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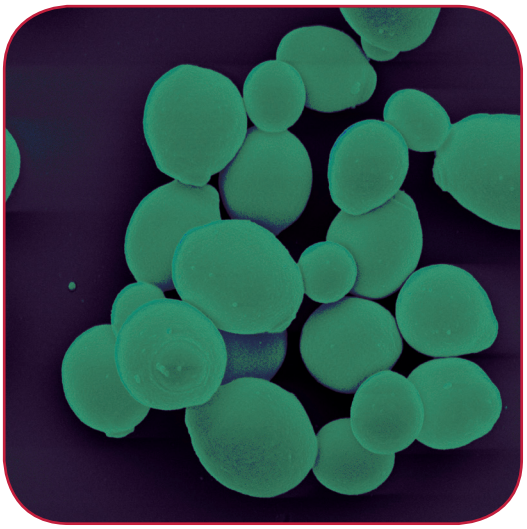

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LETTER

Priority coding for scheduling pain medication consultations: a simple tool supporting both efficient allocation and sustainability of use of resources

Key words
- sustainability
- chronic pain
- appropriateness

Advances in medicine lead to a fragmentation of knowledge, so that for many pathologies patients require visits with medical specialists. The role of the general practitioner (GP) is therefore increasingly one of "gatekeeper" to the specialized hospital world. This situation can create inequities in access to medical care, especially in waiting times, that negatively impact patients with lower education levels and fewer economic resources [1]. Limited access to pain therapy is particularly impactful, as untreated pain has substantial clinical, social and economic implications and reduces patients' autonomy [2].

In Italy, access to some specialist consultations and procedures is managed via a priority system that sets maximum waiting times according to four grades of clinical severity [3]. However, pain therapy consultations are not included in this system. This deficit was recently addressed by Friuli Venezia Giulia, a northeastern Italian region of about 1.1 million inhabitants. In 2018, the region implemented a set of priority criteria for prescribing specialist visits for pain medication [4]. GPs score priority by combining data from a patient's subject pain assessment (on a numerical rating scale), the DN4 scale (douleur neuropatique 4), and opioid doses, and then either send the patient to the emergency room or prescribe a specialist visit to be held within 10, 30 or 180 days. The combination of these three evaluation scales establishes the priority assignment for patients.

Despite the implementation of this system in Friuli-Venezia Giulia, the agreed waiting times have not always been respected. Since it was not possible for the region to simply increase the availability of outpatient specialist appointments, it was decided to examine GPs' use of the priority system and the appropriateness of their prescriptions. To this aim, an online tool was implemented in which the pain therapist indicates, for every examination, whether the priority indicated by the GP is correct and, if not, what should have been the correct score. This tool allows administrators to monitor use of the priority system; a first analysis of the data revealed a concordance rate of around 55%. Moreover, the tool helps identify GPs who routinely err in their assessments.

We believe that the tool implemented by Friuli Venezia Giulia for pain medication management can be adapted to other outpatient services whose public access is subject to priority criteria, to help ensure correct application of the criteria and to identify GPs who would benefit from training.

An added benefit is that the tool will help direct training activities to those local areas (aggregates of GPs) where they are most needed, relieving better-trained GPs from having to undergo unnecessary training. In a proactive perspective of continuous improvement in the quality of basic care, without unnecessarily increasing the offer of hospital services, we recommend that other regional health care services adopt a similar approach for the efficient allocation and sustainability of use of medical resources.

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EDITORIAL
Why research in medicine needs a step back?

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Discovery needs luck, invention, intellect - none can do without the other
Johann Wolfgang Goethe, a discoverer himself

Financial Times in 2008 published an apparently provocative article with the title "Drug research needs serendipity" [1]. In this visionary article the authors state that in the last two decades the Pharma industry did not introduce in the market new drugs that showed some effectiveness against major diseases, despite the huge investment; and they tried to propose some explanation for this. In their own words "What went wrong? The answer, we suggest, is the mis-measure of uncertainty, as academic researchers underestimated the fragility of their scientific knowledge, while pharmaceuticals executives overestimated their ability to domesticate scientific research"; and again "Medical research is particularly hampered by the scarcity of good animal models for most human disease, as well as by the tendency of academic science to focus on the 'bits and pieces' of life – DNA, proteins, cultured cells – rather than on the integrative analysis of entire organisms, which can be more difficult to study". I guess all the readers of these few sentences should realize that pharmaceutical industries have failed in their investment and the authors of the article provide an explanation: the academic researchers have overestimated their findings and the headquarters of the Pharmas were wrongly pretty sure to manage for a perfect control of what came from the scientific research. One paradox of this low effectiveness of the new drugs is that a new field in pharmacology is to discover the off targetting of the known drugs through their side effects [2] and this is leading to think about the use of drugs designed to be specific for a disease for the treatment of other diseases. But this is not surprising being the vast majority of the drugs that pioneered the pharmacology of neurologic diseases thought for other uses [3]. From the whole of this dreadful awareness some doubts may originate on the future of the current research, that is going without breaks in the same direction. However, the authors provide a possible attempt to adjust the sight, to course correct the way, all in all to change the strategy of research in medicine in order to get to results that really may change the health and therefore the fate of the whole humanity. They wrote that in the past the majority of the discoveries were done mostly through serendipity. In fact, serendipity was a fairly common occurrence in science. However, some information is needed on the origin of the word "serendipity". The term was coined by Horace Walpole on January 28th of 1754 in one of the letters written to his friend Horace Mann. Walpole coined the term influenced by the reading of *The three princes of serendip* by the Persian poet Kushrau. In the story three princes are expelled from Serendip (then Ceylon and in modern day Sri Lanka), and along their travels they make discoveries due to sheer luck. The story of how Walpole had access to this literary piece is in itself full of serendipitous anecdotes and difficulties, however what is important to know for our purposes is that "serendipity" originally referred to discoveries by good luck or happy accidents [3].

We had clear examples in the past of discoveries that really changed the natural history of devastating diseases, such as infectious diseases, through incidental findings, that we can well re-call amazing moments of serendipity. The most known example of this is the discovery of penicillin. Fleming was studying *Staphylococcus* when one of his culture plates had become contaminated and developed a mold that created a bacteria-free circle. Then he found within the mold a substance very active against the vast majority of the bacteria infecting the human beings [4]. However, the Fleming's example is the most known but for sure not the only one we can provide. One other example, while much less known, is that of the 1931 Nobel Prize Otto H. Warburg. He left overnight some plates containing tumor cells seeded in culture medium in the laboratory's incubator with the usual 37 °C and O_2/CO_2 atmosphere. The morning after he realized that the O_2 dropped down within the incubator, expecting to find all the cells dead due to the hypoxic conditions; while the cells were pretty well and after an initial astonishment he thought that probably cancer cells did not need oxygen to live. After a series of experiments his conclusion was that differ-

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ently to normal cells cancer cells do not need oxygen for their metabolism, while they fermentate sugar producing lactate, thus contributing to extracellular acidification. Warburg has become a mentor for scientists thinking that tumor acidity is a common phenotype of cancers, and that an antiacidic therapy is at list to be implemented with the current anti-cancer approach. The Warburg's discovery on tumor metabolism convinced me that it is crucial in science to have a look to what is occurring with an open mind; not thinking that what apparently looks a failure of your experiment is actually a failure, but hopefully something that may represent a discovery [5, 6]. Therefore, we should identify serendipity as part of the scientific process. With this approach my group of research got to nonmainstream discoveries, including the evidence that proton pump inhibitors (PPI) have a clear anti-tumor effect and improve the effectiveness of other drugs as well, in turn leading to clinical studies supporting the use of PPI in treatment of patients with different cancers [7-19]; that tumor cells face off the low nutrient supply of tumors by feeding on other cells, thus becoming cannibal [20-24], and sharing this activity with unicellular microorganisms [23], together with sharing specific cannibalism-related genes [24]; that cancer cells under the pressure of a very hostile microenvironment release a huge amount of nanovesicles that are spilled over the body, representing the major determinants of tumor metastasis [25-38].

Max Planck said "Science progresses not because scientists change their minds, but rather because scientists attached to erroneous views die, and are replaced" and Otto Warburg used the same words when he realized the lack of acceptance of his ideas. Probably, we should re-think to research in medicine with a mind sufficiently unbiased from mainstream infrastructures, probably paying more attention to potential "unexpected discoveries". Probably, serendipity should be considered an essential part of the scientific method and, particularly, a tool for progress, and it should be taken as a rational approach to scientific practice, an attitude, and a happy accident. We should not think to serendipity as merely a luck, or chance, or happenstance; rather to a process in which a fortunate event leads to a discovery of a new solution for a problem unexpectedly.

It is hard to talk about a nonmainstream approach in *Research in medicine* [39], but this article was written with the hope to give only a contribute aimed at triggering a new deal in science in which to think and watch will represent a successful strategy.

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COMMENTARY
Epidemiological research as a driver of prevention: the Sibaté study

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Abstract
Although asbestos exposure and risks can be prevented, only five countries in Latin America have banned asbestos, including Colombia. Beginning in 2011, a collaboration between the Istituto Superiore di Sanità in Italy and Universidad de los Andes in Colombia was established, bringing together relevant expertise aiming to improve our understanding of the asbestos problem. An important result of this collaboration was a recently published study conducted in Sibaté, Colombia, a municipality where an asbestos-cement facility has operated since 1942. The evidence collected suggests the presence of a mesothelioma cluster in Sibaté. Landfilled zones with an underground layer of friable asbestos were also discovered in the urban area of the municipality. The importance of this type of collaboration can go beyond understanding the impact of asbestos at the local level, which is crucial, and may also contribute in solving unanswered questions of the problem in countries that banned asbestos decades ago.

Key words
- asbestos
- Colombia
- international cooperation
- mesothelioma
- global public health

INTRODUCTION

Asbestos is among the 120 agents classified by the International Agency for Research on Cancer as human carcinogens [1]. However, and this makes asbestos different compared to most carcinogens, asbestos exposure can be prevented because it is economically and technically feasible to substitute the material with non-carcinogenic agents, which is especially evident for both construction and automotive products (two of the major uses of asbestos in the world). Hence, more than 67 countries have banned asbestos (IBAS, International Ban Asbestos Secretariat; 2019. www.ibasecretariat.org.). In Latin America, only five countries have banned asbestos: Argentina, Chile, Honduras, Uruguay, and Colombia (IBAS, International Ban Asbestos Secretariat; 2019. www.ibasecretariat.org). Currently Latin American countries represent 10% of the world asbestos consumption [2].

A decision of the Supreme Court of Brazil in November 2017 in theory banned asbestos, but the decision has not been fully implemented and is facing legal challenges (IBAS, International Ban Asbestos Secretariat; 2019. www.ibasecretariat.org.).

In Colombia asbestos use has been historically concentrated in supplying products for two sectors: construction and automotive. In construction, there have been 5 major asbestos-cement facilities, three of these owned by the same company, located in Sibaté, Cundinamarca (circa 1942), Cali, Valle (circa 1944), and Barranquilla, Atlántico (circa 1944) (Eternit, www.eternit.com.co/historia). The other two asbestos-cement facilities are located in Manizales, Caldas, owned by different companies (circa 1967 and 1982) (Etex, www.etex.com.co/acerca-de-etex; Toptec, www.toptec.com.co/index.php/quienes-somos). Among the asbestos-cement products distributed in the country there are corrugated sheets, pipelines, and tanks for water storage. All the asbestos-cement facilities previously described are still in operation, and they all claim they have finished the technology reconversion that allows them to manufacture asbestos-free construction products. In the automotive sector, an asbestos friction product facility located in Bogotá, DC, still produces asbestos containing brake pads, linings, and blocks, as well as asbestos-containing clutch disks (circa 1960) (Incolbest, www.incolbest.com/la-empresa/historia/). There is also an active chrysotile asbestos mine in Campamento, Antioquia. The recent asbestos ban in Colombia comes in force in January 1st, 2021, when the use, production, distribution, mining, and exports of asbestos mineral

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and asbestos containing products will cease [3].

The negative legacy of asbestos containing products distributed in Colombia has not been fully quantified and understood. Between 2009 and 2016 asbestos consumption in Colombia ranged between 11 907 and 24 822 tons per year [4]. Information about how much asbestos has been distributed in the country comes mostly from industry itself: more than 5 million homes have asbestos-cement products, 300 million square meters of corrugated asbestos cement sheets have been installed, and more than 3 million vehicles use asbestos-containing friction products [2]. Over 75 years of operation of the asbestos industry in the country, 11 million tons of both corrugated asbestos cement sheets and pipelines have been introduced in the country [5]. The distribution of all these asbestos containing products (ACP) in the country has created a concerning risk of asbestos exposure for the entire population, resulting in a complex technological and economic challenge for the proper removal and disposal of these ACP.

THE RATIONALE FOR INTERNATIONAL SCIENTIFIC COOPERATION

A collaboration between researchers from Istituto Superiore di Sanità (ISS) and Universidad de los Andes (Uniandes) in Bogotá started in 2011 in the frame of the Latin American Chapter during the annual conference of the International Society for Environmental Epidemiology (ISEE). The asbestos issue has been identified as a key topic of common interest for bilateral scientific cooperation, because it represents a national and global public health threat in both countries. For a detailed discussion of this point the reader is referred to Marsili & Comba, 2013; Marsili *et al*., 2014, Marsili *et al*., 2017 [6-8]. In particular, the Italo-Colombian collaboration was motivated by the major past asbestos production and use in Italy, which caused heavy environmental and health impacts currently requiring public investments for environmental remediation and health prevention actions, and the history of asbestos production and use in Colombia, which represents an emerging public health threat and an environmental concern. In 2015, during the ISEE annual conference held in Brazil, we jointly organized a symposium on "Prevention of asbestos-related disease in Latin America", also with the contributions from Brazilian researchers. Since 2015, we have been strengthening our cooperation to make available scientific evidence of asbestos risks in Colombia with the goal of fostering the adoption of prevention initiatives shared with affected communities [2, 9] and scientifically support the prohibition of the use of asbestos in Colombia.

In 2017 we decided to formalize the scientific collaboration through a Memorandum of Understanding (MoU) between ISS and Uniandes (September 2017-September 2019) aimed to "Foster, coordinate, develop and promote scientific and technological multidisciplinary research, in order to contribute to research and outreach activities that pertain to environmental and public health issues of common interest between the two participating organizations, namely the prevention of asbestos related disease". The two partners agreed in recognizing the mutual benefits of this cooperation, providing an institutional frame to the collaborative research, supporting training initiatives and exchanges of scientific information and documentation, fostering participation in research projects and co-authorship in scientific publications.

The recently published study concerning the Sibaté Municipality, one of the five locations of asbestos-cement industries in Colombia, [4] is a relevant example characterizing this international scientific cooperation, relying on the mutual recognition of knowledge and skills of the two cooperating partners, who work together sharing corroborated scientific methodologies in public health, environmental and social sciences. The common objective is creating awareness on an emerging public health issue in Colombia for the benefit of asbestos exposed communities and the improvement of environmental quality in the country.

THE SIBATÉ STUDY

Sibaté is a municipality located 25 km southwest from Bogotá, the capital of the country. An asbestos-cement facility located 5 km north of the urban area of Sibaté has been in operation since 1942, Ramos-Bonilla *et al*., 2019 [4]. For years inhabitants from Sibaté have been complaining that a large number of asbestos related diseases (ARD) were being diagnosed in the town. An interdisciplinary group of researchers from Universidad de los Andes (Colombia), the Istituto Superiore di Sanità (Italy), the French National Research Institute for Development (IRD), Fundación Santa Fe de Bogotá (Colombia), and researchers from the Universities of Rome (La Sapienza), of Turin, and of Bologna, conducted the study that aimed to both determine if the evidence supported the health complaints of the community, and to identify potential asbestos exposure sources in the town [4].

Initially a survey to identify potential cases diagnosed with ARD was implemented door-to-door, in four neighborhoods that people reported were built in the proximity of landfilled areas that might contain asbestos. Three hundred and fifty five surveys were completed, and 29 self-reported mesothelioma cases were identified [4]. For validation purposes, it was possible to obtain copy of the medical records for 17 of these cases. All these cases had lived at some moment of their lives in Sibaté. A panel of 5 physicians from Fundación Santa Fe de Bogotá (i.e., 3 pathologists, a thoracic surgeon, and a radiologist), and a pathologist from the University of Rome La Sapienza examined the medical records following the guidelines of the Italian Mesothelioma Registry (ReNaM), and classified 15 cases as Certain Malignant Pleural Mesothelioma (MPM), one as Probable, and one as Not Mesothelioma [4]. Thirteen of the certain MPM cases were diagnosed between 2008 and 2017, and only two had experienced some type of past professional exposure. The young age of diagnosis was a unique and concerning characteristic, Ramos-Bonilla *et al*., 2019 [4]. Among these 13 MPM cases, 9 were diagnosed while living in Sibaté. Based on these 9 cases, the age-standardized MPM incidence rate for Sibaté is 3.1 x 10^5 persons-year for males, and 1.6 x 10^5 persons-year

for females. Both figures are among the highest in the world compared to those reported by IARC [4].

Regarding potential sources of asbestos exposure, a soil sampling campaign was conducted in areas identified as potential landfilled zones. In three of the four points where soil samples were collected, an underground layer of friable asbestos was identified, at a depth ranging between 35 and 110 cm. It is estimated that the landfills were created between 1975 and 1990 [4]. On top of landfilled zones, a public school and sports facilities were built. The history, extension, and frontiers of this asbestos layer require further investigation. The evidence suggests that a cluster of MPM is present in Sibaté. It is urgent to elaborate a risk management plan for the town, including procedures for conducting excavations that could disrupt the asbestos layer.

When considering the possible contribution of the publication of the Sibaté study on the ongoing process of transition of the Colombian economy towards a model of economic development that does not require the use of asbestos, some comments are warranted. First of all, an economic model subsuming the presence of asbestos is economically viable for industry only if it does not imply recognition and quantification of environmental, health and social costs. Once the latter are estimated, the economic "convenience" of asbestos used is severely challenged. This was originally understood by the Nordic countries in Europe, namely by Sweden, when the use of asbestos was prohibited in 1982 (see for a historical reconstruction of that event the paper by Järvholm and Burdorf, 2015 [10]). In a few words, in the mid Seventies the available scientific evidence was sufficient for understanding that the use of asbestos was simply not sustainable in a society that would not ignore its costs in terms of health impact and environmental cleanup.

Other countries, like Italy, did not reach this decision so timely as the Nordic countries. The Act that introduced the prohibition of the industrial use of asbestos came in 1992, after a long political debate, and, surprisingly enough, there was a peak of asbestos consumption in the last decade before the ban [11, 12]. This is believed to be the reason why we still experience an increasing rate of mesothelioma mortality in the Italian male population [13].

Even if the political decision to terminate asbestos use was not so timely and sharp, it reflected a societal change of perception about the sustainability of asbestos use, including the rejection of the false theory of its "*safe use*". A major contribution to this shift of paradigms came from a set of epidemiological studies published in the Eighties that brought to light two unexpected problems. First, the health impact of asbestos in the manufacture of asbestos-cement products was much worse than previously suspected [14]; second, there were unexpected excesses of asbestos-related diseases, namely of mesothelioma, in occupations where such impacts were not previously suspected, like in railway carriages production and repair [15] and in non-asbestos textile industries, because of the widespread use of jute bags, previously containing asbestos, to pack rags and other textiles to be recycled [16].

It is now too early to assess the contribution that the Sibaté study may have in the design of strategies to address the negative legacy of asbestos products distributed in Colombia, something that is required by the law that banned asbestos. This was the first study that analyzed the potential impact of asbestos at the population level, and could guide future studies in other Colombian communities settled in the area of influence of asbestos facilities. Some aspects of the Sibaté study, like the relatively young age of the mesothelioma cases and the highly demanding cleanup intervention needed for reducing risk of exposure in Sibaté, might turn out to motivate the public opinion in a most relevant way.

Even so, in this unavoidable uncertainty, one final notation appears to be warranted. Two recent Italian studies [17, 18] have shown that the (past) use of asbestos-cement byproducts in paving roads and courtyards is causally associated with the occurrence of mesothelioma in relatively young subjects without any occupational exposure. The Sibaté study has the potential to throw further light on this issue by addressing the etiological role of asbestos-cement byproducts and asbestos residues used to fill depressed areas and drain water intrusions of an adjacent reservoir, activities that were conducted within the urban area of the municipality. Thus the Sibaté study, besides assessing the health impact of asbestos in an industrial area of Colombia, might also contribute to a scientific debate ongoing in countries that have banned asbestos several decades ago.

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 the conduct and findings of this study.

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Additive manufacturing of reconstruction devices for maxillofacial surgery: design and accuracy assessment of a mandibular plate prototype

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Abstract

Additive manufacturing (AM) presents unique opportunities for medical applications and in particular in maxillofacial surgery for developing patient specific implants. The quality assessment of additive manufactured products is an essential aspect for the real introduction in health services. In this framework, the purpose of the present study is to investigate the possibility of developing prototypes of mandibular plates as preoperative surgical planning models, by verification of design, analysis of internal structure integrity and evaluation of the effects of variables involved in AM processes. A PolyJet three-dimensional (3D) printing system is used in the study due to its very fine resolution.

The computer aided design (CAD) models of the implants were converted to stereolithography (STL) file formats in different STL conversion resolutions and then printed using commercial prototyping polymers to observe the effect of model resolution. Finite element analysis (FEA) was conducted to study the capability of the designed mandibular plate to support the involved biomechanical loads. Micro-computed tomography (micro-CT) analysis was performed to verify the dimensions and the internal defects of the printed objects, considering that the presence of defects can affect the quality and compromise the final performance. Results were analyzed to understand the effect of the 3D printing process flow conditions on the obtained prototypes. Relative error in reference to the CAD models mainly evidenced the difference in resolution due to STL files and the effect of the design. No anomalies and defects were detected inside the evaluated samples.

Key words

- additive manufacturing
- 3D printing
- micro-computed tomography
- mandibular plate
- maxillofacial surgery

INTRODUCTION

Healthcare innovations require a great attention in the assessment of all the aspects related to the quality of devices and applications in order to consider the introduction in health services.

Maxillofacial surgery (MFS) includes a series of different subfields, such as craniofacial corrective surgery, orthognathic surgery, maxillofacial trauma, reconstructive surgery and maxillofacial oncological surgery [1, 2]. In general, the main aim of MFS surgery is to restore the normal anatomical structure and function after a trauma, an oncological resection or a facial malformation.

As evidenced by some authors [3-5], 20-42% of all facial bone trauma are mandibular fractures. Indeed, many studies have been carried out to investigate the efficacy of different techniques in the treatment of mandibular defects and fractures [6], proposing mandibular plates as osteosynthesis devices for the stabilization, reconstruction and rigid fixation of cranio-maxillofacial fractures. Different types of reconstructive plates and screws, usually manufactured by traditional processes, and mainly made of metallic alloys, have been proposed and compared [6-8], also considering systems with multidirectional screw placement [5]. However, it has to be taken into account that the reconstruction of mandibular defects has to restore not only facial aesthetic form but also functions of speech and mastication [9, 10]. Moreover, the main benefits in the use of reconstruction systems are achieved by a right choice of the device in terms of geometry, thickness, and dimensions, fitting the specific clinical patient conditions that, in the case of mandibular applications, can be various and not

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always respected using series-manufactured products. Consequently, some devices have to be adjusted during implantation by surgery manipulation in order to fit the clinical case and/or the fracture position. This step introduces critical aspects and high risk factors that are, in some cases, correlated only to the surgeon experience and, thus, difficult to control and improve.

For these reasons, the use of computer-aided design/computer-aided manufacturing (CAD/CAM) method, which includes virtual surgical planning and rapid-prototyping procedures for the design and manufacture of the customized surgical devices [11, 12], is gaining a lot of attention, presenting great applicability in MFS sector. This type of approaches changes, in some cases completely, the surgeons' method to work, hospital processes in the management of a patient, and clinical procedures. It could represent a change from large-scale centralized production to local production models, saving shipping time by allowing production at the site of use.

In particular, additive manufacturing (AM), also called 3D printing [13], presents several applications in surgery, primarily in maxillofacial sector (50%), to produce anatomic models (71.5%), surgical guides (25.3%), and implants (9.5%) [14]. The production of mandible preoperative models by AM, to simulate reconstruction plates prior to mandibular resection or to better visualize the effect of surgery operation, was found to be a useful technique [15]. Indeed, the use of mandible models allows reducing the operating time [16] and improving the esthetic outcome with respect to conventional mandibular reconstruction [17]. Most of the preoperative approaches consider only the creation of mandible model by rapid prototyping in order to determine the pre-bending and the position of serial reconstruction plates [17-19]. At the same time, custom designed implants are becoming the best option for reconstruction of craniofacial defects [20]. The combination of mandible models and custom reconstructive plates, also designed using the original external cortical bone, results promising to simulate surgery with respect to conventional methods [12, 21].

The cost of CAD/CAM method for mandibular reconstruction is recovered by gains in terms of surgical time, quality of reconstruction, and reduced complications [22]. Indeed, Resnick *et al.* [23] evidenced that virtual surgical planning and 3D printing of surgical splints are becoming the standard of care for orthognathic surgery and this option is less expensive than standard planning.

AM could have the potential to allow the realization of patient-matched or specific custom made implantable devices, based on patient anatomy and pathology, but respecting all quality and regulatory aspects, usually checked for traditional medical devices. At the state of art, it is a good solution for preoperative models, useful in the validation phase of new designs in short time, to optimize the device design also adapting it to the patient anatomy. Imaging devices are providing new capabilities to the AM industry by converting the image stacks into solid models that can be used for implant or device production [13, 24]. Moreover, worldwide, the sale of AM products and services is expected to grow rapidly and the industry is forecasted to be worth over $ 6 billion by 2019 [25]. When hospitals and healthcare systems will start to include reimbursement for 3D printing performed in clinical context for patients, it will be probably an explosion of applications by healthcare professionals. Thus, it is necessary to assess the products, in particular for the adoption in public health systems.

A current limitation for AM is that the methodology for the assessment of additive manufactured products is not well defined. This aspect is relevant for the manufactures, for the assessors but also for considering the introduction of 3D printing services and products in healthcare systems. Various aspects should be considered in the quality assessment: the design of the CAD model, the quality of representation of the original model by the STL files [26], the direction of printing [26, 27], the selection of the printer and the printing material. Thus, the accuracy [28], the repeatability and the reproducibility for additive manufactured products represent critical aspects that need to be investigated in details.

In this context and considering the potential impact of AM in MFS, the purpose of the present study was to investigate the appropriateness of a PolyJet system in developing mandibular plates prototypes mainly usable during the design validation phase and as preoperative surgical planning models. PolyJet has one of the finest resolutions among the current commercial 3D printers and is capable of producing application-ready parts.

The present study includes the design of selected models by CAD, the realization of them by AM PolyJet system using two different commercial materials (i.e. Stratasys VeroBlue (VB) and Stratasys VeroClear (VC)). Finite element analysis (FEA) and micro-computed tomography (micro-CT) analyses were performed to verify the appropriateness of the design. FEA was carried out to verify the capability of the designed mandibular plates to support the involved biomechanical loads and the micro-CT to measure the actual dimensions and to capture the internal structure of the printed objects. Design modifications were elaborated in order to evaluate possible improvement in the accuracy and resolution of 3D printed objects, taking into account that steps such as converting CAD models to STL format cause loss in information and affect the final product quality.

METHODS

Mandibular plate models: design and FEA

A plate model for medial and lateral fixation (L-shape, dimensions 22×10 mm^2, Model1) was designed using Solidworks 2016. Dimensions and geometry were defined considering many commercially available devices usually applied in MFS for mandibular reconstruction [29]. A second design (Model2) was created properly modifying Model1, in order to verify the possibility to improve curved printed surfaces (*Figure 1*). *Figure 1* illustrates the CAD models, where the main differences are localized in the critical sections that are marked as A, B and C (see also *Figure 2*). Model1 has smaller sections than those in Model2 (relative difference of about

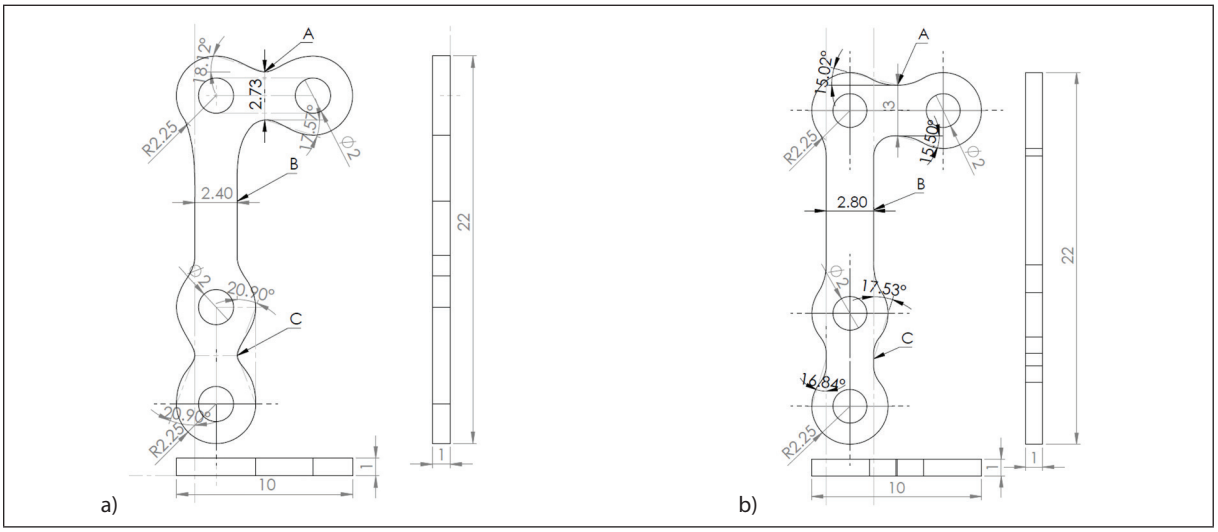

Figure 1
CAD models with dimensions in mm: a) Model1, b) Model2. A, B and C indicate the critical sections which differ between Model1 and Model2.

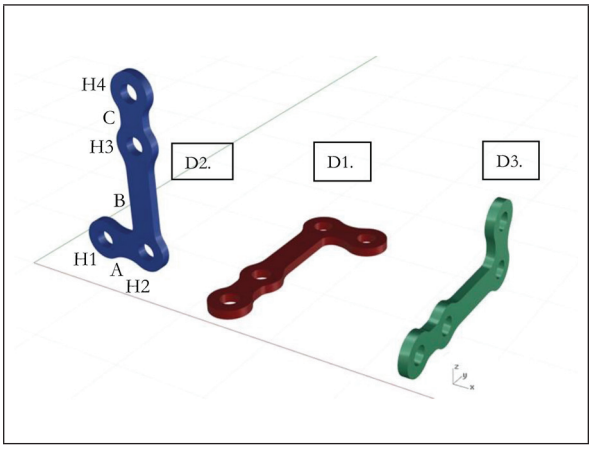

Figure 2
Printing orientations (D1, D2, D3) and points of interests (A, B, C, H1, H2, H3 and H4) marked on the left plate model.

10% in A and 16% in B). The external edges in A and C sections were designed with a difference in curvature angles of about 2°-3° in A and 3.5°-4° in C. Model2 has more straight edges in the critical sections, particularly in C. Two STL files were exported with different resolutions – "Fine" and "Coarse" – for each design, in order to analyze possible modifications in printed objects based on the STL file resolution. "Fine" and "Coarse" are two preset options in SolidWorks software for exporting files to STL format, although higher resolution is possible by using "Custom" settings. The Fine model has higher number of polygons, about 50% more that the Coarse one: Model1-fine has 1212 polygons; Model2-fine has 1144 polygons. Static mechanical simulations were performed by ANSYS – Mechanical. The objects were designed as prototypes for design validation and with a possible application in preoperative surgical planning on mandible models. Therefore, the simulated entity of loading was defined considering the application in a normal mandible under physiological occlusal loading [30-32]. Different types of meshing were implemented with particular attention to the area close to the plate holes. The meshing with fine parameters was selected for curvature (Model2; 2136 elements, 12056 nodes) and the considered loading and constraints conditions were:

- Loading-case1: force of 100 N, compression loads on plate upper surface; plate back surface fixed.
- Loading-case2: force of 100 N, compression loads on screws positions; plate back surface fixed (*Figure 3*).
- Loading-case3: displacement of 10 mm/min in the middle area (edge) of the upper surface [33]; end of plate back surfaces fixed.

The material properties considered in simulations were: Young Modulus: 2500 MPa, density: 1.18 g/cm^3 and tensile strength: 60 MPa, which were obtained from the datasheets of the commercial materials selected for the mandibular plates printing, i.e. Stratasys VeroBlue (VB) and Stratasys VeroClear (VC).

3D Printing process and materials

A Stratasys Object30 Pro PolyJet system was used to print the developed models. Two different commercial materials, i.e. VB and VC, were employed [34]. The printer has a declared resolution of 600 dpi along X- and Y-axes and 900 dpi along Z-axis and an accuracy of 0.1 mm with a minimum layer thickness of 16 µm for the VC material and of 28 µm for other commercial materials.

VB was used only for Model1, whereas VC, with glossy surface refinement, was used for both models. Three different printing directions were considered, i.e. D1, D2 and D3 (*Figure 2*) in a preliminary evaluation to define the optimal printing orientation.

Micro-computed tomography analysis

3D micro-CT scans were performed using the Skyscan 1172, a high-resolution scanner.

The objects analyzed in this study were acquired by oversize scansions due to the length of the samples. The

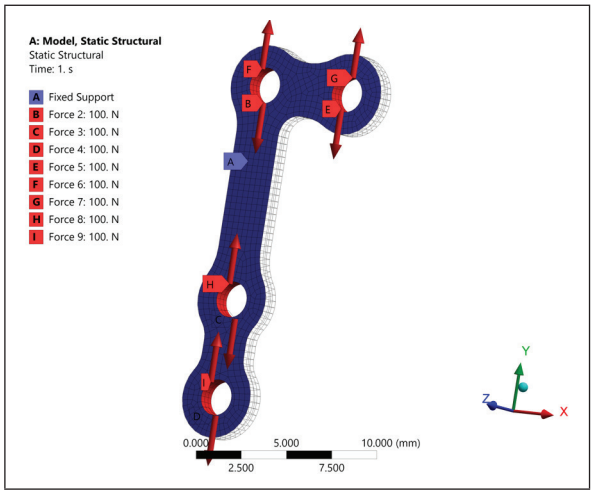

Figure 3
FEA Loading-case2, Model2.

main acquisition parameters were the following ones: source voltage 44 kV, source current 222 µA, image pixel size 12 µm, rotation step 0.3° for the objects printed with VC material. For Model1 printed by VB, the parameters are 47 kV, 212 µA, 13.6 µm, 0.4° ; 44 kV, 222 µA, 5 µm, 0.1° for the Coarse and the Fine models, respectively. For all acquisitions, no filter was used. A dedicated software, SkyScan NRecon, was used to reconstruct the cross section images (slices) of the objects. The slices were evaluated by the software Skyscan CT-Analyser to obtain the 3D models and morphometric parameters. The volume rendering program, Skyscan CTvox, was used to display the 3D object from reconstructed slices.

RESULTS

First, the geometry of the designed models of mandibular plates (Model1 and Model2) was optimized to avoid stress concentrations and ensure the uniformity of stress distribution by performing simulations by FEA. In particular, the applicability of the designed plates as prototypes to be used in preoperative surgical planning on mandible models was evaluated, considering loading and constraints conditions related to a normal mandible under physiological occlusal loading. The considered different loading cases underline the main conditions to investigate the plate models resistance, in particular around holes that have to fit screws.

In *Figure 3* and *Figure 4*, FEA results related to the loading-case2 (compression on screws positions, *Figure 3*) for Model1 and Model2 are reported, respectively. The stress distribution and deformation are similar for both designs, in all the considered loading cases, and only those obtained for Model2 are reported in *Table 1*, where the maximum values of von Mises stress, principal stress, shear stress and the total deformation are compared for the three different loading cases. The magnitude of stress is appropriate for the PolyJet material used in printing the specimens. Loads applied in loading-case2 (compression on screws positions) create stress concentration around holes. *Figure 4* shows total deformation in Model1 and von Mises stress in Model2, for loading-case2. Loading-case3 (displacement in the middle area of the plate upper surface) is useful to investigate the stress distribution correlated to a displacement applied on the plate, simulating the effect of the bending test on the plate [33]. Stress concentration was not observed in the designed models under these loading conditions. Thus, the designed models and the considered materials are appropriate to be manufactured by additive manufacturing.

For the manufacturing process, the printing orientation was investigated in a preliminary evaluation. Three different printing directions, i.e. D1, D2 and D3 (*Figure 2*), were considered in order to identify the optimal one. D2 and D3 configurations resulted inappropriate for this type of geometry, for several reasons: it was necessary to use a great amount of support to print the objects; the objects presented some discontinuities due to the support material; the time necessary in printing

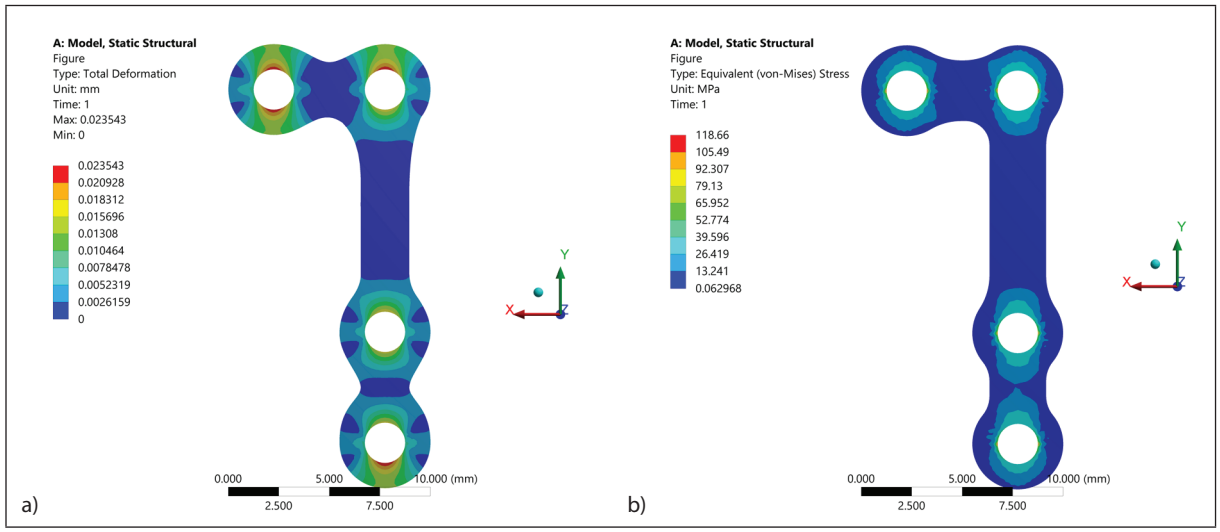

Figure 4
Loading-case2: a) Model1 – Total deformation; b) Model2 – von Mises stress.

Table 1
Finite element analysis (FEA) results for Model2

FEA	Model2			
Loading case	**Max. von Mises stress (MPa)**	**Max. principal stress (MPa)**	**Max. shear stress (MPa)**	**Total deformation (mm)**
Case1	1.8	0.2	1.0	0.0006
Case2	118.6	130.8	64.1	0.0235
Case3	29.7	41.1	15.3	0.1704

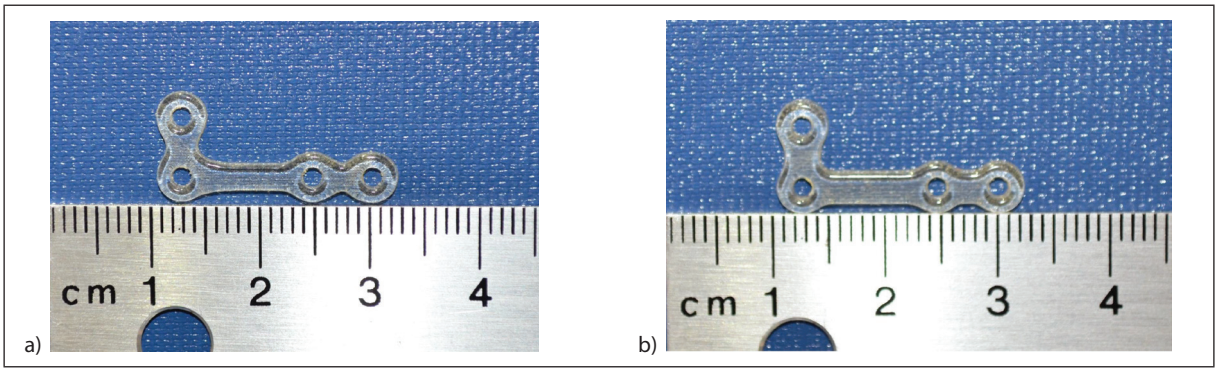

Figure 5
Models printed for STL Fine, configuration D1: a) Model1, b) Model2.

was about twice that required in the D1 configuration. Thus, in the study only the models printed in D1 configuration (*Figure 2*), where the object is printed flat on the 3D printer build plate, are discussed.

Model1 was first printed using Coarse and Fine STL files and VB material in printing direction D1. It was observed that the dimensions of the part at the backside were not accurate as the front side. The same Model1 printed with VC material, that has a specified printing resolution better than VB, did not present this problem at the back surface when printed in the same configuration. As an example, *Figure 5* shows Model1 and Model2 prototypes printed with STL Fine and configuration D1, using VC material, evidencing the respect of the designed shape and dimensions.

In order to evaluate the printing quality, the obtained dimensions were measured on models reconstructed by micro-CT analysis at points of interest marked in Figure 2 (H1, H2, H3 and H4), as well as the object thickness. A representative set of micro-CT images is shown in *Figure 6*, where slices reconstructed by micro-CT in the area of the hole H3 for Model1_VB, and in the B-area for Model2_VC are reported. The entire object (Model1_VC) is reconstructed in *Figure 6c* for measurement. The graph in *Figure 7* shows the relative error calculated in reference to CAD models' dimensions (three repetitions to consider spatial snap resolution) obtained by software measurements.

Morphological 3D analysis of printed samples was performed in reference to the selection, B-volume, for

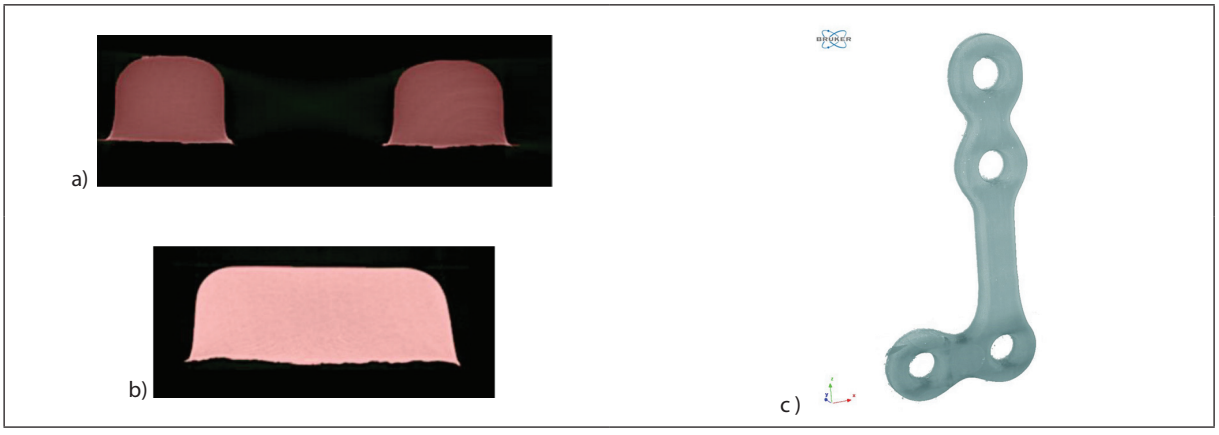

Figure 6
Micro-CT analysis: a) Model1_VeroBlue, H3 selection; b) Model2_VeroClear, B-selection; c) Model1_VeroClear reconstructed.

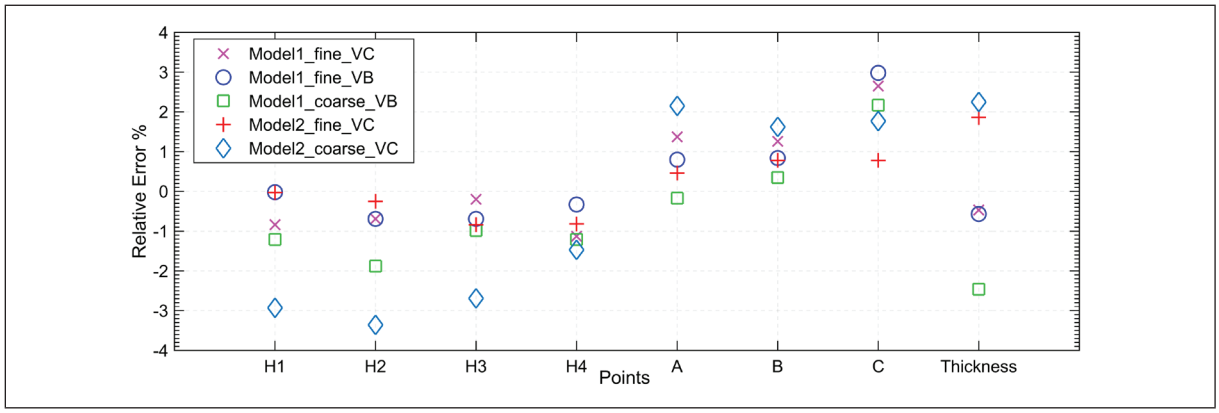

Figure 7
Relative error percent in points of interests for Model1 and Model2, Fine and Coarse, materials VB and VC; reference CAD dimensions.

a number of reconstructed layers equivalent to a distance in z of 0.360 mm (*Figure 2*). *Table 2* reports the morphological data for the samples printed by using VC material. In the analyzed models, the enclosed porosity is not observed and it well represents the uniformity of PolyJet material, whereas the surface porosity values are mainly correlated to the residual support material around the models that is detectable by micro-CT analysis. The structural thickness values were comparable, demonstrating the uniformity of the structure and the analysis did not detect voids inside the solid parts.

DISCUSSION

In planning a surgery operation or in developing a specific device to optimize the medical intervention, it is necessary to consider the variability introduced by the use of a technology, e.g. to find if it is possible to reproduce the same operation at the same conditions. Thus, the suitability of an additive manufacturing design software and printing system in developing prototypes of mandibular plates was investigated, by verification of design, internal structure integrity and evaluation of the effects of variables involved in AM processes. The used PolyJet is considered appropriate to also reproduce anatomic details due to its fine resolution [35, 36] and is commonly used to develop prototypes and preoperative surgical planning models. It is worthy to underline that the printing direction influences the result and represents a quality aspect that has to be controlled, also requiring different process times. Moreover, this is one of the aspects that are correlated to cybersecurity challenges and to the difficulty of defending conventional intellectual property of AM products [37]. For this reason, three different directions were considered, where the D1 print orientation resulted in the best quality part for both investigated models. The directions D2 and D3 showed surface discontinuities in the part.

The preliminary simulations performed in this study showed that the plate designs, as well as the model materials properties, can be considered appropriate for the realization of mandibular plates prototypes (*Figure 3* and *Figure 4*, *Table 1*). Indeed, FEA is a useful method to predict properties of 3D printed objects in reference to design and materials [38].

Micro-CT analysis was carried out to verify the internal structure of the printed objects and the accuracy of the designed geometry. Micro-CT is considered as one of the major tools for the product quality assessment and for the quality control of AM products and materials [39]. In fact, the FDA in the guidance about technical considerations for additive manufactured medical devices [40] indicates micro-CT as appropriate methodology for the verification of geometry, morphology, and some performance characteristics of printed products.

The micro-CT results obtained for the investigated specimens showed no anomalies or defects inside the printed objects for both the materials (VB and VC) used for printing (*Figure 6*). However, a different curvature along edges was recognized around holes of the objects printed with VB material. For the same material, the printed plates presented an amount of residual support that affects the dimensional measurements obtained by micro-CT because the support was made of the VB material as the plate.

Table 2
Morphological 3D Analysis, Volume-B (Δz = 0.360 mm) for Model1 and Model2 printed with VeroClear material

Model	Object volume	Structural thickness	Closed porosity P_{cl}	Open porosity P_{op}
	(mm³)	(mm)	(%)	(%)
Model1_fine	0.922	0.372	0.0000	45.26
Model2_fine_r1	1.148	0.367	0.0000	26.70
Model2_fine_r2	1.130	0.370	0.0001	52.39
Model2_coarse	1.083	0.371	0.0000	80.36

Morphological 3D analysis performed for the printed objects allowed to investigate their internal structure and to identify possible defects. The distribution of the used material during the printing process was uniform in all printed samples. The micro-CT analysis showed that for the designed models the critical aspect is correlated to respect the flat upper surface. Indeed, for the Model1 printed with VB the flat upper surface presented a curvature not included in the design, but due to the material deposition during printing. By using VC material, this aspect was less evident even if present. The use of VC instead of VB improved the back surface of the objects and it allowed obtaining a surface as well-defined, accurate and smooth as the upper one. Thus, the used PolyJet materials seem to be similar in resolution, considering Model1 printed with both materials and STL Fine.

Misalignment due to the position of samples during the acquisition by micro-CT could affect dimensional measures, even if the possible error is minimal. For this reason, during reconstruction, care was taken to limit this effect. Besides this limitation, measurements checked by DataViewer software are representative and useful to investigate the accuracy of printed objects and the conformity to the designed CAD models.

The difference in accuracy between STL files was more evident for the Model2_VC. As shown in *Figure* 7, the difference was greater around holes with a maximum value of error of about 3% in H2, whereas it was less evident for Model1_VB. In general, the relative dimensional error reaches a maximum value of -3.36% in H2 for Model2_coarse_VC and 2.98% in C for Model1_fine_VB. The negative value means that the hole of the printed object is smaller than the designed dimension. For this kind of object design, characterized by curvatures, the major complexity is associated with dimensions and shapes of holes designed for precision fit with screws. In the evaluation of the error value, residual amount of the support material needs to be accounted for because it could be included in the obtained measurements due to its similar gray value to the object material. Minimum relative errors, i.e. 0.02% and 0.03 %, were obtained at the hole H1 for Model1_fine_VB and for Model2_fine_VC, respectively.

By the analysis of dimensions in the sections A and C, where the curvature was modified, it is possible to underline the improvement in design from Model1 to Model2. In Model2, these sections resulted with a relative error less than that revealed in Model1, with a maximum in C, where for Model1_fine_VC the relative error was 2.65% and for Model2_fine_VC the relative error was 0.78%. At these locations, the improvement in designs was more effective.

The thickness of the models was well preserved on the average and presented the maximum relative error in the Model1_coarse_VB (i.e. -2.46%) and the minimum value in Model1_fine_VC (i.e. -0.47%). The thickness of Model1 specimens resulted in general smaller than the designed thickness, vice versa for Model2 (*Figure* 7). A similar trend is detected for the holes dimensions that resulted smaller than the designed dimensions and vice versa for the sections A, B, C. The length resulted with an average relative error of 0.6%, considering all printed objects.

Finally, the repeatability of print quality of the objects is an aspect that requires more investigation. In tests conducted for Model2, i.e. a repetition of the STL Fine analyzed in direction D1 (Model2_fine_r1, Model2_fine_r2), it was noted that there are dimensional and morphological differences in printed objects correlated to the same model and printed at the same time, in the same conditions (*Table 2*). Further investigations are required to quantify the uncertainty in printing an object multiple times using the same printer and printing conditions.

All of the aspects underlined and discussed in this paper are relevant in considering the feasibility of using AM for the realization of prototypes, to perform a verification of a design and in particular to use printed objects in a medical context, also only in a preoperative surgical step.

CONCLUSIONS

In this study, two CAD models of a specific mandibular plate design were printed by an additive manufacturing system using two different materials to investigate the appropriateness of the AM technique in developing prototypes of mandibular plates for the validation of new designs and potentially as components in preoperative surgical planning models. The obtained design, the internal structure integrity and the effects of some AM process variables were investigated.

No anomalies and defects inside the printed samples structure were identified by micro-CT scanning for all models and both materials used. The shape of the models was well preserved and the surface of the specimens resulted in uniform finish, particularly in the case of VC material. Relative errors in reference to the CAD models obtained for all models showed in particular the difference in resolution due to STL files and the effect of the design. The micro-CT analysis showed that a critical aspect is to preserve the flat upper surface of the designed models.

Thus, this study demonstrates the feasibility to use AM for the realization of mandibular plates' prototypes, and preoperative surgical planning models, by identification of dimensional errors, material defects, the model that best fits the initial geometry designed and the effects of printing parameters that can compromise the use of 3D printed products.

The quality assessment of additive manufactured products is essential for medical applications. The methodology used for the assessment is applicable to others surgical planning models, to the manufacturers of custom-made devices and in general to assess additive manufactured medical applications in health services and national healthcare systems.

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Prevalence and attitudes to HIV testing among adults visiting public outpatient clinics in Rome: results of the MeDi (Measuring health Disparities in HIV prevention) survey. Part 1

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Abstract

Background. It is estimated that, in Italy, 12 000-18 000 (11-13% of 130 000) HIV-infected subjects are not aware of their serostatus. People in this condition may visit the healthcare system multiple times without being diagnosed. If tested on one of these occasions, they could modify their high-risk behaviours and benefit from treatment, factors that reduce HIV transmission. In Italy, no data on HIV testing in the general population are available so far and little is known on the relationship between socioeconomic determinants (at individual and neighbourhood levels) and testing uptake.

Methods. A large anonymous survey was performed in 2012-2014 on more than 10 000 individuals 18-59 years old who underwent 21 public ambulatories in Rome to determine the proportion of subjects tested for HIV and factors related to testing uptake. Subjects' socio-demographic characteristics, sexual orientation, number of sexual partners, HIV risk behaviour, HIV testing uptake were collected by a self-administered questionnaire. Level of area deprivation was measured at the postal code level by the index of social disadvantage (ISD). Multilevel Poisson regressions were carried out to take heterogeneity between clusters (post code and clinics) into account.

Results. Among people participating in the study, 58.1% of subjects self-reported to have been tested at least once for HIV. Those who had one high risk behaviour for HIV-infection were 11% more likely to test than those not reporting any, and subjects who had had a STI (sexually-transmitted-infection) in the past were 12% more likely to test than those who had not had a STI. However only 44% (54% among subjects aged 18-35 years) of those with self-reported risks of contracting HIV had been tested at least once in life. This percentage increases, as expected, with the level of education, but, even so, about 40% of university educated subjects self-reporting risks of contracting HIV had never undergone an HIV test.

Conclusions. This study highlights that, while the percentage of subjects tested is even higher than observed in other western nations, only 44% of subjects, self-reporting risks of contracting HIV, had tested at least once in life and about 40% of university educated subjects self reporting risks of contracting HIV had never tested.

Key words
- HIV
- HIV testing
- SEP
- deprivation
- Italy
- urban

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BACKGROUND

Anti-retroviral therapy (ART) has resulted in substantial reductions of HIV/AIDS-related morbidity and mortality, which allowed not only to achieve but also to exceed, in 2000, the AIDS targets of Millennium Development Goal 6 [1]. Building on those achievements, UNAIDS set the ambitious target of ending the AIDS epidemic by 2030 [2].

Mathematical modelling suggests that to achieve this target it is necessary that, by 2020, 90% of people living with HIV know their status, 90% of people on treatment achieve viral suppression and the number of new infections is reduced by 75% [2].

Different strategies have been proposed to increase the proportion of persons living with HIV that are aware of their status. In the United States, the Centers for Disease Control and Prevention proposed in 2006 to test all individuals aged 13 to 64 years coming into contact with the health system, at least once during life independent from any risk assessment [3]. In Europe, it has been proposed to test routinely individuals presenting with an "HIV indicator disease" such as infections that share with HIV a common mode of transmission (subjects presenting with symptoms indicative of sexually transmitted infections or with reported high-risk behaviour) or whose onset is favoured by HIV-induced immunodeficiency, and any other medical condition associated with an undiagnosed HIV prevalence greater than 0.1% [4, 5]. However, the European MSM Internet Survey, found that in a sample of Italian men who had had sex with another man (MSM) in the last year, 28.9% had never tested for HIV [6]. A European pilot study conducted in 2010, which analysed, among others also Italian data, found that only 56.3% of subjects with STI had tested for HIV at least once lifetime [7], despite most of them being likely to see a doctor years before the diagnosis of HIV. Furthermore, a study carried out in 2011, found that only 37.4% of injecting drug users had tested for HIV in the previous 12 months [8].

