecotoxicity or eco-genotoxicity is detected there is the need to integrate the information acquired within the HIA and investigate the pollutants released in greater detail; in case of acute ecotoxicity the alert level is higher; 3) if ecotoxicity and eco-genotoxicity are detected, that is the maximum warning, there is the need to develop and apply adequate risk reduction measures such as more advanced treatment systems.

The ecotoxicological assessment must be site specific and the choice of bioassays will be different in relation to the environmental information acquired on site, also in relation to the human epidemiological and toxicological information required by the HIA procedure.

PRELIMINARY RESULTS OF THE APPLICATION

The Italian National Institute of Health (ISS) has evaluated several projects in the first 3 years, the requests for HIA procedures belong mainly to power-fired gas plants (90%) distributed throughout the whole Italian territory: 54% the northern, 14% the southern, 14% the central part of Italy, 18% Sardinia and Sicily. The HIA projects concern the extension, renovation and partial or total replacement of the old plants. The majority of them referred to the conversion of coal-fired power plants into natural gas-fired power plants, 10% of

Table 1
List of the main bioassays applied by the enterprises

Surface inland waters	Transitional waters	Marine waters	Soil
		Ecotoxicological bioassay	
Daphnia magna - Acute immobilisation test (OECD 202)	*Artemia franciscana* - Acute test (APAT CNR IRSA 29 2003)	Marine algae Chronic assay (UNI EN ISO 1053-2016)	Earthworms - Acute test (OECD 207)
Selenastrum capricornutum - Freshwater Alga and Cyanobacteria - Growth inhibition test (OECD 201)	*Phaeodactylum tricornutum* - Marine algal Growth inhibition test (UNI EN ISO 1053:2016)	*Vibrio fischeri* - Determination of the inhibitory effect of water samples on the light emission luminescent bacteria test (ISO 11348-3)	*Lepidium sativum, Sorgum saccaratum, Sinapis alba* - Determination of the inhibition of germination and root elongation (UNICHIM N. 1651: 2003)
Zebrafish embryos test (OECD 236)	Fish embryos (i.e. *Dicentrarchus labrax)* (OECD 236)	Sea urchin - Acute embryo test with (EPA/600/R-95/136)	
		Eco-genotoxicological assay	
Comet assay (validated methods)	Comet Assay (OECD 489)	MicroNucleus Test on mytilus	Comet Assay (validated methods)
	Bacterial reverse mutation test - Ames test (OECD 471)		Bacterial reverse mutation test - Ames test (OECD 471)

the projects are related to steel industries. At the beginning of the assessment the ecotoxicological approach was never applied neither in the scoping phase nor in the monitoring phase as required by the guideline, this was expected due to the novelty of the procedure. In the second submission, 80% of enterprises have delivered a robust, integrated and detailed documentation in relation to the ecotoxicological assessment.

The typology of bioassays applied by the enterprises until now cover different trophic levels and also endpoints such as genotoxicity, embryotoxicity and neurotoxicity *(Table 1)*. The bioassays applied are mainly acute and chronic aquatic and terrestrial bioassays *in vivo* (e.g., test with earthworms, algae, crustaceans, fish embryos), eco-genotoxicological assays such as the Ames test, Comet Assay and the micronucleus tests. For the marine sites the methods applied are mainly the same that are foreseen by the Italian National Decree about dredging activities N.173/2016, integrated by an eco-genotoxicological assay.

CASE STUDY

Power plants for regasification of liquefied natural gas (LNG) are proposed now in Italy due to the urgent energy demand; in particular the government has recently approved the installation of an FSRU (Floating Storage and Regasification Unit) in the city of Ravenna on the Adriatic Sea in Central Italy. In the context of the HIA that has been carried out for this plant a complete ecotoxicological assessment has been recommended in order to prevent the possible effects for the ecosystems and indirectly for human health. In this assessment EBM will be applied in coastal marine waterbodies (included sediments) to detect the potential effects of the FSRU and in surface waters and soils that are located in proximity of the pipelines for the furniture of the gas. The EBM applied are *in vivo* and *in vitro* assays and will cover several endpoints included genotoxicity and embryotoxicity, they will be performed in the phases of scoping and monitoring of the health impact assessment.

CONCLUSIONS AND FUTURE PERSPECTIVES

The ISS has issued several opinions on HIA submitted in the last 3 years. In agreement with the Ministry of Health, the ISS has also organized training courses, events and specific meetings with stakeholders, to increase awareness and facilitate the application of guidelines. The enterprises have acquired all information and understood the importance of ecotoxicology in this context. Several enterprises have already applied the ecotoxicological assessment in the scoping phase submitting a complete monitoring plan. In the recent European Commission proposal for the protection water resources is included the need to apply EBMs for the detection of endocrine disrupting effects, this is an important step towards the inclusion of ecotoxicity for the evaluation of the status of European aquatic ecosystems [14]. In Italy there is availability of several EBMs that can cover a large range of effects. The economic effort of the EBMs can be advantageous in many cases in comparison to the chemical analysis, the capacity of Italian and European labs to use them is increasing. In future a more accurate procedure about the setting of trigger values/evaluation criteria for the interpretation of the results will be necessary, this is also an important step that will be considered at European level in the context of the EU Water Framework Directive. It is expected in future also an increasing of the use of *in vitro* methods in compliance with the ethical testing requirements. The inclusion of the ecotoxicological aspects in the HIA in Italy will have an important role as early warning and screening system and will contribute

to apply the appropriate preventive measures needed to eliminate or reduce the potential effects and impacts of the industrial chemical emissions on human health.

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Live biotherapeutic products and their regulatory framework in Italy and Europe

Giovanna Franciosa[1], Serena Guida[2], Maria Jesus Gomez Miguel[1] and Christina Von Hunolstein[1]

[1]*Centro Nazionale per il Controllo dei Farmaci, Istituto Superiore di Sanità, Rome, Italy*
[2]*Agenzia Italiana del Farmaco, Rome, Italy*

Abstract
In Italy and Europe, live microorganisms-containing products meant to be used by vulnerable or sick people for preventing or curing a disease are defined as live biotherapeutic products and are regulated as biological drugs. As such, they must undergo extensive quality, safety and efficacy testing and evaluation before receiving a marketing authorization. This review describes the regulatory framework of live biotherapeutic products with special focus on the European Pharmacopoeia monograph 3053 that set mandatory requirements for this kind of medicines, including verification of the number of live microorganisms and absence of certain contamination indicator microorganisms. The other product categories that may contain live microorganisms are also described, with brief references to the overlaps possibly occurring between the different categories.

Key words
- live biotherapeutic products
- regulatory requirements
- human microbiota
- safety

THE HUMAN MICROBIOTA AND THE POTENTIAL RELATIONSHIP WITH HEALTH STATUS

Over the past two decades, the application of omics technologies – whole genome sequencing, transcriptomics, metabolomics, and proteomics – to the study of human physiology and pathology, as well as to the understanding of microbial diversity, has gradually revealed the existence of a postnatally acquired organ within the human body, consisting of a resident complex microbial community [1, 2]. This microbial population includes eubacteria, archea, virus, fungi, yeasts and protozoa, in numbers that exceed at least 10 times those of the human body cells, and is commonly referred to as human microbiota, or microbiome when comprehensive of DNA agents such as phages and plasmids, proteins and metabolites, and the whole surrounding environment [3, 4]. It is partly established during birth by maternal vertical transmission; then, it further develops in early life through natural microbial colonization events that occur in the human body sites directly communicating with the outside, i.e., the gastro-intestinal tract, the skin, the naso-pharyngeal mucosa, the uro-genital mucosa, and the conjunctiva [5, 6]. From an ecological point of view, the human body and its microbiota evolve in a dynamic and mutualistic relationship, with the former providing an optimal niche for the bacteria to survive in, and the latter playing important roles in the maintenance of the host homeostasis [7, 8].

Defining the microbiota composition of the different human body sites in health and disease is currently an active research area. The focus of most studies is the intestine, as it is the largest interface with the external environment and the main reservoir of microorganisms in the human body, containing till 10^{14} bacteria of more than 1000 different species which approximately correspond to two third of the total human microbiota [9, 10].

Data indicate that the gut microbiota composition shows the highest intra- and inter-individual variability during the first three years of life, being shaped by various factors, such as delivery mode, diet (breast- or formula-feeding, introduction of solid food), genotype, environment, geographical and cultural factors, infections and use of antibiotics. Subsequently, it starts to resemble an adult-like composition that persists through childhood, adolescence and adulthood, although small changes constantly occur in relation to dietary modifications, hygiene, use of drugs, physical stress, etc. [4, 5, 11-13].

The sum of factors influencing the gut microbiota composition during the course of life accounts for the considerable variation of bacteria genera and species among individuals; however, the same bacteria phyla have been shown to prevail in all individuals, i.e., *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Protebacteria,*

Address for correspondence: Giovanna Franciosa, Centro Nazionale per il Controllo dei Farmaci, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: giovanna.franciosa@iss.it.

most strains being harmless commensals in the healthy human gut microbiota [14, 15].

A constant and balanced gut microbiota composition (eubiosis) provides positive effects to general health by directly contributing to metabolic functions, protection against pathogens and immune system stimulation [10, 11, 16-18]. Besides, there is increasing evidence of possible complex bidirectional signalling between the gut microbiota and other organs, such as the brain, the lung and the skin (generically defined as gut-brain, gut-lung and gut-skin axes), as indicated by the occurrence of intestinal complications during neurological, respiratory or skin disorders, and *vice versa* [10, 11, 19-21]. The bidirectional communication would be accomplished on one way by metabolites and endotoxins from the inflamed gut reaching the different organs via the blood circulation, and on the other way by products from the inflammatory processes in the organs acting on the gut microbiota [9, 11, 18].

In accordance, aberrant gut microbiota composition (dysbiosis) and the consequent imbalance of the host-microbes relationship may not only be directly implicated in the pathogenesis of gastrointestinal-related and autoimmune diseases, all characterized by over-responsiveness of the immune system, such as inflammatory bowel disease, colorectal cancer, obesity, type 2 diabetes, celiac disease, rheumatoid arthritis, etc. [5, 21]. In fact, dysbiosis is also increasingly recognized in *i)* brain and nervous system diseases (e.g., autism spectrum disorders, Alzheimer's disease), *ii)* lung diseases (e.g., asthma, cystic fibrosis, COVID-19 disease), and *iii)* skin disorders (e.g., psoriasis, atopic dermatitis), etc. [19, 20-23].

Microbial diversity loss with shift towards certain species has commonly been observed in the microbiota of diseased patients compared to those of healthy people, even though whether dysbiosis is the cause or rather the consequence of the disease often remains unclear [10, 24, 25].

Although less studied, the microbiota of body sites other than the intestine have also been hypothesized to play important roles in human health and disease: for instance, dysbiosis of the maternal vagina might be a risk factor for pre-term birth, while dysbiosis of skin, mouth or respiratory tract mucosa have been associated with conditions such as wound infection and acne, dental caries and other periodontal diseases, and sinusitis and pneumonia, respectively, just to name a few [20, 26-28].

CATEGORIES OF LIVE MICROORGANISMS-CONTAINING PRODUCTS FOR HUMAN USE

The increasing awareness of the important influence of eubiosis and dysbiosis in health and disease, has strongly promoted extensive research on the possibility of modulating the human microbiota for preventive and/or therapeutic purposes.

Consequently, the search for live microorganisms conferring health benefits to the human host has expanded exponentially, as well as their potential applications. In parallel, while the traditional delivery vehicles for live beneficial microorganisms were essentially fermented dairy products, they now include at least four different product categories, i.e., food and dietary supplements, medical foods, cosmetics and drugs *(Table 1)* [29-36]. Although live microorganisms may also be contained in biocides, this product category will not be discussed in this review as it is not intended for direct human use, but rather to control organisms that are harmful to human health [37]. The category of medical devices will not be considered as well, because live bacteria have expressly been excluded from medical devices according to recent European regulation [38].

All product categories of *Table 1* fall under distinct regulatory pathways in Europe.

This overview will specifically focus on the medicine category – referred to as "live biotherapeutic products" (LBPs), i.e., medicinal products containing live microorganisms as active substance(s) – and the related regulation in Italy and Europe, with brief reference to the other non-medicinal product categories regulations. Critical aspects as well as implementation issues will also be discussed.

LBPs AND THE NEED FOR THEIR REGULATION AND CONTROL

LBPs differ from the other product categories of *Table 1* because of the intended use, i.e., to prevent or cure a disease, and the intended target population, as they are aimed at individuals prone to develop a pathology or at sick people [39].

The American Food and Drug Administration (FDA) introduced the term "LBPs" in 2010 and subsequently specified, to clearly distinguish between the drug and food categories, that a product that is no longer used as a food with specific characteristics of nutritional content, taste and flavor, but for other physiological purposes, becomes a drug [40-42].

In 2019, the term LBPs was adopted by the European Pharmacopoeia (Ph. Eur.) that defined them as "medicinal products containing live microorganisms (bacteria or yeasts) for human use", and also detailed the possible administration routes (oral or vaginal) and the different types of pharmaceutical forms [34].

Similarly to the other product categories of *Table 1*, LBPs most frequently include – but are not limited to – lactic acid bacteria, bifidobacteria, bacilli and yeast strains, alone or in combination. As the same microbial or yeast strains can often be present in the different product categories, it has been suggested that, in order to stress the distinct categories purposes, different names should be applied to the beneficial microorganisms that they contain [43]. In this view, the term "probiotics", defined as "live microorganisms that when administered in adequate amounts confer a health benefit on the host" [32], would be restricted to the food and dietary supplements category, whereas alternative terms have been proposed for the microorganisms in LBPs, e.g., "biotherapeutic agents" or "pharmaceutical probiotics" or "pharmabiotics", although they have not yet been validated [35, 36].

The differentiation between the "probiotics" and "pharmabiotics" terms has relevant implications for safety assessment. In fact, the main pre-requisite to

Table 1
Product categories that may contain live beneficial microorganisms

PRODUCT CATEGORY	Product characteristics and definitions (according to EU legislation)	Live beneficial microorganisms designation	Mode of administration	Target population	Intended use
Food	Food and beverages Dietary supplements Infant formula	Probiotics[a]	Oral	Healthy people	To retain and improve health and well-being
Medical food (Foods for special medical purposes)	Food specially processed or formulated and intended for the dietary management of patients, including infants, to be used under medical supervision [b]	Probiotics[a]	Oral or enteral, under the supervision of a physician	Patients with a disease that requires dietary management	Specific dietary management of a disease that has distinctive nutritional needs
Cosmetic products	Any substance or mixture intended to be placed in contact to the external parts of the human body (epidermis, hair, nails, lips, teeth) [c]	Probiotics[a]	Topical	Healthy people	Care of skin, hair, teeth, nails Cleaning Keeping in good condition
Drugs (Live biotherapeutic products, LBPs)	Medicinal products containing live microorganisms (bacteria or yeasts) for human use [d]	Pharmabiotics[e]	Oral or vaginal	Sick people or people prone to develop a pathology	To treat or prevent a disease

[a] [32], [b] [33], [c] [30], [d] [38], [e] Proposed (not yet validated) definition [35, 36].

qualify live microorganisms in foods as probiotics – provided their correct and unequivocal identification and accurate characterization – is their long history of safe use, implying consumption by healthy individuals; conversely, each pharmabiotic microorganism in pharmaceutical products requires a proper safety assessment, including *in vitro* and *in vivo* studies, and clinical trials in human volunteers [31, 44-46].

Despite the recommendation of maintaining the different terms, the term "probiotics" is still often used to include the microorganisms contained in LBPs as well as in the other product categories of *Table 1*, thus causing misleading overlaps between products, as discussed later in the text.

The intended use and target population are indeed the main focal points for the regulatory status of a certain product category [39, 40].

At the European level, the Directive 2004/27/EC – amending the Directive 2001/83/EC – describes a *medicinal product* as "(a) any substance or combination of substances having properties for treating or preventing disease in human beings and (b) any substance or combination of substances which can be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action or to making a medical diagnosis" [47].

Falling within this definition, LBPs are to be considered medicinal products and, as such, they can specifically rely on regulatory concepts available for other biological medicines – including vaccines, blood and plasma-derived medicinal products, and advanced therapy medicinal products – requiring to undergo rigorous evaluation of quality, safety and efficacy, in order to obtain a marketing authorization (MA).

Quality aspects such as batch-to-batch consistency, the influence of upscaling process on yield and potency, and the stability of the final product should be closely monitored during production of LBPs. As for other pharmaceutical products, the quality of LBPs must be ensured during pharmaceutical development and production through the application of good manufacturing practices (GMPs); their clinical efficacy should be confirmed by independent trials of acceptable quality (double-blinded, randomized, placebo-controlled trials), performed with specific lots produced under GMP conditions, and applied to a well-defined patient population, using established treatment conditions, dosage, and defined and validated primary endpoint(s).

Challenges to the evaluation process of LBPs may be related to issues such as animal-to-human translation and/or to the suitability of certain preclinical animal models.

As with any drug, also for LBPs the safety assessment is based on a risk analysis which primarily involves the identification of risks and the assessment of the probability of their occurrence and impact. On this basis, the risk must be managed by adopting measures to avoid, mitigate or accept its effects, and constantly monitored over time.

However, as above mentioned, the safety evaluation of LBPs needs further special consideration compared to traditional medicines, due to the peculiar biological properties and mode of action of the live microorganisms that they contain [48].

