ISSN 0021-2571 (print) · 2384-8553 (online) | Coden: AISSAW 59 (No. 4) | 245-318(2023)

ANNALI
DELL'ISTITUTO SUPERIORE DI SANITÀ

Volume **59** No. **4**
2023

A SCIENCE JOURNAL *for* PUBLIC HEALTH

ISTITUTO SUPERIORE DI SANITÀ
www.iss.it

Ann Ist Super Sanità 2023 | Vol. 59, No. 4

Annali
dell'Istituto Superiore di Sanità
A SCIENCE JOURNAL FOR PUBLIC HEALTH

Publication
Annali dell'Istituto Superiore di Sanità is published quarterly and in special issues.
Annali is an open access journal listed in the Directory of Open Access Journals (DOAJ) and is freely available at www.iss.it/annali - https://annali.iss.it

Annali dell'Istituto Superiore di Sanità is indexed in
- CAB
- CHEMABS
- EMBASE/Excerpta Medica
- FSTA
- MEDLINE
- ProQuest
- SCOPUS
- WEB OF SCIENCE

The Journal Impact Factor is 2.1

Annali Editorial Office
Scientific Communication Service
Istituto Superiore di Sanità
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Publishing support
Il Pensiero Scientifico Editore, Rome
Via San Giovanni Valdarno 8, 00138 Rome, Italy
www.pensiero.it

Subscription information & terms
Il Pensiero Scientifico Editore
Tel.: +39 06 86282324 Fax: 06 86282250
E-mail: abbonamenti@pensiero.it

Year 2023
Italy individual subscription € 57,00 | Italy institutional subscription € 67,00.
Other countries € 67,00
Each quarterly issue € 21,00

Responsibility for the contents and opinions expressed on this journal rests solely with the Author(s).

ISSN 0021-2571 (print), 2384-8553 (online)
Coden: AISSAW 59 (No. 4)

Reg. Stampa - Tribunale di Roma, n. 482 del 29 ottobre 1985 (cartaceo); n. 121 del 16 maggio 2014 (online)

MISTO
Carta da fonti gestite in maniera responsabile
FSC® C017635

Printed in December 2023 by Ti Printing s.r.l.
Via Case Rosse 23, 00131 Rome, Italy

Ann Ist Super Sanità 2023 | Vol. 59, No. 4

Annali
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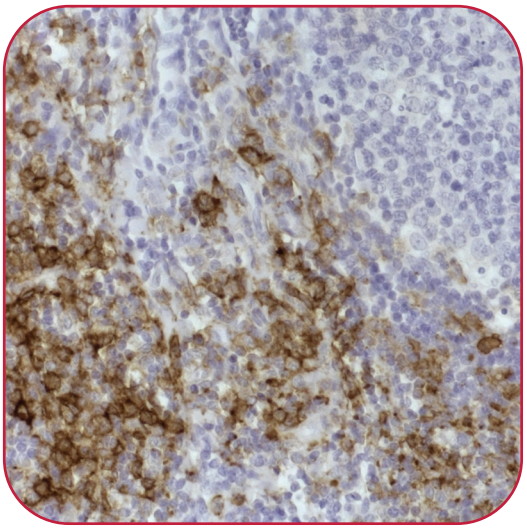
Graphic design of the cover: Massimo Delle Femmine, Istituto Superiore di Sanità

The photograph is an immunohistochemical staining showing the perifollicular distribution of CD138+ plasma cells in the human palatine tonsil. Nuclei are stainined with hematoxylin.
Image is provided by Barbara Serafini, Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy.



ANNALI
dell'Istituto Superiore di Sanità

Vol. 59, No. 4 2023

Contents

Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 245-246
DOI: 10.4415/ANN_23_04_01

EDITORIAL
Between social and healthcare: a necessary synergy

Carlo Petrini[1] and Enrico Alleva[2]

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In Italy, the parliamentary process for the adoption of a law on the prevention of discrimination and the protection of the rights of people suffering from oncological diseases has been completed.

The "oncological oblivion law", requested by civil society, the scientific community, the country's administrative and political leaders, is also part of the implementation of the recommendation European Parliament resolution of 16 February 2022 on strengthening Europe in the fight against cancer [1].

According to the text, people recovered from an oncological pathology have the right not to provide information and not to be the subject of investigations on their previous pathological condition. Furthermore, as regards access to banking, financial and insurance services, the request for information on the state of health of the policyholder relating to oncological pathologies already dealt with is not permitted, if the active treatment has been concluded for more than ten years without episodes of recurrence. This period is reduced by half if the pathology manifested itself before the age of twenty-one.

The text also provides that this information cannot be acquired from sources other than the contracting party and, if they are available from the operator or the intermediary, they cannot be used to determine the contractual conditions.

In Italy the number of people alive after about ten years from a cancer diagnosis is significant: almost 6% of the population (about 1 million people).

The impact of the law, therefore, is also relevant in terms of the size of the population involved.

Also significant is the fact that the term "healed" is used in the law. In oncology, the term "cured" has so far been rarely used, preferring terms such as "long-term survivor" or even "survivor". In fact, it has long been known that there are patients who can, with a reasonable probability, be considered cured. The fact that a subject who has had an oncological pathology can be considered cured represents a radical paradigm shift: from "incurable cancer" to "chronic pathology cancer from which one can be cured".

From an ethical point of view, it is important to consider that the right to have previous oncological pathologies forgotten is based on the protection of personal identity, i.e., in art. 2 of the Constitution. The right consists, in particular, of the right not to provide information about one's past health status with regard to oncological diseases.

The "right to be forgotten" has some analogy with the "right to be left alone", which is the first formulation of the right to privacy: Warren SD and Brandeis LD used it in 1890 in an article entitled "The right to privacy" published in the Harvard Law Reviews [2].

The right to be medically forgotten, however, should not be confused with the right to erasure of one's personal data from the internet, which is grounded in Article 17 of the European Regulation no. 679 of 2017.

Thus, this first aspect, namely the protection of personal identity, concerns a foundation of ethics: the dignity of the person.

From an ethical point of view, the law is also significant because it highlights the many dimensions of health: not only the absence of disease, but also the possibility of living in normal conditions. Therefore, it is not only the principle of beneficence (doing people's physical good) that must be applied: it is also necessary to restore people's autonomy and to treat them with justice and fairness, to give everyone what they need.

This also corresponds to an increasingly deep-rooted awareness that the health dimension and the social dimension are closely intertwined.

Oncological oblivion also calls for reflection on another important notion in bioethics: vulnerability. Considering certain groups as "vulnerable populations" in relation to the normal condition makes little sense: we are all vulnerable, but certain categories of people are in a particularly vulnerable condition. In the case of cancer diseases, for many these special conditions are reversible: one can return to ordinary conditions.

Thus, in general, the law is significant for the protection of the rights of persons who have faced and

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overcome an oncological disease. It is, in fact, aimed at ensuring these persons fair and equal treatment with other persons in the context of financial, banking, and insurance activities, and also in the employment sphere, with measures relating to access to competitive selection procedures and recruitment and relating to active policies for entering and remaining at work. From an ethical point of view, it is significant that, unlike similar regulations in other countries, the Italian regulation also intervenes in the family sphere: recognition of suitability for adoption can no longer be denied to those who have recovered from cancer.

With this legislation, the aim is to ensure that clinical recovery also translates into the full exercise of civil and social rights, without being penalised because of one's previous health condition.

Conflict of interest statement

The Authors declare that there are no conflicts of interest.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 247-250
DOI: 10.4415/ANN_23_04_02

COMMENTARY
From mass vaccination to personalized vaccinology? The COVID-19 case

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**up to May 2023*

Abstract
In recent times, especially as a result of the experience gained worldwide with the COVID19 pandemic vaccination campaigns, the personalization of vaccination strategies is becoming increasingly important. This does not yet mean bringing precision medicine and genomics approaches into immunization campaigns, but where there is more than one vaccine against the same disease, there is a need to identify criteria for personalizing vaccination.
Vaccination strategies based on prescription appropriateness – whenever is possible – can lead to more effective immune response, reduced rates of adverse events, increased public confidence in vaccination and higher vaccination coverage, contributing to a decrease of morbidity and mortality related to preventable diseases.

Key words
- personalized vaccinology
- vaccination campaigns
- national immunization plans

INTRODUCTION

Scientific progress is not an accumulation of knowledge aimed at discovering the truth, but an alternation between standard scientific discovery and scientific revolutions, starting with a group of elements that tend to be articulated and specialized in what Thomas Kuhn described as "paradigm shift" [1]. As Sir Muir Gray argued, shifts in healthcare are more likely to result from new ways of thinking rather than new technologies [2]. However, unprecedented scientific and technological innovation has revolutionized healthcare in the last 40 years. In particular, the advent of genomics and digital data science in healthcare research, with the consequent exponential growth of analytical and diagnostic capabilities in clinical practice, led to what is known as personalized medicine.

According to the National Research Council, "personalized medicine" is an older term with a similar meaning to "precision medicine" [3]. Personalized medicine is a medical model that aims to provide prevention and treatment strategies tailored to defined groups of individuals. To date, there is no universally accepted definition. The European Union Health Ministers in their Council conclusions, published in December 2015, provided the following definition on personalized medicine: "A medical model using characterization of individuals genotypes and phenotypes (e.g., molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, and/or for determining the predisposition to disease and/or to deliver timely and targeted prevention" [4].

From the perspective of the population, precision medicine has promoted a profound reflection – in recent years – on what has been called precision public health, previously defined in the literature as an analytical resource for policymakers and a useful paradigm for directing healthcare interventions towards disadvantaged social groups through granular data [5].

In analogy to precision medicine, precision public health can be also described as an innovative domain for developing data-driven approaches to public health interventions, encompassing both pharmaceutical and non-pharmaceutical interventions, as partially experienced in their implementation during the COVID-19 pandemic [6, 7].

Although most advances in personalized medicine and public health regard the field of individualized medical treatment, several factors can undoubtedly trigger a paradigm shift in modern vaccinology also in the post-mass vaccination campaign against COVID-19.

THE COVID-19 VACCINATION CAMPAIGN EXPERIENCE

The COVID-19 global pandemic has represented a health and socio-economic challenge with few precedents in human history. Vaccination was the most effective intervention to control the spread of the virus and, consequently, to save lives and protect the health

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of the population. In general, it can take 4 to 15 years to develop an effective and safe vaccine; however, starting with the first cases of COVID-19 detected in Wuhan, China, researchers have quickly identified the genome of the SARS-CoV-2 virus and developed viable vaccine candidates using new sequencing methods. Vaccines against SARS-CoV-2 use different technologies, such as mRNA, viral vectors, protein subunits, and inactivated virus. During the COVID-19 pandemic, clinical trials started within 5 months after the first reported cases, leading to the development of effective vaccines, and to "fast-track" authorisation within less than 12 months after virus isolation. The first authorised vaccines were produced with a modified RNA technology encoding a version of the SARS-CoV-2 spike protein capable of inducing neutralising antibody responses. The quick development of vaccines with mRNA technology is considered a triumph for preventive medicine and modern vaccinology [8].

A successful worldwide mass-vaccination campaign followed the authorization of several vaccines based on different technological platforms. To summarize, during anti-COVID-19 national immunization campaigns, for the first time: i) several effective vaccines, mostly based on innovative mRNA platforms, were developed in less than 12 months; ii) the entire world population was affected simultaneously by such a large and rapid immunization program; iii) health policy decisions and immunization strategies were updated on the basis of real world data (RWD) and real world evidence (RWE).

During mass vaccination campaigns, all types of available vaccines were used, and the offer could not be differentiated on the basis of individual's characteristics. Because of limited knowledge, the choice of target groups tended to be updated over time as a result of empirical experience. For example, the use of more effective vaccines, such as mRNA vaccines, was initially prioritized to those at highest risk, while vectored vaccines were preferentially recommended to healthy younger individuals, but after the observation of rare severe adverse events, such as the so-called vaccine-induced thrombotic thrombocytopenia (VITT), following the administration of adenovirus vector vaccines, these vaccines were preferentially recommended to older people, binding their use among younger people to epidemiological driven risk benefits analysis [9].

Nowadays, the end of the acute pandemic phase forces us to reconsider the modalities of COVID-19 vaccine offer, taking in account the so-called personalized vaccinology, extensively theorized, and described by Gregory Poland and colleagues as "vaccinology 3.0, (…) able to provide the right vaccine to the right patient – for the right reason and at the right dose" [10].

FROM THE ONE-SIZE-FITS-ALL APPROACH TO PERSONALIZED VACCINOLOGY

The standard medical practice in vaccinology is to universally deliver the same set of vaccines/vaccinations to the entire population (*one-size-fits-all approach*), in the absence of a contraindication, with several generalizations supporting this approach [10]. It also assumes that everyone is at approximately the same level of risk against the disease being prevented, and that the vaccine dose amount and number of doses needed to develop immunity are the same across the population. The major weakness of this approach is that it ignores individual variability in disease risk/immunologic response, and any genetic propensity for reactogenicity, as well as differences in the dose amount needed for protection [10, 11].

Different variables could influence the effectiveness of a vaccine or the propensity to adverse events such as: age, gender, race/ethnicity, immune status, size (body mass index), lifestyles, medical condition, comorbidities, and genetic profile. Some of these listed factors are identifiable and therefore predictable. Among these, one of the most important factors in determining the antibody response is undoubtedly the age and state of the immune system, which is a fundamental endogenous factor in the response to natural infections and vaccinations. Immunogenetic variation might one day lead to new products designed to minimize vaccine failure. Such host variability may depend on a multiplicity of immune response genes encoding products needed to generate antibodies, T cell receptors, or Human Leukocyte Antigen, HLA loci. Furthermore, gene polymorphism may also explain inter-individual variation due to other functions involved in the response to vaccines. Up to now, vaccine immunogenetics is still under-studied, and most information derives from studies targeting immune response to the measles vaccine [12]. All this information is included in the immune response theory, as defined by Gregory Poland and the Mayo Clinic group, which is the necessary basis for vaccinomics and adversomics [10, 13, 14].

Recently, Valdés-Fernández *et al*. offered a comprehensive review of genetic variants affecting immune response constituents that can influence individual responses to SARS-CoV-2 vaccines. They also discussed the potential public health implications of differing SARS-CoV-2 vaccine effectiveness across population groups [15]. Moreover, during COVID-19 vaccination campaign in Germany in 2021, subject-specific differences in COVID-19 vaccine reactogenicity and work absenteeism after vaccination were observed in a large survey of healthcare workers [16].

The traditional public health population-level paradigm and the emerging individual-level paradigm, which acknowledges genetically encoded unique individual variability in response to biologic agents, are creating a new kind of "tension" in the field of vaccinology. Personalized screening prior to vaccination could be made possible, one day, in order to identify these variables. This would result in the delivery of the right vaccine to the right person, at the right dose, at the right time [10-12]. A system biology approach might also favour the capacity to predict immune responses and adverse reactions, favouring the development of personalized vaccines [14, 17, 18].

However, at the moment, such predictive tests are still not available nor validated and, in any case, cannot be used on a large scale; this is a strong limit to the current feasibility of a personalized approach to vaccine prophylaxis. Furthermore, other challenges still exist.

Table 1
Pros and Cons of "one-size-fits-all" vaccination approach and personalized vaccinology

	Pros	Cons
"One-size-fits-all" vaccination approach	• Ease of planning large national campaigns for macro targets (e.g., age and risk categories)	• Risk/benefit ratio only applicable on a population level, not on an individual level
Personalized vaccinology	• Improved predictability of reactogenicity, resulting in decreased adverse effects and increased vaccine effectiveness, which can also generate positive social outcomes (i.e., less work or school absenteeism)	• Increased costs and potential delays due to pre-vaccination tests, which could hinder access to vaccinations and lower uptake • Potential use of individual-level data to discriminate against certain population groups

There are problems with high costs for genetic-based assays, the complexity of data analysis and interpretation, as well as inertia on the part of current vaccine producers and health authorities, which contribute altogether to postpone the possible transition to new paradigms in the field of vaccinology [17, 19].

On the other hand, a promising field of personalized vaccinology is represented by therapeutic vaccines against cancer. In particular, the rapid mapping of somatic mutations within cancer cells genome is now possible and may lead to the identification of cancer-specific epitopes that can be recognized by autologous T cells. This may favour the selection of specific vaccine targets. Since cancer-specific neoantigens are often unique to each patient's cancer, a personalized development of immunotherapeutic products is required [20, 21].

CONCLUSIONS

The mass vaccination approach, which is absolutely needed in a pandemic phase, when morbidity and mortality rates are high, has inevitable side effects at the individual and community level, and needs to be promptly critically revised in the post-pandemic era, when the clinical impact of the disease – along with risk perception – tends to decrease. At that point in time, a new mindset (i.e., using the best vaccine only for individuals at risk for a specific event) prevails. This kind of approach has been planned by the main European Countries in their national immunization COVID-19 campaign for the 2023/2024 season, where the recommendation for vaccination has been made for specific subgroups of people characterized by specific risk factors (e.g., age, diseases, frailty) [22].

Hopefully, this strategy may also be useful in dealing with hesitation towards vaccines, which is particularly topical after a couple of years in which vaccines and immunisation have been in the spotlight like never before, and citizens have been psychologically stressed by restrictions due to efforts to manage the COVID-19 pandemic. This does not mean that a unique vaccine should be developed for each one, but just that different types of vaccine – possibly based on different platforms – should be prioritized to different population subgroups, as already happens for influenza. In fact, current scientific knowledge is still limited, and pre-vaccination tests are neither reliable nor affordable.

Thus, we may move now from the mass vaccination approach, in which whatever vaccine is available is given to as many people as possible on the basis of a simple risk-benefit analysis, to approaches based on the possibility of differentiating between individuals at higher and lower risk of adverse events and severe disease, using simple variables such as age and gender, even within a restricted range of age classes. Pros and cons of one vaccination strategy versus the other are listed in *Table 1*. Of course, this is possible only in case different types of vaccines are available. Times are not mature for a real personalized vaccinology, but vaccination strategies based on prescription appropriateness – whenever is possible – can lead to more effective immune response, reduced rates of adverse events, increased public confidence in vaccination and higher vaccination coverage, contributing to a decrease of morbidity and mortality related to preventable diseases. It is important that those called upon to support decisions on vaccination campaigns, in particular NITAGs, take these elements into account [23]. To this end, it is necessary to improve training, curricula and professional skills in the field of vaccinology at all levels and among all health professionals.

Conflict of interest statement

The Authors declare that there are no conflicts of interest.

Received on 14 June 2023.
Accepted on 5 October 2023.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 251-259
DOI: 10.4415/ANN_23_04_03

Do demographic and socio-economic factors predict Sense of Coherence among university students?

Guglielmo Bonaccorsi[1], Patrizio Zanobini[1], Claudia Cosma[2], Primo Buscemi[2], Sonia Paoli[2], Vieri Lastrucci[3], Valerio Ferro Allodola[1], Andrea Moscadelli[2], Orkan Okan[4], Kevin Dadaczynski[5] and Chiara Lorini[1]

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Abstract

Introduction. The COVID-19 pandemic and related containment measures have been threatful for psychological well-being, particularly for young people such as university students. Sense of Coherence (SoC) can help in coping with stressful and anxiety-provoking situations.

Aim. The aim of this study is to describe the levels of SoC and to investigate the socio-economic, and demographic predictors in a sample of students attending Florence University, in the timespan between August, 17th and October, 3rd 2020.

Method and results. The cross-sectional online survey was completed by 2,996 students. Higher levels of SoC have been found among males and for respondents reporting a better socioeconomic condition. Regarding the dimensions of SoC, lower levels were reported for comprehensibility and manageability, higher for meaningfulness.

Conclusions. These results reinforce the need to plan and implement health promotion interventions aimed to support and sustain university students in general and specifically those at higher risk of low level of SoC.