Health risk behaviour that have an impact on HIV transmission and health literacy are often socially patterned [9], with low socio-economic position (SEP) individuals being more likely to engage in high risk sexual behaviour. Unsurprisingly, low SEP individuals and families are more likely to concentrate in deprived neighbourhoods which have also less resources and services, so neighbourhoods can thwart individual's likelihood of HIV testing and compound their disadvantage beyond personal circumstances [9, 10]. But the effect of neighbourhood context on testing uptake has received little attention in Italy, so far.

We carried out a survey of the general adult population living in Rome and accessing some outpatient clinics of the local health units, between January 2012 to November 2014 to determine the proportion of subjects tested for HIV and factors related to testing uptake. Specific aims of the survey were: 1) to estimate the proportion of subjects who underwent HIV testing in the population living in Rome who attended one of the outpatient clinics included in the study; 2) to assess whether the participation in HIV testing varied across segments of the population defined by categories of risk for STI; 3) to evaluate the association between participation in HIV testing, subjects' socio-demographic characteristics and the socioeconomic deprivation of their area of residence; and 4) to explain geographical heterogeneity in HIV testing if present. To the best of our knowledge, no other data on HIV testing in the general population are available in Italy, so far.

MATERIALS AND METHODS

MeDi (Measuring health Disparities in HIV prevention) survey

The MeDi survey was conducted in 2012-2014 as part of the Italian Ministry of Health HIV/AIDS projects to provide baseline information on existing levels of positive health behaviours and HIV related risk factors, through a self-completion questionnaire, against which changes could be monitored.

Setting

The study was carried out within public outpatient clinics based either in hospitals or local health units to retrieve a sample as representative as possible of the general population and at the same time preserve subjects' privacy.

Sampling strategy

A list containing the number of accesses (medical examinations, clinical and diagnostic tests) for the outpatient clinics of the local health units (ASL) in the city of Rome for the year 2009, was made available by the regional health authority. From this list, clinics providing only specialistic care or which were located outside of the metropolitan area were excluded, thus leaving a total of 81 outpatient clinics. Out of them, 41 were selected and 21 agreed to participate in the study. For each of them the number of questionnaires to be collected was determined on the basis of the number of accesses in 2009 to obtain a sample of 20 000 questionnaires (the size of the sample was determined to show changes in HIV prevalence and enable detailed age, sex and multi-factor analysis, an objective not addressed in this paper).

Study population

All men and women, aged 18 to 59 years, resident in the Roman metropolitan area, attending the selected clinics from January 2012 to November 2014 were handed in a self-completion questionnaire by *ad hoc* trained study personnel (14) present in each clinic at given scheduled times.

Questionnaire

The MeDi questionnaire was developed by the authors building on previous research (an Italian version of the questionnaire is contained in the Supplementary Material available online) [11, 12]. Participants were asked details about their socio-demographic characteristics (gender, nationality, age, duration of stay in Rome, postal code of the area of residence, educational level, occupation, marital status, duration of stable relationships, health exemption tickets and pregnancy status),

their sexual orientation, the number of sexual partners they had had in the last six months, over the past five years and lifetime and were asked to indicate whether they had ever been tested for HIV. Those ever tested were asked to report the number of tests taken and the year of the testing.

HIV risk behaviour was evaluated by asking subjects whether they had ever been in one or more situations at high risk for HIV transmission. Two lists of hypothetical situations were provided. The first one included: "I have used injective drugs", "I have had sex under the effect of alcohol or drugs", "I have had anal intercourse without a condom", "I have given or received money in exchange for sex"; the second one included: "I have had multiple sexual partners over the same period", "my partner has had multiple sexual partners over the same period", "I have not used a condom during the last intercourse with a casual partner", "I have not used a condom during sexual intercourse with a HIV positive partner".

Participants were also asked whether they had ever suffered from chlamydia, gonorrhea, syphilis, herpes genitalis and genital warts.

Pilot study

The questionnaire and the study procedures were field tested on the first 300 subjects enrolled in the study to investigate questions comprehensions/acceptability and train the 14 field workers (i.e. random call back, check that the returned data had the required high standards).

Data management and statistical analysis

All questionnaires were registered in *ad hoc* database and the dataset was cleaned and ready in December 2016.

Crude, age and gender specific, and age-standardized percentages of ever having performed HIV testing were calculated using the 2012 European population provided by Eurostat as reference [13].

Fisher exact chi square tests were computed to investigate the association between HIV testing uptake and possible determinants/predictors variables such as socio-demographic characteristics, sexual behaviour, STI in the past, and different levels of social disadvantage in the area where the participant was living (see below for description). Poisson regression models were used to produce unbiased prevalence ratios estimates [14]. A test for linear trend was carried out, if necessary, across strata of ordinal categorical variables, including them as "continuous" variables in a Poisson model. Poisson regressions, with stepwise selection, were carried out to identify independent predictor variables from those with a p-value < 0.20 at the univariate analysis.

Within and between clusters (post code and clinics) variances were investigated using a multilevel framework. Since the variance at the postal code level was not significant in a null non –hierarchical multilevel model, in which subjects simultaneously belonged to outpatient clinics and postcodes of residence, a model with a random term at the clinic level only was carried out (i). To this model were added in the following order: individual (ii) and contextual level covariates (iii) as identified with the stepwise procedure, the random slopes for contextual variables (iv) and the cross level interaction terms between deprivation and strata of age, sex, sexual orientation and SEP (v). Only significant effects (from log-likelihood ratio test) were retained. We assumed that: outpatient clinics were exchangeable with the remaining random sample of outpatient clinics, individuals were independent within clusters. Similar analyses were also carried out excluding people who reported to have performed HIV-tests only because of pregnancy or blood donation in order to identify diagnostic testing. A secondary analysis was carried out to investigate if uptake of HIV testing in subjects at high risks of contracting HIV varied by age-class, sex, strata of educational attainment and employment categories. Subjects reporting at least one risk behaviour and/or had had a STI in the past were defined as "high risk"; those not reporting a risk behaviour and/or a STI were considered "low risk".

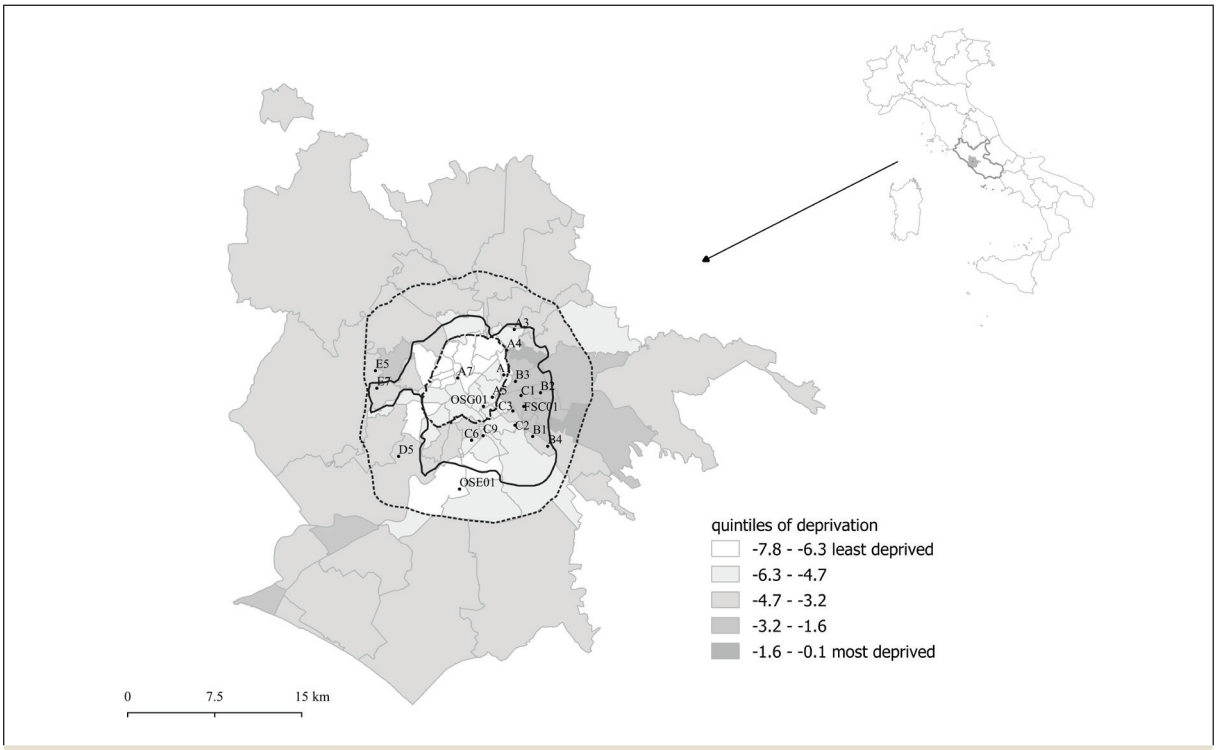
Statistical analyses were carried out in Stata 13 [15].

Index of social disadvantage (ISD) by postal codes

The ISD [16] was used to provide a measure of deprivation in Rome. This index was developed by the "Ufficio Metropolitano di Statistica" and the "Ufficio di Statistica di Roma Capitale" to produce a statistical report on the Roman metropolitan area and it is obtained by summing the unweighted z-scores for the following census variables: unemployment, employment, youth concentration and schooling [16]. Since survey data were measured for postal codes while social deprivation indicators collected by the Census Office were available for census sections and the two geographies are non-overlapping, the ISD deprivation index was re-aggregated from census section to postal codes polygons by areal interpolation in "Quantum" GIS (QGIS) [17]. Postal codes define geographical areas, which may be potentially heterogeneous in terms of social and physical characteristics, but divide the city of Rome into areas of a similar population size (median = 15 977; iqr = 12 539; 19 393) which means that the analyses do not concentrate on small population groups and do not ignore the different experiences of people living in densely inhabited areas. The ISD was categorized in quintiles of frequencies (population weighted).

Characteristics of outpatient clinics

Clinics were classified according to whether they were located within a hospital or not (district facilities) and to whether the amount of prescriptions provided by all clinics combined in the year 2009 was above or below the median as: small size clinics within district facilities (annual amount of prescriptions below 12 000 in 2009); medium size clinics within district facilities (amount of prescriptions of 12 000 or greater); and hospital based outpatient clinics. Clinics were also classified, according to the proportion of prescriptions exempted from the co-pay fee for low income in the year 2009 to the total number of prescriptions for the same year, in tertiles of frequency (population weighted) of co-pay fee for low income as: clinics with a proportion of co-pay fee exemption for low income below 1.1%; between 1.1 and 1.4%; and of 1.4% or more (see *Figure 1*).



id	ASL	Name	Postal code	Type of clinic	Volume of prescription exempt from the co-pay fee for low income	N. of questionnaires			
						Handed in	Data entered	Valid	Refusals
A1	A	SA	00198	medium size within district	low	952	952	793	135
A3	A	LM	00141	medium size within district	low	243	183	179	46
A4	A	NM	00162	low size within district	low	409	409	391	77
A5	A	LZ	00185	low size within district	medium	471	471	447	63
A7	A	CN	00186	low size within district	low	178	178	163	51
B1	B	CR	00174	medium size within district	high	1551	1549	1346	436
B2	B	BR	00171	medium size within district	high	1354	1059	1015	261
B3	B	CB	00157	medium size within district	high	850	850	802	126
B4	B	AN	00174	medium size within district	medium	411	338	308	131
C1	C	SC	00176	medium size within district	high	2096	2080	1941	414
C2	C	DN	00179	medium size within district	low	399	391	365	42
C4	C	NU	00181	medium size within district	medium	776	569	562	127
C6	C	NM	00145	low size within district	low	572	572	467	138
C9	C	ML	00147	low size within district	low	121	121	99	58
D4	D	CN	00164	low size within district	high	451	301	300	0
D5	D	CR	00148	low size within district	high	352	352	350	34
E5	E	BC	00167	low size within district	low	524	524	516	112
E7	E	MN	00166	low size within district	low	344	197	195	74
FSC	C	VN	00177	hospital based	medium	1675	984	824	299
OSE	C	EG	00144	hospital based	low	1323	974	745	400
OSG	D	GN	00184	hospital based	low	353	182	180	92

ASL: Local Health Authorities as defined in 2009 (some of these were aggregated in 2015). Clinics were classified as: small size clinics within district facilities (annual amount of prescriptions below 12 000 in 2009); medium size clinics within district facilities (amount of prescriptions of 12 000 or greater); and hospital-based outpatient clinics. Prescriptions exempted from the co-pay fee for low income in the year 2009 to the total number of prescriptions for the same year: - clinics with a proportion of co-pay fee exemption for low income below 1.1%, - between 1.1 and 1.4% and - of 1.4% or more.

Figure 1

Index of social disadvantage (ISD) by postal codes in the metropolitan area of Rome. The dotted line indicates the city ring road – GRA, the dashed line the railway ring and the solid line the green band (urban area subject to traffic restrictions for polluting vehicles). The area delineated by the ring road is the one in which the study was carried out. The points represent the clinics participating in the study. The table at the bottom of the Figure shows the characteristics of the outpatient clinics included in the study (participating in the MeDi survey between January 2012 and November 2014).

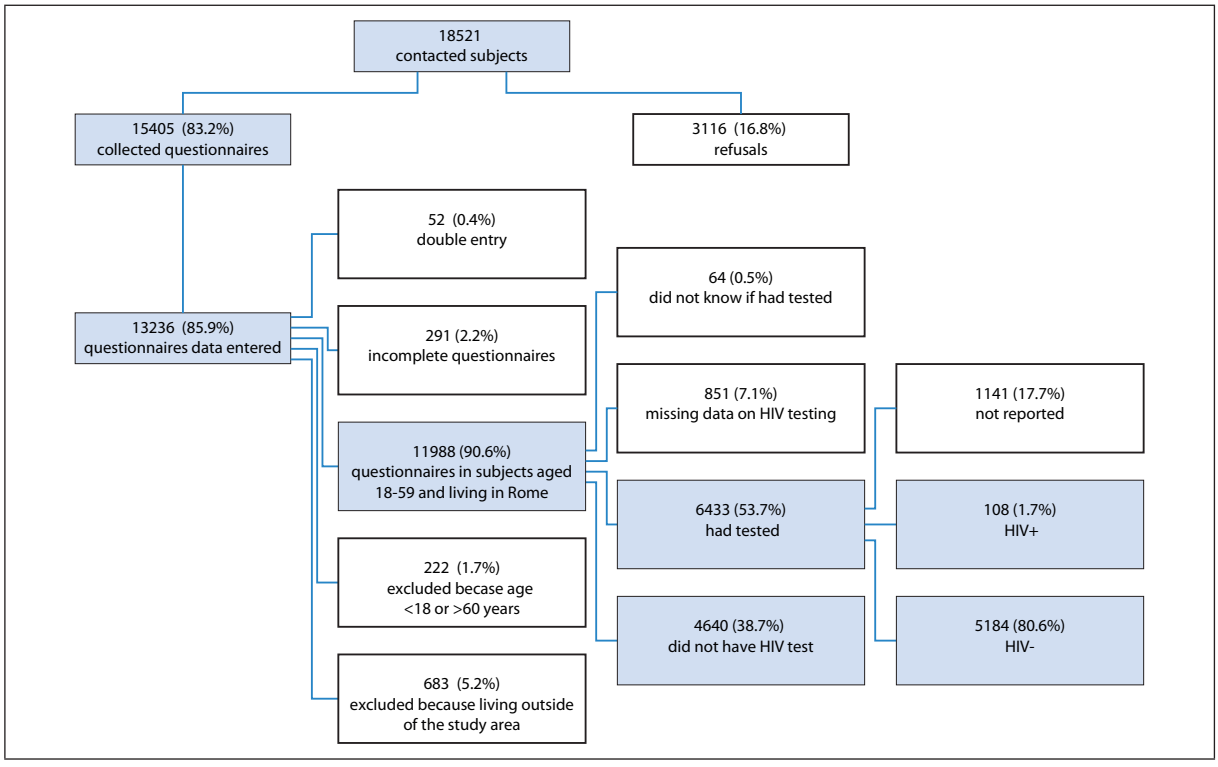


Figure 2
Flow chart of the study population: men and women aged 18-59 living in Rome and participating in the MeDi survey between January 2012 and November 2014.

Ethical issues and approval from the Ethics Committee

The survey was approved by the Ethics Committee of Istituto Superiore di Sanità, Rome, Italy n. CE/12/338, date 7/5/2012. Each subject was also asked to formally consent to participate in the study.

RESULTS

Response rate

As shown in *Figure 2*, 18 521 subjects were contacted and 3116 (average response rate: 83.2%) refused to participate. Of the 15 405 questionnaires that were handed in, some were not returned, others were returned blank or incomplete. Overall, 13 236 valid questionnaires were data entered and out of them 11 988 met the survey eligibility criteria (see *Figure 2*).

Non respondents were asked about their age and sex. The age distribution of subjects who did not participate in the study was comparable to that observed in non participants. Non participants were significantly more likely to be males than participants (non participant males 41.7%; participant males 33.0% $p < 0.001$).

Subjects' characteristics

Figure 2 shows the flow of the study population involved in the MeDi survey between January 2012 and November 2014. Out of 11 988 subjects, 6433 (53.7%) had undergone HIV testing at least one time ever (1973 men and 4409 women); 64 (0.5%) did not know whether or not they had undergone HIV testing and 851 (7.1%) did not answer. These subjects were excluded from analyses, leaving a total of 11 073 subjects (3650 men and 7320 women) aged between 18 and 59 years (median 38; iqr: 30-46). Characteristics of the study population evaluated are reported in *Table 1*. Fifty-one point two percent of them had a high school diploma, 15% were unemployed by at least one year and 15.9% were exempt from paying the health ticket because of low income. Seventy-five point six percent of subjects were heterosexuals, 74.1% were in a stable relationship, 50.1% were married or cohabiting, 80.0% had had up to 3 sexual partners in their life, with a median of 1 partner in the last 6 (iqr: 0-10) months and of 2 (iqr: 0-511) lifetime.

Prevalence of HIV testing for any reason

Overall, crude prevalence of having performed at least one HIV-testing was 58.1% (95% CI: 57.2%; 59.0%), as well as that age standardized (95% CI: 53.8; 56.4).

Subjects who underwent HIV testing were more likely to be 35-49 years old (67.4%), women (60.2%), homosexuals/lesbians (67.4%), highly educated (61.8%), in a stable relationship (62.5%) and had had a STI in the past (66.6%) (*Table 1*).

Respectively, 59.3% and 43.8% of subjects who reported having had one or more high risk behaviours for HIV tested at least once for HIV, compared to 59.0% of those not reporting any. In particular, the chance to test for HIV was lower for subjects who had high risk sexual relationships, compared to those not having it. At the area level, prevalences of HIV testing for any reason increased at increasing levels of deprivation

Table 1
Socio-demographic characteristics of the 11 073 men and women (aged 18-59 years) participating in the MeDi survey from January 2012 to November 2014 by having ever/never performed a HIV test (for any reason and after excluding tests for pregnancy and blood donation); Rome, Italy

	All		Any reason			Excluded pregnancy and blood donors			Blood donors			Pregnancy		
	Freq.	col (%)	Freq.	row (%)	p	Freq.	row (%)	p	Freq.	row (%)	p	Freq.	row (%)	p
Individual level variables														
Gender					<0.001			<0.001			<0.001			<0.001
Female	7325	66.2	4409	60.2		3262	44.5		1080	14.7		370	5.1	
Male	3650	33.0	1973	54.1		1085	29.7		888	24.3		0	0.0	
Not reported	98	0.9	51	52.0		38	38.8		13	13.3		0	0.0	
Years of age					<0.001			<0.001			<0.001			<0.001
18-34	4317	39.0	2100	48.6		1394	32.3		691	16.0		86	2.0	
35-49	4946	44.7	3334	67.4		2413	48.8		881	17.8		237	4.8	
50-64	1777	16.1	978	55.0		561	31.6		407	22.9		45	2.5	
Not reported	33	0.3	21	63.6		17	51.5		2	6.1		2	6.1	
Marital status					<0.001			<0.001			0.316			<0.001
Single	4251	38.4	1952	45.9		1216	28.6		732	17.2		38	0.9	
Married/ cohabiting	5545	50.1	3707	66.9		2632	47.5		1023	18.5		281	5.1	
Separated/ widowed	1152	10.4	714	62.0		495	43.0		208	18.1		49	4.3	
Not reported	125	1.1	60	48.0		42	33.6		18	14.4		2	1.6	
Educational attainment					<0.001			<0.001			0.001			0.065
Low	1415	12.8	778	55.0		568	40.1		200	14.1		31	2.2	
Medium	5665	51.2	3196	56.4		2123	37.5		1038	18.3		205	3.6	
High	3921	35.4	2423	61.8		1670	42.6		731	18.6		132	3.4	
Not reported	72	0.7	36	50.0		24	33.3		12	16.7		2	2.8	
Occupation					<0.001			<0.001			<0.001			0.003
Unemployed	1663	15.0	937	56.3		702	42.2		230	13.8		39	2.4	
Employed	4808	43.4	3014	62.7		2047	42.6		941	19.6		190	4.0	
Self-employed	1627	14.7	927	57.0		604	37.1		318	19.6		39	2.4	
Other	2926	26.4	1530	52.3		1017	34.8		483	16.5		99	3.4	
Not reported	49	0.4	25	51.0		15	30.6		9	18.4		3	6.1	
Sexual orientation					0.006			<0.001			0.013			0.130
Heterosexual	8368	75.6	4853	58.0		3336	39.9		1463	17.5		283	3.4	
Homosexuals/ lesbians	239	2.2	161	67.4		121	50.6		40	16.7		2	0.8	
Bisexual	249	2.3	146	58.6		103	41.4		43	17.3		11	4.4	
Other	320	2.9	203	63.4		153	47.8		46	14.4		7	2.2	
Not reported	1897	17.1	1070	56.4		672	35.4		389	20.5		67	3.5	
Stable partner					<0.001			<0.001			0.160			<0.001
No	2691	24.3	1205	44.8		736	27.4		465	17.3		25	0.9	
Yes	8209	74.1	5130	62.5		3574	43.5		1493	18.2		342	4.2	
Not reported	173	1.6	98	56.7		75	43.4		23	13.3		3	1.7	

Continues

Table 1
Continued

	All		Any reason			Excluded pregnancy and blood donors			Blood donors			Pregnancy		
	Freq.	col (%)	Freq.	row (%)	p	Freq.	row (%)	p	Freq.	row (%)	p	Freq.	row (%)	p
Number of partners in the last 6 months					<0.001			<0.001			0.025			<0.001
0-1	9076	82.0	5536	61.0		3824	42.1		1648	18.2		348	3.8	
2-3	768	6.9	381	49.6		237	30.9		142	18.5		14	1.8	
4-5	146	1.3	64	43.8		39	26.7		25	17.1		0	0.0	
5+	184	1.7	83	45.1		66	35.9		17	9.2		0	0.0	
Not reported	899	8.1	369	41.1		219	24.4		149	16.6		8	0.9	
Number of partners in the last 5 years					<0.001			<0.001			0.317			<0.001
0-1	7619	68.8	4823	63.3		3391	44.5		1369	18.0		319	4.2	
2-3	1531	13.8	746	48.7		471	30.8		273	17.8		37	2.4	
4-5	444	4.0	212	47.8		119	26.8		93	21.0		4	0.9	
6-9	284	2.6	122	43.0		72	25.4		50	17.6		2	0.7	
10+	433	3.9	204	47.1		139	32.1		64	14.8		1	0.2	
Not reported	762	6.9	326	42.8		193	25.3		132	17.3		7	0.9	
Number of partners lifetime					<0.001			<0.001			0.316			<0.001
0-1	7530	68.0	4526	60.1		3158	41.9		1314	17.5		227	3.0	
2-3	1335	12.1	749	56.1		498	37.3		241	18.1		71	5.3	
4-5	704	6.4	386	54.8		254	36.1		131	18.6		29	4.1	
6-10	858	7.8	473	55.1		308	35.9		164	19.1		32	3.7	
11-19	224	2.0	116	51.8		66	29.5		49	21.9		8	3.6	
20+	144	1.3	85	59.0		52	36.1		33	22.9		3	2.1	
Not reported	278	2.5	98	35.3		49	17.6		49	17.6		0	0.0	
High risk sexual behaviours					<0.001			<0.001			<0.001			<0.001
None	7467	67.4	4407	59.0		3011	40.3		1351	18.1		288	3.9	
One	1690	15.3	1002	59.3		700	41.4		297	17.6		33	2.0	
More than one	928	8.4	406	43.8		290	31.3		116	12.5		11	1.2	
Not reported	988	8.9	618	62.6		384	38.9		217	22.0		38	3.9	
Partner had multiple partners					<0.001			<0.001			<0.001			<0.001
No	9681	87.4	5762	59.5		3905	40.3		1791	18.5		352	3.6	
Yes	1084	9.8	493	45.5		344	31.7		149	13.8		17	1.6	
Not reported	308	2.8	178	57.8		136	44.2		41	13.3		1	0.3	
Risky sexual behaviours					0.011			0.004			0.101			<0.001
No	3292	29.7	1842	56.0		1232	37.4		589	17.9		81	2.5	
Yes	7473	67.5	4413	59.1		3017	40.4		1351	18.1		288	3.9	
Not reported	308	2.8	178	57.8		136	44.2		41	13.3		1	0.3	
History of STI					<0.001			<0.001			0.086			0.763
No	10335	93.3	5942	57.5		4010	38.8		1870	18.1		348	3.4	
Yes	730	6.6	486	66.6		372	51.0		109	14.9		22	3.0	
Not reported	8	0.1	5	62.5		3	37.5		2	25.0		0	0.0	

Continues

Table 1
Continued

	All		Any reason			Excluded pregnancy and blood donors			Blood donors			Pregnancy		
	Freq.	col (%)	Freq.	row (%)	p	Freq.	row (%)	p	Freq.	row (%)	p	Freq.	row (%)	p
Contextual level variables														
Index of social deprivation - area level[a]					<0.001			<0.001			0.294			0.247
Medium (-5.8/-4.5)	3056	27.6	1779	58.2		1192	39.0		571	18.7		102	3.3	
Low(< -5.8)	3692	33.3	1976	53.5		1292	35.0		663	18.0		110	3.0	
High (>4.5)	4325	39.1	2678	61.9		1901	43.9		747	17.3		158	3.6	
Type of clinic					<0.001			<0.001			<0.001			0.002
low prescription volume (<12 000)	4844	43.8	2425	50.1		1593	32.9		809	16.7		158	3.3	
high prescription volume (≥12 000)	4761	43.0	2942	61.8		2149	45.1		760	16.0		141	3.0	
hospital outpatient clinics	1468	13.3	1066	72.6		643	43.8		412	28.1		71	4.8	
Health care low income card - area level[a]					<0.001			<0.001			<0.001			0.018
Low (<1.1%)	3778	34.1	2139	56.6		1363	36.1		763	20.2		145	3.8	
Median (1.1%-1.4%)	3738	33.8	2486	66.5		1754	46.9		696	18.6		130	3.5	
High (≥1.4)	3557	32.1	1808	50.8		1268	35.7		522	14.7		95	2.7	

[a]in tertiles (population weighted); STI: sexually transmitted infection.

while it varied from 50.1% in small clinics to 72.6% in the hospitals.

Results from the random effect multivariable Poisson models, reported in *Table 2*, were in line with the univariate analysis. Men were 6% less likely to test for HIV than women (prevalence ratio (PR): 0.94; 95% CI: 0.89; 0.99). Homosexuals/lesbians and bisexuals had prevalence rates for HIV testing 1.37 (95% CI: 1.17; 1.61) and 1.24 (95% CI: 1.05; 1.47) times that of heterosexuals. Prevalences were 1.29 (95% CI: 1.20; 1.39) and 1.33 (95% CI: 1.21; 1.46) times higher for married/cohabiting and separated/widowed than for single men and women. In the same way, subjects in a stable relationship were about 1.15 (PR: 1.15; 95% CI: 1.06; 1.24) times more likely to test for HIV than their counterparts who were not in a stable relationship.

HIV testing prevalence increased linearly from lowest to highest levels of education, but it reached significance only in the latter category (PR: 1.11; 95% CI: 1.02; 1.21; p linear trend = 0.004).

Those who reported having had one high risk behaviours for contracting HIV infection were 11% (PR: 1.11; 95% CI: 1.04; 1.20) more likely to test for HIV than those who had none of them. Subjects who had had a STI in the past were 1.12 (PR: 1.12; 95% CI: 1.02; 1.23) times more likely to test for HIV than those not reporting any STI. At the area level, compared to outpatient clinics with a volume of prescriptions below 12 000 per year, in the clinics with a greater number of prescriptions (PR: 1.88; 95% CI: 1.30; 2.73) and in the hospitals (PR: 1.42; 95% CI: 1.10; 1.82), the prevalence of subjects ever tested for HIV was more than 40% higher. Clinics with a high *vs* low proportion of co-fee exemption to the total number of prescriptions had a PR of 0.52 (95% CI: 0.37; 0.74). After excluding tests carried out for donation or pregnancy, the results obtained were in the same direction as those for the entire population. Subjects not reporting being (or not) in a stable relationship were as likely to test for HIV as those in a stable relationship.

When markers of sexual risk behaviours were used to approximate risk we found 44.1% of subjects with self-reported risks of contracting HIV (subjects reporting at least one risk behaviour and/or had had a STI in the past) had tested at least once in life. This percentage was 54.1% at the age of 18-35 years, 39.6% for the high educated and 36.6% for the homosexuals/lesbians, bisexual and other sexual orientations combined.

DISCUSSION

Using data from a large survey performed in 2012-2014 on more than 10 000 individuals 18-59 year olds who underwent public ambulatories visits we found that 58.1% of adults reported to have been tested at least once for HIV in Rome, Italy. This percentage is higher than that reported in other countries. In Britain, the Natsal-3 found that in 2010-2012, 18.1% of men and 23.2% of women 16-74 years old reporting sexual experience, had tested for HIV for diagnostic purposes (excluding testing in the context of blood donation) [18]. In the United States, the 2013 National Health Interview Survey (NHIS) and the 2013 Behavioural

Table 2

Prevalence ratio (PR) of HIV testing (for any reason and after exclusion of pregnancy and blood donation) by socioeconomic and demographic characteristics of 11 073 men and women (aged 18-59 years) participating in the MeDi survey from January 2012 to November 2014. Results from random intercept and random slope multivariable Poisson models of HIV testing

	Any reason				Excluded pregnancy and blood donors			
	PR	95% CI		p	PR	95% CI		p
Individual level variables								
Gender								
Female	1				1			
Male	0.94	0.89	0.99	0.027	0.71	0.66	0.76	<0.001
Not reported	0.99	0.76	1.31	0.963	1.05	0.76	1.45	0.755
Years of age								
18-34	1				1			
35-49	1.18	1.11	1.25	<0.001	1.22	1.13	1.31	<0.001
50-59	0.94	0.86	1.02	0.154	0.81	0.72	0.90	<0.001
Not reported	0.95	0.61	1.46	0.798	0.98	0.61	1.59	0.937
Marital status								
Single	1				1			
Married/cohabiting	1.29	1.20	1.39	<0.0011	1.45	1.33	1.58	<0.001
Separated/widowed	1.33	1.21	1.46	<0.001	1.48	1.32	1.66	<0.001
Not reported	1.05	0.80	1.37	0.735	1.21	0.88	1.67	0.237
Educational attainment								
Low	1				1			
Medium	1.03	0.95	1.11	0.504	0.93	0.85	1.02	0.132
High	1.11	1.02	1.20	0.019	0.99	0.89	1.09	0.810
Not reported	0.85	0.52	1.40	0.518	0.71	0.38	1.33	0.285
Sexual orientation								
Heterosexual	1				1			
Homosexuals/lesbians	1.37	1.17	1.61	<0.001	1.69	1.40	2.03	<0.001
Bisexual	1.24	1.05	1.47	0.013	1.29	1.06	1.58	0.013
Other	1.11	0.96	1.28	0.169	1.21	1.03	1.43	0.022
Not reported	1.03	0.94	1.12	0.518	1.06	0.95	1.18	0.280
Stable partner								
No	1				1			
Yes	1.15	1.06	1.24	<0.001	1.21	1.10	1.34	<0.001
Not reported	1.10	0.89	1.36	0.388	1.29	1.01	1.64	0.044
High risk sexual behaviours								
None	1				1			
One	1.11	1.04	1.20	0.003	1.24	1.13	1.35	<0.001
More than one	1.06	0.95	1.19	0.263	1.31	1.15	1.49	<0.001
Not reported	1.01	0.92	1.10	0.870	0.92	0.82	1.02	0.124
History of STI								
No	1				1			
Yes	1.12	1.02	1.23	0.021	1.20	1.08	1.34	0.001
Not reported	0.90	0.37	2.17	0.818	0.67	0.21	2.07	0.483
Contextual level variables								
Type of clinic								
Low prescription volume (<12 000)	1				1			
High prescription volume (≥12 000)	1.88	1.30	2.73	0.001	2.23	0.78	6.41	0.136
Hospital outpatient clinics	1.42	1.10	1.82	0.007	1.09	0.94	1.27	0.260
Health care low income card-areaa level								
Low (<1.1%)	1				1			
Median (1.1%-1.4%)	1.03	0.82	1.29	0.831	0.86	0.47	1.58	0.635
High (≥1.4)	0.52	0.37	0.74	<0.001	0.33	0.12	0.92	0.034
Var (health care low income card):	0.00338	0.01490	0.56573		0.07795	0.02972	0.20444	
Var (clinic level):	0.02666	0.06910	0.21815		0.08988	0.03973	0.20331	

Index of social deprivation-area level was not included in the multivariate analyses. [a]in tertiles (population weighted); STI: sexually transmitted infection.

Risk Factor Surveillance System (BRFSS) estimated that 42.2-45.0% of 18-64 years old US residents had ever tested for HIV [19, 20].

We found that those who had one high risk behaviour for contracting HIV infection were 11% more likely to test for HIV than those not reporting any, and subjects who had had a STI in the past were 12% more likely to test for HIV than those who had never had a STI. However, when markers of sexual risk behaviours were used to approximate risk we found that only 44.1% of those with self-reported risks of contracting HIV had tested at least once in life. This percentage was even higher among subjects aged 18-35 years (54.1%). A cross-sectional survey carried out in 2008, reported that 73% of newly diagnosed individuals in Belgium, Estonia, Finland and Portugal had not previously tested for HIV due to a low perception of risk [21]. Hoyos *et al*. also found that 46.5% of subjects classified as high risk considered themselves to be at low risk for HIV infection [22].

We also found that homosexuals/lesbians were more likely than heterosexuals to test for HIV, still 37% of them (homosexuals/lesbians/bisexual/other combined) had never tested for HIV and this proportion was of similar magnitude in men with a high or low risk profile. To this regard, the Natsal-3 survey found that 84.8% of MSM reporting recent unsafe sex rated themselves as low risk for HIV infection [18]. Similarly, a qualitative study found that more than half of HIV positive MSM were surprised by their diagnosis and believed themselves to have only practiced safe sex [23].

Other factors associated with HIV testing in our study included being female, in a stable relationship/ married/ cohabiting and highly educated. The BRFSS for the state of Georgia found that participants that attained educational levels greater than high school tested more than those with a lesser education [24]. In the same way, a survey carried out in 2011 in a sample of 1568 delivering women enrolled in 36 maternal hospitals in the Lazio region, found that women who missed test were of lower education level, with a lower HIV-knowledge score and fewer visits during pregnancy [25]. Still, in our study, about 40% of the highly educated self reporting risks of contracting HIV had never tested.

At the area level the highest prevalence of subjects ever tested for HIV was observed in the hospitals and in high volume clinics. These, perhaps, are characterized by better quality of care and more resources for HIV testing. We found no evidence that HIV testing was associated with neighbourhood deprivation after adjustment for SEP at the individual level. Overall, our results are in line with a study carried out in a large urban US city which found that income inequality and socioeconomic deprivation were associated with higher rates of late HIV diagnosis only in crude models (not adjusted for covariates) [26].

Limitations and strengths

Some limitations can be highlighted: 1) the MeDi data are self-reported and may be subject to biases such as social desirability or recall bias and underreporting of risk behaviours associated with HIV; 2) HIV testing performed was also self reported; 3) reasons for not testing were not explored; 4) the study was conducted in local and hospital based outpatient clinics and we cannot exclude that the prevalence of HIV-testing could have been different for those not accessing the outpatient clinics in the study period. In particular, it appears from *Figure 1* that some part of the city may be less well represented. However, medical appointments are centrally managed by a phone booking system which identifies the first available place in any clinic in Rome. For this reason, we have collected the postal code of the area of residence and re-allocated each subject accordingly; 5) the survey was based on non-institutionalized populations. Incarcerated persons may have higher risks for HIV. However, some subjects self reported to have been tested in correctional facilities before the survey took place; 6) the sampling frame was the Roman metropolitan area, rural/sub-urban areas outside of the metropolitan belt were not represented. Because of these limitations, the results might be either underestimated or overestimated when generalized to other populations.

Our data are the only one available in Rome and the only one to examine socio-demographic factors related to HIV testing. A strength of the present study is the utilization of data from a large survey of the general population with a response rate as high as 83%.

Conclusions and implications

From our study it emerges that while the percentage of subjects tested is even higher than observed in other western nations, only 44% of subjects self reporting risks of contracting HIV (54% among subjects aged 18-35 years) had tested at least once in life. This percentage decreases, as expected, with the level of education, but even so, about 40% of university educated subjects self reporting risks of contracting HIV had never tested. We do not know why this happens but certainly the current system based on proposing the test based on risk, either self-perceived or defined by a doctor, does not allow reaching all patients in need to be tested.

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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HIV prevalence among adults in Rome: results of the MeDi (Measuring health Disparities in HIV prevention) survey. Part 2

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Abstract

Background. In Italy, out of 60 millions of inhabitants, 3000 (2700-4000) new HIV infections are estimated each year. As combined antiretroviral therapy (ART) prolongs life for HIV sufferers, the prevalence of HIV-infection is likely to increase over time. Few studies have assessed factors associated with being HIV positive in people accessing public outpatient clinics and, in particular, the influence of socio-economic circumstances on HIV prevalence. This study aims to evaluate the association between subjects' serostatus and socio-economic determinants measured at the individual and neighbourhood levels.

Methods. Data from a large anonymous survey performed in 2012-2014 on more than 10 000 individuals 18-59 years old who underwent 21 public ambulatories in Rome were analysed. Subjects' socio-demographic characteristics, sexual orientation, number of sexual partners, HIV risk behaviour and HIV testing uptake were collected by a self-administered questionnaire. Level of area deprivation was measured at the postal code level by the index of social disadvantage (ISD). Multilevel Poisson regressions were carried out to take heterogeneity between clusters (post code and clinics) into account.

Results. Self-reported HIV-prevalence was 2.0% among subjects ever been tested (13.7% for the homosexual/lesbians 7.0% for the bisexual and 1.3% for the heterosexual). About 1% of subjects self-identified as low risk was HIV infected. This prevalence increased up to 2% in the age group 18-34 and up to 5% in the non-heterosexuals (i.e. self- identified homosexuals/lesbians and bisexuals). At the individual level, HIV-prevalence decreased linearly from lowest to highest levels of education. Living in a deprived neighbourhood was not associated with HIV-infection.

Conclusions. Our study confirms high HIV prevalences among homosexuals/lesbians. Some infections occur in subjects who do not report high risk behaviours for HIV transmission.

Key words

- HIV
- HIV testing
- SEP
- deprivation
- Italy
- urban

BACKGROUND

HIV prevalence has increased in Italy since 1995. In 2012, out of a population of 60 million, it is estimated that 123 000 (115 000-145 000) subjects live with HIV infection [1], 11-13% of whom non-diagnosed [2]. It is also estimated that 3000 (2700-4000) new cases occur every year [1]. 36.6% of them have a number of CD4 cells below 200 cell/ml [3]. This proportion increases to

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52.7% in the population of 50 years of age or older and to 46.5% in the heterosexual men [3].

The demography of HIV infection has changed since the beginning of the epidemic and, while incidences among homosexuals and injective drug users remain high, new cases occur in heterosexual men and older subjects [3] in particular among the poor.

Studies carried out in western countries suggest that individual socio-economic-position (SEP) and area deprivation might be related to timing of diagnosis and risk of infection [4-9]. At the individual level low literacy, poverty, relationship instability caused by economic stress, unemployment and incarceration can encourage the uptake of risky behaviours. At the neighbourhood level, residential social displacement, segregation (e.g. attending lower quality schools, high crime rate), inequities in environmental resources and psychological influences (e.g. HIV-related stigma, minority stress) concentrate poverty and any attributes correlated with it including HIV infection. Geographical clustering of HIV infections among populations of low SEP may expose subjects to higher transmission rates than individual circumstances alone would indicate. Few studies, at least in Italy, have investigated the connection between socio-economic deprivation and risk of HIV infection. To fill this gap, we carried out a survey of the general adult population living in Rome, between January 2012 to November 2014 to determine the proportion of subjects tested for HIV and factors related to testing uptake. Rationale of the MeDi survey was that of providing baseline information on existing levels of positive health behaviours and HIV related risk factors. Specific aims of this article were: 1) to estimate the prevalence of HIV seropositivity in the population living in Rome who attended one of the outpatient clinics included in the study; and 2) to evaluate the association between HIV serostatus, subjects' socio-economic characteristics and the deprivation of their area of residence.

MATERIALS AND METHODS

Data sources

Data on 5292, 18-59 years old men and women living in Rome who reported HIV testing results and participated in the MeDi (Measuring health Disparities in HIV prevention) survey were used for this analysis.

MeDi survey

The MeDi study is a cross section survey (see "Prevalence and Attitudes to HIV testing among adults in Rome" where the survey methods are fully described [10]) which collected self-reported data on health perception, life satisfaction, access to health care and at risk sexual behaviour in a sample of more than 10 000 individuals aged 18-59 years attending public outpatient clinics in Rome between 2012-2014.

The MeDi questionnaire was developed by the authors building on previous research [11, 12]. Respondents were asked about their socio-demographic characteristics (gender, nationality, age, duration of stay in Rome, postal code of the area of residence, educational level, occupation, marital status, duration of stable relationships, health exemption tickets and pregnancy status), their sexual orientation, the number of sexual partners they had had in the last six months, over the past five years and lifetime and whether they had ever been tested for HIV. HIV risk behaviour was evaluated by asking subjects whether they had ever been in one or more situations at high risk for HIV transmission. Two lists of hypothetical situations were provided. The first one included: "I have used injective drugs", "I have had sex under the effect of alcohol or drugs", "I have had anal intercourse without a condom", "I have given or received money in exchange for sex"; the second one included: "I have had multiple sexual partners over the same period", "my partner has had multiple sexual partners over the same period", "I have not used a condom during the last intercourse with a casual partner", "I have not used a condom during sexual intercourse with a HIV positive partner". Participants were also asked whether they had ever suffered from chlamydia, gonorrhea, syphilis, herpes genitalis and genital warts.

Neighbourhood characteristics

The Index of social disadvantage (ISD) was developed by the "Ufficio Metropolitano di Statistica" and the "Ufficio di Statistica di Roma Capitale" to produce a statistical report on the Roman metropolitan area as the sum of the unweighted z-scores for the following census variables: unemployment, employment, youth concentration and schooling [13]. The ISD was re-aggregated from census section to postal codes polygons by areal interpolation in "Quantum" GIS(QGIS) [14].

Postal codes were also classified in tertiles of frequency according to the proportion of HIV+ subjects within each postal code.

Characteristics of outpatient clinics

Clinics were classified according to whether they were located within a hospital or not (district facilities) and to whether the amount of prescriptions provided by all clinics combined in the year 2009 was above or below the median as: small size clinics within district facilities (annual amount of prescriptions below 12 000 in 2009); medium size clinics within district facilities (amount of prescriptions of 12 000 or greater) and; hospital based outpatient clinics. Clinics were also classified according to the proportion of prescriptions exempted from the co-pay fee for low income in the year 2009 to the total number of prescriptions for the same year in tertiles of frequency (population weighted) of co-pay fee for low income as: clinics with a proportion of co-pay fee exemption for low income below 1.1%; between 1.1 and 1.4%; and of 1.4% or more.

Ethics and funding

The survey was approved by the Ethics Committee of Istituto Superiore di Sanità, Rome, Italy n. CE/12/338, 07/05/2012. Each subject was also asked to formally consent to participate in the study. The study was funded by the Ministry of Health as part of the HIV/AIDS projects.

Statistical analysis

Crude, age and gender specific, and age-standardized prevalences of self reported HIV serostatus were cal-

culated. As reference population, we used the 2012 Eurostat European population, stratified by age-group [15]. Fisher exact chi-square tests were computed to investigate the association between HIV serostatus and possible determinants/predictors variables such as socio-demographic characteristics, sexual behaviour, sexually transmitted infections (STI) in the past, and different levels of social disadvantage in the area where the participant was living (see below for description).

Poisson regression models were used to produce unbiased prevalence ratios estimate [16]. A test for linear trend was carried out, if necessary, across strata of ordinal categorical variables, including them as "continuous" variables in a Poisson model. Poisson regressions, with stepwise selection, were carried out to identify independent predictor variables from those with a p-value <0.20 at the univariate analysis.

Within and between clusters (post code and clinics) variances were investigated using a multilevel framework. Since the variance at the postal code level and clinics levels was not significant, a Poisson model with no random terms was carried out (i). To this model were added in the following order: individual (ii) and contextual level covariates (iii) as identified with the stepwise procedure, (iii) and the cross level interaction terms between deprivation and strata of age, sex, sexual orientation and SEP (iv). Only significant effects (from log-likelihood ratio test) were retained. A secondary analysis was carried out to estimate the prevalence of HIV infection in subjects who reported that they did never engage in HIV risk behaviours and had not had a STI in the past. Subjects reporting at least one risk behaviour and/or had had a STI in the past were defined as "high risk"; those not reporting a risk behaviour and / or a STI were considered "low risk". Statistical analyses were carried out in Stata 13 [17].

RESULTS

Figure 1 shows the population involved in this study. Socio-demographic characteristics of subjects by HIV serostatus, are reported in *Table 1*. Out of 6433 tested subjects, 5292 reported HIV results (5184 were HIV- and 108 HIV+) with a crude prevalence of 2.0% (95% CI: 1.7%; 2.5%). Median ages were 39 years (iqr. 33-46) for the HIV- and 35 years (iqr: 31-45) for the HIV+. Age and sex specific prevalences were 3.1% (95%CI: 2.3%; 4.1%) for males and 1.6% (95% CI: 1.2%; 2.1%) for females, 3.2% (95% CI: 2.4%; 4.1%) for the age group 18-34, 1.4% (95% CI: 0.9%; 1.8%) for the age group 35-49 and 2.0% (95% CI: 1.0%; 3.3%) for the age group 50-59. Age standardized prevalence was 2.0% (95% CI: 1.5%; 2.8%).

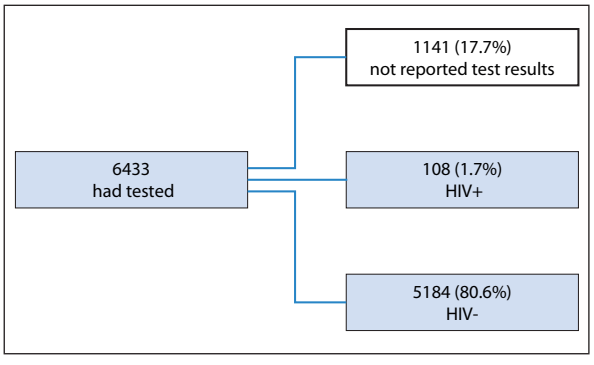

Figure 1
Flow chart of the study population: men and women participating at the MeDi survey between January 2012 and November 2014 who underwent HIV testing and reported test results.

Prevalences of HIV infection were 13.7% for the homosexuals/lesbians, 7.0% for the bisexual, and 1.3% for the heterosexuals (18 HIV+ subjects did not report any information about their sexual orientation). Four point five percent of the low educated and about 1.7% of subjects with a medium or higher educational attainment were HIV+. Five point six percent of subjects who had had 2 or more partners in the past six months, 5.4% of those who had had a STI in the past, 6.9% of those who reported having had more than one high risk behaviours for HIV and 3.5% of those living in areas with a high prevalence of HIV were HIV+.

Results from the multivariable analysis of age, educational attainment, sexual orientation, stable partnership, risky sexual behaviours, STI and HIV prevalence at area level (all had p values <0.2 at the univariate analysis) resembled those obtained from the univariate analysis (see *Table 1*). Prevalence ratios (PR) were 0.62 (95%: 0.40; 0.95) and 0.82 (95%: 0.45; 1.51) respectively for the age groups 35-49 and 50-59 compared to the age group 18-34. HIV prevalence showed a graded association across the educational range decreasing linearly from lowest to highest levels (medium: PR: 0.42; 95% CI: 0.26; 0.68; high: PR: 0.36; 95% CI: 0.21; 0.60; p linear trend: <0.001). Compared to heterosexuals, homosexuals/lesbians, bisexuals and other sexual orientations were respectively 5.38 (95% CI: 3.02; 9.59), 1.99 (95% CI: 0.92; 4.34) and 2.62 (95% CI: 1.26-5.47) times more likely to be HIV+. Those who had had a STI in the past had a PR of 2.00 (95% CI: 1.23; 3.26) compared to those who had never had it. Prevalences increased linearly with the increase in the number of high risk situations (one high risk situation: PR 1.99: 95% CI: 1.21; 3.25; PR: more than one risk situation: 2.44: 95% CI: 1.38; 4.32; p linear trend: <0.040). Subjects living in neighbourhoods with a medium/ high HIV prevalence were more likely to be HIV+ than those living in low prevalence neighbourhoods (medium prevalence neighbourhoods PR: 3.07; 95% CI: 1.72; 5.48; high prevalence neighbourhoods PR: 2.98; 95% CI: 1.65; 5.41).

Secondary analyses restricted to those who did not engage in any risk behaviour yielded prevalences of 1.6% (95% CI: 0.8%; 2.7%) for men and of 1.1% (95% CI: 0.7%; 1.6%) for women. Prevalences were 2.1% (1.3%; 3.1%), 0.1% (0.0%; 1.3%) and 1.1% (0.0%; 2.6%) for the age groups 18-34, 35-49 and 50-59. The homosexuals/lesbians, the bisexuals and the other groups combined had a HIV prevalence of 4.9% (95% CI: 2.1%; 9.4%) while the heterosexuals had a prevalence of 0.8% (95% CI: 0.5%; 0.1%). Prevalences were 0.1 (95% CI: 0.0; 1.4) and 2.2 (95% CI: 1.1;3.9) in the high and low educated.

DISCUSSION

Using data from a large survey performed in 2012-2014 on more than 10 000 individuals 18-59 years old

Table 1
Prevalence ratio (PR) of HIV serostatus by socioeconomic and demographic characteristics of 5292 HIV tested men and women (aged 18-59 years) participating in the MeDi survey from January 2012 to November 2014. Results from univariate and multivariable Poisson models of HIV serostatus

	HIV-uninfected		HIV-infected						
	N.	(%)	N.	(%)	p	PR	95% CI		p
Individual level variables									
Gender					0.002	NI			
Female	3688	98.40	60	1.60					
Male	1448	96.86	47	3.14					
Not reported	48	97.96	1	2.04					
Years of age					0.001				
18-34	1615	96.82	53	3.18		1			
35-49	2857	98.62	40	1.38		0.62	0.40	0.95	0.028
50-64	694	98.02	14	1.98		0.82	0.45	1.51	0.530
Not reported	16	100.00	0	0.00		0.00	0.00		
Marital status					0.002	NI			
Single	1453	96.87	47	3.13					
Married/cohabiting	3108	98.57	45	1.43					
Separated/widowed	572	97.61	14	2.39					
Not reported	49	98.00	1	2.04					
Educational attainment					<0.001				
Low	587	95.45	28	4.55		1			
Medium	2588	98.29	45	1.71		0.42	0.26	0.68	<0.001
High	1977	98.31	34	1.69		0.36	0.21	0.60	<0.001
Not reported	13	100.00	0	0.00		0.00	0.00		
Occupation					0.011	NI			
Unemployed	767	97.34	21	2.66					
Employed	2469	98.52	37	1.48					
Self-employed	743	96.62	26	3.38					
Other	1181	98.09	23	1.91					
Not reported	22	100.00	0	0.00					
Sexual orientation					<0.001				
Heterosexual	3949	98.70	52	1.3		1			
Homosexuals/lesbians	120	86.33	19	13.67		5.38	3.02	9.59	<0.001
Bisexual	120	93.02	9	6.98		1.99	0.92	4.34	0.082
Other	147	94.23	9	5.77		2.62	1.26	5.47	0.010
Not reported	846	97.92	18	2.08		2.00	1.15	3.48	0.014
Stable partner					<0.001				
No	881	95.66	40	4.34		1			
Yes	4233	98.51	64	1.49		0.65	0.42	0.99	0.048
Not reported	68	95.77	3	4.23		0.97	0.29	3.21	0.963
Number of partners in the last 6 months					<0.001	NI			
0-1	4514	98.34	76	1.66					
2-3	282	95.59	13	4.41					
4-5	41	95.35	2	4.65					
5+	67	90.54	7	9.46					
Not reported	278	96.86	9	3.14					

Continues

Table 1
Continued

	HIV-uninfected		HIV-infected						
	N.	(%)	N.	(%)	p	PR	95% CI		p
Number of partners in the last 5 years					<0.001	NI			
0-1	3909	98.44	62	1.56					
2-3	612	97.61	15	2.39					
4-5	162	98.18	3	1.82					
6-9	97	97.98	2	2.02					
10+	157	90.23	17	9.77					
Not reported	245	96.84	8	3.16					
Number of partners lifetime					0.003	NI			
0-1	3562	97.43	94	2.57					
2-3	631	99.21	5	0.79					
4-5	328	99.70	1	0.30					
6-10	415	99.28	3	0.72					
11-19	100	99.01	1	0.99					
20+	74	98.67	1	1.33					
Not reported	72	97.3	2	2.70					
High risk sexual behaviours					<0.001				
None	3623	98.80	44	1.20		1			
One	825	96.27	32	3.73		1.99	1.21	3.25	0.006
More than one	336	93.07	25	6.93		2.44	1.38	4.32	0.002
Not reported	398	98.51	6	1.49		1.06	0.44	2.51	0.902
History of STI					<0.001				
No	4778	98.27	84	1.73		1			
Yes	404	94.61	23	5.39		2.00	1.23	3.26	0.005
Contextual level variables									
Index of social deprivation - area level[a]					0.704	NI			
Medium (-5.8./-4.5)	2399	98.12	46	1.88					
Low (< -5.8)	613	97.61	15	2.39					
High (>4.5)	2170	97.92	46	2.08					
Type of clinic					0.059	NI			
Low prescription volume (<12000)	2008	98.19	37	1.81					
High prescription volume (≥12000)	2310	97.51	59	2.49					
Hospital outpatient clinics	864	98.74	11	1.26					
Health care low income card-areaa level					0.444	NI			
Low (<1.1%)	1783	98.07	35	1.93					
Median (1.1%-1.4%)	1941	98.18	36	1.82					
High (≥1.4)	1458	97.59	36	2.41					
Prevalence of HIV-area level[a]					<0.001				
Low (<1.3%)	2023	99.22	16	0.78		1			
Median (1.3%-2.6%)	1959	97.66	47	2.34		3.07	1.72	5.48	<0.001
High (>2.6%)	1200	96.46	44	3.54		2.98	1.65	5.41	<0.001

NI: not included in the final model; [a] in tertiles; STI = sexually transmitted infections.

who underwent a visit in a public health care facility we found a prevalence of people reporting to be HIV-infected of 2.0% (95% CI: 1.5%; 2.8%) among those who declared to have ever been tested. This result is in line with a study carried out in 2012 which found that the overall estimated prevalence of HIV among subjects with known serostatus and linked to care was 2.2% in the Lazio region [18]. We found that the prevalences of HIV was 8.4 for homosexuals/lesbians and bisexuals (13.7% for the homosexuals/lesbians, 7.0% for the bisexual) and 1.3% for the heterosexual. In the same way, the 2010 EMIS study (European MSM Internet Survey) found that the self reported prevalence for men who have sex with another men (MSM) in Italy was 9.7% [19, 20]. A large-scale bio-behavioural survey implemented across 13 European cities, the Sialon II study, also found, in Verona (Italy), a measured prevalence of 9.6 among MSM between 2010-2014 [21], while in other European cities, estimates ranged between 2.4% in Stockholm to 18.0% in Bucharest [21, 22].