THE SAFETY CONCERNS RELATED TO LIVE MICROORGANISMS FOR HUMAN USE

Two main safety concerns have been raised on the intentional use of large amounts of live microorganisms:

a) the possibility of adverse side effects due to their translocation into the blood circulation, particularly in higher risk people, and *b)* their potential role as reservoir of antibiotic resistance genes or putative virulence/toxin genes [49-51]. While there is growing evidence on the occurrence of severe infectious adverse events in vulnerable populations, the second safety aspect is more theoretical since so far it has not been supported by clinical evidence but only by *in vitro* and *in vivo* studies.

a. Infectious complications associated to the use of live microorganisms

A large number of randomized double-blind placebo controlled human trials have been carried out to prove the efficacy of probiotics, as recommended by the FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Foods [52, 53]. However, most studies have been conducted with healthy individuals, while only a few assessed their safety for vulnerable groups of people, such as critically ill or immunocompromised patients, elderly people, premature infants, pregnant women, etc.; moreover, it has been noted that even when the target population was vulnerable people, adverse events were not adequately assessed in the follow-ups of clinical trials and/or insufficiently reported or documented [45, 53-56]. For these reasons, the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) has recently recommended that any adverse events specifically linked to the consumption of live microorganisms-containing products should be appropriately recorded in a dedicated register maintained by health authorities [57].

Notably, for a proper drug safety assessment, at least two clinical trials with the same primary endpoint should be independently conducted in distinct centers [58].

As a matter of fact, the administration of large amounts of live microorganisms to vulnerable people, often in combination with or after excessive use of antibiotics, has occasionally been correlated to infectious complications in the treated patients: *Table 2* describes the microorganisms most frequently associated with infectious complications and the underlying conditions identified in the vulnerable population groups, according to most recently published systematic reviews, meta-analyses and single case reports [45, 50, 54, 56, 59-74].

In several cases, the microorganism isolated from the patient was shown by molecular techniques to be identical to the one administered, confirming that it was indeed the infection source [66, 67, 70-74].

Leaking intestinal mucosa barrier and/or immunosuppression in the vulnerable individuals have been suggested as possible predisposing factors contributing to the infectious progression, even though the underlying mechanisms remain unclear [24].

The adhesion properties of the microorganisms, that are normally a selection criteria for probiotics, allowing them to increase persistence and colonization in the host gut, but also critical for microbial pathogens, have been hypothesized to act as a virulence factor in vulnerable individuals, by increasing the microorganism translocation from the intestine to the blood stream [50, 58].

Of note, several cases of fungemia occurring in intensive care units, mainly due to the yeast *Saccharomyces cerevisiae var. boulardii,* were caused by the accidental contamination of the central intravenous catheter of hospitalized patients who were either being treated with preparations containing that microorganism or were infected by spread from other roommate(s) who were receiving such preparations. *S. cerevisiae var. boulardii* is indeed a spore-forming yeast rapidly spreading into the environment, especially in the absence of high standard hygienic practices [70, 73-75].

Although in most studies no deaths were reported, others describe variable mortality rates, even though whether death had resulted from the infectious complications due to the treatment or from the underlying conditions of the patients was unclear in some cases: in general, the factors associated with higher mortalities were the severity of patient conditions and immunosuppression or the presence of co-morbidities, while lower mortalities were associated to prompt therapy with effective antibiotics [50, 54, 56, 59, 60].

It must be highlighted that the incidence of infectious complications related to the use of live microorganisms in vulnerable people appears to be extremely low and

Table 2
Infection cases associated with so-called "probiotic therapy", often coupled with antibiotic therapy[a]

Microorganisms involved (in order of decreasing frequency)	Underlying conditions of treated patients (in order of decreasing frequency)
• *Saccharomyces spp* [b] *(S. cerevisiae var. boulardii)* • *Lactobacillus spp. (L. rhamnosus, L. delbruecki subsp. bulgaricus, L. acidophilus, L. paracasei, L. reuteri, L. gasseri)* • *Bifidobacterium spp. (B. breve, B. longum subsp. infantis)* • *Bacillus spp. (B. clausii)* • *Lactococcus spp. (L. lactis)*	• Immunocompromised patients often admitted to ICU (cancer, organ transplantation, surgical intervention, AIDS, hepatic cyrrhosis, diabetes, etc.) • Pre-term newborns or newborns with pathological conditions • Healthy seniors • Children with short gut syndrome • Non-immunocompromised people suffering from diarrhea • Non-immunocompromised people suffering from ulcerative colitis • Non-immunocompromised people suffering from dental abscess

[a][45, 50, 54, 56, 59-74], [b]Infections either caused by direct ingestion or catheter contamination.

largely exceeded by the reported positive effects of the same live cultures in healthy population [50, 54, 76].

In any case, although the studies are very heterogeneous for characteristics of the treated population groups (age, illness, received therapy previous to treatment, etc.), live microorganisms that were used, and dose and duration of treatments, altogether the results clearly indicate an increased risk of developing infections in the vulnerable groups treated with the live microbial preparations compared to the untreated patients.

Thus, despite overwhelming evidence that live probiotic microorganisms are effective and can safely be taken by healthy individuals, a careful analysis of the risk-benefit ratio should be applied by clinicians before recommending any products containing live microorganisms, either a food, a supplement or a medicine, to vulnerable patients, who on their side should be fully informed prior to treatment. In particular, measures to ensure the safe handling and administration of live microorganisms-based formulations to hospitalized and seriously ill patients should be implemented by the hospital staff.

b. Potential transfer of antibiotic resistance genes or putative virulence/toxin genes

Antibiotic resistance (AR) could be a selective advantage for probiotic/pharmabiotic microorganisms, especially those used in combination with antibiotics to re-equilibrate the intestinal microbiota, as they would have better chances to survive, colonize the host gut and also contemporarily exclude other antibiotic-resistant bacteria by competition; on the other hand, given that the gut microbiota is one of the main hot spots for horizontal gene transfers, the concomitant presence in the intestine of broad-spectrum antibiotics and antibiotic-resistant live microorganisms might exert a selective pressure potentially leading to AR spread to opportunistic pathogenic bacteria strains, thus seriously reducing the therapeutic treatments of infections [51, 77, 78].

For this reason, demonstration that the AR is intrinsic (non-transferable), and not extrinsic (acquired) is required for both probiotics and pharmabiotics by the respective EU regulating authorities, i.e., EFSA and Ph. Eur. [34, 79]. In particular, the AR genes should not be located within potentially mobile genetic elements such as plasmids, transposons, integrons, and bacteriophages in order to exclude the possibility that they are horizontally transferred from probiotics/pharmabiotics to commensal or pathogenic bacteria, or *viceversa*.

Several studies, some of which using metagenomics approaches, have shown that probiotic strains may harbor AR genes [51, 80-83]: yet, the presence of AR genes in a microorganism does not always result in resistance, since point mutations, deletions or insertions in the genes could negatively affect the gene expression. Thus, concurrent testing for phenotypic resistance would be appropriate: however, another challenge is posed by the fact that the minimum inhibitory concentrations cut-off values for most antibiotics used to test the probiotic strains are not always available or standardized [51, 78].

Nonetheless, neither detection of AR genes nor phenotypic evaluation of AR indicate whether the AR genes may indeed be transferable.

The location of AR genes within potentially mobile genetic elements, such as plasmids or transposons, which could contribute to spread, has been demonstrated in strains belonging to typical probiotic/pharmabiotic genera, including lactobacilli and bifidobacteria strains [80, 83, 84].

More importantly, a number of studies have shown the transfer of AR genes between microbial genera commonly used for human (or animal) use (especially lactic acid bacteria) and pathogenic strains. For instance: (a) the tetracycline *tet*(M) resistance gene could be horizontally transferred *in vitro* from some *Lactobacillus spp*. (namely, *L. plantarum*, *L. sakei* and *L. alimentarius*) to *Enterococcus faecalis* and *Lactococcus lactis*, and from *L. delbruecki bulgaricus* to *Listeria monocytogenes* [85]; (b) the erythromycin (*ermB*) resistance gene was shown to transfer from *L. plantarum* to *E. faecalis* in the gastrointestinal tract of rats [86]; (c) the genetic transfer of the vancomycin (*vanA*) resistance and ampicillin (*amp*) resistance genes between probiotic strains of *Enterococcus faecium* and probiotic strains of *L. acidophilus* has been demonstrated *in vivo*, in the gut of mice [87].

The potential dissemination of toxin/virulence genes among probiotic/pharmabiotic microorganisms is also of concern, as it could lead to the emergence of bacteria or yeast strains with new pathogenic potentials. In fact, similarly to the AR genes, the genes encoding any putative pathogenic factors (such as adhesion factors, cytolysins, damaging enzymes, and biogenic amines) could be transferred through mobile genetic elements between commensal and pathogenic bacteria and probiotic/pharmabiotic microorganisms, in response to any environmental pressure within the dynamic microbiota of the different human body sites [51, 77, 88].

Higher awareness of the above-described critical safety aspects potentially related to the consumption of probiotics/pharmabiotics is necessary to lead to more careful advising by clinical professionals and more prudential purchasing by consumers; it also demands for increasing regulation and control of the products that contain live microorganisms.

Ph. Eur. MONOGRAPH 3053

In Europe, testing and compliance to the standards detailed within the Ph. Eur. compendia is a basic requirement for the manufacturing and release of pharmaceutical ingredients and drug products.

In 2019, Ph. Eur. released a general monograph on *Live biotherapeutic products for human use* (3053), as well as two accompanying general chapters: *Microbial examination of live biotherapeutic products (LBP): test for enumeration of microbial contaminants* (2.6.36) and *Microbiological examination of live biotherapeutic products: test for specified microorganisms* (2.6.38) [34, 89, 90].

In particular, the Ph. Eur. monograph 3053 describes the quality and safety requirements for LBPs during production and in the finished lots [34].

These requirements specifically concern (a) the pro-

duction method and (b) the microorganisms (bacteria or yeasts) used:

(a) the production method must ensure constant final yields and microbial viability maintenance through the whole process; furthermore, the number of microbial subcultures from the master seed lot must not exceed that used for LBP production and shown to be satisfactory in the clinical trials. Consistent minimization or removal of any impurities or adventitious agents is also required during production;

(b) the microorganism(s) to be used must accurately be identified, and characterization at the strain level is required: the origin of strain(s), any subsequent manipulations, and description of the culture media used to grow the microorganism(s) should be provided. The tests used to characterize the phenotype and genotype of strain(s) must be detailed, and the stability of phenotypes and genotypes demonstrated. As above mentioned, besides the determination of their antibiotic susceptibility, the absence of any antibiotic resistance genes potentially transferable to the human microbiota is required. Furthermore, any virulence factor in the microorganism "should be investigated and evaluated with respect to safety".

As an additional quality requirement for those products to be orally administered, survivability of the microbial strain(s) in the human gut must be demonstrated by *in vitro* gastric acid and bile resistance testing.

Since microbial seed lot system is required to be used during production, it is recommended that it contains no adventitious agents or other impurities, and that any replacing seed lots are fully characterized. The sterility of the culture media must be ensured along with the absence of ingredients known to cause toxic, allergic or other undesirable effects; if inclusion of such ingredients is necessary, demonstration that the residual amount in the final lot does not affect product safety must be provided. Growth and harvesting must be performed under appropriate conditions. Stability data should be established for each intermediate product.

Tests to be performed on the final lots include the identification of each microorganism by proper methods, as well as the determination of the number of each live microorganisms (potency) by a suitable microbial enumeration test, and expressed as CFU/g, CFU/ml, CFU/unit: the resulting number must not be less than the stated range.

Concerning the microbiological quality of LBPs, the monograph 3053 indicates the acceptance criteria for aerobic microbial contamination counts (AMCC) and yeast and moulds contamination counts (YMCC) for all LBP products, whereas absence of *Escherichia coli* in those to be orally administered, and of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* in those for vaginal use is required.

The appearance, and the pH and water activity values of the final lots must comply with the specifications established for the products.

Regarding the storage conditions, it is recommended that liquid LBPs are not frozen, in order to maintain the full viability of the microorganisms.

Labels must state the name of strains, the potency of each strain (expressed as CFU/g, CFU/ml/CFU/unit or as viable cells/ml), the route of administration, the storage conditions, the expiry date, the name of any stabilizers and other excipients. For freeze-dried preparations, labels must also state the indications on reconstitution before use with name, composition and volume of the liquid to be added, and finally the storage conditions and expiration after the product has been reconstituted [34].

THE REGULATORY SITUATION FOR LBPs IN ITALY

As for other medicinal products, in order to obtain a MA for LBPs in Italy and Europe, the company has to submit an application, consisting of a dossier containing information on the chemical-pharmaceutical, preclinical and clinical aspects, structured according to a standardized format (CTD – Common Technical Document). The data and studies submitted to support the application for MA must comply with guidelines defined at international level (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use – ICH M4 document) [91]. The CTD includes 5 modules, of which module 1 contains the administrative information and prescribing information; module 2, the information summaries; module 3, the quality characterization of the product; module 4, the non-clinical study reports; and module 5, the clinical study reports.

Importantly, the CTD must provide the information concerning the product characteristics, labelling and package leaflet. Moreover, application of the good manufacturing practices (GMPs) according to the Commission Directive EU 2017/1572 during the product manufacturing must be demonstrated in the CTD [92].

According to the European legislation, LBPs can apply to a MA by either a National authorization procedure or by European procedures, the latter involving several EU regulatory authorities in the scientific assessment of the CTD provided by the applicant. Currently, all LBPs marketed in Italy have been licensed at national level.

For most of them, the MA was issued before the implementation of Directive 2001/83/EC, but during the renewal procedure submitted after the national transposition of this directive in 2006, the LBPs underwent a critical review, in order to achieve compliance of the authorization in Italy with the requirements of the European Union legislation.

LBPs AND PROBIOTIC SUPPLEMENTS: SIMILARITIES AND OVERLAPS

Despite the sharp demarcation existing between the regulations of the food/dietary supplements, medical foods, cosmetics and drugs categories *(Table 1)*, overlapping of products still occurs, most frequently between LBPs (drugs) and probiotic food supplements.

At the EU level, the food supplements fall within the scope of the general food law, i.e. Regulation (EU) 2017/625 [93]. With specific regard to the microorganisms contained in the supplements, EFSA has released

Table 3
Comparison of products containing the same microorganism (*Saccharomyces boulardii*) in similar amounts that are marketed in Italy either as LBPs or as probiotic supplements

Product features	LBP	Probiotic supplement
Information to patients	Patient information leaflet: - included in the medicine package; - publicly available, together with the Summary of Product Characteristics, on the web site of the Italian Medicines Agency (AIFA)[a]	No patient information leaflet included in the supplement package The product is listed in the Italian Register of dietary supplements[b], but information is only available on the manufacturer website
Product form	Capsule	Capsule
Product content	Each capsule contains: - Active substance: *S. boulardii,* 5 billion live microorganisms - Excipients: lactose, magnesium stearate, gelatin, titanium dioxide	Two capsules contain: - *S. boulardii* (MYA796), 10 billion; - *Enterococcus faecium* (SGEf01), 2 billion; - Magnesium hydroxide 124 mg; Zinc 4 mg.
Indications	- Prophylaxis and treatment of intestinal dysmicrobism and disvitaminosis due to antibiotic use - Prophylaxis and treatment of traveler's diarrhea - Treatment of acute diarrhea of different origins - Treatment of Irritable Bowel Syndrome - Treatment of candidiasis of the gastrointestinal tract	- Restoration of the intestinal flora equilibrium, specially following antibiotic or chemotherapeutic treatments - Zinc contributes to normal activity of the immune system
Dosage and use	- 1 or 2 capsules, twice daily - Do not take with hot or alcoholic drinks - capsule must be swollen intact	- 2 capsules daily, before or during meals - For children under six years, the content of the capsule can be solubilized in water or in other drinks
Warnings	Do not take the product if you: - are allergic to *S. boulardii* or other ingredients contained in the medicine - are allergic to yeast - are under treatment with anti-fungal/mycosis medicine - are an immunocompromised patient or if you are in hospital	- Do not exceed the recommended dose of capsules - The food supplements are not intended to replace a balanced diet - Keep out of reach of children under three years

LBPs: live biotherapeutic products.
[a]https://farmaci.agenziafarmaco.gov.it/bancadatifarmaci/home.
[b]https://www.salute.gov.it/portale/temi/p2_6.jsp?id=3668&area=Alimenti+particolari+e+integratori&menu=registri.

Table 4
Example of products with similar brand names from the same company that are sold in Italy as live biotherapeutic products (LBPs) or as probiotic supplements (PSs)

Product brand name (LBP or PS)	Microorganisms and quantities Other active principles	Indications
X[a] (LBP)	*Bacillus clausii* poly-antibiotic-resistant spores (strains SIN, O/C, T, N/R) 1 billion	- Prevention and treatment of intestinal disorders related to alterations of the intestinal microflora causing diarrhoea, abdominal pain and disvitaminosis - Restoration of the intestinal microflora during antibiotic or chemotherapy treatments - Treatment of acute or chronic gastrointestinal diseases of breast-fed children
X-A[b] (PS)	*Bacillus clausii* (strain SIN) 6 billion Zinc, Selenium	Restoration of the intestinal microflora balance
X-B[b] (PS)	*Lactobacillus acidophilus* strain LA-5 1 billion *Bifidobacterium animalis subsp. lactis* strain BB-12 2 billion Mint and coriander extracts	Fight abdominal swelling and tension, aerophagia, and imbalance of the intestinal microflora
X-C[b] (PS)	*Bifidobacterium lactis* strain HN019 ATCC SD5674 1 billion Fructo-oligosaccharides (FOS)	Restoration of the intestinal microflora balance and facilitation of the intestinal transit
X-D[b] (PS)	*Saccharomyces boulardi* 6 billion Vitamins A, D, B6, B9 and B12	Restoration of the intestinal microflora balance

[a]X: same name for the five (LBP and PS) products.
[b]A, B, C, D: specific designations differentiating the PS products on the bases of the claimed functions.

and regularly updates a list of microorganisms that are given the "quality presumption of safety" (QPS) status based on long tradition of safe use, absence of virulence/pathogenic factors which could harm the host, and absence of any potentially transferable antibiotic resistance genes [94, 95]. The accurate identification and characterization of the microorganisms is necessary to fulfill all requirements. The QPS approach was developed to provide a safety pre-assessment of microorganisms to be intentionally added to food and feed: since the QPS status is granted at the species level for bacteria and yeasts, it is recommended that any safety concern be excluded at the strain level [95]. As an example, the *Enterococcus faecium* species is excluded from the QPS evaluation because some of its members have been associated to nosocomial infections: however, certain beneficial *E. faecium* strains that are sensitive to the ampicillin antibiotic and do not contain any genetic elements commonly found in the nosocomial strains, as required by EFSA, may be used in food and feed supplements, as well as in medicines [95-97].