Key words

- university students
- Sense of Coherence
- cluster analysis
- financial situation

INTRODUCTION

In the past two years life and psychological wellbeing has been violently stricken by COVID-19 pandemic [1]. Since the beginning of the pandemic, physical distance represented one of the most effective containment measures and on March 9th, 2020, the stay-at-home order was extended to the entire Italian population [2]. This lockdown forced the closure of schools and universities and the reorganization of lessons and examinations via online meetings, compelling young people to engage with digital communication technologies to maintain relationships with peers and teachers during the Italian curfew [3]. Quarantine, which was adopted to contain the virus spread and new infections, seriously affected behavioral routines and was particularly hard to accept for students who usually spent much of their time in social situations, like in schools or universities [1].

The pandemic and the related containment measures were, indeed, threatful for psychological well-being and the new living condition strongly affected university students, who showed risks of mental disorders, such as anxiety, depression, and even suicidal ideation [4]. Therefore, pandemic gave new importance to the concept of resilience defined as a characteristic of people which protects themselves from stress and depression and help to adapt to unpleasant circumstances. It is a developable capacity that changes over time [5, 6].

An indicator of resilience in dealing with critical events is the Sense of Coherence (SoC), which has a strong relationship with health [7]. Indeed, it emerges that some people maintain themselves healthy despite their dramatic experiences, thanks to the good view they have of their life and of the essence of their existence [8-11]. Antonovsky theorized that SoC developed during lifetime and that socio economic status, network education, and culture seem to strongly influence indi-

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vidual SoC level too. SoC is often considered to be a stable entity that since childhood completely develops around the age of 30 [9-11]. However, recent studies have questioned this stability of SoC: high SoC tents to be more stable than a lower SoC [12] and, under certain conditions (such as mental health promotion efforts, etc.) changes might be possible in adulthood [13].

In summary, SoC could be conceptualized as the extent to which life is understood as more or less comprehensible, meaningful, and manageable by different individuals and lets one mobilize resources to cope with stressors. Individuals having a higher SoC are more capable of facing and handling hard situations, such as the pandemic could be. University students with high SoC usually experience fewer daily unpleasant events than students with low SoC and experience these events as less stressful [14, 15].

Therefore, it appears that, according to the theoretical approaches [9], socio-economic and demographic factors, such as the economic conditions and the social position of the family, play a significant role in the formation of the SoC. These theoretical principles have been also confirmed by the findings of several researches that have identified the economic wealth as a factor that can, both directly and indirectly, contribute to have a strong SoC [16].

Biennium 2020-2021 represented a real challenge to university students' resilience and SoC even because it has introduced hard financial pressure, changed living conditions and *modus vivendi* [17]. Since Universities face to face lessons were largely replaced by remote teaching those with limited digital resources have been negatively affected at most [18].

Following the radical changes caused by the COVID-19 pandemic, an international group of researchers from all over the world launched the COVID-HL network [19] (see https://covid-hl.eu/) which aims to establish an open science and research community to foster research in the field of health literacy, health information, and digital health.

The aim of this study, as part of the COVID-HL University Students Survey, is to describe the level of SoC and to investigate socio-demographic and socio-economic predictors in a sample of students from Florence University, in the timespan between the first and the second Italian wave of COVID-19 pandemic.

MATERIALS AND METHODS

Study design

This cross-sectional study was designed and conducted in accordance with the Helsinki declaration and approved by the Ethics Committee of the University of Florence (n. 108, 2020/07/07).

Data were collected using a questionnaire developed by Dadaczynski *et al.* [20]. In Florence, the COVID-HL University Students Survey was conducted sharing the online questionnaire with the students of all the courses (bachelor, master, PhD, Postgraduate School) through the institutional email. In addition, the survey was advertised on the University of Florence web pages and social networks. There were no exclusion criteria, apart from not being a Florence University student.

In 2020, the students enrolled at the University of Florence were about 50,000 and a convenience sample of 2,996 students filled in the questionnaire. The questionnaires were administered from August 17th to October 3rd, 2020. Filling in the questionnaire was voluntary. In accordance with the European Regulation 2016/679 and the Legislative Decree 101/2018, all the data have been processed anonymously and cannot be attributed to a specific person.

Questionnaire

The questionnaire included either existing validated scales adapted to the COVID-19 pandemic or newly developed scales. In particular, the following data was collected: sociodemographic information; life situation and future anxiety; digital health literacy and information seeking behavior; personal health situation.

The questionnaire, proposed in English language [20] was translated into Italian using a standard procedure: two independent native English speakers translated the questionnaire in Italian language, then two independent native Italian speakers back-translated the two versions into English. The four versions (two in Italian and two in English languages) were assessed and discussed by the research group to verify any discrepancies emerging from the process.

For the purposes of this study, the following sections of the questionnaire were included in the analysis: sociodemographic characteristics (sex, age, country of origin, study course), general economic situation (subjective social status, satisfaction with the financial situation, how they finance their studies), SoC.

Specifically, the socioeconomic status was measured using the MacArthur Scale of Subjective Social Status (SSS), which assesses the person's perceived position in a social hierarchy: it is a ten-point scale (from 1 to 10) with the top (higher points) representing wealthy people, with a prestigious job and high level of education while at the bottom (lower points) were placed poor people with less prestigious job and lower education [21, 22]. According to the score, three categories of SSS were identified: low, medium, and high SSS.

Satisfaction with the financial situation was investigated with the question "How sufficient do you consider the money at your disposal?", with the following response options: not sufficient (score=1), less sufficient (score=2), sufficient (score=3), completely sufficient (score=4).

The source for financing the students' study was assessed through a specific question ("How do you primarily finance your studies?") with 6 possible responses ("support by parents", "students' grant", "employment during the semester", "employment during the semester brake", "scholarship", "other").

To measure the SoC, the work-related SoC instrument developed by Vogt *et al.* [23] was used, adapting the initial question to life in general ("How do you personally find your current life situation in general") instead of working condition. The primary study that used the tool and to which we are referring to as a good internal consistency (Cronbach alpha=0.83) and factorial analyses showed that the scale has a three-factor

structure with the sub-dimensions of comprehensibility, manageability and meaningfulness. Before the pandemic there are studies that used Vogt *et al.* original instrument [23] which present the differences respect the instrument used here regarding a single word of the introduction question that is "work situation" turned in "life situation" so: "How do you personally find your current job and work situation in general?" becomes in our version: "How do you personally find your current life situation in general?". It included nine bipolar adjectives (items) that could be rated on a seven-point semantic differential scale, with higher values indicating a higher SoC [22]. The nine items refer to the three dimensions of SoC: four items for "comprehensibility" (items n. 1, 3, 6 and 9), two items for "manageability" (items n. 4 and 7), and three items for "meaningfulness" (items n. 2, 5 and 8). The Italian version of the SoC scale presented a good internal consistency (Cronbach's alpha: 0.874). Considering the subscales, two presented a good or acceptable-to-good internal consistency (for "comprehensibility" and "meaningfulness": Cronbach's alpha >0.71) while for the other one (i.e., "manageability") the Cronbach's alfa was lower than 0.71. However, as also discussed by Dadaczynski *et al.* [20], it could be considered as sufficient due to the low number of items included in this subscale. A SoC scale score and three subscale scores were then calculated as the mean value of the item scores.

Statistical analyses

Normality of continuous variables was assessed using Kolmogorov-Smirnov test.

Continuous variables were described using mean and standard deviation (SD), or median and interquartile range (IQR) as appropriate. Categorical variables were expressed as percentages.

Since no cut-off values was previously defined to identify different levels of SoC, a hierarchical cluster analysis was conducted using the Ward's minimum variance method to identify groups with different level of SoC. At each step, Ward's minimum variance criterion allows to agglomerate two clusters into one such that the within-class variance of the partition thereby obtained is minimum, and the between-class variance of the partition obtained is to be maximized [24]. The Ward's method was used to identify groups on the basis of the scores obtained on the items of the SoC scale. The optimal number of clusters was selected through the visual examination of the dendrogram. To confirm the goodness of the identified clusters, the association of the distribution of the scores of each subscale of SoC, as well as of those of the entire SoC scale, was assessed using ANOVA test or Kruskal-Wallis, as appropriate.

According with the theorical models on Salutogenesis [25], bivariate analyses were conducted to investigate the association between the SoC and the following potential predictors: sex (male, female), age (<=median value, >median value), country of origin (Italy, others), SSS (low, medium, high), satisfaction for the financial situation (not sufficient, less sufficient, sufficient, completely sufficient), parents as primary financial support for study (yes, no), employment as primary financial support for study (yes, no). In particular, ANOVA or Kruskal-Wallis test were used to test association with respect to SoC scores – either as subscales or as total scale – while Fisher's exact test was used with respect to SoC categories according to the results of the cluster analysis.

Then, multivariate analyses were conducted, including – as independent variables – the potential predictors with statistically significant associations with SoC at the univariate analysis (sex, age, country of origin, SSS, satisfaction for the financial situation, employment as primarily financial support for study). In particular:

- four different models of multivariate linear regression were performed, considering respectively the score at comprehensibility (model 1), manageability (model 2), meaningfulness (model 3) subscales, and at the total SoC scale (model 4) as dependent variables;
- a multivariate multinomial logistic regression (an extension of binary logistic regression that allows for more than two categories of the dependent or outcome variable) was performed to assess the predictors of SoC as categorical dependent variable (i.e., the three categories identified using the cluster analysis).

For all the analyses, the alpha level was considered as significant at 0.05. The analyses were performed using IBM SPSS 27.0 and Stata.

RESULTS

Descriptive analysis of the sample

As a whole, a convenience sample of 2,996 university students participated in the study and completed the online survey, of whom 68% were female. The median age was 22 (IQR: 20-24; range: 18-70 years).

Ninety-two percent was born in Italy, 1.7% in Albania, 5.8% in other countries (China, Romania, Poland, Cameroon, Russia). Fifteen percent attended a course of study in the Medical or Health Sciences area, 13% in Engineering, 11% in Humanities, 10% in Economics/Statistics, 9% in Architecture/Urban and Environmental Sciences, 5% in Education Sciences, while the remaining 37% were engaged in other training fields. Most of the students (62%, n=1,862) attended a bachelor's degree program, 37% (n=1,111) attended a master's degree course, 1% a PhD, or a post-doc program.

More than 80% claimed to be financially supported by their parents for their studies, 22% gained resources by working during the semesters, 12% reported to work during the summer and winter holidays (i.e., during the semester brake), 10% obtained funding from a scholarship. The SSS median score was 6 (IQR: 5-7; range: 1-10); 13.6% presented a low SSS, 69.1% a medium SSS and 17.3% a high SSS. About 70% (69.7%, n=2,088) considered their financial resources "completely sufficient" (18.9%, n=567) or "sufficient" (50.8%, n=1,521), while 30.3% (n=908) considered their resources "less sufficient" (24.3%, n=728) or "not sufficient" (6%, n=180).

Descriptive analysis of SoC

Figure a available online as *Supplementary Material*, reported the responses to the items included in the SoC scale, while *Table 1* reported the descriptive statistics of each item, subscales, as well as of the entire SoC scale.

Considering the item responses, the lower values (i.e., lower SoC) were reported for the item 9 (unpredictable *vs* predictable; 6.7% of score 0, 14.4% of score 1, mean score: 2.8±1.5), followed by the item 7 (unclear *vs* clear; 6% of score 0, 11.9% of score 1, mean score: 3.7±1.7). On the contrary, the higher values (i.e., higher SoC) were reported for the item 5 (insignificant *vs* significant; 19.7% of score 6, 27.5% of score 5, mean score: 4.1±1.5), the item 2 (meaningless *vs* meaningful; 19.1% of score 6, 29.4% of score 5, mean score: 4.2±1.5), and the item 1 (unmanageable *vs* manageable; 12.8% of score 6, 27.3% of score 5, mean score: 3.9±1.4).

In *Figure b* available online as *Supplementary Material,* the cluster dendrogram of SoC items is reported. Using visual examination, a three-cluster solution was chosen: cluster A (n=861; 28.7%), B (n=1,398; 46.7%) and C (n=737; 24.6%). The score distribution of each subscale, as well as of the entire scale, significantly differed by cluster (Kruskal-Wallis test <0.001). In particular, the scores were lower for cluster A and higher for cluster C (*Figure 1*). In detail, cluster A corresponds to a low SoC, cluster B to a medium SoC, cluster C to a high SoC.

Predictors of SoC

At the bivariate analyses, sex, age, country of origin, SSS level, satisfaction with the financial situation and employment as the primary finance of study were statistically significantly associated with SoC ($p<0.05$), either considering each dimension (comprehensibility, manageability and meaningfulness), the total SoC score, or the cluster classification obtained on the single items (low SoC – cluster A, medium SoC – cluster B, high SoC – cluster C), with the exception of manageability with respect to both sex and employment as the primary finance of study (*Table 2* and *Table a* available online as *Supplementary Material*). In particular, each dimension of SoC (comprehensibility, manageability and meaningfulness), the total SoC score, and the cluster classification obtained on the single items presented consistent results: SoC was higher among males, students ≤22 years old, with higher SSS, higher satisfaction about the financial situation, and no employment as the primary source to finance the study.

Due to the results at the bivariate analysis, the variable "parents as primary financial support" was not included in the multivariate analyses.

Table 3 reported the results at the multivariate linear regression analyses. Considering these results, all the included variables presented statistically significant associations with the outcome variables (i.e., for model 1: score at the Comprehensibility subscale; for model 2: score at the Manageability subscale; for model 3: score at the Meaningfulness subscale; for model 3: total score at SoC scale), at least with one category, with the exclusion of sex and country of origin for model 3. In particular, the scores at each subscale and at the total scale significantly increased among students with medium or high SSS (with respect to those with low SSS), less sufficient, sufficient, or completely sufficient satisfaction with the financial situation (with respect to those who have declared it as not sufficient), and no employment as the primary source to finance the study. Conversely, the score significantly decreased among students older than 22 years (with respect to those younger). Moreover, for models 1, 2 and 4, the scores significantly decreased among female students (with respect to males) and increased among those for whom Italy is the country of origin (with respect to other countries).

Multivariate multinomial logistic regression analysis confirmed the results of linear regression analysis (*Table b* available online as *Supplementary Material*). In particular, the risk relative ratio (RRR) of presenting medium SoC (cluster B) with respect to low SoC (cluster A) significantly increased with increasing SSS, satisfaction with the financial situation, and among those students not declaring employment as the primary financial source of study. Similar results were observed

Table 1
Sense of Coherence (SoC): descriptive statistics of each item, subscales, total scale

Items, subscales, or scale		Mean	SD	Median	IQ range
Single items	SoC1. Unmanageable - Manageable	3.9	1.4	4	3 - 5
	SoC2. Meaningless - Meaningful	4.17	1.5	4	3 - 5
	SoC3. Unstructured - Structured	3.7	1.5	4	3 - 5
	SoC4. Impossible to influence - Easy to influence	3.6	1.4	4	3 - 5
	SoC5. Insignificant - Significant	4.1	1.5	4	3 - 5
	SoC6. Unclear - Clear	3.3	1.6	3	2 - 5
	SoC7. Uncontrollable - Controllable	3.7	1.4	4	3 - 5
	SoC8. Unrewarding - Rewarding	3.6	1.6	4	3 - 5
	SoC9. Unpredictable - Predictable	2.8	1.5	3	2 - 4
Subscales	Comprehensibility	3.4	1.2	3.5	2.5 - 4.25
	Manageability	3.7	1.1	4.5	3 - 4.5
	Meaningfulness	4.0	1.4	4	3 - 5
Scale	SoC	3.7	1.1	3.7	2.9 - 4.4

SD: standard deviation; IQ: interquartile.

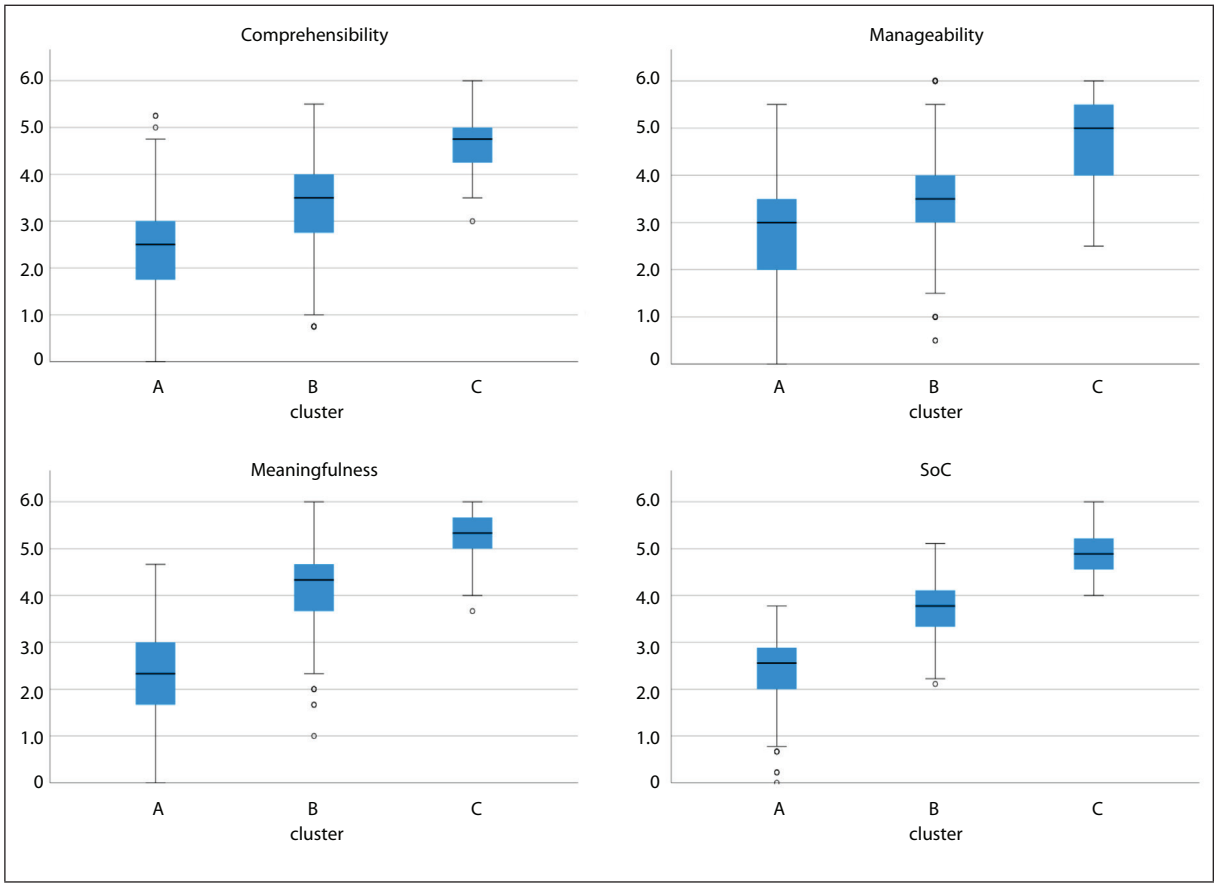

Figure 1
Boxplots of SoC (Sense of Coherence) scores (subscales and total scale) by cluster. Kruskal-Wallis test <0.001 for each subscale, as well as for the total scale.

considering the RRR of presenting high SoC (cluster C) with respect to low SoC (cluster A), although in this case significant associations were observed also for sex and age class (RRR<1 for females and for students older than 22 years old) and the RRR values indicated stronger relationships.