People with one or more risky sexual behaviour or who had had a STI in the past were especially at risk, but also, about 1% of men and women self identified as low risk (who did not engage in HIV risk behaviour and who had not had a STI in the past) was HIV infected. This prevalence increased up to 2% in the age group 18-34 and in the low educated and was of 5% in homosexuals/lesbians and bisexuals. Similarly, a qualitative study found that more than half of HIV positive MSM were surprised by their diagnosis and believed themselves to have only practiced safe sex [23]. Our results are also consistent with findings from other studies which observe that about one third of HIV infections among MSM occur within main partnerships [24].

We found that there is an inverse association between education and HIV prevalence, being subjects with lower levels of education at higher risk of HIV infection than their highly educated counterparts in line with the observed socio-economic patterning of sexual risk behaviours (e.g. having more sexual partners or a partner with several partners). This result may be also influenced by a less efficient use of the health services (e.g. an increased use of emergency department and hospitals, and a lower use of prevention services) among the low educated [25-28] which, in the long run, increases HIV progression and risk of death [29] .

At the area level, we observed that subjects living in neighbourhoods with a medium/ high HIV prevalence were more likely to be HIV+ than those living in low prevalence neighbourhoods. Some studies argued that, the greater exposure to a high HIV prevalence pool of individuals may foster HIV epidemic in some strata of the population (e.g. ethnic groups) [30]. We found no evidence that living in a deprived neighbourhood increases HIV risk overall and for any population strata considered (age groups, sexes, SEP, sexual orientation). A study carried out among individuals aged 13 years and older residing in 37 US states found that HIV diagnosis rates increased as community deprivation decreased [7], but the effect differed for various strata of the population and was negligible for white males. Additionally, a study carried out in US cities also found that from 1990 to 2000, HIV incidence was highest amongst higher-income, more educated individuals but transitioned to a poverty- and low-education-dependent risk after 2000 [6].

Limitations and strengths

Some limitations can be highlighted: 1) the MeDi data are self-reported and may be subject to biases such as social desirability or recall bias and underreporting of risk behaviours associated with HIV; 2) HIV serostatus was also self reported; 3) the study was conducted in local and hospital based out-patient clinics and we cannot exclude that the prevalence of HIV could have been different for those not accessing the outpatient clinics in the study period; 4) the survey was based on non-institutionalized populations and excluded all subjects living in nursing homes, prison or long-term-care facilities at the time in which the survey took place. Incarcerated persons may have higher risks for HIV. However, some subject self reported to have been tested in correctional facilities before the survey took place; 5) the sampling frame was the Roman metropolitan area, rural/suburban areas outside of the metropolitan belt were not represented. Because of these limitations, the results might be either underestimated or overestimated when generalized to other populations; 6) few people have social activities only in an area defined by the postal code of residence and their life could be affected also by socioeconomic determinants of other places (e.g. work places) which were not taken into account in our work.

A strength of the present study is the utilization of data from a large survey of the general population with a response rate as high as 83%.

CONCLUSIONS AND IMPLICATIONS

Our study confirms high HIV prevalences among homosexuals/lesbians. Prevention efforts are not adequately reaching them in Rome and there is a real possibility that the HIV epidemic may further expand since some infections occur, presumably within main partnership, in subjects who do not report high risk behaviours for HIV transmission. It is believed that tackling poverty can lead to a reduction in HIV transmission. However, we did not find support for this at the postcode level, but this does not exclude that such a relationship exists for other indicators and other geographical levels [31].

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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ORIGINAL ARTICLES AND REVIEWS

Rehabilitation strategies for low anterior resection syndrome. A systematic review

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Abstract

Objective. To summarize the evidence in the literature about rehabilitative treatments that reduce low anterior resection syndrome (LARS) symptoms in patients who underwent surgery for colorectal cancer.

Methods. We have search in PubMed, Cochrane Central Register of Controlled Trials, Cumulative Index of Nursing and Allied Health and Scopus databases. Studies selected were limited to those including only patient undergone low rectal resection with sphincter preservation and with pre-post assessment with a LARS score. Five articles fit the criteria.

Results. The percutaneous tibial nerve stimulation demonstrated moderate results and sacral nerve stimulation was found to be the best treatment with greater symptom improvement. Only one study considered sexual and urinary problems in the outcomes assessment.

Conclusions. In clinical practice patients should evaluate with the LARS and other score for evaluation of urinary and sexual problems. Future research must be implemented with higher quality studies to identify the least invasive and most effective treatment/s.

Key words

- low anterior resection syndrome
- rehabilitation
- therapy
- treatments
- quality of life

INTRODUCTION

Colorectal cancer is the third most common cancer worldwide [1]. Although surgical treatment has improved in recent years, patients who have a sphincter-preserving operation may experience symptoms that can affect their quality of life (QOL) [2-4]. Eighty percent of patients who undergo a low or very low anterior rectal resection will experience fecal or flatus urgency or incontinence, frequent bowel movements, bowel fragmentation, difficulties emptying, incomplete evacuation, and increased gas postoperatively [2]. This suite of symptoms is referred to as low anterior resection syndrome (LARS) [2, 3]. Anatomical, nervous/sensory, and muscular changes [2-4], the new sphincter's functional capacity [5], pelvic floor functionality [4], colon motility [6], post-prandial response [7], and the new rectum's compliance [8] are involved in LARS.

Although LARS has been recognized for years, a rigorous scientific definition of this syndrome never has been developed. Conventionally, it is defined as a bowel disorder following rectal resection that affects patient QOL [4]. After surgical treatment of a colorectal cancer, LARS appears immediately, becomes more evident in the first several months, and stabilizes after approximately one to two years [7].

The incidence of LARS in Europe is approximately 52% in patients with low rectal surgical treatment [9] and the approach to treat it is conservative, consisting of an appropriate diet, fiber intake, mass-forming agents [10] and enemas [11]. Nonetheless, LARS has adverse effects on patient satisfaction and QOL. The literature demonstrates the availability of different rehabilitation treatments [10, 12, 13-15], but currently there is no evidence of the best rehabilitative intervention to improve LARS patients' symptoms.

Therefore, the goal of this paper was to summarize the evidence available in the literature on different rehabilitative treatments to reduce LARS symptoms in patients who have undergone surgery for colorectal cancer.

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METHODOLOGY

Study design

A systematic literature review was conducted according to Lefebvre *et al.* (2013) [16] and Liberati *et al.* (2009) [17] methodologies.

Literature search strategy

To identify relevant studies, we searched the following databases: Pubmed, Cochrane Central Register of Controlled Trials (CCTR), Cumulative Index of Nursing and Allied Health (CINAHL), and Scopus. The PICOS (Participants, Interventions, Comparisons, Outcomes, and Study Design), respectively: patients who underwent low or very low anterior rectal resection for rectal neoplasm (P), were treated with rehabilitation therapy (I) by comparison to standard care (C), and had fecal incontinence or bowel dysfunctions that compromise their quality of life (O). The study designs considered were randomized clinical trials and cohort studies. Search terms were "low anterior resection syndrome," "rectal neoplasms," "rectal cancer," "rehabilitation," "therapy," "treatments," "process assessment," "biofeedback," "electrical stimulation," "disability evaluation," "early intervention," "rehabilitation nursing," "fecal incontinence," "quality of life," "bowel dysfunction," and "outcome." The MESH terms were combined with free terms and Boolean operators (AND, OR) to include all possible combinations. Thereafter, a manual search was conducted using a snowball sampling technique [17].

The primary end-points of this systematic review were LARS score and QOL. Secondary end-points were anal pressure and urinary and sexual symptoms.

Inclusion and exclusion criteria

Studies included were those published through October 2018 that had abstracts available and included only adult patients (>18 years) who had undergone low rectal resection with sphincter preservation. As a large proportion of the elderly public, particularly in the 50 to 79 years age group, exhibits major LARS in the absence of any surgical procedure, only studies with pre-post treatment assessment with a LARS [18] score to identify patient with LARS were included.

Data extraction and risk of bias

Two researchers evaluated the studies independently. In the first phase, titles, abstracts, and full texts were analyzed to establish the studies' relevance to the research questions; the risk of bias was assessed using the Edward Score [19] for observational studies. The 11 items of the assessment tool are as follows: definition of aims; sample formation; description of inclusion and exclusion criteria; description of subject characteristics; power calculation; objectivity of outcome measures used; adequacy of follow-up; adequacy of analysis (intention to treat); adjustment for baseline differences between groups; appropriate unit of allocation to groups; randomization method. Each item is scored from 0 to 2 for adequacy and a high score indicates a study's high methodological quality. The total score possible ranges from 0 to 22 for experimental studies or from 0 to 16 for observational studies. In the second phase, the researchers combined the data and resolved any discrepancies by consensus.

RESULTS

Search results

We selected 439 articles, 186 of which were duplicates. Three researchers analyzed the remaining 254 studies independently and 5 articles that fit the criteria were selected *(Figure 1)*.

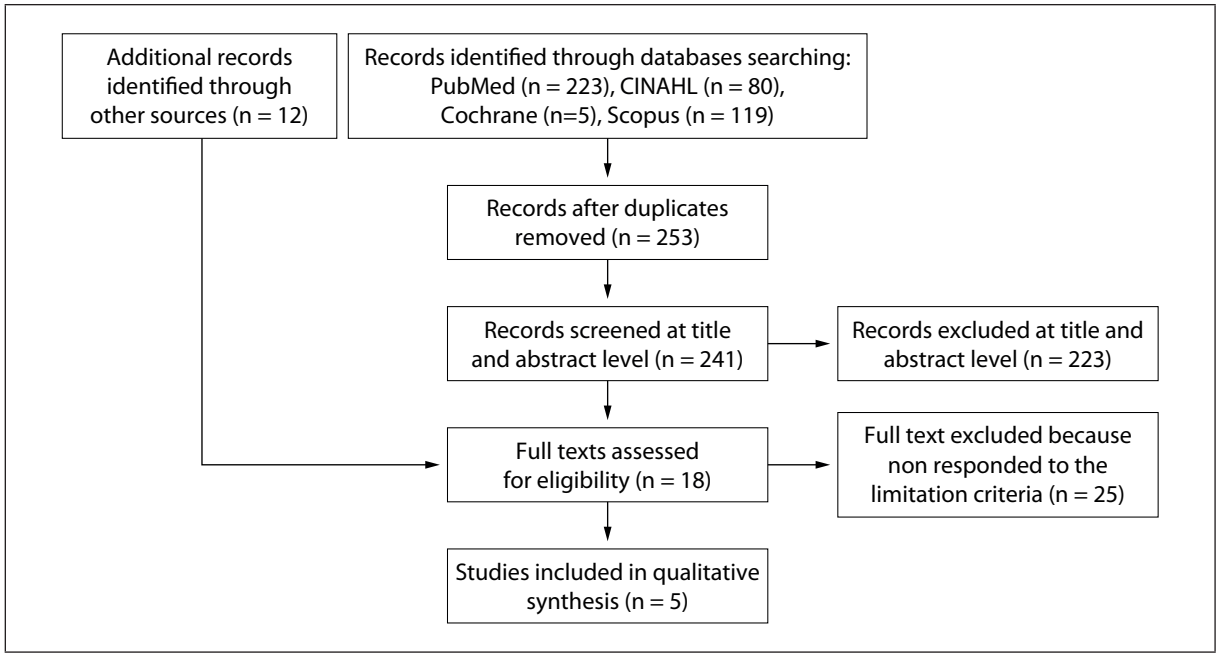

Figure 1
PRISMA Flow Diagram.

Table 1
Methodological quality of included studies

		Altomare *et al.* 2017 [23]	D'Hondt *et al.* 2017 [21]	Eftaiha *et al.* 2017 [20]	Mege *et al.* 2017 [22]	Vigorita *et al.* 2017 [24]
1. Aims/ outcomes	2: explicitly described in article	2	2	2	2	2
	1: implied in article					
	0: unclear					
2. Sample formation	2: random	1	1	0	1	1
	1: quasi-random, sequential series, or total available					
	0: selected, historical, other					
3. Inclusion/ exclusion	2: criteria clearly described	2	2	2	0	2
	1: implied by patient characteristics, setting					
	0: unclear					
4. Sample characteristics	2: three or four characteristics	2	2	2	2	2
	1: one or two characteristics					
	0: no characteristics					
5. Power of study calculated	2: yes, appropriate	0	0	0	0	0
	1: yes, inappropriate					
	0: no					
6. Outcome measures	2: objective or validated scale	2	2	2	2	2
	1: subjective/self-report					
	0: not explicit					
7. Follow-up	2: >90% of patients enrolled/approached	2	2	2	2	2
	1: 80%-90% of patients					
	0: <80% of patients/no information					
8. Analysis	2: intention to treat/including all available data	2	2	2	2	2
	1: excluding dropouts but evidence of bias adjusted or no bias evident					
	0: excluding dropouts and no attention to bias					
Total score (0-16) for observational studies		**13/16**	**13/16**	**12/16**	**11/16**	**13/16**
9. Baseline differences	2: none or adjusted					
	1: differences unadjusted					
	0: no information					
10. Unit of allocation	2: appropriate					
	1: nearly					
	0: inappropriate					
11. Randomization	2: random and concealed					
	1: random but not concealed					
	0: randomization before protocol exclusions, or no information					
Total score (0-22) for experimental studies						

Risk of bias in the studies

The description of the studies' quality assessment and risk of bias are provided in *Table 1*. The cohort studies included demonstrated a low quality in selection criteria [20-24], and no study included a statistical power analysis [20-24].

Interventions to reduce LARS symptoms

Table 2 provides a summary of the studies' characteristics. The rehabilitative treatments used in the studies included sacral nerve stimulation (SNS) [20-22] and percutaneous tibial nerve stimulation (PTNS) [23, 24].

Sacral nerve stimulation intervention

Three studies addressed SNS [20-22] with a total of 43 participants; one was [21] prospective and the others [20-22] retrospective. Patients included had undergone a surgical procedure for rectal cancer [20, 21] and

Table 2
Data extraction of included studies

Author, year of publication, country	Aim	Study design	Sample	Instrument	Intervention/ description	Results
Altomare et al. 2017 [23] **Italy and Spain**	To test effectiveness of PTNS in FI and UI in LARS.	Prospective Follow up: 6 months	Method: convenience N: 21 Age: average 66 ± 5.8 years Gender: M 47.6% (10/21), F 52.4% (11/21) Inclusion criteria: postoperative after rectal cancer free from anastomotic complications, age over 18 years, LARS score ≥ 20, LARS duration for at least 12 months, failure of conservative therapy (diet, medical treatments and BF) Surgical procedure: LAR in laparotomy 71.4% (15/21), 28.6% (6/21) laparoscopy Temporary stoma: 66.6% (14/21) Anastomosis type: 28.5% (6/21) EtE, 71.5% (15/21) StE Perioperative treatment: preoperative 47,6% (10/21), postoperative 61,9% (13/21) CT	LARS score (Emmertsen and Laurberg 2012) TAPE score (Altomare *et al.* 2017) FIQL (Rockwood *et al.* 2000) St Marks score (Vaizey *et al.* 1999) ODS score (Altomare *et al.* 2008). ICIQ-SF score (Tubaro *et al.* 2006). IUGA-Revised (PISQIR) (Rogers *et al.* 2013) for woman IIEF-5 Questionnaire (SHIM) (Rosen *et al.* 1999) and PEDT score (Symond *et al.* 2007) for man Baden-Walker score for genital prolapse (Baden and Walker 1972) Urinary retention Anorectal manometry	PTNS (12 sessions - 2 per week for the first 4 weeks, and 1 per week for the last 4 weeks - of 30 minutes. Stimulation parameters were set at 200 µs pulse width and 20 Hz frequency. Stimulation was gradually increased until sensory and/ or motor response were seen and set at a well-tolerated intensity)	The median LARS-score significantly decreased from 32 (IQ ranges 30-38) to 27 (IQ ranges 17- 37, p=0.009) The TAPE-score significantly improved from a median value of 55 (IQ ranges 49- 64.6) to 58 (IQ ranges 51-70, p=0.004). 43% (9/21) of patients reported a significant (>50%) improvement in the St Marks faecal incontinence score 50% (3/6) of patients showed an improvement in ICIQ-SF for the urinary incontinence score The ODS score only improved significantly (>50%) in 3 of the 21 cases and its median value was 9.5 (IQ ranges 7-14) before and 8.5 (IQ ranges 4.7-10.7) after PTNS (p= 0.05) Urinary retention was absent in all patients and no female patients complained of genital prolapse All the male patients except two and all the eleven women were not sexually active before and after treatment. Therefore, no changes were observed concerning sexual activity after PTNS The median FIQL score at 6 months follow-up however, did not show significant changes in any domain (lifestyle 2.7 *vs* 2.8, p=0.468, coping 1.6 *vs* 1.4 p=0.808, depression 1.7 *vs* 1.9 p=0.56, embarrassment 1 *vs* 1.3 p=0.27). The median resting and squeezing anal pressures did not change significantly before and after PTNS (33 (range 8-52) *vs* 35 (range 10-66) mmHg for resting pressure, and 49 (range 32-124) *vs* 46 (range 34-125) mmHg for squeeze pressure. Recto anal inhibitory reflex was present in 5 patients Drop out: none

Continues

other diseases, such as ulcerative recto-colitis [22].

Patients underwent low resection rectum (LAR) [20-22] with laparoscopic [20, 21] and laparotomic [22] approaches, and received different treatments, including chemotherapy [20, 21], and/or radiotherapy [22]. The authors included patients unresponsive to conservative therapy, such as diarrheal drugs [20-22], diet [20], biofeedback (BF) [20], pelvic floor muscle exercises (PFME) [21], or PFME and BF combined [22].

With respect to the outcome assessment [20-22], ev-

Table 2
Continued

Author, year of publication, country	Aim	Study design	Sample	Instrument	Intervention/ description	Results
D'Hondt et al. 2017 [21] **Belgium**	To investigate the impact of SNS on all symptoms of LARS	Prospective Follow up: 9 months (1-13)	Method: convenience N: 15 Age: average: 77.66 Gender: M 73.3% (11/15), F 26.7% (4/15) Inclusion criteria: surgical treatment for rectal cancer with LARS, unresponsive to conservative therapy (such as ant diarrheal drugs and pelvic floor physiotherapy). Surgical procedure: LAR 100% with TME 86.6% (13/15) and with PME 13.4% (2/15); in laparotomy 53.3% (8/15), in laparoscopy 46.7% (7/15) Temporary stoma: 26.7% (10/15) Anastomosis type: EtE 26,6% (4/15) StE 20% (3/15) and JP 53.4% (8/15) Perioperative treatment: preoperative 80% (12/15) and postoperative CT 86.6% (13/15)	LARS score (Emmertsen and Laurberg 2012). CCF-FI (Jorge and Wexner 1993). Manometry, colpo-cysto-defecography, ultrasound of the anal sphincter	SNS (First, the efficacy of the neurostimulation for the patient was verified through percutaneous nerve evaluation (PNE). The lead was introduced through the third sacral foramen and tested for 2 weeks)	The mean Wexner scores decreased from 17.7 to 4.6 (Z=2.93; p=0.003) The mean LARS score dropped from 36.9 to 11.4 (Z=2.93; p=0.003) Drop out: 4/15 Four patients, all with major LARS, had insufficient response to the PNE procedure
Eftaiha et al. 2017 [20] **Canada and USA**	To test the efficacy of SNS in LARS.	Retrospective Follow up:19.5 months (4-42)	Method: convenience N: 12 Age: average 67.8 ± 10.8 Gender: M 50% (6/12), F 50% (6/12) Inclusion criteria: surgical treatment of rectal cancer, with LARS, unresponsive to BF, fiber, anti-mobility agents for at least 12 months Surgical procedure: LAR in laparotomy 71.4% (15/21) and laparoscopy 28.6% (6/21) Temporary stoma: ND Anastomosis type: CA 58.3% (7/12) and CR 41.7% (5/12) Peri operative treatment: preparative CT 19% (4/21)	LARS score (Emmertsen and Laurberg 2012) CCF-FI (Jorge and Wexner 1993) Bowel diary Anal manometry, endo-anal ultrasound and defaecography	SNS (after failure of BF, fiber, anti-motility agents) 12 months)	At a median follow-up of 19.5 months, there were significant improvements in CCF-FI and in the LARS Scores (p=0.001) Drop out: none

Continues

ery study used the Cleveland Clinic Florida Fecal Incontinence (CCF-FI) [25], while diaries [20] for bowel habit assessment and the fecal incontinence quality of life score (FIQL) [26] were used for the QOL assessment [22].

The longest follow-up was 19 months (range 4-42) [20] and the shortest 9 (1-13) [21]. The outcomes demonstrated a statistically significant improvement in LARS syndrome [20-22], fecal incontinence (FI), and QOL [22].

Percutaneous tibial nerve stimulation

Two prospective studies [23, 24] that included 31 patients addressed PTNS to assess its efficacy in improving LARS, QOL, fecal and urinary incontinence, sexual dysfunction, and obstructed defecation. Patients underwent perioperative chemotherapy [23] or preoperative chemotherapy combined with postoperative radiotherapy [24]. In each study [23, 24], patients were included after conservative therapy failed (diet, drug, and BF).

Two studies [23, 34] used FIQL [26] to assess QOL;

Table 2
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Author, year of publication, country	Aim	Study design	Sample	Instrument	Intervention/ description	Results
Mege et al. 2017 [22] **France**	To analyze the effectiveness of SNS on poor functional results and on the QOL, after different colorectal resections.	Retrospective Follow up: 18 (3.5-91) months	Method: convenience N: 16 Age: average 53 Gender: M 43.8% (7/11), F 56.2% (9/16) Inclusion criteria: surgical treatment for rectal cancer or other diseases, unresponsive to conservative therapy (i.e. medications influencing stool consistency, pelvic floor rehabilitation and biofeedback) Surgical procedure: TP 44% (7/16), LAR with TME 37% (6/16), LE 9% (3/16) Anastomosis type: IPAA 44% (7/16), CA 37% (6/16) CR 19% (3/16) Temporary stoma: 81.2% (13/16) Perioperative treatment: preoperative 25% (4/16) and postoperative CT 12.5% (2/16)	LARS score (Emmertsen and Laurberg 2012) CCF-FI (Jorge and Wexner 1993). FIQL score (Rockwood *et al.* 2000). Endo-anal ultrasonography or pelvic Magnetic Resonance	SNS (ND)	In PC patient fecal urgency had improvement from 5 to 0 (p=0.02) and bowel frequency from 11 to 5 (p=0.004); FI from 4 to 1.8 (p=0.03) In CA and CR anastomosis bowel frequency decreased from 10 to 2 (p=0.04), FI from 5 to 0.6 (p=0.02) while bowel urgency from 2 a 4.8 (p=0.86) FIQL significantly improved Drop out: 2/16
Vigorita et al. 2017 [24] **Spain**	To evaluate the effectiveness of treatment with PTNS in LARS and to identify predictors of the outcome	Prospective Follow up: 6 week	Method: convenience N: 10 Age: average 62 years Gender: M 60% (6/10), F 40% (4/10) Inclusion criteria: patients > 18 years old, surgical treatment for rectal cancer with LARS, unresponsive to conservative therapy (i.e. medications, fiber and biofeedback) Surgical procedure: LAR Anastomosis type: ND Perioperative treatment: CT 80% (8/10). Preoperative 50% (5/10) and postoperative RT	LARS score (Emmertsen and Laurberg 2012) CCF-FI (Jorge and Wexner 1993) FIQL score (Rockwood *et al.* 2000) Bowel diary Endo-anal ultrasound and anal manometry	PTNS (All patients had 2 PTNS sessions per week - 30 min for 6 weeks. Therefore patient with an improvement had second phase of PTNS)	LARS Score improvement has observed in 50% of patients with total resolution of LARS in 20% (2/10) FI decreased in 70% (7/10) of the patients. The median Wexner score decreased from 14 (IQR 10.75–18.5) to 10 (IQR 6.5–18) after treatment (p = 0.034) A significant improvement in FIQL score in lifestyle, depression, and daily defecation urgency (p=0.05). Drop out: 3/10 Only seven patients (70%) responded positively after the first phase and received the second phase of treatment. After the second phase, three patients with major LARS symptoms became classified as minor LARS and two patients with minor LARS had resolution of the syndrome. Two patients did not show improvement in LARSS; however, they demonstrated an improvement in defecation diary and FIQL Of the three patients who did not respond, two were from the major LARS group and one from the minor LARS group

Continues

Table 2
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F = female; M = male; ND = non described; FI = fecal incontinence; UI = urinary incontinence; QOL = quality of life.
Surgical procedure: LAR = low anterior resection; LARS = low anterior resection syndrome; LE = left hemicolectomy; PEC = percutaneous endoscopic cecostomy; PME = partial mesorectal excision; TME = total mesorectal excision; TP = total proctolectomy.
Anastomosis type: CA = coloanal anastomosis; CR = colorectal anastomosis; EtE = end-to-end anastomosis; HS = hand-sewn anastomosis; IPAA = ileal pouch-anal anastomosis; JP = j-pouch anastomosis; StE = side-to-end anastomosis.
Treatments: AE = anterograde enema; BF = biofeedback; CT = chemotherapy; PNE = percutaneous nerve evaluation; PTNS = percutaneous tibial nerve stimulation; RT = radiotherapy; SNS = Sacral Nerve Stimulation.
Scales:
Baden-Walker score ((Baden and Walker 1972): for genital prolapse assessment.
Cleveland Clinic Florida Fecal Incontinence (CCF-FI) scoring system or Wexner Fecal Incontinence Score (Jorge JM and Wexner SD 1993): to assess fecal incontinence. The range score from 0-8 (midle incontinence), 9-14 (moderate incontinence), 15-20 (severe incontinence).
Fecal Incontinence Quality of Life score (FIQL) (Rockwood *et al.* 2000): to assess fecal incontinence quality of life: divided in 4 dimensions (Depression, Embarrassment, Life Style and Coping). The score range from 1 (low quality of life) to 4 (good quality of life).
Gastrointestinal Quality of Life Index (GIQLI) (Slimet *al.* 1999): to assess quality of life in physical status, emotions, social integration, and the effect of medical treatment. The score ranging from 0 to 144 (excellent).
International Index of Erectile Function (IIEF-5) (Rosen *et al.* 1999): diagnostic tool for erectile dysfunction.
International Consultation on Incontinence Questionnaires Short Form (ICIQ-SF) (Tubaro *et al.* 2006): for assessment of urinary incontinence.
Low Anterior Resection Syndrome score (LARS) (Emmertsen *et al.* 2012): to assess LARS syndrome. The range score was from 0 to 42: 0 to 20 (no LARS), 21 to 29 (minor LARS), and 30 to 42 (major LARS).
Obstructed Defecation Syndrome (ODS) (Altomare *et al.* 2008): to assess obstructed defecation.
Pelvic Organ Prolapse/Incontinence Sexual Questionnaire IUGA-Revised (PISQIR) (Rogers *et al.* 2013): questionnaire for sexual dysfunction.
Premature Ejaculation Diagnostic Tool (PEDT) (Symond *et al.* 2017): for assessment sexual disfunction in man.
St. Mark's score (Vaizey *et al.* 1999): to assess fecal incontinence. The score range from 0 (continence) to 24 (incontinence).
Three Axial Perineal Evaluation score (TAPE) (Atlomare *et al.* 2017): to assess pelvic floor function evaluation (urinary, fecal and sexual) normal value 100% of hexagonal area.
Urinary retention: was scored according to the volume of urine retention if present (score 0 when absent, 1 if >50 ml, 2 if between 50-100ml and 3 if > 100ml of urine).

one used the CCF-FI [25] and bowel diary to evaluate fecal incontinence and bowel movement; another [23] adopted the St Marks score [27] for fecal incontinence assessment, while the other studies adopted the three axial perineal evaluation (TAPE) score [28, 29] (comprehensive of the obstructed defecation syndrome (ODS) score [30], the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF) score [31], the Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire (PISQIR) (IUGA-Revised) [32], the International Index of Erectile Function (IIEF-5) Questionnaire (SHIM) [33], the Premature Ejaculation Diagnostic Tool (PEDT) score [34], and the Baden-Walker score [35] to measure obstructed defecation, urinary incontinence, sexual dysfunctions, and genital prolapse. Urinary retention was scored according to the volume of urine retention, if present (score 0 when absent, 1 if > 50 ml, 2 if between 50-100 ml, and 3 if > 100 ml of urine) and FI with the anal manometry for pressure assessment [23]. Follow up occurred from 6 weeks [24] to 6 months [23].

Patients reported a significant improvement in fecal and urinary incontinence; obstructive defecation improved in only three patients, and because the sample was not sexually active, sexual function was not assessed [23]. There were no significant changes in the FIQL and in anal pressure after PTNS [23]. In one study [24], all patients underwent PTNS in the first phase of the study; however, only those who experienced an improvement in incontinence (70%) were included in the second phase, and three patients showed no LARS improvements. In this study, QOL improved in lifestyle, depression, daily defecation, and urgency.

DISCUSSION

Eighty percent of patients who undergo a low or very low rectal anterior resection with sphincter preservation will experience LARS [2], a syndrome that has adverse effects on patients' QOL, in the postoperative period [2-4].

Currently, no indications of the best rehabilitative interventions are available, and thus, we conducted this review to identify the treatments that reduce LARS symptoms best in patients who have undergone surgery.

Methodological issues

The methodological quality of the studies selected differed significantly and all had a small sample size. Two used a retrospective design [20-22] that may have introduced a recall bias because patients must remember their symptoms. Moreover, the prospective studies used follow-up times that ranged from 6 weeks [24] to 91 months [22]. The literature underscored the fact that LARS appears immediately after surgery, becomes more evident during the first several months, and stabilizes after approximately one to two years [7]; with this assumption, follow-up times up to two years should be appropriate. At the same time, we should consider the results of studies with shorter follow-up times with caution, because patients likely will experience further improvements in outcomes. Moreover, the studies with long follow-ups may be associated with a high percentage of drop out and the possibility of confounding variables biases, such as age or changes in clinical conditions [36], and the author can attribute treatment efficacy or failure to the level of the anastomosis from the anal verge (lower anastomosis might have worst results) and preoperative radiotherapy [24]. Therefore, these factors' potential roles must be explored in future studies, which also should include larger samples to assess the differences in treatment results with respect to the surgical procedure and perioperative treatments.

The studies' inclusion criteria also differed greatly. One source of bias may be the inclusion of patient with a temporary stoma. The presence of a temporary

ileostomy is a risk factor for bowel dysfunction following surgery [37, 38] because prolonged inactivity of the pelvic floor and sphincter complex contributes to LARS [39-40].

The researchers used different instruments to assess LARS outcomes, QOL, and incontinence, and thus, the results of the studies are not comparable; moreover, a meta-analysis cannot be conducted. Therefore, we should consider the outcomes evaluations with caution. In addition, the LARS score simply is a screening instrument [21] and does not consider urinary and sexual dysfunction, which are included as symptoms in LARS syndrome [41, 42]. With this assumption, LARS [18] scores always should be accompanied by the TAPE score [28, 29].

Treatments to reduce LARS symptoms

We selected studies that included adult patients who underwent low rectum resection with sphincter preservation and with pre/post LARS evaluation. The treatments analyzed were the SNS [20-22] and PTNS [23, 24].

The patients recruited were treated first with conservative therapy, including constipating medications, physiotherapeutic pelvic floor exercises, and biofeedback, all of which have been demonstrated effective in improving FI, bowel movement [43-45], and QOL [43-45]. We need stronger evidence about conservative therapy's efficacy and which outcomes it improves most effectively; these findings should indicate whether to submit patients for conservative therapy initially, and then, in cases of inefficacy, for other treatments.

Two studies [23, 24] considered PTNS treatment. One [23] concluded that it offers a moderate improvement in LARS syndrome, but argued that it can be associated with the time or the level of the anastomosis and preoperative treatment, while the other study [24] included a sample of only 10 patients and considered only those who showed improvement after the first phase of PTNS in the outcome assessment. Among these, two demonstrated no improvement in the LARS score, but QOL and defecation habits did improve [24]. Future studies should investigate more thoroughly the roles the anastomosis level and type and preoperative treatment (chemotherapy and radiotherapy) play in the LARS rehabilitation process.

Three studies addressed SNS efficacy in improving LARS [20-22]. One found improvement in such FI and LARS symptoms as clustering and urgency. The second [21] demonstrated that SNS is effective for LARS and FI, fragmentation, and urgency, but four patients, all with major LARS, had insufficient responses to the treatment. The final study [22] was multi-centric, but retrospective, with a small sample, and found improvement in FI, LARS scores, stool frequency, and urgency in 86% of cases. In the outcome evaluation, these authors [20-22] also used manometry [20-22], colpocysto-defecography [20-22], ultrasound of the anal sphincter [20-22], anorectal dyssynergy [22], sphincter electromyogram [22], and magnetic resonance [22], and not simply self-reports or medical records alone. However, there were no post treatment data on these evaluations.

It is difficult to judge which treatment is the best, because only two studies considered PTNS [23, 24] and only one [23] evaluated patient outcomes thoroughly and extensively. Moreover, the authors argued about moderate improvement [23] or excluded patients who did not improve during the first phase of the study from the second phase of PTNS [24]. Among the three studies that evaluated SNS [20-22], two demonstrated improved outcomes, although they were retrospective [2,22]. Our conclusion seems to confirm Ramage *et al.* finding, in which the authors reported a significant improvement of LARS symptoms in 74% of patients after SNS. However, this technique's high cost also must be considered [46].

This review has several limitations. We consulted only certain databases and included only those studies with abstracts available. Therefore, despite the systematic approach we used to identify studies, these conditions may have excluded some publications. Lastly, by including only studies with pre and post treatment LARS score evaluations, we may have excluded from our review studies that reported potentially valid treatments.

Implications for practice and further research

Future studies must be multi-centric RCTs with larger samples of patients who have failed to respond to conservative therapy. Further, great attention should be given to the patient inclusion criteria. Because the LARS etiology is multifactorial [47, 48], we must consider patient groups that represent every surgical procedure and perioperative treatment.

Moreover, the follow up time should be approximately two years, because LARS symptoms have stabilized by that time [7].

In clinical practice patients should evaluate with the LARS [18] and TAPE scores [28, 29] in pre and post treatment, and the FIQL [26] should be considered in the QOL assessment. Only two studies [32, 33] consider the urinary and sexual dysfunction which are included as symptoms in LARS syndrome [41, 42]. Physician should take into consideration any patient problem and should include in their interventions also the caregivers. In addition, our findings highlight the importance to include in education programs the best rehabilitation strategies for the patient affected of LARS.

CONCLUSION

Most patients who undergo a low or very low rectal anterior resection experience LARS postoperatively, a syndrome that has significant adverse effects on their satisfaction and QOL. From our review, SNS seems to be the most effective treatment for LARS symptoms and is less expensive than is PTNS; however, PTNS is a minimally invasive procedure [23].

Future research must be implemented with higher quality studies and with pre-post treatment assessment with LARS scores. This will allow us to develop the most effective intervention protocol, with the goal of identifying the least invasive and most effective treatment for LARS.

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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ORIGINAL ARTICLES AND REVIEWS

Syringomyelia and Chiari Syndrome Registry: advances in epidemiology, clinical phenotypes and natural history based on a North Western Italy cohort

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Abstract

Background. Syringomyelia and Chiari Syndrome are classified as rare diseases, but current known occurrence in Europe is missing. The increased ability to diagnose these pathologies by magnetic resonance imaging and its widespread availability has led to an increase of reported cases, often asymptomatic, with the need to standardize definitions, diagnostic criteria and treatments.

Aims. We present shared Interregional Recommendations developed with the primary aim to estimate Syringomyelia and Chiari Syndrome prevalence and incidence in North Western Italy, with special reference to symptomatic forms.

Methods. An agreement for the standardization of definitions, classifications, diagnostic criteria and surgical Recommendations was reached by the multidisciplinary Interregional Piemonte and Valle d'Aosta Chiari-Syringomyelia Consortium (Delphi method); next, in 2011 a census for Syringomyelia and Chiari Malformation was performed through the Interregional Piemonte and Valle d'Aosta Rare Disease Registry, integrated by a dedicated form in order to estimate prevalence and incidence.

Results. 436 patients, 292 females, met shared interregional diagnostic criteria. Syringomyelia prevalence was estimated in 4.84:100 000; Chiari Malformation prevalence was 7.74:100 000; incidence was 0.82:100 000 and 3.08:100 000 respectively. Demographics, neuroradiological parameters and aetiology were reported (in symptomatic and asymptomatic forms). Finally, symptoms and signs, familiar and natural history were analyzed.

Conclusions. First Italian epidemiological data (prevalence, incidence) on Chiari and syringomyelia was collected, according to shared diagnostic Recommendations. Future perspectives include the adoption of these Recommendations at national level to standardize the access to diagnosis and care process and promote multicenter clinical trials.

Key words
- epidemiology
- rare disease
- Syringomyelia
- Chiari Malformation
- recommendations

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INTRODUCTION

Syringomyelia (Syr) is morphologically defined at magnetic resonance imaging (MRI) as the presence of single or multiple fluid-filled cavity (syrinx) within the parenchyma of the spinal cord and/or the bulb (Syringobulbia) and classified as a rare disease (ORPHA3280). About 50% of Syr patients have severe neurological damage, chronic-progressive disability with complete loss of independence. Prognostically speaking, even more unfavourable is the presence of Syringobulbia (swallowing and breathing bulbar centers involved). Before 1968, neurologists diagnosed Syr only by typical neurological symptoms. Since the introduction of CT myelography and MRI techniques for spinal cord imaging, the diagnosis has become easier, and many cases have been reported. In studies conducted before the advent of modern neuroimaging, prevalence ranged from 3.3 to 8.5/100 000 [1-3]; after the advent of MRI estimated prevalence ranged from 1.9 to 8.4/100 000 [4, 5].

Chiari Malformation (CM) includes a heterogeneous group of abnormalities characterized by the caudal cerebellum ptosis through the foramen magnum; clinical manifestations define the Arnold-Chiari or Chiari Syndrome (CS). The malformation can cause a wide variety of neurological symptoms, often vague or nonspecific, such as headaches, ocular disturbances, otoneurologic disturbances, lower cranial nerve signs, cerebellar ataxia, or spasticity [6]. Onset of symptoms is usually in the third decade of life. However, many individuals with CM remain asymptomatic even later in life. A US study estimated 400 000 patients affected by Chiari type 1 Malformation (CMI), the most common CM type. The epidemiology of CMI malformation has been scarcely investigated. The true population prevalence of CMI is unknown and there are no studies on CMI incidence. A retrospective analysis of more than 22 000 neuroimaging diagnoses [7] provided indirect data of CMI prevalence, estimable in 1:1280 (0.77%). When CM is defined by cerebellar tonsil position 5 mm or more below the foramen magnum, imaging prevalence studies estimate CM prevalence at between 0.24 and 3.6% of the population [8]; the discrepancy between these estimates is a result of the different age groups analysed, substantially higher in children and young adults compared with older adults. In Italy and in the European Community the CMI is classified as a rare disease (ORPHA268882), but current known occurrence data in Europe is missing.

The increased ability to diagnose CM and Syr by MRI and its widespread availability has led to an increase of reported cases, often asymptomatic or minimally symptomatic, with the need to standardize definitions, diagnostic criteria and treatments.

In 2001 DM 279/2001, National Law [9] set up the Italian National Network for rare diseases, to deal with the prevention, surveillance, diagnosis and treatment of rare diseases (RD), including Arnold-Chiari or Chiari Syndrome; the same law activated the RD National Registry located at the Istituto Superiore di Sanità, which is expected to receive epidemiological data from Regional Registries. A Regional Decree (2/3/2004, n. 22-11870) established the Piemonte Regional Network for the prevention, screening, diagnosis and therapy of RD. Moreover, it obliges the Hospital Units to report RD in the Regional Registry [10], with epidemiological and legislative purposes (i.e. to regulate the access on exemption path for RD). In 2005 because of the low prevalence estimates, the potential clinical severity in chronically debilitating nature and the resulting significant expense for its treatment, Syr was inserted as RD involving the nervous system in Piemonte and Valle d'Aosta [11]. Now, with the adoption of the new National law on Essential Assistance Levels [12], Syr is recognized also as RD in all Italian regions, but previously this was recognized as RD only in Piemonte, Valle d'Aosta, Toscana and Marche; for this reason Italian epidemiological data on Syr was not available in the RD National Registry.

Syr and CM are classified as rare diseases on Orphanet, the International reference portal for RD and orphan drugs, but current prevalence data is missing.

Guidelines on diagnostic criteria and case definition are missing, and consequently estimation of the prevalence in Piemonte and Valle d'Aosta for symptomatic and asymptomatic forms are missing.

In 2008 a consortium dedicated to the study of Syr and CM began its activity in the Piemonte and Valle D'Aosta regions, as part of the Rare Diseases Network of the Italian National Health Service, with the aims to standardize/share definitions, classifications, care and diagnostic approaches.

The consortium, named the Interregional Chiari-Syringomyelia Consortium (CSC), was composed by clinicians (neurologists, neurosurgeons, neuroradiologists, physiatrists, neuro-urologists, psychologists, speech pathologists, spinal surgeons, pain specialists), experts of public health for RD, and patient association representatives [13]. In 2010 the CSC Recommendations were proposed: some of these indications derived from the outcomes of the CSC meetings, others were from the First International Chiari Consensus Conference, held in Milan in 2009 [14].

Diagnostic, Surgical and Rehabilitative Recommendations were approved by the members of Technical-Specialized Task-force supporting the Regional Center of Coordination for RD and by the following scientific societies: Italian Society of Neurology, Piemonte and Valle d'Aosta section; Italian Society of Neurologists, Neurosurgeons, Neuroradiologists, Piemonte-Valle d'Aosta-Liguria section; Italian Society of Physical Medicine and Rehabilitation, Piemonte section [15]. The document was published as technical-scientific integration of the Regional Legislation (DGR n. 95-13748, 29 March 2010), specifying the Institution of the first Center of Expertise for Chiari and Syringomyelia in Torino [16, 17].

In this study, based on the Interregional Recommendations, we present the first estimation of prevalence and incidence of Syr and CM in North Western Italy (Piemonte and Valle d'Aosta), with special reference to symptomatic forms (Symptomatic Syringomyelia: SS; Chiari Syndrome: CS).

MATERIAL AND METHODS

Interregional recommendations

In order to estimate the epidemiological indices (prevalence and incidence), the diagnostic criteria used

for case definition was based on the Interregional Diagnostic, Surgical and Rehabilitative Recommendations. The Recommendations were developed following several steps: a) collecting all available evidence on clinical studies related to Syr and CM. The following databases were queried for literature review on Syr and CM: Medline (PubMed interface, www.pubmed.gov), Cochrane Library (Health Library of Piedmont, www.bvspiemonte.it), National Guidelines Clearinghouse (www.guideline.gov). The literature searching strategy was conducted by combining: the Medical Subject Heading (MeSH): "Arnold-Chiari Malformation" or "Syringomyelia" or "Arnold-Chiari Malformation" AND "Syringomyelia"; Publication type (PT): "Systematic Reviews", "Practice Guideline", "Meta-Analysis", "Randomized Controlled Trial"; b) assessing studies for relevance. All studies were selected and critically evaluated. Results with low evidence level were restricted for date (last 10 years) and language (English, French, German, Spanish, Italian); these were also selected and critically evaluated; c) categorising the evidence. Each expert member expressed an evaluation of the articles, giving a score between 0 (strongly disagree) and 10 (strongly agree), according to the Delphi method [18]. If there was an agreement, the document was revisited in textual form and submitted to the working group for the final approval and subsequent drafting of the ultimate document; for evaluation lower or equal to 7 it was necessary to propose an alternative text version and relative notes. If no agreement was reached, the document came back to the recommendation authors with comments for necessary changes and resubmitted to the experts involved. When an agreement was reached, the document in the preliminary form was prepared and submitted to the judgment of the entire experts group for final consensus. A description of the sections of the Recommendations including classifications, radiological and clinical definitions, surgical and conservative indications are reported in *Table 1* and *Table 2*.

Census study

According to these Recommendations, in 2011 a Syr and Chiari census study was performed through the Interregional Piemonte and Valle d'Aosta Registry of RD, integrated by a dedicated Case Report Form, reported in *Figure 1*; the CSC form was developed by the Chiari-Syringomyelia Consortium and filled out by every specialist (neurologist or neurosurgeon) involved in the diagnosis.

We integrate the data extracted from the RD Registry (minimal data set) with the CSC form in order to estimate Syr and Chiari prevalence and incidence; moreover, the CSC form enriched the information providing further socio-demographics and clinical data. The study was approved by the Local Ethic Committee ("Prospective collaborative epidemiologic, clinic and genetic study in CM with and without Syr, hereditary connective tissue disorders and tethered cord", Protocol n. 7837, 1/2/2010, Città della Salute e della Scienza di Torino Hospital, Turin). All partecipants gave signed informed consent at the time of inclusion in the census study.

Statistical analysis

Up to 31 December 2011, the Syr and Chiari age standardized prevalence and the incidence rate in 2011 with 95% confidence intervals (95% CI) in Piemonte and Valle d'Aosta Regions was estimated. The prevalence (number of alive diagnoses up to 2011 year) and the incidence (number of new reported cases in 2011 year) were estimated using both the Interregional Piemonte and Valle d'Aosta Rare Disease Registry and the CSC census data for symptomatic forms. The CSC census data was also used to analyse asymptomatic forms. The standardization was performed by a direct method using the Italian census population at 1st January 2011 year (respectively 4 457 335 inhabitants in Piemonte and 128 230 in Valle d'Aosta, 4 585 565 total population), according to ISTAT census data (2012) [18, 19]. Confidence intervals were calculated assuming a Poisson distribution.

Basic statistics in terms of frequencies (absolute and percentage values) were calculated for: socio-demographic data, age at diagnosis, age at survey, diagnostic delay, MRI parameters (morphology/level), diagnoses, associated conditions, neurological symptoms/signs, types of surgery.

All statistical analyses were performed using the Stata statistical software (StataCorp. Statistical Software: Release 7.0. College Station, TX: Stata Corporation. 2001).

RESULTS

Census results

Using CSC form, 436 patients (292 females and 144 males) met shared diagnostic criteria for Syr and/or CM (*Table 3*). Demographics, MRI parameters (morphology/level), diagnoses, associated conditions, estimated prevalence and incidence data were reported respectively in 347 CM patients and in 217 patients affected by Syr. Percentage of symptomatic Chiari (CS) and symptomatic Syr (SS) are represented in *Figure 2*. In 2011 the estimated Syr prevalence is 4.84:100 000 and incidence is 0.82:100 000; the estimated CM prevalence is 7.74:100 000, incidence 3.08:100 000 (*Table 4* and *Table 5*).

Description of clinical manifestations

Major neurological symptoms in CS and in SS were respectively: headache (48% and 28%), cervical pain (30% and 24%), loss of balance (30% and 18%). Major neurological signs in CS and in SS respectively were: sensory disorders (48% and 70%), motor disorders (32% and 60%), cranial nerves (41% and 40%), autonomic bladder disorders (14% and 19%). Neuropathic pain, defined on the basis of a DN4 questionnaire score higher or equal than 4 [30], was 19% in CS and 32% in SS. Familiar history was positive in 5% in CS and in 2% in SS (familiar forms); pregnancy was carried out by vaginal delivery in 67% and 55% respectively in CS and SS, while by caesarean delivery (general anaesthesia) in 23% and 35%. Scoliosis was reported in 29% of all CM and 32% of all Syr; 43 patients (25%) presented with scoliosis, CM1 and Syr.

In our study females are prevalent in all groups (*Table*

Table 1
Diagnostic Recommendations for Chiari 1 Malformation and Syringomyelia (elaborated by the Interregional Chiari and Syringomyelia Consortium)

Chiari Malformation (CM) classification
CM is a congenital anomaly of the cerebellum associated or not with neural tube defects
1. CM I: paraxial mesoderm disorder, with abnormalities of the posterior cranial fossa (mostly small) and the consequent descent of the cerebellar tonsils
   CMI-A: with Syr in MRI
   CMI-B: without Syr in MRI
2. CM II: associated with myelomeningocele (prevalent in childhood), hydrocephalus, and, less frequently, hydrosyringomyelia Other types of intracranial defects (hypoplastic tentorium cerebelli, cranial lacunae, anomalies of the Sylvius aqueduct) may exist
3. CM III: intracranial defects associated with Chiari II Malformation (very rare and severe form)
4. CM IV: cerebellar aplasia or hypoplasia, associated with aplasia of the tentorium cerebelli [21]

Chiari Malformation: "subtypes" classification
1. Classical CMI + craniosynostosis + osteopetrosis
2. CMII + Tethered Cord Syndrome (TCS)
3. CMI + inherited disorders of connective tissue-HDCT (i.e. Ehlers-Danlos syndrome)
4. Hypertension intracranial + hydrocephalus + space occupying process
5. Hypotension intraspinal CSF + lumbo-peritoneal shunting [22]

Chiari Malformation (neuroradiological) definition
According to IHS diagnostic criteria (the second updated edition of "International Classification of Headache Disorders", code 7.7), cerebellar tonsillar herniation is defined by one of the following on craniocervical MRI:
- ≥ 5 mm caudal descent of the cerebellar tonsils
- ≥ 3 mm caudal descent of the cerebellar tonsils plus at least one of the following indicators of crowding of the subarachnoid space in the area of the craniocervical junction:
- compression of the CSF spaces posterior and lateral to the cerebellum
- reduced height of the supraocciput
- increased slope of the tentorium
- kinking of the medulla oblongata [23]

Chiari Syndrome definition
CS is the clinical manifestation (symptoms and signs) of CM (radiologically defined), or "symptomatic Chiari".
Clinical diagnostic criteria (symptoms and neurological signs) are:
1. Headache, according to IHS diagnostic criteria characterised by at least one of the following criteria:
   - precipitated by cough and/or Valsalva manoeuvre
   - occipital and/or sub-occipital headache
   - associated with symptoms and/or signs of brainstem, cerebellar and/or cervical cord dysfunction
2. Otoneurogical symptoms and/or signs (eg, dizziness, disequilibrium, sensations of alteration in ear pressure, hypacusia or hyperacusia, down-beat nystagmus, oscillopsia)
3. Transient visual symptoms (spark photopsias, visual blurring, diplopia or transient visual field deficits)
4. Demonstration of clinical signs relevant to cervical cord, brainstem or lower cranial nerves or of ataxia or dysmetria

Notes: for the diagnosis of Chiari Syndrome, in addition to the typical headache (criterion 1), neurological symptoms/signs (at least two of criteria 2-4), evidence of posterior fossa dysfunction, are mandatory [23]

Syringomyelia/Hydromyelia: classification and definition
1. Type I: with obstruction of the foramen magnum and dilation of the central spinal canal
A) Associated with CMI
B) Associated with other obstructive lesions of the foramen magnum
2. Type II: syringomyelia without obstruction of the foramen magnum, or idiopathic
3. Type III: syringomyelia with other diseases of the spinal cord
   A) Spinal cord tumours (usually intraspinal)
   B) Traumatic myelopathy
   C) Spinal arachnoiditis and pachymeningitis
   D) Myelomalacia due to compression of the spinal cord (tumour, spondylosis)
4. Type IV: pure hydromyelia, developmental widening of the central canal of the spinal cord, usually associated with hydrocephalus

Notes: 1) The diagnosis of Syringomyelia-Syringobulbia is attributable by neurologists or neurosurgeons in the presence of Syrinx/Syringobulbia at MRI in addition to spinal/bulbar signs related to the syrinx level. The clinical criteria are mandatory; 2) Hydromyelia is an intramedullary, centrally located, non-enhancing, slit-like cavitation, often localized short-segment and occurring in a non-enlarged or only slightly enlarged spinal cord ("idiopathic localized hydromyelia"); clinically, patients present without neurological deficits but unspecific pain syndromes; they lack electrophysiological alterations and progressive signs/symptoms specifically related to the spinal cord [24].

3), and in particular in the CM group (68%). A slight prevalence of employed compared to unemployed (student and retired person) is present in the Syr group. Maybe this is due to the small sample size in the pediatric subgroup in Syr (≤18 yrs, students = 11%) compared to CM (20%) with average age at diagnosis of 34 yrs (lower than 36 yrs of Syr group). This may have a significant social impact for the high prevalence of symptomatic forms in Syr (62%), potentially severely disabling, compared with a lower percentage (40%) of Chiari symptomatic forms (*Figure 2*, *Tables 4*, *5*). Percentage in the over 60 yrs subgroup (retired person) is similar in both groups (18% CM and 22% Syr, *Table 3*).

Among CM, isolated form (CMI-B) is more frequent

Table 2
Surgical Recommendations for Chiari 1 Malformation and Syringomyelia (elaborated by the Interregional Chiari and Syringomyelia Consortium)

CSC Surgical Recommendations
- CM I-B symptomatic (CS isolated): children and adults with headache (typical) + auditory/cerebellar/spinal/visual signs
- CM I-A (CM I + Syr): children and adults, symptomatic and asymptomatic, especially in the case of
  1) holocord syringomyelia
  2) evolutionary trend (clinical/MRI worsening),
  3) central syringe and Vaquero Index >0.5 [25] or eccentric syringe
  4) syringomyelia with syringobulbia (spinal/bulbar signes)

Notes:1 In children with CMI-A surgical indications are larger, even if asymptomatic (prognostic value of early surgery: disappearance/reduction of syringe), while in children with CM I-B (without syringe) surgical indications are not clear in asymptomatic forms ("wait and see", with clinical and neuroradiological follow-up); 2) Asymptomatic and isolated Syringomyelia: in children and in adults surgical indications are not clear; if symptomatic forms, no consensus for surgery; 3) Post-traumatic Syringomyelia: no indication for direct decompression at the time of initial injury; a strong recommendation for surgical intervention in the presence (setting) of motor neurologic deterioration; a weak recommendation against surgical intervention for patients developing sensory loss/pain syndrome or for asymptomatic but expanding syrinx [26, 27].

Neurosurgical strategies
- CM I-A (with syrinx) and CMI-B symptomatic
  First Line: C1 occipito-cervical decompression with dura opening and dural plastic
  In children with isolated CM, surgery can be limited to the bone decompression (delamination of the atlanto-occipital ligament), without duraplasty [28, 29]
- CM 1 and hydrocephalus
  First Line: third ventriculostomy by endoscopy
  Second Line: osteo-dural decompression of the posterior fossa
- Re-interventions
  For patients developing neurological deterioration and expanding syringe (failure of first/second Line surgery)

Notes:1) Surgical efficacy is inversely proportional to the number of treatments; 2) Section of the filum terminale (in presence of "occult" tethered cord) is not a procedure of choice in the treatment of Chiari Syndrome; spinal cord detethering in CMI is accepted only when a real tethered cord is associated.

(58%), while CMI-A type (CMI and Syr) is just 36%, according to literature data reporting association ranges 32-74%. CM type 2 (CMII) is reported in 1%; other associated conditions, such as retroflexed odontoid, hydrocephalus, Klippel-Feil, Tethered Cord Syndrome, are reported in 5% of the cohort. Syr type I (associated to CM1) is the prevalent clinical phenotype (59%), while isolated Syr is at 41% (18% pure hydromyelia, 14% secondary and 9% idiopathic). Males are more symptomatic than females in both symptomatic groups (47% in CS, 64% in SS), even if the estimations on gender and on measure of tonsillar herniation don't identify associated risk factors in CS patients.