Furthermore, Italy and some other EU countries have set national guidelines on probiotics and prebiotics complementing the European regulations: for example, the Italian guidelines propose that the identification and characterization of bacteria and yeasts is always made at the strain level [98]. In addition, the Regulation (EC) No 1924/2006 on nutrition and health claims made on food also applies to the supplements containing live microorganisms [99]. According to the interpretation of this regulation by the EU Commission, the term "probiotic" is a health claim, as it implies a beneficial effect on health. This could be misleading for consumers if "probiotic" is used on labels, unless sustained by adequate scientific evidence. As a result of this interpretation, since 2007 EFSA has only approved the use of the term "probiotic" for a single yogurt containing *Streptococcus thermophilus* and *Lactobacillus delbruecki subsp. bulgaricus* (minimum 10^8 CFU/g) for which the claim "it improves lactose digestion" was satisfactorily demonstrated [100]. Since this interpretation of Regulation (EC) No 1924/2006 was increasingly deemed too restrictive, in recent years divergent interpretations on the use of the "probiotic" term were given in the EU Member States: consequently, in some countries including Italy its use has been allowed in the labeling and advertising of food and supplements, provided that specific conditions are fulfilled [98].

In summary, similarly to LBPs, the probiotic supplements are also regulated by complex rules, the former as drugs and the latter as foods.

Several aspects of the two distinct food and drug regulations are comparable, such as most requirements for live microorganisms and their quantities in the products. However, a remarkable difference is that LBPs must undergo extensive pharmacodynamics, pharmacokinetic, safety and efficacy testing and evaluation prior to receiving MA, while in most countries including Italy the probiotic supplements, as other dietary supplements, are not subjected to any pre-marketing authorization. In fact, they just need to be notified to the competent health authority that evaluates the general compliance of the product content and label with the food legislation (the Italian Register of dietary supplements is available at: www.salute.gov.it/portale/temi/p2_6.jsp?id=3668&area=Alimenti+particolari+e+integratori&menu=registri).

The fact that the procedure to get MA is much faster for probiotic food supplements compared to LBPs clarifies the reason why the probiotic food supplements largely exceed LBPs in the market, and is also at the basis of some drawbacks.

For instance, in Italy products of different brands containing the same microorganism(s) in similar quantities may actually be sold over the counter in the drugstores either as LBPs or as probiotic supplements, with the latter being easily available in the grocery shops, too *(Table 3)*. This implies that less information on the product characteristics and on safety warnings are available for the probiotic supplements compared to the LBPs counterparts, because the specific requirements for labeling and package leaflets that apply to LBPs are not needed for supplements, and this could represent a risk for diseased costumers purchasing them.

In Italy, this scenario is further complicated by the fact that some LBPs and probiotic supplements from the same company are marketed with very similar brand names, despite the fact that they contain distinct microorganisms and/or different quantities of the same microorganism, and are intended for different purposes *(Table 4)*.

CONCLUSIONS

In this review, considerable differences have emerged between the regulatory frameworks of LBPs and probiotic food supplements, even though both products and the related regulations also share several similarities, including frequently the same microorganisms in similar amounts. It must be mentioned that in Italy both LBPs and probiotic food supplements are sold over the counter in the drug stores, with difficulties in distinguishing between them for most customers and sometimes for the physicians, too. For these reasons, further improvement and harmonization between the regulations of LBPs and probiotic food supplements would merit consideration. This would properly orient the physicians and increase end users confidence besides implementing research in the field and ultimately supporting the manufacturers to invest in new products development.

Authors' contributions

All Authors conceived the study. GF wrote the manuscript, CvH critically revised the draft, MJG and SG contributed to the implementation of the final version of the manuscript.

Conflict of interest statement

The Authors declare that they have no conflict of interest.

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Effects of phthalates on marine organisms: cytotoxicity and genotoxicity of mono-(2-ethylhexyl)-phthalate (MEHP) on European sea bass (*Dicentrarchus labrax*) embryonic cell line

Chiara Molino*, Silvia Filippi*, Giada Giovani, Alessandra Caccia, Roberta Meschini and Dario Angeletti

Dipartimento di Scienze Ecologiche e Biologiche, Università della Tuscia, Viterbo, Italy
**These Authors contributed equally to this work*

Abstract

Introduction. Mono-(2-ethylhexyl) phthalate (MEHP) represents a toxicological risk for marine organisms due to its widespread presence in aquatic environments.

Methods. MEHP effects on cell viability, cell death and genotoxicity were investigated on the DLEC cell line, derived from early embryos of the European sea bass *Dicentrarchus labrax* L.

Results. A dose-dependent cytotoxic effect, with no induction of necrotic process, except at its highest concentration, was observed. Moreover, chromosomal instability was detected, both in binucleated and mononucleated cells, coupled with a minor inhibition of cell proliferation, whereas genomic instability was not revealed. To our knowledge, the overall results suggest the first evidence of a possible aneugenic effect of this compound in the DLEC cell line, that is the induction of chromosomal loss events without the induction of primary DNA damage.

Conclusions. MEHP should be considered more harmful than its parent compound DEHP, because it induces genomic instability in the DLEC cell line without triggering cell death.

Key words

- MEHP
- European sea bass cell line
- cytotoxicity
- genotoxicity
- micronuclei

INTRODUCTION

Phthalates (PAEs) are generally used in plastics, fertilizer, pesticides, toys, cosmetics and other industries and they can promote the plasticity, durability and strength of materials [1, 2]. Dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzylbuthl phthalate (BzBP), dicyclohexylphthalate (DCHP), di-2-ethylhexyl phthalate (DEHP), diisobutyl phthalate (DiBP), diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), dinhexyl phthalate (DnHP), and di-n-octyl phthalate (DnOP) are the most commonly used PAEs in consumer products [3]. In aquatic environments, PAEs can be readily degraded by hydrolysis, photodegradation and microbial degradation [4, 5], or they can come into direct contact with aquatic organisms, entering the food chain and being transported through the trophic levels, ultimately becoming a threat to humans as consumers of aquatic resources [5-7]. When PAEs are ingested by organisms, they are easily metabolized [5]. Most PAEs metabolites are fat-soluble and can be stored in biological tissues for long times [3, 5], up to 6 months, eventually becoming toxic to the organisms [5, 8].

Among PAEs metabolites, mono-(2-ethylhexyl) phthalate (MEHP) is one of the most studied due to its widespread presence in aquatic environments and its toxicological risk [5]. Indeed, several studies have reported that the average concentration of MEHP in superficial neustonic/planktonic samples of the Tyrrhenian Sea ranged from 29.17 ng/g to 93.37 ng/g [9], whereas its concentration in samples collected from the Sea of Cortez (La Paz Bay) ranged from 13.08 ng/g to 13.69 ng/g [9]. Recent studies have also shown that fresh algae and cyanobacteria produce and re-

Address for correspondence: Roberta Meschini, Dipartimento di Scienze Ecologiche e Biologiche, Università della Tuscia, Largo dell'Università, 01100 Viterbo, Italy. E-mail: meschini@unitus.it.

lease MEHP under natural conditions, metabolizing the parent compound, DEHP, uptaken directly from the aquatic environment [6]. Nevertheless, European regulations concern the parent compound DEHP and not its primary metabolite MEHP [10]. Thus, to date, a limit for exposure to MEHP in the aquatic environment has not yet been identified.

Similarly to most phthalates, MEHP can adversely affect the developmental and reproductive functions of several organisms, alter the number of offspring produced, reduce hatching success and disrupt larval development [5, 11-13]. Among its major effects, MEHP is known to cause the impairment of reproductive success [14], in particular interfering with androgenic activity [12, 15] and the expression of both sex hormone receptors [12, 14] and steroidogenesis-related genes [12, 15, 16]. Furthermore, MEHP is known to induce apoptosis [13], and have genotoxic, mutagenic, and carcinogenic effects [5, 17] on human and rodent cell lines. However, to our knowledge, very little data is available on the toxic and genotoxic effects of MEHP on aquatic organisms, especially on marine fishes [12].

The European sea bass (*Dicentrarchus labrax*, L. 1758) is an euryhaline marine teleost, which primarily inhabits estuaries, lagoons and coastal waters. This species is of high commercial and recreational value and is one of the most cultivated by the aquaculture industry in the Mediterranean basin [18]. In addition, *D. labrax* plays an important role in trophic networks, and in particular those of a large number of European estuaries and coastal areas [19]. As are most carnivorous species, *D. labrax* is highly exposed to the ingestion of anthropogenic pollutants [6]. Thus, the European sea bass is considered a good bioindicator of marine pollution [19]. In recent decades, fish cell lines have shown to be a reliable tool to assess the cytotoxicity, genotoxicity, gene regulation, virology and tumorigenesis of many pollutants [19, 20]. To date, the European sea bass embryonic cell line (DLEC) [20, 21], formed by fibroblast-like adherent cells, has been shown to be a useful instrument for *in vitro* assessment of toxic compounds [20].

In our previous study [20], we demonstrated that DEHP has a toxic effect on the DLEC cell line, resulting in a significant decrease in cell viability, a moderate increase in DNA strand break, and a dose-dependent increase in the frequency of micronuclei (MN) coupled with a significant and progressive decrease in cell proliferation. Considering that different studies [5, 12, 13] have shown the high toxicity of MEHP, the objective of this study was to evaluate the potential adverse effects of increasing concentrations of MEHP on the DLEC cell line, using specific *in vitro* tests to evaluate MEHP cytotoxicity, genotoxicity, and potential mutagenicity. The DLEC cell line was chosen because of the economical and environmental value of *D. labrax*, and also because of its easy maintenance in laboratory conditions and reliability for *in vitro* applications [21].

MEHP is the primary biologically active metabolite of DEHP, the latter having a short half-life. Moreover, since MEHP remains in the aquatic ecosystem for long periods [22], its occurrence and effects in the aquatic environment have been targeted as biomarkers [12] as well as a tracer of the intake of microplastics due to its high concentration in the blubber of stranded fin whales [9]. Besides, quite high MEHP concentrations have also been found in human samples [23] such as maternal serum (42.6 µM) and umbilical cord serum (37.5 µM). Finally, the accumulative exposure could be even higher considering the continuous contact of the organisms with this phthalate. In this context, MEHP effects should be more thoroughly investigated since exposure to MEHP poses a risk not only to aquatic organisms but to humans as well.

MATERIALS AND METHODS

Chemicals

Leibovitz (L-15) medium without L-glutamine, phosphate buffer saline (PBS) without calcium and magnesium and L-glutamine were purchased from Lonza, Italy. Penicillin/streptomycin, trypsin-EDTA in PBS without calcium, magnesium and phenol red were acquired from EuroClone, Italy. MEHP, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), cytochalasin B (1200 µg/mL), trypan blue solution (0.4%), dimethyl sulfoxide (DMSO), and sodium dodecyl sulfate (SDS) were bought from Sigma-Aldrich, Italy, while foetal bovine serum (FBS) was purchased from Invitrogen, Italy. MEHP was dissolved in DMSO to obtain a stock solution of 100 mM

Cell culture and MEHP treatments

The European sea bass (*Dicentrarchus labrax* L.) embryonic (DLEC) cell line [21] was used to assess MEHP cytotoxicity and genotoxicity. DLEC cells were maintained at 20-22 °C, without CO_2, in L-15 medium supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin.

For the positive control 100 µM H_2O_2 was used, while the solvent sample was treated with 1% DMSO for 24 h. Cells were treated for 24 h with different MEHP concentrations ranging from 0.5 to 100 µM. The working concentrations were freshly prepared in DMSO before treatments from MEHP stock solutions. DMSO never exceeded 1% v/v for both treatments and solvent control. For both cytotoxicity and genotoxicity assays, two independent experiments were performed.

Cell viability assay and cell death

DLEC cells were treated with MEHP for 24 h at concentrations of 1, 5, 10, 50 and 100 µM to assess both cell viability and cell death. Cytotoxicity of MEHP was evaluated as per the standard protocols by MTT and Trypan Blue Exclusion (TBE) assays [20, 24]. Briefly, for the former, MTT solution (0.5 mg/ml per well) was added at the end of MEHP treatment and, after additional 3 h of incubation at 21 °C, cells were lysed (10% SDS, 0.6% acetic acid in DMSO) to dissolve the formazan crystals. The spectrophotometer DTX 880 Multimode Detector (Beckman Coulter) was used to measure optical density. Instead, for the TBE assay, cells were harvested after MEHP treatment and cell suspensions were mixed with Trypan Blue solution (1/1, v/v) for 5 minutes, seeded on a slide and evaluated under an optical microscope.

Cell death was measured by fluorescence microscopy, evaluating the pattern of chromatin fragmentation [20, 25, 26]. At the end of MEHP treatment, cells were harvested and cell suspensions were stained with a combination of Fluorescein Di-Acetate (FDA, 0.75 mg/mL), Propidium Iodide (PI, 0.25 mg/mL), and Hoechst (HO, 0.1 mg/mL) dyes before cell death analysis.

Single cell gel electrophoresis

The DLEC cell line was treated with 1, 5, 10, 50 and 100 µM of MEHP for 24 h and the standard alkaline (pH>13) SCGE was performed according to previous works [20, 27]. After slide preparation and cell lysis (2.5 M NaCl, 10 mM Tris-HCl, 100 mM EDTA, pH 10, with 1% Triton X-100 and 10% DMSO freshly added), electrophoresis was conducted for 20 minutes at 25 V and 300 mA at 4 °C preceded by a 15-minute incubation in electrophoresis buffer (1 mM EDTA, 300 mM NaOH, pH 13) to allow DNA unwinding. Slides were then neutralized (0.4 M Tris-HCl, pH 7.5), and stained with ethidium bromide (20 µg/mL, 50 µL). Nucleoids were examined at 400× magnification with a fluorescence microscope (Axioskop 2, Zeiss) associated with a Comet assay III program. For each experimental point, three operators scored a total of 300 randomly-selected cells. Computer-generated % DNA in the tail (tail intensity, TI) values were used to evaluate the amount of primary DNA damage.

Cytokinesis-block micronucleus assay

The DLEC cell line was treated with 0.5, 1, 5, and 10 µM of MEHP for 24 h. The cytokinesis-block micronucleus (CBMN) assay was carried out with the standard technique proposed by Fenech [28]. Cytochalasin B was added after MEHP treatment, lowering the concentration to 2 µg/mL and arresting cell cytokinesis for 48 h. Harvesting and fixing were carried out as previously described [20, 29]. Slides were stained for 10 min with 5% Giemsa. Micronuclei (MN) were scored in both 1000-mononucleated and 1000-binucleated cells with intact cytoplasm for each experimental point. Cell cycle progression analysis was assessed calculating the cytokinesis block proliferation index (CBPI) as previously described [20, 30]. Subsequently, the percentage of cytostasis was calculated according to Lorge and coworkers [30].

Statistical analysis

The comparison between MEHP treatments and solvent was performed by carrying out one-way ANOVA, followed by Sidak's or Tukey's multiple comparisons post-test, for viability tests (MTT, TBE) and apoptotic/necrotic cell death assay; by means of χ^2-test for the cytostatic effect (CBMN); and using Student's *t*-test for paired samples for mean TI (Comet assay) and yield of micronuclei per cell (CBMN). The statistical significance for H_2O_2 and solvent samples was evaluated by comparison to untreated control (medium) as mentioned above. The level for statistical significance was set at $p<0.05$.