DISCUSSION

Worldwide, the pandemic situation has drastically changed the way of life university students suffered from drastic modifications to their daily life both at university routine as well as at personal level [17-19] and this is particularly alarming if we consider that they represent a group at risk of mental health consequences mainly for the uncertainty of their job perspectives [4] and for their future perspectives. An individual characteristic that could have played a determining role in contrasting negative effect on mental health status during the pandemic is represented by SoC in its three different dimensions [23]. Starting from this point of view, we conducted a cross-sectional study aimed to evaluate the level of SoC of Florence University students and identify its predictors. From our results emerged that lower levels of SoC were reported for the comprehensibility and manageability subscales, either considering the whole sample or stratifying it by sex and socio-economic indicators. Starting from these results, we can argue that information about COVID-19 and the management of personal health during the pandemic have played a relevant role in the university students' perception of life situation and have influenced their SoC especially within the domain of comprehensibility and manageability. In fact, Dadaczynski *et al.* [19] highlighted that a significant proportion of university students may not perceive external information as clear and ordered, revealing difficulties in finding information about COVID-19 and evaluating their reliability. This statement calls to mind a huge problem strongly related to COVID-19 pandemic, that is infodemic [26, 27], meant as the large amount and conflicting information about the coronavirus, that has contributed to mental stress caused by the spread of the pandemic [28, 29]. Nonetheless, the absence of data describing SoC of Florentine university students before the COVID-19 pandemic do not allow us to make concluding remarks. Other studies agreed about the relation between SoC level, socio-economic situation, sex, and mental well-being, generally showing that SoC improves with age and it is negatively associated with female sex, and high levels of stress and bas socio-economic situation [8, 30-32].

We argue that although students maintained high level of meaningfulness and resilience, at the beginning of the second wave of the pandemic (when the survey has been conducted) they did not exactly know how to

Table 2
SoC (Sense of Coherence): subscale and total scale scores by predictors

Variables (predictors)		Comprehensibility mean ± SD; median	Manageability mean ± SD; median	Meaningfulness mean ± SD; median	SoC mean ± SD; median
Sex	Males	3.55 ± 1.17; 3.75	3.81 ± 1.07; 4	3.95 ± 1.38; 4	3.74 ± 10.7; 3.78
	Females	3.36 ± 1.15; 3.25	3.61 ± 1.11; 3.5	3.97 ± 1.34; 4	3.62 ± 1.05; 3.67
	*p-value**	*<0.001*	*<0.001*	*0.951*	*0.002*
Age	≤22	3.52 ± 1.12; 3.5	3.75 ± 1.06; 4	4.05 ± 1.31; 4.33	3.75 ± 1.02; 3.78
	>22	3.30 ± 1.19; 3.25	3.58 ± 1.13; 3.5	3.86 ± 1.39; 4	3.55 ± 1.08; 3.55
	*p-value**	*<0.001*	*0.009*	*0.001*	*<0.001*
Country of origin	Italy	3.46 ± 1.15; 3.5	3.70 ± 1.10; 3.5	3.99 ± 1.35; 4.33	3.69 ± 1.05; 3.78
	Others	2.98 ± 1.20; 3	3.36 ± 0.99; 3.5	3.62 ± 1.36; 3.67	3.28 ± 1.04; 3.11
	*p-value**	*<0.001*	*<0.001*	*<0.001*	*<0.001*
SSS	Low	2.72 ± 1.10; 2.75	3.22 ± 1.06; 3	3.14 ± 1.41; 3.33	2.98 ± 1; 3
	Medium	3.43 ± 1.09; 3.5	3.69 ± 1.08; 3.5	4.0 ± 1.29; 4.33	3.67 ± 0.99; 3.67
	High	3.94 ± 1.18; 4	3.99 ± 1.1; 4	4.48 ± 1.28; 4.67	4.13 ± 1.05; 4.33
	*p-value**	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Satisfaction with the financial situation	Not sufficient	2.63 ± 1.08; 2.75	3.23 ± 1.03; 3.5	3.26 ± 1.43; 3.33	2.97 ± 1.01; 3
	Less sufficient	3.00 ± 1.07; 3	3.47 ± 1.12; 3.5	3.65 ± 1.31; 3.67	3.31 ± 1.00; 3.33
	Sufficient	3.49 ± 1.08; 3.5	3.70 ± 1.05; 3.5	4.03 ± 1.30; 4.33	3.72 ± 0.99; 3.78
	Completely sufficient	4.04 ± 1.12; 4.25	4.02 ± 1.10; 4	4.41 ± 1.31; 4.67	4.16 ± 1.03; 4.33
	*p-value**	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Financial support - by parents	Yes	3.44 ± 1.14; 3.5	3.68 ± 1.10; 3.5	3.95 ± 1.36; 4	3.66 ± 1.05; 3.67
	No	3.33 ± 1.22; 3.25	3.68 ± 1.12; 3.5	4.03 ± 1.33; 4.33	3.64 ± 1.07; 3.67
	*p-value**	0.085	0.865	0.250	0.675
Financial support - employment	Yes	3.29 ± 1.15; 3.2	3.64 ± 1.08; 3.5	3.93 ± 1.34; 4	3.58 ± 1.03; 3.67
	No	3.47 ± 1.16; 3.5	3.69 ± 1.11; 3.5	3.98 ± 1.36; 4.33	3.69 ± 1.06; 3.78
	*p-value**	*<0.001*	*0.313*	*0.355*	*0.015*

SSS: MacArthur Scale of Subjective Social Status; *Kruskal-Wallis test.

manage their current life. This probably happened because of the specific context of pandemic in which they were living. Information about limitations rules and related to health management often appeared as unclear or contradictory; moreover, the laws enacted by the states to face the pandemic leave no room for coping an own strategy, so as to let them feeling unable to address everyday life problems [33-35]. In fact, at the beginning of the summer 2020, the COVID-19 pandemic appeared to be under control, and many restrictions had been dropped. But, starting from the middle of August, an increase in the number of new cases was again observed, especially related to holiday places and recreational contexts, which then gave origin, with the end of September 2020, to the second wave. Future similar evaluations could be useful to assess the effects of the other pandemic waves and, more generally, of long-term exposure to the pandemic, with respect to SoC.

From the multivariate analyses of our data, it emerged that SoC was higher among students who were males, younger than 22 years old, with higher SSS, more satisfied with their financial situation, and who do not declare their employment as the primary source of financing of their study. Our analysis highlighted that the role of socio-economic determinants of SoC are consistent considering the different dimensions of the SoC scale (comprehensibility, manageability, and meaningfulness), the total SoC score, and the cluster classification obtained on the single items.

The economic situation and the link with SoC, female sex and wellbeing have also been investigated in Germany [19], where a low SSS among university students is correlated with low wellbeing, female sex, more health complaints and greater anxiety for the future. The relationship between economic situation and wellbeing appears also in an Australian study [17], after the impact that the pandemic had with students' finances; in fact, since Australian students use to have an employment as a source of money, many of them lost their jobs during 2020 and 2021. A research conduct in Spain showed that the economic status was positively related to SoC, but the relationship between SoC and family support was greater [36].

These data reinforce the idea that SoC is strongly influenced by the context of life and in particular by the socio-economic situation in which an individual was

Table 3
Multivariate linear regression models (n=2,996)

		Model 1 Outcome: comprehensibility			Model 2 Outcome: manageability			Model 3 Outcome: meaningfulness			Model 4 Outcome: SoC		
		coeff	95% CI	p	coeff	95% CI	p	coeff	95% CI	p	coeff	95% CI	p
Sex	Males	ref	-	-	ref	-	-	ref	-	-	ref	-	-
	Females	-0.14	-0.22; -0.06	0.001	-0.17	-0.25; -0.09	<0.001	0.05	-0.05; 0.15	0.343	-0.85	-0.16; -0.01	0.027
Age	≤22	ref	-	-	ref	-	-	ref	-	-	ref	-	-
	>22	-0.11	-0.19; -0.04	0.004	-0.14	-0.22; -0.06	0.001	-0.11	-0.20; -0.01	0.027	-0.12	-0.19; -0.04	0.001
Country of origin	Others	ref	-	-	ref	-	-	ref	-	-	ref	-	-
	Italy	0.18	0.03; 0.33	0.018	0.18	0.03; 0.33	0.018	0.11	-0.07; 0.29	0.228	0.16	0.02; 0.29	0.024
SSS	Low	ref	-	-	ref	-	-	ref	-	-	ref	-	-
	Medium	0.44	0.32; 0.56	<0.001	0.33	0.21; 0.45	<0.001	0.68	0.54; 0.83	<0.001	0.50	0.39; 0.61	<0.001
	High	0.79	0.64; 0.94	<0.001	0.56	0.41; 0.71	<0.001	1.07	0.89; 1.25	<0.001	0.84	0.70; 0.97	<0.001
Satisfaction with the financial situation	Not sufficient	ref	-	-	ref	-	-	ref	-	-	ref	-	-
	Less sufficient	0.28	0.10; 0.45	0.002	0.17	-0.01; 0.34	0.06	0.28	0.06; 0.49	0.011	0.25	0.09; 0.41	0.002
	Sufficient	0.65	0.47; 0.82	<0.001	0.31	0.13; 0.48	<0.001	0.52	0.30; 0.73	<0.001	0.53	0.37; 0.69	<0.001
	Completely sufficient	1.09	0.90; 1.28	<0.001	0.54	0.35; 0.73	<0.001	0.78	0.55; 1.01	<0.001	0.86	0.69; 1.03	<0.001
Financial support - employment	Yes	ref	-	-	ref	-	-	ref	-	-	ref	-	-
	No	0.10	0.01; 0.18	0.030	0.12	0.03; 0.21	0.007	0.18	0.07; 0.29	0.001	0.13	005; 0.21	0.002

CI: confidence interval; coeff: coefficient; ref: reference category; SoC: Sense of Coherence; SSS: MacArthur Scale of Subjective Social Status.

born and grows, besides being unequally distributed among students with different social background. Poor social support has a strong influence on the quality of life of young people and these factors can change the way they take risks and make decisions. In fact, it was observed that the scarcity of resources (poor family and social support, waste of money and time) negatively affects people's stress levels: in this sense, a younger adult with poor resources may be pushed to make worse decisions for his own health and wellbeing [37]. Since the SoC construct is mainly formed in young adulthood and adolescence and improves with age [8], it is important to consider that good socioeconomic status at this stage of life is a good starting point and can contribute to higher SoC, lower stress levels, and better quality of life.

Regarding the association between female sex and low level of SoC, some data suggested that females are more scared by COVID-19 than males [14, 17]. On the other hand, it was observed that in general females pay more attention, are more sensitive and critical in the evaluation of the accuracy of digital information on the new coronavirus and the consequences it generates [35, 38, 39]. Probably, female university students perceived the pandemic situation as more dangerous and complex than males, and this affected their level of SoC in general and, above all, the manageability dimension.

Finally, regarding the role of age on individual SoC, our results suggested that younger students showed higher SoC. Despite this association, in the literature there is not agreement about the role of age on the individual SoC. An interesting point of view is proposed by Nilsson *et al.* [8] that investigated how psychological well-being changed in relation to age. Regarding the association that we found and that emphasized the positive role of younger age, we supposed that older students who were closer to the end of the university pathway, were more worried and stressed about how they will have to manage their life after degree, especially in the context of COVID-19 pandemic.

Our study presented several limitations. All university students were invited to participate in the study but only about 3,000 students decided to participate in the survey; no data on non-respondents are available, so selection bias cannot be excluded. For this reason, the sample could not be certainly considered as representative of the Florentine university students, which limits the generalizability of the results. Finally, cross sectional study design does not allow to interpret the associations as causal. On the other hand, the strong methodological approach in analyzing SoC (also considering classification according to cluster analysis instead) and its predictors using (with different approaches) constitute a strength.

CONCLUSIONS

Despite the huge impact of the pandemic on university students' lives, there are few studies that have investigated their mental health status and personal characteristics to forefront this critical situation. Investigating university students' SoC during the pandemic could allow to better understand how students live and perceive the current situation, as well as the limits and strengths they use to recover from this situation. Additionally, it appears urgent to identify specific groups of university

students that are more at risk of lower SoC and with fewer personal resources to deal with this situation and maintain a propositional perspective.

The results obtained through the present cross-sectional study conducted in a sample of university students from the University of Florence at the beginning of the second wave of the COVID-19 pandemic highlighted the role of demographic and economic determinants of SoC; in particular, older females with lower socio-economic condition presented worse 'trust in life', with possible negative impact on health and well-being.

These results reinforce the need to plan and implement health promotion interventions aimed to support and sustain university students in general and specifically those at higher risk, favoring the improvement of their SoC. In particular, there is a need to act upon those factors that have been identified as relevant in the promotion of university students' SoC level [40].

Furthermore, recently researchers stated that the creation of an enabling environment for youngers through financial support promotes general health status, mental health, decisions, and behaviors [40]. In particular, specific examples of financial support for university students could be tax reliefs, such as tax cuts of university rates.

Regarding the group of students at risk of low SoC, specific intervention to sustain them could involve those who are perceived as influential by others. This strategy seems to be efficient taking into account what are the characteristics of an enabling environment during COVID-19 pandemic that have been identified by the experts [18]. Inviting influential university students to say how they perceive COVID-19 situation and what are the strategy they use to forefront mental stress, and anxiety, and solve problems could be an efficient contribution for others to assume new points of view and perceive the situation differently, less stressful, and more manageable.

In conclusion, this research highlights SoC as another factor affected by the pandemic, critical for health, especially for students in lower SES. It is also another dimension to be considered when planning intervention devoted to university student wellbeing such as EUniWell (https://www.euniwell.eu/).

Acknowledgments

The Authors wish to thank the University of Florence – both the students and the administrative staff – for the interest and the participation.

Ethics

This cross-sectional study was designed and conducted in accordance with the Helsinki declaration and approved by the Ethics Committee of the University of Florence (n. 108, 2020/07/07).

Conflict of interest statement

None.

Received on 9 May 2023.
Accepted on 4 August 2023.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 260-266
DOI: 10.4415/ANN_23_04_04

Estimation of ethanol and methanol exposure through jarred fruit purees

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Abstract

Introduction. Chemicals in foods enter the human body from early life likely posing chronic toxic health risks in the future. This study aimed to estimate the exposure to ethanol and methanol in children consuming an acceptable daily amount of fruit purees.
Methods. Different fruit purees were purchased and measured for methanol and ethanol by using HS-GC. The exposure dose of these alcohols was calculated based on a consumption of 125-250 g of fruit purees in children weighing 7, 12 and 16 kg.
Results. The highest methanol was found in carrot-apple puree (29.07 mg/dL) and ethanol in peach-banana puree (42.07 mg/dL). Daily methanol exposure was estimated between 4.54 and 6.06, and ethanol between 6.57 and 8.76 mg/kg bw.
Conclusions. Our results show higher exposure doses of methanol and ethanol than allowable ones (methanol 2 and ethanol 6 mg/kg/day) in children consuming fruit purees. This should be handled as a public health risk and further comprehensive studies should be enrolled on the chronic toxic effects of food-derived alcohols. Besides, food-derived exposure to toxic chemicals from early life should be more questioned by physicians (in assessing chronic diseases), and related authorities should establish a sustainable, safe, and healthy food production policy.

Key words
- children
- ethanol
- fruit puree
- methanol

INTRODUCTION

Human beings are exposed to various chemical substances through nutrition from the mother's womb. In addition to genetic and phenotypic effects, chemicals taken into the body for a long time, albeit in low amounts, can affect the health status of people throughout their lives. The most important of these are chemicals that are exposed during infancy and childhood when development and growth are rapid. In the healthy and balanced diet of children, ready-made complementary foods mainly based on fruits and vegetables (e.g., jarred baby foods and fruit purees) are often preferred and most of them are consumed from the 4-6 months of life [1]. Infants (0-9 months) require three times more energy than an adult (30-59 years) and eat more food per kilogram of body weight (bw) than adults which make them more vulnerable to toxic effects of hazardous chemicals [2].

Industrial food products designed for infants and children available on markets are regulated by some directives in several countries especially in European Union (EU) to monitor dietary exposure to potentially toxic chemicals in children. These directives include use of additives and materials (coming into contact with foods), presence of contaminants and other toxic substances (e.g., pesticides), and measures about hygiene [3]. However, no provisions about methanol and ethanol as well as some endocrine disrupters in baby foods are available.

Ethanol can be found in foods as a natural fermentation product produced by the action of alcohol dehydrogenase from pyruvate under hypoxic conditions, as well as added to foods as a solvent, preservative and flavoring agent in the food industry. Alcoholic beverages contain more than a certain ratio (>3-5%, v/v) which must be stated on product labels. If the ethanol content is below this specified rate, these products are considered soft drinks or non-alcoholic foods and no labeling about the alcohol content is mandatory [4, 5]. Ethanol is also a commonly used excipient in medications and although its safety limits in pediatric formulations are regulated, some countries question replacing them with alcohol-free formulations since higher ethanol levels than proposed by international guides were found on some pediatric formulations' leaflets [6].

Present data on the effect of ethanol in children is based mainly on acute poisoning cases or studies enrolled in adults or animals. Ethanol may cause acute poisoning symptoms in children at a level of 0.3 g/kg and death at 3 g/kg. There is inadequate data about the effect of low dose ethanol exposure in pediatric population. Committee for Human Medicinal Products (CHMP) proposed not to use traditional herbal medications in children younger than two years, and

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suggested a critical blood alcohol concentration (BAC) of 0.125 g/L at which psychomotor impairment can appear, and calculated a max ethanol dose of 1.5 g for 6 years old (20 kg) child [7]. The blood ethanol should not exceed 1 mg/dL (or 0.01 g/L or a dose of 6 mg/kg) in children less than 6 years old. According to CHMP (questions and answers on ethanol in the context of the revision of the guideline on "Excipients in the Label and Package Leaflet of Medicinal Products for Human Use") French Medicines Agency and Food and Drug Administration (FDA) suggested not to use ethanol in pediatric medicines unless necessary and the World Health Organization (WHO) recommended an ethanol limit less than 0.5% in over the counter products prepared for children under 6 years old [8].

Methanol is a commonly used industrial solvent, and a raw material for the production of several compounds such as formaldehyde, acetic acid, and methyl tertiary butyl ether. It is also frequently utilized as a sample extraction solvent in routine or research laboratories. Dietary methanol is consumed via fruits and fruit-based products, vegetables, fermented beverages and aspartame (artificial sweetener). It is present in plants as both free alcohol and a major component of pectin. Pectin is a gelling agent and contains methyl esters of polygalacturonic acid. The utilization of methyl esterase during industrial food processing leads to the release of methanol by the decomposition of methyl esters. The European Food Safety Authority (EFSA) suggested a reduction of the maximum permitted level (10 g/L) of pectin in baby foods, and moreover, the Scientific Committee on Food (SCF) suggested not to use pectin in baby foods [9]. Daily ingestion of 90% methylated pectin (2,000-2,600 mg/kg bw) via foods can induce significant exposure to methanol (91.6-119.4 mg/kg bw) which may threaten an infant's health. It is considered that all methanol is transformed to formaldehyde and then to formaldehyde acetal which may lead to an increase in the intracellular formaldehyde level at a rate of 70% by 91.6 mg/kg per day methanol. This amount of formaldehyde exceeds the upper limit of natural level (blood steady state concentration: 2.25 mg/L in rats, 2.6 mg/L in human; intracellular level: 12 mg/L in human) [9, 10]. Methanol is also produced endogenously during normal metabolism including the amino acid and methanol metabolism, one carbon pool, P450-linked demethylations, and lipid peroxidation and exist in all body fluids due to its water solubility [10, 11]. It is claimed that methanol is a neurodevelopmental toxicant leading to autistic disorders if ingested by pregnant women, where this toxicity is particularly associated with formaldehyde [12]. Formaldehyde is defined as a class I carcinogen. It can be built in various parts of the body (blood vessels, brain, heart, breast, skin, bone) by the action of aldehyde dehydrogenase 1 (ADH 1) [13]. Formic acid is produced rapidly from formaldehyde (half-life: ~1 min) by the activity of formaldehyde dehydrogenase and transformed to carbon dioxide through the action of formyl-tetrahydrofolate (THF) synthetase and formyl-THF dehydrogenase where THF (coenzyme of folic acid) deficiency may lead to formic acid accumulation and then acidosis [14]. Thus, the oxidation of formic acid varies among individuals (even in different ages) and species (twice as slow in humans compared to rats) depending on the availability of folate [15, 16].