Hydromyelia patients (Pure-Hydromyelia included) are less than a third of Syr and are mostly asymptomatic (82%); this result on MRI morphology confirms the trend of Hydromyelia in presenting a low risk of clinical evolution towards symptomatic forms (18%).

Negative prognostic factors in the Syr group, with higher percentage (> 50%) of symptomatic patients (SS), are identified (*Table 5*). MRI syrinx distribution: cranial level (syringobulbia 100%, cervical 61%) in focal/single cavity; MRI syrinx extension (multilevel or olocorde 81%); aetiology (Secondary 77% and Primary 74%). In CS (*Table 4*) positive prognostic factor (poor/any clinical evolution) at MRI morphology (as tonsillar herniation length ≥5 mm/3-4 mm/<3 mm) is a tonsillar descent <3 mm (100% in ACM), while a descent ≥5 mm is not a significant prognostic indicator to clinical evolution in CS.

Among different clinical phenotypes: CMII is the less frequent and the most severe form (100% CS); CMI-A and other associated condition forms are symptomatic in approximately 60% of cases; CMI-B (isolated Chiari) is mostly asymptomatic with just 25% of CS (*Table 4*).

A high percentage of SS patients has sensory and motor disturbances (respectively 70% and 60%); neuropathic pain is relevant in Syr (32%), much more frequent than in CS group (19%). Percentage of autonomic disturbances (bladder dysfunction) is similar in both groups (19% SS *vs* 14% CS). Familiar forms are reported, confirming a role for genetic factors in the disease pathogenesis.

DISCUSSION

This study reports diagnostic and surgical Recommendations for Chiari and Syr, according to the International Consensus Conference in Milan in 2009, including a panel of experts, developed by the Interregional Piemonte and Valle d'Aosta Chiari and Syringomyelia Consortium [13, 14, 15].

Based on these diagnostic Recommendations, the first Italian epidemiological study for CM and Syr was designed to estimate prevalence and incidence in symptomatic and asymptomatic forms.

Our census study involved patients diagnosed in Piemonte and Valle d'Aosta hospitals, with a total population of 4 484 469 inhabitants, with a 99% Caucasian ethnic group. The prevalence estimation for Syr was similar to that reported in a New Zealand study [4] relative to prevalence in Caucasians (5.4/100 000), but was higher than the results of a nationwide survey in Japan, where prevalence was estimated in 1.9/100 000 [5]. We reported, to our knowledge, the first incidence estimates for Syr and Chiari in literature at international level.

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CHIARI-SYRINGOMYELIA CENSUS FORM

Center Name:	Country:	Date: / /
Patient:	Birthplace:	Birth date: / /
Residence:	Gender: ☐F ☐ M	Occupational status: ☐ Employed ☐Unemployed*
Partecipant Race: ☐White	☐ Black ☐ Hispanic ☐ Asian	☐ Other: _____

Medical history and family
MC/Syr familiarity: ☐YES ☐ NO (if yes, affected relative: _____)
Scoliosis: ☐YES ☐ NO
Pregnancy: ☐YES ☐NO (if yes: ☐physiological delivery ☐general anaesthesia)
Surgery: ☐MC ☐ Syr ☐ MC+Syr Number of surgery: ___
- Date : / /
- PFD**: ☐YES ☐ NO
- (if yes: ☐bony ☐osteo-dural ☐ osteo-dural+ duraplasty ☐osteo-dural+ tonsillar coagulation)
- Other Type: _____

Pathological MRI: ☐Brain ☐Spinal cord ☐Brain+spinal cord

Signs and symptoms:
☐Headache ☐Loss of balance ☐Cervical pain ☐ Dysphagia ☐ Nystagmus ☐ Neuropathic pain
☐Hyposthenia UL *** ☐Hyposthenia LL**** ☐ Upper MTN signs***** ☐ Lower MTN signs*****
☐Paraparesis ☐ Tetraparesis ☐Paraplegia ☐ Tetraplegia ☐ Spasticity ☐Hypoaesthesia UL***
☐Hypoaesthesia LL**** ☐ Sensory Levels ☐ Paraesthesia ☐Urge-incontinence ☐ Incontinence

Diagnosis date : / /
☐CMI (Tonsillar herniation : ☐ <3mm ☐ 3-4mm ☐ ≥5mm)
☐CMII (Myelomeningocele: ☐YES ☐ NO)
☐Chiari Syndrome
☐ Primary Syringomyelia ☐Idiophatic Syringomyelia ☐Secondary Syringomyelia
(level: ☐cervical ☐dorsal ☐cervical-dorsal)
☐Syringobulbia
☐Hydromyelia (level: ☐cervical ☐dorsal ☐cervico-dorsal)
☐Hydrocephalus ☐ TCS ☐ Occult TCS ☐Retroflexed Odontoid ☐ Klippel-Feil

*Unemployed person, retired persons, students; **PFD: Posterior Fossa Decompression;
*** UL: upper limbs; ****LL: lower limbs; ***** MTN: motoneuron

Figure 1
Dedicated clinical Consortium form developed by the Interregional Chiari and Syringomyelia Consortium, including medical hystory, familial, radiological, clinical and diagnostic data.

The etiology of CM1 malformation is, at present, poorly understood. In some cases, CM1 may be associated with connective tissue diseases like Ehlers-Danhlos [22]. In the remaining cases, a multifactorial inheritance is the most likely explanation of the disease. Recently, a role for genetic factors in the disease pathogenesis has been suggested.

In CM patients, recent studies have revealed the presence of a small posterior fossa (PF) leading to a cramped cerebellum and herniation of the tonsils into the top of the spinal column. Based on examination of skull radiographs, Aydin *et al.* found that the posterior fossa was smaller and shallower in patients with CM1 malformation than in controls; the ratio of the posterior fossa with supratentorial volumes on MR images is smaller in CM1 patients than in controls, and those with smaller posterior fossa developed symptoms earlier and were more likely to respond to decompressive surgery. Experimentally-induced small posterior fossa was also found to lead to tonsillar herniation. So, it

Table 3
Summary of demographic and clinical data (gender, age, occupational status, surgery) in all patients and in CM/Syr patient groups; percentages in brackets

		Patient Group		
		All	CM[a]	Syr[a]
		n = 436	n = 347	n = 217
Gender (%)	M	144 (33)	111 (32)	78 (36)
	F	292 (67)	236 (68)	139 (64)
Occupational status (%)	Employed	192 (44)	132 (38)	111 (51)
	Unemployed[b]	244 (56)	215 (62)	106 (49)
Age (%)	Pediatric (≤18 yrs)	70 (16)	69 (20)	24 (11)
	Adult (18-60 yrs)	283 (65)	215 (62)	145 (67)
	Over 60 yrs	83 (19)	63 (18)	48 (22)
Surgery (%)	Yes	113 (26)	107 (31)	67 (31)
	No	323 (74)	240 (69)	150 (69)

[a] CM/Syr patient group includes associated forms: 128 patients with CMI/II+Syr; [b] Unemployed person, retired persons, students. *Abbreviations* - CM: Chiari Malformation; Syr: Syringomyelia.

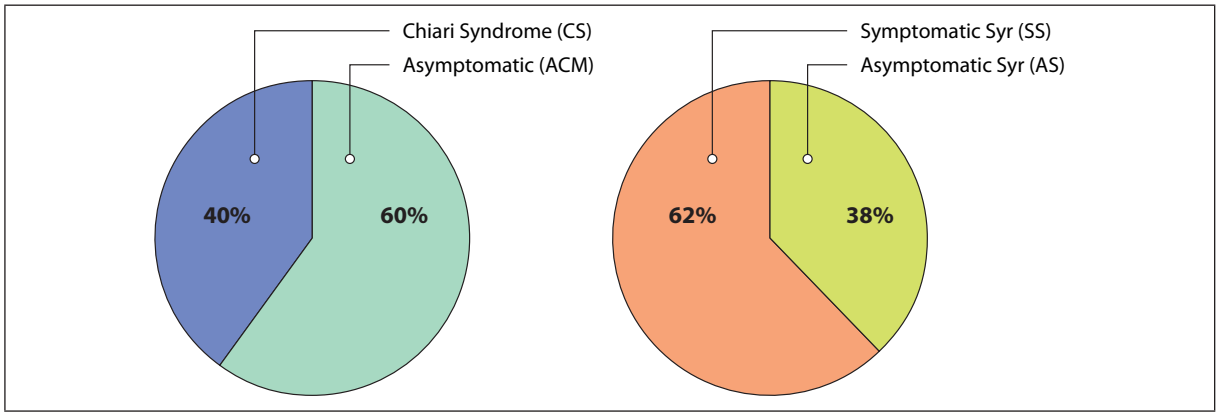

Figure 2
Percentages of symptomatic/asymptomatic forms in Chiari and Syringomyelia: SS are prevalent (62%) whereas CS are only 40%.

has been postulated that the pathogenesis of CM1 involves underdevelopment of the occipital bone, perhaps due to abnormal development of the occipital somite originating from the paraxial mesoderm, resulting in overcrowding in the posterior fossa [31]. In some families, the CM1-S phenotype is inherited as autosomal dominat trait. Genomewide linkage analyses of several families with CM1 identified candidate loci on chromosome 15q21.1-q22.3 (maximum 2-point nonparametric exponential lod score of 3.33 at rs744318) and on chromosome 9q22.31 (maximum multipoint parametric lod score of 3.05 between rs1000735 and rs2895201). Speer *et al.* postulated that an underlying gene responsible for CMI/Syr may have pleiotropic effects that influence posterior fossa volume, other skull bone abnormalities, the extent of cerebellar tonsil herniation, and the formation of Syr [32]. At present, however, the number and the type of genes involved in CM1 with or without Syr are unclear.

The indications, optimal timing, and type of surgical intervention to treat Syr associated with CM1 are unclear; prospective, controlled trials are lacking. Approximately 1/3 of untreated patients with Syr have minimal or no neurologic progression [33]. Progressive motor deficits and dysesthesias tend to respond more favourably to surgical intervention than sensory deficits. Greater syrinx size may predict a beneficial surgical response. Surgical intervention is suggested in patients with progressive motor deficits and a large syrinx. Suboccipital decompression, to alter the CSF flow and pressure dynamics, is considered the most successful technique [34, 35]. Williams advocate concurrent syringe-arachnoid shunting [36-38].

The strength of the study is that our estimations are based on a population based registry such as the Interregional Piemonte and Valle d'Aosta Rare Diseases Registry, integrated by the specific clinical Consortium form. A clear and standardized criteria for clinical inclusion was adopted.

Moreover, the dissemination of the shared recommendations has led to a greater awareness in the diagnostic process, especially improving its appropriateness of symptomatic versus asymptomatic forms.

A limitation of the study is the geographical local

Table 4
Demographic, radiological and prevalence/incidence data in CS and ACM patients

	Total CM (CM) n = 347	Symptomatic-Chiari Syndrome (CS) n = 139	Asymptomatic (ACM) n = 208
Age (%)	69 (20)	20 (29)	49 (71)
Pediatric (≤18 yrs)	215 (62)	87 (40)	128 (60)
Adult (18-60 yrs)	63 (18)	32 (51)	31 (49)
Over 60 yrs			
Gender (%)			
Male	111 (32)	52 (47)	59 (53)
Female	236 (68)	87 (37)	149 (63)
MRI Morphology (%)			
Tonsillar herniation ≥5mm	323 (93)	137 (42)	186 (58)
Tonsillar herniation 3-4mm	14 (4)	12 (17)	2 (83)
Tonsillar herniation ≤3 mm	10 (3)	0	10 (100)
Types (%)			
CMI A - CMI+Syr	125 (36)	75 (60)	50 (40)
CMI B-isolated	201 (58)	50 (25)	151 (75)
CMII + Myelomeningocele [a]	4 (1)	4 (100)	0 (0)
Other associated conditions [b]	17 (5)	10 (59)	7 (41)
Prevalence [c] [x100 000] and relative 95% Confidence Intervals	7.74 (6.965-8.596)	3.10 (2.625-3.659)	4.64 (4.049-5.313)
Gender			
Male	5.13 (4.260-6.177)	2.40 (1.833-3.151)	2.73 (2.114-3.517)
Female	10.17(8.952-11.55)	3.75 (3.039-4.624)	6.42 (5.469-7.537)
Age			
Pediatric (≤18 yrs)	9.42 (7.441-11.951)	2.73 (1.767-4.216)	6.69 (5.058-8.839)
Adult (18-60 yrs)	8.74 (7.650-9.992)	3.54 (2.868-4.363)	5.21 (4.378-6.188)
Over 60 yrs	4.87 (3.810-6.235)	2.47 (1.754-3.495)	2.40 (1.690-3.404)
2011 Incidence [d] [x100 000] and relative 95% Confidence Intervals	3.08 (2.605-3.635)	1.23 (0.942-1.596)	1.85 (1.493-2.294)
Gender			
Male	2.36 (1.793-3.099)	1.25 (0.858-1.816)	1.11 (0.745-1.650)
Female	3.75 (3.039-4.624)	1.21 (0.835-1.744)	2.54 (1.971-3.279)
Age			
Pediatric (≤18 yrs)	4.09 (2.868-5.844)	1.09 (0.553-2.154)	3.00 (1.983-4.546)
Adult (18-60 yrs)	3.54 (2.868-4.363)	1.47 (1.057-2.027)	2.07 (1.577-2.727)
Over 60 yrs	1.62 (1.063-2.484)	0.85 (0.475-1.524)	0.77 (0.420-1.424)

[a]CMII + Myelomeningocele: 75% of patients present also Syr; [b]Other associated conditions: 41% Retroflexed Odontoid, 24% Hydrocephalus, 24% Klippel-Feil, 11% TCS; [c]Prevalence cases/100 000 population who were alive in 2011 (ISTAT data); [d]New reported cases /100 000 population who were alive in 2011 (ISTAT data).
Abbreviations - CM: Chiari Malformation; Syr: Syringomyelia; TCS: Tethered Cord Syndrome.

extension of the census, restricted to only a few Italian regions (Piemonte and Valle d'Aosta), but with standardized access to the Syr and CM diagnosis and with availability of epidemiological data, also for Syr, included in the registry. More analytic and association analyses will need to be performed in the future.

CONCLUSIONS

The systematization of few known facts and the dissemination of guidelines or, failing these, of recommendations, as the result of a rational consensus by experts, represents a valuable tool for knowledge transfer drawn from biomedical and social and healthcare practices. We propose: adoption of Consortium Recommendations at national level to standardize the accessibility to the diagnosis and care process; moreover, the extend the methodology of census study in the national context to complete Italian epidemiologic data on Chiari and Syr. The estimated prevalence at national level could have a great impact in the field of rationalization of diagnostic costs and reduction of unnecessary hospitalizations/surgeries. We believe shared Interregional

Table 5
Demographic, radiological and Prevalence/Incidence data in Symptomatic Syr (SS) and in Asymptomatic Syr (AS) patients

	Total (Syr) n = 217	Symptomatic Syr (SS) n = 135	Asymptomatic (AS) n = 82
Age (%)	24 (11)	9 (37)	15 (63)
Pediatric (≤18 yrs)	145 (67)	96 (66)	49 (34)
Adult (18-60 yrs)	48 (22)	30 (62)	18 (38)
Over 60 yrs			
Gender (%)			
Male	78 (36)	50 (64)	28 (36)
Female	139 (64)	85 (61)	54 (39)
MRI Morphology (%)			
Syr	158 (73)	123 (78)	35 (22)
Hydro	59 (27)	12 (20)	47 (80)
MRI Distribution (%)			
Syringobulbia	2 (1)	2 (100)	0
Syr/Hydro cervical	54 (25)	33 (61)	21 (39)
Syr/Hydro thoracic	52 (24)	12 (23)	40 (77)
Syr/Hydro cervical-thoracic	109 (50)	88 (81)	21 (19)
Aetiology (%)			
Type I-primary Syr	128 (59)	95 (74)	33 (26)
Type II-idiopathic Syr	20 (9)	10 (50)	10 (50)
Type III-secondary Syr	30 (14)	23 (77)	7 (23)
Type IV - pure Hydro	39 (18)	7 (18)	32 (82)
Prevalence[a] [x100 000] and relative 95% Confidence Intervals	4.84 (4.124-5.527)	3.01 (2.544-3.563)	1.83 (1.473-2.269)
Gender			
Male	3.60 (2.889-4.499)	2.31 (1.753-3.046)	1.29 (0.895-1.870)
Female	5.99 (5.073-7.071)	3.66 (2.962-4.528)	2.33 (1.783-3.036)
Age			
Pediatric (≤18 yrs)	3.28 (2.201-4.873)	1.23 (0.646-2.334)	2.05 (1.240-3.378)
Adult (18-60 yrs)	5.90 (5.012-6.937)	3.90 (3.197-4.767)	2.00 (1.507-2.634)
Over 60 yrs	3.71 (2.801-4.923)	2.32 (1.626-3.313)	1.39 (0.881-2.201)
2011 Incidence[b] [x100 000] and relative 95% Confidence Intervals	0.82 (0.599-1.137)	0.51 (0.342-0.770)	0.31 (0.186-0.5524)
Gender			
Male	0.60 (0.351-1.028)	0.46 (0.251-0.851)	0.14 (0.047-0.408)
Female	1.03 (0.695-1.539)	0.56 (0.327-0.958)	0.47 (0.265-0.849)
Age			
Pediatric (≤18 yrs)	0.82 (0.375-1.786)	0.14 (0.024-0.773)	0.68 (0.291-1.597)
Adult (18-60 yrs)	1.06 (0.722-1.549)	0.81 (0.526-1.256)	0.25 (0.112-0.532)
Over 60 yrs	0.39 (0.165-0.906)	0.16 (0.042-0.564)	0.23 (0.079-0.682)

[a] Prevalence cases/100 000 population who were alive in 2011 (ISTAT data); [b] New Reported cases /100 000 population who were alive in 2011 (ISTAT data);
Abbreviations: *Syr* - Syringomyelia; *Hydro* - Hydromyelia.

Recommendations will help the promotion of national/international clinical research, i.e. multi-center prospective study to evaluate surgery efficacy in different clinical phenotypes (CMI with or without Syr/HDCT/TCS).

Finally, the design and the implementation of a specific registry dedicated to Syr and CM will contribute to better understanding the natural history of patients affected by these conditions. In fact, the European Commission is supporting European Reference Networks for implementing new registries on RDs. The collaboration and the strong linkage of activities at regional level with other initiatives at European level, such as European Reference Networks, will provide additional opportunities in the research and clinical aspect of Syr and CM.

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Authors' contributions statement

PC: study design, focus group analysis, data interpretation, literature analysis, draft and final revision of the manuscript. DG, PP: data collection, focus group analysis, final revision. GP, LM, LV: final revision of the manuscript. GM: study design and statistical analysis. SB, DR: literature analysis, data collection, final revision. YK: data interpretation, collaboration to the preparation and final revision of the manuscript. DT: data collection, literature analysis, final revision of the manuscript. The authors read and approved the final manuscript.

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Long-term consequences in survivors of critical illness. Analysis of incidence and risk factors

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Abstract

Aim. This study investigates the incidence of long-term consequences in survivors of critical illness 6 months after ICU care. A retrospective analysis of the risk factors was also completed.

Methods. A mixed-method design was used. A qualitative design was used in the questionnaire study (phase 1), and a quantitative design was used for the retrospective study (phase 2).

Results. 116 patients were interviewed. Forty-eight patients (41.4%) reported at least one long-term consequence 6 months after ICU discharge. The most frequent consequences were anxiety (n = 33, 28.4%), depression (n = 32, 27.6%) and chronic pain (n = 24, 20.7%). The interview showed the concurrent caseness of PTSD, anxiety and depression in 14 (12.1%) patients. Observed risk factors were age > 60 years (OR = 2.65, IC = 1.23-5.69; p = 0.0119), trauma diagnosis (OR = 5.3, IC = 1.60-17.76; p = 0.0033), length of mechanical ventilation > 7 days (OR = 2.18, IC = 1-4.74; p = 0.0471) length of ICU stay > 10 days (OR = 2.47, IC = 1.16-5.26; p = 0.0185) and clinical conditions at the ICU admission. The quality of life score was lower if the respondent had long-term consequences.

Discussion. A high incidence of long-term consequences is found in survivors of critical illness. In future, studies that investigate interventions to prevent these issues after ICU care are need.

Key words
- intensive care
- outcome assessment
- risk factors
- survivors

INTRODUCTION

Critical illness is recognized as being associated with a number of detrimental long-term sequelae that can impact the health of people for many years after discharge from an intensive care unit (ICU) [1].

These long-term sequelae are now recognized as cognitive impairment, psychological disability and ICU-acquired neuromuscular weakness [2-4].

Cognitive impairment has been reported to occur on average in 25-75% of ICU survivors, in the form of disturbed memory, amnesia and Alzheimer's disease [2, 3, 5]. The major risk factors associated with it are hypoxia (ARDS, cardiac arrest), respiratory failure, severe sepsis, trauma, requiring prolonged mechanical ventilation, use of renal replacement therapy, acute respiratory distress syndrome (ARDS), delirium during ICU stay and prior cognitive impairment (older age, pre-existing cognitive deficits) [6, 7].

Psychological disability has been reported to occur on average in 1-62% of ICU survivors, in the form of depression, anxiety, and post-traumatic stress disorder (PTSD) [3, 6, 8]. The major risk factors are same as for cognitive impairment and also include the use of sedation and analgesia in ICU, female gender, lower education level, and pre-existing disability [8-10].

ICU-acquired neuromuscular weakness is the most common form of physical impairment occurring more than 25% of ICU survivors (poor mobility, recurrent

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falls, or quadri or tetra paresis) [4, 11]. The major risk factors include prolonged mechanical ventilation (> 7 days), sepsis, multisystem organ failure, as well as prolonged duration of the lenght of ICU stay [12, 13].

Quality of life, chronic pain, psychological and psychiatric factors, physical fitness, functional capacity, are the long-term outcomes more commonly investigated in intensive care research [14-16].

The problem and the need for follow-up studies is increasingly relevant. A number of risk factors for individual long-term consequences in critical care survivors have been investigated. However, there is a lack of studies that analysing different symptoms after ICU care to date in critical care research. Furthermore, the studies available today do not pay attention to the risk factors that can be analysed before and during intensive care. In other words, if there are ICU-related risk or general risk factors. The present study was carried out maintaining the hypothesis that long-term consequences in survivors of critical illness is influenced not only by the ICU care but also by the clinical and general conditions at the time of ICU admission.

Aim

This study investigates the incidence of long-term consequences in survivors of critical illness 6 months after ICU care. A retrospective analysis of the risk factors was also completed.

Study design

For this study, a qualitative design was used in the questionnaire study (phase 1), and a quantitative design was used for the retrospective study (phase 2).

A questionnaire study is a research consisting of a series of questions (or other types of prompts) for the purpose of gathering information from respondents. Although questionnaires are often designed for statistical analysis of the responses, this is not always the case. Surveys and questionnaires are the most common technique for collecting quantitative or qualitative data.

A retrospective study uses existing data that have been recorded for reasons other than research. A retrospective case series is the description of a group of cases with a new or unusual disease or treatment. Therefore, a retrospective study design should never be used when a prospective design is feasible. However, a retrospective study looks backwards and examines exposure to suspected risk or protection factors in relation to an outcome that is established at the start of the study.

Patients received a letter introducing the study at ICU discharge. The letter explained that they might receive a phone call from the study team and provided contact details for the study office. Written consent was obtained by the nursing staff at the time of discharge, or at the follow-up visit.

MATERIALS AND METHODS

Setting

The study was single centered, based in an Italian adult 8-bed ICU in a 950-bed secondary hospital in Northern Italy (Azienda Socio Sanitaria Territoriale di Lecco). This hospital is the largest and most important in terms of numbers and economic size of the territory. Each year Lecco hospital carries out about 35 000 admissions, almost 15 000 surgical procedures, about 3 000 000 outpatient appointments and around 80 000 emergency room visits. The hospital admits more than 350 patients to the general ICU per year.

Common conditions that are treated within the ICU include acute respiratory distress syndrome (ARDS), post-operative surgical, trauma, multiple organ failure and sepsis. The unit made up of dedicated full-time intensivists (registered nurses and medical doctors) trained in adult multidisciplinary medicine; 24 registered nurses and 12 medical anaesthesiologists working full-time in the department, (4 registered nurses on each shift, 2 medical doctors morning-afternoon and 1 on night shift).

Participant selection

All patients aged at least 18, admitted to Lecco Hospital ICU from 1 January 2018 to 30 November 2018, were eligible to be included in the study. The interview was carried out between June 2018 and May 2019 (6 months after ICU discharged). Patients were included in the study only if they were able to communicate at the time of the interview.

Phase 1: Questionnaire study

The first aim was addressed by the use of standardized questionnaires or interviews to collect data. A short one-on-one interview was designed for the purpose of investigating presence of anxiety, insomnia, depression, chronic pain, Post-Traumatic Stress Disorders, fatigue and quality of life. The interview was administered by an ICU registered nurse who was involved in the study. Face-to-face administration of the questionnaires was chosen to increase the response rate. The interview was conducted in a dedicated room within the intensive care unit department of the Lecco Hospital.

We used the previously validated Hamilton Anxiety Rating Scale (HAM-A) for the assessment of anxiety [17]; the Insomnia severity index (ISI) for the assessment of insomnia [18]; the Patient Health Questionnaire (PHQ-9) for the assessment of depression [19]; the Brief Pain Inventory (BPI) for the assessment of chronic pain [20]; the Post-Traumatic Stress Disorder Check List -Civilian (PCL-C) for the assessment of PTSD [21]; the Revised-Piper Fatigue Scale (PFS-R) for the assessment of fatigue [22] and Euroqol 5D instrument (EQ-5D) for the assessment of perceived quality of life [23].

These instruments can be both self-administered and administered in person, as we did in our study.

Phase 2: Retrospective study

After the interview, patients' clinical data were obtained from their electronic medical records (*Margherita3 2010 form*) stored at the ICU. The electronic medical records were used to obtain the data required to complete the retrospective study, including all risk factors and outcomes investigated.

The independent variables examined were defined in the research protocol and consisted of the risk factors

for long-term consequences highlighted previously in the literature. These included patient age, APACHE II and SOFA score, admission diagnosis, gender, ICU LOS, use of renal placement therapy, severe sepsis and ARDS.

Interpretation of instrument scores

All instruments used during the interview have specific cut-offs that indicate, based on the scores, the absence or presence (mild, moderate or severe/intense) of the assessed symptom.

Hamilton Anxiety Rating Scale: total score range of 0-56, < 17 indicates mild entity, 18-24 mild to moderate and 25-30 moderate to severe.

Insomnia severity index: total score range of 0-28, absence of insomnia (0 -7), insomnia below the threshold (8-14), moderate insomnia (15-21) and severe insomnia (22-28).

Patient health questionnaire: total score range of 0-27, absent (0-4), sub-threshold depression (5-9), mild major depression (10-14), moderate major depression (15-19) and severe major depression (20-27).

Brief pain inventory: This instrument has different items. For this study we considered the item 3) please rate your pain by ticking the box beside the number that best describes your pain at its worst in the last 24 hours; 4) please rate your pain by ticking the box beside the number that best describes your pain at its least in the last 24 hours; 5) please rate your pain by ticking the box beside the number that best describes your pain on the average, and 6) please rate your pain by ticking the box beside the number that tells how much pain you have right now. Each item has a total score range of 0-10, no pain (0-3), mild or moderate pain (4-6) and intense pain (7-10).

Post-Traumatic Stress Disorder Check List – Civilian: total severity score 0-80. For this instrument we considered a PCL-C score ≥ 45 defined PTSD caseness.

Revised-Piper Fatigue Scale: for the final score, the scores of all the items of each subscale specific are added and divided by the number of items (n = 22), absent (0), mild (1-3), moderate (4-6), severe (7-10).

Euroqol 5D: three different levels of problem severity within each of five health domains. The levels are none, moderate and severe/extreme (coded 1 through 3, respectively), whilst the domains are mobility, capacity for self-care, conduct of usual activities, pain/discomfort and anxiety/depression, ordered as such. The conscious health states are therefore limited to 243 severity/domain vectors, ranging from 11111 (no problems in any domain) to 33333 (severe problems in all five domains). Having located the current health state, the respondent then evaluates his or her health using a visual analogue scale (VAS). This is a vertical, calibrated, line, bounded at 0 ("worst imaginable health state") and at 100 ("best imaginable health state").

Data analysis

The data were analysed using the Statistical Package for SS version 21.0 (SPSS Inc., Chicago, IL, USA).

Patients' demographic and clinical characteristics were analysed by using descriptive statistics and presented as numbers and percentages for the categoric variables and means (M) and standard deviations (±) for the continuous variables.

Comparisons between groups were performed with the chi-square test for the categorical data and with Student's t-test for the continuous data.

Variables were included in the analysis only if they were statistically significant at $p < 0.05$.

For the multivariate analysis, logistic regression with backward stepwise elimination by using the likelihood test statistic was used to assess potential predictors of development of long-term consequences in survivors of critical illness. For the univariate analysis, a Mann-Whitney *U* test was performed for comparisons between the continuous variables.

Odds, ratios and the 95% confidence intervals were calculated for each risk factor regarding ICU admission.

Relative risk and the 95% confidence intervals were calculated for each variable analysed during the follow-up interview.

As a number of studies have suggested that the risk of developing disability after ICU discharge, ranges from 1 to 62 percent (%) [3, 6, 8] we needed at least 100 patients in total. 6 months post ICU discharge, the sample provided sufficient patients to achieve this number, allowing for mortality and loss to follow-up.

Statistical significance for the identification of independent risk factors was set at $p < 0.05$.

Ethical statement

The project was promoted by the Azienda Socio Sanitaria Territoriale di Lecco and the study protocol was approved by the Human Research Ethics Committee of Brianza.

All participants provided their informed written consent to participate at the time of interview.

Consent was obtained by the nursing staff.

RESULTS

Three hundred eight patients were admitted to Lecco Hospital ICU between 1 January 2018 and 30 November 2018. Six months post ICU discharge, the participation rate in the study was 116 (37.66%) *(Table 1)*. Of these patients, 74 (63.8%) were males, with a mean age of 67.2 ± 13.49 years, and 42 (36.2%) were females, with a mean age of 62 ± 11.6 years. Most patients were surgical patients (n = 69, 59.5%).

Long-term consequences

Forty-eight patients (41.4%) reported at least one long-term consequences.

Twenty patients (17.2%) had 3 consequences, fifteen (12.9%) 2 consequences, and thirteen (11.2%) 4 consequences.

The most frequently were anxiety (n = 33, 28.4%) depression (n = 32, 27.6%) and chronic pain (n = 24, 20.7%) *(Table 2)*.

The interview showed the concurrent caseness of PTSD, anxiety and depression in 14 (12.1%) patients.

Patients with PTSD had an increased anxiety and depression risk (RR = 6.16, IC = 2.72-13.97; $p < 0.001$).

Table 1
Demographic and clinical characteristics of patients included in the study

Characteristics	(n = 116)
Age, mean (±) y	57.12 (12.9)
Gender, n (%)	
Male	74 (63.8)
Female	42 (36.2)
Weight, mean (±), kg	74.12 (13.6)
BMI, mean (±)	25.8 (3.59)
APACHE II, mean (±)	13.2 (5.6)
SOFA at ICU admission, mean (±)	5.8 (3.9)
Diagnosis, n (%)	
Medical	31 (26.7)
Surgical	69 (59.5)
Trauma	16 (13.8)
Advanced cardiovascular support, n (%)	14 (12.1)
Advanced respiratory support, n (%)	82 (70.7)
Neurological support, n (%)	2 (1.7)
Renal support, n (%)	8 (6.9)
Septicaemia and septic shock, n (%)	6 (5.2)
Acute pancreatitis, n (%)	5 (4.3)
Diabetic ketoacidosis, n (%)	2 (1.7)
Acute myocardial infarction, n (%)	4 (3.5)

BMI (body mass index); ASA (American society of anesthesiologists) physical status classification system before surgery; APACHE II (acute physiology and chronic health evaluation II) it is applied within 24 hours of admission of a patient to an ICU; SOFA (sepsis-related organ failure assessment score) scoring system is useful in predicting the clinical outcomes of critically ill patients, it is applied within 24 hours of admission.

Patients with anxiety had an increased depression risk (RR = 0.66, IC = 0.47-0.95; p = 0.0475).

Patients with chronic pain had an increased fatigue risk (RR = 3.50, IC = 1.34-9.11; p = 0.0089).

No other significant association was observed between the variables analyzed during the interview.

Quality of life

Sixty-two different EQ-5D vectors were represented in this recruitment sample, although 11111 (no health problems in any of the five domains) was the most frequently cited, by 58.6 per cent (n = 68) of subjects. For the individuals recording the 11111 health state, the mean EQ VAS score was 86.0 (±11.9).

More severe health problems in any dimension gave rise to a lower EQ VAS value for self-reported health. For any given EQ-5D health state classification, the EQ VAS score was lower if the respondent had pain, fatigue, insomnia, was likely to be anxious and/or depressed as assessed by the HAM-A and PHQ-9 or if they showed severe symptoms of PTSD.

For the 48 individuals with long-term consequences the mean EQ VAS score was 58.0 (± 16.7).

Risk factors in the intensive care unit

Table 3 highlights the results of the subgroup analysis, investigating the risk factors for the long-term consequences analysed in our study.

Significant risk factors with odds, ratios and the 95% confidence intervals are presented.

The significant risk factors for the 48 patients observed were age ≥ 60 years (OR = 2.65, IC = 1.23-5.69; p = 0.0119), trauma diagnosis (OR = 5.3, IC = 1.60-17.76; p = 0.0033), length of mechanical ventilation ≥ 7 days (OR = 2.18, IC = 1-4.74; p = 0.0471) length of ICU stay ≥ 10 days (OR = 2.47, IC = 1.16-5.26; p = 0.0185).

In addition, the severity of the patient at the time of admission to the Intensive Care Unit is to be reported among the risk factors. Indeed, the data APACHE II score ≥ 15 (OR = 2.64, IC = 1.06-6.53; p = 0.0328) and SOFA score ≥ 10 (OR = 2.7, IC = 1.06-6.89; p = 0.0340) at the ICU admission are also significant.

DISCUSSION

We present a result of self-reported anxiety, depression, insomnia, chronic pain, PTSD, fatigue and quality of life of ICU survivors to date. A high burden of post-ICU psychopathological issues was reported in about 4 respondents in 10. A high degree of symptom and long-term consequences concurrency between these six conditions was observed.

Long-term consequences are increasingly recognized as a problem in survivors of critical illness.

This study reported that 40% about of patients were experiencing chronic symptoms at least 6 months after ICU discharge. These consequences have a negative impact on the quality of life perceived by the patients themselves.

Our findings are comparable with incidence of persistent psychopathological issues in longer-term follow-up studies of survivors of ARDS where both the overall incidence of anxiety, depression and PTSD of psychopathological issues are similar [24]. However, we observed a reduction in chronic pain (20.7%) compared to 40% in a previous study [16].

Table 2
Distribution of anxiety, insomnia, depression, chronic pain, PTSD and fatigue, 6 months after ICU discharge

Variable, n (%)	No caseness	Mild symptoms	Moderate symptoms	Severe symptoms
Anxiety[a]	83 (71.6)	22 (18.9)	6 (5.2)	5 (4.3)
Insomnia[b]	96 (82.8)	9 (7.8)	4 (3.4)	7 (6.1)
Depression[c]	84 (72.4)	12 (10.3)	14 (12.1)	6 (5.2)
Chronic pain[d]	92 (79.3)	-	16 (13.8)	8 (6.9)
PTSD[e]	101 (87.1)	-	---	15 (12.9)
Fatigue[f]	98 (84.5)	11 (9.5)	5 (4.3)	2 (1.7)

[a]Hamilton Anxiety Rating Scale (HAM-A); [b]Insomnia severity index (ISI), [c]Patient health questionnaire (PHQ-9), [d]Chronic pain (BPI), and [e]Post-Traumatic Stress Disorder Check List-Civilian (PCL-C), [f]Fatigue (PFS-R).

Table 3
Risk factors of patients which at least one of long-term consequences

Risk factors	Patients with long-term consequences n = 48	Patients without long-term consequences n = 68	OR (95% CI)	p-value
Age (years) > 60, n (%)	26 (54.2)	21 (30.9)	2.65 (1.23-5.69)	0.0119
APACHE II > 15, n (%)	15 (31.3)	10 (14.7)	2.64 (1.06-6.53)	0.0328
SOFA > 10, n (%)	14 (29.2)	9 (13.3)	2.7 (1.06-6.89)	0.0340
Gender, n (%)				
Female	19 (39.6)	23 (33.8%)	1.28 (0.60-2.76)	0.5250
Reason for admission, n (%)				
Surgical	25 (52.1)	44 (64.7%)	0.59 (0.28-1.26)	0.1726
Non-surgical	11 (22.9)	20 (29.4%)	0.71 (0.30-1.67)	0.4362
Trauma	12 (25)	4 (5.9%)	5.3 (1.60-17.76)	0.0033
MV (days) > 7, n (%)	22 (45.8)	19 (27.9)	2.18 (1-4.74)	0.0471
ICU LOS > 10, n (%)	29 (60.4)	26 (38.2)	2.47 (1.16-5.26)	0.0185
Use of renal replacement therapy, n (%)	5 (10.4%)	3 (4.4%)	2.52 (0.57-11.1)	0.2087
Severe sepsis, n (%)	5 (10.4)	2 (2.9)	3.84 (0.7-20.68)	0.0959
ARDS	6 (12.5)	11 (16.2)	0.74 (0.25-2.16)	0.5813
Total of patients; n (%)	48 (41.4%)	68 (58.6%)		

MV = mechanical ventilation;
LOS = length of stay;
OR = odds ratio;
For comparisons, an indipendent sample *Chi-square test* were used.

In line with the literature, age, trauma, length of mechanical ventilation and length of ICU stay were the major risk factors highlighted. In addition to what is described in the literature, age and clinical conditions at the time of admission influence the outcome 6 months after ICU discharge. Under a clinical and public health perspective, these observations potentially inform and support targeted interventions aimed at reducing the occurrence of adverse outcome after ICU discharge. The identified risk factors can be targeted in order to improve the health trajectories of subjects surviving critical illnesses. Given the greatest risks that emerged among elderly patients and with comorbidity, it is necessary to set specific therapeutic pathways for these patients at the time of discharge. Early identification of patients at greater risk means reducing the impact that these long-term consequences have on people's health and on their psycho-social sphere.

Therefore, clinicians' priorities and standards of therapy for the prevention of long-term consequences should take into account the potential effects on patient health, with the goal of improving long-term outcomes through early and effective treatment.

The set of these long-term consequences, after ICU treatment, is often recognized as post-intensive care syndrome (PICS) [25, 26]. Healthcare professionals involved in the follow-up activity and assessment of all survivors of ICU should be aware of the co-occurrence of psychopathological conditions as part of PICS [25].

PICS has cognitive, psychiatric and physical components [26] and describes the consequences that remain in the surviving the critical illness and it is due to the associated neuropsychological, physical and functional disability [26]. However, its exact prevalence remains unknown.

It is important to study the chronicity and consequences of patients discharged from the ICU due to negative factors of public health [27]. Often in the post-ICU population, the observed association between depression and mortality can in part explained by the severity of chronic illness both pre-discharge and post-discharge. However, we did not adjust these factors in this study. The best knowledge, an association between long-term consequences and an increased rate of mortality after discharge from ICU has not been demonstrated previously.

Collecting data on pre-morbid psychological and medical co-morbidities would also be essential in terms of understanding the risk factors for developing set of these long-term consequences, as current illness severity scoring, organ support information and severity scoring is clearly insufficient when it comes to understanding which individuals are at greatest risk [28].

ICU survivors are known to experience impairment in cognition or psychological health and physical function [28]. However, there is a lack of studies that have examined the association among two or more of these variables. We suggest that using methodologies and standardized instruments in ICU survivor populations has the potential to contribute to the development of treatments, preventative strategies and screening guidelines, for this clinically condition.

Study limitation

The main limitations of the study are that the risk factors data were collected retrospectively and that the loss to interview exceeded 15%, which may have introduced an attrition bias. In addition, this study is a single-center study with a limited number of patients included.

The manuscript refers to an interesting issue concerning public health, but it covers a limited number of patients all from the same hospital, and is therefore not representative of a broader situation.

This study had limited access to pre-morbid condi-

tions, specifically pre-existing psychopathological conditions. Indeed, patients with pre-existing psychological and psychiatric conditions are at higher risk of both developing new symptoms and worsening existing problems following treatment in the ICU. At the same time, patients were not asked if they suffered from insomnia and chronic pain before their ICU admission.

Any data concerning the type /destination of discharge (e.g. other department, house, rehabilitation service) was not collected.

Furthermore, having simultaneously found several symptoms in the same subjects, it was not possible to carry out a stratification of the risk factors for each individual clinical condition emerged at the follow-up.

CONCLUSION

A high incidence of long-term consequences is found in survivors of critical illness. The major risk factors are increasing age, clinical and general conditions at the time of admission (APACHE II ≥ 15 or SOFA ≥ 10), prolonged ICU stay and mechanical ventilation.

These results concur with the findings of a number of previous studies but also highlight areas for further research.

In future, potentially beneficial research would include studies that investigate various clinical therapeutic interventions to prevent these long-term consequences experienced by patients after ICU care.

In conclusion we suggest informing patients at the time of hospital discharge of: the palliative care network, the role of specialist pain physician, the role of the psychologist and the counselling psychology. In addition, it is important to clarify the positive effect of early patient care in reducing the long-term consequences and related disabilities.

An awareness of the risk factors for the onset of long-term consequences allows the healthcare professionals caring for the patient to potentially address contributing factors, such as sheltered discharge and the patient's early acceptance by a multidisciplinary team of physician, nurses and psychologists.

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Conflict of interest statement

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Effectiveness of psycho-educational intervention to promote mental health focused on emotional intelligence in middle-school

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Abstract

Purpose. The specific "outcome-oriented" pattern of the emotional intelligence (EI) should be considered of capital importance for teenagers in order to promote mental health. Nevertheless it is rarely evaluated because a specific tool, useful for routinely use, is not available. In this paper the authors describe the effectiveness of a new approach of public health to improve the EI "outcome-oriented", by a specific index.

Design. A comparison of two samples: experimental (i.e. applying the program) *vs* control group, without randomization.

Setting. 12 classes belonging to 3 different schools.

Subjects. A sample of 276 students, 146 (53%) belonging to the experimental classes.

Intervention. A program of 20 meetings, once a week, based on the handbook *Intervento psicoeducativo per la promozione del benessere psicologico e dell'intelligenza emotiva nelle scuole* (Psycho-educational intervention for promoting psychological well-being and emotional intelligence at school) in order to stimulate a "peer to peer student approach".

Measures. Index of emotional intelligence (15 items), inventory idea questionnaire (19 items), learning ability questionnaire (6 items).

Analysis. Nonparametric tests were used.

Results. The authors found significant statistical differences at the conclusion of the study for all considered measures.

Conclusion. The results show a remarkable and positive impact of the approach above all on the "outcome-oriented" EI. Significant results were also observed about the indicator concerning irrational beliefs. The same significant results were found about learning abilities (goal definition, problem-solving and communication skills). The main limit is the study design (lack of randomization). Further evaluation is needed.

Key words

- mental health promotion
- emotional intelligence
- cooperative learning
- school
- psycho-educational structured approach

INTRODUCTION

Adolescence is the most important period for laying the foundations of psychological well-being. The estimated prevalence of worldwide mental health problems among young people associated to school failure, delinquency and substance misuse is 10-20% [1, 2]. School is considered one of the most important contexts for the promotion of mental health [3, 4]; this environment offers the best opportunity for this initiative [3, 4]. Promoting positive mental health may provide young people with the necessary life skills and resources to accomplish their potential and to deal with adversities [3]. This kind of initiative may be effective especially if health promotion programs are undertaken as part of school activities that improve social and life skills [5-7] and include active forms of learning, time focused on reaching realistic learning goals and lesson plans written in a handbook [8]. It is important for these programs to focus on Life skills [7, 9], because they are considered psychosocial competences that help people to be more aware in the process of decision-making, problem solving, critical and creative thinking, effective communication and healthy relationships, in order to manage their lives in a healthy and productive manner; the most important competences are self-efficacy, problem-solving, empathy and coping strategies [10].

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Training for Life Skills [11] is effective if "active forms of learning are used" with "sufficient time focused on reaching explicit learning goals" [12] according to the theory of the Social Skill Training, the most effective intervention to acquire skills in the field of mental health [13]. The method of Social Skill Training is founded on the psycho-educational approach which suggests a procedure based on small direct instructional steps to be dealt with in sequence in order to be mastered. Written lesson plans, including suggestions for skill acquisition practices, are very useful for this purpose [14, 8].

The Italian National Institute of Health has developed a handbook [5] that is a psycho-educational guide for facilitators in order to promote mental health among high school students. It was designed to reach all students, regardless of their level of risk in developing emotional/behavioural problems, with the aim to promote self-efficacy, psychological well-being, and satisfaction with life [15]. The core component of the handbook is training in the form of structured problem-solving and teaching skills that enable students to improve emotional intelligence to cope satisfactorily with psychosocial problems and stress in their life. It was inspired by Goleman's model of emotional intelligence [16] and Falloon's psycho-educational approach [17], in which psychiatric patients and their families are trained to use structured problem-solving to address problems that cause them the most stressful situations in their life. Therefore, it greatly emphasises structured problem-solving and regulation, and utilization of emotional information techniques. In addition, the handbook places a high level of importance on defining personal goals and using communication skills [18]. These last two skills make the approach significant and innovative, because promoting students' active involvement in taking decisions concerning their individual objectives could give them greater control over their lives and better personal and social functioning.

A preliminary study was carried out to evaluate the effectiveness of the handbook implementation, and although positive results were found [19], the authors considered them not satisfactory as expected. In that study, the handbook implementation was evaluated through a quasi-experimental study design involving four high school classes (two of which acting as a control), including 79 students aged 14 to 16 years (15.35 ± 0.68). The results showed improvement in perceived self-efficacy ($p \leq 0.001$), but limited improvement in behavioural and psychosocial problems, as assessed by Strengths and Difficulties Questionnaire (SDQ; Goodman, 2001) and well-being as assessed by the Health and Wellness Questionnaire [18]. The authors thought that future studies in which students practice assiduously and apply targeted skills outside the classroom should obtain better outcomes. This was an important issue because in the handbook programme regular practice was a key component of effective skills acquisition. For that purpose, the authors considered appropriate to incorporate a notebook in the handbook to stimulate homework in order to allow students to consolidate skills outside the classroom. This notebook mainly consisted of exercises to be conducted on a daily basis at home and some space to report what the students experienced in applying the exercises. As a consequence, in a second effectiveness study involving 10 high school classrooms (5 of which acting as a control), including in total 162 students aged 14 to 16 years, the results showed an improvement in self-efficacy and well-being, as well as an improvement in applying communication and problem solving techniques to solve psychological and interpersonal relationships problems. The results also showed a decrease of some behaviours at risk, such as involvement in fights and smoking [20]. After this encouraging performance, the authors speculated that the handbook equipped with the notebook might also enter in practice in middle-school, for pupils younger than 15 years, considering that late childhood and puberty are critical moments of opportunity for building skills and positive habits [7]. For this purpose, some few changes in the wording of the handbook were made; essentially, the topics and the examples were provided in a jargon more suitable to middle-school students aged 12-14 years. Lastly, a preliminary study was carried out to compare the outcomes obtained from 91 high school students with those of 38 middle school students who used the new version of the handbook [21]. As with the previous study, the results showed improvements in communication and problems solving skills and subjective well-being both in middle and high school students [21].

However, in all the above mentioned studies, the Emotional Intelligence (EI), which is a key competence covered in the handbook [5, 21], was not assessed because a easy tool to be used in non clinical settings was not available in Italy. The available instruments were questionnaires or interviews for assessing the emotional intelligence quotient, such as the Emotional Quotient Inventory (EQ-I) [22] or its characteristics related to personality organization, such as Trait Emotional Intelligence Questionnaire (TEIQue) [23]. These instruments were too long and elaborated especially for clinical purposes. For the authors, an ideal EI instrument for assessing mental health educational programs had to be comprehensive, (*i.e.*, covering all relevant domains of emotional intelligence: knowing your emotions, managing your own emotions, using emotions to motivate yourself, recognising the emotions of other people, managing relationships); but at the same time easy and speed to administer; *outcome* oriented (i.e., particularly able to assess the ability to use emotions for achieving personal goals) [24]. Accordingly, a tool called Index of Emotional Intelligence (IEI) was developed and validated by the authors in the attempt to give an original answer to this issue [25].

In order to corroborate the preliminary results, which suggested that the handbook for middle school produced significant positive effects on targeted attitudes about life skills, and determine whether or no it would also produced significant positive effects on targeted emotional competencies (*i.e.*, emotional intelligence, and abilities to recognize and modify dysfunctional thoughts that precede, accompany, and follow unpleasant emotions), in the 2018-2019 school year, the authors performed a pre-post test study design with a con-

trol group in 3 Italian middle schools. The short-term results are reported in the present paper.

METHOD

The handbook for middle-school

The theoretical basis of the handbook is the "salutogenic" approach as suggested by the World Health Organization (WHO) [7]; the handbook consists of four main modules: defining goals, problem-solving, effective communication, recognizing emotions and coping with anger. According to these modules, the main contents of the handbook [23] address skills such as defining personal goals; using structured problem solving; adopting effective communication skills; using negotiation for improving interpersonal relationships; coping with stress and anger; resolving conflict; recognizing and modifying negative dysfunctional beliefs that precede, accompany, and follow unpleasant emotions *(available online a Supplementary Material)*. The handbook mainly consists of exercises to be practiced at school and at home. The handbook includes several work-units which are articulated in the following steps: description of the unit's content; emotional Roll Call; a random check of the homework among 2-3 students, with a focus on their personal goals; 3-4 students in turn read the content of the unit and the instructions to perform exercises (2-3 exercises) in a small group (60% of total time is dedicated to role-playing), followed by feedback; homework assignment to be performed by using the notebook. By way of an example, the content of the *problem solving* work-unit is synthetically reported *(available online a Supplementary Material)*. The cooperative learning methodology is a "peer to peer student approach" supervised by a trained facilitator. The handbook is designed so that students can take turns in reading brief sections with facilitators coordinating group discussion on the key points and their specific relevance to students in the classroom.

Implementation of the handbook

The implementation was held in the classroom during regular school hours and each work-unit of the handbook in the vast majority of cases needed one-hour session a week. Sessions were coordinated by facilitators, psychologists/pedagogists who were trained by the handbook's authors; specifically they completed the training through a one-day session and also received a guide with practical information. It is worth noting that the role of facilitators was stimulating the active participation of all students in classroom and ensuring that sessions were conducted as described in the handbook.

Study design

Twelve classes of 3 middle schools volunteered to participate in the study (four classes in each school). In each school, two classes were identified for being included in the experimental group and others two for inclusion in the control group.

The total sample consisted of 276 middle-school students (51% females; mean age = 12.7 ± 0.6; range = 11-15): six experimental classes (146 students, 54% females) and six classes as a control group (130 students, 48% females).

The schools and the classes were chosen on the basis of voluntary participation by headmasters (the principals of the schools) and teachers, without any particular criteria or preferences. School councils of the participating schools approved the study. As the study concerned a psycho-educational intervention, it needed no formal approval by the Ethical Committee of the National Institute of Health, which was nevertheless consulted and gave informal authorization.

The study was worked out according to the international guidelines and ethical codes of the Belmont and Oviedo chart as well as the first section, paragraph 9 of the Italian ethical code of the Order of Psychologists. Prior to participation, students' parents signed written informed consent.

Instruments

Students attending the classes completed twice some self-administered assessment instruments (see later), before and after the handbook implementation. They had to generate a secret password which had to be memorized and reported on all the instruments.

Index of Emotional Intelligence, IEI [25]. The IEI assesses, by 15 items, the ability to regulate emotions in a flexible, adaptive way, oriented to personal goals. It uses a 4 level Likert-Scale, ranging from strongly disagree to strongly agree *(see Figure 1)*. The minimum score is 15 (very low), the maximum is 60 (very high), with a central value of 37.5. The internal consistency is alpha 0.72.

Inventory Idea Questionnaire [26]. It consists of 19 items related to irrational/dysfunctional beliefs. It uses a 4 level Likert scale ranging from 1 (strongly agree) to 4 (strongly disagree) for each item. The minimum score is 19 (the greatest IB), the maximum 76 (the greatest functional beliefs). The internal consistency is alpha 0.84.

Learning Abilities Questionnaire (LAQ). This tool, *ad hoc* developed, with items constructed to assess learning levels of the most important skills covered by the handbook (as reported by the students on the basis of case-vignettes), in total 6 skills through 6 items, one for each skill: a) item 1, for smart-goal definition with a score ranging from 0 (no ability) to 5 (greatest ability); b) items 2-5, for the four communication skills (each of them made up of 4 components). The score is 1 if the specific component is known, otherwise it is 0. For each communication skill the score ranges from 0 to 4. For all communication skills therefore the total score ranges from 0 to 16; c) item 6, for problem solving. For each of the six steps known the score is 1. As a consequence, the score ranges from 0 (no steps are known) to 6 (all steps are known). Finally, the *LAQ* total score ranges from 0 to 27 (5 points for the first item plus 16 points for the four items of communication skills plus 6 points for the item of problem-solving).

Statistical analysis

For all variables nonparametric tests were used. The Mann-Whitney U-Test was used to compare scores between groups (experimental versus control) at pre- and

post-implementation. The Wilcoxon signed-rank test was used to compare scores obtained in the pre- and post-implementation for each group (experimental and control). All statistical analyses were performed using SPSS software version 25 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

RESULTS

Sample

All students completed both pre- and post-tests. There were no immigrants; there were only 5 foreigners, who had been living in Italy for more than 8 years. The percentage of divorced parents was 12%, equally distributed in the classes. There was socio-economic homogeneity in the sample.

Experimental vs control group

IEI: no significant statistical differences were found between the experimental group *vs* control, before (mean ranks = 139.8 *vs* = 137.0; U = 9305.0; p = 0.77). Significant differences were found at the conclusion of the study (mean ranks = 149.1 *vs* =126.5; U = 7918.0; p < 0.01). At pre-post comparison in the control group the mean-rank Wilcoxon test was 4.30 (p = 0.001); in the experimental group was 7.18 (p = 0.001), therefore an improvement was observed in both experimental and control groups with regard to emotional intelligence.

Idea Inventory Questionnaire: no statistical significant differences were found between the groups before (mean ranks = 142.1 *vs* = 134.5; U = 8967.5; p = 0.43). Significant differences were found at the conclusion of the study (mean ranks = 149.1 *vs* = 126.6; U = 7938.5; p < 0.05). At pre-post comparison in the control group the mean-rank Wilcoxon test was 2.47 (p < 0.01); in the experimental group was 2.7 (p < 0.01), therefore an improvement was observed in both experimental and control groups with regard to irrational/dysfunctional beliefs.

LAQ: no statistical significant differences were found between the groups before the study for all variables. Significant differences at the conclusion of the study were found for all variables:

- *goal definition* (mean ranks = 148.3 *vs* 127.5; U = 8055.5; p < 0.05). At pre-post comparison in the control group the mean-rank Wilcoxon test was 0.94 (p = 0.35), in the experimental group was 2.21 (p = 0.027);
- *expressing positive feelings* (mean ranks = 163.7 *vs* 110.2; U = 5811.0; p < 0.01). At pre-post comparison in the control group the mean-rank Wilcoxon test was -1.0 (p = 0.32), in the experimental group was 6.96 (p < 0.001);
- *making requests* (mean ranks = 163.0 *vs* 110.9; U = 5909.0; p < 0.01). At pre-post comparison in the control group the mean-rank Wilcoxon test was -1.24 (p = 0.21), in the experimental group was 4.97 (p < 0.001);
- *expressing unpleasant feelings* (mean ranks = 166.0 *vs* 107.6; U = 5468.0; p < 0.01). At pre-post comparison in the control group the mean-rank Wilcoxon test was -1.55 (p = 0.13), in the experimental group was 6.58 (p < 0.001);
- *active listening* (mean ranks = 158.2 *vs* 116.4; U = 6610.0; p < 0.01). At pre-post comparison in the control group the mean-rank test was -0.33 (p = 0.74), in the experimental group was 6.15 (p < 0.001);
- *problem-solving* (mean ranks = 144.2 *vs* 132.2; U = 8664.0; p < 0.05). At pre-post comparison in the control group the mean-rank Wilcoxon test was -2.89 (p = 0.004), in the experimental group was 2.11 (p = 0.035); therefore an improvement was observed in the experimental group and a worsening in the control group with regard to problem solving.