RESULTS

Cell viability and cell death

Results of cell viability measured by MTT and TBE assays after 24 h of MEHP treatment are illustrated in *Figure 1*. After treatment with different MEHP concen-

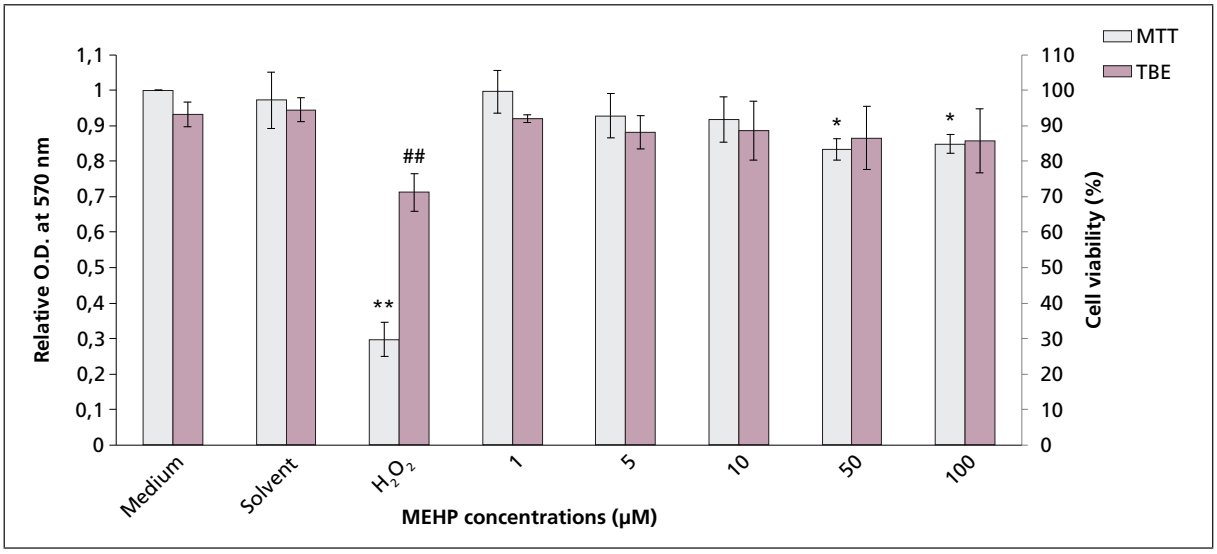

Figure 1

Cell viability determined through MTT and TBE assays in DLEC cell line exposed to MEHP. Results of MTT are displayed as a mean of the optical density (570 nm). MEHP treatment O.D. values were normalized to the solvent and are shown as mean ± SD of two experiments. Results of TBE are presented, at each treatment level, as the percent of viable cells out of the total cells and are displayed as mean ± SD of two experiments. One-way ANOVA significance: *$p<0.05$ MEHP treated *vs* solvent; **$p<0.01$ H_2O_2 vs untreated control (medium); ##$p<0.01$ H_2O_2 vs untreated control (medium).

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TBE: Trypan Blue Exclusion; DLEC: European sea bass embryonic cell line; MEHP: Mono-(2-ethylhexyl) phthalate; O.D.: Optical Density; SD: Standard Deviation.

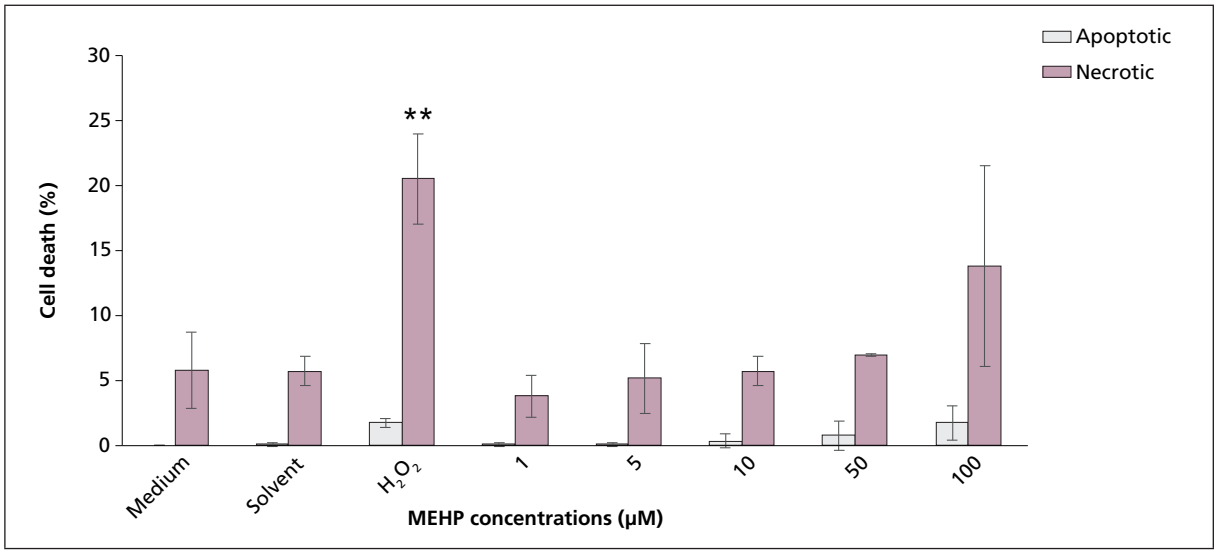

Figure 2
Percentage of apoptosis and necrosis in MEHP-treated DLEC cells. For each treatment results are shown as means ± SD of two independent experiments. One-way ANOVA significance: **$p<0.01$ H_2O_2 *vs* untreated control (medium).
MEHP: Mono-(2-ethylhexyl) phthalate; DLEC: European sea bass embryonic cell line.

trations, a dose-dependent reduction in DLEC viability was observed in both assays. In the MTT assay, survival rates of MEHP treatments significantly decrease, with respect to the solvent, only at the higher concentrations of 50 µM and 100 µM ($p<0.05$ and $p<0.0001$, respectively; F=189.4; DF=7), whereas in the TBE assay, decrease in survival resulted not to be statistically significant. In both assays, the solvent showed no effect on cell survival in DLEC. Conversely, treatment with H_2O_2 decreased cell survival to 30% ($p<0.0001$; F=3.409; DF=7) and 71.2% ($p=0.0535$; F=3.409; DF=7), respectively in the MTT and TBE assays.

Figure 2 shows the apoptosis induction in DLEC cells treated with MEHP. No induction of necrotic and apoptotic cells was observed in the untreated control and solvent samples. In MEHP treatments, induction of both necrosis and apoptosis were not statistically significant in comparison to the solvent. Conversely, with respect to the untreated control, in H_2O_2 treatment a statistically-significant induction of necrotic cells ($p=0.0001$; F=21; DF=7) and no increase in apoptotic cells were detected.

Comet assay

Table 1 illustrates the results of the mean TI values representing the induction of primary DNA damage in DLEC cells treated with MEHP. A TI of 5.63 was detected in the untreated control, while an increase in the TI value was observed in the solvent (TI=6.45), which was albeit not significant when compared to the untreated control. When compared to the solvent sample, treatment with MEHP did not induce any increase in TI values. The frequency of DNA damage was significantly increased by the H_2O_2 treatment (TI=30.50; $p<0.01$).

CBMN assay

Table 2 shows the induction of chromosomal damage as measured by CBMN assay. In binucleated cells, no difference in both the frequency of MN and CBPI values was observed in the solvent sample when compared to the untreated control. Conversely, treatment with MEHP revealed a dose-dependent and statistically-significant ($p<0.01$) increase in the yield of MN per cell at all MEHP concentrations with respect to the solvent. Moreover, a cytotoxic effect was detected as a decrease of CBPI values ($p<0.01$) and an increase in the percentage of cytostasis. Similarly, a statistically-significant increase in the frequency of MN ($p<0.01$) and a cytotoxic effect ($p<0.01$) were detected in H_2O_2 treatment in comparison to the untreated control.

As a further end-point to distinguish between a clas-

Table 1
Tail intensity (%) values obtained by the Comet assay in DLEC cell line treated for 24 h with MEHP. Data are presented as means ± SD of two independent experiments

Cell Line	Medium	Solvent	H_2O_2	MEHP concentrations (µM)				
				1	5	10	50	100
DLEC	5.63±1.14	6.45±1.44	30.50±5.70 §§	7.05±0.66	6.59±0.80	6.37±1.50	6.23±1.20	5.31±0.70

Significance of Student's *t*-test (ts): §§ $p\leq0.01$ H_2O_2 *vs* untreated control (medium). SD: standard deviation.
DLEC: European sea bass embryonic cell line; MEHP: Mono-(2-ethylexyl) phthalate.

Table 2
Induction of micronuclei (MN) in binucleated and mononucleated cells, cytokinesis block proliferation index (CBPI) and % of cytostasis in DLEC cell line treated for 24 h with MEHP and harvested after 48 h of cyto-B. Data are presented as means ± SE of two independent experiments

Treatment	MN/1000 BN±SE	ts	CBPI±SE	χ_2	% Cytostasis±SE	MN/1000 Mono±SE	ts
Medium	12.0±0.05		1.36±0.0003		0±0.00	10.3±0.06	
Solvent	13.2±0.08	NS	1.36±0.0006	NS	0±0.01	11.3±0.09	NS
H_2O_2	35.8±0.05	§§	1.26±0.0001	§§	26.3±0.07	15.2±0.01	§
0.5 µM	20.7±0.08	**	1.32±0.0002	**	12.6±0.11	22.0±0.09	**
1 µM	25.3±0.03	**	1.31±0.0001	**	13.6±0.16	28.3±0.05	**
5 µM	32.8±0.20	**	1.28±0.0002	**	22.3±0.12	40.3±0.05	**
10 µM	42.7±0.26	**	1.29±0.0005	**	19.4±0.02	50.7±0.11	**

Significance of *t*-Student test (ts) and Chi-squared test (χ_2): NS: not significant; **$p \leq 0.01$ MEHP treated *vs* solvent; $p \leq 0.05$ and §§$p \leq 0.01$ H_2O_2 *vs* untreated control (medium). SE: standard error.
DLEC: European sea bass embryonic cell line; MEHP: Mono-(2-ethylexyl) phthalate.

togenic or aneugenic effect of MEHP, the analysis of MN was performed in mononucleated cells as well. In the solvent sample, no difference in the frequency of MN was observed. With regard to MEHP treatments, a statistically-significant dose-dependent ($p<0.01$) increase in the frequency of MN, when compared to the solvent, was detected. When compared to the untreated control, H_2O_2 treatment caused a statistically significant ($p<0.05$) increase in the yield of MN, albeit lower with respect to MEHP.

DISCUSSION

The presence of phthalates in the marine environment has aroused great concern for aquatic organisms, due to the growing threat posed by plastic marine litter. Currently, it is unclear whether PAEs bioaccumulate and/or biomagnify through the trophic chain, and whether their metabolites may exhibit higher toxicity than their precursors. However, recent studies have shown that PAE metabolites, such as monobutyl phthalate, are easily stored in fat and biological tissues, reflecting a continuous and, thus, chronic exposure to living organisms [3, 5, 8]. Therefore, the assessment of their toxicity cannot be ignored. MEHP, as a DEHP primary metabolite, is one of the most studied pollutants since it is responsible for many of the effects of its parent compound [31]. For many organisms, including humans, MEHP toxic effects have been widely evaluated both *in vivo* and *in vitro*. Conversely, the *in vitro* toxicity of MEHP in cells deriving from marine fishes has been poorly investigated. Therefore, in the present work, the potential cytotoxic and genotoxic response to MEHP treatment on the European sea bass embryonic cell line, DLEC, has been analysed.

The cytotoxicity of MEHP, evaluated through the MTT and TBE assays, resulted in a significant decrease in cell viability only at the highest concentrations in the MTT assay. Given these results, MEHP displayed a minor cytotoxic effect with respect to DEHP in the DLEC cell line [20]. The variability observed in H_2O_2 cell survival results could probably be due to a different sensitivity of the MTT and TBE assays [32]. Cytotoxicity results are also sustained by a not significant induction of apoptosis and necrosis at all tested concentrations of this metabolite. Since the DLEC cell line lack of metabolic activation [15] the results suggest a direct cytotoxic effect of MEHP. In literature, large differences in sensitivity to MEHP and in its resulting cytotoxicity have been noted in both human and rodent cell lines [31]. Some *in vitro* studies have reported a variety of evidence of cytotoxicity starting from low concentrations of MEHP. For example, Erkekoğlu and collaborators [33, 34] reported a decrease in cell viability within 24 h, in MA-10 (mouse Leydig tumour) and LNCaP (human prostatic cancer) cell lines starting from 3 µM of MEHP treatment. On the other hand, other studies detected scarce cytotoxic effects of MEHP on rodent or human cell lines, if not at higher concentrations. In GCs (rat ovarian granulosa) and HepG2 (human liver) cell lines, a significant decrease in cell viability at 50 µM MEHP has been reported [14, 35], whereas HRT-8/SVneo (human placenta) cell lines were more resistant with a decrease in cell viability at a MEHP concentration of 180 µM [13]. The decreased viability of both HepG2 and HRT-8/SVneo was associated with an increase in apoptotic cells starting from 100 µM MEHP [13, 35]. Great variability in MEHP toxicity ranges can also occur within the same cell line. Indeed, other studies on the MA-10 cell line reported significantly-different results compared to Erkekoğlu and collaborators [33, 34], by finding a cytotoxic response starting at 300 µM [16] or at even higher concentrations, such as 1 to 3 mM of MEHP [15, 36], coupled with occasional encounters of apoptotic cell bodies in all MEHP tested treatments [36]. The reason for these discrepancies might lie in several factors: differences in the experimental designs, cell culturing conditions, cell density/number, cell source, purity of the MEHP, employment of secondary compounds [34], as well as the different responses of the diverse cell lines to this metabolite.

MEHP genotoxicity was assessed by both the Comet and the CBMN assays. The Comet assay is a sensitive test able to identify DNA strand breaks typically induced by clastogenic agents, while CBMN detects

both clastogenic and aneugenic effects, the latter being the induction of chromosomal loss events without the induction of DNA strand breaks. The results of the Comet assay do not suggest a genotoxic effect of this phthalate in DLEC cells. Indeed, the level of primary DNA damage, in terms of strand breaks revealed by the Comet assay, was not increased by MEHP, while H_2O_2, a clastogenic agent, caused a high and significant induction of DNA strand breaks. This outcome suggests that MEHP does not have a clastogenic effect in the DLEC cell line. Conversely, in the CBMN assay, MEHP induced a dose-dependent enhancement of MN, not only in the binucleated but also in the mononucleated cells, strongly suggesting an aneugenic action of this compound in the DLEC cell line. As reported in the literature, the possible cellular targets of MEHP triggering chromosome malsegregation could be the organization of the meiotic spindle and the assembly of actin [37]. Moreover, the same authors reported altered 5mC and H3K4me2 levels and a significant elevation of oxidative stress after MEHP treatment; it was recently suggested that the latter might have a role in chromosome alignment [38]. The genotoxic effects of DEHP and MEHP have been investigated in a number of different tissues and with various genotoxicity assays [5, 31]. MEHP genotoxic potential has been investigated in several studies by means of the Comet assay. For instance, Erkekoğlu and collaborators [33, 34] reported high levels of DNA damage associated with an increase in both Tail Moment and Tail Intensity by several folds at very low concentrations of MEHP (3 µM). Other authors detected an enhancement of DNA migration only at higher concentrations of MEHP [17, 39] and also a relationship between urinary concentrations of phthalate metabolites, including MEHP, and sperm DNA damage in humans [40]. However, to our current knowledge, no studies have been conducted on the induction of micronuclei by MEHP. The micronucleus assay is a methodology that makes it possible to obtain a measure of both chromosome breaks and whole chromosome loss [28]. With the CBMN assay, it is possible to detect between 60% and 90% of acentric fragments and, in combination with kinetochore/centromere detection or other genotoxicity assays (e.g. Comet assay), it is an optimal procedure for measuring whole chromosome loss events [28]. Moreover, scoring MN in mononucleated cells could be a further end-point able to distinguish agents with clastogenic action from aneugenic ones [41]. Indeed, Elhajouji and collaborators [41] first, and later Kirkland [42], demonstrated that increasing MN in mononucleated cells is a clear and sensitive index for detection of aneugenic compounds. Therefore, given the results of both the Comet and CBMN assays, it can be hypothesized that the dose-dependent increase in the frequency of MN caused by MEHP treatments on the DLEC cell line could represent chromosomal loss events rather than chromosomal breaks. Thus, this is the first experimental evidence of an aneugenic effect of MEHP.

In the present study, we demonstrated that MEHP pose a great risk for the European sea bass; since MEHP caused genomic instability to DLEC cell line it is possible that it may cause even more harm under natural condition, where this contaminant can accumulate in fish tissues and have a direct effect on target organs, such as the liver [43, 44]. In a recent study, Barboza and colleagues [7] have detected the presence of microplastic in the gastrointestinal tract, gills and also in the dorsal muscle of 150 commercial fishes (42% in *D. labrax*, n=50). The presence of microplastics in commercially important fish tissues may presents a risk to human health, due to the potential transfer of microplastics to humans, but also the potential toxicity of contaminants associated to plastic items [7, 18]. In humans, it is well known that phthalates in general can cause the disruption of endocrine function [18] and sperm DNA damage [40], however, information on the transfer of these pollutants to humans through the diet is still poorly investigated [18]. Since different studies showed that both wild and aquaculture fishes can bioaccumulate plastic additives, such as bisphenols and phthalates, in their tissues [43, 44], it is important to increase our knowledge on human absorption, distribution and metabolism of MEHP through fish ingestion, information indispensable for human health risk assessment.

CONCLUSION

The current study highlights a difference between the cytotoxic and genotoxic effects of DEHP and MEHP on the European sea bass embryonic cell line. In our previous study [20], we found that the cytotoxicity of DEHP was much higher compared to that caused by MEHP, its primary metabolite. Even the induction of apoptosis and necrosis was significantly higher after treatment with the precursor compound when compared to MEHP as well as the induction of MN. Thus, MEHP should probably be considered even more harmful than its precursor, because it induces genomic instability in the DLEC cell line at lower concentrations without triggering cell death. To conclude, this study underlines that MEHP, which is ubiquitous to the marine ecosystem as its parent compound, is an even more hazardous pollutant for the European sea bass, as well as for other marine organisms, and may represent an even greater risk for human health.