In this study, it was aimed to evaluate whether infants and children are at risk of food-derived chemicals, such as methanol and ethanol. Hence, ethanol and methanol ingredients of jarred fruit purees were measured, and the daily exposure amounts of these alcohols were estimated.

MATERIAL AND METHODS

Chemicals

All chemicals and reagents used in laboratory works were of analytical grade. Methanol and ethanol as standard solutions, and n-propanol as internal standard solution were obtained from Merck. Ultrapure water was obtained from Medical Industrial Systems Minipure (MES Mp) water system (Turkey).

Food samples

Different jarred fruit purees (n=12) under different brand names (n=4, each 125 g) were purchased from supermarkets. The jars were held at room temperature (max: 1h) until opening for alcohol measurements and closed and put into the refrigerator after sampling.

Sample preparation and instrumental analysis

All fruit puree samples (800 µL) were mixed with internal standard (200 µL from 40 mg/dL n-propanol) in a 20 mL headspace vial and analyzed using headspace gas chromatography – flame ionization detector (HS-GC-FID). The instrumental properties of the device are presented in *Table 1*. Every batch (12 samples) of

Table 1
Instrumental properties of headspace gas chromatography

Equipment	Agilent, 7890B GC and 7694E headspace (HS)
Detector	Flame ionization detector (dual detector)
Carrier gas	Helium
Detector gas	Hydrogen and dry air
Column	DB-ALC1 and DBA-ALC2 (30 m / 0.320 mm / 1.80 µm, 30 m / 0.320 mm / 1.2 µm)
Mode	Split (ratio 20:1)
Injection time	1 min
Injection temperature	250 °C
Vial temperature	70 °C
Loop temperature	80 °C
Transfer line temperature	90 °C
Pressure build-up time	3 min
Detector temperature	260 °C
Analysis time	6 min
Thermostatic temperature	80 °C
Thermostation time	15 min

HS: headspace; GC: Gas chromatography; DB-ALC1: Alcohol analysis coloumn1; DB-ALC2: Alcohol analysis coloumn2.

the analysis involved negative and positive control samples at the beginning and at the end. Each sample was measured twice and the mean value was obtained.

Calibration and validation of the method

Standard (pure) ethanol and methanol solutions (diluted with distilled water) at increasing concentrations between 1-100 mg/dL were used for drawing the calibration curves of ethanol and methanol. Matrix based calibration was not possible because of no available reference standard materials for fruit purees. But we prepared a sample pool of fruit purees from different jars and used it as quality control (QC) sample in validation studies. Validation of the method was evaluated by using three different (low, mid, and high concentrated) QC samples by means of linearity (r^2), sensitivity (limit of detection, LOD, limit of quantification, LOQ), repeatability (relative standard deviation, RSD), accuracy (bias), recovery and carryover based on the guideline related to method validation [17]. Repeatability and accuracy were tested by 5 runs in a day (intra-day), and every day up to 5 days (inter-day). Sensitivity was determined by ten-time measurements of the lowest quality control sample in the same batch. Selectivity was evaluated by the runs of mixed alcohol (ethanol, methanol, acetone, isopropyl alcohol, and toluene) spiked QC and blank samples (non-spiked QC and standard solution samples) each with three repeats. Recovery was calculated from the measurements (n=3) of QC and standard samples for three different concentrations. Before using the pooled puree sample its initial (existing) alcohol content was measured and considered during the final calculations. Carryover was tested by repeated analysis of high concentrated (above and near the highest calibration point) spiked samples and a blank sample (n=3) consecutively, where the blank sample should not show any acceptable peak for targeted analytes.

Statistics

Alcohol ingredients of fruit puree samples were determined by HS-GC software tool according to validated calibration curve of the method. Further calculations were performed using MS Excel program. Average daily intake of alcohols via fruit purees for kids weighting between 7 and 16 kg (~4 month-4 years old) was estimated by considering a consumption of one (125 g) and two jars (250 g) of fruit purees daily. Daily exposure amounts of methanol and alcohol were presented as mg/kg bw.

RESULTS

Acceptable values were achieved by method validation studies. The mean bias value was 2.74% for methanol, 4.10% for ethanol, and RSD was 1.36% for methanol, 1.53% for ethanol after intra- and inter-day analysis of QC samples (low, mid, and high concentrated, n=5). LOD and LOQ were found 0.47 and 1.58 mg/dL for methanol, 1.34 and 4.5 mg/dL for ethanol respectively. The method was linear between 2.96 and 94.8 mg/dL with a calibration coefficient (r^2) of 0.9995. No carryover was observed after a high concentrated sample analysis, as well as no interference after analyzing multiple alcohols in the same sample, all analytes appeared separately in their own scheduled time (retention time). Recovery was found between 82 and 112% for three different concentrations. Methanol and ethanol contents of fruit purees, the total methanol and ethanol ingredient of each fruit puree jar and estimated daily exposure levels of children are summarized in *Table 2* and *Table 3*. It is supposed that small kids around 7 kg or 4-6 months old may consume one jar (125 g) of fruit puree in a day, whereas older kids around 12 and 16 kg or 3-4 years old may consume at least two jars daily. Considering these conjectures small kids may consume methanol between 8.01 and 36.34 mg/day (1.14-5.19 mg/kg bw) and ethanol between 6.48 and 52.59 mg/day

Table 2
Methanol ingredients of fruit purees and estimated daily exposure level by consumption of one or two jars based on different body weights (bw)

Fruit puree	Methanol mg/dL	Methanol in one jar (mg)	Daily exposure mg/kg bw (7 kg)	Methanol in two jars (mg)	Daily exposure mg/kg bw (12 kg)	Daily exposure mg/kg bw (16 kg)
1 (plum 1)	7.96	9.95	1.42	19.90	1.66	1.24
2 (peach and banana)	15.35	19.19	2.74	38.38	3.20	2.40
3 (apple)	9.12	11.40	1.63	22.80	1.90	1.43
4 (peach and apple)	10.55	13.19	1.88	26.38	2.20	1.65
5 (apple and banana)	10.05	12.56	1.79	25.13	2.09	1.57
6 (mixed fruits)	7.43	9.29	1.33	18.58	1.55	1.16
7 (apple 2)	6.41	8.01	1.14	16.03	1.34	1.00
8 (banana, mandarin and apple)	7.37	9.21	1.32	18.43	1.54	1.15
9 (plum 2)	6.85	8.56	1.22	17.13	1.43	1.07
10 (apple and pear)	17.67	22.09	3.16	44.18	3.68	2.76
11 (carrot and apple)	29.07	36.34	5.19	72.68	6.06	4.54
12 (apple and peach)	11.51	14.39	2.06	28.78	2.40	1.80

Table 3
Ethanol ingredients of fruit purees and estimated daily exposure level by consumption of one or two jars based on different body weights (bw)

Fruit puree	Ethanol mg/dL	Ethanol in one jar (mg)	Daily exposure mg/kg bw (7 kg)	Ethanol in two jars (mg)	Daily exposure mg/kg bw (12 kg)	Daily exposure mg/kg bw (16 kg)
1 (plum 1)	18.89	23.61	3.37	47.23	3.94	2.95
2 (peach and banana)	42.07	52.59	7.51	105.18	8.76	6.57
3 (apple)	ND	-	-	-	-	-
4 (peach and apple)	5.18	6.48	0.93	12.95	1.08	0.81
5 (apple and banana)	11.59	14.49	2.07	28.98	2.41	1.81
6 (mixed fruits)	7.78	9.73	1.39	19.45	1.62	1.22
7 (apple 2)	5.53	6.91	0.99	13.83	1.15	0.86
8 (banana, mandarin and apple)	8.03	10.04	1.43	20.08	1.67	1.25
9 (plum 2)	15.15	18.94	2.71	37.88	3.16	2.37
10 (apple and pear)	15.70	19.63	2.80	39.25	3.27	2.45
11 (carrot and apple)	ND	-	-	-	-	-
12 (apple and peach)	14.18	17.73	2.53	35.45	2.95	2.22

ND: not detected; -: below the limit of quantification (LOQ).

(0.93-7.51 mg/kg bw), older kids may consume methanol between 16 and 73 mg/day (1.34-6.06 mg/kg bw for 12 kg and 1-4.54 mg/kg bw for 16 kg kids) and ethanol between 12.95 and 105.18 mg/day (1.08-8.76 mg/kg bw for 12 kg and 0.81-6.57 mg/kg bw for 16 kg kids). The highest methanol ingredient was measured in carrot-apple puree (29.07 mg/dL) in which ethanol was not detected, followed by apple-pear (17.67 mg/dL), peach-banana (15.35 mg/dL), and apple-peach (11.51 mg/dL) purees. The highest ethanol was measured in peach-banana (42.07 mg/dL), followed by plum 1 (18.89 mg/dL), apple-pear (15.70 mg/dL), apple-peach (14.18 mg/dL) and apple-banana (11.59 mg/dL) purees. It seems that apple containing purees involve methanol (more content of pectin), whereas banana containing purees involve ethanol (more fermentation during ripening) primarily.

DISCUSSION

Acute methanol exposures of human can pose visual problems (from blurred vision to blindness), neurological symptoms (persistent motor dysfunction), metabolic acidosis, and dermatitis through dermal contact. Chronic methanol exposure (oral or inhalation) can cause gastric problems, visual disturbances, conjunctivitis, blindness, headache, giddiness, nausea, and insomnia in humans [18-20]. When these symptoms are forefront and methanol exposure is evident by the individual's statement necessary treatment and preventive measures can be taken. But chronic methanol intake, in low doses, through nutrition especially beginning from an early age may lead to some health problems or may contribute to the emergence of chronic diseases in older ages where the effect of food-derived methanol is not considered. The industrial production of food, the degradation of pectin in fruits and vegetables, and the hydrolysis of aspartame, a sweetener, release methanol which may pose a potential health risk to human most notably infants and children [9, 21]. A study suggested that the mothers of autistic-born children were exposed to higher dietary methanol (142.31 mg/week) than mothers of non-autistic children indicating intrauterine toxicity of methanol [22].

According to the data of EFSA [21] on the methanol composition based on pectin contents in different foods, fruit and fruit products contain 531 mg/kg methanol which corresponds to 53.1 mg methanol intake for a child consuming 100 g of these foods. Given the methanol content in fruit purees (3,673 mg) evaluated in this study and fruit products mentioned in the EFSA opinion, the maximum daily methanol consumption would exceed the human MADL (maximum allowable dose level, 23 mg/day) suggested by the Office of Environmental Health Hazard Assessment (OEHHA) (https://oehha.ca.gov/proposition-65/crnr/proposed-specific-regulatory-levels-chemicals-causing-reproductive-toxicity) even if children consume these foods in acceptable quantities.

The EFSA Panel estimated the methanol exposure via baby foods as 43.2 mg/kg bw (in case of 1,084 mg/kg bw/day pectin exposure, 95th percentile) for infants younger than 16 weeks and 50% lower exposure for infants older than 16 weeks. This required them to take precautions on reducing the maximum permissible pectin level in special foods for infants and children in order to prevent toxic exposure to methanol [9]. Methanol consumption via fruit purees corresponds to a maximum of 5.19-6.06 mg/kg bw methanol exposure for children weighing 7-12 kg (16 weeks-4 years); these values are lower than those estimated by the EFSA Panel. However, given that infants or children may ingest different types of foods that contribute to the release of different amounts of methanol in their bodies, exposure may rise up to the amounts specified by EFSA. Moreover, the

individual differences in the metabolism of methanol into its toxic metabolites formaldehyde and formate via aldehyde dehydrogenases along with the availability of folate are also important factors in the chronic toxicity of methanol [16]. ADH, in humans, is the main actor in oxidation of alcohols (ethanol and methanol) especially in low dose exposures. Children below 5 years old have reduced ADH activity. It is claimed that infants have 20-50% of adult ADH activity indicating that some infants may use catalase to eliminate methanol especially in high exposures [23, 24]. But little is known about the metabolism of methanol in infants since catalase has not been observed in adult methanol metabolism. Several studies in rats, mice, and monkeys have shown some cellular effects of methanol, especially in liver and brain tissues after 90 days of chronic exposure [25-28]. Some carcinogenicity studies in animals revealed increased incidence in cancer diseases involving ear duct, bone, and hemolymphoreticular tumors [25] .

The endogenous blood methanol level is considered to be below 0.25 mg/dL. The Environmental Protection Agency (EPA) (https://iris.epa.gov/static/pdfs/0305tr.pdf) recommended an oral reference dose of 2 mg/kg methanol per day for humans. Considering this data, approximately half of the fruit purees evaluated in this study exceed the allowable methanol dose when eaten in specified amounts by children. CPMP used the Widmark equation to make approximate assumptions on pediatric blood alcohol [8]. So, the same formula may also be applied to methanol having similar pharmacokinetic properties. A 12 kg child consuming 72.68 mg of methanol (carrot-apple puree) will have a blood methanol level of around 1.1 mg/dL, 4 times higher than normal, consistent with previous estimated findings (0.76 to 1.11 mg/dL) for fruit and fruit products [29].

It is known that acute or chronic exposure to ethanol poses a health risk. It affects various systems and can contribute to different diseases such as cancer, diabetes mellitus, cardiovascular and neurological disorders. The main oxidation pathway of ethanol results in the production of acetaldehyde (toxic, carcinogenic) followed by acetate, through reactions catalyzed by ADH and acetaldehyde dehydrogenase [30]. An alternative way uses cytochrome enzymes where oxygen radicals are the by-product and may contribute to oxidative damage [31]. As in methanol, there are genetic variations in ethanol metabolizing enzymes among people even genders and different ages which clarifies why the same amount of alcohol causes different effects among individuals. Our knowledge on ethanol metabolism and ethanol exposure consequences in children are obtained from poisoning cases or studies enrolled in adults or animals [25, 32]. There are some studies that measured the ethanol contents of various nonalcoholic foods or beverages and estimated the exposure amount and discussed their consequences to remark on the topic that nonalcoholic considered foods or products are not alcohol-free. Ethanol ingredients of flavored beverages and soft drinks were found between 0 and 0.096% w/v. This was explained with that ethanol is a commonly used chemical as a carrier of volatiles and flavoring materials in the production of beverages and with artificial flavoring agents containing ethanol [4]. Various fruit juices such as grape, apple and orange juices were found to contain considerable amounts of ethanol, up to 0.77 g/L, and led to an average ethanol exposure of 10.3 mg/kg bw/day which is higher than the critical dose (6 mg/kg bw) accepted for children under 6 years old [33]. In a recent study, a similar result was also found for fruit juices where the blood ethanol concentration they posed was also estimated. The estimated ethanol exposures in children were above the risky level when they consume certain amounts of fruits or fruit juices [29]. Also, in this study, we found that fruit purees, the most preferred food for small kids in many countries, could lead to an ethanol exposure of up to 8.76 mg/kg bw per day considering a minimum consumption amount in children weighing between 7 and 16 kg (*Table 3*). The approximate blood ethanol level of a 12 kg weighing child consuming 105.18 mg ethanol (peach-banana puree) will be 1.46 mg/dL exceeding the suggested normal level (1mg/dL) [8].

Initially, both methanol and ethanol are oxidized with the same enzyme (ADH) which has a greater affinity (20 folds) for ethanol than methanol. In addition to this, children under 5 years have low ADH activity than adults. So, children are more vulnerable to chronic toxic effects of alcohols than older ones. But considering that methanol (more toxic than ethanol) almost exists with ethanol in most of purees evaluated in the current work, its toxic consequences would be reduced by ethanol (due to high enzyme affinity), which is relatively less dangerous. However, methanol derived from food additives would not be prevented by ethanol and metabolized by entering additional pathways (cytochrome P and catalase) and would play role in the occurrence of chronic health problems in children's future life. Assuming that fruit purees are made from ripe fruits, methanol may accumulate by the action of pectin methylesterase and ethanol by the yeast fermentation of sugars during the ripening of these fruits and then may be transferred to kids by nutrition [14].

CONCLUSIONS

Long-term and continuous consumption of fruits and vegetables, especially industrially produced ones, can create more alcohol exposure (namely methanol and ethanol) than expected contributing to gradual cell and tissue damage, and playing an additive role in the etiology of some chronic diseases (autism, liver diseases, cancer, diabetes, neurological disorders, etc.) along with the presence of metabolic and genetic predispositions (e.g., folate deficiency, ADH variations). In this pilot study made on a limited number of samples ethanol and methanol exposure levels of children (7-16 kg or 4 months-4 years) consuming estimated amounts of different fruit purees were evaluated. The results showed that the maximum methanol consumption was 73 mg/day, exceeding the level suggested by OEHHA, and ethanol consumption 105 mg/day, leading to exceed the blood ethanol level recommended by CHMP. Based on this findings and current evidences [34-36] and the different susceptibility of children to certain chemicals present in food products, significant regulations should be put into effect.

In conclusion, we have to pay attention to what and how much food we and our children eat. In order to produce natural and safe foods, especially for infants, and to eliminate chronic food-borne toxicity, authorities should determine food ingredients, make regulations regarding food additives, preservatives and contaminants, and constantly inspect them. In addition, more comprehensive research on industrial foods for infants and children should be performed in future.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

There is no funding associated with the work featured in this article.

Conflict of interest statement

No potential conflict of interest is declared by the Authors. All Authors agree with the final version of the manuscript and approve its submission.

Authors' contributions

MG designed the work, analyzed and interpreted the results, and was the major contributor in writing the manuscript. BB Bayram purchased the samples, analyzed and interpreted the results.

Consent for publication

Not applicable. Our manuscript does not include individual data in any form (images, videos, personal identity) requiring consent to publish.

Received on 27 February 2023.
Accepted on 19 September 2023.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 267-270
DOI: 10.4415/ANN_23_04_05

Exploring the potential of ChatGPT for clinical reasoning and decision-making: a cross-sectional study on the Italian Medical Residency Exam

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Abstract

Background. This study aimed to assess the performance of ChatGPT, a large language model (LLM), on the Italian State Exam for Medical Residency (SSM) test to determine its potential as a tool for medical education and clinical decision-making support.

Materials and methods. A total of 136 questions were obtained from the official SSM test. ChatGPT responses were analyzed and compared to the performance of medical doctors who took the test in 2022. Questions were classified into clinical cases (CC) and notional questions (NQ).

Results. ChatGPT achieved an overall accuracy of 90.44%, with higher performance on clinical cases (92.45%) than on notional questions (89.15%). Compared to medical doctors' scores, ChatGPT performance was higher than 99.6% of the participants.

Conclusions. These results suggest that ChatGPT holds promise as a valuable tool in clinical decision-making, particularly in the context of clinical reasoning. Further research is needed to explore the potential applications and implementation of large language models (LLMs) in medical education and medical practice.

Key words

- ChatGPT
- medical education
- clinical reasoning
- artificial intelligence
- examination performance

INTRODUCTION

In recent years, there has been a growing interest in the application of artificial intelligence (AI) in healthcare. AI technologies have been applied in different fields of medicine, showing promising results [1-3]. AI has the potential to overcome errors made by doctors in decision-making, which are due to lack of knowledge, the so-called "salient distracting clinical features", and irrelevant factors, such as current mood, time since the last meal, or the weather [3, 4]. One promising development in this area is the use of large language models (LLMs), such as ChatGPT, to assist in clinical reasoning and decision-making [5].

ChatGPT is a general LLM developed by OpenAI, an organization founded in December 2015, with the primary objective of promoting responsible and beneficial applications of artificial general intelligence for society, that has been trained on a massive corpus of text data from the internet via reinforcement and supervised learning methods. Unlike traditional rule-based systems, LLMs can process natural language input and generate output similar to human-generated text (https://openai.com/about). ChatGPT, specifically, has garnered significant attention due to its ability to perform a diverse array of natural language tasks, and exhibit evidence of deductive reasoning, the chain of thought, and long-term dependency skills (https://openai.com/).