The scores of both experimental group and control group at T0 (pre-implementation) and T1 (post-implementation) and differences between experimental and control groups at pre- and post-implementation are summarized in *Table 1*.

DISCUSSION AND CONCLUSION

This study confirms, as observed by the same authors previously, that school can be considered one of the more important setting to promote mental health [3, 4].

In this research, attention was focused on improvements of EI outcome-oriented and irrational beliefs to demonstrate the effectiveness of the intervention. At the baseline, the IEI difference between the two groups was not statistically significant (p = 0.77), whereas at the end of the study the difference was significant (p < 0.01). This shows a remarkable and positive impact of the approach on a specific pattern of emotional intelligence, which at this age is of capital importance. The results become more important because EI in general is scarcely considered in promoting mental health programs and in the same way is rarely evaluated [3, 4], despite it is recognized as a positive predictor of social functioning and school success [27]. The Outcome-Oriented "pattern" emphasizes specific emotional, flexible, and target-oriented abilities associated with some adaptation patterns in the living areas of the individuals; among these patterns there is "job performance" [28]. In technical terms, with regard to this peculiar characteristic of EI the authors must point out that the IEI contains a factor structured "self-efficacy meaning", in order to address the ability to use emotions to achieve personal goals. This structural aspect is well represented by some items, one of them for instance is "I think I am able to overcome problems".

In the present study, the irrational beliefs were also considered a very important emotional and cognitive dimension linked to school success [29, 30]. Significant results were observed on this indicator (p < 0.05). It is also likely that EI and irrational beliefs are closely interconnected, because it is hard to become emotionally intelligent without being capable of thinking in a functional way and controlling emotions. According to Di Pietro [29] the principles of emotional education, not the psychotherapy, should already be learned in a playful way starting from elementary school, as suggested by the handbook here presented.

Lastly, it is confirmed that the implementation of the handbook had an impact on the knowledge of the steps

Table 1
Median outcome scores and ranges of the scores of participating students (n = 276)

Variable	Group	N	Pre-implementation Median (range); mean ranks	Post-implementation Median (range); mean ranks
			U-test; p value	*U-test; p value*
IEI	Experimental	146	37 (25-53); 139.8	42 (28-59);149.1
	Control	130	39 (21-53); 137.0	41 (25-56);126.5
			9305.0; 0.77	*7918.0; < 0.01*
Idea Inventory	Experimental	146	47 (26-68); 142.1	49 (32-74);149.1
	Control	130	47 (23-71); 134.5	49 (30-68);126.5
			8967.5; 0.43	*7938.5; < 0.05*
LAQ- Goal definition	Experimental	146	0 (0-4);134.8	1 (0-5);148.3
	Control	130	0 (0-3);142.70 0 (0-3);127.5	
			8948.0; 0.17	*8055.5; < 0.05*
LAQ- Expressing positive feelings	Experimental	146	1 (0-2); 134.8	1 (0-4); 163.7
	Control	130	1 (0-3); 142.6	1 (0-2); 110.2
			8950.5; 0.29	*5811.0; < 0.01*
LAQ- Making requests	Experimental	146	1 (0-2); 140.8	1 (0-4); 163.0
	Control	130	1 (0-2); 135.9	1 (0-2); 110.9
			9832.5; 0.55	*5909.0; < 0.01*
LAQ- Expressing unpleasant feelings	Experimental	146	0 (0-2); 138.5	1 (0-5); 166.0
	Control	130	0 (0-2); 138.50 (0-3); 107.6	
			9490.5; 0.99	*5468.0; < 0.01*
LAQ- Active listening	Experimental	146	0 (0-1); 136.9	1 (0-5); 158.2
	Control	130	0 (0-2); 140.3	0 (0-1); 116.4
			9254.0; 0.19	*6610.0; < 0.01*
LAQ- Problem solving	Experimental	146	0 (0-3); 135.20 (0-4); 144.2	
	Control	130	0 (0-3); 142.2	0 (0-1); 132.2
			9007.0; 0.09	*8664.0; < 0.05*

to be dealt with in sequence for improving goal definition, effective communication and problem solving. The positive impact is satisfactory, given the statistically significant difference between the experimental and control group.

This study has mainly investigated the impact of intervention on EI "outcome-oriented". This aspect represents the core point of this work. With this study, the authors confirmed the effectiveness of the handbook, which has been conceived to promote health in the way suggested by "gaining health" programmes. It is important to pinpoint that this intervention to promote mental health is based on a structured and systematic continuous psycho-education, bearing in mind that psycho-education and salutogenesis are the key components of public health promotion and share the same principles and actions [31, 32].

The limit of this study is the study design, which did not take into account randomization and therefore there may be a generalization defect. The lack of this kind of methodology was due to feasibility reasons and therefore further evaluation is needed.

Another limitation is that differences between pre- and post-implementation scores are small in absolute values, also because the scores improved for both experimental and control groups, although to a lesser extent in the control group compared to the experimental group, on the instruments which assessed EI and irrational beliefs. An introduction of contamination within the schools, between experimental and control classes, may have played a role in this, weakening or diluting the implementation effects. The control classes within the schools may have become aware of the implementation processes by knowledge transfer from the experimental classes, either inadvertently or intentionally as students may have discussed their experiences. Nevertheless, the implementation performance in the experimental group is encouraging and provides preliminary evidence of its efficacy.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Malaria in pediatric age in the Piedmont Region

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Abstract

Introduction. Pediatric malaria, even in countries considered as free as Italy, is an important problem of public health because children have a high variability of the clinical picture. The objective of this brief note is to determine the incidence of pediatric malaria in the Piedmont Region during the period 1989-2015.

Materials and methods. All cases of pediatric malaria notified were considered thanks to the regional information flow over the period 1989-2015. Cases of congenital malaria, unconfirmed malaria cases, and aged 14 and older were excluded of the study.

Results. In Piedmont in the period 1989-2015, pediatric malaria accounts for 8.8% of the total (172/1946 cases). 74% of patients are of foreign nationality, to which must be added the 14% represented by those born in Italy from foreign parents, while it is 100% the fraction of patients who have made a trip to the abroad. The notification of cases is greater in the autumn months. Only 7.6% of the sample carried out a complete chemoprophylaxis. In 79% of cases, the primary care physician advised chemoprophylaxis on trips to endemic areas.

Conclusions. At present, lacking an effective vaccine, the prevention and implementation of standard precautions such as chemoprophylaxis, represent the safest strategy to put into practice to eradicate the disease especially for the groups at greater risk as visiting friends and relatives.

Key words
- malaria
- pediatric malaria

INTRODUCTION

Malaria is a disease caused by Plasmodium protozoans (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium Knowlesi*) transmitted to humans by the bite of some species of female mosquito of the genus Anopheles. *Plasmodium falciparum* is the most prevalent malaria parasite in sub-Saharan Africa (100%), the Western Pacific (72%), Eastern Mediterranean (69%) and South East Asia (63%), while *Plasmodium vivax* is the dominant malaria species in the Americas (74%) [1].

The symptoms, depending on the plasmodium species, appears 7-15 or more days after the bite of the infected mosquito. They are consistent in fever, often very high, headache, vomiting, diarrhea, sweating and shaking chills, all symptoms, at least existing, common to any flu syndrome or other infections.

Malaria cases are classified according to the terminology adopted by the WHO in 1963, as follows: a case of malaria is considered to be imported when the infection was contracted in a country other than that in which it is diagnosed; autochthonous when it is locally contracted. Among the autochthonous cases are defined induced, those caused by transfusions or other form of parenteral inoculation (transplants, nosocomial infections, etc.); introduced the secondary cases locally contracted after the bite of an indigenous mosquito infected on an importation case (bearer of gametocytes) or contracted with the bite of an infected mosquito accidentally imported (malaria from luggage, from the airport). A cryptic case is an isolated case of malaria that after an adequate epidemiological investigation cannot be classified in any of the categories described above. In the period 2013-2017 in Italy there were 3805 cases of malarial cases, of which 12 cases were autochthonous, 4 induced and 8 cryptic, with a peak of 7 cases occurred in summer 2017 that created a great concern for public health [1].

WHO's World Malaria Report has estimated that in 2017 the number of total cases was around 219 million, with about 435 000 deaths [2]. Although it represents one of the most widespread diseases in the world, 92%

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of cases and 93% of deaths occurred on the African continent.

However, in the latest years the spread of malaria has considerably reduced thanks to increasing fight and control plans and since 2016 WHO announced that the autochthonous transmission of malaria in European states was eradicated [3].

Yet, because of global migrator fluxes' increase and growing number of international travelers, in non- endemic countries malaria continues to be the most important import disease [4].

Worldwide, imported malaria is defined as a malaria infection acquired in an endemic area of malaria but diagnosed in no endemic country. Italy is included by WHO among malaria-free countries since 1970 but the attention remains very high because cases imported into areas that are no longer endemic may represent infection's sources due to the presence of competent vectors on the territory, allowing the reactivation of a local transmission, as recently happened in Greece [5].

In areas of high malaria transmission, children under the age of 5 are particularly vulnerable to infection and develop the disease in severe form. In 2017, 61% of deaths (266 000) affected this age group [2]. Pediatric malaria is an important public health problem because clinical picture is highly variable for children, and often not easily attributable to the disease. Children develop more severe disease's forms especially before the age of 5, with a greater probability of death than adults [6].

The objective is to determine the incidence of pediatric malaria in the region of Piedmont in the period 1989-2015. Secondary objectives are the determination of the incidence of malaria cases among European and Non-European citizens, the determination of the percentage of malaria cases despite chemoprophylaxis and the determination of the increase in notifications post holidays periods.

MATERIALS AND METHODS

The study evaluated all cases of pediatric malaria notified and recovered through in Piedmont thanks to regional information flow in the period 1989-2015. The composition of the study sample included all confirmed cases of malaria in the age group up to 14 years, regardless of nationality and sex. The exclusion criteria applied were: unconfirmed cases of malaria, age over 14 years, congenital malaria cases. The analysis provided a quantitative stratification for some characteristics: nationality, notification month of malaria cases, travel destination and fulfillment prophylaxis. The notification forms analyzed did not allow us to describe the clinical course and the severity of the individual case. In fact it was not possible to consult the medical records. Furthermore, another aspect that could not be investigated was the possible diagnostic delay.

CASE DEFINITION

The criteria adopted for the identification of the case are those provided by the Italian Ministry of Health [7]: a case of malaria is defined as a symptomatic, paucisintomatic or asymptomatic individual in which there is the presence of malarial parasites with diagnostic methods that have clinics, epidemiological or laboratory criteria. The classification offers the possibility of having a probable case, in which the clinical and epidemiological criteria are met, and a confirmed case, in which the laboratory criterion (microscopic confirmation) is also satisfied. Only confirmed cases were considered in this study.

RESULTS

Among 1946 malaria cases notified in Piedmont in the period 1989-2015, pediatric malaria represents 8.8 % of total (n. 172), the case-fatality rate is 0.58 %, due to a single case of death in the study sample. The most represented species of *Plasmodium protozoans* was the *Plasmodium falciparum* (150 cases) *Plasmodium vivax* (3 cases), *Plasmodium ovale* (8 cases); there were also 3 cases of mixed forms and 9 cases in which the agent is not specified. The stratification of the sample by citizenship has shown that the largest share of patients is of foreign nationality. They represent 74% (n. 127) of the sample, while 12% (n. 21) of the sample is represented by Italian citizens and 14% (n. 24) by children born in Italy from foreign parents. The 100% of the sample is the share of patient who travelled abroad. The country of destination was North e West Africa for over 95% of cases, around 60% of the notifications, in fact, come from people who have visited the Ivory Coast and Nigeria. There were several reasons behind those travels, but in the most cases it was for returning to the country of origin, *Table 1* shows the destination and the reason for the trip and the use of chemoprophylaxis.

August, September and October and partly even November and January are the months in which the largest number of diagnosis occurred, as a consequence of people returning from journeys taken during school summer and winter breaks *(Figure 1)*.

The notification forms analyzed starting from 2003 are enriched with some information: type of trip, type and method of chemoprophylaxis and operator who recommended chemoprophylaxis. Considering that the data is partial, related to 131 cases, the data we present are not to be considered general.

Only 7.6% of the sample received a complete chemoprophylaxis. Moreover, it should be considered that 26% of malaria cases are not preventable by a prophylaxis, as they concern that share of children reaching Italy via immigration. Mefloquine was the most used molecule in either complete or partial chemoprophylaxis. It was the family doctor (general practitioner/family pediatrician) that in most cases (79%) recommended a chemoprophylaxis before a trip to endemic areas.

DISCUSSION

According to Surveillance Atlas of Infectious Diseases data of the European Center for Disease Prevention and Control (ECDC) for 2017, 8401 cases of malaria were reported in the EU/EEA, 8393 (99.9%) of which were confirmed and almost all cases of malaria reported by EU countries for 2017 have been imported [8].

France reported the highest number of cases, followed by the United Kingdom, Germany and Italy.

All countries that report the greatest number of cases have historical, economic, linguistic and cultural

Table 1
Reason and destination of travel and use of malaria chemoprophylaxis*

Destination	
Benin	3
Burkina Faso	16
Cameroon	10
Congo	8
Ivory Coast	27
Ghana	9
Equatorial Guinea	3
India	3
Kenya	2
Liberia	1
Madagascar	2
Mali	4
Nigeria	73
Not reported	1
Senegal	9
Togo	1

Reasons	
Immigration	26 (15%)
Parental work	2 (1.2%)
Residence	3 (1.7%)
Return of origin country	102 (59.3%)
Tourism	20 (11.6%)
Other	2 (1.2%)
Does not know	17 (9.9%)
Percentage of sick people who have carried out chemoprophylaxis during travel*	
Complete chemoprophylaxis	10 (7.6%)
Missed doses	6 (4.6%)
Not executed	65 (49.6%)
Interrupted	12 (9.1%)
Late	3 (2.2%)
Not remember	30 (22.9%)
Partial chemoprophylaxis	5 (3.8%)

Type of prophylaxis	
Atovaquone/Proguanil	2
Mefloquine	33
Quinine	1
None	65
Not remeber	30
Subdivision by citizenship	
Italian	21 (12%)
Foreign	127 (74%)
Born in Italy from foreign parents	24 (14%)
Clinicians who recommended chemoprophylaxis	
Travel medicine surgery	26 (20%)
Family Doctor	103 (79%)
Does not know	2 (1.5%)

*Reference period 2003-2015 131 cases.

links with endemic areas, particularly in Africa and the Americas. Most cases of malaria imported into France and the United Kingdom are linked to travel routes from West Africa [4]. Italy was the fourth country in terms of number of notifications, after France, United Kingdom and Germany, with 830 reported malaria cases, of which about 12% of pediatric malaria cases.

Although the Member States of the European Union have been free from malaria since 1975, the presence of the vector of the genus Anopheles is frequently documented [9]. Italy in particular seems to remain a risk zone due to the vector competence of some species of Anophele (*Anopheles superpictus*, *A. sacharovi* and *A. labranchiae*) and the high malarious degree [10].

Over the last 10 years occasional cases of indigenous malaria were reported in several European countries. In almost all cases it was "airport malaria" [9]. However, malaria is more frequently introduced in Europe through importation, which has multiple variable: increased international travels, especially in tropical and subtropical areas, more immigrants from countries where malaria is endemic, and the role of visiting friends and relatives (VFRs).

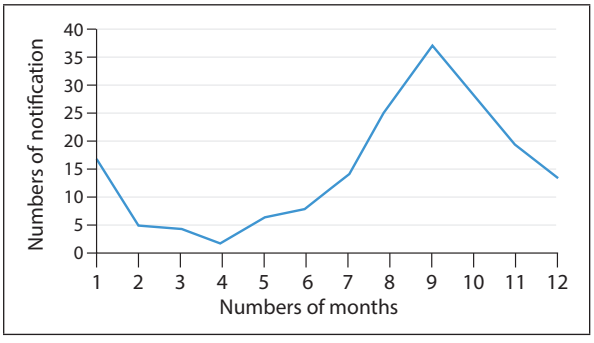

Figure 1
Diagnosis of malaria by month, cumulative 1989-2015 (n. 172 pediatric cases in the Piedmont Region, Italy).

VFRs are individuals from low-income countries who having settled in high-income countries periodically go back to their native country to visit relatives and friends. This population of immigrants alone counts for 50% of international travelers [10]. They represent a group of travelers particularly at risk with regard to travel-related diseases, more than other kind of travelers (tourists, business-travelers) [11].

VFRs visit rural areas more frequently and stay there longer than those who travel in risk area for tourism or business, and probably in healthier conditions. Furthermore, with regard to malaria, VFRs may have a lower perception of risk, believing that they have a permanent immunity to infection, acquiring with birth in the endemic country, without considering the potential for a relapse of the infection. This leads to a lower employment of available preventive measures: repellents, mosquito nets, air conditioning and chemoprophylaxis [12], and also for their children. Even the subpopulations belonging to the group of traveler VFRs, like pregnant women and pediatric individuals, seems to be slightly sensitive about preventive measures [13].

VFRs may represent up to 70% of imported malaria cases in developed countries [11]. Data analyzed in this study also show how the greater incidence of pediatric malaria occurs during periods corresponding to school summer holidays (August, September and October) and Christmas festivities (December and January), during which VFRs often go back to their native country *(Figure 1)*.

CONCLUSION

Imported malaria represents one of the most diagnosed diseases in developed countries, but at the same time one of the most preventable if standard precau-

tions were always adopted. VFRs are one of the group most at risk of contracting malaria and importing it in developed countries. This multiethnic and diverse category should represent the main target of awareness policies of the actors in charge of the purpose. However, there seems to be many implementation difficulties: VFRs often consider chemoprophylaxis as an almost exaggerated precaution for a disease that is been known since childhood [14]; furthermore, socio-economic difficulties, lack of knowledge of prevention tools and lack of knowledge of the correct and timely use of health and social services.

Prevention and awareness-raising strategy should thus be extensive and systematic, especially for VFRs individuals who are integrated into society in developed countries. Many actors shall be involved: general practitioner, family pediatrician, pharmacists, primary school teachers. Furthermore, an extensive information might be offered also in workplaces and places of entertainment.

In Europe, malaria chemoprophylaxis is recommended only for travelers in malaria endemic countries, which are classified into different groups to determine the most effective drug regimen [15]. The choice of prophylactic drugs and prevention measures also depend mainly on the local epidemiology of malaria, on the duration of potential exposure to vectors, on the model of resistance to parasites, on the level and seasonality of transmission, on tolerance to prophylactic drugs, from age and pregnancy.

Currently, in the absence of an effective vaccine, prevention and implementation of standard precaution represent the safest strategy to put into practice to eradicate malaria.

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 the conduct and findings of this study.

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ORIGINAL ARTICLES AND REVIEWS

Evolution of Italian laws banning trafficking, use and abuse of psychotropic drugs

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Abstract
The penalty system implemented by Italian law still represents a barrier against psychoactive drugs and drug addiction, especially at a time when the age of first consumption has considerably dropped. Presidential Decree n. 309 of October 9, 1990 entitled "Consolidation of the laws governing drugs and psychotropic substances, the prevention, treatment and rehabilitation of drug addicts", and referred to as Presidential Decree 309/90, is the reference text for the cultivation, production, trade and use of narcotics and other psychoactive substances in Italy. The Presidential Decree has its origins in the now-forgotten law of December 22, 1975, n. 685, amended by law 162/90, which provided a draft of the current Presidential Decree 309/90. The current text has been amended numerous times over the years.

Key words
- Presidential Decree 309/90
- psychoactive drug
- NPS
- legislative evolution
- Italy

INTRODUCTION

In ancient times, there was no moral problem regarding the use of psychoactive substances, as they represented a fundamental part of the relationship to the Gods, a link with medicine, and a spiritual connection with the body. Psychoactive substances were closely linked to the religious experience. They were mainly used as an entheogenic agent during rituals of vision and communication with the divine: peyote ("bread of the gods") in Mexico, ayahuasca ("liana of spirits") in the Amazon and Central America, iboga ("miraculous plant" or "tree of knowledge") in West Africa, kawa ("bitter, pungent, sour drink") in the South Pacific, and cannabis in the East. Drugs were also used by philosophers, for their stimulant and exhilarating properties. The ancient Greeks used them to cheer up their banquets (hemp, henbane, opium, mandrake). In the *Odyssey* of Homer, the author tells that Helen gave wine mixed with opium to Telemachus, the son of Ulysses, to alleviate his pain. Even the ancient Romans used psychoactive substances. With the development of the Christian religion, drugs started being regarded as "evil", and their use, either for medical or religious purpose, was severely punished. With the discovery of the New World, coca and tobacco were imported into Europe from Americas. In Europe, the development of scientific disciplines such as chemistry, pharmacology and medicine, restored the status of psychoactive drugs as active ingredients or excipients of medicines. In the second half of the 19th century, the use of drugs in Europe, either for recreative or medical use, spread considerably. Opium was used in pharmacies, even for children, due to its relaxing properties. Cocaine was preferred by intellectuals because it was regarded as a substance capable of amplifying critical and creative thoughts. In 1897, Bayer marketed heroin as a medicine to treat cough, respiratory problems and to fight morphine dependence. A year later, heroin was already a huge commercial success and was exported to more than twenty countries. In the 1930s, a number of "new psychoactive substances (NPS)", as they were so called at the time, were synthetized in laboratories and marketed. For instance, amphetamines were prescribed to treat depression and the excessive use of hypnotic medicines; they were also used in the military sphere because of their anorectic and psychostimulating properties. In 1943, the "lysergic acid diethylamide", or LSD, a hallucinogenic substance whose precursor can be extracted from the fungus ergot, was synthesised and was initially used as a stimulant in psychotherapy and to treat alcohol addiction. This review decribes for the first time the evolution of the National legislation on psychoactive substances since the first laws at the beginning of 20th Century till the Presidential Decree 309 of 1990 and subsequent amendements.

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METHODOLOGY

A wide-ranging search of relevant scientific literature has been performed using multidisciplinary databases (Scopus, PubMed, PubMedCentral, Research Gate, Medline, Google Scholar) and in legal search engines (NORMATTIVA, Regulatory archive of the Ministry of Health) with the aim to gather all relevant laws regarding psychoactive substances banning that were published from 1920 until December 2019. The used search keys were: Presidential Decree 309/90, Psychoactive Drug, NPS, Legislative Evolution. The search was limited to English language and Italian language materials, and all articles have been independently revised for content by three of the authors to verify their relevance within the framework of the present review. Only the articles that were ultimately considered relevant by at least two of the paper authors have been chosen.

RESULTS

The Italian legislation has always considered the production and illicit trafficking of drugs a crime, adopting repressive measures and always more incisive sanctions on the basis of International Conventions. The drug legislation was initially inspired by the prohibitionist point of view, although part of the public opinion called for separate treatment of cannabis and its derivatives (so-called soft drugs), asking for their legalization. The first drug law of February 18, 1923, n. 396, was part of a political-social context radically different from now, and considered drug use as a "vice".

However, it provided for the punishability of the consumers only if their conduct threatened public order. Morphine, cocaine and other "poisonous substances that in small doses give narcotic action" were controlled, identified and included in a specific list [2]. The 1930 Rocco Code established a number of measures aiming at repressing the trade and use of illegal drugs and the facilitation of use [3]. The subsequent reform resulted in the decree-law n. 151 of January 15, 1934, which repealed the previous law and absorbed some of the provisions of the penal code [4]. This regulatory framework remained in force until the introduction of the law of October 22, 1954, n. 1041, "Law on Narcotics - Discipline of their production, trade and employment" (law n. 1041, www.gazzettaufficiale.it/eli/id/1954/11/12/054U1041/sg), which severely punished all possible conducts concerning the narcotics, including detention for personal use [5]. This law took into account the control of cultivation, production and trade of the generally defined "psychotropic drugs" with particular attention on opiates, and severely punished personal use outside medical prescription. The spread of psychoactive substances in Italy began at the end of the 1960s, and has reached great proportions over the following decade. Given the inadequacy of the legislation, which was characterized by a merely repressive approach to the problem, providing for the punishability of the consumer who was considered as a delinquent, a political intervention was asked to the civil society that also took into account the social and health aspects of the drug phenomenon.

Subsequently, the law "Disciplina degli stupefacenti e sostanze psicotrope. Prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza. (Regulation of narcotic drugs and psychotropic substances. Prevention, treatment and rehabilitation of the related states of drug addiction)" was introduced on December 12, 1975 [6] (law n. 685, www.gazzettaufficiale.it/eli/id/1975/12/30/075U0685/sg). For the first time, the law recognized the possibility of not punishing simple drug users for their own consumption. However, there was no precise definition of the concept of "small quantity", which was therefore left to the complete discretion of the judges. For this reason, the Court of Cassation dictated guidelines to reach consensus, defining the concept of "small quantity" of drug that encompassed the notion of drug addiction. Furthermore, to reach a sentence of acquittal or conviction, it was required that, the magistrate, through scientific investigations, should understand the number of drug doses in addition to the nature and composition of the substance. During the 1980s, the trend in drug use reached worrisome proportions, with a significant increase of drug-related fatalities involving drug overdoses, road accidents under the influence, the onset of drug-related diseases (AIDS, viral hepatitis) and the increase drug-related crime. During the 1990s, Italy and most European countries faced the increase and spread of psychoactive substances using law enforcement, prevention policies (training and education in schools) and treatment and social rehabilitation programs. The need to cope with the increase and diffusion of drugs brought about a new turning point in the evolution of Italian legislation, achieved by the with law n. 162 (Vassalli-Russo-Jervolino) [7] "Aggiornamento, modifiche ed integrazioni della legge 22 dicembre 1975, n. 685, recante disciplina degli stupefacenti e sostanze psicotrope, prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza. (Updating, modification and integration of law n. 685 of December 22, 1975, regulating narcotic drugs and psychotropic substances, prevention, treatment and rehabilitation of the relative states of drug addiction)" of June 26, 1990 (law n. 162, www.gazzettaufficiale.it/eli/id/1990/06/26/090G0197/sg) [7] which provided a draft of the current Presidential Decree 309/90 [1]. This law restored the sanctions against holders of small quantities for personal non-therapeutic use. As a legislative instrument of intervention, the regulations in force were collected in a special consolidated text, the Presidential Decree 309/90, on the subject of the discipline of narcotic drugs and psychotropic substances, prevention, treatment and rehabilitation of drug addicts. With the Presidential Decree 309/90, the laws n. 685 and n. 162 and the applicative decrees issued by the Ministry of Health were unified, in order to align themselves on the resolutions adopted by the Member States of the European Community.

PRESIDENTIAL DECREE N. 309 OF OCTOBER 9, 1990

The law of June 26, 1990 (n. 162) [7] introduced into the December 12, 1975 (law n. 685) [6] numerous modifications, suppressions, substitutions and insertions of articles and paragraphs. It was therefore necessary to reorganize the whole discipline through the elaboration

of a "single text", which was adopted with the Presidential Decree 309/90 [1]. Laws n. 685 and n. 162 [7] were thus unified with the implementing decrees issued by the Ministry of Health. Before April 1993 popular referendum, personal use was linked to the quantity of the substance used, which should not, however, exceed the "average daily dose". Art. 73 was based on the system of double track sanctions, which made a distinction between "soft drugs" and "hard drugs". These drugs were divided into six tables. Tables 1 and 3 listed the so-called hard drugs (e.g., ecstasy or coca leaves). Tables 1 and 4 listed the so-called soft drugs (e.g., cannabis and derivatives). Finally, Tables 5 and 6 included products used for therapeutic purposes and containing substances mentioned in Tables 1-4, for their potential for abuse and dependence (e.g., anxiolytics, antidepressants and psychostimulants).

The Presidential Decree n. 309 of October 9, 1990: "Testo unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope, prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza. (Consolidation of the laws governing drugs and psychotropic substances, the prevention, treatment and rehabilitation of drug addicts)", (www.gazzettaufficiale.it/eli/id/1990/10/31/090G0363/sg) [1] referred as Presidential Decree 309/90, came into force in 1990, concerning the legal discipline of narcotic drugs and psychotropic substances together with rules for the prevention, treatment and rehabilitation of the relative states of drug addiction. It was divided into 12 different parts:

- *Title I* - Authorities and schedules (articles 1-16)
- *Title II* - Authorization and permits (articles 17-25-bis)
- *Title III* - Provisions relative to the cultivation and production, manufacture, use and wholesaling of narcotic and psychotropic substances (articles 26-37)
- *Title IV* - Provisions distribution (articles 38-49)
- *Title V* - Import, export and transit (articles 50-59)
- *Title VI* - Documentation and custody (articles 60-68)
- *Title VII* - Specific provisions governing the substances listed in tables IV, V and VI (articles 69-71)
- *Title VIII* - Penalties for illicit activities (articles 72-103)
- *Title IX* - Information and educational measures (articles 104-112)
- *Title X* - Regional, provincial and local authorities' responsibilities. Services for drug addiction (articles 113-119)
- *Title XI* - Preventive, curative and rehabilitation measures (articles 120-126)
- *Title XII* - Final provisions (articles 127-136)

Annexes to the Single Text on Narcotic Drugs

Title I indicates and reserves the responsibility for guiding and promoting the general policy of control, prevention and intervention in the sector of the National Coordination Committee for Action against Drugs, and determines the general supervisory functions of the Ministry of Health and the Ministry of Internal Affairs, as well as the particular powers of the Regions. The surveillance and control activities of the Police Forces are then established, as well as the tasks of information and operational coordination of the Central Directorate for Anti-drug Services, a joint association under direct orders of the Ministry of Internal Affairs. Articles 13 and 14 indicate the criteria for establishing the 6 Tables containing the list of controlled substances ordered by potential hazard, on the basis of which the criminal and administrative norms (Title I-VIII) apply differentiated punitive sanctions. *Titles II, III, IV, V, VI and VII* examine all aspects of the cultivation, production, manufacture, distribution, use, import and transit of psychoactive drugs, laying down specific requirements for authorisation, documentation and communication of data and information. The evolution of the criminal law on psychoactive substances is detailed in *Title VIII* and deals specifically with the measures of "repression" of illicit activities related to psychoactive substances, regarding both the treatment applied to the consumer and the limits of the criminal relevance of the conduct of detention. *Titles IX, X and XI* constitute a very articulated text that can be considered as the guiding principle of the law to give great effectiveness to the prevention, treatment and reintegration. In this context, a provision operating in the fields of information, education, medical and social assistance has been established. The *annexes* consist of Tables. The first four Tables list all psychoactive drugs and psychotropic substances connected to the system of sanctions for illicit use and placed under International and National Control (Tables I and III major sanctions; Tables II and IV minor sanctions). The first four Tables also include preparations containing the substances listed in each of these Tables. The Table of Medicinal Products indicates the medicinal drugs, with particular reference to the prescriptions of the medicinal products for pain therapy and the medicinal products used in the course of treatment for the cessation of addictions, based on narcotic and psychotropic active substances of current therapeutic use for human or veterinary use. The Table of Medicinal Products is divided into five sections A, B, C, D and E. In these Tables medicinal drugs are distributed according to their potential for abuse. The Tables also indicate the dispensing regime. Medicinal drugs with simplified prescriptive procedures are included in Annex IIIa. The evolution of the criminal law on psychoactive substances, currently represented by the Presidential Decree 309/90, has mainly considered four aspects: the legal treatment of the simple drug consumer, the limits of criminal relevance concerning drug detention, the public intervention in prevention, treatment/rehabilitation and the inclusion of new psychoactive substances in the Tables of banned compounds. On the other hand, the regulatory system for the detection of psychoactive substances has remained substantially unchanged over time. This regulatory system for psychoactive substances is characterised by the absence of a general notion of "drug". This means that the drug is not considered in terms of its origin (vegetable or synthetic), consumption method (e.g., chewing, smoking, injection, oral intake) and ability to cause alterations in interpersonal relationships and with the environment. The difficulty found in the identification of a univocal notion has therefore induced the legislator to prefer the

"Tabular System". In fact, in the Presidential Decree, only the substances included in annex Tables of the Presidential Decree 309/90 are considered as "psychoactive substances". The Presidential Decree n. 309 of 1990 has been the first exhaustive act on the regulation of psychoactive substances traffick and use in Italy.

AMENDEMENTS OF THE PRESIDENTIAL DECREE 309/90 DURING THE YEARS

Referendum of 1993

A turning point came with Presidential Decree of June 5, 1993 "Abrogazione parziale, a seguito di referendum popolare, del testo unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope, prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza, approvato con decreto del Presidente della Repubblica 9 ottobre 1990, n. 309. (Partial repeal, following a popular referendum, of the consolidated text of the laws on the discipline of narcotic drugs and psychotropic substances, prevention, treatment and rehabilitation of the relative states of drug addiction, approved by Presidential Decree n. 309 of 9 October 1990)" (law n. 171, www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=1993-06-05&atto.codiceRedazionale=093G0238&elenco30giorni=false) [8], which amended 309/90. In particular, Article 72, paragraph 1, which contained the prohibition of personal use and any unauthorised use of drugs and psychotropic substances, was repealed. By repealing Article 75, paragraph 1, and Article 78 paragraph 1, letter b and c, the notion of dose limit ("average daily dose") was also abolished. The positive effects of 309/90 – i.e. a reduction in the number of drug-related deaths, an increase in the number of people entering community detoxification protocols, the seizure of ever greater quantities of drugs – were therefore hampered by the referendum promoted and won by the Radicals in 1993, as only the distribution of drugs remained illegal. Since the referendum, and until 2006, even the detention of large quantities of drugs was criminally irrelevant. In these terms, the jurisprudence had been oriented, considering acceptable the possession of tens of grams of heroin, and even the transfer aimed at "group consumption". The regulatory framework had become both lax and unnecessarily rigorous. Lax at the user/drug dealer interface: without proof of sale, there was no illegal limit for possession. Unnecessarily rigorous at the recovery: in several cases, the drug addict who completed his stay in prison could be reincarcerated, even for minor crimes and despite rehabilitation, thus frustrating the efforts for recovery.

Decree of September 23, 2004

With the implementation of European Commission Directive 2003/101/EC of November 3, 2003 [9], and the Decree of September 23, 2004 "Attuazione della direttiva 2003/101/CE del 3 novembre 2003 della Commissione europea, per quanto concerne la classificazione ed i valori di soglia di alcune sostanze soggette a controllo, con sostituzione degli allegati I e III al testo unico delle leggi sulla disciplina delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309, come modificato dal decreto legislativo 12 aprile 1996, n. 258, recante il recepimento della direttiva 92/109/CEE relativa alla fabbricazione e all'immissione in commercio di talune sostanze impiegate nella fabbricazione illecita di stupefacenti o di sostanze psicotrope. (Implementation of European Commission Directive 2003/101/EC of November 3, 2003, regarding the classification and threshold values of certain substances subject to control, replacing Annexes I and III of the consolidated text of the laws on the regulation of narcotic drugs and psychotropic substances, referred to in Presidential Decree n. 309 of October 9, 1990, as amended by Legislative Decree n. 258 of April 12, 1996, transposing Directive 92/109/EEC to the manufacture and the marketing of certain substances used in the illicit manufacture of narcotic drugs and psychotropic substances)" (www.myttex.net/forum/attachment.php?aid=1590) [10], regarding the classification and threshold values of certain substances subject to control, Annexes I and III of the consolidated text of the laws governing narcotic drugs and psychotropic substances were replaced by the following: Category I (1-phenyl-2-propanone, N-acetylanthranilic acid, lsosafrol cis and trans, 3,4-methylenedioxyphenylpropan-2-one, piperonal, safrole, ephedrine, pseudoephedrine, norephedrine, ergomethrine, ergotamine and lysergic acid), Category II (acetic anhydride, phenylacetic acid, anthranilic acid, piperidine and potassium permanganate) and Category III (hydrochloric acid, sulfuric acid, toluene, ethyl ether, acetone and methyl ethyl ketone).

Law February 2, 2001 (n. 12) "Standards to facilitate the use of opioid analgesic drugs in the treatment of pain"

Further amendments followed with two important laws: law of February 8, 2001 (law n. 12, www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2001-06-16&atto.codiceRedazionale=001A6713&elenco30giorni=false) [11] entitled: "Norme per agevolare l'impiego dei farmaci analgesici oppiacei nella terapia del dolore – Indicazioni applicative. Rules to facilitate the use of opioid analgesic drugs in pain therapy – Application guidelines" and law of February 21, 2006 "Conversione in legge, con modificazioni del decreto-legge 30 dicembre 2005, n. 272, recante misure urgenti per garantire la sicurezza ed i finanziamenti per le prossime Olimpiadi invernali, nonché la funzionalità dell'Amministrazione dell'interno. Disposizioni per favorire il recupero di tossicodipendenti recidivi. (Conversion into law, with amendments to decree-law n. 272 of December 30, 2005, containing urgent measures to guarantee the safety and funding for the next Winter Olympics, as well as the functionality of the internal administration. Provisions to encourage the recovery of recidivist drug addicts)". For the first time, By law n. 12, by the more and more pressing requests from patient associations in terminal phase were received with the aim of facilitating prescription and administration the following active substances (Annex III-bis): buprenorphine, codeine, dihydrocodeine, fentanyl, hydrocodone,

hydromorphone, methadone, morphine, oxychodone, oxymorphone, in pain treatments for patients suffering from severe pain due to neoplastic or degenerative pathology. The offences, in prescription and dispensation, were all decriminalized and penalties were reduced to administrative pecuniary sanctions.

Law February 21, 2006, (n. 49)

The deppest revision of 309/90 was brought with the law of February 21, 2006, (law n. 49 - www.gazzettaufficiale.it/eli/gu/2006/02/27/48/so/45/sg/pdf [12] "Conversione in legge, con modificazioni del decreto-legge 30 dicembre 2005, n. 272, recante misure urgenti per garantire la sicurezza ed i finanziamenti per le prossime Olimpiadi invernali, nonché la funzionalità dell'Amministrazione dell'interno. Disposizioni per favorire il recupero di tossicodipendenti recidivi. (Conversion into law, with amendments to decree-law n. 272 of December 30, 2005, containing urgent measures to guarantee the safety and funding for the next Winter Olympics, as well as the functionality of the internal administration. Provisions to encourage the recovery of recidivist drug addicts)", conversion of decree-law December 30, 2005 (n. 272) [13].

With the law n. 49, also known as Fini-Giovanardi, all psychoactive substances that did not find any therapeutic use were included in a single Table that did not discriminate "soft" and "hard" drugs for personal use, resulting in equal sanctions. The former six Tables were reduced to two, of which only the second, subdivided into sections A to E by dependence liability, was relevant from a therapeutic and legal point of view. The rigorous choice of equalisation had, however, been mitigated by the reduction of the minimum penalty and the existence of mitigating circumstances referred to in art. 73, paragraph 5. The new system of sanctions – administrative and criminal – aimed to combine prevention, repression and recovery, assuming that drug use is not an innocuous exercise of freedom, but an act of rejection of the most basic duties of the individual towards the different communities in which he lives. The punishment of drug possession was reintroduced and a boundary was established between detention, which represents an administrative offence, and detention, which constitutes a criminal offence. The boundary was no longer represented by the "small quantity", which was subjective and arbitrary, nor the "average daily dose", but an objective quantitative Table for substance: if the drug in possession exceeded a certain limit, criminal sanctions were applied; if the quantity was below that limit, administrative sanctions were applied (suspension of the driving licence, weapon licence, passport, residence permit for tourists, and administrative detention of the scooter in use). With the new law, the criminal sanctions were gradual. For users who had committed a minor crime, a completely new measure was introduced if the person did not want to go through rehabilitation and had already been granted a sentence suspension: instead being incarcerated and upon request, convicted people could do public utility work for the entire duration of the prison sentence. Confirming existing provisions, which were made more appropriate to the seriousness of the crimes, recovery was favored from the moment when pre-trial detention was ordered: detention could be avoided by going under house arrest and starting, under certain conditions, a therapeutic program. To have a better chance of undergoing therapy, the possibility of suspending the execution of the final prison sentence was widened: the limit of punishment allowing suspension was raised from 4 to 6 years of imprisonment, which allowed a larger number of drug addicts to go through rehabilitation. The reform also had a profound impact on the management of drugs. It was important for both the doctor and the pharmacist to replace the old "two copies" prescription with the "tracing" one, reserving the use of only one category of medicines (Table II, Section A) and a maximum lenght of 30 days. In order to limit illicit activities associated with therapeutic use, the new discipline provided a draft of a copy of the prescription, limited to section A, which the patient must hold as proof of legal possession of the drug preparation. Galenic preparations may then be prepared only with the narcotic active ingredients listed in Section B of Table II, and the validity of the galenic and renewable prescription was limited to 30 days. The extension of section E prescriptions could not exceed three times in the 30 days of validity. A vouchers-purchase bulletin is necessary for cumulative orders. Although official, this bulletin is not printed by the State Polygraph and can be downloaded and printed directly from the Ministry's website and coexists with the old official model bulletin, which can only be used for one substance or preparation. Like all laws, the discipline of narcotics also needed rules of application and interpretation. It has been clarified that the discharge from the register of substances and preparations for destruction can only be made when the pharmacist loses the material possession of narcotic drugs to no availing itself of the possible entrustment by the Local Healthcare Units after drawing up a special report (Ministerial Health Note May 31, 2006 N. D.G.F.D.M/VIII/P/I.8d.q/20116). It has also been made clear that the compilation, of the voucher-purchase bulletin can be carried out also by a pharmacist other than the holder or director, provided that it was authorized for this purpose by formal act (Ministerial Health Note May 20, 2008 N. 0019201-P- 20/05/2008 DGFDM). Finally, as far as the model of the entry and exit register is concerned, the Ministry has clarified that the number of pages can be different from 200. This clarification therefore allows for registers with less than 200 pages to be set up and, consequently, to replace them more frequently, thus being able to destroy the prescriptions downloaded two years after the last registration, with an obvious advantage for the confidentiality of personal data.

Ministerial Decree of April 18, 2007

Subsequently, Ministerial Decree of April 18, 2007 "Aggiornamento e completamento delle Tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope e relative composizioni medicinali, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309, e successive modificazioni ed integrazioni, recante

il testo unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope e di prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza". (Update and completion of the Tables in the Presidential Decree n. 309/90, www.trovanorme.salute.gov.it/norme/dettaglioAtto?completo=si&id=23064) [14], was amended by the decree of April 29, 2007. The prescription of opioid analgesic medicinal products (Annex III-bis) was no longer limited to the treatment of severe pain caused by neoplastic or degenerative pathology. Therefore, these medicinal products can be used for the therapy of the severe pain (postoperative, trauma...), regardless of the origin of the pain itself. New substances were also added to Table I: total opium alkaloids, beta-hydroxymethyl-3-fentanyl, buprenorphine, intermediate destromoramide, (+) - 1 - methyl - lysergic acid diethylamide, morphine methyl bromide and other morphine derivatives with {pentavalent nitrogen} including N-oxymorphine derivatives (such as N-oxycodeine). For the same decree, the following substances were also removed from Table I: ethylcyclidine and ethyl ester of 4-phenylpiperidin-4-carboxylic acid. In the same Table, the common name of messalina has also been replaced by the common name of mescaline. New substances were also added to Table II, section B: delta-9-tetrahydrocannabinol, trans-delta-9-tetrahydrocannabinol and nabilone, while tramadol and the compositions containing this substance were removed from section B and D of the same Table. Moreover, medicinal compositions for uses other than the injectable ones containing destropropoxyfen in association with other active ingredients were moved from section D to section E of Table II.

The Ordinances of the Vice-Minister Fazio (June 16 and July 2, 2009)

Additional ministerial intervention consisted of the Ministerial Ordinance of June 16, 2009 "Iscrizione temporanea di alcune composizioni medicinali nella Tabella II, sezione D, allegata al testo unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope e di prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza. (Temporary entry of certain medicinal compositions in Table II, Section D, attached to the consolidated text of laws on the regulation of narcotic drugs and psychotropic substances and on the prevention, treatment and rehabilitation of the relative states of drug addiction)" (www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2009-06-20&atto.codiceRedazionale=09A07142&elenco30giorni=false) [15] with substances such as opioids, certain pharmaceutical forms, to a limited extent, and authorized analgesic substances, were transferred from section A to section D of Table II (buprenorphine, codeine, dihydrocodeine, fentanyl, hydrocodone, hydromorphone, oxycodone, morphine, oxymorphone). The measure, which was followed by another Ordinance on July 2, 2009 "Supplements to the Ordinance of 16 June 2009 on "Integrazioni all'ordinanza 16 giugno 2009, recante Iscrizione temporanea di alcune composizioni medicinali nella tabella II, sezione D allegata al testo unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope e di prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza. (Temporary entry of certain medicinal compositions in Table II, Section D attached to the consolidated text of laws on the discipline of narcotic drugs and psychotropic substances and on the prevention, treatment and rehabilitation of their states of drug addiction" (www.gazzettaufficiale.it/eli/id/2009/07/08/09A08111/sg) [16] involved the following practical effects for physicians and pharmacists:

- use of personal prescription of the doctor or the National Healthcare System;
- overcoming, without limits, of the dose for thirty days of treatment (except in the case of National Healthcare System prescription);
- deletion of input and output recording;
- the obligation to store psychoactive drugs in a closed locker no longer applies;
- purchase without filing the bulletin (in the sole case of purchase from a wholesaler);
- destruction of prescriptions retained after six months;

However, with the Ministerial Ordinance of July 2, 2009, the obligation to identify the purchaser in the case of personal prescription was introduced. The goal was to avoid the circulation of falsified prescriptions without possibility to trace the person responsible for the presentation. The obligation to send to the Local Healthcare Unit and the Order of Pharmacists, by the end of each month, a summary communication of the prescriptions sent privately (white prescription) in the previous month was also provided for.

Law March 15, 2010 (law n. 38) "Arrangements to ensure access to palliative care and pain therapy"

After a process that lasted almost two years, law of March 15, 2010 (law n. 38, www.gazzettaufficiale.it/gunewsletter/dettaglio.jsp?service=1&datagu=2010-03-19&task=dettaglio&numgu=65&redaz=010G0056&tmstp=1269600292070) [17], entitled "Disposizioni per garantire l'accesso alle cure palliative e alla terapia del dolore. (Provisions to ensure access to palliative care and pain therapy)", was published in the Official Gazette n. 65 of March 19, 2010. The law had a very strong impact on the prescription and dispensing of medicines used in pain therapy. The rules were contained in art. 10 entitled "Simplification of procedures for access to medicines used in pain therapy" and intervened significantly on various articles of Presidential Decree n. 309 of October 9, 1990, which regulates narcotic drugs and psychotropic substances. The following amendments were enforced on April 3, 2010.

Article 14 (Table). The amendment to Article 14 provided for the inclusion in Section D of Table II of all medicinal products containing substances listed in Annex III-bis to Presidential Decree 309/90, namely: buprenorphine, codeine, dihydrocodeine, fentanyl, hydrocodone, hydromorphone, methadone, morphine, oxycodone, oxymorphone, provided that they are not parenteral preparations. The Ministry of Health was forced to amend the Table because the provision of law could be fully implemented.

Article 25-bis (destruction). Article 25-bis greatly

simplified the procedures for the destruction of psychoactive substances. The destruction could be carried out by allowed private companies which had to provide a report to be transmitted to the Local Heathcare Unit by the Pharmacy Director.

Article 38 (vouchers). The amendment to art. 38 removed the requirement for a written request to purchase medicinal products from Sections D and E, when purchased from suppliers other than wholesalers. In essence, the obligation to complete the bulletin only remained for medicinal products belonging to sections A, B and C, in all procurement scenarios.

Articles 41 and 43 (receivers). Indication of the addressees of simplified forms for prescription analgesic medicinal products (art. 41 and 43) passed from patients affected by severe pain during pathology neoplastic or degenerative to that more generic and extensive of patients who had access to palliative care and pain therapy.

Article 43 (medical prescription book). Paragraph 4-bis Article 43 provided for the possibility of the prescription of medicinal products belonging to section A, to be on the health service's medical preascription book national. However, it was obvious that, if the doctor prescribed the medicine of section A not under National Healthcare System control, only the medical prescription book must be used to Ministerial tracing.

Article 45 (buyer's documents). With regard to the obligations of the pharmacist in the dispensation of Section A medicinal products (art. 45, 1st paragraph), it should be ensured that (the pharmacist) the identity of the purchaser and took note of the extremes of an identification document from transcribe on the prescription to the following forecast that noted on the prescription the first name, last name and contact details of the buyer's identification document.

Article 45 (excess therapy). The legislator wanted to overcome the following problem: the pharmacist still sent out the prescriptions that should prescribe a quantity which, in relationship to the indicated dosage, theoretically exceeded the maximum limit of 30-day therapy, where the surplus was due to the number of contained dosage units in the packages on the market. The pharmacist was also allowed to reduce the number of packages to fit in with the requirements of the 30 days of therapy, taking into account however, the previous forecast was present and communicating to the doctor.

Article 45 (fulfilments). Paragraph 6-bis, added to Article 45, concerned the pharmacist's obligations if presented with a prescription, so-called white, prescribing medicines which, according to the ordinance of 16 June 2009 and this law, have passed, and will pass, from section A to section D.

Article 45 (reduced delivery). For all prescribed medicinal products and belonging to Sections A to E, the possibility was provided for, on request of the patient, reduced delivery than the prescribed quantity by giving communication to the doctor, or but in any case by the end of the year. 30-day period of validity of the prescription and reporting on it the quantities delivered from time to time (Article 45, paragraph 10-bis).

Article 60 (logbook). The amendment to Article 60 reduced to two years the conservation period of the entry and exit register date of last registration. In this way, the conservation time of the prescriptions of sections A, B and C (two years) was brought into line with the conservation time of the register and, obviously, of all the accompanying documents (bulletins, vouchers, reports of destruction, theft, etc.). In addition, art. 60, as amended, provided that the number of pages could be different from two hundred and adequate to the amount of drugs that would normally taken over and sold.

Article 68 (decriminalizations). Finally, with the addition of paragraph 1-bis to art. 68, the irregularities found relating to breaches of the regulations on record keeping were decriminalised.

Sentence n. 32/2014 of the Constitutional Court

With decree-law of March 20, 2014 "Disposizioni urgenti in materia di disciplina degli stupefacenti e sostanze psicotrope, prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309, nonché di impiego di medicinali meno onerosi da parte del Servizio Sanitario Nazionale. (Urgent provisions concerning the regulation of narcotic drugs and psychotropic substances, prevention, treatment and rehabilitation of the relative states of drug addiction, as per Presidential Decree n. 309 of 9 October 1990, as well as the use of less onerous medicines by the National Healthcare Service)", law n. 36, (www.gazzettaufficiale.it/eli/id/2014/3/21/14G00047/sg) [18], several amendments were made to the Presidential Decree n. 309/90, following sentence of 2014, n. 32 (www.cortecostituzionale.it/actionSchedaPronuncia.do?anno=2014&numero=32) [19], of the Constitutional Court, which reinstated the distinction between "soft" and "hard" drugs. Compared to the original single Table of Drugs, the decree-law returned to four Tables (plus a Table of "Medicines"), and considered separately cannabis and its derivatives, which ended up in the Second Table. The second exception concerned the sanctions: as a result of the combined effect of the new decree and the ruling of the Constitutional Court, the penalty system of the law n. 162 was revised. In this concern the penalties for imprisonment in case of possession of significant cannabis quantities and the trafficking of cannabis and its derivatives were again significantly reduced.

Moreover, a new Table of medicinal products was established to allow complete continuity in the production, prescription, and distribution of psychoactive medicinal products, with particular reference to the prescriptions of pain therapy medicinal products and medicinal products used in the course of treatment for the cessation of addictions. The modalities of prescription and dispensing therefore remained unchanged for all therapies with psychoactive drugs; the modalities of management of the medicines by the operators of the pharmaceutical sector also remained unchanged.

Therapeutic use of cannabinoids in Italy

Ministerial Decree 98/2007 "Aggiornamento e completamento delle Tabelle contenenti l'indicazione

delle sostanze stupefacenti e psicotrope e relative composizioni medicinali, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309, e successive modificazioni ed integrazioni, recante il testo unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope e di prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza. (Update and completion of the Tables containing the indication of narcotic and psychotropic substances and related medicinal compositions" as per Presidential Decree n. 309 of 9 October 1990 and subsequent amendments and additions, containing the consolidated text of the laws on the regulation of narcotic drugs and psychotropic substances and on the prevention, treatment, rehabilitation of their states of drug dependence) (www.trovanorme.salute.gov.it/norme/dettaglioAtto?completo=si&id=23064) has recognized the therapeutic properties of delta-9-tetrahydrocannabinol (THC), the main active ingredient of cannabis, and two other analogs of synthetic origin (dronabinol and nabilone). These substances are listed in Table II section B (art. 2), which lists the substances that can be used in therapy and prescribed according to Article 72, paragraph 2, of the consolidated law 309/90.

With the Ministerial Decree n. 33 of 2013, an update of the Tables containing the indication of narcotic and psychoactive substances was made, inserting in Table II section B the "medicinal products of vegetable origin based on cannabis (herbal substances and preparations, including extracts and tinctures" (www.gazzettaufficiale.it/eli/id/2013/02/08/13A00942/sg).

To the present day, the current classification of cannabis and derivatives, is finally dictated by the law n. 79/2014 (www.gazzettaufficiale.it/eli/id/2014/05/20/14G00090/sg), which confirms the therapeutic function of the substances mentioned above and therefore their inclusion in Table II section B, now renamed "Table of Medicines". However, the law also classifies tetrahydrocannabinol (THC) and its analogues in Table I (most dangerous substances), and cannabis and its derivatives – oil, resin, leaves and inflorescences – in Table II (substances with a lower risk of addiction).

Production, distribution and use of medical cannabis

According to art. 26 of Presidential Decree 309/90 "(…) the cultivation of the plants included in Table I and II of art. 14 (…) is forbidden in the territory of the State", however "the Minister of Health can authorize university institutes and public laboratories with institutional research purposes, to cultivate the abovementioned plants for scientific, experimental or didactic purposes". Thanks to this exception, the Ministry of Health and the Ministry of Defence signed an agreement on September 18, 2014, under which the military chemical-pharmaceutical plant (SCFM) in Florence now carries out the operations of cultivation and manufacture of the active substance of plant origin based on cannabis. It also provides for the packaging and distribution, upon request of the Regions and Autonomous Provinces, to local pharmacies or hospital pharmacies for the preparation of magisterial preparations, to be dispensed on presentation of a non-repeatable prescription, in order to meet the needs of the assisted population. The active herbal substance is therefore supplied by the SCFM or can be found abroad, following a series of procedures regulated by the decree of February 11, 1997 entitled "Methods of importing specialty medicines registered abroad", which allows the importation of drugs not distributed in the Italian Health circuit.

The Ministerial Decree of November 9, 2015 "Functions of the State Agency for cannabis provided for in Articles 23 and 28 of the Single Convention on Narcotic Drugs of 1961, as amended in 1972" (www.gazzettaufficiale.it/eli/id/2015/11/30/15A08888/sg) has laid down more detailed provisions on the subject. Article 1 has identified the precise functions of the Ministry of Health as the State Agency for cannabis, which:

- authorises the cultivation of cannabis plants for use in the manufacture of herbal medicinal products based on cannabis;
- identifies the areas of use for such cultivation;
- imports, exports and distributes across the national territory, i.e. it authorises the import, export, wholesale distribution and maintenance of stocks of cannabis plants and material;
- provides for the determination of the production quotas of active substances of plant origin based on cannabis on the basis of the requests of the Regions and Autonomous Provinces and informs the International Narcotics Control Board (INCB) at the United Nations.