Authors' contributions

CM contributed conducting both cytotoxicity and genotoxicity assays, drawing Figures and Tables, performing data analysis, statistical analysis, writing the first draft and revising the final draft of the manuscript; SF contributed designing the experimental work, collaborating in both cytotoxicity and genotoxicity assays, performing data and statistical analyses and revising the final draft of the manuscript; GG contributed revising the final draft and bibliography of the manuscript; AC contributed collaborating in both cytotoxicity and genotoxicity assays; RM contributed to the conception and design of the work, interpretation of the data and revising the final draft of the manuscript; DA contributed to the conception and design of the work, interpretation of the data and revising the first draft of the manuscript; all the Authors approved the final version of the manuscript to be published.

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Conflict of interest statement

None

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Exploratory study on the endogenous ouabain in idiopathic pulmonary arterial hypertension patients

Valerio Manfrini[1], Roberto Badagliacca[2], Elisabetta Messaggio[3], Roberto Poscia[4], Roberto Torre[4], Paolo Manunta[3]* and Carmine Dario Vizza[2]*

[1]Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Rome, Italy
[2]Dipartimento di Scienze Cliniche Internistiche, Anestesiologiche e Cardiovascolari, Sapienza Università di Roma, Rome, Italy
[3]Unità di Genomica delle Malattie Renali e dell'Ipertensione, Scuola di Specializzazione in Nefrologia, Università "Vita-Salute" San Raffaele di Milano, Milan, Italy
[4]Unità Ricerca Clinica e Clinical Competence, Direzione Generale Azienda Policlinico Umberto I di Roma, Rome, Italy
**These Authors share last authorship*

Abstract

Introduction. Endogenous ouabain (EO) is a steroid hormone secreted by the adrenal glands associated with adverse cardiovascular outcomes. However, EO plays other roles as brain protection against traumatic injury and seems involved in the adaptive response to hypoxia. Recently, we detected, for the first time, EO in a healthy human group of acute hypoxia and diving animals.

Methods. This study complements the above as we considered a human model of chronic hypoxia. The aim is to detect EO in five idiopathic pulmonary arterial hypertension patients.

Results and Discussion. We found that these patients had higher plasma concentrations of EO than control subjects. In addition, EO plasma concentrations were negatively correlated with the mean pulmonary arterial pressure and total pulmonary vascular resistance. The results could suggest that high concentrations of EO are predictive of better adaptation of the right ventricular afterload.

Conclusion. Although the results are preliminary, they can represent a helpful hint for future investigations for possible therapeutic and diagnostic approaches.

Key words

- hypoxia
- endogenous ouabain
- pulmonary arterial hypertension
- chronic disease

INTRODUCTION

Acute and chronic exposure to hypoxia reveals a range of cognitive and behavioral deficiencies [1]. However, possible explanations for the intact cognitive functioning found in mountaineers, in some clinical conditions, or apnea divers include neuroprotective factors [2] and adaptive response to low-oxygen states [3]. Among the neuroprotective factors, the endogenous ouabain (EO), a stress-related hormone secreted by the adrenal glands [4], seems to be involved in the adaptive response to hypoxia. EO is a cardiac glycoside structurally similar to digoxin [5]. Digoxin-like immunoreactive substances have been found in sleep obstructive apnea syndrome patients [6].

In Manfrini, *et al.* [7], we detected, for the first time, plasma EO in a healthy human model of acute hypoxia (18 elite apnea divers) and 31 diving animals (common bottlenose dolphins, phocids and otariids, and loggerhead sea turtles) which perform many short or long apneas without reporting neurological damages.

Human pulmonary arterial hypertension (PAH) is a pathophysiological disorder that may involve multiple clinical conditions and can complicate most cardiovascular and respiratory diseases. This disorder is characterized by right-heart catheterization showing precapillary pulmonary hypertension with a mean pulmonary artery pressure (mPAP) of >25 mmHg and a normal pulmonary artery wedge pressure of <15 mmHg [8, 9]. The classification of pulmonary hypertension (PH) has undergone a series of changes since the first classification proposed in 1973 designated only two categories: primary and secondary PH, which depend on the presence or absence of identifiable causes or risk factors [10, 11]. According to the clinical classification of PH from

Address for correspondence: Valerio Manfrini, Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Rome, Italy. E-mail: valemanfro@gmail.com.

Table 1
Variables collected in IPAH (idiopathic pulmonary arterial hypertension) patients

N	Code	Age (yrs)	H (m)	W (kg)	EO (pM)	BSA (m^2)	6MWT (m)	SO_2 (%)	PAPm (mmHg)	CF (bpm)	CI ($l/min/m^2$)	PVRtot (WU)
1	IPAH40	46	1.76	101	396	2.17	590	97.0	28	85	2.40	5.18
2	IPAH41	31	1.78	55	264	1.68	410	90.0	94	90	2.20	25.26
3	IPAH42	78	1.60	80	312	1.83	300	88.0	40	82	2.90	7.50
4	IPAH47	59	1.63	80	290	1.86	468	93.7	57	71	2.37	12.95
5	IPAH48	55	1.72	72	523	1.85	500	94.2	21	70	2.90	4.00

H: height; W: weight; EO: endogenous ouabain; pM: picomolar; BSA: body surface area; 6-MWT: 6-minute walking test; SO_2: oxygen saturation; PAPm: mean pulmonary arterial pressure; CF: cardiac frequency; CI: cardiac index; PVRtot: total pulmonary vascular resistance (WU wood units are preferred to dynes.s.cm-5).

the 4th World Health Organization (WHO) symposium (2008), held in Dana Point, California, PAH can be idiopathic (IPAH) caused by unknown reasons, heritable/familiar or associated with other medical conditions such as connective tissue disease, HIV infection, portal hypertension (liver disease), sickle cell disease and congenital heart disease. Therefore, IPAH is a rare disease characterized by a progressive increase in pulmonary vascular resistance that leads to right heart failure. The right ventricle (RV) inability to adapt to post-load increasing is a crucial prognostic factor characterized by an increase in RV size, a decrease in systolic function, and consequent hypoxia increasing over time [12]. In adulthood, IPAH is more frequent in females than in males (1.6:1), probably due to hormones [13] and (or) genetic and autoimmune mechanisms [14]. However, we enrolled male patients as they were the only subjects available for the study at the time of data collection.

This study aims to investigate whether EO is detectable in the plasma of IPAH patients affected by chronic hypoxia.

METHODS

This study was approved by the Ethics Committee of Sapienza University (Policlinico Umberto I) in Rome, Italy (Ref. No. 4468; Protocol No. 303/17) and adhered to the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines. It was conducted in compliance with the protocol, data protection regulations, and all other regulatory requirements, as appropriate (STROBE). Each participant provided a written informed consent form before enrolling in the study.

Five Caucasian males were involved in this study. For each subject, we collected age, height, weight, EO plasma concentration, and specific variables to evaluate the severity of IPAH pathology *(Table 1)*. Blood sample collection for these patients was performed during their routine health monitoring. IPAH patients were selected from the Clinic database of the Pulmonary Hypertension Center of Policlinico Umberto I, Rome, Italy. Regarding the control subjects, we considered the EO concentrations of twenty-six (26) healthy subjects, which matched IPAH patients by gender, age, and ethnicity without any experience of breath-holding studied in Manfrini, et al. [7].

Blood sampling was carried out between 09:00 a.m. and 12:00 p.m. by collecting 4-6 mL of venous blood into EDTA collection tubes. Plasma was separated by centrifugation at 3,000 rpm for 15 minutes at room temperature and stored in a cryovial at -80 °C until analysis. Plasma EO is quantified by a Scintillation Proximity Assay (SPA) using Yttrium Silicate (YSi) beads (Perkin Elmer, Hebron, KY, USA) conjugated with a secondary antibody. These beads contain an embedded scintillant that emits light when bound with EO tritiated through the primary antibody. They are counted in a β-counter. The CPM (counts per minute) readings of the samples are translated, by software, into EO values. Please refers to Manfrini, *et al*. [7] for all details of this analysis.

Statistical analysis was performed using R version 3.2.1 (www.r-project.org) [15] and code programmed to specific studies and methodological approaches. Descriptive data were reported in jitter plots that show the median (thick line), the limits of the 95% confidence interval (thin line), the minimum and maximum values (short horizontal lines), and each value (small spots).

Data were studied using bivariate analysis to look for linear correlations between EO concentrations and the specific variables of IPAH pathology. The degree of correlation was assessed by the coefficient per rank of Spearman (ρs) that can take values between -1 (negative correlation) and +1 (positive correlation), 0 (no linear correlation). The Spearman coefficient has greater robustness than the Pearson coefficient for any outliers and deviations from the normality of the data.

Comparisons of endogenous ouabain concentrations between groups were performed by using the analysis of covariance (ANCOVA) to correct the analysis for confounding biometric data according to the linear correlation analysis previously performed. *Post-hoc* analysis was corrected by the Bonferroni method to contain type I error.

RESULTS

The plasma EO concentrations and specific markers of pathology severity are shown in *Table 1*. We ran a preliminary and exploratory correlation analysis among EO plasma concentrations and the markers collected. We found that EO concentrations were negatively correlated with the mean pulmonary arterial pressure (PAPm)

(ρs=-1, p<0.05) and the total pulmonary vascular resistance (PVRtot) (ρs=-1, p<0.05). Also, a positive correlation trend was observed with the cardiac index (CI) (ρs=0.82, p=0.09). These results are consistent with those found in Manfrini *et al*. [7], where comparisons of plasma EO concentrations between controls and diver groups showed a statistically significant category effect (p=0.034). In this study, plasma EO concentrations were significantly higher in IPAH patients than in the same control subjects. This comparison showed a statistically significant category effect ($p<10^{-6}$) *(Figure 1)*.

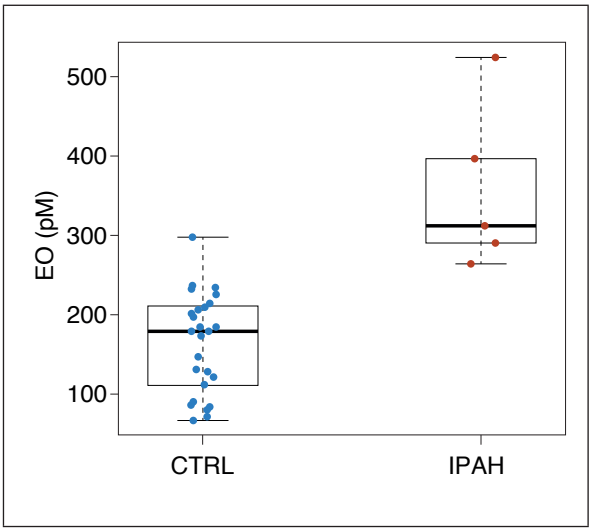

Figure 1
Jitter plots of plasma EO concentrations in 26 control subjects (CTRL) studied in Manfrini and colleagues [7] and 5 IPAH patients. EO is significantly higher ($p<10^{-6}$) in IPAH patients as compared to controls. Bars represent means ± standard deviation (SD); EO: endogenous ouabain; pM: picomolar; IPAH: idiophatic pulmonary arterial hypertension.

DISCUSSION

This study is preliminary, so we first measured EO in a peculiar hypoxia pathologic human model. IPAH patients are good models as they are exposed to chronic low oxygen levels [12]. We observed that IPAH patients had higher EO values than the control subjects without any breath-holding experience (no divers, no other sports activity involving apnea). This result is in line with data showing that EO plasma concentrations are higher in some clinical conditions that often occur in comorbidity with IPAH, like heart failure [16] and acute kidney injury [17]. It is known that the ouabain inhibits the activity of the Na+/K+-ATPase pump. The cell energy consumption by this enzymatic system is high [18, 19]. In these patients, the activity reduction of the pump might be a compensatory strategy to reduce cell energy consumption to adapt the ion balance and increase the resistance to low oxygen levels.

Although the number of IPAH patients was small, we explored correlations between EO and some specific clinical markers of disease severity. The limited sample size and the lack of long-term monitoring of the EO plasma concentrations do not allow reaching conclusions and make the analysis not very powerful. However, the choice to carry out this analysis, in any case, is motivated by the essentially descriptive and preliminary nature of the study in which even slight trends can be suggestive of hypotheses of interest. We found that EO plasma concentrations negatively correlated to the average pulmonary arterial pressure and total pulmonary vascular resistance and positively correlated (trend) to the cardiac index, a marker of healthy cardiac output. These findings, even though preliminary, could suggest that high concentrations of EO are predictive of a better adaptation of the right ventricular afterload.

CONCLUSIONS

These results, if confirmed with a larger sample, could have an impact on clinical practice as higher EO plasma concentrations might represent a positive prognostic index in IPAH patients and a helpful hint for future investigations involving other pathological conditions, for example, obstructive sleep apnea for possible therapeutic and diagnostic approaches.

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Authors' contributions

VM performed the experiment, analyzed the data, and wrote the manuscript; RB conceived the idea of the study and contributed to the study design; EM measured EO concentration in all samples and helped write the manuscript; RP supervised IPAH patient recruitment; RT executed IPAH patient recruitment and blood sampling; PM and CDV supervised the whole study and revised the manuscript. All Authors approved the manuscript and the version to be published.

At the time of data collection and analysis, Valerio Manfrini's affiliation was the Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Rome, Italy.

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Conflicts of interest statement

The Authors declare no conflict of interest.

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Knowledge, attitudes and practices regarding HIV/AIDS and STIs among youths and key populations in informal settlements of Nairobi, Kenya

Susanna Caminada[1], Francesca Maria Carrani[1], Marco Simonelli[2], Simonetta Crateri[2], Judith Mwikali Musyoka[3], Richard Muga[3], Eric Isyaho Mulinya[3], Catherine Onguti[3], Grazia Orsolato[4] and Maria Elena Tosti[2]

[1]*Dipartimento di Sanità Pubblica e Malattie Infettive, Sapienza Università di Roma, Rome, Italy*
[2]*Centro Nazionale per la Salute Globale, Istituto Superiore di Sanità, Rome, Italy*
[3]*NO ONE OUT, Nairobi Office, Brescia, Italy*
[4]*Medicus Mundi Italia, Nairobi Office, Brescia, Italy*

Abstract
Kenya is home to one of the worst HIV/AIDS epidemics, with higher prevalence rates in youths in urban slums. We conducted a cross-sectional mixed-methods study in Nairobi informal settlements. The aim was to investigate knowledge, attitudes and behaviours of this marginalized community, and to identify, with a bottom-up approach, the most appropriate interventions to increase the utilization of HIV/STIs services. Preliminary qualitative research was used to draw questionnaires, which assessed: STIs/HIV/AIDS knowledge, attitudes, and behaviours; access and barriers to STIs/HIV/AIDS services; perceived quality of services; the impact of COVID-19. One thousand and fifty-four respondents completed the questionnaire. 48.3% were youth in the community, 23% youth in school, 16.8% young mothers, 6.9% drug users and 5% people attending a technical-vocational training. We found unsatisfactory knowledge of STIs/HIV/AIDS transmission and prevention, and low condom use, mainly due to difficult access, poverty, and gender-based violence. We also found limited use of health services, and lack of trust due to poor attitude of the staff. COVID-19 has widened barriers to access to health services. To reach this population, it is necessary to implement educational interventions, facilitate access to free condoms, and train health centre staff to be more welcoming. Respondents found proximity strategies more efficient, including door-to-door testing and community outreach.

Key words
- HIV/AIDS
- sexual and reproductive health
- global health
- health service delivery

INTRODUCTION

HIV/AIDS and other sexually transmitted infections (STIs) are of major public health concern worldwide, accounting for more than 2.3 million people dying per year and 1 million newly infected each day [1]. Although global statistics reveal a general decline in AIDS related deaths and new HIV infections thanks to the concerted efforts of various stakeholders, the toll of HIV/AIDS continues to be harsh in developing countries, especially in Eastern and Southern Africa [2]; other STIs disproportionately affect low-income and middle-income countries as well [3]. STIs, in addition to being key epidemiological markers of unprotected sex, contribute adversely to sexual, reproductive and maternal-child health; lead to pelvic inflammatory disease, genital malignancies and infertility and increase the risk of HIV acquisition and transmission [1, 4].

The youth are especially prone to HIV infection as well as other sexually transmitted infections: young people accounted for 27% of HIV infections in 2020 [2, 5, 6]. This disproportionate impact on adolescents and young adults is a result of a lack of correct health information and age-appropriate comprehensive sexuality education, leading to engagement in risky behaviours, and a lack of knowledge and access to adequate reproductive health service, due for instance to fear of shame and stigma or to parental consent barrier [2, 7-11]. Sixty percent of the adolescents living with HIV reside in Eastern and Southern Africa [11].

Address for corrispondence: Susanna Caminada, Dipartimento di Sanità Pubblica e Malattie Infettive, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Rome, Italy. E-mail: susanna.caminada@uniroma1.it.

Similarly, due to the even higher probability of high-risk practices, marginalization and lack of adequate access to services, five key population (KP) groups are particularly vulnerable to HIV/AIDS and other STIs. These include: men who have sex with men, sex workers, transgender people, people who inject drugs and incarcerated people [2]. Not only do young people constitute a large percentage of most-at-risk groups, but they also frequently have higher HIV infection rates within these groups [12-15].

Kenya is home to one of the world's worst HIV and AIDS epidemics, with 1.4 million of people infected. The prevalence rate is 4.0% in the age group 15-49 [16]. Although the country did significant progress in fighting HIV in the last years, there is still a considerable number of new infections among youths, especially girls and KPs [17]. Previous studies carried out in the country found high numbers of STIs and HIV among young women [18] and street-connected youth [19]. According to the estimates of Kenya's Ministry of Health, in 2018 new infections in the youth population were disproportionately recorded in women: in the age group of 15-19 years, new HIV infections stand at 70% for women and 30% for men; in the age group 20-24, at 62% for women and 38% for men [20].