Although initially it seemed that research on ChatGPT's role in clinical settings was sparse, recent literature indicates a burgeoning interest in this area. Studies like Liu *et al*. [6] highlighted the utility of ChatGPT in routine clinical practice. At the same time, Ferdush *et al*. delved into the broader implications, applications, and limitations of ChatGPT in clinical decision support [6, 7]. Particularly notable is the work by Alessandri Bonetti *et al.*, which specifically investigated ChatGPT's performance on the Italian Residency Admission National Exam, drawing comparisons to a vast cohort of medical graduates [8].

The purpose of this study is to determine its potential as a tool for clinical decision-making support, by assessing the performance of ChatGPT on a test performed by graduated medical students to become medical resi-

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dents (the Italian State Exam for Medical Residency (SSM) test). Specifically, we aimed to evaluate the accuracy of ChatGPT responses to questions from the SSM test, and compare its performance to that of medical doctors who had taken the test in 2022.

METHODS

Artificial intelligence

ChatGPT is a language model developed by OpenAI (https://openai.com/blog/chatgpt). It uses advanced self-attention mechanisms and a vast corpus of training data to generate natural language responses in a conversational context. ChatGPT is especially adept at handling complex dependencies over long distances and can produce coherent and contextually appropriate responses. While it has been trained on vast amounts of text data from the internet, it operates in a standalone mode once trained. This means that ChatGPT cannot actively access or browse the internet post-training. All responses it generates come from its internal knowledge, based on the data it was initially trained on, until its last update in 2021. Thus, any insights or information it provides reflects its training data and not from real-time online searches (https://openai.com/blog/chatgpt). Consequently, all responses are generated internally, based on the abstract relationships between input words in the neural network.

Dataset

The Italian State Exam for Medical Residency (SSM) is a comprehensive standardized testing program covering all topics in physicians' fund of knowledge (https://www.universitaly.it/). The difficulty and complexity of questions in the SSM test are highly standardized and regulated, making it an ideal input substrate for AI testing. A total of 140 publicly-available multiple-choice practice questions were obtained from the official website of the SSM released in July 2022, which ensured that all inputs represented accurate out-of-training samples for the GPT-3.5 model. To verify this, a random sample of questions was checked to ensure that none of the answers, explanations, or related content were available on Google before January 1, 2022, representing the last date accessible to the ChatGPT training dataset. Any questions containing visual assets such as clinical images, medical photography, and graphs were removed (questions 13, 83, 84, 85), resulting in 136 items available for encoding. Questions were classified into two categories: clinical case (CC) and notional question (NQ). Two research operators blindly assigned the test questions to one of these categories and a third one resolved discordant assignments. All the researchers involved in this task are licensed physicians. A total of 17 items (12.5% of the dataset) required arbitration. The final dataset consisted of 136 questions, of which 83 NQ questions and 53 CC questions.

Input and output

Questions were formatted into single multiple-choice answers. A new chat session was started in ChatGPT for each entry to reduce memory retention bias. In case of elusive, unclear answers, a single attempt was made to force the AI to answer with one of the options available. The input phrase "Answer with the correct option only" was used to do so. It was coded as incorrect if the answer was still elusive or unclear. Given that the SSM test is written in Italian, all the inputs were submitted in Italian on March 6, 2023.

Statistical analysis

Firstly, AI outputs were dichotomized (1=correct; 0=incorrect). Then, the overall score, clinical case score and notional question score were calculated. Adjusted score on a 140-point scale to compare overall results was calculated with the criteria of the official SSM test, awarding 1 point for each correct answer and -0.25 for the incorrect ones. The overall scores of the medical doctors (MDs) were anonymously retrieved from the official ministerial website. A descriptive analysis of the data was performed. AI score was compared to the mean and median scores of medical doctors who took the same test in 2022. Percentile distribution was calculated to locate the AI-adjusted overall score.

RESULTS

ChatGPT answered 90.44% of the questions correctly. It scored slightly higher on clinical cases compared to notional questions (92.45% *vs* 89.15%). The score adjusted on a 140-point scale was 123.27 following the SSM test criteria of evaluation. A detailed description of the AI answer scores is provided in *Table 1*.

Regarding the distribution of MDs scores, the mean value was 79.42/140, and the median value was 80.75/140 out of a total population of 15,869 participants. The quartile distribution is provided in *Figure 1*.

Analyzing percentile distribution, ChatGPT scored higher than 99.6% of the MDs who took the SSM test in 2022.

DISCUSSION

The present study investigated the feasibility of using ChatGPT as a clinical reasoning and decision-making tool. ChatGPT performance on the SSM test, a standardized assessment for evaluating clinical reasoning skills and medical knowledge in medical doctors entering residency programs, was assessed.

Table 1
Descriptive statistics of ChatGPT answers to the SSM test

	Overall score	NQ score	CC score	Adj score by SSM criteria	Adj score by SSM test criteria on a 140 scale
ChatGPT answers	123/136 (90.44%)	74/83 (89.15%)	49/53 (92.45%)	119.75/136 (88.05%)	123.27/140 (88.05%)

NQ: notional question; CC: clinical cases; SSM: Italian State Exam for Medical Residency.

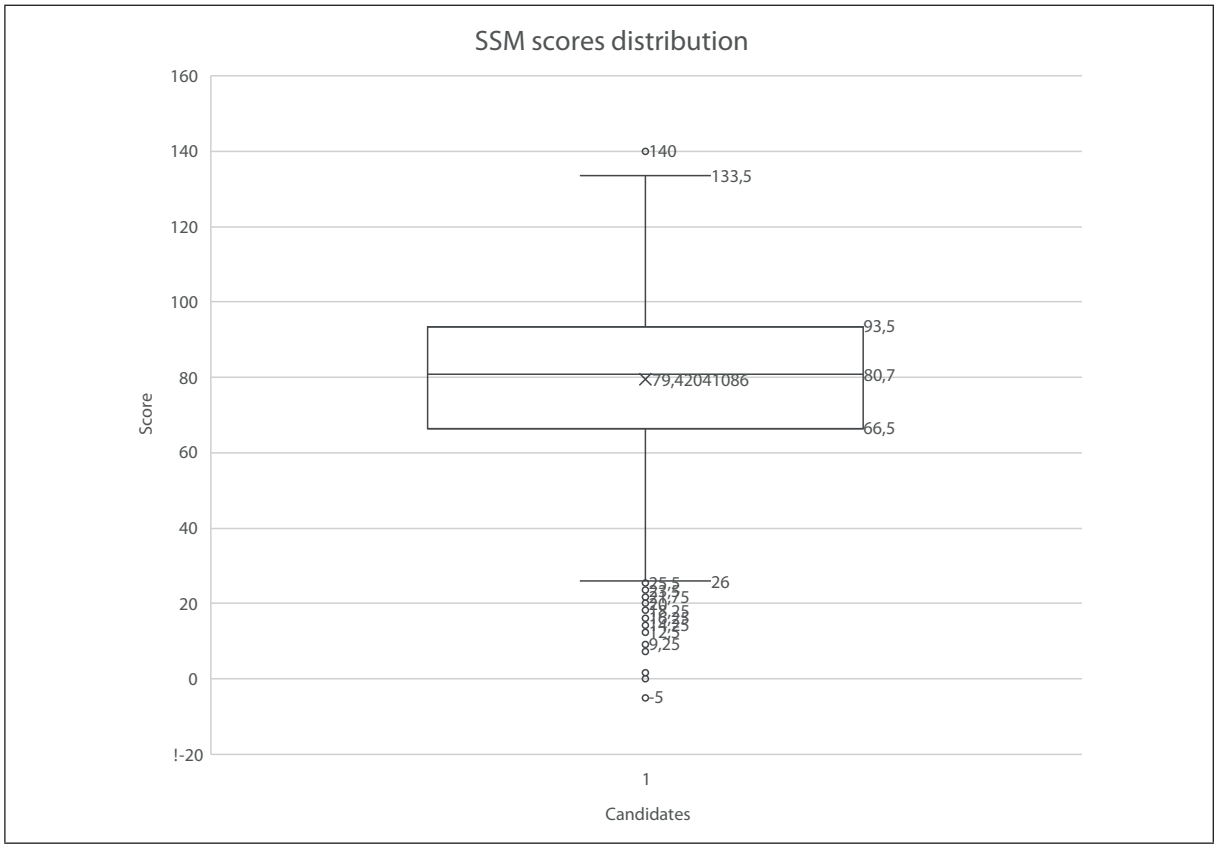

Figure 1
Quartile distribution of SSM test score in 2022. SSM: Italian State Exam for Medical Residency.

Our results demonstrated that ChatGPT achieved an overall score of 90.44% on the SSM test, higher than other studies on different datasets. A study conducted by Gilson *et al*. examined ChatGPT's abilities in the medical field by evaluating its performance on the NBME-Free-Step-1 dataset, which is a part of the USMLE (United States Medical Licensing Examination) [9]. According to their report, ChatGPT exceeded the 60% threshold, which is equivalent to a passing score for a third-year medical student. Additionally, the study highlighted ChatGPT's proficiency in providing logical and informative context for most of its responses. These findings suggest that ChatGPT has the potential to be a valuable medical education tool, capable of enhancing and potentially transforming the learning process. Compared to our findings, the improved performance on the SSM test can be determined by the type of questions, the language in which the test was administered, variations in exam structure, such as the balance between multiple-choice and descriptive questions, and the different region-specific fine-tuning of ChatGPT.

This high level of accuracy is particularly encouraging given the complexity of the SSM test, which requires to integrate knowledge from multiple sources, making diagnostic decisions, and prioritizing patient care. Compared to the doctors' results of the 2022 test, ChatGPT achieved a higher score than 99.6% of the participants.

In a recent study by Bonetti *et al*. [8], ChatGPT was found to correctly answer 122 out of 140 questions on the Italian Residency Admission National Test, positioning it in the top 98.8th percentile among 15,869 medical graduates. Notably, they observed errors in ten questions evaluating direct basic science medical knowledge and in eight questions gauging applied clinical knowledge. Logical errors appeared in two instances, while informational errors were more frequent, noted in 16 instances. These comparative insights from the two studies, both based on the Italian Residency Admission National Test, underscore the remarkable potential of ChatGPT in medical examinations and clinical decision-making.

Given the performance results of ChatGPT on clinical case scenarios, and the emerging evidence of AI applications [10, 11], LLMs have been proven to have the potential to be an effective support for physicians in clinical decisions. However, to become valuable and helpful tools in support of healthcare professionals, LLMs should be tested and validated, and the sources with which ChatGPT constructs the answers must be clear and evident. Moreover, ChatGPT has limited knowledge of the world and events after 2021, with the risk of a lack of information about innovation in clinical practice and diagnosis.

ChatGPT is a useful tool for quickly summarizing the latest medical knowledge, echoing the philosophy of Evidence-Based Medicine but with greater immediacy. While its prowess in quickly synthesizing information is undeniable, over-reliance on these tools may

lead to the solidification of certain medical practices, especially in contexts that foster defensive medicine. As underscored by Beaulieu-Jones *et al.*, while AI aids in decision-making, the physician's adaptive judgment and experience remain irreplaceable, ensuring nuanced and dynamic clinical decisions [12].

This study has some limitations. Firstly, the sample size is relatively small, as the AI was tested only on the SSM test, including 136 multiple-choice questions. Secondly, the test was written in Italian, and the results may not be generalizable to other languages. Finally, the study only evaluated ChatGPT performance on the SSM test and did not evaluate its potential in every area of medical knowledge.

CONCLUSIONS

This study provides preliminary evidence that ChatGPT has the potential to support clinical decisions, particularly in the context of clinical reasoning and decision-making. The results show that ChatGPT achieved an overall accuracy of 90.44% on the SSM test, which is a promising indication of its ability to handle complex medical concepts and generate contextually appropriate responses. Future studies should focus on understanding the modalities in which LLMs, such as ChatGPT can be implemented in real clinical decision-making scenarios.

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.

Received on 28 June 2023.
Accepted on 22 September 2023.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 271-279
DOI: 10.4415/ANN_23_04_06

Effectiveness of different local actions to control vitamin D prescription in Italy

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Abstract

Introduction. In the last decade, the significant expenditure and consumption increase of vitamin D in Italy led some regions to adopt strategies to improve prescribing appropriateness and contain expenditure.

Materials and methods. Using the statistical analysis method of interrupted time series for consumption and expenditure of cholecalciferol, different types of interventions adopted in four Italian regions and their efficacy were evaluated.

Results. Molise achieved the best results by adopting a health professionals' education program in addition to a prescriber-sanction system. Emilia-Romagna also opted for a medical education strategy, but the results were less relevant due to the lack of penalties. Lazio obtained a slowdown in consumption growth by targeting on the utilization of lower-cost per defined daily dose (DDD) packs and adopting a therapeutic plan. Sardinia showed a decrease in expenditure by adopting a target threshold of lower-cost formulation.

Conclusion. The reimbursement of the lowest-cost packs within the National Health Service (NHS) undoubtedly influences spending trend, but it does not solve prescriptive inappropriateness.

Key words

- cholecalciferol
- expenditure
- intervention
- prescriptive appropriateness
- interrupted time series

INTRODUCTION

Vitamin D's role in bone tissue physiology has long been known, specifically in promoting formation and mineralization processes [1].

In the clinical context, there is a general agreement that vitamin D contributes to bone health and, when combined with calcium, if indicated, protects against bone demineralization, especially in the elderly [2-5]. Several observational studies on cardiomyopathy, neoplasms, and degenerative diseases found a worse health status in people with low 25-OH vitamin D levels. These findings prompted the development of randomized clinical trials (RCTs) to evaluate the potential extra-skeletal effects of vitamin D supplementation. However, the results of numerous RCTs did not confirm these assumptions but outlined ineffectiveness areas of vitamin D supplementation, especially in oncology and cardiology. In these studies, despite the use of relatively high doses (2,000 IU/day and 100,000 IU/month), the treated people did not have advantages in preventing events compared to the placebo group [6-10]. Furthermore, the absence of unique indications for defining the vitamin D dose to be prescribed and the lack of a shared results' interpretation of the 25-hydroxyvitamin D blood assay have raised important critical issues related to vitamin D [11-18]. There is an ongoing debate in the scientific community regarding which serum level of 25-OH-cholecalciferol should define vitamin D deficiency and whether to carry out a preventive supplementation. Normal levels of 25-OH vitamin D also do not have univocal values in the different international guidelines, generating further confusion [19, 20].

In Italy, since 2005, vitamin D and analogues have been subject to medical prescription and reimbursed by the NHS for treating and preventing vitamin D deficiency.

However, in the last few years, vitamin D consumption has steadily increased to the point that the Italian Medicines Agency (AIFA) has assessed vitamin D as a

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drug that requires extra oversight in terms of expenditure, consumption and clinical evidence in 2014. The observed increase in prescriptions suggested inappropriate prescriptive phenomena, primarily associated with its use in extra-skeletal diseases [21-23].

Therefore, between 2015 and 2017, targeted measures were adopted in some Italian regions to promote appropriate use and/or to control cholecalciferol's expenditure, the most prescribed drug. These implemented interventions can be classified into three main categories: 1) healthcare professional education program, 2) prescriptive appropriateness intervention, and 3) expenditure control and rationalization measures.

The first group includes information campaigns aimed at general practitioners to improve the appropriateness of medicines prescriptions, such as audits, dissemination of fact sheets about how medicines are used in clinical practice, or in-depth courses. The second group includes the compilation of treatment data sheets or the presence of a regional committee authorizing the therapy. The third category includes measures of expenditure control and rationalization to promote greater use of lower-cost packs.

This analysis aims to describe the different types of intervention implemented to regulate cholecalciferol prescription in four Italian regions (Lazio, Molise, Emilia-Romagna, and Sardinia) at different times and to verify whether their adoption was associated with a statistically significant change in the trend of consumption and expenditure. The analysis also provides an overview of spending and prescribing vitamin D and analogues in Italy from 2013 to 2019.

MATERIALS AND METHODS

Data

The data analyzed were retrieved from the Medicines Utilization Monitoring Centre (OsMed) flow for the regions of Lazio, Molise, Emilia-Romagna, and Sardinia. OsMed is an information flow of pharmaceutical prescriptions, established according to Italian Law 448/1998 and provided through pharmacies affiliated with the National Health Service (NHS), records the number of packs, the total expenditure, and consumption of reimbursed drugs in Italy stratified by region and month.

The active substances considered for this analysis were selected using the Anatomical Therapeutic Chemical classification at the fifth level (ATC V) in the pharmacological class of vitamin D and analogues of the marketed compounds commonly used in clinical practice: ergocalciferol (ATC A11CC01), dihydrotachysterol (A11CC02), alfacalcidol (A11CC03), calcitriol (A11CC04), cholecalciferol (A11CC05), calcifediol (A11CC06). Cholecalciferol is also available in fixed-dose combinations with calcium (A12AX). The consumption was expressed by defined daily dose (DDD) which represents the maintenance dose per day of therapy, in adult subjects, related to the main therapeutic indication of the substance (therefore it is a standard unit and not the recommended dose for the single patient). The DDD used to calculate cholecalciferol consumption is 20 mcg, according to the one chosen by WHO [24].

Expenditure and consumption data stratified by region, month, and active substance were analyzed for the period 2013-2019.

Statistical method

Monthly data about cholecalciferol expenditure and consumption, expressed as DDDs per 1,000 inhabitants/day, have been extracted and analyzed using the Interrupted Time Series (ITS) statistical method [25-29]. Segmented regression models were used, assuming a linear relationship between the dependent variable, (i.e., drug use or expenditure) and the explanatory variable, which was always time. To evaluate the adopted interventions that were implemented, the focus was mainly on consumption data, while outlay data were also analyzed by considering monthly per capita expenditure as a dependent variable.

The method evaluates several parameters before and after the regional actions.

The following model was used to estimate the parameters:

$$y_t = b_0 + b_1 time + b_2 dummy + b_3 time_{post} + b_4 seasonality + v_t \quad (1)$$

y_t represents the dependent variable of monthly cholecalciferol consumption (expressed as DDDs per 1,000 inhabitants/day) or per capita expenditure; b_0 is the value at the beginning of the period; b_1 is the parameter associated with time and measures the overall trend over the period considered (how much the dependent variable average increases, if $b_1 > 0$ or decreases if $b_1 < 0$ each month). The parameter b_2 is associated with a dichotomic variable that is equal to zero before the regional actions and equals to one in the following months, measuring the immediate impact of the intervention on the average consumption level (it indicates how far the line is moved up or down because of the resolution introduction). The parameter b_3 is associated with time starting from regional resolution introduction and measures the effect of these actions, or rather, the trend-changing produced (b_3 value indicates trend changing of the dependent variable as a result of provision introduction. Therefore, the value of $b_1 + b_3$ measures how much the dependent variable each month grows, if $b_1 + b_3 > 0$ or decreases if $b_1 + b_3 < 0$ dependent on the resolution introduction). Finally, the parameter b_4 is associated with the seasonal component and v_t represents the residual component.

A preliminary analysis was performed on the observed data for each region to assess:

- whether it was correct to assume a linear trend as suggested by equation (1) in Model 1, which implies a linear relationship between dependent variable y and explanatory variable t (time);
- whether it was necessary to incorporate a parameter to account for the seasonal component in the model. In all the regions, a systematic decrease in consumption data was observed in August, and hence the parameter b_4 associated with the $dummy=1$ was considered for this month, while it was set to zero for all other months.

To verify the effect of introducing the regional provisions through the evaluation of ITS models, successive steps were carried out, and the parameters of alternative models were estimated.