A technical annex to the Ministerial Decree has dealed with the most practical issues:

- the dosage of the medicinal product is determined, as well as the the mean of production and distribution to pharmacies;
- States that treatments with cannabis cannot be considered "a therapy in the strict sense of the word", but only to palliate the standard treatments when they have not produced the desired effects or have caused side effects that cannot be tolerated;
- the possible therapeutic uses of cannabis are exhaustively indicated for diseases involving spasticity and pain (multiple sclerosis, spinal cord injury); chronic pain; nausea and vomiting caused by chemotherapy, radiotherapy and HIV therapy; with appetite-stimulating effect in cachexia, anorexia, oncology or AIDS patients; with hypotensive effect in glaucoma; for reduction of involuntary bodily and facial movements in Tourette's syndrome);
- a phytosurveillance system will be set up, and therapeutic responses to cannabis will be monitored, thanks to reports made by health professionals to the Istituto Superiore di Sanità about any suspected adverse reactions following administration.

Currently, Italy allows the exclusive therapeutic use of cannabinoids in the form of magisterial preparations based on cannabis, i.e. galenic drugs (e.g., tinctures, infusions, oils, extracts) prepared by the pharmacist in his laboratory. The only drug of synthetic origin, based on cannabinoids, and authorised for trade by Italian Medicines Agency is Sativex, which can only be used in the symptomatic treatment of muscle spasms in patients

with multiple sclerosis. The latter is a class H drug, and can therefore be supplied at the expense of the NHS only in the hospital environment and dispensed through hospital pharmacies and the territorial system.

New Psychoactive Drugs (NPS)

All narcotic drugs and psychoactive substances are now listed in Tables I, II, III and IV, which account for the substances with strong addictive liability and potential for abuse in decreasing order; these Tables are amended whenever there is a need to introduce or remove a new substance or change its location. These Tables, linked to the system of sanctions for illicit use, list the narcotic and psychoactive substances placed under International and National control. The Table of medicinal products, itself divided into 5 sections (A, B, C, D, E), displays the medicinal products containing narcotic and psychotropic active substances and currently used for therapeutic purposes in human or veterinary medicine, and exemptions for doctors, pharmacists and operators in the pharmaceutical sector. In addition, Annex III-bis of the same decree contains the medicines used in pain therapies, which benefit from simplified prescriptive procedures, marked in the Tables with a double asterisk.

Since July 2007, by separate decrees of the Ministry of Health entitled "Update of the Tables containing the indication of narcotic and psychoactive substances, as per Presidential Decree n. 309/90, and subsequent amendments and additions". Many new psychoactive substances *(Table 1 of this article)*, have been included in Table I [20-56].

"Substances with an analogy" were also included in Table I, and include substances with similar chemical structure or effects to the substances already present in the same Table (2-amino-1-phenyl-1-propanone, indazol-3-carboxamide, indol-3-carboxamide, 3-benzoylindole, 3-phenylacetylindole, 3-(1-naphthoyl)indole). Bupropion and pyrovalerone have been excluded from Table I. In Table II, herbal medicinal products based

Table 1
New psychoactive substances included in Table I, Presidential Decree n. 309790, since 2007

Cathinones	Phenethylamines	Synthetic cannabinoids	New synthetic opioids
4-MEC	2C-E	XLR-11	Furanylfentanil
Ethylone	25H-NBOMe	5F-Apinaca	3-Phenylpropanoylfentanyl
Buphedrone	2C-H	5F-APP-Pica	4-Fluoroisobutyrfentanyl (4F-iBF)
Pentedrone	25E-NBOMe	5F-APP-Pinaca	Benzylfentanyl
Alpha-PVT	4Cl-iBF	5F-PB22	Benzoylfentanyl
4F-NEB	25B-NBF	AB-Chminaca	Carfentanyl
Alpha-PHP	25B-NBOH	AB-Fubinaca	Cyclopentylfentanyl
Alpha-PVP	DOC	ADB-Chminaca	Cyclopropylfentanyl
BK-2C-B	3,4-DMA NBOMe	ADB-Fubinaca	Benzodioxolefentanyl
Isopentedron	4-EA NBOMe	APP-Fubinaca	Methoxyacetylfentanyl
Methylone	HHMA	BB-22	Tetrahydrofuranylfentanyl (THF-F)
bk-MBDB	HMA	Cumil-5F-Pinaca	Tetramethylcyclopropanfentanyl
Amfepramone	HMMA	MDMB-Chmica	Thiophenefentanyl
3.4-Methylenedioxypyrovalerone (MDPV)	25B-NBOMe	CP 47,497	Butyrfentanil
Mephedrone	25C-NBOMe	CP 47.497-homologous C8	2-fluorofentanyl
	25I-NBOMe	AM-694	U-47700
	4-Methylamphetamine	JWH-250	Acryloylfentanyl
	4-fluoroamphetamine	JWH-122	3-Methylfentanyl
		JWH-018	3-Methylthiofentanyl
		JWH-073	Acetylfentanil
			Morphine-N-Oxide
			Ocfentanil
Benzofurans	**Arylcyclohexylamines**	**Tryptamines**	AH-7921
6-EAPB	Deschloro-N-ethyl-ketamine	5MeO-MIPT	MT-45
	3-MeO-2-Oxo-PCE	ETH-LAD	6-MAM
		5-MeO-EIPT	3-MAM
		DALT	Oripavine

Continues

Table 1
Continued

Aminoindanes	Benzodiazepines	Piperidines and pyrrolidines	Piperazines
MDAI	Flubromazolam	Ethylphenidate	Benzylpiperazine
Amphetamines	**Plants and extracts**	**Arylalkylamines**	**Others**
4-FMA	Ibogaine	MPA	G-130
5-EAPB	Mitragynine	2-MABB	PRE-084
Fenbutrazate	Mitragyna Speciosa	6-IT	2-MeO-difenidine
Phentermine	Tabernanthe Iboga	5-APB NBOMe	4,4-dimethylaminorex
Mazindol	Argyreia nervosa seeds	6-APB	Afloqualone
	Ipomoea violacea seeds	5-APB	MMQ
	Rivea corymbosa seeds	6-APDB	W-18
		5-APDB	Phendimetrazine
		5-IT	Nandrolone

on cannabis in Section B (substances and vegetable preparations, including extracts and dyes), nandrolone in Section A and tapentadol in Section D (previously included in Table II Section A) have been included respectively. Amfepramone and phentermine, have been excluded from Table II Section B. In Table IV, fonazepam (benzodiazepine analogue of flunitrazepam), methylmorphonate (methylphenidate anologue), sufentanil for sublingual use, etizolam and meprobamato have been included. In the Table of Medicinal Products, section D, lormetazepam has been included to the section "Compositions for use parenteral". In addition, the use of propilesedrine, except for the manufacture of barbesaclone, has been excluded. Moreover, the following substances have been excluded from the Table of Medicinal Products, Section B (dextropropoxyphene, fenproporex, mefenorex, meprobamate and tetrazepam), section C (dextropropoxyphene) and section E (meprobamate, dextropropoxyphene and tetrazepam). Conversely, cannabis-based herbal medicinal products have been included as usable in pain therapy in the Table of Medicinal Products, Section B. In Annex III-bis, tapentadol has been added to the list, previously included in Table II Section A. These decisions have been taken "to protect public health, in view of the risks associated with the use and spread of new psychoactive substances onto the international market", as the world of drugs is evolving every day, and has considerably changed in the last 10 years, with different forms of addiction, substances and modes of consumption.

CONCLUSION

The areas addressed offer an opportunity to recall that, in the Italian law, the penalty system for drug use and trafficking is based on Tables, and illicit conduct concerning (only) substances that are included in the "Tables" referred to in Articles 13 and 14 of Presidential Decree n. 309/90 are punished. The fact that the Ministry of Health, an administrative authority, has been entrusted with the task of drawing up and amending the "Tables" determines the construction of the criminal cases concerning narcotic or psychoactive substances as "blank criminal regulations", in which the sanction is determined by a legislative act, while the illegal conduct is only partly described, since it must be specified by the Ministerial Decree governing the individual substances. The adoption of the tabular system of substances subject to control determines that only conduct concerning substances included in the "Tables" may be sanctioned, both criminally and administratively. It is worth repeating the need for the complete and timely updating of these Tables, to avoid the effect of punishing conduct involving substances that, although dangerous, have not been tabulated. The problem arises in all its emergence for the so-called new psychoactive drugs (NPS) freely available on the Internet, although they produce stimulating and hallucinogenic effects similar to those of banned substances. They are obtained by modifying the chemical structures of the main illegal substances to obtain new substances that are not controlled and, for this reason, are called "legal highs". They are unregulated and untested. Given that the chemicals in these drugs are constantly changing to stay ahead of the law, it's possible to receive a very different product from batch to batch, even if the packaging and name are the same. The new molecules detected by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in 2018 are about sixty. Some of these have already been detected on Italian users, but as hundreds of other types are not yet listed in the "Tables" updated by the Ministry. Old and new generations of drugs are passing on the baton at a faster rate than bureaucratic procedures, and Italy is struggling.

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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44. Italia. Decreto del Ministero della Salute, 25 giugno 2013. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Inserimento nella Tabella I delle sostanze 6- (2-aminopropil) benzofurano (6-APB); 5-(2-aminopropil)benzofurano (5-APB); 6-(2-aminopropil)-2,3-diidrobenzofurano (6-APDB) e 5-(2-aminopropil)-2,3-diidrobenzofurano (5-APDB). Gazzetta Ufficiale – Serie Generale n. 158, 8 luglio 2013.
45. Italia. Decreto del Ministero della Salute, 8 gennaio 2015. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Inserimento nella Tabella IV delle sostanze etizolam e meprobamato e nella Tabella dei medicinali, Sezione D, dei medicinali ad uso parenterale a base di lormetazepam. Gazzetta Ufficiale – Serie Generale n. 22, 28 gennaio 2015.
46. Italia. Decreto del Ministero della Salute, 10 febbraio 2015. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni. Inserimento nella tabella I delle sostanze 4-iodo-2,5-dimetossi-N-(-2-metossibenzil) fenetilammina (25I-NBOMe) e 3,4-dicloro-N-(1-(dimetilammino)cicloesil)metil] benzamide (AH-7921), 1-cicloesil-4-(1,2-difeniletil)-piperazina (MT-45). Gazzetta Ufficiale – Serie Generale n. 48, 27 febbraio 2015.
47. Italia. Decreto del Ministero della Salute, 4 febbraio 2016. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Gazzetta Ufficiale – Serie Generale n. 41, 19 febbraio 2016.
48. Italia. Decreto del Ministero della Salute, 10 febbraio 2016. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Inserimento nella Tabella I delle sostanze HHMA, HMA e HMMA. Gazzetta Ufficiale – Serie Generale n. 51, 2 marzo 2016.
49. Italia. Decreto del Ministero della Salute, 1 agosto 2016. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre, n.309 e successive modificazioni e integrazioni. Inserimenti e ricollocazione di sostanze stupefacenti o psicotrope nelle tabelle I e IV, nella tabella dei medicinali sezioni A-B-D e nell'allegato III bis. Gazzetta Ufficiale – Serie Generale n. 187, 11 agosto 2016.
50. Italia. Decreto del Ministero della Salute, 13 marzo 2017. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Inserimento nella tabella I delle sostanze DOC e Acriloilfentanil. Gazzetta Ufficiale – Serie Generale n. 70, 24 marzo 2017.
51. Italia. Decreto del Ministero della Salute, 18 maggio 2018. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Inserimento nella tabella I e nella tabella IV di nuove sos-

tanze psicoattive. Gazzetta Ufficiale – Serie Generale n. 126, 1 giugno 2018.
52. Italia. Decreto del Ministero della Salute, 18 maggio 2018. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Inserimento nella Tabella I delle sostanze: XLR-11, 2C-E, 25H-NBOMe, 2C-H, 25E-NBOMe, 4-FMA, 6-EAPB, 5-EAPB, furanilfentanil. Gazzetta Ufficiale – Serie Generale n. 126, 1 giugno 2018.
53. Italia. Decreto del Ministero della Salute, 25 giugno 2018. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Inserimento nella tabella I delle sostanze Bufedrone, Pentedrone e Alfa-PVT. Gazzetta Ufficiale – Serie Generale n. 160, 12 luglio 2018.
54. Italia. Decreto del Ministero della Salute, 25 giugno 2018. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Inserimento nella tabella I delle sostanze Butirfentanil, 4-MEC, Etilone, MPA, 5F-APINACA. Gazzetta Ufficiale – Serie Generale n. 160, 12 luglio 2018.
55. Italia. Decreto del Ministero della Salute, 25 giugno 2018. Aggiornamento dell'elenco dei medicinali di cui all'Allegato III-bis del decreto del Presidente della Repubblica 9 ottobre 1990, n. 309. Gazzetta Ufficiale – Serie Generale n. 160, 12 luglio 2018.
56. Italia. Decreto del Ministero della Salute, 12 ottobre 2018. Aggiornamento delle tabelle contenenti l'indicazione delle sostanze stupefacenti e psicotrope, di cui al decreto del Presidente della Repubblica 9 ottobre 1990, n. 309 e successive modificazioni e integrazioni. Inserimento nella Tabella I delle sostanze: 3-Fenilpropanoilfentanil, 4-Fluoroisobutirfentanil (4F-iBF), Benzodiossolfentanil, Benzilfentanil, Benzoilfentanil, Carfentanil, Ciclopentilfentanil, Ciclopropilfentanil, Metossiacetilfentanil, Tetraidrofuranilfentanil (THF-F), Tetrametilciclopropanfentanil e Tiofenefentanil. Gazzetta Ufficiale – Serie Generale n. 255, 2 novembre 2018.

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ORIGINAL ARTICLES AND REVIEWS

Prevalence and correlates of food insecurity among children in high-income European countries. A systematic review

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Abstract

Background. In Europe, there is not routinely collected data on children's food insecurity. Indirect data show that food insecurity is on the rise in Europe, which may have a great impact on children's health.

Methods. Considering that, we systematically reviewed any evidence coming from European countries in the last 10 years that reported the prevalence and correlates of food insecurity among children, intending to serve as a starting point for policymakers and guidelines.

Results. We report worrying prevalence rates of food insecurity among children from 9 studies. There is a lack of evidence regarding this issue in many EU countries, especially Eastern Europe. Hence, the need for increased attention towards food insecurity among children in European countries.

Conclusions. Achieving food security means designing targeted policies and interventions, both at a national and EU level. Policymakers and governments should make the appropriate efforts to deliver food security as a public good.

Key words

- food insecurity/security
- households
- children
- prevalence
- Europe

INTRODUCTION

Food insecurity (FI) is an increasing public health issue in the world, affecting even developed countries [1, 2]. According to Food and Agriculture Organization (FAO), food insecurity is on the rise starting from mid-2014, with nearly 1 in 10 people in the world living in this condition [3].

That is even more worrying when talking about children, who, if affected, may be in danger, not only for present, but, also, future adverse health outcomes, since their developing brains and bodies can suffer long-term negative consequences [4].

Food security is achieved when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. As it is seen, food security is a broad concept. It includes not only the quality of food and food shortage but also physical, social and economic access to food as well as food preferences [5]. The likelihood of experiencing food insecurity is higher among households with children, those who rely on social assistance, renters, households headed by a single parent, without a university degree, a divorced person, younger persons and most importantly, those with lower income [2]. Other factors may be the region of residence and family composition [6].

Food insecurity is worrying because of the negative consequences on children's health through physiopathological pathways such as a compromised dietary intake and being a strong stressor [7]. Furthermore, food insecurity is strictly connected to nutritional insecurity (sufficient calories but insufficient or unbalanced essential oligo-nutrients) and poor food safety (unsafe environments, poor hygiene, lack of clean water), creating a complex relationship and converging on a series of negative health outcomes.

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FI is associated with worse diet quality, micronutrient deficiency, poor health status and stress situations [7]. Iron or zinc deficiencies, associated with food insecurity, may impair learning, delay cognitive development and decrease productivity and academic achievements [8]. These children have a higher probability of presenting cardio-metabolic risk factors [9] and chronic conditions, particularly asthma, depression and suicidal ideation in adolescence [3], as well as dental caries [10] anemia, hypercholesteremia and hypertension [11]. As for the relationship between food insecurity and children's BMI, literature is ambivalent. While some studies have found a positive association between food insecurity and obesity [12], in other cases it is concluded that this correlation doesn't exist [13].

Unlike Europe, household food security is routinely monitored in the USA and Canada using the Household Food Security Survey Module (HFSM) through nationally representative surveys, providing accurate prevalence data on child food insecurity and its determinants [1, 2]. In Europe data regarding this issue come from reports published by FAO, UNICEF and Eurostat surveys, or from a limited number of studies conducted in only a few European countries [3, 14]. In most cases the tool used to measure food security is not HFSM. This implies that the duration of exposure is not specified, nor multiple dimensions of food insecurity captured, such as hunger or insecure access to sufficient quantities of food, as are measured by the HFSM [2]. Furthermore, in Europe, there is a lack of studies assessing the impact of food insecurity on children's health.

The importance of having these data rises from warnings that food insecurity has been increasing in Europe since the 2008 recession [15]. An indirect indication of this, is the proliferation of food banks since 2010, as observed in the UK [16], Greece, Spain, and France [17].

The evidence gap regarding food insecurity among children in a national and European level and the impact that it has on their health might be a missed opportunity to act. This evidence could guide future policies concerning food insecurity among children, both at a national or EU level. Considering that, we aimed at summarizing any evidence coming from European countries in the last 10 years that reported the prevalence and correlates of children living in food insecure households.

METHODS

Search strategy

We searched three major databases (Web of Science, PubMed, Scopus), looking for relevant articles published between January 1st, 2009 and June 1st, 2019. We considered this time frame in order to have information on children's food insecurity following the economic crisis that started 10 years ago in Europe. In PubMed, we searched the title/abstract of the articles using the keywords *"food", "nutrition", "nourishment", "supply", "adequacy", "access", "security", "insecurity", "utilization", "availability", "poverty", "hunger", "children", "pediatric", "adolescent", "teenager", "kid", "Europe"* using the Boolean operators *AND, OR*. The search was restricted to only humans and full-text availability. We adapted the search strategy for the other two databases.

Study selection and inclusion/exclusion criteria

Articles were imported to Excel and the duplicates were removed. The screening process was carried out separately by two reviewers (DZ, FC) and was divided in two rounds. During the first round, pertinent articles were selected based on titles/abstracts. Then, the full texts of these articles were obtained and entirely read and those satisfying all the inclusion criteria were included. We included studies that assessed the prevalence of children (until 18 years old), living in food insecure households in high-income countries in Europe (European Union). We included only these countries because of their economic and social similarities. Studies assessing food insecurity in children with disabilities or health problems were excluded. Cross-sectional studies were considered, as well as longitudinal ones, as long as the prevalence of food insecurity was measured at a specific time frame (i.e. baseline). We excluded articles that were not original studies and were not published in a peer-reviewed journal. Disagreements were resolved by consensus. The reference lists of the included studies were hand-searched to look for additional articles. If data from the same sample was reported in different studies, we decided to include the most recent one.

Data extraction and synthesis

Data extraction was performed independently by two reviewers (DZ, FC). A data extraction form (see *Table 1*) was used retrieving the following information for each eligible article:

- Study identification: first author, title, publication year
- Study characteristics: study period, country, design, sample
- Households' characteristics: demographic, socioeconomic status (SES), children's age
- Food insecurity measurement method, reported by who
- Food insecurity prevalence
- Socio-economic and health correlates of food insecurity.

Due to high heterogeneity between studies, especially in the population that was studied, a narrative synthesis was conducted. This included summarizing the characteristics of the studies and households, reporting the prevalence of children living in food insecure households and investigating its relationship with characteristics of the households, children's age and the tool used to assess food insecurity.

Quality assessment

Two examiners (DZ, FC) separately used the Newcastle Ottawa Scale, modified and adapted for cross-sectional studies, [18] and the Newcastle Ottawa Scale for cohort studies, [19] evaluating the selection process, comparability, outcome and appropriateness of statistical analysis. The final scores were compared, and discrepancies were resolved by consensus.

We used the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement, [20] as a guide for this review.

The protocol of this systematic review was registered in PROSPERO, registration number: CRD42018109745.

RESULTS

Characteristics of the included studies

Our search strategy produced a total number of 2105 articles. The results of the screening process are given in *Figure 1*. After the screening process, only 9 studies were included in the review (*Table 1*). The quality score in the final sample ranged from 6 to 8 (*Supplementary material 1, available online*).

Eight (89%) were cross sectional studies [21-28], while one (11%) was longitudinal [29]. One study (11%) was conducted in Spain [21], one (11%) in Germany [22], one (11%) in Greenland [23], one (11%) in Greece [26], two (22%) in France [24, 25] and three (35%) in the United Kingdom (UK) [27-29]. Six studies (67%) [23-27, 29] explored also the variables associated to household food insecurity, while two studies (22%) [23, 29] reported health correlates of this condition.

Characteristics of the households

Three studies (33%) [21, 23, 26] had directly recruited children and, then, retrieved information from their caregivers or from the children themselves, when age pertinent, for a total of 28 029 children. The rest had included households with at least one child in them, for a total of 3342 households.

Participants were living in mainly urban locations (67%), with 3 studies (33%) [23, 27, 29] having a mixed location. Four studies (45%) [21, 23, 25, 29] included households with different socio-economic status (SES), others were focused only on low or very low SES households.

Children's age varied from 1 to 18 years old. One study (11%) included the age category 1-5 years old [29], one (11%) the category 6-11 [28], two (22%) the category 12-18 years old [21, 23], and four (45%) more than one of these categories [24-27]. One study (11%) did not report children's age [22].

Measurement of food insecurity

Food insecurity was measured using the HFSM in all the studies, except one [23]. Based on 18 questions, this questionnaire classifies the households as high, marginal, low and very low food security [30]. Five studies (55%) used the full version of HFSM [29, 24-26, 28], one (11%) used the 6-items adapted version [27] and one (11%) the 8-items adapted version [22]. When food insecurity was reported by children them-

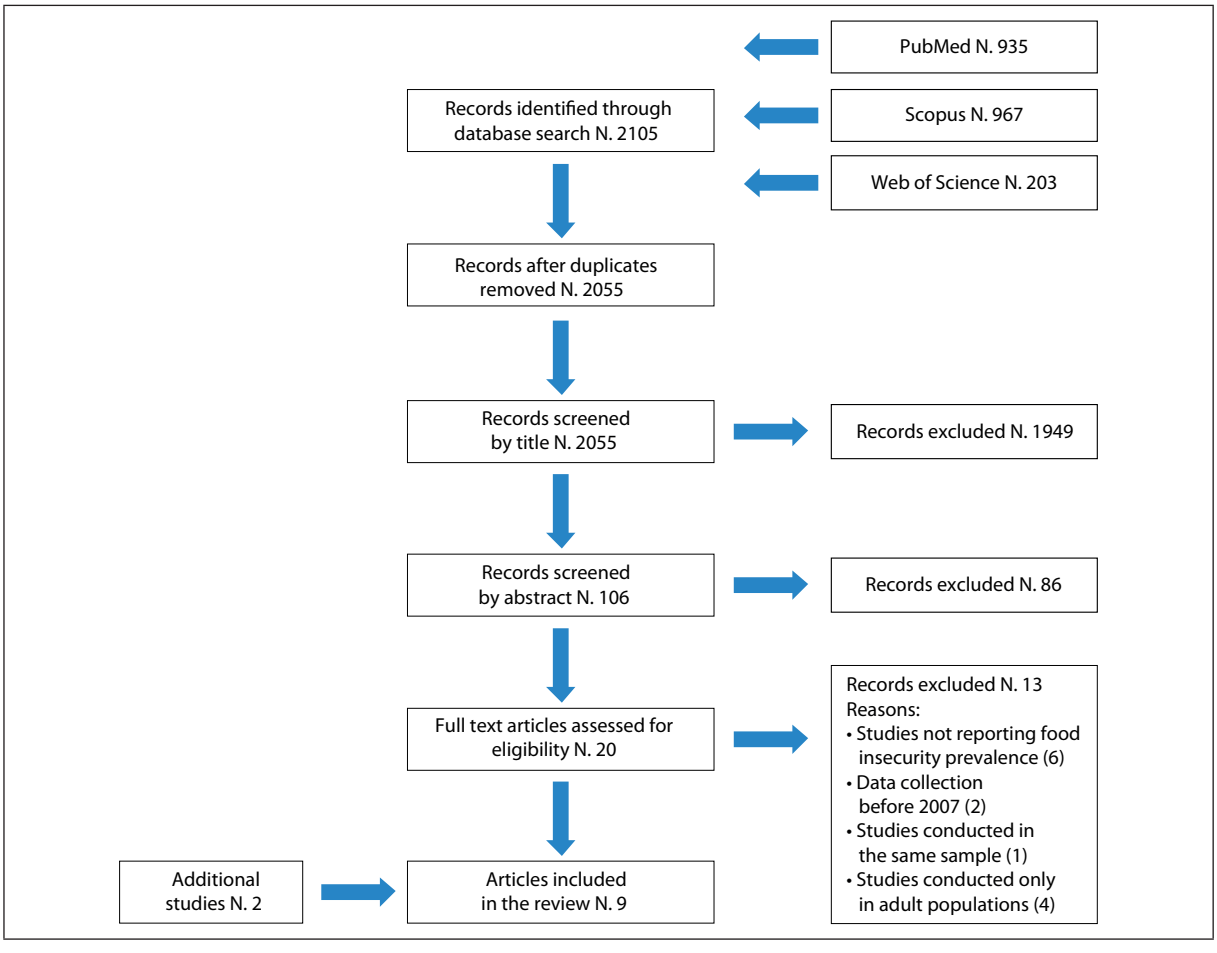

Figure 1
Flowchart of the screening process of the articles included in the review.

Table 1
Extraction form reporting relevant information from all the included articles in the systematic review

Author	Title	Period	Publication	Country	Design	Sample	Setting	Location
Shankar-Krishnan, et al.	Spanish adaptation and validation of the Child Food Security Survey Module (CFSSM-S)	2017	2018	Spain	Cross sectional	426 adolescents	Secondary public school in Terrassa, Catalonia	Urban
Depa et al.	Prevalence of food insecurity among food bank users in Germany and its association with population characteristics	2015	2018	Germany	Cross sectional	217 households with children	Foodbank users in Stuttgart, Berlin, Karlsruh	Urban
Niclasen et al.	Adverse health effects of experiencing food insecurity among Greenlandic school children	2010	2013	Greenland	Cross sectional	2254 students in the 5th-10th grade from Health Behaviour in School-aged Children (HBSC) survey	School grades 5-10	Mixed
Yang et al.	Association of food security status with overweight and dietary intake: exploration of White British and Pakistani-origin families in the Born in Bradford cohort	2010	2018	England	Longitudinal	1104 mothers from Born in Bradford Study	Bradford Royal Infirmary for universal oral glucose tolerance testing at 26-28 weeks gestation.	Mixed
Martin-Fernandez et al.	Food Insecurity in Homeless Families in the Paris Region (France): Results from the ENFAMS Survey	2013	2018	France	Cross sectional	772 homeless families living in facilities in Paris from the ENFAMS Survey	Accommodation facilities in the Paris region	Urban
Martin-Fernandez et al.	Prevalence and socioeconomic and geographical inequalities of household food insecurity in the Paris region, France, 2010	2010	2013	France	Cross-sectional analysis	1139 households with children among 3006 households from SIRS (a French acronym for "health, inequalities and social ruptures")	Face to face interviews at home	Urban
Petralias et al.	The impact of a school food aid program on household food insecurity	2012-2013	2016	Greece	Cross sectional (before after)	25 349 students	162 elementary and secondary schools	Urban
Long et al.	The impact of holiday clubs on household food insecurity. A pilot study	2015	2017	UK	Cross sectional pilot study	38 families	Holiday clubs held in schools or church halls	Mixed
Harvey	When I go to bed hungry and sleep, I'm not hungry": Children and parents' experiences of food insecurity	2013	2016	London, UK	Cross sectional	72 parents	UK Charity that provides support for deprived children	Urban

Continues

*The age of children included in the studies was ordered according to the stages of children's development: toddlers and pre-schoolers (aged 1-5 years), children (aged 6-11 years), and adolescents (aged 12-18 years)
SES: Socio-economic status
HFSM: Household Food Security Module
USDA: United States Department of Agriculture
FI: Food Insecurity
LFS: Low Food Security
VLFS: Very Low Food Security

Table 1
Continued

Author	Ses	Children's age*	Tools (test/scores/ questionnaire)	Reported by	Prevalence	Variables associated with FI	Health outcomes
Shankar-Krishnan et al.	Mixed	12 to17 years (mean 13.8)	Child Food Security Survey Module-Spanish version	Children	FI 18.3%: 1.9% VLFS 16.4% LFS	/	/
Depa et al.	Low	No info	8 questions U.S. Household food Security Survey Module (US HFSSM	Parents	FI 35%: 5.5% VLFS 29.5% LFS	/	/
Niclasen et al.	Mixed	Children aged 11-17	Answers to the following question: "Some young people go to school or to bed hungry because there is not enough food at home. How often does this happen to you?"	Children	37.7% of boys and 26.5% of girls	Boys, younger children, coming from low SES households experienced food insecurity more often.	Children experiencing food insecurity had poorer self-rated health (OR=1.60), more physical symptoms, like headache, stomach ache or backache (OR=1.34) and more medicine use (OR=1.79)
Yang et al.	Mixed	1-4 years old	United States Department of Agriculture (USDA) 18-items Household Food Security Module (HFSM)	Mothers	FI: 9%	Ethnicity	Food insecurity was associated with dietary intakes, consuming more sugar-sweetened beverages and savoury snacks and less vegetables. Obesity was more frequent among food insecure children
Martin-Fernandez et al.	Very Low	Less than 13 years old	United States Department of Agriculture (USDA) 18-items HFSM	Parents	FI 43.1%: 9.8% VLFS 33.3% LFS	Residential instability, single parenthood, having more than three children, parents' depressive symptoms, housing in social hostels, and difficult access to cheap or free food were associated with food insecurity in children	/
Martin-Fernandez et al.	Mixed	Less than 18 years old	United States Department of Agriculture (USDA) 18-items HFSM	Parents	FI 9.1%: 3.8% VLFS 5.5% LFS	Household type, umber of children in the household, household head's age, socio-occupational category and education level, income and neighbourhood socioeconomic status were associated with food insecurity	/
Petralias et al.	Low	Mean age 10.4	United States Department of Agriculture (USDA) 18-items HFSM	Parents	64.2% of households: 26.9% VLFS 37.3% LFS	Middle and high school children, with higher number of siblings, with unmarried parents or living only with their mother and whose parents had lower education level or were unemployed experienced food insecurity more often.	/
Long et al.	Low	Ages 2-18	Six- item short adapted form of the HFSM	Parents	FI 42%: 24% VLFS 18% LFS	Ethnicity, unemployment, lower income and larger households were associated with food insecurity	/
Harvey	Low	Aged 5-11 years	18 questions Food Security Survey Module	Parents	FI 100%: 86% VLFS 14% LFS	/	/

*The age of children included in the studies was ordered according to the stages of children's development: toddlers and pre-schoolers (aged 1-5 years), children (aged 6-11 years), and adolescents (aged 12-18 years)
SES: Socio-economic status
HFSM: Household Food Security Module
USDA: United States Department of Agriculture
FI: Food Security
LFS: Low Food Security
VLFS: Very Low Food Security

selves, the 9-items form adapted for children was used [21].

The only study that did not use this questionnaire, asked the following question: "Some young people go to school or to bed hungry because there is not enough food at home. How often does this happen to you?" [23].

Prevalence of food insecurity among children

Prevalence of low food security ranged from 5.5% in a study conducted in the general population in France [25], to 43.3% in a study conducted in homeless families in the same country [24]. Very low food security ranged widely from 1.9% in a study in Spain [21], to 86% in a study conducted in deprived families receiving charity in England, UK [28]. The ranges of food insecurity in total (low and very low) went from 9% in a study conducted in the general population in England, UK [29], to 100% in a study conducted in deprived families receiving charity in the same country [28].

The two studies conducted in France reported a very different prevalence of food insecurity among children; 9.1% [25] *vs* 43.1% [24]. Also, studies conducted in the UK, reported high differences in prevalence values of food insecurity, going from 9% [29] to 42% [27] and 100% [28].

In the studies conducted in households with mixed SES, food insecurity prevalence ranged from 9% [29], to 37.7% [23], compared to the studies conducted in those with low or very low SES, where the prevalence of food insecurity ranged from 35% [22] to 100% [28].

Only two studies used a short version of the HFSM reporting food insecurity prevalence rates of 42% [27] and 35% [22]. The five studies that used the full version of the HFSM reported prevalence rates ranging widely from 9% [29]-100% [28]. In the two studies where food insecurity was reported by children themselves, prevalence rates were 18.3% [21] and 37.7% [23].

The study conducted in the age category 1-5 years old reported a prevalence of 9% [29]. Food insecurity prevalence in the study conducted in the age category 6-11 was 100% [28], while in the age category 12-18, 18.3% [21] and 37.7% [23]. The studies that included more than one of these categories reported a prevalence rate of 43.1% [24], 9.1% [25], 64.2% [26], and 42% [27].

Correlates of food insecurity

Among socio-economic correlates of food insecurity in children there were: low income [23, 25, 27], households with single parents [24, 26], with a higher number of children [24-26], household's structure [25, 27], parents' occupation [25-27] and education level [25, 26], household head's age [25], depressive symptoms in parents [24] and ethnicity [27, 29]. Among children's characteristics, younger ones [23, 26] and boys [23] were more often experiencing food insecurity. Children living in food insecure households had more physical symptoms, such as headache, stomachache or backache, used medicines more often and had in general poorer health [23]. They also consumed more sugar-sweetened beverages and savory snacks and fewer vegetables and experienced more often obesity [29].

DISCUSSION

In this review, we aimed at summarizing evidence from European countries on the prevalence and correlates of food insecurity among children in the last 10 years. Compared to other developed countries like the USA [1] or Canada [2], where food insecurity is monitored using the HFSM, providing accurate data on child food insecurity prevalence and determinants, in Europe, there is not routinely collected data on food insecurity. Following the economic downturns in Europe, there has been a rise in food insecurity in the population, including children [14]. But, since systematic national monitoring of food insecurity in Europe does not yet exist, there is only indirect data coming from food banks, reports published by FAO or UNICEF [3, 14] and a small number of studies conducted in a limited number of European countries. In fact, we included in our systematic review only nine studies reporting the prevalence and correlates of food insecurity among children, conducted in the UK, France, Germany, Greece, Spain and Greenland. This lack of studies could be because of less recognition of the concepts or lack of established measurements. Furthermore, the included studies were all from western Europe (except Greece), confirming a lack of evidence regarding this issue in east Europe countries. A study analyzing the trends of food insecurity in Europe after the 2008 crises concluded that there has been an increase in food insecurity in the general population, with the highest overall rates coming from eastern Europe, so studies addressing food insecurity among children in these countries are warranted [30].

The included studies report worrying prevalence rates, but, since there is no routinely collected data, we couldn't conclude about the trend of child food insecurity in Europe during the past 10 years. Prevalence varied widely between studies. This may be attributed to the differences in data collection and sample, geographical setting, family's SES, the method used to assess food insecurity, children's age.

As for the geographical location, as suggested by Bernell *et al.* [31], there's a higher likelihood of experiencing food insecurity in an urban location compared to a rural one. In this review, most studies (67%) were conducted in urban locations and the rest in mixed locations, without the possibility to stratify if it was rural or urban. So, it was not possible to say anything conclusive by comparing rural to urban locations. Further studies in Europe should evaluate the association between food insecurity and geographical location.

Half of the studies were focused on low-income households since family's income is most frequently associated with household food insecurity, although the population of poor and food insecure people overlap but are not identical [32]. In the USA, people with an income below the poverty line are 3.5 times more likely to experience food insecurity [33]. In our review, studies that included only low-income households with children reported the highest prevalence of food insecurity, with a range from 35% [22]-100% [28], compared to households with mixed SES, where prevalence ranged from 9% [29]-37.7% [23]. This im-

plies that the family's income remains a strong predictor of food insecurity and interventions to mitigate this condition should focus on low-income households with children.

In almost all studies, the tool used to assess food insecurity was the HFSM. Short versions of this questionnaire have been validated and used, minimizing the respondent fatigue and cost of administration [34]. One hesitation over using a shorter questionnaire, categorizing households as food insecure based on only one affirmative response, may be the increased number of false positives [35]. In two studies food insecurity was reported by children, since research has shown that children experience food insecurity more frequently than their parents think. Nord *et al.* conclude that caregivers' reports of adolescents' food insecurity do not agree with adolescents' own reports [35]. Using different versions of the HFSM is a reason for heterogeneity and we may have a situation of over-reporting when the short adapted versions were used, so the comparison was impossible.

Food insecurity is not experienced the same during a child's life. Some studies report that households with more severe food insecurity are also more likely to have older children compared to households reporting moderate or low food insecurity [1]. Almost half of the studies included in this review involved children of all ages, without the possibility to stratify for age category, so it was not possible to conclude about this possible association. Future studies should focus on stratifying by age when measuring food insecurity in children.

There are four included studies with a very high prevalence of food insecurity among households with children [24, 26-28], conducted in very low SES households and populations that are specifically susceptible to food insecurity, like homeless persons or foodbank users. One of these studies was conducted in Greece, in a low socio-economic status population during the economic crisis. It is known that Greece was one of the most affected countries by the crisis, so this high prevalence is expected [26].

As for correlates of food insecurity, only a few studies addressed this issue, along with reporting the prevalence of food insecurity. Among household characteristics associated to food insecurity there were low income [23, 25, 27], households with single parents [24, 26], with a higher number of children [24-26], household's structure [25, 27], parents' occupation [25-27], lower education [25, 26], head household's age [25], depressive symptoms in parents [24]. Ethnicity, as a factor correlated to food insecurity, was seen in only two studies [27, 29]. In a growing ethnic diversity Europe, future studies should address this evidence gap.

Among children's characteristics, younger ones [23, 26] and boys [23] were more often experiencing food insecurity. Anyways, age and sex need to be further investigated as correlates of food insecurity in children, since only two and one among the included studies, respectively, had explored the possible association.

As seen, there are only a few studies reporting these correlates and the evidence is not strong enough to give definite conclusions as happens, for example, in the USA or Canada where there is ample evidence on the correlates of food insecurity in children [1, 2, 6, 36]. Knowing the populations at risk for food insecurity is important, to appropriately assist the more vulnerable children and prevent the negative health outcomes.

Only two of the studies included in the review explored the impact of food insecurity on children's health, reporting in general poorer health among food insecure children. [23] There is a need for more studies to explore the impact of food insecurity on children's health. Since food insecurity has long-term effects that may be seen even after many years, longitudinal studies would be appropriate to better explore the possible association.

This review has some limits. The included studies had, generally, a medium quality. Some of them did not give a good estimation of food insecurity in children since the majority were conducted in a specific population group, lacking representativeness and generalizability. Hence, the need for studies that could be representative of the general population. As in all systematic reviews, publication bias may be an issue, leading to the loss of small unpublished studies.

On the other hand, we used a comprehensive and rigorous methodological approach to identify every possible study reporting the prevalence of food insecurity among children living in Europe. To our knowledge, this is the first review reporting prevalence data of children living in food insecure households in Europe. It includes data from high-income countries and comes at a moment when food insecurity is considered a public health issue. The fact that countries with the same SES were considered is another strong point accounting for lack of heterogeneity regarding this aspect.

Implications for public health and policymakers

There is a need for evidence and increased attention towards this public health issue in Europe. Because of this lack of evidence, the establishment of a monitoring system of food insecurity becomes a priority for food and nutrition policies. We recommend monitoring the situation by annual surveys and conducting research regarding who and why certain groups are affected. Longitudinal studies are needed to explore the impacts of food insecurity on children's health. More studies should focus on the stress pathway of food insecurity, and not only the nutrition one, as well as assess the relationship between food insecurity, nutrition insecurity and food safety. Achieving food security means designing targeted policies and interventions, both at a national and EU level. Policymakers and governments should make the appropriate efforts to deliver food security as a public good. Considering the high prevalence of food insecurity among children in Europe and the impact it has on their health, this systematic review could serve as a starting point for framing guidelines on screening for food insecurity among children.

Declaration of interests

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Effects of a practice-focused nutrition intervention in Hungarian adolescents

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Abstract

Introduction. This work evaluated the impact of a nutrition intervention in school children of 6th and 7th grade and assessed whether changes persisted after the summer break.

Materials and methods. Eight classes of Hungarian adolescents (45% boys; 12.6 ± 0.1 years) were randomized into intervention (n = 117) and control (n = 112) groups. The 9-month long intervention included: 1) weekly classroom-based education with strong focus on practical elements such as tasting and meal preparation; 2) five sessions of after-school cooking classes (open to children, parents and grandparents); and 3) online education materials. Anthropometric parameters (weight, height, waist circumference and body fat), aerobic fitness (Cooper test, 20-meter shuttle run test), nutrition knowledge and behaviors (questionnaires) were measured three times at baseline, post-intervention and after the summer holiday.

Results. Slight improvement in dietary knowledge and habits from baseline to post-intervention which did not persist after summer. Aerobic fitness increased in the intervention group, while did not change among controls. Anthropometric parameters remained unchanged in the intervention group, but waist circumference increased in controls, particularly in summer.

Conclusions. Findings suggest a positive impact of this intervention. Measures to mitigate unhealthy changes during the summer break are needed.

Key words

- school-based
- nutrition
- education
- obesity
- adolescents

INTRODUCTION

Publications on malnutrition in the form of obesity and nutrient deficiency presented alarming figures both for European adults [1, 2] and children [3, 4]. Similarly to other parts of Europe, Hungary has a high rate of child obesity [5]. Compliance with dietary recommendations is also poor [6]; and low level of physical activity and aerobic fitness is highly presented, particularly in older age groups (NETFIT).

Because of their current and lifetime impact, these risk factors need to be addressed via a range of approaches from individual to environmental interventions [7-9]. Among environmental settings, school has a unique role and huge potential to reach out a large number of children and to promote healthy behaviors [10]. In Hungary, a number of regulations have been introduced in the last ten years aiming to create an enabling school environment [11]. Three mandatory regulations apply nationwide: the introduction of daily physical education (PE). classes in the academic year of 2012/2013 in a step-up implementation system, the national standards for public catering which came into force in 2015 and the prohibition of selling of those foods and drinks in schools that are subject to the Public Health Product Tax. Besides, within the framework of the EU School Fruit, Vegetables and Milk Scheme students from 2157 primary schools (90% of all schools) get fresh fruit and vegetable daily [12]. Finally, since the academic year of 2016/2017, the education on biology and health including lessons on healthy diet and nutrition was also introduced from grade 7th on a stepwise manner.

Adolescence, defined as the transitional phase between childhood and adulthood, is one of the critical intervention periods. This is the time when young people begin to make their own choices and decisions related to their diet and health, as well as to establish lifestyle habits that will carry over into adulthood [9]. Besides, the prevalence of overweight and obesity as well as lifestyle-related risk factors are high at this age [6].

Recent evidence shows that school-based education interventions have the potential to improve children's weight status, knowledge and lifestyles if running for a sufficient duration and targeting also the family members [13]. However, only limited information is available to understand whether these programs can increase cardiovascular fitness and whether the improvements last

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after the summer holiday. This study therefore aimed to develop a whole-year school-based nutrition intervention for adolescents with an emphasis on practical education, active participation and family involvement, and to evaluate its effects on participants' dietary knowledge and behavior, as well as on weight status and aerobic fitness using an intervention-control study design. Knowing that inadequate intake of nutrients and calories can impair exercise performance [14]; in the present work it was hypothesized that improving diet quality in adolescents would possibly increase their aerobic capacity. The secondary objective was to study whether these changes would persist after the summer holiday.

MATERIALS AND METHODS

Study population and recruitment strategies

Budaors is a town in Pest county with a total population of 28 394 inhabitants. It is one of the most developed towns in Hungary, with four state-owned primary schools. For the purpose of this study, two primary schools were enrolled. Participating schools had similar socio-demographic characteristics. One had a total of 808 students from grade 1st to 8th; with an average classroom size of 30 students. The other school was smaller with a total number of 463 students, and with an average classroom size of 26 pupils.

From the two enrolled schools, a total of eight classes were selected from grade 6th and 7th (two 6th and two 7th grade classes from each school). Classes in each grade were then randomized into intervention or control groups.

Recruitment of the study population took place in September 2015 during the registration period. Parents were contacted and informed about the purpose and processes of the study during the first parents' meeting of the academic year. All parents agreed to participate in the study and were contacted for completing the baseline parental questionnaire. All study participants gave their informed consent for inclusion before participating in the study. The work was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Scientific and Research Ethics Committee of the Medical Research Council (8776-1/2016/EKU).

Description of the intervention

The intervention included three main components: 1. weekly classroom-based education (25 to 45 minutes long); 2. five sessions of after-school cooking classes (open to the entire family); and 3. online education materials distributed via e-mails and social media. Children in grades 6 and 7 within the intervention classes received the combination of all components, whereas children in the control classes continued their usual curriculum.

The weekly classroom-based education developed in this study included both theoretical and practical parts and were led by the same trained dietician in each intervention class. A total of 27 interactive sessions were delivered over the period of 9 months. Sessions started with the theoretical part followed by a tasting or meal preparation activity. During the first academic semester tasted foods were prepared by the dietician in advance. Meanwhile, in the second semester, children prepared the foods in the schools' small kitchen unit as part of the session with the help of the dietician. Topics covered within the education sessions included the principles of healthy nutrition, relation between nutrition and health, the role of different nutrients, importance of different meals (i.e. breakfast, lunch, dinner and snacks), healthy snacking, role and recommended amount of different food groups, labelling, and healthy party tips. Games and tasting were incorporated to reinforce main messages of each session. Detailed description of each session is presented as a supplementary file.

After-school cooking classes were offered five times in the second semester and were attended by children, parents and grandparents. They aimed on one hand to educate caregivers, but also to increase the involvement of children in meal preparation and cooking. Similarly to classroom-based activities, these sessions had both theoretical and practical parts, but here more emphasis was put on practice. Activities were organized in the schools' small kitchen unit and typically lasted 1 or 2 hours. Sessions addressed the following questions and topics: "*How to make kids eat fruits and vegetables?* (2 sessions)", "*How to reduce dietary risk factors?*", "*The importance of breakfast*" and "*How to reduce added sugar?*" *(see Supplementary Material available online).*

Recipes posted on Facebook or sent via e-mail completed the intervention and strengthened its family-involvement component.

Data collection procedures

Information on students' age, gender, dietary knowledge and behavior were collected by a 29-item questionnaire. Children completed the questionnaire with the assistance of a trained field worker in their classroom. In the present paper we analyzed the following parts of the questionnaire: 1) age and gender; 2) nutrition knowledge section (6 questions) which covered the areas of healthy nutrition in general, recommended amount of fruits and vegetables, recommended fluid intake, energy drinks and sugary drinks; and 3) dietary behaviour section (10 questions) which assessed the frequency of breakfast skipping, number of meals, frequency of eating fast food, daily fluid intake, as well as the frequency of dairy products, fruits, vegetables, sweets, energy drinks and sugary drinks consumption. For the purpose of the current paper, nutrition knowledge and dietary behaviour questions were separately analyzed. Each correct answer for the nutrition knowledge questions was allocated a score of 1 point; all others received 0. The total knowledge score ranged between 0 and 6 points with higher scores indicating a better nutrition knowledge. For the dietary behaviour questions, favorable options were scored as 1 point and others got 0. Thus, the total behavior score ranged from 0 to 10 points with a higher score reflecting healthier dietary behaviour.

Anthropometric measurements of children (weight, height, waist circumference, fat mass) were obtained using standard protocols and equipments by a trained dietician with the support of the physical education teachers. Children's verbal permission was requested before taking the measurements. Weight was measured

to the nearest 0.1 kg in light indoor clothing with bare feet, using portable digital calibrated scales (OMRON BF 511) and height was measured standing upright, without shoes, to the nearest 0.1 cm using a 2M wall-mounted stadiometer roll-up height measurer. Fat mass was estimated by using the same equipments as for the weight measurements (OMRON BF 511). These scales use 8-sensor single-frequency bioelectrical impedance analysis technology and offering hand-to-foot measurements to improve accuracy [15]. A non-stretchable measuring tape (Seca 201) was used to measure waist circumference of children above the iliac crest to the nearest 0.1 cm, with the subject standing and after normal expiration [16]. Anthropometric measurements were taken only once for each child.

Physical fitness assessments followed the Hungarian National Student Fitness Test (NETFIT) protocol [17], which now serves as a compulsory fitness assessment for all Hungarian schools. NETFIT test has four parts: 1) body composition and weight status incl. weight, height and body composition measurements; 2) aerobic fitness incl. 20-meter shuttle run test [18]; 3) muscle strength and endurance incl. paced curl-up, trunk lift, paced push-up, handgrip strength and standing long jump tests; and 4) flexibility incl. back-saver sit-and-reach test. Evaluation is based on health-related, criterion-referenced youth fitness standards. In the present study, the NETFIT protocol was completed with a Cooper test which was designed to measure aerobic fitness [19]. The results of this 12 minutes long running test can be correlated with VO_2 max values. The longer distance is covered in 12 minutes the higher is the estimated VO_2 max value, and therefore higher is the estimated aerobic capacity. So, both in Cooper and in 20 meters shuttle run tests higher values indicating a better fitness status.

Data analysis

All data was processed anonymously. EpiData Entry 3.1 software was used for data entry, which included built-in range (e.g. outliers, out of range values) and consistency checks for validation. In order to get the exact age of each child, the birth date was subtracted from the measurement date then variables with age in years were created. Body mass index (BMI) (kg/m^2) was calculated by dividing the weight (kg) over the height squared (m^2). Children were classified as underweight, normal weight, overweight or obese based on the age- and gender-specific IOTF cut-offs [20]. Descriptive statistics were performed and presented as means and standard error (SE) for continuous variables and as frequencies and proportions for categorical variables. At baseline, between-group differences (i.e. intervention versus control groups) in age, anthropometric characteristics, knowledge and behaviour scores and fitness status of participants were assessed using Student's t-test. For categorical variables chi-square tests were applied. Paired t-tests were used to compare independently the differences between baseline, post-intervention and follow up within each of the intervention and control groups (within-group differences). In addition, between-group differences (intervention *vs* control groups) in mean changes were evaluated using Student's t-tests. As gender distribution was significantly different at baseline between the intervention and control groups, generalized linear regression analyses were conducted to test the effect of gender on mean changes in anthropometric parameters, nutrition knowledge score, dietary habits score and in aerobic fitness status. We also tested whether our conclusions were biased by an interaction between gender and group status (i.e. intervention or control), but no interaction was identified. A p-value of < 0.05 was considered statistically significant.

RESULTS

Data were collected at baseline from 232 children, 226 children completed the post-intervention tests (97.4%) and 203 participated in the follow-up evaluation (87.5%) *(Figure 1)*. At the end, three children were excluded from the analysis due to the large number of missing data, so the current analysis is based on the data of 229 children.

At baseline, mean age of all enrolled children was 12.6 ± 0.1 years and gender distribution was slightly unequal (44.5% boys and 55.5% girls). Significant differences were observed between the intervention and control group at baseline ($p = 0.003$) in gender distribution (i.e. lower proportion of boys in the intervention group), BMI (i.e. slightly higher mean BMI in the intervention group), number of children with obesity (i.e. higher proportion in the intervention group) and aerobic fitness status (i.e. lower aerobic fitness in the intervention group). However, age, waist circumference, fat mass, nutrition knowledge score and dietary habits score did not differ significantly between the two groups *(Table 1)*.

Table 2 presents between-group differences (intervention *vs* control) with respect to mean change from baseline to post-intervention and from baseline to follow-up in anthropometric parameters, nutrition knowledge and dietary habits scores as well as in aerobic fitness tests. No significant changes in BMI, waist circumference or fat mass were noted in the intervention group, while waist circumference increased in the control group from baseline to follow-up (67.5 ± 1.0 cm *vs* 72.8 ± 1.0 cm, $p < 0.001$). The number of children with obesity decreased from 8 to 7 in the intervention group, while increased from 2 to 7 among the control children from baseline to post-intervention. By the end of the summer break, there were 6 children with obesity in both groups. The nutrition knowledge score slightly decreased in the control group, but an increase was observed in the intervention group from baseline to post-intervention (3.79 ± 0.1 point *vs* 4.14 ± 0.1 point, $p = 0.04$). However, the score decreased back to baseline level by the time of the follow-up. Similarly, there was a small increase in the dietary habits score in the intervention group from baseline to post-intervention (4.35 ± 0.2 point *vs* 4.5 ± 0.2; $p = 0.02$). However, the favorable change did not persist after the summer holiday. In contrast, in the control group the score decreased from baseline to post-intervention and remained low by the time of the follow-up. Aerobic fitness improved significantly from baseline to post-intervention in the intervention group (Cooper test: 2020.3 ± 39.9 m *vs* 2256.3 ± 39 m, $p < 0.001$ and 20-me-

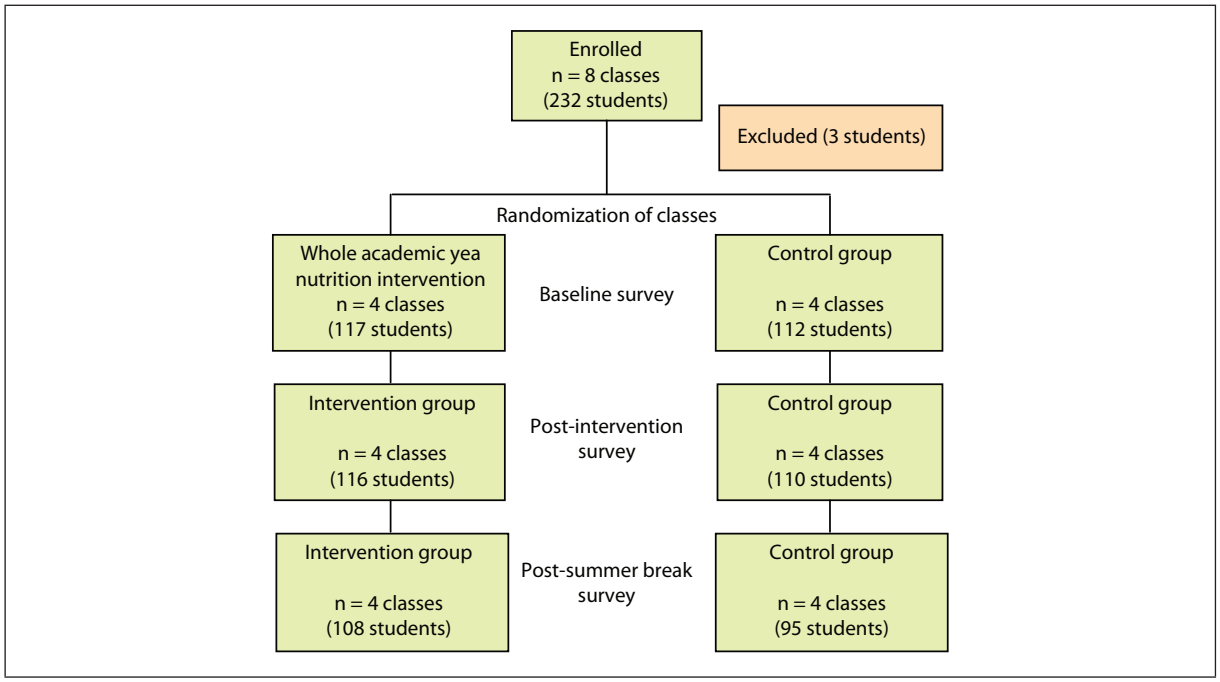

Figure 1
Total number of classes and participants in the intervention and control groups at the time of the three assessments (i.e. at baseline, post-intervention and after the summer break).