In Kenya, as elsewhere, HIV/AIDS prevalence rates in urban areas are higher than in rural areas [21-23], with strong intra-urban differences, especially in Nairobi, where the prevalence of HIV was much higher among slum residents compared with non-slum urban residents [24-26]. The increased vulnerability to HIV infection among the urban poor seems due to risky sexual practices, economic hardships, lower social cohesion, overcrowding, lack of security, multiple sexual partnerships, and early initiation of sex [27], but also very low awareness of HIV status [28], causing low access to testing and treatment. Misinformation and misconceptions about modes of transmission and treatments remain a widespread phenomenon [29, 30]. This is exacerbated by the lack of healthcare facilities and services, due to the informal and marginalized nature of these settlements [31-35].

Knowledge, attitudes and practices (KAPs) surveys regarding HIV/AIDS and STIs is one of the cornerstones in the fight against these diseases [8]: evaluating target targets' KAPs can help in designing appropriate and tailored prevention strategies, defining a baseline to evaluate the success of prevention strategies.

This present study was conducted within the project "Be free! Integration among community and health system for a youth population free from HIV and stigma" implemented by the Italian NGO Medicus Mundi Italia - MMI in collaboration with NO ONE OUT, an NGO based in Kenya, for which the Italian National Institute of Health (Istituto Superiore di Sanità - ISS) provided technical-scientific assistance for its design, coordination, and implementation. The "Be Free" Project aims at contributing to the national strategy to fight and end HIV, as envisaged by Kenya Vision 2030. The initiative aims at promoting the integration between communities and public health system, in order to guarantee access to prevention services and HIV treatment for communities who live in the most hard-to-reach and marginalized areas, and to reduce the stigma and discrimination caused by HIV among adolescents and young people, particularly in women and KP in urban informal settlements.

The aim of the study, in addition to investigating knowledge attitudes and behaviours of youths, was to try to identify, with a bottom-up approach, the most appropriate interventions and practical solutions that might be implemented in the future, in order to increase the utilization of HIV/STIs testing services addressed to youths and KP.

METHODS

This is a cross-sectional study carried out from June to August 2021 in four sub-counties in Nairobi: Mathare, Embakasi North/West, Ruaraka, Kamukunji. These are informal settlements, poor in terms of incomes, assets, access to resources and environmental conditions. Target groups included: *i)* adolescents and young people in school; *ii)* adolescents and young people in the community; *iii)* young mothers; *iv)* vulnerable KPs, especially drug users. The survey was conducted with a mix of qualitative and quantitative methods. Initially, Focus Group Discussions (FGDs) were conducted to explore youth attitudes toward STIs and to design a more specific quantitative questionnaire. The questionnaire was administered to a convenience sample of youths, young mothers and KPs, selected through the collaboration of the co-researchers with the Community Health Volunteers (CHVs) engaged by the project in 10 Health Facilities of these urban sub-counties, with the aim to evaluate their KAPs regarding HIV/AIDS and STIs. Being a hard-to-reach community, participants were directly recruited in the proximity of the health facilities. The knowledge of the local context by the CHVs and the trust they hold from the local population and, in particular, from the young people living in these areas, was the key element ensuring the participants' adherence to the study.

Before the study took place, all participants were provided with information about the study's methods and objectives, emphasising that the responses would only be used to improve the health service delivery according to the needs of the population. The participation in both the FGDs and the questionnaire was completely voluntary. Verbal or signed informed consent was obtained from all respondents before the start of the investigation. Questionnaires were completed anonymously. The study was performed in line with the principles of the Declaration of Helsinki.

Focus Group Discussions

The first activity was the organisation of qualitative FGDs, whose questions have been developed in English and translated in Swahili. FGDs were conducted by three co-researchers (2 males, 1 female): one FDG in each of the four target groups, for a total of 12 FDGs (with 6 to 8 participant each). These were conducted in the selected areas by local researchers, under the supervision of a local supervisor, selected by the NGOs involved in the project.

The questionnaire

The questionnaire was developed by the researchers in Italy in collaboration with the local researchers in Nairobi, considering the reports of the FGDs. The content of the questionnaire was based on handbooks [36, 37] scientific literature [8, 23], and on the experience of similar project previously conducted by some research group members [38-40].

The questionnaire was pre-tested before administration by all co-authors and by local researchers to verify language, flow, clarity, readability and completeness, together with acceptability and response alternatives. It was made of 36 questions, grouped into five sections: i) socio-demographic characteristics (8 questions); ii) STIs/HIV/AIDS knowledge and source of information (3 questions); iii) STIs/HIV/AIDS behaviours (5 questions); iv) knowledge, access and barriers to STIs/HIV/AIDS test and treatment (including questions on healthcare providers' perceived attitude - 9 questions); v) youth centres and training activities on sexual and reproductive health, STIs and HIV (6 questions). An additional set of 5 questions concerning the impact of COVID-19 on daily life and STIs/HIV prevention programs was added due to the context in which the research was conducted. All 36 questions were multiple-choice, but 21 of them had a possible open-ended "other" answer.

The questionnaire was in English. It was self-administered, with the assistance of field survey workers (CHVs involved in the project), in case of need for translation purposes. The CHVs were trained by local researchers in collaboration with supervisors in Italy (remotely) and in Nairobi, together with the project partners in Kenya. The local researchers coordinated and supported the survey workers in all phases of the implementation. Data collection was completely anonymous.

Definitions and statistical analysis

Door-to-door testing/service in the community: testing strategy that sends an HIV Testing and Services counsellor door-to-door in the area of interest, accompanied by a CHV.

Outreach testing in the community: provision of free medical check-ups and general clinical management at no costs by healthcare workers who reach hotspots of KP after mapping out the area.

Inreach testing in the community: organization of a public health center aimed at capturing the population that cannot be reached over week days, with openings on weekends or public holidays, when the health facilities are closed to public and people feel free to express themselves.

Level of education was categorized, in the analysis, in two groups: low and high education, the latter including those who completed secondary school or had higher education.

Descriptive statistical tests on the items included in the questionnaire were performed. Continuous variables were expressed as median and interquartile range, whereas categorical variables were expressed as proportions. Differences in proportions between groups were evaluated by the chi-squared test, or with the exact Fisher test where appropriate. p-values<0.05 were considered statistically significant and all tests were two-sided. All analyses were performed with STATA version 16 (StataCorp LLC 4905 Lakeway Drive College Station, Texas, USA).

RESULTS

Study population

One thousand and fifty-four respondents completed the questionnaire; 55.6% were females and the median age was 22 years old (IQR 19-25). The target groups surveyed were mostly youth in the community (48.3% of the sample), followed by youth in school (23%), young mothers (16.8%), drug users (6.9%) and people who have attended a TVET education (Technical and Vocational Education and Training, 5%). Socio-demographic characteristics of the respondents, stratified by gender, are reported in *Table 1*.

STI/HIV/AIDS knowledge and source of information

Regarding STIs/HIV/AIDS knowledge, 96% of youths of reproductive age report having heard about HIV/AIDS, with no statistically significant differences by gender or population group. Seventy-eight percent report being aware of STI's complications, with a greater degree of knowledge about complications being shown by females (83.9% *vs* 76.5% by male, p=0.003), members of the "youth in school" (87.8%), "young mother" (83.5%) and people with TVET education (83.2%). However, many respondents do not recognize most of the possible complications of STIs: while about half reported infertility and pregnancy complications and 40% pelvic pain, only 29% reported cancer. The knowledge regarding STIs/HIV/AIDS complications is independent of education level except for infertility, recognised by 65.7% of people with higher education (p=0.008) (data not shown).

The main sources of information on HIV and STIs reported by respondents are social media (44.8%), followed by TV (39.3%), school (38.9%), family/friends (34.7%) and radio (34.6%). Less frequently reported are sexual and reproductive health trainings (27.0%) and very few respondents report turning to religious leaders as sources of information (6.4%). No significant difference is observed in information sources by gender. A higher education is significantly associated with a higher use of social media (49.4% *vs* 30.8% in the low education group, p<0.001), school (43.1% *vs* 26.1%, p<0.001) and other training courses (30.4% *vs* 16.7%, p<0.001), while respondents with lower education rely significantly more on family (44% *vs* 31.8% in the high education group, p<0.001), radio (41.2% *vs* 32.4%, p=0.10), and TV, although not significantly. Addressing religious leaders is not associated with a lower education but it is more frequent in the "TVET education" group (13.2%). Drug users report using mainly TV (52.8%) and radio (50%) as sources of information.

STI/HIV/AIDS behaviours and attitudes

Table 2 shows the reported attitudes towards risky behaviours. Ninety-four percent of the respondents report knowing how to protect themselves from HIV infection,

Table 1
Socio-demographic characteristics of the study population (absolute and relative frequencies), by gender (HIV/AIDS and STIs) in Nairobi, Kenya

Characteristics	Gender			p-value
	Male	Female	Total	
	N (%)	N (%)	N (%)	
Age				
13-19	116 (26.3)	171 (30.8)	287 (28.8)	0.291
20-24	229 (51.9)	273 (49.2)	502 (50.4)	
25-40	96 (21.8)	111 (20.0)	207 (20.8)	
Population				
Youth in school	117 (25.2)	125 (21.4)	242 (23.0)	<0.001
Drug user	51 (11.0)	21 (3.6)	72 (6.9)	
Youth in community	268 (57.6)	239 (40.9)	507 (48.3)	
Young mother	-	176 (30.1)	176 (16.8)	
TVET education	29 (6.2)	24 (4.1)	53 (5.0)	
Level of education				
None	18 (3.8)	16 (2.7)	34 (3.2)	0.242
Primary school	75 (16.0)	124 (21.2)	199 (18.9)	
Secondary school	254 (54.3)	304 (52.0)	558 (53.0)	
Higher education	111 (23.7)	127 (21.7)	238 (22.6)	
Adult education	10 (2.1)	14 (2.4)	24 (2.3)	
Marital status				
Married	74 (16.0)	132 (22.7)	206 (19.7)	<0.001
Never married	362 (78.4)	382 (65.8)	744 (71.3)	
Separated	24 (5.2)	62 (10.7)	86 (8.2)	
Widowed	2 (0.4)	5 (0.9)	7 (0.7)	
Occupation				
Student	159 (34.0)	180 (30.8)	339 (32.2)	0.039
Wage labourer	82 (17.6)	80 (13.7)	162 (15.4)	
Formal employee	24 (5.1)	26 (4.4)	50 (4.7)	
Farmer	2 (0.4)	4 (0.7)	6 (0.6)	
Unemployed	129 (27.6)	217 (37.1)	346 (32.9)	
Self-employed	71 (15.2)	78 (13.3)	149 (14.2)	
Total	**468 (44.4)**	**586 (55.6)**	**1,054 (100.0)**	

with no significant difference by gender, target group or level of education; 60.9% recognize as effective using male condom, 38.5% abstinence, 32.3% partner fidelity, 22.8% female condom, and 12.7% PREP/PEP. However, more than half of the sample (51.6%) report having been previously engaged in risky behaviours.

Self-reported risky behaviours are more frequently found among males (significantly after the exclusion of "no answer"), drug users and subjects with a lower education level. The most provided reason for having previously engaged in risky behaviours is the lack of money/unemployment/poverty (41.1%), reported, in particular, by almost 35% of the employed people. 23.6% of respondents report, as a reason, the shame in asking the partner to use a condom or the fear of losing the partner or being beaten up, with no difference by sex (p=0.678). 18.8% report difficulty in finding available condoms and almost 14.5% a lack of trust and the discomfort given by the condom. Only 2.4% attribute responsibility for their behaviour to peer influence.

Twenty percent of respondents admit being unaware that their behaviour was at risk for HIV infection. When asked to identify the riskiest behaviours for the transmission, 64.5% answer unprotected sexual intercourse; 53.1% having several sexual partners, while 27.9% attribute it to the use of drugs. Few identify anal intercourse (15.5%) or homosexual intercourse between men (14.2%). Fourteen percent are aware of mother-to-child transmission and only 0.5% recognized transfusions as a possible risk.

Regarding condom use, as shown in *Table 2*, 45% of the sample report not always using condoms when having sex, this percentage being higher among women (48%, p=0.022). Reasons for this behaviour include the

Table 2
Absolute and relative frequencies of self-reported risky behaviours and attitudes towards test and treatment for HIV/STIs, by gender in Nairobi, Kenya

STIs/HIV/AIDS behaviours	Total		% in males	% in females	p-value
	N	%			
Have you ever had any risky behaviour?					
Yes	543	51.6	55.3	48.5	0.088
No	456	43.3	39.7	46.1	
No answer	54	5.1	4.9	5.3	
Do you know how to protect yourself from STIs and HIV/AIDS?					
Yes	988	93.7	93.4	93.9	0.717
No	40	3.8	3.6	3.9	
No answer	27	2.6	3.0	2.2	
If "Yes", which one do you use?					
Male condom	602	60.9	74.2	50.4	<0.001
Abstinence	380	38.5	34.5	41.6	0.022
Faithfulness to one single partner	319	32.3	23.3	39.4	<0.001
Female condom	225	22.8	11.6	31.6	<0.001
PREP/PEP	125	12.6	8.7	15.8	0.001
Do you always use condoms when having sexual intercourse?					
Yes	441	41.8	46.5	38.0	0.022
No	473	44.8	40.9	48.0	
No answer	138	13.4	12.6	14.0	
Why do you /young people have difficulties in getting tests and treatments for HIV/STIs from hospitals and clinics?					
Lack of privacy (fear of bumping into someone you/they know)	503	47.7	43.9	50.3	0.038
Attitude of health workers	361	34.3	31.6	36.2	0.116
Long queues in hospitals and clinics	310	29.4	30.9	28.2	0.328
Lack of information	205	19.4	20.5	18.6	0.446
Facilities are far away/cost of transports	71	6.7	5.8	7.5	0.259
Fear of finding out you are positive	30	2.8	3.4	2.4	0.321
Too expensive	27	2.6	3.6	1.7	0.050
Stigma, discrimination, peer pressure	19	1.8	1.7	1.9	0.835
Other reasons	17	1.6	1.7	1.5	0.924

STI: sexually transmitted infection.

following: condoms make the intercourse less pleasant (39.3%); condoms bring a sense of infidelity (34.2%); shame/stigma/discomfort given by buying condoms from pharmacists or health care providers (25.8%); condoms are not readily available (14.8%); the price of condoms is too high (6.6%); the influence of religious leaders (4.9%). Although 61% consider condoms as a tool for protection, less than half of the respondents report using it. Lower levels of education are significantly associated with a lower usage of condoms (41.3% versus 50.1% in the high educational group, p=0.016).

Regarding the stigma against individuals with HIV/AIDS, 84.3% acknowledge that they are normal people whereas 5.3% blame them for their illness; 3.2% say that one cannot share utensils, glasses and cutlery, or the bed with these people; 2.2% think that they cannot have children. No differences are observed by target group nor by gender. Instead, differences in stigma do exist regarding the education level: among people without education only 55.9% say they are normal people and significantly higher percentages think they are cursed or will die soon.

Knowledge, access and barriers to STIs/HIV/AIDS test and treatment

Most of the respondents (95.7%) report knowing where to get tested and treated for STIs/HIV/AIDS, with no significant difference by gender, target group or level of education. Among these, 69.5% claim they would turn to public hospitals and clinics in case of need; 56.3% to VCTs (Voluntary Counselling and Testing) and 23.4% to private services. Only 16.4% state

that they would seek help from Youth Friendly Services (YFS).

Only 39.8% report being aware of the availability of effective treatments for HIV/AIDS, independently from the gender and level of education of the respondents; of all groups, the least aware are young mothers (34.5%).

In case of infection, 88.0% of respondents claim they would take medications from hospitals and clinics and 7.2% from pharmacies, while 3.8% would rely on self-medication. 2.6% would turn to religious leaders, 0.6% to traditional physicians; 7 people respond that they would kill themselves in the event of infection. Only 0.9% claim they would seek counselling and psychosocial support.

As shown in *Table 2*, one of the main problems in getting tests and treatments seems to be related to lack of privacy and lack of confidentiality from the staff. There are no significant differences between male and female, except for lack of privacy (reported more frequently by women (p=0.038%), in particular young mothers (p=0.002). Drug users say a problem is the attitude of health workers (44.4%, p=0.021, in comparison to the other target groups).

Regarding the frequency of testing, according to 68.9% of respondents, it would be appropriate to be tested for HIV regularly, 31.3% only after engaging in risky behaviour, 12.8% before entering a stable relationship, 8.1% if you have symptoms and 4.5% only before having children.

As shown in *Table 3*, when asked about possible strategies and ways to increase access to HIV/STIs services, half of the respondents to the questionnaire identify "door-to-door testing/service in the community" as the most effective, followed by outreach testing and increasing the offer of YFS.

More than half of respondents (55.1%), with no significant difference by gender or level of education, report that health care providers' attitudes towards young people who come to them for counselling, testing, and treatment are "not good". Among these, the most frequently reported reasons are: they ask too many questions (24.2%), they are judging (23.2%), they are unfriendly or rude (22.7%), and they do not keep confidentiality (18.2%). Drug users complain most about the attitude of the operators (70.4%), followed by young mothers (59.7%).