The AUTOREG procedure of SAS software was used for estimate the parameters, test and control for autocorrelation on the y_t residues that could distort the parameters estimate. The choice of the most suitable model (among A, B, C, D, E, F, G) to explain consumption or expenditure data was based on the Akaike Information Criteria (AIC) index *(see Supplementary Material "Mathematical formulas of statistical models" available online)*.

Every region represents a specific case, both for the type of resolution adopted and for the time of its introduction, therefore separate models for each region were developed and analyzed. Particularly for Emilia-Romagna, Lazio, and Molise, where the resolutions were aimed at improving prescription appropriateness, the analysis focused on consumption data, and the statistical models have been formulated taking into consideration as dependent variable y_t = DDDs per 1,000 inhabitants/day. A second set of models was estimated to assess whether, in addition to an eventual impact on consumption, the regional decisions also led to a reduction in spending, with monthly expenditure per capita as a dependent variable. The dependent variables' logarithmic transformations resulted in a better choice in the model specification. In contrast, for Sardinia, where the resolution aimed at reducing vitamin D medicines outlay, monthly per capita expenditure was considered as the dependent variable, and then consumption data were analyzed. The characteristics of the actions adopted by each region are summarized in *Table 1*.

RESULTS

Overview of expenditure, consumption and prescription of vitamin D and analogues in Italy for the period 2013-2019

The context analysis of NHS prescription of medicines containing vitamin D and analogues showed that in the last years, cholecalciferol, as a single component, was the most prescribed active ingredient in the analyzed period. Its expenditure and consumption also grew exponentially over time. In 2013, cholecalciferol expenditure amounted to 73.1 million euros (1.22 euros per capita) representing 57.1% of all medicines containing vitamin D and analogues outlay. In 2019, this value increased to 281.3 million euros (4.70 euros per capita), representing more than 80% of the expenditure. Its use also steadily grew, from 51.8 DDDs per 1,000 inhabitants/day in 2013 to 159.9 DDDs in 2019, recording a Compound Annual Growth Rate (CAGR) of about 20.7% (see *Table S1* and *Figure S1 in the Supplementary Material available online)*.

The international comparison of the outpatient expenditure of the first ten active substances, published in the National Report on Medicine Use in Italy of 2019 [21], outlined how cholecalciferol is the first drug in Italy. In other European countries analyzed, this drug was ranked between the 37th in Belgium and the 151st in France [21].

In Italy, cholecalciferol is available in three formulations (oral drops, vials for both injection or oral consumption, and oral solution bottle), each with different costs per pack. In 2019, the two bottles pack of 50,000 IU oral solution was the most expensive (14.3 euros), followed by the single bottle pack of 50,000 IU (8.1 euros per pack), the two bottles pack of 25,000 IU (7.8 euros per pack) and by the oral drops pack with dosing syringe (5.4 euros per pack). The analysis of the average cost per 1,000 IU, stratified by formulation and dosage, confirms this trend. However, the 25,000 IU single bottle pack (oral solution) represents the most expensive (0.205 euros per 1,000 IU; see *Table S2* and *Figure S2 in the Supplementary Material available online*).

In 2019, the total number of cholecalciferol packs prescribed was almost 35 million, with bottle packages accounting for about 80% of total expenditure, drop formulations represented 17.3%, and injectable solutions were only 2.9%. Thus, consumption was mainly directed towards packages with a higher average cost per 1,000 IU. Compared to the previous year, this annual regional expenditure and consumption trends of cholecalciferol showed remarkable growth in almost all Italian regions. Campania recorded the highest prescription increase (+34.5%), compared to the national average of +16.2%. Molise was the only region with a reversed trend, showing a decrease in consumption of about 3.4% between 2017 and 2018 and 9% between

Table 1
Intervention adopted by Lazio, Molise, Emilia Romagna and Sardinia in the period 2015-2017

Region	Date of action	Action adopted	Type of intervention
Lazio	July 2017	Prescriptive appropriateness indicator for cholecalciferol with the goal of increasing the prescription of lowest-cost packs to 70% of the total. Introduction of therapeutic plan for prescription in adults and assessment of prescribing appropriateness by a commission for the first prescription.	Appropriateness control and expenditure rationalization.
Molise	December 2017	Introduction of prescribing treatment form for cholecalciferol prescription. Healthcare professionals' education. Sanctioning actions to prescribers.	Appropriateness control and education of healthcare.
Emilia-Romagna	March 2016	Adoption of an information kit.	Education of healthcare professionals.
Sardinia	November 2015	Expenditure indicator with the goal of achieving a target of 70% calculated as a ratio between oral/intramuscular DDDs of 100-300,000 IU formulations and total DDDs.	Expenditure rationalization.

2018 and 2019, probably linked to the effect of the adopted resolution *(see Figure S3 e S4 in the Supplementary Material available online)*

TYPE AND EFFECTS OF REGIONAL INTERVENTIONS

Lazio

In July 2017, the authorities of the Lazio region introduced a prescriptive appropriateness indicator for cholecalciferol, with the goal of increasing the use of lowest-cost packs per DDD. The regional objective was to increase the prescription of lowest-cost packs to 70% of the total. Moreover, a therapeutic plan was implemented for cholecalciferol prescription in adults to optimize its use in this population group [30]. The therapeutic plan had to be drawn up by clinicians, and a special commission had to evaluate each patient's first prescription appropriateness. This provision can be defined as based on both appropriateness control and expenditure rationalization.

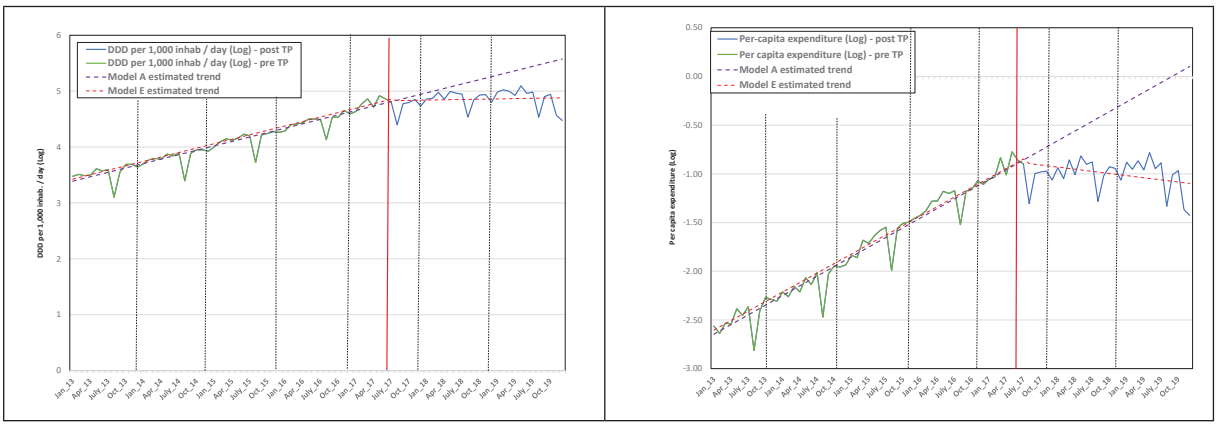
For the period 2013-2019, the monthly consumption data expressed as logarithms follow an approximately linear trend. Based on the AIC index, E is the best model explaining real consumption data. It shows a reduction in use and a stabilization trend compared to the data observed before the provision *(Figure 1)*. The monthly data of per capita expenditure, expressed in logarithm, were used to conduct the same analysis. Evaluating the expenditure trend and the AIC index, model E best represents the data after the intervention. The expenditure model also underlined a positive effect of the Lazio measure, which caused a decreasing trend. The results highlight a slowdown in consumption growth, moving from a monthly increase of 2.6%, expressed as DDDs per 1,000 inhabitants/day before the regional action, to about +0.03 after it. This trend corresponds to a monthly expenditure stabilization from +3.3% to -0.7%, respectively, before and after the regional provision.

Molise

In December 2017, Molise transposed the guidelines about vitamin D supplementation, integrating those of the Society for Osteoporosis, Mineral Metabolism and Bone Diseases (SIOMMS) regarding the prevention and treatment of vitamin D deficiency. The region also introduced a prescribing treatment form for cholecalciferol prescription [31] with the aim of regulating, decreasing, and optimizing cholecalciferol use, combining a healthcare professionals' educational program with a prescriptive appropriateness control intervention. The added value of Molise's intervention is the provision of sanctioning actions committed by the director of the regional health government against non-compliant prescribers. Using the logarithmic transformation of the variable under study (DDDs per 1,000 inhabitants/day), the exponential curve becomes almost linear. Based on the AIC index, model G best explained the consumption data, showing not only a decrease in the use average level, but also a change in the direction of drug consumption which decreases *(Figure 2)*. The same analysis was carried out on per capita expenditure monthly data for the period 2013-2019, expressed as a logarithm. By comparing the various models, model E was found to be the best fit for the data, and all variables included in the model are statistically significant. This model underlines the positive effect of the regional intervention, which caused a lowering of the average level and a reversal trend after the introduction of guidelines and the regional form. It also highlights a marked effect on consumption, going from a +3% monthly use increase, expressed as DDDs per 1,000 inhabitants/day before the regional measure, to a negative value of about -1.1%. Similarly, the monthly per capita expenditure trend moves from +3.6% to -2.7%, respectively, before and after the regional resolution.

Emilia-Romagna

In March 2016, Emilia-Romagna adopted and disseminated an information kit about the role of vitamin

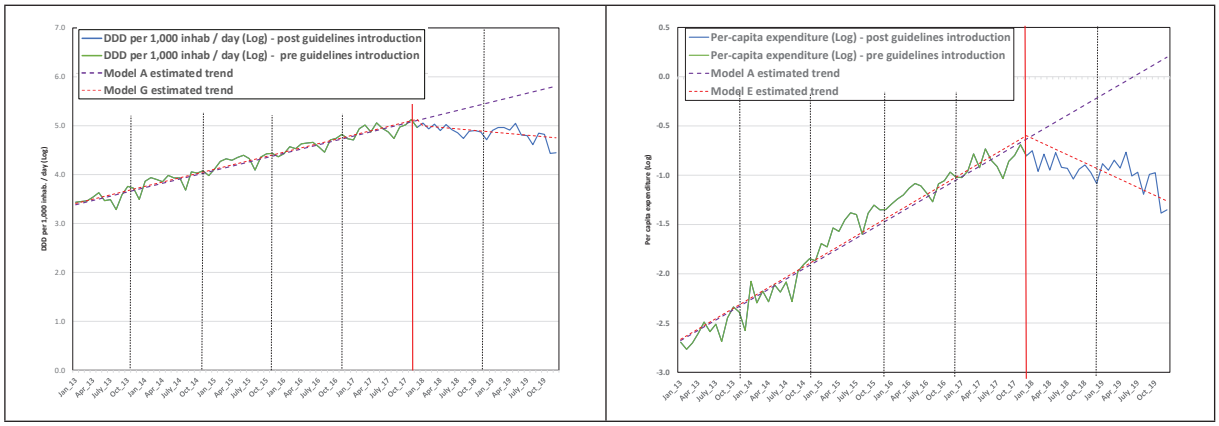


(a) (b)

Figure 1

Lazio - Comparison between real data and trend estimated by models on consumption (DDD per 1,000 inhabitants/day) and per capita expenditure.

The green and the blue line show the real tendency of DDDs per 1,000 inhabitants/day (a) or per capita expenditure (b) for the period between 2013-2019. The red vertical line indicates the timing of the introduction of therapeutic plan (TP) in July 2017. The dashed lines show the trends according to statistical models before (purple line) and after (red line) the regional action.



(a) (b)

Figure 2

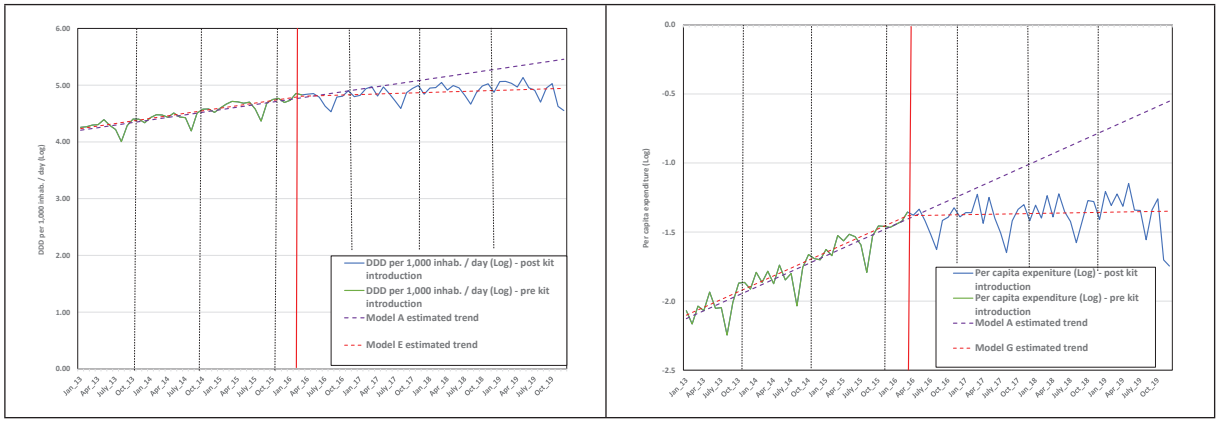
Molise - Comparison between real data and trend estimated by models on consumption (DDD per 1,000 inhabitants/day) and per capita expenditure.

The green and the blue line show the real tendency of DDDs per 1,000 inhabitants/day (a) or per capita expenditure (b) for the period between 2013-2019. The red vertical line indicates the timing of the introduction of guidelines form for cholecalciferol prescription in December 2017. The dashed lines show the trends according to statistical models before (purple line) and after (red line) the regional action.

D in clinical practice, aiming to evaluate and compare available effectiveness evidence. The topics of clinical interest addressed particularly the best behavioral advice to give clinicians, when to use blood assays are necessary and how they should be, at what levels should be started, which kind of medicines should be used, and what treatment patterns are most appropriate. Through the diffusion of the information kit, the region intended to provide some useful indications based on scientific evidence for a better vitamin D and analogues prescription, with the objective to reduce and optimize consumption [32]. This measure was focused on educating healthcare professionals. Using the logarithmic transformation of the variable under study (DDDs per 1,000 inhabitants/day), the exponential curve becomes almost linear. Based on the AIC index, the most suitable model for explaining consumption data is E. It underlines that after the information kit diffusion, the cholecalciferol use has been reduced and shows a stabilization trend. Monthly per capita expenditure data were converted into logarithms and used for the same analysis. Model E is the best data set model, where all the evaluated variables are statistically significant. It shows how, after the information kit introduction, there is a similar effect on consumption trend *(Figure 3)*. The results show a stabilization of consumption and expenditure. In fact, the growth moved from an increase of about +1.5% DDDs every month, before the information kit's diffusion, to a value of about +0.3% monthly after the intervention. Furthermore, the per capita expenditure trend changed from an increase (+1.9%) to a constant value (+0.1).

Sardinia

In November 2015, Sardinia approved a regional intervention defining specific actions to rationalize and contain pharmaceutical expenditure and improve pre-



(a) (b)

Figure 3

Emilia-Romagna - Comparison between real data and trend estimated by models on consumption (DDD per 1,000 inhabitants/day) and per capita expenditure.

The green and the blue line show the real tendency of DDDs per 1,000 inhabitants/day (a) or per capita expenditure (b) for the period between 2013-2019. The red vertical line indicates the timing of the adoption of the information kit in March 2016. The dashed lines show the trends according to statistical models before (purple line) and after (red line) the regional action.

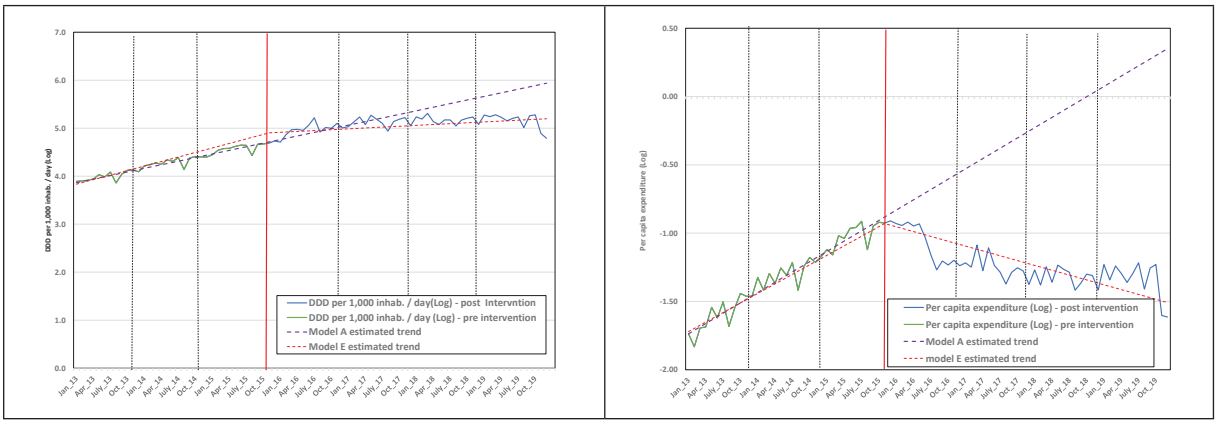
scribing appropriateness of vitamin D. The resolution established that each Local Health Unit should achieve a specific target value higher than 70% by the end of 2016, calculated as a ratio between oral/intramuscular DDDs of 100-300,000 IU formulations and total DDDs [33]. The provision clarifies that "the goal aimed to limit inappropriate use of cholecalciferol is to contain the user level of formulations different from the intramuscular or oral vials of 100/300,000 IU, within 30% of total dispensed doses". The lower annual gross expenditure was estimated at 1.5 million euros, corresponding to about 1.2 million of NHS net expenditure. The intervention aimed to reduce the higher unit price prescription of cholecalciferol and promote the use of lower-cost packs. Aggregate expenditure data indicated an effect of the intervention, showing an increasing trend until 2015 and then a marked decrease from 2016.

Unlike other regions, the Sardinia model was primarily based on cholecalciferol's per capita expenditure data, as the measure was exclusively geared towards reducing outlay. Using the logarithmic transformation of the variable under study (monthly per capita expenditure), the curve from exponential becomes almost linear. Once the linearity was verified through the data series graphic representation, the most suitable model based on the value of the AIC index was chosen. Model E is the best data set model and highlights a reduction in usage trend after the regional resolution approval. The same analysis for consumption data was carried out. Data, expressed as the log transformation of DDDs per 1,000 inhabitants/day, shows an evident impact on use reduction, even if there are fluctuations challenging to explain, particularly since 2016, the period after the intervention approval *(Figure 4a)*.

In summary, the results highlight a remarkable decrease in expenditure due to the regional intervention adopted *(Figure 4b)*. Per capita expenditure moved from a monthly increase of about 2.4% to a reduction of about 1.2%. Instead, consumption values increased from 100.2 DDDs per 1,000 inhabitants/day in 2015 to 175.0 in 2019. However, in the last year, there was a reduction of about 0.7% *(see Table S3 in the Supplementary Material available online)*. Probably, there was a gradual transition to the cheaper formulations (increasing use of oral or intramuscular formulation of 100,000 IU and a great reduction of formulation of 25,000 IU). The consumption analysis *(Figure 5)* shows that since the measure's introduction, there has been a fall in total packs, particularly of 25,000 IU oral solution single bottle packs. The consumption, expressed as total DDDs/1,000 inhabitants per day and stratified by formulation, shows an important growth of 100,000 IU packs (6 vials of injectable/oral solution) and 50,000 IU (oral solution two bottles' packs). Finally, different dosages determine different therapy days (five days for 25,000 packs, ten days for 50,000, and 60 days for 100,000), which could explain the fluctuations recorded.