Table 1
Baseline characteristics of intervention and control children participated in the school-based nutrition intervention in Budaors n = 229)

Dependent variables	Intervention group (n = 117)	Control group (n = 112)	*p*-value
Boys-girls, n (%)	41-76 (35-65%)	61-51 (54.5-45.5%)	*0.003*
Age (years)	12.5 ± 0.1	12.6 ± 0.1	0.572
Weight (kg)	51.7 ± 1.2	49.6 ± 1.1	0.179
Height (cm)	158.9 ± 0.8	159.7 ± 0.7	0.386
BMI (kg/m^2)	20.4 ± 0.4*	19.3 ± 0.3**	*0.037*
Weight status – overweight, n (%)	21 (18.4%)*	22 (20.6%)**	0.613
Weight status – obese, n (%)	8 (7%)*	2 (1.9%)**	0.073
Waist circumference (cm)	68.2 ± 0.9	67.5 ± 1.0	0.645
Fat mass (%)	20.4 ± 0.9	18.3 ± 0.7	0.063
Nutrition knowledge score[#]	3.8 ± 0.1	3.9 ± 0.1	0.479
Dietary habits score[§]	4.6 ± 0.2	4.2 ± 0.2	0.140
Cooper test (m)	2020.3 ± 39.9	2134.1 ± 41.2	*0.048*
20-m shuttle run test	37.1 ± 1.5	41.7 ± 1.7	*0.046*

Unless other is noted, numbers are means ± standard errors (SE). Clustered independent t-tests were used to compare continouos variables and chi-square tests were applied for categorical variables. BMI, body mass index. *n = 114. **n = 105. [#]Total score ranged from 0 to 6. [§]Total score ranged from 0 to 10.

ter shuttle run test: 37.1 ± 1.5 *vs* 43.4 ± 1.6, $p = 0.004$). There was inconsistency between the Cooper and the 20-meter shuttle run test results from post-intervention to follow up as mean Cooper test result decreased but 20-meter shuttle run test result increased (Cooper test: 2173.6 ± 41.9 m, 20-meter shuttle run test: 45.9 ± 1.9). Significant changes were not observed in aerobic fitness in the control group.

To study whether these changes were the results of the unbalanced gender distribution between the two groups or can be considered as intervention effects we made a gender adjusted regression analysis *(Table 3)*. Changes in nutrition knowledge score, dietary habits score and in Cooper test from baseline to post-intervention remained significant after adjusted for gender. Besides, after adjusting for gender, changes in waist circumference and 20-m shuttle run test achieved statistical significance from baseline to follow-up.

Table 2
Between-group differences (intervention *vs* control) in mean change of anthropometric parameters, nutrition knowledge score, dietary habits score and aerobic fitness status of study participants (n = 229)

Dependent variables	From baseline to post-intervention		*p*-value	From baseline to follow-up		*p*-value
	Intervention	Control		Intervention	Control	
Weight (kg)	2.7 ± 0.3	3.6 ± 0.3	*0.039*	4.6 ± 0.4	5.4 ± 0.4	0.166
Height (cm)	2.6 ± 0.2	2.8 ± 0.2	0.487	4.7 ± 0.3	5.5 ± 0.4	0.097
BMI (kg/m^2)	0.4 ± 0.1	0.7 ± 0.1	0.083	0.6 ± 0.1	0.7 ± 0.2	0.560
Waist circumference (cm)	1.1 ± 0.5	2.2 ± 0.4	0.084	1.8 ± 0.5	6.5 ± 0.7	*0.000*
Fat mass (%)	0.9 ± 0.5	0.4 ± 0.4	0.385	0.4 ± 0.5	-0.03 ± 0.6	0.556
Nutrition knowledge score	0.3 ± 0.2	-0.3 ± 0.2	*0.004*	-0.1 ± 0.2	-0.5 ± 0.2	0.136
Dietary habits score	0.2 ± 0.2	-0.6 ± 0.2	*0.021*	-0.5 ± 0.2	-0.8 ± 0.2	0.271
Cooper test (m)	236.2 ± 28.3	22.8 ± 31.7	*0.000*	138.8 ± 39	136.6 ± 48.3	0.972
20-m shuttle run test	5.5 ± 0.9	3.9 ± 1.1	0.270	8.4 ± 1.3	2.7 ± 1.5	*0.005*

Numbers are means ± standard errors (SE). BMI, body mass index.

Table 3
Results of gender adjusted regression analysis based on general lineal model for two kinds of time interval: from baseline to post-intervention and from baseline to follow-up. Intervention coefficient shows difference of change regarding the intervention group compare to the control group

Dependent variables	From baseline to post-intervention			From baseline to follow-up		
	Intervention coefficient	SE	p-value	Intervention coefficient	SE	*p*-value
Weight (kg)	-0.694	0.418	0.097	-0.325	0.520	0.532
Height (cm)	-0.016	0.280	0.954	-0.218	0.397	0.582
BMI (kg/m^2)	-0.271	0.164	0.099	-0.103	0.204	0.615
Waist circumference (cm)	-1.012	0.668	0.130	-4.541	0.811	*0.000*
Fat mass (%)	0.207	0.600	0.730	-0.204	0.741	0.783
Nutrition knowledge score	0.723	0.237	*0.002*	0.487	0.272	0.085
Dietary habits score	0.713	0.318	*0.025*	0.453	0.313	0.148
Cooper test (m)	215.07	43.17	*0.000*	-3.832	62.490	0.951
20-m shuttle run test	1.858	1.490	0.212	5.679	2.035	*0.005*

DISCUSSION

This work evaluated the impact of a whole school year nutrition intervention on nutrition knowledge, dietary behavior as well as on nutritional and fitness status in Hungarian adolescents. Findings suggest small beneficial changes in dietary knowledge and habits from baseline to post-intervention, which could not be sustained by the end of the summer holiday. In contrast, significant improvements were noted in aerobic fitness in the intervention group which could be detected even at the follow-up. Finally, an increase was found in waist circumference and number of children with obesity in the control group while these parameters did not change among the intervention group participants.

Increasing knowledge by nutrition education is thought to be an effective measure for establishing healthy dietary habits throughout the lifespan [21]. At baseline, the average nutrition knowledge score both in the intervention and control groups was around 3.8 out of 6. Given that the six questions were simple and basic; this is relatively low. Nutrition knowledge of adolescents is often inadequate [22, 23] which is an issue as without sufficient knowledge of this subject they cannot understand the impact of their choices on overall health. This poor knowledge was reflected in the low dietary habit scores. The association between dietary knowledge and behavior is well known and provides the theoretical basis for nutrition education interventions [21]. In the presented work we were able to slightly increase knowledge and improve dietary habits in the intervention group by the end of the 9-month program, while we detected a mild decrease both in knowledge and behavior among the control group for this period. However, these beneficial effects could not be maintained during the summer holiday. These intervention effects on dietary knowledge and habits are consistent with the results of previous nutrition education interventions implemented in the school setting among adolescents [24-27], however only two of these studies included a follow up [26, 27]; and none of the works included explicit post-summer holi-

day assessments. Public health professionals all over the world are continuously working on improving children's diet. Despite the field's best efforts, not much progress has been made on this area [2]. One reason for this failure may be that health promotion and disease prevention programs, like ours, are mainly delivered during the academic period and much less activities are happening during the summer break. To move forward, public health professionals should develop strategies to mitigate unhealthy changes during summer.

Schools are the most important locations for implementing nutrition related educational programs among children and adolescents [28]. However, the evidence on the effective elements of these interventions on dietary habits is inconsistent [29]. Whole academic year duration, strong focus on practical education and involvement of parents and grandparents are those elements that we consider as the most effective ingredients of this program. Other studies also reinforced the importance of building partnerships with parents to complement school-based education programs [30]. In the current work we offered after school cooking lessons for the entire family including children, parents and grandparents. Although participation rate was low, those who attended the extra classes found it very useful. Online education materials and recipes have also targeted the parents. Beside family involvement, practical hands-on experience seems to be also essential for realizing positive behavioral changes in youth [28, 31]. Strategies suggested by adolescents to support healthy diet are, among others, greater cooking involvement, adolescent-specific recipe books and greater parental and peers support [29]. Consequently, the program described here put high emphasize on adding practical elements to every classes. An experimental approach was chosen to provide opportunities to learn about and become familiar with a variety of minimal processed foods. Tasting new foods or ingredients, developing skills in food preparation and making it an enjoyable activity were also considered as key strategies in our study. Few previous interventions have included cooking classes to improve diets in adolescents. For example, in a recent review, only two interventions involved both nutrition education and cooking classes, while only other two included tasting sessions parallel with cooking [32]. Cooking should be re-integrated into the academic curriculum as a compulsory component. Particularly, as lack of cooking skills is one of the most commonly mentioned barriers by adolescents to preparing food at home [33], and consequently they turn more often to less healthy options such as convenience foods or eating out [34].

The association between athletic performance and nutrition is well-known from the literature [35]. Although one's fitness level cannot be increased simple by eating better, unhealthy nutrition can be a limiting factor for performance and recovery [35]. Therefore, in this study we hypothesized that if we improve adolescents' dietary habits it may influence their aerobic capacity. So far, only few publications focused on the relationship between diet and physical fitness in children. Chung and colleagues emphasized the benefits of balanced diet on fitness levels in 6-12 years old children [36]. However, authors noted that beyond diet there are several other factors that affect physical fitness level. As for interventions, according to our knowledge, no other studies assessed the effects of a program with only nutrition-focused activities on physical fitness in children. Da Silva *et al*. performed a study in Brazilian school children and found a marked improvement in fitness, however the intervention included structured physical activities together with the nutritional education element [37]. In this study, the results showed significant improvements in aerobic fitness tests in the intervention group both from baseline to post-intervention and from baseline to follow-up, while no significant changes were noted in the control group. Due to the lack of data in the literature, it was not possible to compare these findings with other results.

One the other hand, the average of BMI, waist circumference and fat mass did not change in the intervention group; whereas in the control group there was a mean increment of 6.5 cm in waist circumference ($p < 0.001$) at the time of the follow-up. Other school-based nutrition education studies conducted in adolescents have shown mixed results with respect to anthropometric outcomes [24, 25]. Differences in results between studies can be attributed to a number of factors, such as differences in intervention elements or variations in participants' baseline characteristics. The increase in mean waist circumference in the control group is remarkable and deserves some thoughts. Adolescence can be considered a critical time for the onset of obesity [38]. Waist circumference has been considered as an important data for visceral fat accumulation, however assessment should be performed in adolescents according to their pubertal staging, since changes are strongly influenced by sexual maturation [39]. In our study, we did not collect data on pubertal stage thus this analysis could not be performed. But we assumed that sexual maturation could explain this sudden increase.

Our study has some limitations that warrant considerations. The gender difference at baseline between the intervention and control groups could led to a selection bias. Sex differences in body composition (i.e. fat and muscle mass) as well as in aerobic capacity is well known from the literature [40, 41]. These differences are more remarkable during adolescents than in children due to the impact of sex specific hormones. Given that in our study the intervention group had higher mean BMI and lower aerobic fitness at baseline, the beneficial effects of the intervention on these parameters can be considered as more significant. Second, as only limited number of completed parental questionnaire were sent back to the research group we were not able to properly analyze and present these results. Parental nutrition knowledge and dietary habits have strong impact on the diet of their offspring [42, 43]. Therefore, it would have been better if we would be able to capture beneficial changes among the participants' parents. Third, dietary habits, one of the most important outcome of our work, were self-reported and therefore are subject to recall bias. However, currently questionnaire based surveys are still the most frequently used instruments to evaluate dietary habits [44], so we were not in a position to deliver objectively measured data in this regard. Finally, the

intervention in its current form is quite labour and resource intense which diminishes its potential to transfer the work for a different target group in other context. However, some elements needed less resources such as the distribution of the online educational materials; while others, like the developed education material, could be built into the regular school curriculum after slight modification to reduce the costs. The curriculum of the current nutrition intervention can also complement the national implementation of the EU School Fruit, Vegetables and Milk Scheme in which funding is available for educational measures [12]. Despite these limitations, this study certainly provides tools to include in future works in this area.

CONCLUSIONS

This work evaluated the impact of a whole school year nutrition intervention in Hungarian adolescents. Small beneficial changes in dietary knowledge and habits from baseline to post-intervention were observed which did not persist by the end of the summer holiday. In addition, remarkable increase was found in waist circumference and number of children with obesity in the control group during summer. These findings have important implications for practice as weight gain and unhealthy lifestyle changes during summer can undermine the beneficial effects of school-based interventions. Moving forward, experts need to identify ways to avoid unhealthy shifts in diet, physical activity and sleep during the summer break. In contrast, for the first time, significant improvements were detected in aerobic fitness of children after a nutrition-focused intervention. Future research may continue to study and validate this possible impact and to understand the underlying mechanisms.

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Authors' contribution statement

HT developed the protocol under the supervision of EM and organized and carried out the field work. HT also drafted the manuscript. EM supervised the data collection and coordinated the field work. VAK critically revised the manuscript. All authors had final approval of the submitted version.

Conflict of interest statement

The authors declare no conflict of interest.

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Social cognition deficit and genetic vulnerability to schizophrenia in 22q11 deletion syndrome

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Abstract

Introduction. 22q11.2 microdeletion syndrome (22q11DS) is associated with a 25% risk of psychotic onset.

Materials and methods. The sample consist of 120 subjects: 39 schizophrenics (SCZ); 20 siblings of schizophrenic patients (SIB); 34 22q11DS non-psychotic patients (DEL); 17 22q11DS psychotic patients (DEL_scz); 30 control subjects (CS). Social cognition was evaluated with the awareness of social interference test. Intelligence Quotient (IQ) was calculated with Wechsler Adult Intelligence Scale. TASIT (Awareness of Social Inference Test) performance was analyzed via MANOVA, including IQ as covariate.

Results. Group and IQ showed significant effect ($p < 0.001$; $p = 0.037$). The only TASIT variables where IQ showed no effect were paradoxical sarcasm; sincerity; lie. In sincerity, CS group shows a better performance than both 22q11DS groups ($p < 0.05$). In paradoxical sarcasm and lie, CS group performed better than each clinical group ($p < 0.05$). Regarding lie, DEL group was worst also respect to SCZ group ($p = 0.029$).

Conclusions. Our results show a specific social cognition deficit in 22q11DS and schizophrenia.

Key words

- schizophrenia
- 22q11 deletion syndrome
- social cognition
- psychosis

INTRODUCTION

22q11.2 deletion syndrome, also known as velocardiofacial syndrome or DiGeorge syndrome, is the most common microdeletion in humans. This autosomal dominant deletion determines a syndrome which is expressed in 1:4000 live birth [1]. It has a 100% of penetrance but the phenotipic expression is highly variable. Indeed, with 180 characteristics associated, the syndrome is one of the most protean. Some of the most common manifestations of the 22q11DS are: facial dysmorphias, cardiovascular congenital abnormalities, palatal defects, thymus hypoplasia with primary immunodeficiency, ipoparathyroidism [2]. Organs and tissues affected have all origin during embryo development from neural crest cells [3]. The microdeletion of the band 11.2 in the chromosome 22, according to literature, gives a 25% risk of developing a psychotic disorder [4]. More recently, the prevalence of schizophrenia spectrum disorders in 22q11DS has been attested from 23% to 43%, depending on the study. In this deletion syndrome neurobiological factors seem to play a key role in determining psychosis onset, while other triggers, which have a prominent role in idiopathic schizophrenia, have in this case a minor part. Recognizing those factors in a simplified model of schizophrenia, respect to the complex multifactorial model of the disorder in the general population appears as a needful opportunity. During the last decade 22q11.2 deletion syndrome (22q11DS) has been studied as the best genetic and biological model of vulnerability to schizophrenia, which provides a unique method to unveil the etiopathogenesis of psychosis and to arrange new strategies of prevention. The first clinical documentation of the syndrome dates back to 1978 [2], but it is only from 1992, when the first report on psychiatric manifestations associated was published, that the attention of research is focused on cognitive and behavioural phenotypic aspects [5]. Studies on animal models showed that many of the deleted genes in 22q11DS are physiologically expressed during the cerebral development and are responsible for a correct neurogenesis. The abnormal brain maturation consequent to the aplodeficiency of those genes may be the biological cause of

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the behavioural, neurocognitive and psychopathological phenotype expressed [6, 7]. The neurocognitive profile has been well-defined and it appears highly variable both inter-individuals than during the life of a single subject. During infancy, motor delay and language difficulties are commonly observed [8]. In the school age learning disabilities are frequent. The cognitive functions more often compromised are mathematical reasoning [9] and visuo-spatial abilities [10]; attention, executive functions and working memory deficits have been frequently reported [11]. Most of the patients has a borderline cognitive delay (IQ from 70 to 84), while only one third has a moderate mental retardation [12]. Temperamental and behavioral typical aspects are social difficulties, impulsiveness or shyness [8]. Also the psychiatric phenotype of the syndrome has been now clearly described [13]; individuals have significantly higher incidence rate compared to general population for several mental disorders [14]. Previous studies indicated that up to one third of adolescent/young adults develops a schizophrenic or schizoaffective disorder [14].

Schizophrenia is considered the most severe of all psychiatric pathologies, occurring in 1% of the general population. It has a multifactorial pathogenesis. The genetic component is of evident importance: the risk of illness with a schizophrenic sibling is 8.5%, 13.8% with a parent and 36.6% when both parents are affected. The concordance rate in twins is 57.7% for the homozygous and 5.6% to 12% for the dizygotic. In second grade relatives of schizophrenics, the percentages are referred to range from 2% to 2.8% [15]. It is relevant that in 22q11DS psychotic symptoms and correlated manifestations, included neurocognitive profile, are not different from schizophrenia characteristics in general population: studies didn't find any difference in onset age, positive or negative symptoms and global functioning [16, 4]. It has been estimated that deletion 22q11.2 is responsible for 0.75% of schizophrenia cases in the general population [4]. However, mutations or polymorphisms in genes which map in the 11.2 band of chromosome 22 could contribute more widely to determine vulnerability to schizophrenia in general population.

Patients with 22q11 deletion syndrome (22q11DS) offers a homogeneous population with a genetic risk of schizophrenia whose study could help in identifying schizophrenia endophenotypes with better accuracy and validity. The deleted region could permit to detect the genes that might be involved in the neurodevelopmental and functional alterations that are risk factors for schizophrenia. Amongst the genes of the deleted region, several have been identified, some of which are present in a mutated form also in patients affected with schizophrenia. Reticulon 4 Receptor (RTN4R) encodes for a protein which inhibits axonal sprouting and is involved in neuronal plasticity [17, 18]. DiGeorge Critical Region gene 8 (DGCR8) it's a gene involved in regulating the genetic transcription through miRNA. DGCR8 deficiencies can cause alterations in the morphology of synapses in the prefrontal cortex [19], hippocampus [20], thalamocortical pathway [21, 22] and throughout the connections between the lateral thalamus and the amygdala [23]. Proline dehydrogenase 1 (PRODH) and catechol-O-methyltransferase (COMT) are enzyme-encoding genes involved with the metabolism of neurotransmitters [24]; in both cases the enzymatic deficiency leads to an increase in dopaminergic transmission. PRODH causes an indirect increase of dopamine as compensatory mechanism to glutamate deficiency, normally produced by proline metabolism [25]. COMT on the other hand is directly involved with biogenic amine degradation including dopamine. PRODH and COMT mutations have been correlated to the development of schizophrenia not linked to DiGeorge Syndrome. The reduced expression of these genes is also associated with negative symptoms and social withdrawal in schizophrenia [26].

Social cognition deficits are a well-known cognitive characteristic of schizophrenia and it is well established that social dysfunction is also a common feature of the 22q11DS profile [8]. Social cognition consists of a wide spectrum of functions that control social interactions with other people; it is the result of a set of mental operations organized in domains, Theory of Mind being the main one (ToM), which is the ability to comprehend other people's mental functions through deducing their states of mind [27]. Bora *et al*. found very interesting data through meta-analysis on the studies that inquired into the performance task on ToM in subjects at their first psychotic episode, high clinical risk and high genetic risk [28]. Results show a deficiency comparable to those of chronic patients for the first group, while the performance across the other two groups was intermediate between sanity tests and patients at their first episode. The other components of the social cognition construct are: social perception and social knowledge, as the abilities to understand society rules and roles and the nature of relationships between people and of goals that guide social interactions; attributional bias, or how people deduce the reasons of others' actions; emotional processing, as the way people recognize emotions. It has been observed that social cognition, usually considered as a whole, has an important role in quality of life in schizophrenia patients [29]. The Italian Network for Research on Psychoses [30] found through a network analysis that social cognition deficits are most of the core of schizophrenia, more than positive, negative, and disorganization symptoms. These results highlight the importance of social cognition interventions, such as social skills training, to improve outcome of schizophrenia patients.

The paradigm of social cognition has become ever more studying in 22q11DS [31]. Weinberger *et al.* [32] found more severe deficits in social cognition in psychotic respect to non-psychotic subjects. Jalbrzikowski *et al*. [33] observed a correlation between ToM and positive symptoms. However executive functions and global intellectual functioning could have an important role in social cognition deficit; it has been demonstrated that a basic dysfunction that implies a global intellectual and executive deficit, lead to a weak social cognition [34]. Several findings connected neurocognition and social cognition of 22q11.2 DS schizophrenic patients [31]. Facial emotion recognition deficit, apparently due to altered visual processing in 22q11DS have a key role in impaired social cognition as well [31].

The aim of the present study is to investigate social cognition deficit in an adult sample of 22q11DS patients, for the first time in literature comparing directly to schizophrenic patients and their siblings, in order to evaluate the role of this cognitive deficit in the genetic vulnerability to psychosis. Our aim is also to disentangle neurocognition deficit from social cognition performance, evaluating the sample also in general intelligence.

MATERIALS AND METHODS

Our sample consists of 140 subjects, consecutively enrolled in Policlinico Umberto I, Sapienza University of Rome, divided in 5 groups: schizophrenic patients negative for 22q11DS (SCZ, N = 20); siblings of schizophrenic patients (SIB, N = 20); 22q11DS subjects with no diagnosis of psychosis (DEL, N = 34); 22q11DS patients with diagnosis of psychotic disorder (DEL_scz, N = 17); control subjects (CS, N = 30). Patients were clinically monitored at our outpatients' services specialised in psychotic disorders and psychiatry disorders in 22q11DS. Healthy controls joined the study through word of mouth. All subjects signed an informed consent approved by Policlinico Umberto I Ethical Committee (Rome, Italy). Data from SCZ and SIB groups were also used in an Italian multicentric study conducted by Italian Network for Research on Psychoses. Diagnosis of psychotic disorder was made employing the structured clinical interview for DSM-IV – patient version (SCID-I-P). Genetic diagnosis was ascertained through fluorescent in situ hybridization (FISH). Exclusion criteria for SCZ group were: brain injuries; neurological disorders; substance abuse. Inclusion criteria for DEL group consisted in: age between 18 and 65 years; absence of psychotic symptoms; deletion of band 11.2 in chromosome 22 confirmed by FISH. Exclusion criteria for DEL group were: brain injuries; neurological disorders; substance abuse. Inclusion criteria for CS and SIB groups were age between 18 and 65 years. Exclusion criteria for CS and SIB groups were: diagnosis of psychiatric disorder in axis I or II; brain injuries; neurological disorders, substance abuse; other medical conditions. General intelligence was assessed in all subjects through IQ measurement by the Wechsler Adult Intelligence Scale (WAIS). For all schizophrenic patients, clinical information was obtained on positive and negative symptoms severity with Positive and Negative Symptoms Scale (PANSS) [34]. Social cognition was evaluated through the Awareness of Social Inference Test (TASIT) [35], which is a ToM test where is requested identification of thoughts, feelings, and intentions of characters of video vignettes, and consists of seven scales (positive emotions, negative emotions, sincere, simple sarcasm, paradoxical sarcasm, sarcasm enriched, lie), organized into three sections: emotion recognition; social inference (minimal); social inference (enriched).

Statistical analysis was conducted on IBM software SPSS (version 24). Differences between groups for continuous variables were calculated with ANOVA and post-hoc test were corrected for multiple comparisons. For categorical variables χ^2 test was used. TASIT performance was compared between groups by means of Multivariate ANOVA, entering in the model all test scales and as nuisance covariates gender, age and IQ. Correlation analysis between TASIT performance and PANSS scores was run with Pearson' partial correlation entering QI as nuisance covariate.

RESULTS

Regarding demographical characteristics *(Table 1)*, samples showed significant difference in mean age ($F = 16.183$; $p < 0.001$). SIB group was significantly older respect to each other group (SIB *vs* CS $p < 0.001$; SIB *vs* SCZ $p = 0.021$; SIB *vs* DEL_scz $p < 0.001$; SIB *vs* DEL $p < 0.001$). Moreover, SCZ group was older respect to DEL group ($p < 0.001$). Groups differed also in gender composition ($X^2 = 14.543$, $p = 0.006$), as CS group had a higher proportion of females. Anova analysis for IQ showed significant differences between groups ($F = 16.854$ $p < 0.001$). Post-hoc analyses revealed a higher QI in CS group respect to the others (CS *vs* SIB $p = 0.015$; CS *vs* SCZ $p < 0.001$; CS *vs* DEL_scz $p < 0.001$; CS *vs* DEL $p < 0.001$). SIB had higher mean IQ level respect to DEL_scz group ($p = 0.009$). No differences in mean IQ was observed between clinical groups SCZ, DEL and DEL_scz *(Table 1)*. No differences were observed in PANSS scores or illness duration between DEL_scz and SCZ groups *(Table 1)*.

Analysis of TASIT performance revealed that the

Table 1
Socio-demographical and clinical characteristics of the sample

Variables	CS (n = 30)		SIB (n = 20)		SCZ (n = 39)		DEL_scz (n = 17)		DEL (n = 34)		Statistics	*p*
Sex (%)	M	F	M	F	M	F	M	F	M	F		
	30	70	70	30	56.4	43.6	76.5	23.5	67.6	32.4	$\chi^2 = 14.543$	0.006*
Age (mean ± sd)	28.9 ± 7.5		42.7 ± 12.9		35 ± 9.9		27.5 ± 6.7		24.3 ± 6.9		F =16.183	< 0.001*
IQ (mean ± sd)	113.5 ± 10.8		99.4 ± 8.8		90.4 ± 18		82.5 ± 19.6		89.1 ± 15.1		F =16.854	< 0.001*
PANSS pos					13.4 ± 4.2		14.2 ± 6.1				t = -0.525	0.602
PANSS neg					20.1 ± 7.3		16.8 ± 6				t = 1.637	0.107
PANSS gen					35.2 ± 8.1		32.3 ± 8.9				t = 1.087	0.282
DoI (mean ± sd)					10.6 ± 8		7.1 ± 5				t = 1.33	0.187

CS: control subjects; SIB: siblings of schizophrenic patients; SCZ: schizophrenics; DEL_scz: psychotic patients; DEL: non psychotic patients.
Sd: standard deviation; IQ: intelligent quotient; pos: positive symptoms; neg: negative symptoms; gen: general psychopathology. *statistical significance.

model corrected for nuisance covariates was statistically significant for all TASIT scales (Wilks' Lambda 0.516; F = 12.948; p < 0.001). Group (Wilks' Lambda 0.379; F = 3.825; p < 0.001) and IQ (Wilks' Lambda 0.869; F = 2.07; p = 0.037) variables showed a significant effect. Group effect was significant for all TASIT scales: positive emotions (F = 6.243; p < 0.001; partial Eta^2 = 0.159); negative emotions (F = 8.2017; p < 0.001; partial Eta^2 = 0.199); emotion recognition (F = 10.258; p < 0.001; partial Eta^2 = 0.237); sincere (F = 3.224; p = 0.015; partial Eta^2 = 0.089); simple sarcasm (F = 11.081; p < 0.001; partial Eta^2 = 0.251); paradoxical sarcasm (F = 17.495; p < 0.001; partial Eta^2 = 0.346), social inference (minimal) (F = 17.945; p < 0.001; partial Eta^2 = 0.352); sarcasm enriched (F = 5.779; p < 0.001; partial Eta^2 = 0.149); lie (F = 10.9; p < 0.001; partial Eta^2 = 0.248); social inference (enriched) (F = 15.853; p < 0.001; partial Eta^2 = 0.325). Age showed a significant effect on the following TASIT scales: paradoxical sarcasm (F = 4.166; p = 0.043; partial Eta^2 = 0.031); social inference (minimal) (F = 5.199; p = 0.024; partial Eta^2 = 0.038); sarcasm enriched (F = 6.903; p = 0.010; partial Eta^2 = 0.050). IQ presented a significant effect on the following TASIT scales: positive emotions (F = 6.320; p = 0.013; partial Eta^2 = 0.046); negative emotions (F = 5.825; p = 0.017; partial Eta^2 = 0.042); emotion recognition (F = 9.274; p = 0.003; partial Eta^2 = 0.066); simple sarcasm (F = 4.381; p = 0.038; partial Eta^2 = 0.032); social inference (minimal) (F = 4.586; p = 0.034; partial Eta^2 = 0.034); sarcasm enriched (F = 10.357; p = 0.002); social inference (enriched) (F = 7.641; p = 0.007).

Post-hoc were conducted for TASIT scales were a significant effect of group was found, without the effect of other nuisance covariates.

CS group showed a significantly better performance in sincere scale of TASIT respect to DEL and DEL_scz groups (respectively p = 0.015; p = 0.049) *(Figure 1)*.

In paradoxical sarcasm scale SIB and CS groups had significantly higher score than SCZ, DEL and DEL_scz groups (CS *vs* SCZ p < 0.001; CS *vs* DEL p < 0.001; CS *vs* DEL_scz p < 0.001; SIB *vs* SCZ p < 0.001; SIB *vs* DEL p < 0.001; SIB *vs* DEL_scz p < 0.001) *(Figure 2)*.

For lie scale a significant better performance of SIB and CS group was observed respect to the other groups (CS *vs* SCZ p = 0.025; CS *vs* DEL p < 0.001; CS *vs* DEL_scz p < 0.001; SIB *vs* SCZ p = 0.026; SIB *vs* DEL p < 0.001; SIB *vs* DEL_scz p < 0.001). Moreover, the SCZ group had a significantly higher score respect to DEL group (SCZ *vs* DEL p = 0.029) *(Figure 3)*.

Partial correlations in SCZ group showed a negative correlation between PANSS Positive Symptoms subscale and Sarcasm Enriched TASIS scale (r = -0.337; p = 0.038) and significant negative correlations between PANSS Negative Symptoms subscale and the following TASIT scales: positive emotions (r = -0.363; p = 0.025); negative emotions (r = -0.482; p = 0.002); emotion recognition (r = -0.557; < 0.001); paradoxical sarcasm (r = -0.438; p = 0.006); social inference (minimal) (r = -0.429; p = 0.007); lie (r = -0.451; p = 0.004); social inference (enriched) (r = -0.449; 0.005). A significant negative correlation was found in DEL_scz group between PANSS Negative Symptoms subscale and social inference (enriched) TASIT scale performance (r = -0.525; p = 0.037). Moreover, in this group, PANSS General Psychopathology subscale score showed a significant negative correlation with TASIT lie scale performance (r = -0.561; p = 0.023).

DISCUSSION

The sample consisted of 140 subjects, divided in 5 groups: 30 healthy subjects with no psychiatric diagnosis, genetic predisposition or familiarity for these conditions (CS); 20 first grade relatives of schizophrenic patients (SIB); 39 patients affected with schizophrenia, non-carriers of the 22q11.2 chromosome microdeletion (SCZ); 34 subjects with 22q11.2 deletion syndrome with no psychotic symptoms (DEL); 17 subjects with 22q11.2 deletion syndrome and diagnosed with a psychotic disorder (DEL_scz).

The family members group is the one with the highest average age, being partially composed of schizophrenic patients' parents (4 subjects out of 20). The SCZ group, having in average 11 years duration of illness, resulted significantly older than DEL, consisting of patients who came under our observation during their early-late adolescence in order to assess risk factors and plan the psychotic onset prevention. No significant age gap has been observed between SCZ and DEL_scz groups.

Besides a slight majority of female subjects in the control group, no significant gender differences have been observed within the groups.

A statistically relevant IQ gap between CS and the other three clinical groups has been observed, in line with literature. However the CS group generally shows a significantly higher IQ compared to the SIB group too; this information could be interpreted with the presence of an intermediate cognitive phenotype in family members in comparison to their schizophrenic relatives. The SIB group also shows a higher IQ than DEL_scz patients, which could be due to a particular severity of cognitive retardation in psychotic patients affected with deletion. These results are coherent with literature. There are no relevant IQ gaps amongst the three clinical groups (SCZ, DEL, DEL_scz). IQ has been taken into account in analyzing TASIT performance differences, with the purpose of discerning the global deficiency of neuro-cognitive skills observed in clinical groups from the specific social cognition deficiency that our study aims to demonstrate being associated to psychosis genetic vulnerability. IQ corrected MANOVA results highlight a significant difference in performance for the following TASIT scales: sincerity; paradoxical sarcasm; lie. The control group shows a significantly better performance in recognizing Sincerity compared to 22q11.2 DS affected patients, regardless of psychosis diagnosis. For TASIT sincere clips, where congruence exists between what the actors are literally saying and the paralinguistic and facial cues, in a previous article [29], schizophrenic subjects do not show differences compared to the control group. Our results show that this specific ToM alteration is peculiar to patients affected with DiGeorge Syndrome. Our hypothesis is that this deficiency is solely observed in DEL groups of the study as it is strictly dependent on social perception anomalies

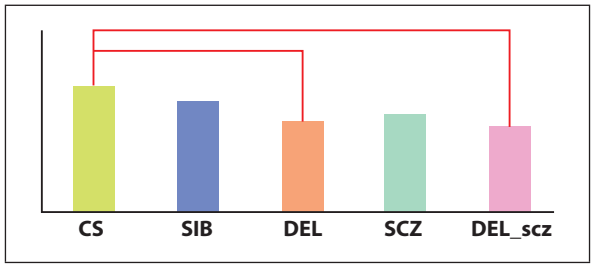

Figure 1
Mean score in TASIT sincere scale for each group. Red lines show significant post-hoc comparisons.
TASIT: Awareness of Social Inference Test; CS: control subjects; SIB: siblings of schizophrenic patients; DEL: non psychotic patients; SCZ: schizophrenics; DEL_scz: psychotic patients.

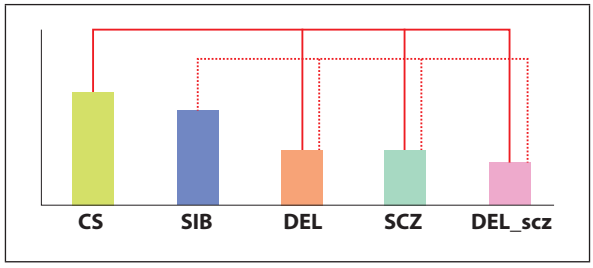

Figure 2
Mean score in TASIT paradoxical sarcasm scale for each group. Red lines show significant post-hoc comparisons.
TASIT: Awareness of Social Inference Test; CS: control subjects; SIB: siblings of schizophrenic patients; DEL: non psychotic patients; SCZ: schizophrenics; DEL_scz: psychotic patients.

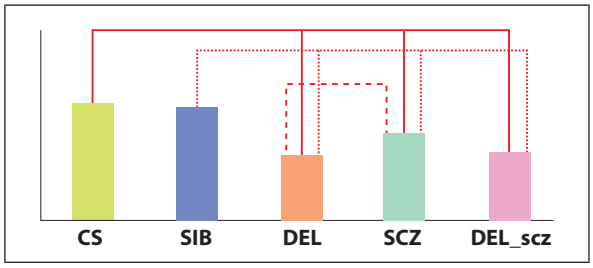

Figure 3
Mean score in TASIT lie scale for each group. Red lines show significant post-hoc comparisons.
TASIT: Awareness of Social Inference Test; CS: control subjects; SIB: siblings of schizophrenic patients; DEL: non psychotic patients; SCZ: schizophrenics; DEL_scz: psychotic patients.

linked to difficulties in processing faces already demonstrated for 22q11.2 deletion syndrome [36].

To evaluate paradoxical sarcasm, some simple scenes are used which only acquire sense if the patient is able to perceive the sarcasm involved in the dialogues (wherein there is no correspondence between what the actors say and what they refer to through their paraverbal language and facial expressions). On this TASIT scale, control and family members groups scored significantly higher than all three clinical groups. Whilst schizophrenic patients' family members perform similarly to healthy subjects, schizophrenic patients groups, both 22q11.2 DS carriers and non-carriers, and 22q11.2 DS patients not affected with psychosis, performed significantly worst in this socio-cognitive function compared to healthy subjects. Hence the recognition of paradoxical sarcasm is particularly compromised in both 22q11.2 DS and schizophrenia, and it might be related to genetic causes of schizophrenia. In comprehending lies, coherently, both control and sibling groups performed significantly better than the other three groups. The SCZ group shows in addition a significantly better performance compared to 22q11.2 DS non-psychotic patients. This result suggests that this specific deficit is representatively prominent in 22q11.2 deletion syndrome, regardless of a diagnosis of schizophrenia.

There are no studies in literature that compare, for socio-cognitive skills, patients affected with schizophrenia or 22q11.2 DS subjects with healthy subjects and first grade family members of schizophrenic patients. Our study is the first in literature to compare all these categories. Resuming our results found specific deficit in interpreting sincere social situations in 22q11DS. Instead, deficit in understanding situation in which people express with paradoxical sarcasm or lying is shared among schizophrenia and 22q11DS. It appears that having both conditions worst this kind of ToM and social perception deficit. We can argue then that this deficit is not depending from a general intelligence gap respect to controls. Other studies found a lack of correlation between IQ and emotion recognition in the 22q11DS, while it was observed in individuals with other developmental disorders such as ASD [37]. However we expected a such deficit, if on a genetic basis, to be present also in SIB group. This could be due to the fact that our SIB group it's composite most of parents, so adult people that had not expressed the psychosis phenotype and evidently not carrying the same risk factors of 22q11DS. In this sense, social cognition deficit appear to be associated with the genetic risk to schizophrenia linked to mutations of genes in the 22q11.2 band. Recently, Antshel *et al*. [38] found that deficits in emotion recognition, in addition to other cognitive functions such as set shifting and reading decoding, were present before the transition to prodromal/overt psychosis in 22q11DS group. Another study found that individuals with 22q11DS showed lower abilities than healthy controls to correctly recognize facial emotions. Authors suggested this difference could be due to abnormal faces recognition in 22q11DS. Studies employing eyetracking infact have consistently shown different patterns of visual exploration during face-processing tasks: compared to typical and idiopathic developmentally delayed control groups, patients with 22q11DS were shown to spend less time on the eyes and more time on the mouth or the nose when examining faces [39]. Moreover they found impairments of specific component of cognitive ToM (*i.e.*, perspective-taking abilities). They interpreted the results as the perspective-taking abilities might have been influenced by higher-order cognitive difficulties, as perspective-taking was shown to engage working memory or cognitive control processes.

The systematic application of tests investigating Social Cognition may contribute to the diagnostic phase

and enable the monitoring of the effect of a rehabilitating intervention.

Performing longitudinal evaluation studies of socio-cognitive skills, in deletion patients, could prove useful to identify possible clinical predictive markers more susceptible to the development of disorders in the schizophrenia spectrum. Because 22q11.2 DS patients are studied since childhood, researches in this field could identify markers that may have a predictive function of the future course of the schizophrenia [33]. In that sense, negative and positive symptoms predictors were studied; the most viable marker for the negative symptoms seems to be impairment of executive functions, while for the positive symptoms it would be the impoverishment in social cognition, particularly ToM alterations, which could build the foundations for the development of interpretative aspects of reality that can trigger delusional symptoms.

Regarding patients affected with schizophrenia but not 22q11 deletion carriers, in literature there are several studies highlighting a correlation between ToM alterations and the insurgence of positive symptoms, whilst no correlation with negative symptoms has been found. The presence of this set of data in literature can serve as a support to the hypothesis of a possible causal correlation between ToM and the subsequent development of psychotic disorder with a prevalent delusional component in the symptomatology.

Our study showed that in SCZ group PANSS positive symptoms subscale shows a negative correlation with sarcasm enriched TASIS scale while PANSS negative symptoms subscale had significant negative correlations with the following TASIT scales: positive emotions; negative emotions; emotion recognition; paradoxical sarcasm; social inference (minimal); lie; social inference (enriched). In DEL_scz group a significant negative correlation between PANSS negative symptoms subscale and social inference (enriched) TASIT scale performance was found. Moreover, in this group, PANSS general psychopathology subscale score shows a significant negative correlation with TASIT lie scale performance. A poorer performance in social cognition is hence associated with more severe negative psychotic symptoms. We may infer that patients with more severe negative severe symptoms tend to be more isolated socially and suffer affective flattening, leading to impaired ability at tuning with other people's emotions and inner state. Nevertheless, given the previously discussed data regarding deficiencies in social cognition in both non-psychotic deletion patients and on a qualitative degree in first grade family members of psychotic patients, it is possible to hypothesize a primary deficiency in social cognition, which leads to the deterioration of social functioning, even before the onset of schizophrenia. Psychotic symptoms will then probably aggravate those deficiencies.

CONCLUSIONS

Our results showed a specific deficit, not influenced by general intelligence impairment, of social cognition in 22q11DS and in schizophrenia, both idiopathic that 22q11DS-linked. Non-psychotic 22q11DS subjects showed similar severity in social cognition deficit to those with schizophrenia. It is possible to argue that genetic alterations in 22q11DS determine a social cognitive deficit, that is more frankly evident after psychosis onset. Social cognition deterioration could be considered an endophenotype of schizophrenia, linked to the genetic etiology of the illness.

Limitation of the present study are the small sample size and the cross-sectional design. Other limits consist in the absence of relevant clinical data, such as treatment and pharmacological interventions. Future study should examine longitudinally neurocognitive functioning of 22q11DS population.

Conflict of interest statements

The authors declare to have no conflicts of interest to disclose.

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Application of effect-based methods (EBMs) in a river basin: a preliminary study in Central Italy

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Abstract

Introduction. Effect-based methods (EBMs), i.e. *in vitro* and *in vivo* bioassays, represent innovative tools for the effect detection of environmental chemical pollutants on living organisms. The aim of this study was to evaluate the water quality of a river ecosystem implementing two *in vivo* bioassays on target freshwater animal species: the crustacean *Daphnia magna* and the small fish *Danio rerio*, also known as zebrafish.

Materials and methods. The methods applied in this study, i.e. the *Daphnia* sp. Acute Immobilisation assay and the Fish Embryo Acute Toxicity (FET) test, are commonly used in water quality research and their application in short-term ecotoxicity detection is suggested by recent European projects. Two sampling sites were chosen in the urban part of the Tiber River in Rome, while a third one was chosen as a reference site in the Farfa River, a tributary upstream of the city. The sites in the Tiber River are potentially affected by different pollution sources, including urban and industrial wastewater discharges, the pesticide release, livestock waste products, and waste dumps.

Results and discussion. The results of the study showed wide differences between the two applied bioassays. The FET test was generally more sensitive in detecting even low effects in all the water samples, but the strongest statistically results were observed with the *D. magna* Acute Immobilisation test. The results of this research confirm the effectiveness of EBMs in investigating and monitoring water chemical pollution, and stress the need for performing further studies, e.g. chemical analyses and other bioassays, to improve the knowledge of the health status of the Tiber River basin.

Conclusions. Further results will aim to support the local authorities in adopting measures to reduce and to eliminate the sources of chemical pollution in the study area.

Key words

- effect-based methods
- *Daphnia magna*
- zebrafish
- chemical pollution
- Tiber River

INTRODUCTION

The Water Framework Directive (WFD-2000/60/EC) commits the European Union (EU) Member States to ensure a good qualitative and quantitative status of all water bodies [1]. The chemical monitoring programmes of the WFD includes a list of different priority chemicals substances (Directive 2013/39/UE) and river specific pollutants that need to be constantly monitored in surface waters [2]. Despite the efforts of the EU to reduce the release of chemicals into the aquatic environment, new emerging pollutants and contaminant mixtures still make water pollution one of the main challenges across Europe [3].

The classical single-chemical risk assessment approach for the management of the chemical pollution in water bodies shows some limitations, as highlighted by recent European projects and monitoring networks [4, 5]. Analysing, detecting and quantifying all the substances in the aquatic environment is very challenging [3, 6]. Furthermore, the chemical pollutants present in the water bodies can combine themselves in mixtures whose effects may not be predictable on the only basis of chemical analyses. However, it is fundamental to investigate the effects of living organisms exposure to pollutants released in water ecosystems in order to understand the impact of chemical pollution on the aquatic biodiversity. It is also necessary to link the observed effects with the cost-effective management objectives. Therefore, in the context of the WFD, a specific activity was foreseen for the elaboration of a technical report [7] on aquatic effect-based tools (e.g. bioassays, biomarkers). As mentioned in the report of the WFD, these tools can be used as it follows:

- as screening tools to aid in the prioritisation of analysis of water bodies;

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- to establish early warning systems;
- to take into account the effects of chemical mixtures or chemicals that are not analysed (e.g. to support investigative monitoring when the reason why the decline of specific species is unknown);
- to provide additional support in water and sediment quality assessment.

In this WFD report, the adoption of apical short-term ecotoxicity bioassays, i.e. fish embryo toxicity and immobilisation of *Daphnia* sp., is recommended [4, 7]. In this research, the southern part of the Tiber River basin was chosen as a case study for a preliminary investigation of the chemical pollution effects in a water ecosystem particularly important from a human perspective. Indeed, the Tiber River is the third-longest river in Italy and the main watercourse in Rome. It rises in the Appennine Mountains in the Emilia-Romagna Region (Fumaiolo Mountain, 1407 m above s.l.) and it flows for 405 km to the southwest through Umbria and Lazio Regions towards the Tyrrhenian Sea. The river represents the largest basin in Central Italy with a drainage area of 17 375 km^2. The total human population living in this geographic area is approximately 4.7 million people. The plan of the Tiber River Basin Authority (TRBA) reports a large land agricultural use of the basin covering about 53% of the surface, while approximately 39% is forested and 5% is urbanised [8]. Thus, it can be assumed the presence of several chemical pollutants, also combined as contaminant mixtures, affecting its entire watercourse, even at very low concentrations. Environmentally hazardous concentrations of certain priority substances, e.g. heavy metals, nonylphenols, polycyclic aromatic hydrocarbons (PAHs), were found both in water and in sediments of the urban stretch of the Tiber River [9-11]. Chemical pollutants widespread into the watercourse represent an ecological risk for the river ecosystem and for the coastal area of the Tyrrhenian Sea, where the Tiber River flows. Previous studies found different organophosphate (OPPs) pesticides in the river and in its estuary, even if mostly in lower concentrations than the guideline values [12]. Moreover, other environmental pollutants were found in the last stretch of the river. For instance, high concentrations of pharmaceuticals, such as the hydroxy-metabolite of the mood-stabilising drug carbamazepine and the non-steroidal anti-inflammatory drug diclofenac, PAHs, pesticides, perfluorinated and polyfluorinated alkyl substances (PFAS), were detected [13]. The occurrence of antibiotics was also recorded [14]. The data of the local environmental agency obtained following the current legislation show that some priority substances, e.g. nickel, exceed the environmental quality standards (EQS) in the Tiber River [15]. Considering the high number of chemical pollutants that may be found in this river basin, and the resulting effects on living organisms, the use of EBMs appears to be a low-cost and sustainable opportunity for the water ecosystem management.

Aim of the study

The purpose of the present study was to apply two effect-based methods (EBMs), i.e. *Daphnia* sp. Acute Immobilisation assay and Fish Embryo Acute Toxicity (FET) test, with a view to investigating the effects of chemical substances in the last stretch of the Tiber River basin on two target freshwater model organisms: the crustacean *Daphnia magna* and the small fish *Danio rerio*, otherwise known as zebrafish. Each of these species can respond to the environmental pollution differently since they belong to separated trophic levels. Therefore, the implementation of bioassays on different organisms allows achieving a better overview of the effects of chemical pollution on water ecosystems. The bioassays used in this research represent common applied experimental procedures in water quality studies. Moreover, the use of these model organisms for environmental monitoring purposes is encouraged by recent European projects [4]. The general aim of this study was to support the effect-based evaluation of the quality status of the urban area of the Tiber River in the city of Rome and one tributary.

MATERIALS AND METHODS

Study area and sampling

The study area was located along the Tiber River basin in the Lazio Region, comprising two different sites along the main watercourse and one tributary *(Figure 1)*. The three selected sites were characterised by different types and levels of pollution, reflecting different anthropogenic pressures, potentially including drift of pesticides, urban and industrial wastewater discharges, waste dumps, and livestock waste products [15]. The southern part of the Tiber River reflects all the contamination sources affecting the river along its stream. Castel Giubileo (CG) is located in the North of Rome (upstream of the city): this site is likely affected by leaching of waste, agricultural and zootechnical discharges as well as the presence of small and medium factories. Mezzocammino (MC) is located in the South of Rome (downstream of the city), about twenty kilometers from the estuary: this site collects the urban discharges of the city of Rome and it is located right after a sewage treatment plant. Farfa River (FA) is a stream that flows into the Tiber River at around fifty kilometers before Rome: it was chosen as a reference site for its good ecological status according to the demands of the WFD [16, 17]. Two sampling campaigns were conducted in these sites: the first was performed during the summer of 2018, while the second was carried out during the summer of 2019. Water samples (2 L) were collected in each site between 0 and 20 cm from the surface and then they were quickly stored at +4 °C. Each sample was filtered at 0.45 µm in order to remove the suspended materials.

Instrumentation

The observations of the daphnids and the photographs of representative zebrafish individuals were performed using a Leica S8AP0 stereo microscope linked to a Basler acA 1300-60 gm camera. The images were acquired thanks to the software Media Recorder™ 4.0 provided by Noldus Information Technology.

Laboratory analyses

Analyses were carried out by the Laboratory of Ecotoxicology of the Unit of Ecosystem and Health of the Italian Institute of Health (Istituto Superiore di Sanità, ISS, Rome). The Laboratory works in quality according

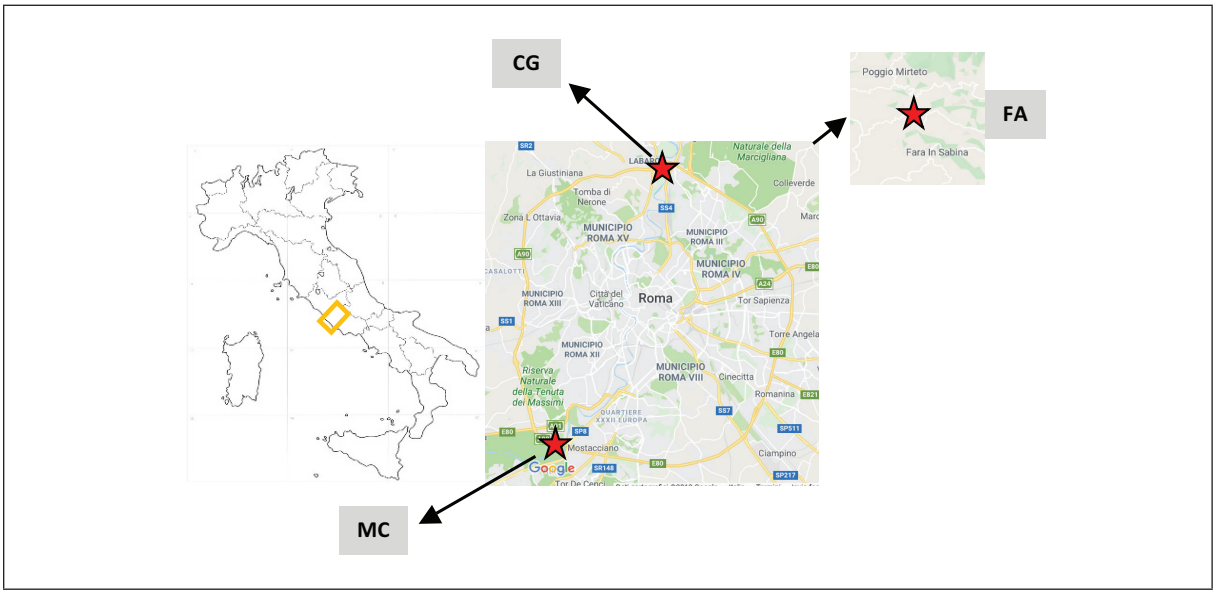

Figure 1
Map of the sampling sites in the study area.
CG: Castel Giubileo; FA: Farfa River; MC: Mezzocammino.

to the UNI CEI EN ISO/IEC 17025 standard [18] and participates in different national and European inter-laboratory comparisons.

Daphnia sp. Acute Immobilisation assay (OECD 202:2004)

The *Daphnia* sp. Acute Immobilisation assay is a screening method using freshwater crustacean daphnid species. In this study, the test was performed starting from the resistant forms of *D. magna*, i.e. ephippia, that were included in the kit named as Daphtoxkit® and developed by the Laboratory for Environmental Toxicology and Aquatic Ecology (LETAE) at the University of Ghent, in Belgium. The experimental procedure application followed the OECD No. 202:2004 guideline [19]. The tests lasted 48 hours. All the physicochemical parameters were measured at the start of tests and after 48 hours. Six independent tests were performed, one for each sample. Three tests were carried out during 2018, while the other three during 2019. Each test was performed in three replicates. Twenty daphnids per sample (no longer than 24 hours after the hatching) were exposed in multi-well plates and they were incubated at 21 ± 1 °C in the dark. Each well contained five daphnids in 10 mL of the water sample. A control was performed exposing twenty daphnids to the test medium. At the end of the experiment, the number of immobilised individuals was recorded. Daphnids were considered immobilised if no directed movement was observed within 15 s after gentle stirring.

Fish Embryo Acute Toxicity (FET) test (OECD 236:2013)

Wild type zebrafish embryos were used to perform the analysis. The bioassay was conducted according to the OECD No. 236:2013 guideline [20]. The embryos were collected from the breeding groups at the Laboratory of Ecotoxicology of the ISS. Breeding fish were maintained in tanks with a loading capacity of 1-L water per fish at 26 ± 1 °C and with a fixed photoperiod of 12:12 (light:dark). Six independent tests were performed, one for each sample. Three tests were carried out during 2018, while the other three during 2019. Each test was performed in two replicates. Zebrafish eggs were exposed in 24-well plates at a developmental stage ranging from 32 to 128 cells of segmentation. Each well contained one egg in 2 mL of the sample. A plate control and internal control were prepared with the test medium. Embryos were kept in dark conditions for four days at 26 ± 1 °C. The morphological observations of the embryos were made at 96 hours post fertilisation (hpf). Four apical observations were recorded as lethal endpoints indicating acute toxicity: coagulation of the embryo, non-detachment of the tail, lack of somite formation, and lack of heartbeat. Sublethal endpoints were also recorded in order to improve the evaluation of the sample toxicity with an enhanced level of detail. The investigated sublethal endpoints were spine deformation, hatching delay, general underdevelopment, absence of pigmentation, eye deformation, tail deformation, fin deformation, low heartbeat, head skeleton malformation, edema.

Statistical analysis

The experimental data were analysed performing non-parametric tests. The Kruskal-Wallis test allowed the comparison among the sampling sites in the two different years (2018 and 2019) and the different time pattern showed by the Delta (values of 2019 – values of 2018). Moreover, the average and the standard deviation were calculated where possible. The statistical analysis was performed with the aid of the SAS® software. Please, see the *Supplementary materials* available on line for a detailed description of the statistical analysis and the related boxplots.

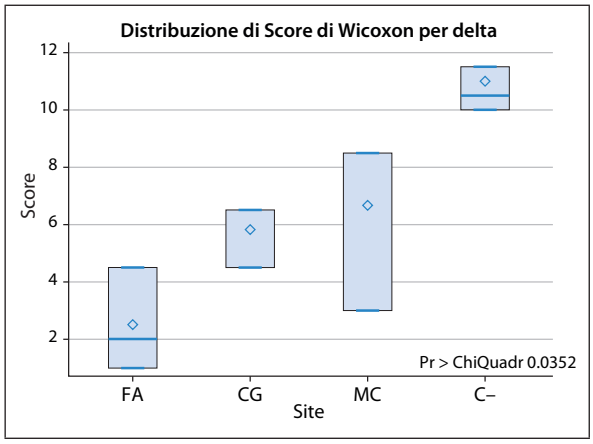

Figure 2
Wilcoxon Score Distribution showing the different time pattern (values of 2019 – values of 2018) indicated by the *delta*. Significance is expressed as Pr > Ch-square and it has be equal to ≤0.05. FA: Farfa River; CG: Castel Gandolfo; MC: Mezzocammino; C-: negative control.