Table 3
Absolute and relative frequencies of responses on strategies to improve access and attitude of HIV/AIDS/STIs services in Nairobi, Kenya

According to you, what would make the access to these services easier?	N	%
Door-to-door testing/service in the community	507	48.1
Outreach testing in the community	379	36.0
More Youth Friendly Services	342	32.4
Self-test kits	257	24.4
Inreach testing in the community	139	13.2
Testing at school	115	10.9
Incentive to pay transport fees	38	3.6
Counselling and advertising	11	1.0
All services to be free of charge	2	0.2
Other	10	0.9
Do you have any suggestion to improve the attitude of health workers/Youth Friendly Services?		
Training	710	67.4
Hiring younger personnel	293	27.8
Availability of CMEs in the centres	139	13.2
Presence of HIV/STI positive people in the centre	128	12.1
Other	36	3.4
No answer	17	1.6

STI: sexually transmitted infection.

Youth centres and training activities on sexual and reproductive health, STIs and HIV

Fifty-five percent of respondents, with no significant difference by gender or level of education, report knowing the YFS that provide counselling, information, education, testing and treatment for STIs and HIV in their area. However, 57.4% of all respondents state they have never been to one (55% of people who have never attended a YFS are not aware of their existence). Level of education is significantly associated with attendance at youth centres: 44.4% of those with a high level of education report having been to one at least once, compared with 32.5% of those with a low level of education (p=0.001). Drug users are less aware of youth centres (50.7% do not know this service exists) and visit them less than other groups (71.4% of them have never attended any YFS).

18.9% of the sample claim there are not enough YFS in the area; 90.3% believe it would be necessary to have more YFS, with no significant difference by gender, target group or level of education; 16.6% think there is a problem of accessibility to these facilities since they are far from their home.

The reasons for which 58.1% report preferring Health Centres over YFS for these services (counselling, information, education, testing and treatment) are primarily because the formers are free (54.5%), have more professional staff (41.4%) and are more accessible in terms of distance (26.2%); it is also reiterated that there are only few YFS (17.2%). However, 41.5% of respondents prefer YFS, especially youths in the community (46.2%), because of the following reasons: they are more youth-friendly (55.7%), the staff is friendlier and maintains confidentiality (48.4%) and is less judging (32.1%).

Only 53.3% of respondents report having participated in sexual education and STI/HIV training activities, with no significant difference between males and females. Level of education is significantly associated with having attended trainings (65.3% of respondents with higher education versus 51.9% of those with lower education, p<0.001). Drug users are the group that participated the least in the trainings (50.8%). Among those who report participating in trainings, only 20.9% received it at school, while 28.9% at health centres/hos-

pitals, 12.5% at NGOs, 6.9% at formal and informal community spaces, 5.4% in the Church, 5.0% within in-reach/outreach programs, and only 1.3% at Youth Centres; 18.6% of respondents reported other places. Those belonging to the "low education" group attend the training in 51.9% of the cases in comparison to the 65.4% of those belonging to the "high education" group. Among the top reasons for not taking part in training activities on the subject, the most common is not being aware of it (54.4%), followed by lack of accessibility (6.7% + 4.2% "it was far from where I live"), not being invited (6.7%) and the lack of time (6.3%). On the other hand, among those who participated in the trainings, the main benefits reported are: receiving sexual and reproductive education (76.4%) and information on family planning (32.2%), followed by receiving sanitary towels (26.5%) and/or financial incentives (15.9%).

COVID-19: knowledge and impact

Among the respondents, the vast majority (98.2%) report having heard of COVID-19 and 98.8% report knowing how to prevent the infection. Among the possible ways of prevention, the most frequently reported are wearing a face mask (92.7%), washing hands (88%), or using hand sanitizer (75.2%), followed by refraining from handshaking and hugging (58.3%) and keeping a distance of 1 meter from other people (56.8%). However, 5 subjects also indicate drinking/eating water, lemon, garlic, or praying.

The primary consequences that COVID -19 has had on youth in the community appear to be job loss and poverty (75.8%), followed by dropping out of school (43.8%), pregnancy (37.6%), violence at home (29.4%) and lack of food (28.1%). 21.1% of respondents report an increase in transactional sex while 9.2% report discontinuation of disease treatment and vaccinations.

Regarding the lack of access to hospitals and clinics for STIs/HIV diagnosis, counselling and treatment, 57.4% report a personal experience with COVID-19 impact. Among these, 43% report fear of getting COVID-19 in the health structures/centres and almost 40% Limitation of people accessing hospitals since they are taking in only small numbers; 30% report fear to be tested for COVID-19. Regarding the activities of the Youth Friendly Services, 53% of respondents complain that they were closed or that anyone without mask cannot be allowed (36%).

DISCUSSION

In this study, we developed and used a questionnaire to collect information about knowledge, attitude, practices, and possible interventions concerning STIs and HIV/AIDS from youths, young mothers, and drug users of informal settlements of Nairobi, Kenya. The content of the questionnaire was based on the results of qualitative research; using the results of FGDs to develop surveys has several advantages, including the possibility of learning about the local context, knowing the language of the target population in order to formulate comprehensible questions, and providing insight into the results of the questionnaires [41]. Moreover, the questionnaire was developed with the constant support and participation of local collaborators, familiar with the setting in which the research was carried out.

The study population was from a marginalised, hard-to-reach and excluded community, disadvantaged in terms of social determinants of health and access to services, and characterised by widespread risky sexual behaviour and high HIV/AIDS prevalence [34, 42, 43]. The target population included, more specifically, highly vulnerable to sexually transmitted diseases groups, such as young people and drug users [5, 44, 45]. The latter, like other KPs, are often criminalized and marginalized, which exposes them to a higher risk of infection and adverse HIV outcomes, since they are often excluded from prevention and treatment services [2, 46]. Reproductive health services targeted at young people are also scarcely widespread or not easily accessible in many African countries [9, 47, 48]. Despite being at a stage of life characterised by impulsivity, increased social relationships and development of self and sexual identity, adolescents are often neglected by the health system [2, 5, 49, 50].

Among young people, there are major inequalities in disadvantage of women, who face discrimination, fewer opportunities for education and health and further difficulties in accessing sexual and reproductive health services, due to harmful gender norms and gender-based violence, especially in sub-Saharan Africa and in slums and informal settlements [2, 51, 52]. STIs and HIV are not the only hazard for this group; therefore, reproductive health services must also consider other social and health issues that young people may face, such as unwanted pregnancies, early forced marriages, and sexual abuse [53, 54].

In Kenya, the Government has adopted the Adolescent Reproductive Health and Development Policy [55] in 2003 to make reproductive health services available, accessible, acceptable and affordable to young people. Afterwards, in 2005, the Division of Reproductive Health of the Ministry of Health has published national guidelines for YFS provision of reproductive health programs, based on the World Health Organization (WHO)'s guidelines [17, 56]. YFS are defined by WHO as services that are accessible, acceptable and appropriate for young people, and meet their individual needs, including keeping convenient opening hours, privacy and confidentiality.

An important foundation for any prevention effort aimed at young people is to provide them with basic information on how to protect themselves and their partners from acquiring STIs and HIV [13, 57]. However, many young people do not have the basic knowledge and skills to prevent themselves from becoming infected with HIV and continue to have insufficient access to adequate information and misconceptions about these diseases [13, 58, 59].

In our sample, the participants' level of knowledge about STIs and HIV/AIDS transmission and prevention was heterogeneous. Although most respondents reported having heard of AIDS and being aware of STIs/HIV complications and routes of transmission, a much lower percentage identified them correctly when asked.

Inadequate sexual and reproductive health knowledge among youths may contribute to stigmatizing tendencies towards those infected and affected by HIV/AIDS and STIs [59]. In our sample, there is in fact considerable stigma towards HIV-positive people; some respondents even stated that they would commit suicide if diagnosed. A similar picture had already emerged from the FGDs, where only a small percentage of participants did not blame people living with HIV, while the widespread idea was that people who tested positive had brought it on themselves or deserved it, as HIV was a curse. As a consequence, people avoid testing themselves fearing to test positive and thus being isolated. Similarly, they avoid telling their partners about their status fearing being left or receiving physical abuse. Stigma, a weak social support network and poor inter-partner relationships are known to contribute to delayed testing [60].

Given the insufficient knowledge of our respondents, educational interventions may need to be implemented. Gender-transformative, age- and culturally- appropriate comprehensive sexuality education is in fact a key component of HIV and STI prevention and empowerment for adolescents and young people [2]; the lack of knowledge about risks and consequences is an important barrier to accessing reproductive health services [57].

Regarding the sources of information, despite significant differences related to the level of education, the relevance of social media and TV among respondents of our sample is considerable. These are in fact powerful means of communication to reach these target populations. While mass media such as TV and radio are known effective tools for changing HIV/AIDS related behaviour among young people in developing countries [13], evidence on the impact of social media interventions in these contexts is still scarce [61]. However, as social media becomes more and more widespread [62], its potential is even greater in these countries [63]: through highly cost-effective interventions, it can provide psychosocial support, build community engagement, increase awareness of HIV testing and services, and promote behaviour change such as spreading condom use [64]. The power of social media lies in the possibility of engaging the very stigmatized and discriminated high-risk individuals by connecting and creating virtual communities [65, 66].

A key role in reproductive health education, especially in developing countries, must be played by schools, which provide a comprehensive and structured opportunity for interventions to achieve high coverage of young people around the time they become sexually active - unlike other initiatives, which are often fragmentary and sporadic [13, 52, 67]. In our sample, only 38.9% of the sample reported receiving information on the topic at school, especially respondents with higher education: perhaps the topic is discussed late in the school curriculum, although to be effective sexual and reproductive health education should be targeted at young people before the onset of sexual activity. From the FGDs preceding the questionnaire, it appeared that some had participated in training activities also organized outside the school. However, two were the major limitations found in these activities: only addressing general health topics without diving deep into the details of sexual and reproductive health and selecting participants based on age (i.e., 12-16 years old), therefore leaving out the youngest population. It is important to overcome inequalities in access to correct information, facilitating the empowerment of individuals and the increase of their health literacy.

Considering a possible underestimation because the behaviour was self-reported, we found a high percentage of people engaging in risky behaviour in our sample, especially women and respondents with lower education. Our findings are consistent with previous studies, in Africa and in Kenya [68, 69]: although many respondents know at least theoretically how to protect themselves and recognize the importance of condoms, few report using them when having sexual encounters. There is thus a gap between theoretical knowledge and practical application in daily life. The reasons are diverse and include social phenomena that are difficult to address, such as gender-based violence; however, among the practicable strategies, there is a need to increase the availability and accessibility of free condoms, considering that many responded that they committed risky behaviour due to lack of money, unemployment, and poverty. Socioeconomic status was in fact found as a significant predictor of consistent use of condoms in another study conducted in urban Kenya [70]. Condom programmes are among the most cost-effective interventions in the HIV response and should be reinforced [2], as many have also stated that they have difficulties in accessing condoms. The economic issue is not exclusively about access to condoms, but, as FGDs and other studies revealed, also about poverty driving young people to engage in transactional sex, which constitutes a further risk factor [30, 71].

Despite the government's efforts to bring young people closer to health services, the results of the questionnaire show only partial knowledge of the offer, limited use of services, and a distinct lack of trust: only 16.4% stated that they would seek help from YFS. The fact that YFS are little known is a major barrier to their use [57]: these services should be promoted and advertised, for instance through mass and social media. Poor access of young people to HIV testing services is consistent with another study conducted in Nairobi slums [23]. It is interesting to note that only 0.9% would seek counselling and psychosocial support in case of HIV/AIDS diagnosis; these are essential services for the support of the diagnosis of these diseases especially in younger groups and should be promoted [72].

One of the main problems in accessing SRH services seems to be related to lack of privacy and lack of confidentiality from the staff. Negative attitudes of service providers, often seen as judgmental or insensitive, are known as one of the most important barriers to youth access to reproductive health services [9, 57, 68, 73]. In Kenya, these attitudes are greatly influenced by religious and cultural backgrounds, professional training and orientation [17]. In our sample, the two most marginalised groups (drug users and young mothers) are the ones who complained most about the attitudes of

the staff, despite being the ones that need confidentiality the most [54]. Recommendations on the implementation of healthcare service provision should be characterized by a prompt, entertaining and welcoming environment that would encourage adolescents to interact freely, including additional training for staff and creating additional space for confidential counselling and examinations [57]. Providers must be sensitized to understand the needs of young people and be able to offer a comprehensive range of services [17, 74]. Staff training is recognized also by our respondents as a key strategy to improve the attitude of the operators of these facilities, to improve their communication skills with young people, increasing confidentiality and avoiding stigma. In addition to that, staff should take specific courses to interact and manage at-risk and fragile populations, as well as patients undergoing HIV/STIs tests or counselling on sensitive topics [74]. In addition, about 30% of our sample thought it would be helpful to hire younger personnel, consistently with previous studies [75, 76]. On the other hand, the presence of people with HIV/STIs in the centre, who might help combat the stigma and contribute to the psychological support of individuals at the time of diagnosis, was not recognised as a particularly effective strategy (only 12.1% of respondents).

Among the service provision methods identified as most efficient by respondents, proximity strategies seem to prevail, including door-to-door testing and community outreach. The facilitator role of outreach community-based approach in improving youth access to services is well acknowledged [30, 77, 78]. Another popular option in our sample was the inreach testing activity (organised either on weekends or public holidays). Again, the need for privacy and confidentiality is reiterated, as in previous studies [30, 79]. This becomes even more relevant for those subgroups that are even more marginalised and difficult to reach, such as drug users. Although our sample is limited and unrepresentative (72 respondents, 52 of whom are male), it is possible to assess the even greater inequalities faced by this group, excluded not only from health services but also from the tools to learn about and prevent the transmission of these infections.

Finally, since the survey was administered in the summer of 2021, a section on the impact of COVID-19 was included. Across the world, the COVID-19 pandemic has disrupted essential health services, including those needed to support the prevention, diagnoses, and treatment of HIV and STIs [1]. The pandemic and the actions taken to control transmission of the infection have put many children out of school, placing them — especially girls — at higher risk of contracting HIV and other STIs [2, 80]. Also in Kenya, everyday life has been dramatically affected by highly restrictive government-imposed measures such as stay-at-home curfews, prohibitions on mobility across national and county boundaries, and strict policing, especially affecting the urban poor and highly marginalized KP [81, 82]. The results of our survey also revealed that the pandemic has had a strong indirect impact, resulting not only from the infection itself but also from the measures undertaken to contain the spreading of the virus, such as the lockdown. The restrictive measures, job losses and the fear of infection affected every aspect of life and access to health services. The pandemic has therefore abruptly interrupted the progress made in recent years and the consequences will be evident in the long term.

Study limitations and strengths

This study has some limitations that should be considered when interpreting the results. First, in order to cope with the difficulties of recruiting respondents in such a marginalized and hard-to-reach population, the sampling strategy was adapted to the context. Social desirability bias may have occurred, especially for self-reported behaviour: participants may not be comfortable disclosing sensitive topics and may therefore underreport risky sexual behaviours. In addition, the presence of the survey worker during the questionnaire completion, which was essential as support for any translation needs, may have impacted the veracity of some responses. However, the anonymity of the questionnaires hopefully encouraged the respondents to be honest in their responses.

Conversely, the most important strength of this study is the mixed-methods design and the development of a highly tailored questionnaire based on the results of qualitative FGDs, in collaboration with local staff, familiar with the context. Additionally, this research/action project provides strategies and practical solutions to implement in order to improve the health services. Lastly, the additional set of questions concerning COVID-19 brings more knowledge about the indirect impact of the pandemic in vulnerable settings.

CONCLUSION

The level of knowledge regarding HIV/AIDS transmission, prevention and control in our sample was not satisfactory; some misconceptions about HIV transmission, self-reported risky behaviours and discriminatory attitudes were observed among participants that call for concern and must be addressed promptly. Despite the efforts of the Kenyan government, we found a lack of awareness and limited access to sexual and reproductive health and youth friendly services.

Despite the methodological limitations of the study, due to the hard-to-reach nature of the target population, our findings call for strengthened and promoted HIV/AIDS/STIs interventions targeting adolescents, young adults, and marginalized KPs in urban informal settlements; these include appropriate comprehensive education, exploiting the potential of social media, promoting and facilitating access to free condoms, training health care workers to be more welcoming and developing proximity strategies.

Our research confirms the importance of investigating the health of groups and communities characterized by social marginality and deprived living conditions. Further studies are needed to evaluate the impact on the target population's KAP and relation with healthcare services after the implementation of the suggested initiatives.

Authors' contributions

Conceptualization: MS, MET; funding acquisition: GO, MS, MET; investigation: JMM, RM, EIM, CO, GO; methodology: MS, MET; data curation: SC, MET; formal analysis: MET; resources: GO; software: SC; writing - original draft: SC, FMC; writing - review & editing: SC, FMC, MS, SC, JMM, RM, EIM, CO, GO, MET.

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Conflict of interest statement

The Authors have declared that no competing interest exists.

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Book Reviews, Notes and Comments

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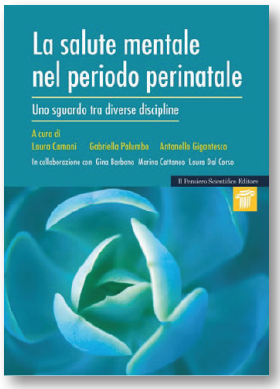

LA SALUTE MENTALE NEL PERIODO PERINATALE
Uno sguardo tra diverse discipline
Laura Camoni, Gabriella Palumbo, Antonella Gigantesco (Eds).
Roma: Il Pensiero Scientifico Editore; 2022.
440 p.
ISBN: 9788849007428
€ 40,00.
[Perinatal mental health: multidisciplinarity as a resource]

Perinatal depression should be considered a public health problem due to its significant prevalence worldwide, in middle- and high-income countries as well as low-income countries [1]. Economic indices, such as the social support network, are associated with the increase in this disorder as can be seen from Italian data thanks to the "surveillance" network promoted by the Istituto Superiore di Sanità (Italian National Institute of Health) [2]. In the years 2019-2022, the percentage of women screened positive for perinatal depression increased by 14 points, from 11.6% in 2019 to 25.5% in the period between November 2021 and April 2022. Such high percentages leave no doubt about the need and priorities to extend screening programs and to have interventions that are effective at the same time to promote a good mother-child relationship and improve the psychological well-being of the future mother as soon as possible, with particular attention to the "family emotional climate". When writing about mother-child relationship it means focusing attention on the relationship already existing during pregnancy since a depressive state, and sometimes the coping mechanisms are not only ineffective but counterproductive (think of the possible use of alcohol to soothe suffering or help sleep), greatly interfere with the state of health of the unborn child as has been known for some time now [3, 4]. Improving the psychological state of the mother, and of family relationships, therefore also means intervening to promote health and prevent any psychopathological problems in the child.