DISCUSSION

The increase in expenditure and consumption of medicines containing vitamin D and analogues, reimbursed by the NHS, between 2013 and 2019, urged some regional interventions aimed at reducing the observed trends. However, the approach taken by each region was very different and resulting in highly variable outcomes. The analysis shows how Molise achieved the best results. Its educational program for health professionals has reduced both expenditure and consumption of cholecalciferol, although penalty-type regional control may have played a key role. Emilia-Romagna also approved an intervention based on medical education, but the results were less notable, likely due to the lack of penalties. In fact, in this region, there is a stabilization in consumption and expenditure trends, but not a decrease. Lazio introduced a prescriptive appropriateness indicator, targeting the use of lower-cost/DDD packs, and adopted a therapeutic plan for cholecalciferol prescription in adults. This action showed positive results

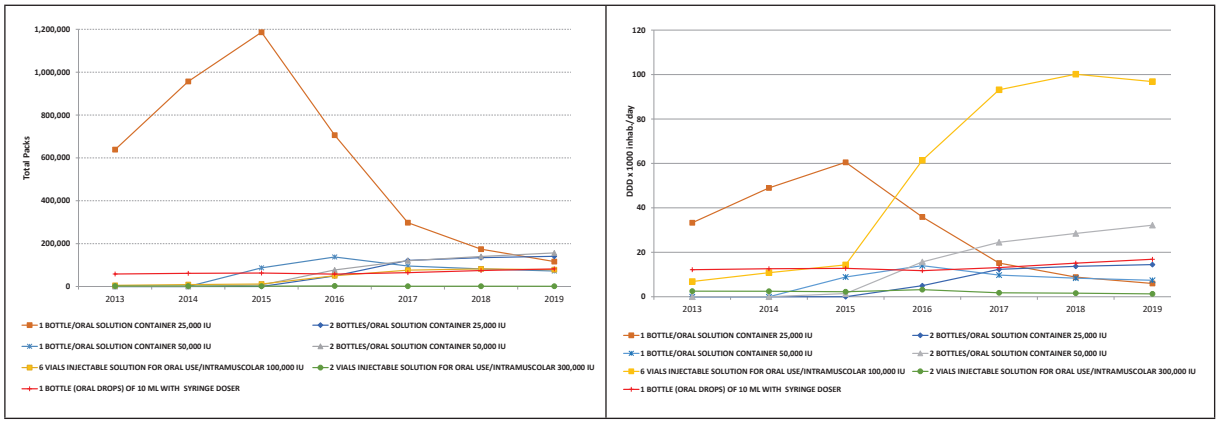


(a) (b)

Figure 4

Sardinia - Comparison between real data and trend estimated by models on consumption (DDD per 1,000 inhabitants/day) and per capita expenditure.

The green and the blue line show the real tendency of DDDs per 1,000 inhabitants/day (a) or per capita expenditure (b) for the period between 2013-2019. The red vertical line indicates the timing of the intervention in November 2015. The dashed lines show the trends according to statistical models before (purple line) and after (red line) the regional action.



(a) (b)

Figure 5
Sardinia - Comparison between consumption and expenditure trend stratified by different formulation (packs DDD x 1,000 inhabitants/day and Total expenditure per pack).
The figure shows the usage trend, expressed as number of packs (a) or DDD per 1,000 inhabitants/day (b), of different formulations of cholecalciferol for the period 2013-2019.

in containing the exponential growth of consumption and stabilizing per capita expenditure. The Sardinia resolution also showed that choosing the lowest cost packages could undoubtedly influence the spending trend, as highlighted by the change in the time course. However, it remains unclear whether this strategy could influence the appropriateness of vitamin D use. In general, there seems to be little mutual learning between regions, and the outcomes do not appear to be conclusive. Therefore, in October 2019, AIFA established the 96 Note to be applied throughout the national territory [34]. The AIFA Notes are a regulatory instrument that defines the drug's therapeutic indications reimbursed by the NHS based on the best evidence of literature efficacy and guarantees appropriateness of use by directing doctors' prescriptions [21].

According to Note 96, the NHS can reimburse medicinal products containing cholecalciferol, cholecalciferol/calcium salts, and calcifediol in two different cases. The first includes institutionalized persons, pregnant or breastfeeding women, and subjects with osteoporosis or osteopathy not eligible for remineralizing therapy, regardless of the 25-OH vitamin D serum levels determination. The second involves patients who need to be identified by a flow chart (Annex 1 of Note 96) to perform 25-OH-cholecalciferol serum assay and then identify the most appropriate drug dose [21, 34]. The Note 96 has been updated based on emerging scientific evidence regarding the lack of effect of vitamin D supplementation in healthy individuals without risk factors for osteoporosis and in the treatment of COVID-19. Some modifications suggested by clinicians and scientific societies have also been included [35]. Moreover, there are certainly persisting issues regarding the appropriate and evidence-based use of vitamin D. It should be noted that the trends analyzed in this study do not consider either supplementation through vitamin supplements or private purchases. It has been estimated that the share of privately purchased medication by citizens reached 20 million in 2019 [22].

CONCLUSION

The article discusses the importance of monitoring the effects of the measures adopted by different regions in Italy (Lazio, Molise, Emilia Romagna and Sardinia) in order to evaluate their effectiveness in inducing important changes in the prescription and expenditure of medicines containing cholecalciferol and understanding which type of action could be the most effective. The findings clearly indicate that the most effective approach involves educating and monitoring prescribers. It is necessary to consider that the costs associated with the various dosages of cholecalciferol are very different, and promoting the use of lower-cost packs could be the milestone to reduce medicines expenditure without compromising patient care, but ensuring the prescriptive appropriateness remains the most important issue to be considered, as showed by Sardinia intervention. Therefore, the prescription appropriateness should be implemented not only to avoid therapeutic abuse and economic waste, but also because prescriber monitoring seems to be a suitable governance mechanism.

The outcomes of regional measures for a single drug class are important in guiding regulatory action nationwide. However, when there is a problem of excess expenditure and consumption involves most of the regions, and only some of them take corrective actions, a central-level intervention is necessary. The AIFA notes have always been an instrument capable of promoting prescribing appropriateness based on the most recent scientific evidence.

Further detailed studies are required to evaluate which types of interventions (regional or national) produce the best results.

Conflict of interest statement

The Authors declare no conflict of interest.

Received on 24 April 2023.
Accepted on 25 September 2023.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 280-285
DOI: 10.4415/ANN_23_04_07

Molecular characterization of *Yersinia enterocolitica* strains to evaluate virulence associated genes

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Abstract

Introduction. *Yersinia enterocolitica* (*Ye*) species is divided into 6 biotypes (BT), 1A, 1B, 2, 3, 4, 5 classified based on biochemical reactions and about 70 serotypes, classified based on the structure of the lipopolysaccharide O-antigen. The BT1A is considered non-pathogenic, while the BT 1B-5 are considered pathogenic.

Methods. Evaluate the distribution of eleven chromosomal and plasmid virulence genes, *ail*, *ystA*, *ystB*, *myfA*, *hreP*, *fes*, *fepD*, *ymoA*, *sat*, *virF* and *yadA*, in 87 *Ye* strains isolated from food, animals and humans, using two SYBR Green real-time PCR platforms.

Results. The main results showed the presence of the *ail* and *ystA* genes in all the pathogenic bioserotypes analyzed. The *ystB*, on the other hand, was identified in all non-pathogenic strains biotype 1A. The target *fes*, *fepD*, *sat* and *hreP* were found in both pathogenic biotypes and in BT1A strains. The *myfA* gene was found in all pathogenic biotype and in some *Ye* BT1A strains. The *virF* and *yadA* plasmid genes were mainly detected in bioserotype 4/O:3 and 2/O:9, while *ymoA* was identified in all strains.

Conclusions. The two molecular platforms could be used to better define some specific molecular targets for the characterization and rapid detection of *Ye* in different sources which important implications for food safety and animal and human health.

Key words

- *Yersinia enterocolitica*
- SYBR Green real-time PCR
- virulence genes

INTRODUCTION

The genus *Yersinia* has been recently classified to *Yersiniaceae* family [1] and comprises of 28 species (https://lpsn.dsmz.de/genus/yersinia), three of which are pathogenic to humans *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis* [2]. *Yersinia enterocolitica* (*Ye*) is Gram-negative and psychrophilic enteropathogen [3]. In fact, *Ye* has an optimal growth temperature between 28-30 °C but is able to growth from 2 and 42 °C [4]. Yersiniosis is usually a self-limiting disease and gastrointestinal symptoms pre-dominate, but extra-intestinal disorders may also appear [5]. Children under 5 years of age, immunocompromised people, and elderly are more likely to get sick and to have a more serious illness [4].

In Europe, yersiniosis is the third most frequently reported foodborne zoonosis in humans with a stable trend in 2015-2019 [6]. In Italy the number of reported cases is very low compared with other EU/EEA member states, because the notification of yersiniosis is voluntary, and the number of cases is probably underestimated [7].

Ye is widely distributed in the environment and pigs are the main reservoir of pathogenic strains, in particular, the bacterium colonized tonsils, lymph nodes and intestines. *Ye* is also isolated from other animals, such

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as cattle, small ruminants, wild animals (e.g., deer) and pets (cats and dogs) [8, 9]. Humans become infected by ingesting undercooked pork contaminated with *Ye*, but also milk and dairy products, vegetables and untreated water [10, 11]. Although pork meat is the major source of pathogenic *Ye*, in recent years, fresh vegetables have been linked to several foodborne outbreaks, particularly spinach and salad [12, 13]. The *Ye* species is highly heterogeneous and is divided into 6 biotypes (BT), 1A, 1B, 2, 3, 4, 5, on the basis of biochemical tests and about 70 serotypes [14]. The biotypes show differing pathogenic potential, in particular: biotype 1A (BT1A) is considered non-pathogenic, biotypes 2-5 weakly pathogenic, and biotype 1B highly pathogenic [15]. Pathogenic *Ye* have historically been defined as carriers of a 70 kb virulence plasmid (pYV), which has genes encoding adhesin A (*yadA*) and a transcriptional regulator gene (*virF*); in addition, at the chromosomal level they harbour virulence genes as *invA* (invasin), *ail* (attachment and invasion locus), *ystA* (stable *Yersinia* toxin A), and *myfA* (mucoid *Yersinia* factor A) [16].

BT1A strains are considered non-pathogenic because they do not have the pYV and some chromosomal virulence genes such as *ail*. However, studies have shown that some biotype 1A strains, particularly those isolated from faeces during a gastrointestinal illness, could be pathogenic [17]. Although BT1A may lack the pYV plasmid, alternative virulence factors, including the thermostable toxin *ystB* and *hreP* [18] may be present. BT1A is the most heterogeneous of the six *Ye* biotypes and includes a wide range of serotypes of which O:5, O:6,30, O:6,31, O:7,8, O:10, as well as not-typable (NT) O strains, are most often reported [19]. The most common bioserotypes causing human yersiniosis in Europe are *Ye* 4/O:3 and 2/O:9 [6].

ISO 10273 (International Organization for Standardization) standard cultural method for the detection and isolation of *Ye* from food samples are laborious and time-consuming to differentiate pathogenic and non-pathogenic strains [20, 21].

To meet the requirement for faster analysis, ISO approved a standard method for rapid identification of pathogenic *Ye* based on detection of the chromosome-localized *ail* gene which is present in all pathogenic bioserotype by real-time PCR [22]. However, recent studies have indicated that in some non-pathogenic 1A strains of *Ye*, the *ail* gene can be detected [23]. Therefore, it would be useful to consider other pathogenicity targets of the strains to have a more complete genomic characterisation. Recently, a study analyzed by real-time PCR the distribution of the *yadA*, *virF*, *inv*, *ystA*, *ystB*, *myfA*, *hreP* and *ymoA* genes in *Ye* strains in order to select useful target genes for assessing the presence of pathogenic *Ye* [18].

The aim of the present study is to extend the examination of the distribution of virulence genes in non-pathogenic and pathogenic *Ye* strains, isolated from animal, food and human samples. Additional molecular targets would allow for a more complete characterization of *Ye* strains, and the evaluation of the distribution of these genes which have virulence potential and are less investigated.

Thus, besides the virulence genes *ail*, *ystA*, *ystB*, *myfA*, *virF* and *yadA*, the other target genes analyzed are: *hreP* (host reactive element), *sat* (streptogramin acetyltransferase), *fepD* (enterochelin transporter ABC), *fes* (enterochelin esterase) and *ymoA* (*Yersinia* modulating protein) [24], using two molecular real-time PCR SYBR Green platforms.

MATERIALS AND METHODS

Identification and typing of bacterial strains

A total of 87 *Ye* strains, 82 isolates from 2005 to 2015 and 5 isolates from 1980 to 1985 in Italy, were identified at biochemical level and subsequently biotyped and serotyped. These strains were isolated from human (n=39), in particular stools (n=24), blood (n=3), appendix (n=1) and unknown (n=11), from animals (n=12), in particular faeces (n=7), amygdala (n=2) and unknown (n=3) and from food (n=36).

Identification of *Ye* species was performed using the API® 20E system (bioMérieux). *Ye* biotyping was carried out according to the ISO 10273 scheme, based on biochemical reactions, in particular: pyrazinamidase and lipase activity, production of indole, production of acids from xylose, trehalose and hydrolysis of esculin. Serotyping was performed using O-antisera for serogroups O:3, O:5, O:8, O:9 and O:27 purchased from Biolife (Biolife Italiana, Milan, Italy).

Genomic DNA extraction

The strains were grown in Tryptone Soy Broth (Biolife Italiana, Milan, Italy) at 30 °C for 24-48h. Two mL of each broth culture was subjected to DNA extraction according to the protocol of Peruzy *et al.* [18]. The extracted DNA was used as a template for real-time PCR.

Real-time PCR-based protocol

For the molecular characterization of *Ye* virulence genes, two SYBR Green real-time PCR platforms were developed with two different thermal profile. The first platform, a PCR reaction in singleplex, containing: 1X SsoAdvanced SYBR Green PCR Master Mix (Bio-Rad), one of the primer (*Table 1*) at different concentrations, of the virulence genes *ail* (250 nM), *ystA* (100 nM), *ystB* (150 nM), *myfA* (300 nM) and 3 µl of DNA in a final volume of 25 µl. The thermal profile was: 95 °C for 5 min, 35 cycles at 95 °C for 10 s and 60 °C for 30 s, followed by a thermal cycle (65-95 °C) necessary for the analysis of the melting curve. The second platform, a PCR reaction in singleplex, containing: 1X SsoAdvanced SYBR Green PCR Master Mix (Bio-Rad, USA), one of the primer (*Table 1*), at different concentrations, of the virulence genes *hreP* (300 nM), and 250 nM of the other virulence genes *virF*, *yadA*, *ymoA*, *fes*, *fepD*, and *sat* and 3 µl of DNA in a final volume of 25 µl. The thermal profile of the reaction was: 95 °C for 5 min, 35 cycles at 95 °C for 60 s, 60 °C for 60 s, 72 °C for 60 s, followed by a thermal cycle (65-95 °C) necessary for the analysis of the melting curve. The specificity of the reaction is given by the detection of the melting temperature (T_m) of the amplification products after the last reaction cycle. The melting curve was visualized with the software MxPro (Mx3005P v 4.00 - Agilent).

Table 1
Primers used for SYBR Green real-time PCR

Gene	Primer sequence (5'->3')	Amplicon size (bp)	Reference
ail	ACTCGATGATAACTGGGGAG	170	[25]
	CCCCCAGTAATCCATAAAGG		
ystA	ATCGACACCAATAACCGCTGAG	79	[26]
	CCAATCACTACTGACTTCGGCT		
ystB	GTACATTAGGCCAAGAGACG	146	[26]
	GCAACATACCTCACAACACC		
myfA	CAGATACACCTGCCTTCCATCT	272	[27]
	CTCGACATATTCCTCAACACGC		
hreP	GCCGCTATGGTGCCTCTGGTGTG	757	[24]
	CCCGCATTGACTCGCCCGTATC		
virF	GGCAGAACAGCAGTCAGACATA	591	[25]
	GGTGAGCATAGAGAATACGTCG		
yadA	TAAGATCAGTGTCTCTGCGGC	747	[28]
	TAGTTATTTGCGATCCCTAGCAC		
ymoA	GACTTTTCTCAGGGGAATAC	330	[29]
	GCTCAACGTTGTGTGTCT		
fes	GCCGGCAGGCACAGCGTAAT	561	[30]
	GGCCAACCCACCCAAAACTT		
fepD	GTGTGATTGCCTTACTATTG	381	[30]
	CGGTCATCCTTTTATTACGG		
sat	CCGATGGTGGGGTTTTCTCAAG	456	[24]
	GGGATTACCGCCGACCACACTA		

RESULTS

The results obtained of serotyping and biotyping of the 87 *Ye* strains have identified four biotypes and several serotype: 1A/O:5 (n=15), 1A/O:8 (n=9), 1A/NT (n=25), 2/O:9 (n=5); 3/O:5,27 (n=1); 4/O:3 (n=32). The results obtained analysing these strains through SYBR Green real-time PCR showed a close association between the *ail* gene, considered an exclusive indicator of pathogenicity, and the *ystA* gene as they were found in all strains belonging to the pathogenic biotypes (*Table 2*). In contrast, *ystB* was found exclusively in all non-pathogenic biotype 1A strains. The presence of the *virF* and *yadA* plasmid genes appears to be exclusive to pathogenic biotypes, found in 42.1% (n=16) of the total of the pathogenic strains (*Table 2*).

The *myfA* gene was detected in all pathogenic strains (n=38), but also in 3 strains of BT1A strains (6.1%). The *fes* and *fepD* genes, which encode for factors capable of capturing and utilizing host iron, were found in synergy with each other in both pathogenic and non-pathogenic biotypes. The *hreP* and sat genes appear present in high percentages in all biotypes analyzed, as is the *ymoA* gene, which is present in 100% of strains (*Table 2*). The distribution of bioserotypes showed the prevalence of 4/O:3 and 2/O:9 mainly in human samples, and 4 of 32 strains of *Ye* 4/O:3 were found in pigs, whereas BT1A was found mainly in a wide variety of food samples, but also, less frequently, in animals and humans (*Table 3*).

DISCUSSION

The results showed a non-uniform distribution of the different target genes in the various *Ye* strains. Chromosomal virulence genes are very important elements that determine the pathogenic capabilities of *Ye*. These include the attachment invasion locus (*ail*), which encodes the outer membrane proteins responsible for adhesion, and is considered the target gene for detection of pathogenic *Ye* according to ISO/TS 18867:2015.

Furthermore, *Ye* has the ability to produce three types of YstI toxins (YstA, YstB and YstC) encoded by *ystA*, *ystB* and *ystC* genes, respectively, which play a crucial role in the origin of diarrhea [31]. As expected, all strains belonging to pathogenic biotypes, analyzed in this work, contain the *ail* gene together with the *ystA* gene. *Ye* BT1A mainly produce the enterotoxin YstB and rarely YstC. The ability of some BT1A strains to cause illness gives indirect evidence that YstB plays an important role in yersiniosis, as suggested in some studies [31, 32]. Indeed, in the present study the *ystB* was detected in 100% of BT1A strains, results in agreement with literature data [24, 33].

The pYV plasmid undoubtedly plays an important role in pathogenicity and carry virulence factors, such as *virF*, a regulatory gene, which encodes the transcriptional activator of several genes, including the plasmid virulence gene *yadA* involved in the *Yersinia* invasion process. However, plasmids are unstable structures, and *Ye* BT1A generally do not harbour plasmids [31, 34].