RESULTS AND DISCUSSION

All the test results met the validity criteria in both the bioassays. Overall, the results showed toxic effects, i.e. lethal and sublethal effects, in the three sampling sites with differences between the applied bioassay and between the sites. However, the acute toxicity was generally weak, i.e. low mortality rate, for the organisms employed in this study. The *D. magna* Acute Immobilisation assay showed a significant difference between the results observed from the samples of 2018 and those of 2019 (*Figure 2*). Based on the average of the results, only the sample FA1 showed significant toxic effects (over 20% of the effect percentage) for the daphnids (*Table 1*). The results of the FET test did not show a significant difference between the samples of 2018 and those of 2019. However, this bioassay detected both lethal and sublethal effects. Indeed, the FET test is able to detect toxic effects even at low concentrations of chemical substances [5, 21]. Only the samples FA1 and MC1 showed significant toxicity (over 20% of the effect percentage) for zebrafish embryos (*Table 2*).

Surprisingly, most of the effects were observed in the organisms exposed to the tributary Farfa River. A possible explanation for the difference of the effects emerged in the Tiber River samples and the Farfa River samples could be related to the release of some pesticides into the tributary or to the use of some pharmaceuticals for veterinary purpose. Indeed, land exploitation for agricultural uses is carried out nearby the torrent, although this is limited to a restricted area. Other explanations could be sought in the presence of physical and chemical parameters in the samples that may have affected the development and survival of the tested organisms. Moreover, the low water flow rate of the Farfa River could amplify the effects of chemical pollutants because they could be more concentrated if compared with those released in the huge water flow of the Tiber River. Furthermore, other sources of pollution could be found in possible illegal wastes or in the presence of a dam upstream the site used for the abstraction of drinking water.

Daphnia *sp. Acute Immobilisation assay*

The results of *Daphnia* sp. Acute Immobilisation assay did not show any acute effect for the two Tiber River samples of MC and CG. The recorded percentage of mobility inhibition ranged between 0% and 20%, for both the campaigns (*Table 1*). The percentage of immobilised individuals recorded was closed to 0% as regards the second campaign (MC2 and CG2). However, the first sample of Farfa River (FA1) weakly affected the motility of daphnids showing an acute toxicity effect, ranging from 15% to 35%, while the second sample (FA2) was not toxic for the crustacean (*Table 1*). Samples that show values ranging between 20% and 50% are considered low toxic on the basis of the scale of toxicity used by Regional Environmental Protection Agency of Lazio – ARPAL. Statistical analysis revealed that the differences between the two campaigns are significant (*Figure 2*). However, considering the low amount of the data, these conclusions have to be treated very carefully and further analyses are needed. The results do not necessarily mean the absence of chemical pollution in the Tiber River since the *D. magna*

Table 1
Results of *Daphnia magna* Immobilisation Assay. The data represent the effect percentage calculated in each test for all the samples, including the negative control. Average and standard deviation are also reported. FA: Farfa River; CG: Castel Gandolfo; MC: Mezzocammino; C-: negative control.

Sampling	Site	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	μ	σ
2018	FA1	30	15	35	26.7	10.4
	CG1	10	10	15	11.7	2.9
	MC 1	5	20	5	10	8.7
	C-	3,3	0	0	1.1	1.9
2019	FA2	5	5	0	3.3	2.9
	CG 2	0	0	0	0	0
	MC2	0	0	0	0	0
	C-	0	0	0	0	0

Table 2
Results of FET Test with *Danio rerio*. The data represent the effect percentage calculated in each test for all the samples, including the negative control. Average is also reported. Lethal and sublethal were calculated separately. NA (i.e. not applicable) means that the negative control was recorded only once for both lethal and sublethal effects. FA: Farfa River; CG: Castel Gandolfo; MC: Mezzocammino; C-: negative control.

Sampling		Site	Test 1 (%)	Test 2 (%)	µ
2018	*Lethal*	FA	15	45	30
		CG	5	5	5
		MC	25	30	27.5
		C-	0	0	0
	Sublethal	FA	0	9	4.5
		CG	0	11	5.5
		MC	13	0	6.5
		C-	NA	NA	
2019	*Lethal*	FA	5	10	7.5
		CG	15	10	12.5
		MC	5	5	5
		C-	0	0	0
	Sublethal	FA	11	6	8.5
		CG	0	6	3
		MC	5	11	8
		C-	NA	NA	

model could not be sensitive to the specific chemical substances and concentrations dissolved into the Tiber River. Furthermore, most of the chemical substances might have been stuck to the organic material that was removed during the sample filtering [22]. The chemical analysis could help in explaining the obtained results and revealing the chemicals in the samples and their concentrations.

FA1 and FA2 showed different effects, ranging from a low degree of toxicity to the absence of toxic effects on development, survival, and motility. The different results obtained with the Farfa River samples in the two sampling campaigns could be due to specific seasonal environmental perturbations as well as different levels of chemical discharge into the watercourses or single pollution phenomena. For instance, the available rainfall data show that the summer of 2018 was much rainier than the summer of 2019, especially in the area of the Farfa River [23]. However, it interesting to notice that a recently published study [24] indicated that the ecological status in a small investigated portion of this stream should be considered as *moderate* instead of *good*, according to the definition provided by the WFD obligations. These data refer to the period of 2018. However, the same study states that the ecological status of the Farfa River is *good* overall. The positive results recorded in the tested samples of the first campaign were weak and contrasting with those emerged in the samples from the second campaign. Therefore, more samplings are needed to confirm or reject these data.

Fish Embryo Acute Toxicity (FET) test

Lethal and sublethal endpoints on zebrafish embryos were observed and recorded in all the tests. The mortality rate was ≤10% in the plate control and internal control. Overall, the number of adverse effects was higher in the samples coming from the summer of 2018. This difference between the two sampling campaigns was due to MC and FA, while the organisms exposed to CG samples did not show any relevant difference between the two investigated periods (*Table 2*). Mortality was observed in all the tested samples for each site. However, a minimum number of lethal effects can be due to the mortality rate characteristic of this species. Indeed, only the embryo mortality percentage greater than 10% is considered significant according to the guideline. FA1 and MC1 registered levels of lethality that may indicate weak toxicity. CG showed a slight difference in lethality between CG 1 and CG2. Sublethal endpoints were also recorded in all the samples (*Table 2*). These observations could reveal the presence of chemicals or chemical mixtures that may not be assessed with the only record of lethal endpoints. Sublethal effects occurred as spine deformation, delay or absence in the hatching and general underdevelopment, and they were recorded in all the samples (*Figure 3*). Specifically, the application of the FET test has been useful in several scientific studies to detect the effects of chemical pollution on living organisms in surface water bodies, even for substances at very low exposure levels [16, 25]. Moreover, the detection of sublethal effects can be linked to some

Figure 3
Sublethal effects detected by the FET test. All the images were obtained at 96 hpf. A) represents a normal developing hatched embryo; B) shows the spine deformity; C) is a non-hatched individual.

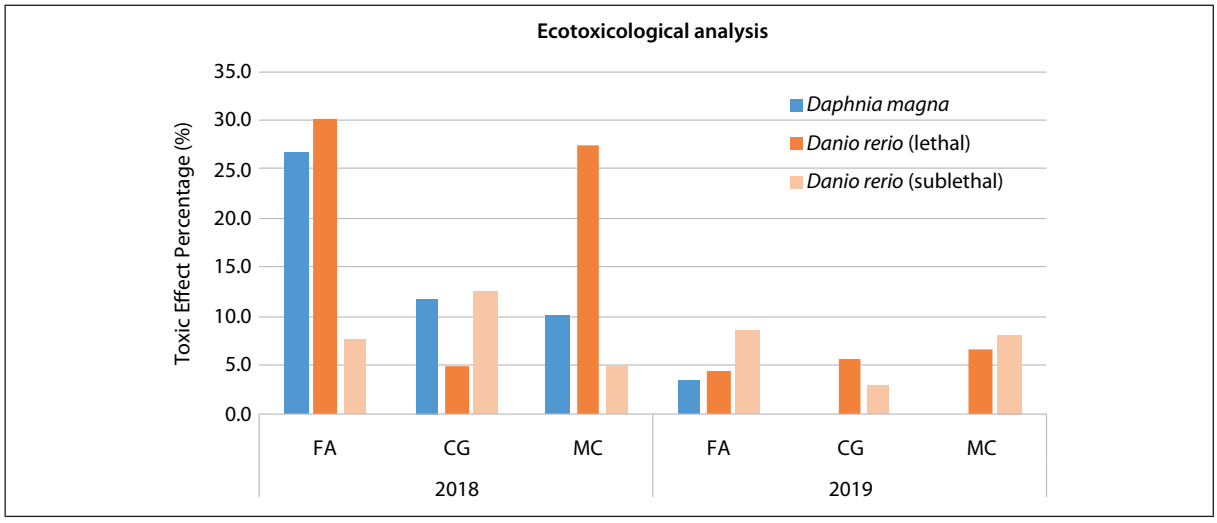

Figure 4
Results of the ecotoxicological analyses applied on three Tiber River basin samples (FA: Farfa, CG: Castel Giubileo, MC: Mezzocammino) in two sampling campaigns (2018-2019).

widespread classes of environmental pollutants and it allows the understanding of the main modes of action (MoAs) of these substances, e.g. DNA toxicity, neurotoxicity, developmental toxicity, cardio-circulatory toxicity [21]. The results of the test were congruent with those shown in *D. Magna* Acute Immobilisation assay, although very weak (*Figure 4*). The higher peaks of toxicity were registered in the organisms exposed to the Farfa River samples. The outcomes of the FET test also showed a certain level of toxicity in the site of MC as expected: this site is indeed located downstream to the city of Rome and it is affected by the pollution load typical for a big city, i.e. small enterprise discharges, waste pollution, personal care products, detergents, heavy metals. MC also receives the emissions of the urban wastewater treatment plant. Further studies integrating other ecotoxicological bioassays and chemical analyses are needed to improve our knowledge about the state of health of the study area.

CONCLUSIONS

Several activities have arisen along the banks of the Tiber River during the centuries, and this water ecosystem has been always relevant for its socio-economic activities and public health. Moreover, the Tiber River could be used in the future as a reservoir for drinking water in the city of Rome, especially considering the global climate change effects, e.g. water scarcity [26, 27]. Therefore, in this context, every new result on the water quality of this river ecosystem should be carefully considered. The effects detected with the FET test can play an important role in future chemical screening of the Tiber River. The sublethal effects detected in all the sampling sites could be due to some classes of environmental pollutants left off the list of the chemical substances included in the European and Italian legislations [28]. Specifically, future studies should explain which sources could be responsible for the water contamination in the Farfa River, and the outcomes should be considered by the policymakers for monitoring and protecting this important naturalistic area. Integrating EBMs, e.g. FET test and *D. Magna* Acute Immobilisation assay, into surveillance, operational and investigative monitoring for water quality management is fundamental to evaluate the hazards of the whole chemical substances widespread in the water bodies. Furthermore, EBMs may reveal risks for natural communities working as environmental early warning systems, as already mentioned by past studies on the Tiber River [29].

It would be essential to combine the morphological observations detected in this study with the methods taking into account different MoAs, e.g. mutagenicity and neurotoxicity, in order to increase the knowledge on the underlined toxicity mechanism. These MoAs have been commonly revealed by bioassays that used samples collected in several European rivers [30]. A better comprehension of the chemical pollution levels in the Tiber River basin, including emerging substances, e.g. pharmaceuticals and pesticides not included in the legislation, would be fundamental to prevent risks for human health when there is a reuse of water for agricultural, aquaculture and drinking purposes.

In conclusion, the recommendation of this study is continuing to apply ecotoxicological bioassays in studying the Tiber River basin in support to the chemical analysis foreseen by the WFD. Future screening of these environmental sites could be essential for better explaining the results of this study, and to identify which substances are potentially responsible for these effects.

Authors contributions

The co-authors had together contributed to the completion of this article. Specifically, it follows their individual contribution. Conceptualisation: WC and IL; methodology: WC, IL, KDD, Ma.Co.; validation: IL, MC; investigation: WC, IL, KDD, MaCo; data cura-

tion: IL, WC; writing – original draft preparation: WC; writing – review and editing: MC, IL, WC; supervision: LM. All authors read and approved the final manuscript.

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Conflicts of interest statement

The authors declare no conflict of interest.

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BRIEF NOTE

Identification of two novel LDLR variants by Next Generation Sequencing

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Abstract

Introduction. Familial hypercholesterolemia (FH) is an autosomal dominant inherited disease characterized by elevated plasma low-density lipoprotein cholesterol (LDL-C). Targeted Next Generation Sequencing (NGS) is a new opportunity to expand the existing pathogenic variants (PVs) spectrum associated to FH. Our aim was to report a diagnostic NGS-based approach to detect variants associated to FH.

Methods. We report two patients: a 48-year-old Asian woman, without known history of hypercholesterolemia and a 46-year-old Caucasian man, with childhood hypercholesterolemia.

Results. An effective NGS-based pipeline, *FH-Devyser kit/Amplicon Suite*, beginning from sequencing to data analysis, did not identify known PVs in the *LDLR*, *APOB*, *APOE, LDLRAP1*, *STAP1* and *PCSK9* genes, but revealed two *novel LDLR* variants (*c.1564A>T*, p.Ile522Phe and *c.1688C>T*, p.Pro563Leu).

Discussion and conclusions. This study showed that an effective NGS-based pipeline led to a definitive diagnosis in two FH families, allowing to plan their therapeutic treatment. Although the functional consequence of the two *LDLR* variants needs to be assessed *in vitro*, the *in silico* analysis and high preservation of the two amino acid positions observed in the LDLR protein, across different animal species, suggest that both variants are deleterious.

Key words

- next generation sequencing
- LDL-cholesterol
- FH-Devyser Kit
- SmartSeq
- *novel LDLR* variants

INTRODUCTION

Three canonical genes underlie autosomal dominant familial hypercholesterolemia (ADFH): low-density lipoprotein receptor (LDLR) (FH-type 1), apolipoprotein B (APOB) (FH-type 2) and pro-protein convertase subtilisin/kexin 9 (PCSK9) (FH-type 3). Other genes, like signal transducing adaptor family member 1 (STAP1) (FH-type 4) and apolipoprotein E (APOE) (FH-type 5) have been associated to some rare FH forms [1]. In addition, FH-like phenotypes, with a recessive transmission, are extremely rare. This group of disorders includes the classic autosomal recessive hypercholesterolemia (ARH) caused by mutations in the Low Density Lipoprotein Receptor Adaptor Protein 1 (*LDLRAP1*) gene [2].

Successful molecular diagnosis depends on the ability of the designated method to identify molecular lesion associated with FH [3]. Next Generation Sequencing (NGS) allows molecular diagnostic laboratories to increase sample throughput, to reduce the turn-around time and to analyse more disease-related genes simultaneously. As a result, recent studies demonstrated that NGS-based assays are very useful and at the same time cost effective for the genetic diagnosis of FH in primary care [4]. However, the main challenge to translate NGS in clinical practice is to develop a simple and robust diagnostics and bioinformatics pipeline, fulfilling the quality control requirements for clinical diagnosis.

We present an effective NGS-based pipeline, from sequencing to data analysis, which identified two unreported *LDLR* variants in two FH families.

CASES' PRESENTATION

Case A

Index case A is a 48-year-old Asian woman, without known history of hypercholesterolemia. During a rou-

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tine blood examination, high LDL-C levels (324 mg/dl) were revealed *(Table 1)*. Deeper insights on her family history disclosed two already deceased brothers from heart attack at the age of 48 and 54, respectively. Her parents' medical history remained unclear. No tendon xanthoma or corneal arcus were diagnosed during physical examination. The patient had no other cardio-vascular (CV) risk factors as smoking, diabetes and CHD history. Her body mass index was 21 kg/m^{2}and she was normotensive.

The patient's Dutch Lipid Clinic Network diagnostic criteria for FH score was 10, indicating a definite FH diagnosis. Therefore, we performed genetic evaluation, according with EAS/ESC guidelines [5]. Furthermore, her 13-year-old son and 14-year-old daughter, whose lipid profile *(Table 1)* was clearly indicative of FH, underwent genetic testing.

Case B

Index case B is a 46-year-old Caucasian man, suffering from hypercholesterolemia since his childhood, in absence of CV history. The patient complained about muscle pain and cramps due to statin therapy, which was, therefore, suspended for about a month. His blood examination showed high LDL-C levels (313 mg/dl), despite ongoing therapy with ezetimibe and fibrate *(Table 1)*. Family history revealed that his mother suffered from severe hypercholesterolemia, even if no familial CV events were reported. No tendon xanthoma or corneal arcus were diagnosed during physical examination. He did not show additional CV risk factors, such as smoking and diabetes, while he presented with a normal BMI of 22 kg/m^2 and normotensive status.

Considering that his Dutch Score was 9, which is related to a definite diagnosis of FH, according with EAS/ESC guidelines, the genetic evaluation was performed.

The two patients gave their written informed consent prior to blood sampling. Genomic DNA was isolated from peripheral blood by an automatic device (MagCore HF16 Plus, Diatech Lab Line, Jesi, Italy). We used 30 ng input DNA for the analysis with the NGS-based method (Devyser FH kit, Devyser, Hägersten, Sweden). All exons, the exon–intron boundaries, 5' and 3' untranslated regions of six FH-related genes (*LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *APOE* and *STAP1)* and polymorphisms, associated with polygenic FH form and statin treatment effects, were examined [6]. The design was based on the human (Hg19) reference genome to generate 200-bp amplicons by using 192 primer pairs designed and mixed in a single tube. The Qubit® 2.0 Fluorometer 8 (Life Technologies) was used to normalize the genomic DNA concentrations to 2 ng/µl. The final libraries were quantified on Qubit®, diluted to a concentration of approximately 7 pM and denatured to ensure the efficient use of the MiSeq Reagent Kit (Illumina, CA, USA).

MATERIALS AND METHODS

NGS pipeline and Sanger sequencing

Each NGS run consisted of eight FH samples loaded on MiSeq Reagent Kit v2 Nano cartridge, using 2 × 150-bp paired-end chemistry, according to manufacturer instructions. MiSeq-generated FASTQ files were downloaded and processed using Amplicon Suite software (SmartSeq s.r.l, Novara, Italy) providing integrated tools for the analysis, visualization and interpretation of NGS data. Proprietary algorithms based on coverage depth and uniformity were applied for copy number variation (CNV) detection in the *LDLR* gene. Sanger sequencing was performed with an Applied Biosystems 3500 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA). Sequence analysis was carried out with the SeqScape Software v2.5. The primers were designed by Primers 3 software (http://primer3.ut.ee/). Their sequences were: forward (F) 5'-ACTGGATCCACAGCAACATCT-3' and reverse (R) 5'-TGGGATTACAGGTGCTTTGAG-3' and (F) 5'-AGCTATTCTCTGTCCTCCCA-3' and (R) 5'-CTTCAGGGAGCAGCTTGG-3', able to amplify and sequence the *LDLR* exons 10 and 11, respectively.

Prediction of variant effects and species sequence alignment

Four different programs were used to predict pathogenicity of the two *LDLR* variants identified in this study: *Polymorphism Phenotyping version 2* (http://genetics.bwh.harvard.edu/pph2/), *Sorting Intolerant From Tolerant* (http://sift.jcvi.org/www/SIFT_enst_submit.html), *Mutation Taster* (http://www.mutationtaster.org) and *Provean* (http://provean.jcvi.org/index.php). In addition, multiple sequence alignment was created using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). The reference sequence used for LDLR protein was P01130.1 (SwissProt).

Table 1
Lipid profile of the two index cases and their family's members. The values beyond the normal reference ranges are highlighted in bold

Lipid fraction	Case A			Case B	Laboratory reference ranges* (mg/dl)
	Proband	Son	Doughter	Proband	-
Total cholesterol	**406**	**319**	**302**	**402**	< 200
HDL-C	66	50	51	73	> 45
Triglycerides	78	62	72	82	20-170
LDL-C	**324**	**258**	**237**	**313**	< 130
Apo-B	**145**	**169**	**167**	85	55-130

*target therapy ranges were instead defined according to ESC/EAS guidelines [5]

RESULTS

FH testing obtained by NGS did not reveal any known PVs in all genes investigated and no bioinformatics algorithms were suggestive for the presence of CNV in the *LDLR* gene. However, we found two *LDLR* variants (*c.1564A > T*, coverage: 325/643X, allele frequency: 50%, p.Ile522Phe) and *c.1688C > T*, coverage: 219/433X, allele frequency: 50%, p.Pro563Leu) in case A and B, respectively *(Figure 1)*. The nomenclature of the variants is based on the *LDLR* sequence (NCBI Reference Sequence: NM_000527.4; *GRCh37*), according to the recommendations of the Human Genome Variation Society (https://www.hgvs.org) *(Table 2)*. Sanger sequencing confirmed the presence of both variants on a second independent patient sample.

These variants were considered as *novel* since they were neither found in *ClinVar* (https://www.ncbi.nlm.nih.gov/clinvar/), *Ensemble Human Genome Mutation* (https://www.ensembl.org/index.html), *ExAC* (http://exac.broadinstitute.org) nor *1000G* (http://www.internationalgenome.org/1000-genomes-browsers/) browsers. In addition, among more than 200 alleles routinely analysed by NGS-based molecular screening, we did not identify the two variants in other FH or healthy individuals.

Other family members were not testable for the variant p.(Pro563Leu). Instead, we conducted family screening on two hypercholesterolemic subjects (the patient's sons) for the variant p.(Ile522Phe). Both subjects resulted to be carriers of the variant, providing evidence for his co-segregation genotype/phenotype.

In silico analysis of the p.(Ile522Phe) and p.(Pro563Leu) variants, performed with four different prediction tools, suggested that both *LDLR* variants are deleterious *(Table 1)*. In addition, the alignment of the amino acids in the LDLR protein across seven different animal species, ranging from *Homo sapiens* to *Ovis aries (sheep*), showed that two amino acids (in humans: Ile522, and Pro563) are highly preserved *(Figure 2)*.

DISCUSSION

FH diagnosis is usually based on clinical features, such as physical findings of tendon xanthomas or corne-

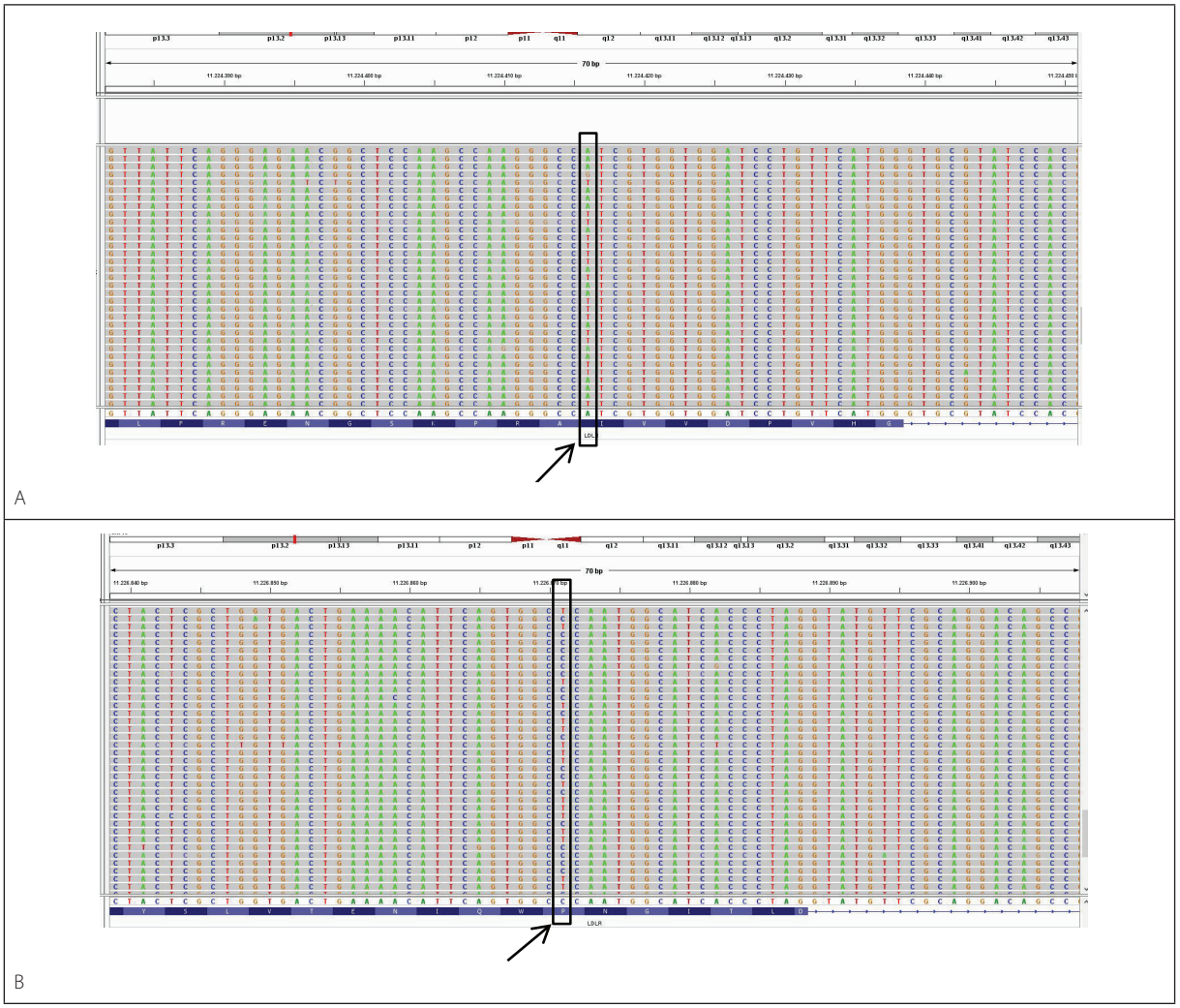

Figure 1
BAM files visualized by the Integrative Genomics Viewer show the reads associated to c.1564A>T, p.(Ile522Phe) (A) and c.1688C>T, p.(Pro563Leu) (B) with a coverage of 325/643X (variant allele frequency: 50%) and 219/433X (variant allele frequency: 50%), respectively. The arrows indicate the nucleotide position of the two LDLR variants.

Table 2
Identities of the two *LDLR* variants and *in silico* prediction of their effect at protein level. Both variants show high scores of pathogenicity for all four software used; following the ACMG guidelines, the *c.1564A>T* and the *c.1688C>T* can be classified as *likely pathogenic* variants

Transcript	Coding impact	HGVS coding	HGVS protein level	Location	Protein position	Splice distance	PolyPhen-2 (range: 0-1)	SIFT (cutoff = 0.05)	Mutation Taster	Provean (cutoff = -2.5)	ACMG classification*
NM_000527.4	M	*c.1564A>T*	I522F p.(Ile522Phe)	exon 10 of 18	206 of 228 (coding)	-23	Probably damaging (score = 0.990)	Damaging (score = 0.001)	Disease causing	Deleterious (score = -3.92)	**Likely pathogenic** (PM1+PM2+ PP1+PP3)
	M	*c.1688C>T*	P563L p.(Pro563Leu)	exon 11 of 18	102 of 119 (coding)	-18	Probably damaging (score = 1)	Damaging (score = 0)	Disease causing	Deleterious (score = -9.39)	**Likely pathogenic** (PM1+PM2+ PM5+PP3)

*Interpretation of variants pathogenicity based on the American the College of Medical Genetics and Genomics (ACMG) recommendations [15], *i.e.*, **PM1**: located in a mutational hot spot and/or in critical functional domain, **PM2**: absent from controls, **PM5**: novel missense change at amino acid residue where a different pathogenic missense change has been seen before, **PP1**: co-segregation with disease in multiple affected family members, **PP3**: multiple lines of computational evidence support a deleterious effect on the gene or gene product.

al arcus, cardio-vascular heart disease history, and high LDL-C concentrations. About 50% of heterozygous FH patients lack obvious phenotypes. Thus, many patients are underdiagnosed until they suffer from acute cardio-vascular events [7]. Previous studies indicated that in many countries less than 1% of FH individuals were correctly diagnosed with FH [8], partly due to the lack of reliable cost-effective genetic testing.

Genetic testing can provide a definitive diagnosis. Possible benefits include individual patient management. For instance, heterozygous FH from CNVs seems to be a more severe phenotype than FH compared to single nucleotide variants. Genetic diagnosis could reduce delays for appropriate treatment, possibly more aggressive LDL-C lowering strategies [9, 10]. Additionally, for some monogenic dyslipidaemias, a genetic diagnosis is needed to ensure funding for newer therapies. This is the case of inhibitors of PCSK9, where, in certain jurisdictions, third party private coverage for the treatment of FH requires a genetic diagnosis [11, 12].

In different FH patient cohorts, PVs in *LDLR* gene are found in more than 85-90% of FH cases and more than 1800 PVs of this gene have been reported and annotated in the UCL database (http://www.ucl.ac.uk/ldlr/LOVDv.1.1.0/)

The heterogeneity of FH-causing variants supports the sequencing-based techniques as the primary methodology to detect the disease and recent advances improved accessibility for diagnostic use. This is the case of the targeted NGS in which only genes specifically involved in FH pathogenesis are screened.

In the present study, targeted sequencing of six genes (*LDLR, APOB, PCSK9, APOE, LDLRAP1* and *STAP1*) performed by an effective NGS-based molecular pipeline (*FH-Devyser Kit/Amplicon Suite software*) allowed to identify two *novel LDLR* variants.

The first, namely *c.1564A>T,* causes substitution of isoleucine for phenylalanine residue (p.Ile522Phe) in the LDL-receptor class B3 domain (486-528aa). The second, *c.1688C>T,* causes substitution of proline for leucine residue (p.Pro563Leu) in the LDL-receptor class B4 domain (529-572 aa). Both variants are located in the very conservative ß-propeller domain, in the YWTD 3 and 4 repeats of the LDLR receptor, respectively [13]. Both protein positions result to be extremely conserved in the species in which they were analysed. For this reason, *in silico* predictions performed with four different tools, using mainly amino acid conservation analysis, suggested that both *LDLR* variants are deleterious. Furthermore, at least in the family with the p.(Ile522Phe) variant, this FH-related mutation segregates with high serum LDL-C levels, linking the variant with the phenotype.

In addition, the p.(Pro563Leu) variant occurs at the same position as other pathogenic missense changes: the *c.1688C>A*, rs879254987, (p.Pro563His) and the *c.1687C*>T, rs879254986, p.(Pro563Ser). Regarding the *c.1564A>T* variant, we underline that it is in the proximity of three missense substitutions: *c.1567G>A,* rs28942080, p.(Val523Met), *c.1567G>T*, rs28942080, p.(Val523Leu) and *c.1561G>A*, rs879254940, p.(Ala521Thr), showing discordant pathogenicity data. We underline that these findings provide further evidence in order to consider p.(Pro563Leu) and (p.Ile522Phe) as deleterious variants.

CONCLUSIONS

In this study, targeted sequencing of six genes (*LDLR, APOB, PCSK9, APOE, LDLRAP1* and *STAP1*) performed by an effective NGS-based molecular approach (*FH-Devyser Kit/Amplicon Suite software)* allowed to identify two *novel LDLR* variants in FH families. Although the functional consequence of the p.(Ile522Phe) and the p.(Pro563Leu) variants remains to be determined *in vitro*, we believe these substitutions should be considered as *"likely pathogenic variants"*.

Finally, we underline that NGS integration with other molecular and cellular techniques, to assess the significance of VUS or *novel* variants, will improve the sensitivity of FH testing entirely, supporting personalized medicine.

Conflict of interest statement

All authors (SM, GM, MDB, EDP, MEO, AP, AU, AG, EC and AM) have read and approved submission

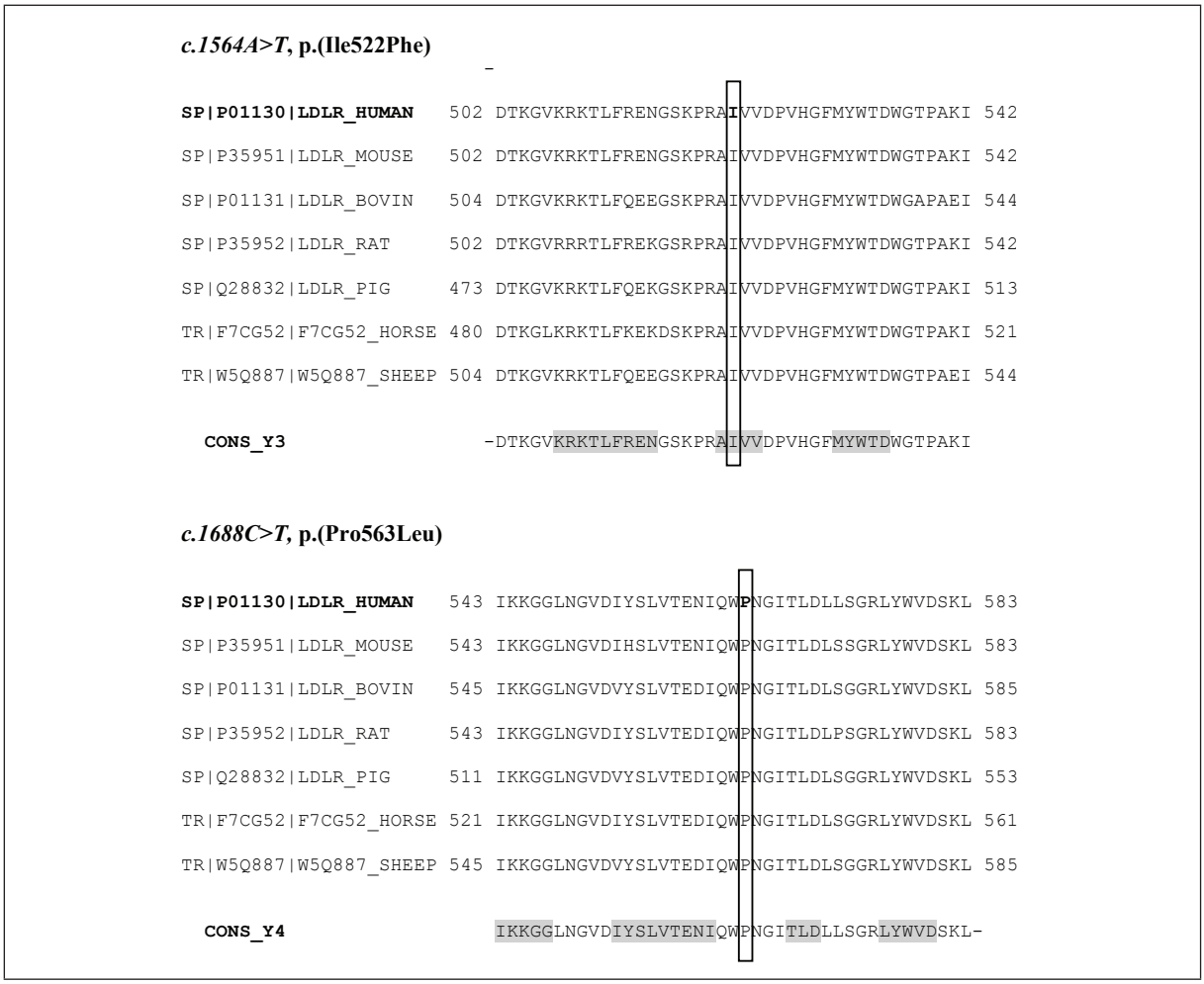

Figure 2
The alignment of the LDLR aminoacids in seven different species and a consensus of 89 YWTD domains (CONS_Y3 and Y4) are shown. The residues predicted to be in b-strand [13,14] are highlighted in grey. The amino acids (SP|P01130|LDLR_HUMAN: Ile522, and Pro563) show high conservation between the species analysed.

of the manuscript. The paper has not been published and is not being considered for publication elsewhere in whole or part in any language. All authors declare that there is no conflict of interests. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Ethical approval

This study was in compliance with the Ethical Principles for Medical Research Involving Human Subjects according to the World Medical Association Declaration of Helsinki and was reported to the Committee of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy.

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Book Reviews, Notes and Comments

Edited by
Federica Napolitani Cheyne

LA MENTE DEL CORVO
Bernd Heinrich
Milano: Adelphi Edizioni
(Animalia, 3); 2019.
556 p. ISBN 9788845934025.
€ 35,00.

[Mind of the raven]

"Everything you always wanted to know about the raven, and never dared to ask". This could be an appropriate title for this effort by Bernd Heinrich, who is rightly so considered a passionate and extremely knowledgeable expert of raven's biology and behavior. We remember his title from 1989 (Ravens in winter), mainly focused on feeding habits of these wonderful birds, and their propensity (or not) to share their food with conspecifics. This time the approach is wider, and Heinrich explores many dimensions of ravens' ecology and ethology, and the tale is full of surprises and fascinating findings. For example, I particularly enjoyed the parts dedicated to pair bonding and the feeding of chicks, as well as the pages which describe the ravens hiding foods, which lead to interesting hypothesis about cognitive abilities of this species. The parallel with the formidable abilities of scrub jays in remembering where the food has been hidden, comes obviously to mind.

The author's approach when studying his subjects is pretty physical: it includes climbing high trees in the early hours of morning, or carrying heavy carcasses of prey animals in the snow, without mentioning remaining motionless for hours in the freezing cold waiting for the ravens to appear. All of this has to do with an attitude towards field ethology that often goes beyond the merely observing animals in nature, but to actually perform behavioural experiments in the field. This is one of the most captivating aspects of this book: the curiosity drives the authors, and his students, to ask questions to those beautiful birds, through simple and provoking modifications of the ravens' environments (modifying number of eggs in the nest, hiding food under the snow, presenting unfamiliar objects, etc.). Heinrich also makes use of a large aviary near his house, where he keeps most of his experimental subjects. A large part of his observations, described in the book, comes from this open-air laboratory.

Through these pages, we learn the characters and motivations of different subjects, when they respond to the stimuli and challenges proposed by the researcher. The biology of the ravens, is described in details through 28 chapters. It seems a lot, the book runs for more than 500 pages, but it is surprising how the pages rarely cease to entertain, and I personally was never tired of hearing another adventure of "Pennabianca" and "Golia". The individuals Heinrich knows best are named, and this is not a case: as a matter of fact, Heinrich offers undeniable observational proofs that the term "personality" and "individual" belongs not only to humans and their primate cousins, but to other seemingly distant animals, such as birds. This is a trend which is now well established in the ethological literature. Personalities traits similar to humans were first described for non-human primates, but now papers are published which describe personality profiles in invertebrates (such as spiders, for example). Therefore, it is really no surprise that individual ravens are described in this book nearly like humans, and the reader finds easy and satisfying to feel empathy for the adventures of these volatiles. However, anthropomorphism is not what this book is about. Bernd Heinrich is a well-respected and known researcher in behavior and ecology: he has studied and published work on insects, environment, general ethology and…running marathons! (Heinrich is a champion of long-distance running).

So, perhaps this book is less didactic that his previous effort on ravens' behavior, but nevertheless its rich hypothesis grounded in behavioural ecology, and predictions derived from the knowledge of birds behavior and evolutionary principles. I found particularly fascinating the description of the relationship between ravens and wolves. The final picture describes a sort of mutualism between the two species, which recall the mutualism existing between local populations of humans and honeyguide birds in Africa.

Mind of the raven (translated in Italian as "La mente del corvo") is a very appropriate title. As a matter of fact, the book is a journey in the mind of these animals. We learn about their motivations, their feelings, and the relation between these and their behavior. It is also laudable the fact that the author, although enthusiastic about the cognitive capacities of these birds, never falls (as said before) in the trap of attributing human-like sentience or reasoning abilities, but he remains within the boundaries of acceptable scientific explanations even for the more impressive, cognitively speaking, ravens' behavioural manifestations.

And then, the final chapter of the book summarises very well the attitude and the underlying feeling which run through the previous pages. Love and passion for these creatures and scientific questions are strongly linked.

Who is the audience for this book? My instinctual answer would be "anybody really". Students, researchers in ecology and evolution, bird-watchers, nature lovers (some of these categories happily overlap). I also think that the book is full of examples, as I have already said before, that could be used in ethology courses to explain

students the "hypothesis-predictions" methodological paradigm to approach the study of animal behavior.

Therefore, this book is entertaining, fascinating, easy to read and engaging (pictures and drawings in the text are very beautiful as well). At the end, it is very hard not to fall in love with these extraordinary birds.

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MALATERRA: COME HANNO AVVELENATO L'ITALIA
Marina Forti
Bari-Roma: Editori Laterza, Giuseppe Laterza & Figli Spa; 2019.
193 p.
ISBN 978-88-581-3259-3.
€ 13,00.

["Malaterra": how they poisoned Italy]

The eco-toxicological and epidemiological research line situation (mainly, abnormal levels of oncological patients, malformations at birth, etc.) represented a pivotal and emerging issue in the last two or three decades. Such a trend undoubtedly occurred at a global level, yet Europe, and particularly some Italian areas, suffered more because of their management difficulties.

The Italian scenario witnesses, in fact, an historical series of "special cases" which are described in the present book. Single chapters are dedicated to the following Italian geographical areas, selected on the basis of their mediatic and eco-toxicological interest: Seveso (summarily quoted) Brescia, the Sacco River area, the Taranto Gulf and its local heavy industries, Porto Marghera (Venice area, chemical pollution in marine coastal zones), Montichiari (a minor community south-east of Brescia), Portoscuso (Sardinia).

They mostly concern notorious ecotoxicological situations, as SICs ("ecologically hot" sites of special EU interest), which already attracted years of biomedical and public health attention.

A major problem concerned, and still concerns, the sometimes "irrational" reaction of the "ordinary people", especially those spending their entire lives within the boundaries of recognized SICs: but, also, specific vulnerable populations living outside those recognised SIC zones, but somehow involved in such a potentially harmful effect. In particular, pregnant women, neonates, early-, middle- and/or late-adolescents, elderly people as well as patients affected by specific pathologies rendering them highly fragile subjects in the case of even short- and/or mild-level exposure to toxicants, including subtle behavioural (e.g. infantile learning, school achievements, etc.) effects caused by neurotoxicants. For these national priorities our ISS (Istituto Superiore di Sanità, Italian National Institute of Health) devoted, since this year, a specific high-level inter-departmental structure named "Infantile health and environmental pollution" with its coordination attributed to the "Environment and health" (DAMS) ISS Department.

The author Marina Forti is a recognized journalist and writer. She is endowed by a double journalistic specialization. Firstly, she is a mature expert of foreign affairs and policies, with a particular interest in contemporary problems experienced by women. The countries she has been travelling and spending consistent periods of time had been and still are Iran, Southern and South-East Asia.

Secondly, her marked interest in "ecological problems" also aroused a long time ago. She was, for several years, columnist and media active in this field, also receiving the prestigious prize "Premiolino" in 1999 for her long effort as an ecology problems reporter. As a whole, her approach is undoubtedly the one of "a serious journalist", including in the various chapters, among various other aspects, a variety of direct interviews with well-selected opinion leaders, soundly representatives of the Italian scientific community: therefore "translating" and condensing their ideas, point of views and methodological approaches. This is (in, of course, a summarized and made "digestible" version for the general readership) a rather fragile attempt, especially when reporting of hot and necessarily alarming issues such as not enough assessed risk or exposure level, the latter in turn often being the result of multiple concurrent toxicants, difficult to disentangle in terms of actual arm. In general, she does prefer a "principle of precaution" approach.

It is however noticeable that Forti in her final formal thanks at the end of her book mentions, among many others, scientific experts of the calibre of epidemiologist Pietro Comba (active for a few decades at ISS and also recipient of the honorary citizenship by Casale Monferrato for his invaluable multi-annual involvement in assessing local mesothelioma spreading) and Gianni Tognoni, active at the Milan-based Istituto Mario Negri, a top institution which for a long time showed a regular complicity with Italian (a more recently European) media world.

Therefore, this book, in reality a kind of collection of Italian special cases, which unfortunately may also attract biased geographical readership, is recommended to policy makers as well as to sensitive yet experienced public health officials. It also contains a rather expanded apparatus of grey literature quotations in its final notes, also including a good sitographic list. It may deserve an English version.

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Publications from International Organizations on Public Health

Edited by
Anna Maria Rossi

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)

Antimicrobial movement from agricultural areas to the environment: The missing link. A role for nuclear techniques. Rome: Food and Agriculture Organization of the United Nations. 2019, 44 p. ISBN 978 92 513 1648 1 Antimicrobials (AM) play a critical role in the treatment of human and animal (aquatic and terrestrial) diseases, which has led to their widespread application and use. Antimicrobial resistance (AMR) is the ability of microorganisms (e.g. bacteria, viruses and some parasites) to stop an antibiotic, such as an antimicrobial, antiviral or antimalarial, from working against them. Globally, about 700 000 deaths per year arise from resistant infections as a result of the fact that antimicrobial drugs have become less effective at killing resistant pathogens. Antimicrobial chemicals that are present in environmental compartments can trigger the development of AMR. These chemicals can also cause antibiotic-resistant bacteria (ARB) to further spread antibiotic resistance genes (ARG) because they may have an evolutionary advantage over non-resistant bacteria. This paper will provide alternative screening methods useful for environmental samples and surveillance approaches in planning such screening efforts. Based on case studies, this paper aims to summarize the current understanding of the occurrence of ARG in the environment, and the antimicrobial movement from agricultural areas to the environment.

Taking a multisectoral, One Health approach: A tripartite guide to addressing zoonotic diseases in countries. Food and Agriculture Organization of the United Nations, World Organisation for Animal Health, World Health Organization 2019, 164 p. ISBN 978 92 513 1236 0 Every day we hear about health challenges at the human-animal-environment interface. Zoonotic diseases such as avian influenza, rabies, Ebola, and Rift Valley fever continue to have major impacts on health, livelihoods, and economies. These health threats cannot be effectively addressed by one sector alone. Multidisciplinary and multisectoral collaboration is needed to tackle them and to reduce their impacts. As a way to support countries in taking a One Health approach to address zoonotic diseases, this guide has been jointly developed by the Tripartite organizations (FAO, OIE, and WHO). It is referred to as the Tripartite Zoonotic Guide (TZG) and it is flexible enough to be used for other health threats at the human-animal-environment interface; for example, food safety and antimicrobial resistance (AMR). The TZG provides principles, best practices and options to assist countries in achieving sustainable and functional collaboration at the human-animal-environment interface.

INTERNATIONAL LABOUR ORGANIZATION (ILO)

Bureau for Employers' Activities (ACT/EMP). **Women in Business and Management: The business case for change.** Geneva: ILO. 2019, 149 p. ISBN 978 92 213 3168 1 This second global report on Women in Business and Management offers new insights into how gender diversity at the top improves organizational performance. These include how the many dimensions of an organization's policies, a gender-balanced workforce and a gender-inclusive culture, among other factors, move the needle for more women to hold decision-making power.

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD)

The digitalisation of science, technology and innovation. Key developments and policies. Paris: OECD. 2020, 182 p. ISBN 978 92 644 2725 9 doi:10.1787/b9e4a2c0-en This report examines digitalisation's effects on science, technology and innovation and the associated consequences for policy. In varied and far-reaching ways, digital technologies are changing how scientists work, collaborate and publish. While examining these developments, this book also assesses the effects of digitalisation on longstanding policy themes, from access to publicly funded research data, to the diffusion of technology and its absorption by firms. New and emerging topics are also explored. These include the roles of artificial intelligence and blockchain in science and production, using digital technology to draw on the collective intelligence of the scientific community, advances in the digitalisation of biotechnology, and possible "dark sides" of digitalisation.

Rochai M, Faldutoi C. **Key questions guiding the process of setting up long-term low-emissions development strategies.** Paris: OECD. 2019, 52 p. doi:10.1787/54c2d2cc-en The Paris Agreement states that all countries should strive to formulate and communicate long-term low greenhouse gas emission development strategies (LT-LEDS) and the Paris Agree-

ment's accompanying decision invites countries to communicate a LT-LEDS by 2020. LT-LEDS are a fundamental tool available to countries to envision low-emission development in alignment with broader sustainability, socio-economic and climate change adaptation goals. This document aims to support countries' efforts in the development of LT-LEDS, as it provides points of reflection for the establishment of an effective process for developing LT-LEDS. The document discusses potential elements to be included in a LT-LEDS; identifies and explores potential linkages between Nationally Determined Contributions (NDCs) and LT-LEDS; examines governance options for setting up a LT-LEDS process and analyses countries' experience to date in developing LT-LEDS. The paper also provides a set of guiding questions useful for the development of LT-LEDS.

Main science and technology indicators. Paris: OECD. 2019, Issue 1 This biannual publication provides a set of indicators that reflect the level and structure of the efforts undertaken by OECD member countries and seven non-member economies (Argentina, People's Republic of China, Romania, Russian Federation, Singapore, South Africa, Chinese Taipei) in the field of science and technology. These data include final or provisional results as well as forecasts established by government authorities. The indicators cover the resources devoted to research and development, patent families and international trade in R&D-intensive industries. Also presented are the underlying economic series used to calculate these indicators. Series are presented for a reference year and for the last six years for which data are available.

UNITED NATIONS PROGRAMME ON HIV/AIDS (UNAIDS)

Cities on the road to success - Good practices in the Fast-Track cities initiative to end AIDS. Geneva: Joint United Nations Programme on HIV/AIDS. December 2019, 96 p. (*UNAIDS/JC2969*). The Fast-Track cities initiative was launched in 2014 when mayors from 26 global cities met and endorsed the Paris Declaration on Fast-Track Cities Ending the AIDS Epidemic (the Paris Declaration). Since then, more than 300 cities and municipalities have endorsed the declaration. Cities play a critical role in both the AIDS epidemic and the response. On the one hand, more than half of the world's population currently lives in cities, and in most countries, cities account for a large and growing proportion of the national HIV burden. Risk and vulnerability to HIV is often higher in cities than rural areas due to a range of factors, such as migration, overcrowding, and social and economic inequalities. Urbanization may also bring about cultural and social changes that provide increased opportunities for HIV risk behaviour, and key populations, who are at higher risk of HIV exposure, are often concentrated in urban areas. On the other hand, cities offer advantages and important opportunities for programming, effective action and innovations to end AIDS. This report describes activities and good practices from a selection of Fast-Track cities that represent a range of experiences: from low-burden, high-income countries in the global north to high- and low-burden countries in Africa, Asia, eastern Europe, Latin America and the Caribbean. These are examples of cities that have addressed barriers to the response and optimized service delivery to all citizens, including marginalized and vulnerable populations. This report also describes innovative approaches that have strengthened HIV prevention and treatment services and improved outcomes in the HIV response.

Global AIDS Monitoring 2020. Indicators for monitoring the 2016 Political Declaration on Ending AIDS. Geneva: Joint United Nations Programme on HIV/AIDS. 2019, 176 p. The purpose of this document is to provide guidance to national AIDS programmes and partners on the use of indicators to measure and report on the country HIV response. It focuses on the five-year period ending in 2021, but it also covers the period of the Sustainable Development Goals (SDGs) (through 2030) and the integration of the global HIV response into the broader development agenda. Although governments have adopted the 2016 Political Declaration on Ending AIDS, its vision extends far beyond the government sector, reaching private industry and labour groups, faith-based organizations, nongovernmental organizations (NGOs) and other civil society entities, including those representing people living with HIV. As indicated in the 2016 Political Declaration on Ending AIDS, a successful AIDS response should be measured by the achievement of concrete, time-bound targets. These guidelines are designed to improve the quality and consistency of data collected at the country level, enhancing the accuracy of the conclusions drawn at the national, regional and global levels. Countries should also develop national and programme indicators that capture the specific goals of both their national strategic plan for HIV and their particular context.

UNITED NATIONS ENVIRONMENT PROGRAMME (UNEP)

Emissions Gap Report 2019. Nairobi: United Nations Environment Programme. 2019, 108 p. This tenth edition of *UNEP Emissions Gap Report* presents the latest data on the expected gap in 2030 for the 1.5°C and 2°C temperature targets of the Paris Agreement. It provides an independent . assessment of scientific studies on current and estimated future greenhouse gas (GHG) emissions and compares these with the emission levels permissible for the world to progress on a least-cost pathway to achieve the goals of the Paris Agreement. This difference between "where we are likely to be and where we need to be" has become known as the 'emissions gap'. As in previous years, this report explores some of the most promising and applicable op-

tions available for countries to bridge the gap, with a focus on how to create transformational change and just transitions. It looks at the potential of the energy transition – particularly in the power, transport and buildings sectors – and efficiency in the use of materials such as iron steel and cement. Reflecting on the report's overall conclusions, it is evident that incremental changes will not be enough and there is a need for rapid and transformational action. It shows that despite a decade of increased focus on climate change, global GHG emissions have not been curbed and the emissions gap is now larger than ever. It is clear that the world cannot afford another decade lost. For the first time, it looks at how large annual cuts would need to be from 2020 to 2030 to stay on track to meeting the Paris goals.

Boileau P, Ekins P, Gupta J. (Eds.) **Global Environment Outlook – GEO-6: healthy planet, healthy people.** Nairobi: United Nations Environment Programme. 2019, 745 p. Job Number: DEW/2214/NA ISBN 978 11 087 0766 4 doi:10.1017/9781108627146 UN Environment's sixth Global Environment Outlook (2019) calls on decision makers to take immediate action to address pressing environmental issues to achieve the Sustainable Development Goals as well as other Internationally Agreed Environment Goals, such as the Paris Agreement. By bringing together a community of hundreds of scientists, peer reviewers and collaborating institutions and partners, the GEO reports build on sound scientific knowledge to provide governments, local authorities, businesses and individual citizens with the information needed to guide societies to a truly sustainable world by 2050. GEO-6 builds on the findings of previous GEO reports, including the six regional assessments (2016), and outlines the current state of the environment, illustrates possible future environmental trends and analyses the effectiveness of policies. This flagship report shows how governments can put the world on the path to a truly sustainable future. It emphasizes that urgent and inclusive action is needed by decision makers at all levels to achieve a healthy planet with healthy people.

WORLD HEALTH ORGANIZATION (WHO)

Inequality monitoring in immunization: a step-by-step manual. Geneva: World Health Organization. 2019, 81 p. CHF 20.00/US $ 20.00 Order Number: 19300407 ISBN 978 92 415 1653 2. The manual is an introductory guide to inequality monitoring in the topic of immunization. Aiming to build capacity for the uptake and improvement of inequality monitoring practices in immunization, the manual was primarily designed for monitoring and evaluation officers for immunization, and may also be of interest for other readers with basic knowledge and experience working with immunization data. It is organized in five sections, illustrating how the five steps (and corresponding sub-steps) of health inequality monitoring apply to immunization. From the first step of determining the scope of monitoring to the fifth step of knowledge translation, contemporary considerations and examples for immunization inequality monitoring are highlighted. Step-by-step guidance, including key questions and best practices for each sub-step, are summarized in a flow chart. Additional information is provided through appendices and a glossary of terms.

WHO Report on the Global Tobacco Epidemic. Geneva: World Health Organization. 2019, 210 p. CHF 40.00/US $ 0.00 ISBN 978 92 415 1620 4. The report tracks the status of the tobacco epidemic and interventions to combat it. It finds that more countries have implemented tobacco control policies, ranging from graphic pack warnings and advertising bans to no smoking areas. About 5 billion people – 65% of the world's population – are covered by at least one comprehensive tobacco control measure, which has more than quadrupled since 2007 when only 1 billion people and 15% of the world's population were covered.

Vaccarella S, Lortet-Tieulent J, Saracci R, *et al.* **Reducing social inequalities in cancer: evidence and priorities for research.** Geneva: World Health Organization. 2019, 272 p. (*IARC Scientific Publication*; 168) CHF 50.00/US $ 50.00 Order Number: 17300168 ISBN 978 92 832 2223 7. This volume summarizes the current scientific evidence and identifies research priorities needed to decrease social inequalities in cancer. The publication, based on the expert knowledge of more than 70 international scientists from multiple disciplines, undertakes a populations-within-populations approach, highlighting the large variations in cancer incidence, survival, and mortality that exist between countries and, within countries, between social groups. Several factors may lead individuals with low social status to adopt unhealthy behaviours, to be exposed to a wider range and a higher intensity of cancer risk factors, and to have reduced access to health-care services, compared with their fellow citizens. A special focus is given to how the phenomenon of inequalities in cancer evolves and is reshaped over time, driven by economic, social, political, legislative, and technological forces; it affects everyone, but the most disadvantaged individuals are particularly hard hit. This publication was developed to serve as a reference for policy-makers and public health officials, linking to specific examples of interventions that may reduce future inequalities in cancer.

Instructions to Authors

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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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