The work of Camoni, Palumbo and Gigantesco (in collaboration with Gina Barbano, Marina Cattaneo, Laura Dal Corso) is characterized as a working tool that responds to the complex problem of this disorder and does so both by clarifying the cultural aspects in depth with a multidisciplinary structure and by providing evidence-based recommendations. For this last aspect, consider the presentation of the work by Professor Jeannette Milgrom, of the Department of Clinical and Health Psychology and Parent-Infant Research Institute (PIRI) of Melbourne University, a pioneer and one of the leading international experts. The book thus originates from a systematic collection of the most prestigious Italian experiences in this sector of health services, universities, and the twenty-year commitment of the researchers of the Istituto Superiore di Sanità also thanks to the provident and visionary Morosini, an innovator in the field of evaluative epidemiology and public health. With a multidisciplinary structure, the various chapters range from anthropology to psychobiology, from genetics to information technology, from suicidology to attachment theory, from psychopathology to economics, and from before conception to the return to working life, thus crossing the perinatal and postpartum periods.

In the first part "Towards parenthood" the themes of the influence that nature, culture, mass media, and stereotyped representations have on the maternal and paternal parental identity are explored. The mother-child and father-child relationship are thus accurately described, from the point of view of attachment theory as well as the difficulties and dangers inherent in the couple when it becomes a family and finally, the relationship between maternal perinatal depression and personality structures. Particular attention is also given to the description of the functioning of same-parent families and the formation of parenting in the aspects relating to the unconscious construction of the parental function relating to sexual orientation, gender identity and the biological or non-biological link with the son.

In the second part "The period of pregnancy", the issues of prenatal attachment are addressed as well as how the mother builds a representation of the child based on sensory and visceral experience. The important recent epidemiological evidence is therefore presented concerning: a) the repercussions that stress and the presence of anxiety or depressive disorders in the mother can have on the mental health of the unborn child; b) the possible biological mechanisms involved in fetal programming and long-term outcomes, including the most current studies on epigenetic mediation; c) the diffusion of depressive and anxiety disorders in the perinatal period, the importance of early screening and treatment of depression in the perinatal period to obtain better health outcomes for the mother, the child and the family unit. The session concludes with the delicate topic of traumatic experiences during childbirth, which can be associated with more severe clinical phenotypes of perinatal depression and reduced levels of resilience.

The third part "The post-partum and the first two years after childbirth" deals with the theme of resilience understood as a continuous process of personal growth,

the adoption of efficient emotional regulation strategies, adaptation and the search for family balance and well-being, also useful for overcoming the vulnerability and daily challenges that pregnancy and postpartum entail. The psychological, genetic and environmental factors that characterize the aetiology of mental disorders in the perinatal period are examined and the theme of violence that takes place within the home, suicide, infanticide and finally, more generally, mourning.

The fourth part "Parenthood and return to work" deals with the topic, sometimes neglected but very relevant, of returning to work after maternity leave and the new technological opportunities to support women by protecting their individual and organizational well-being.

In the fifth part "Taking care of mental health in the perinatal period" there is a broad examination of the treatment of anxiety and depression in the perinatal period from the perspective of the different theoretical approaches (cognitive-behavioural, psychodynamic, psychoeducational both individual and couple, family and group). Updated information on web-based approaches and the use of new technological resources is also provided.

The last part of the volume "An intervention program of proven effectiveness in the practice of national health services in Italy" explores, in particular, the issues being studied by the Istituto Superiore di Sanità. Specifically, it describes a process of taking charge of women with mental health problems in the perinatal period starting from the screening, to continuing with the assessment and the intervention for which efficacy has been demonstrated in practice in Italian services. Finally, ample space is given to a review of the main guidelines on perinatal mental health and the consequences of the economic impact when the problem of the affected parent extends to the family context and especially to the children.

In my opinion, it is a wide-ranging work, which provides the reader interested in really knowing the topic of perinatal mental health going beyond its more usual declinations, an all-round overview of this discipline still in a dynamic evolution.

Franco Veltro
Associazione Italiana per la Diffusione degli Interventi Psicoeducativi in Salute Mentale (AIDIPSaM – APS)
francoveltro@gmail.com

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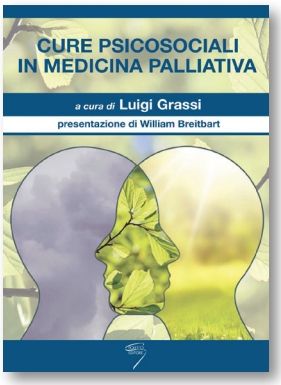

CURE PSICOSOCIALI IN MEDICINA PALLIATIVA
Edited by Luigi Grassi
Milano: Poletto Editore; 2021
256 p.
ISBN: 9788895033921
€ 55,00
[Psycho-social care in palliative medicine]

Palliative care as such is not a discipline but a collection of disciplines including psychology, communication, ethics, and psycho-social rehabilitation that require considerable training and empathy on the part of healthcare professionals.

In such delicate area as palliative care, this publication explores the multiple and pressing needs that must be addressed correctly and competently. This book offers an overview of general aspects, fundamental principles and alleviation in advanced stages of disease and end-of-life situations; it also explores communication and relational strategies, as well as existential and spiritual needs, with a focus on psychological and psychosocial aspects alongside analysis of psychiatric disorders in palliative medicine. An extremely interesting and useful section for professionals discusses psychotherapy intervention with families and the team. The volume closes with special topics such as palliative sedation, suicide, medically assisted euthanasia, pediatric palliative care and alleviation in chronic and degenerative pathologies. On the whole, this book brings together contributions by highly experienced authors and therefore covers the various aspects of emotional, cognitive and behavioral needs that are encountered when facing a palliative care programme.

The same publishing house in 2019 published another book on the subject which is worth mentioning here: *The Italian book of medicine and palliative* care [1]. This is intended for all professionals interested to learn more about this discipline, and to those who already have experience in the field and can use it as a useful source of references and updates.

Anna De Santi
Dipartimento di Neuroscienze
Istituto Superiore di Sanità, Rome, Italy
anna.desanti@iss.it

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DOI: 10.4415/ANN_23_01_14

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 2022. Repurposing food and agricultural policies to make healthy diets more affordable. Rome: Food and Agriculture Organization of the United Nations 2022; 260 p. ISBN 978-92-5-136499-4. The State of Food Security and Nutrition in the World 2022 has been prepared by the FAO Agrifood Economics Division in collaboration with the Statistics Division of the Economic and Social Development Stream and a team of technical experts from the Food and Agriculture Organization of the United Nations (FAO), the International Fund for Agricultural Development (IFAD), the United Nations Children's Fund (UNICEF),the World Food Programme (WFP) and the World Health Organization (WHO). This year's report first presents the latest updates of the food security and nutrition situation around the world, including updated estimates on the cost and affordability of a healthy diet. The report then takes a deep dive into "repurposing food and agricultural policy support to make healthy diets more affordable" through reducing the cost of nutritious foods relative to other foods and people's income, which, in turn, helps countries make more efficient and effective use of – in many cases – limited public resources.

Thinking about the future of food safety. A foresight report. Rome: Food and Agriculture Organization of the United Nations 2022; 158 p. ISBN 978-92-5-135783-5. In this publication, the FAO Food Safety Foresight programme provides an overview of the major global drivers and trends by describing their implications for food safety and for agrifood systems by extrapolation. The various drivers and trends reported include climate change, changing consumer behaviour and preferences, new food sources and production systems, technological advances, microbiome, circular economy, food fraud, among others. The intended audience for this publication is broad – from the policymakers, academia, food business operators, private sector, to the consumers.

UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION (UNESCO)

World heritage glaciers: sentinels of climate change. Paris: UNESCO Publishing 2022; 33 p. ISBN 978-92-3-100557-2. Glaciers are some of the most valuable indicators for understanding climate change. Among the most dramatic evidence that Earth's climate is warming is the retreat and disappearance of glaciers around the world. Closely observing and quantifying this phenomenon is essential to develop effective adaptation responses. Around 18,600 glaciers have been identified in 50 World Heritage sites. These glaciers span an area of about 66,000 km², representing almost 10% of the Earth's glacierized area. Research studies performed with satellite data highlight that these glaciers have been retreating at an accelerating rate since 2000. The most important protective measure to counteract substantial glacier retreat worldwide is to drastically reduce greenhouse gas emissions. If emissions are drastically cut to limit global warming to 1.5°C relative to pre-industrial levels, glaciers in two-thirds of World Heritage sites could be saved. At site level, adaptative measures need to be strengthened to respond to inevitable glacier changes in the near future. These include identifying knowledge gaps and improving monitoring networks, designing and implementing early warning and disaster risk reduction measures, making glaciers a focus of targeted policy, and promoting knowledge exchange, stakeholder engagement and communication. The successful implementation of these measures requires the mobilization of key stakeholders (e.g., governments, civil society, Indigenous Peoples, local communities, and the private sector) to develop sustainable financing and investments, notably through the establishment of an international fund for glacier research and monitoring.

JOINT UNITED NATIONS PROGRAMME ON HIV/AIDS (UNAIDS)

Dangerous inequalities: World AIDS Day report 2022. Geneva: Joint United Nations Programme on HIV/AIDS 2022; 80 p. The world is not on track to end the AIDS pandemic. New infections are rising and AIDS deaths are continuing in too many communities. This report reveals why: inequalities are holding us back. This report calls the world's attention to the painful reality that dangerous inequalities are undermining the AIDS response and jeopardizing the health security of everyone. The report highlights three specific areas of inequality for which concrete action is immediately possible: gender inequalities and harmful masculinities driving HIV; marginalization and criminalization of key populations, and inequalities for children whose lives must matter more than their market share.

Global AIDS Monitoring 2023. Indicators and questions for monitoring progress on the 2021 Political Declaration on HIV and AIDS. Geneva: Joint United Nations Programme on HIV/AIDS 2022; 168 p. This document is a detailed compilation of indicators and a suite of questions on national policies and their implementation designed for use by national AIDS programmes and partners to assess the state of a country's HIV and AIDS response, and to measure progress towards achieving national HIV targets. Countries are encouraged to integrate these indicators and questions into their ongoing monitoring efforts and to report comprehensive national data through the Global AIDS Monitoring (GAM) process. In this way they will contribute to improving understanding of the global response to the HIV epidemic, including progress that has been made towards achieving the commitments and global targets set out in the new United Nations Political Declaration on HIV and AIDS: Ending Inequalities and Getting on Track to End AIDS by 2030, adopted in June 2021,1 and the linked Sustainable Development Goals.

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD)

The COVID-19 Pandemic and the Future of Telemedicine. OECD Health Policy Studies. Paris: OECD Publishing 2023; 180 p. ISBN 9789264484566 (HTML) ISBN 9789264758155 (EPUB) ISBN 9789264420038 (PDF). This report provides an update to a Health Working Paper published in January 2020, which showed that, while care delivered via telemedicine could be both safe and effective, telemedicine services represented only a small fraction of all health care activity and spending. At that time, providers and patients seeking to use telemedicine faced regulatory uncertainty, limited financing and reimbursement, and unclear governance. Just a few weeks later, in response to the unfolding COVID-19 crisis, governments across the OECD adopted broad non-pharmaceutical interventions to limit social contacts and mobility. With in-person care heavily restricted, governments and providers moved quickly to expand remote care services. Consequently, the number of remote consultations skyrocketed. The sudden increase in virtual care has had clear benefits, preserving access to and continuity of care. Yet, it has also laid bare the limits of remote care and added to concerns that some teleconsultations constitute low value care. This report provides an overview of national policies to implement and scale up remote consultations during the COVID-19 pandemic and tries to quantify the resulting boom in the use of telemedicine services; the impact that the massive shift to virtual care has had on health care system performance; and policy priorities for remote care as countries move to a post-acute stage of the COVID-19 pandemic.

INTERNATIONAL LABOUR ORGANIZATION (ILO)

World Employment and Social Outlook: Trends 2023. Geneva: International Labour Organization 2023; 190 p. ISBN 9789220372913 (print) ISBN 9789220372920 (web PDF). This year's Report provides a comprehensive assessment of current decent work deficits and how these have been exacerbated by multiple, overlapping crises in recent years. It analyses global patterns, regional differences, and outcomes across groups of workers. The report provides labour market projections for 2023 and 2024 and presents trends in labour productivity growth, analysing the factors contributing to its decline.

Greening Enterprises: Transforming processes and workplaces. Geneva: International Labour Organization 2022; 223 p. ISBN 978-92-2-032008-2 (print) ISBN 978-92-2-032007-5 (web PDF).
The conduct of enterprises is crucial to the natural environment's well-being and to a just transition. Most enterprises, including small ones, are implementing measures to reduce waste and carbon emissions; in most cases this entails no cost or even a reduction in production costs. This is the first ILO research report focusing specifically on the transition of enterprises. What are enterprises of different sectors and sizes doing to reduce emissions? How are small enterprises in developing countries adapting to the just transition and what was the impact of the COVID-19 crisis on the green transition? What policies promote enterprise productivity and environmental sustainability? These are some of the questions addressed in the report. The report considers the enterprise not only in terms of its production processes but also as a workplace. Through this approach, it has identified a range of measures that enterprises may use to become more environmentally sustainable and that also give workers a role in the process. Sustainable transport, increased resource intensity, waste management, work organization and sustainable food at work are increasingly part of enterprises' efforts to curb their impact on the environment.

WORLD HEALTH ORGANIZATION (WHO)

Improving the lives of people with epilepsy. Technical brief. Geneva: World Health Organization 2022; 43 p. ISBN 978-92-4-006407-2 (electronic version) ISBN 978-92-4-006408-9 (print version). The technical brief presents the key information on epilepsy and recommends actions to policy makers and other stakeholders. Using the concept of levers for change introduced by the Operational Framework for Primary Health Care, it identifies actions on the policy and operational levels that national and local governments, policy-makers, and programme managers across various sectors at national and local levels should take to strengthen services for people with epilepsy using a person-centred approach based on human rights and universal health coverage.

The levers and corresponding actions and resources could also be useful in planning and programming by civil society groups, professional associations, academic institutions, organizations of people with epilepsy and their families and carers, development partners, and global and national funding initiatives. Each country can customize its multisectoral approach to epilepsy according to the setting and its priorities to achieve significant gains for its society and improve the lives of people with epilepsy.

The WHO AWaRe (Access, Watch, Reserve) antibiotic book. Geneva: World Health Organization 2022; 697 p. ISBN 978-92-4-006238-2 (electronic version) ISBN 978-92-4-006239-9 (print version).
Antimicrobial resistance (AMR) is a threat to global health and development and it contributes to millions of deaths worldwide each year. Inappropriate use and overuse of antibiotics are driving an increase in AMR and have a detrimental impact on the effectiveness of these critical medicines. Given the urgency of the threat to human health from AMR, and the many clinical infections for which antibiotics play a lifesaving role, WHO took a pragmatic approach to developing simple, practical guidance on how they should be used. The WHO AWaRe (Access, Watch, Reserve) antibiotic book, produced as an adjunct to WHO's Essential Medicines List (EML), provides concise, evidence-based guidance on the choice of antibiotic, dose, route of administration, and duration of treatment for more than 30 of the most common clinical infections in children and adults in both primary health care and hospital settings. The AWaRe system groups the hundreds of different antibiotics used globally into three simple categories – Access, Watch and Reserve – based on their clinical importance and the risk of their use promoting resistance. The AWaRe antibiotic book provides clear guidance on the choice of antibiotic, formulation, dose, and duration for essential antibiotics for hospital and primary health care settings, including guidance on when not to use antibiotics.

World malaria report 2022. Geneva: World Health Organization 2022; 293 p. ISBN 978-92-4-006489-8 (electronic version) ISBN 978-92-4-006490-4 (print version). Each year, WHO's World malaria report offers in-depth information on the latest trends in malaria control and elimination at global, regional, and country levels. The report highlights progress towards global targets and describes opportunities and challenges for curbing and eliminating the disease. This year's report includes 3 new sections on: global and regional initiatives launched in 2021 and 2022; global malaria surveillance and country-level case studies on surveillance systems assessments; and research and development. The report also includes an expanded section on threats to malaria control, with a focus on the declining effectiveness of insecticide-treated mosquito nets.

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Authors should deal responsibly and effectively with security issues that might be raised by their papers (see: Statement on Scientific Publication and Security *Science* 2003;299:1149).

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Please consult the guidelines (https://researchintegrityjournal.biomedcentral.com/articles/10.1186/s41073-016-0007-6).

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Articles in journal

Bozzuto G, Ruggieri P, Molinari A. Molecular aspects of tumor cell migration and invasion. Ann Ist Super Sanità. 2010;46(1):66-80. doi: 10.4415/ANN_10_01_09

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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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