Table 2
The distribution of genes in *Ye* strains grouped by biotype/serotype

Biotype/serotype (n)	*ail*	*ystA*	*ystB*	*myfA*	*hreP*	*virF*	*yadA*	*fepD*	*fes*	*ymoA*	*sat*
4/O:3 (32)	100%	100%	-	100%	100%	40.1%	40.1%	43.8%	43.8%	100%	96.8%
2/O:9 (5)	100%	100%	-	100%	100%	60.0%	60.0%	100%	100%	100%	100%
3/O:5.27 (1)	100%	100%	-	100%	100%	-	-	100%	100%	100%	100%
1A/NT (25)	-	-	100%	12.0%	72.0%	-	-	96.0%	96.0%	100%	84.0%
1A/O:5 (15)	-	-	100%	-	93.3%	-	-	100%	100%	100%	100%
1A/O:8 (9)	-	-	100%	-	100%	-	-	100%	100%	100%	100%

Table 3
The distribution of different sources (food, animals and human) in *Yersinia enterocolitica* biotypes (BT)

Source (n)	Specific source	BT1A	BT2	BT3	BT4
Animals (12)	Bear	1			
	Bovine	1			
	Sheep	1		1	
	Swine	4			4
Food (36)	Beef	1			
	Chicken meat	4			
	Fish	2			
	Fresh vegetables	2			
	Milk	1			
	Minced meat of beef and pork	3			
	Pork meat	8			
	Ready to eat pork meat	2			
	Ready to eat vegetables	12			
	Sheep meat	1			
Humans (39)	Humans	6	5		28

The results reported in our study confirm that plasmid virulence genes are not present in any strain belonging to the non-pathogenic biotype (BT1A), whereas they are found in about 42.1% of the total pathogenic biotypes, with a different distribution between bioserotype 4/O:3 where plasmid genes are present in 40.1% and bioserotype 2/O:9 where *virF* and *yadA* are present in 60%.

MyfA, plays an important role at the beginning of infection by promoting adhesion to enterocytes. The *myfA* gene has been found in *Ye* strains of bioserotype 4/O:3 isolated from human and pigs [33] and has also been detected in some *Ye* strains of BT1A [24].

These results are confirmed in this study, and we also found *myfA* in all strains of bioserotype 2/O:9 isolated from human and in the only strain belonging to bioserotype 3/O:5,27 isolated from animal.

The *ymoA* gene was present in all *Ye* isolates studied, as reported in several studies [18, 24, 33]. According to recent studies, the *ymoA* gene is the main regulator of *yst* gene expression and other virulence genes [34].

The *hreP* gene, which encodes the bacterial subtilisin/Kexin-like protease, and the *sat* gene, which encodes the acetyltransferase streptogramin, are both present in high percentage in all *Ye* strains. These results are in agreement with data from some other Authors [18, 24], but seem to be in contrast with data reported by Morka *et al.* [33]. Few studies have described the *hreP* gene, encoding for a bacterial protease, relevant for full virulence of *Ye* [35]. Finally, the *fes* and *fepD* genes, involved in iron capture and utilization, contribute to the growth of microorganism [30].

The genes *fepD* and *fes* were found in 100% or percentage near 100% in all isolates of all bioserotypes except for BT4/O:3 where the prevalence of these genes was about 44%. BT1A strains possessing the genes *ystB*, *hreP*, *sat*, *fes*, *fepD* and *myfA* genes may have a virulence potential with respect to causing infections in humans and animals [24, 36]. Although in the study of Campioni and Falcão [36], *myfA* appears to have a higher prevalence in the BT1A strains (55%) than in our study.

Furthermore, the lack of identification of BT1B strains in this work, appears to be in line with what has been reported in the literature regarding the limited presence or absence of this biotype in Europe [4, 6].

Overall, the study seems to confirm what other authors have already pointed out regarding the distribution of these virulence genes in *Ye* strains.

CONCLUSIONS

The isolation and biotyping of *Ye* are currently difficult and time-consuming, but biotyping remains important as a basis for assessing the pathogenicity of isolated strains. The detection of this microorganism by means of molecular biology tools as a real-time PCR allows the quick detection of pathogenic *Ye* in food, animal and human and can be a valid support to classical microbiology techniques. Although, the *ail* gene remains the main virulence marker as reported by ISO/TS 18867:2015, other virulence genes are important in evaluating the pathogenicity of *Ye*, such as the *ystB* gene to identify strains of biotype 1A. Therefore, the use of two SYBR Green real-time PCR platforms in this study, allowed the rapid detection of the eleven virulence genes, in pathogenic biotypes and in BT1A of *Ye*. It also allowed to highlight a diversity in the distribution of virulence genes in *Ye* strains isolated from different sources, which has valuable implications in terms of food safety and animal and human health from a One Health perspective.

Authors' contributions

Conceptualization: ED and EV; methodology: ED; investigation: ED, EV, SF, SL, GF, SB and SO; re-

sources: ED, SF, SL, GF, SB and SO; writing-original draft preparation: EV and ED; writing-review and editing: ED, SF, SL, GF, SB; supervision: ED. All authors have read and agreed to the published version of the manuscript.

Conflict of interest statement

The Authors declare no conflict of interest.

Received on 2 May 2023.
Accepted on 26 September 2023.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 286-294
DOI: 10.4415/ANN_23_04_08

ORIGINAL ARTICLES AND REVIEWS

Testimonies and experiences of patients awaiting transplant and transplanted patients in Italy: a survey aiming to understand their needs

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Abstract

Introduction and methods. In 2020 the Italian National Transplant Centre (Centro Nazionale Trapianti, Istituto Superiore di Sanità, Rome, Italy) launched two online surveys to explore waitlisted and transplanted patients' needs.

Results. The analysis of two-year results shows prevailing feelings of anxiety and discouragement (44.5%) in waitlisted patients. A mere 19.7% expresses feelings of trust and hope. Conversely, in transplanted patients, gratitude (65.7%) predominates. Both transplanted (53.5%) and waitlisted patients (41.5%) worry about organ rejection and complications, SARS-CoV-2 pandemic was an additional concern. The latter report certainty of transplant as their main need (81.3%), followed by psychological support (41.8%) also indicated by 27.9% of transplanted patients, while donor-recipient anonymity is an issue for 31.3% of the transplanted.

Conclusion. Focusing on the needs and, above all, taking them on, means putting the patient at the centre of care and increasing the chances of a better life despite sufferings and preoccupations.

Key words

- testimonies
- needs
- waitlisted patients
- transplanted patients
- national survey

INTRODUCTION

Testimonies and experiences of transplanted or waitlisted patients represent the best approach to understand their needs and implement the necessary actions for their fulfilment: this was the initial goal of the research. However, with the progressive spreading of the SARS-CoV-2 virus, the paradigm inevitably changed.

We had to face a reality nobody was ready for. Hospital professionals were challenged with unpredictable situations of great emotional impact. Outside the healthcare facilities, citizens lived in an unimaginable world of quarantine, social distancing and fear of infection.

Moreover, the widespread feeling of uncertainty, generated by the pandemic, was exacerbated by the perception that our healthcare system, as well as that of other countries [1], was not prepared for an emergency of this magnitude; let alone the dramatic death toll, rate of infections and a never-reassuring global media coverage [2].

All this had a disruptive effect on the entire population with tragic consequences for the most fragile, who also suffered enormously from social isolation and the difficulties of accessing healthcare facilities [3], on top of having to pay the highest direct price in terms of lost lives. No doubt, transplanted or waitlisted subjects might have as well risked and experienced the same hardships encountered on a global scale [4]. The current study should, therefore, be framed within an anomalous and greatly concerning context, which might have affected the experience and feelings of the participants, transforming the initial cognitive objective of the research into a cross-section of such a dramatic reality.

Nevertheless, the themes for reflection offered are numerous and of great interest, along with the propensity shown by a substantial amount of the interviewed participants to interact with the system through their testimony and experience; two sharply different situations, in some respects, for those awaiting transplant and those who already received one. An example is the time on the waiting list which is different for the two groups. On the contrary, in some other matters, feelings and moods are shared by both groups, despite their experiences being completely distinct.

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Many answers prove what has been described and each would be worthy an in-depth debate and consideration. The survey is however still ongoing and the urgency to limit the analysis to those aspects that might be more easily corrected, resulted in the postponement – to the end of the research – of a further analysis of other components affecting the treatment path. It is also possible to go through all answers on the Italian National Transplant Centre's (Centro Nazionale Trapianti, CNT) website at *https://www.trapianti.salute.gov.it/trapianti/homeCnt.jsp*.

MATERIALS AND METHODS

Since April 2020, the CNT – in collaboration with the National Heart Transplant Association (Associazione Trapiantati di Cuore, ATCOM) – launched a random sampling national survey on the experiences of transplanted and waitlisted patients, through two specific questionnaires. The survey is part of a broader research project focused on how transplant patients are managed, from waitlist enrolment to post-transplant follow up. This multi-center, retrospective, observational project was born thanks to a collaboration agreement between ATCOM and CNT, with the initial aim to create the first national register of volunteering associations working in this field and then further investigate the main need of waitlisted and transplanted patients, in order to identify necessary measures for their care pathway.

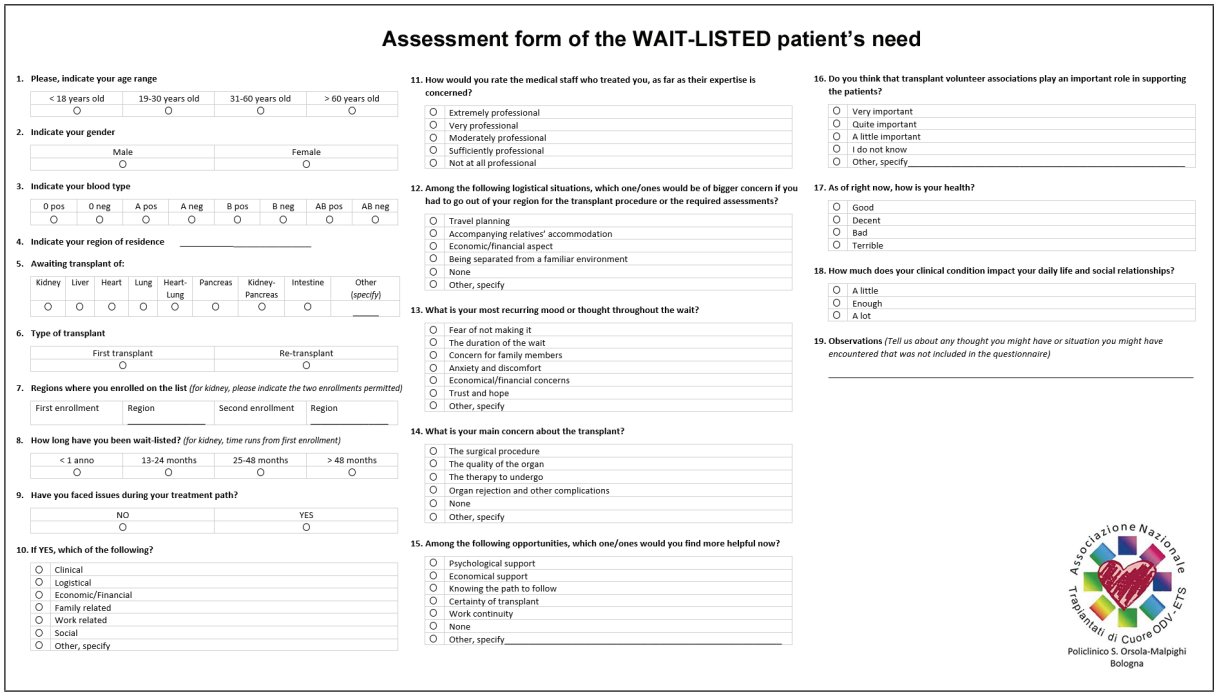
Each questionnaire, accessible online, asks 19 qualitative and quantitative questions, some of demographic nature, some other epidemiological and clinical-managerial and others aimed at exploring anxieties and needs (*Figure 1* and *2*). The latter are those this study specifically addresses. Most questions are closed-ended, while others are mixed and multi-response. The last one is open and it gives the participants the opportunity to express their critical thought and their unconditional opinion. The identification of the survey topics was planned to be translated into well definite questions; its organization is meant to meet a set of rules directed at safeguarding anonymity, standardization, comprehensibility and conformity to the object of the study.

Both were submitted to a pool of experts (psychologists, epidemiologists) in order to evaluate their structure and content. In particular, three basic aspects were verified:

- level of structuring of the questions (closed, semi-structured, open);
- language used (consistency, precision, relevance, simplicity);
- formulation and questions' order.

The initiative was then presented to the actors of the transplant network (regional and transplant centres), so that they could disseminate and support it in their territory. The CNT and the ATCOM made available, on their institutional websites and social media pages, the link to the questionnaires accompanied by a presentation note for further promoting the initiative. These measures have been instrumental in increasing the number of participants, reaching over 50 questionnaires filled in a day.

For these reasons, the deadline for filling the questionnaires was postponed from December 31st, 2020 to December 31st, 2021 especially due to the participants' repeated references to the pandemic. This last aspect led us to modify the initial questionnaires while work was in progress, with the introduction of new specific questions on the topic. We expanded the number



Assessment form of the WAIT-LISTED patient's need

1. **Please, indicate your age range**

< 18 years old	19-30 years old	31-60 years old	> 60 years old
○	○	○	○

2. **Indicate your gender**

Male	Female
○	○

3. **Indicate your blood type**

0 pos	0 neg	A pos	A neg	B pos	B neg	AB pos	AB neg
○	○	○	○	○	○	○	○

4. **Indicate your region of residence** _____

5. **Awaiting transplant of:**

Kidney	Liver	Heart	Lung	Heart-Lung	Pancreas	Kidney-Pancreas	Intestine	Other (*specify*)
○	○	○	○	○	○	○	○	_____

6. **Type of transplant**

First transplant	Re-transplant
○	○

7. **Regions where you enrolled on the list** *(for kidney, please indicate the two enrollments permitted)*

First enrollment	Region	Second enrollment	Region
	_____		_____

8. **How long have you been wait-listed?** *(for kidney, time runs from first enrollment)*

< 1 anno	13-24 months	25-48 months	> 48 months
○	○	○	○

9. **Have you faced issues during your treatment path?**

NO	YES
○	○

10. **If YES, which of the following?**

○ Clinical
○ Logistical
○ Economic/Financial
○ Family related
○ Work related
○ Social
○ Other, specify

11. **How would you rate the medical staff who treated you, as far as their expertise is concerned?**

○ Extremely professional
○ Very professional
○ Moderately professional
○ Sufficiently professional
○ Not at all professional

12. **Among the following logistical situations, which one/ones would be of bigger concern if you had to go out of your region for the transplant procedure or the required assessments?**

○ Travel planning
○ Accompanying relatives' accommodation
○ Economic/financial aspect
○ Being separated from a familiar environment
○ None
○ Other, specify

13. **What is your most recurring mood or thought throughout the wait?**

○ Fear of not making it
○ The duration of the wait
○ Concern for family members
○ Anxiety and discomfort
○ Economical/financial concerns
○ Trust and hope
○ Other, specify

14. **What is your main concern about the transplant?**

○ The surgical procedure
○ The quality of the organ
○ The therapy to undergo
○ Organ rejection and other complications
○ None
○ Other, specify

15. **Among the following opportunities, which one/ones would you find more helpful now?**

○ Psychological support
○ Economical support
○ Knowing the path to follow
○ Certainty of transplant
○ Work continuity
○ None
○ Other, specify_____

16. **Do you think that transplant volunteer associations play an important role in supporting the patients?**

○ Very important
○ Quite important
○ A little important
○ I do not know
○ Other, specify_____

17. **As of right now, how is your health?**

○ Good
○ Decent
○ Bad
○ Terrible

18. **How much does your clinical condition impact your daily life and social relationships?**

○ A little
○ Enough
○ A lot

19. **Observations** *(Tell us about any thought you might have or situation you might have encountered that was not included in the questionnaire)*

_____

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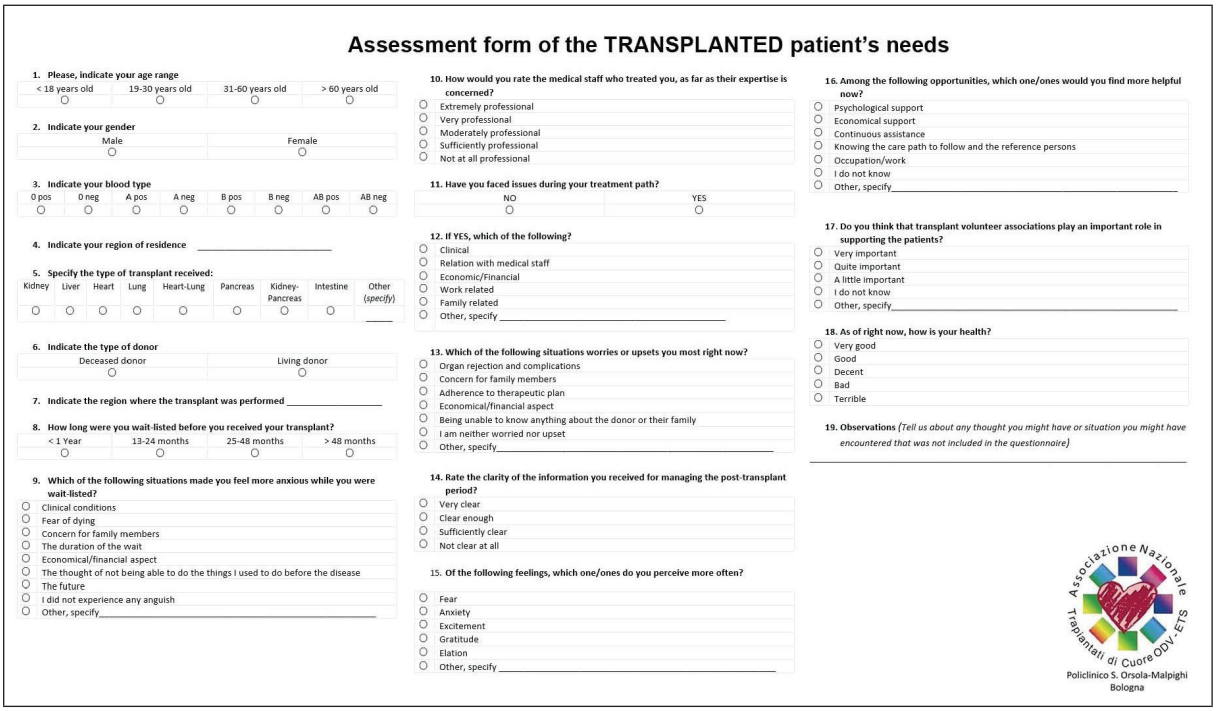
Figure 1
Survey form for the waitlisted patients.



Assessment form of the TRANSPLANTED patient's needs

1. Please, indicate your age range
< 18 years old | 19-30 years old | 31-60 years old | > 60 years old

2. Indicate your gender
Male | Female

3. Indicate your blood type
0 pos | 0 neg | A pos | A neg | B pos | B neg | AB pos | AB neg

4. Indicate your region of residence _____

5. Specify the type of transplant received:
Kidney | Liver | Heart | Lung | Heart-Lung | Pancreas | Kidney-Pancreas | Intestine | Other (specify) _____

6. Indicate the type of donor
Deceased donor | Living donor

7. Indicate the region where the transplant was performed _____

8. How long were you wait-listed before you received your transplant?
< 1 Year | 13-24 months | 25-48 months | > 48 months

9. Which of the following situations made you feel more anxious while you were wait-listed?
- Clinical conditions
- Fear of dying
- Concern for family members
- The duration of the wait
- Economical/financial aspect
- The thought of not being able to do the things I used to do before the disease
- The future
- I did not experience any anguish
- Other, specify_____

10. How would you rate the medical staff who treated you, as far as their expertise is concerned?
- Extremely professional
- Very professional
- Moderately professional
- Sufficiently professional
- Not at all professional

11. Have you faced issues during your treatment path?
NO | YES

12. If YES, which of the following?
- Clinical
- Relation with medical staff
- Economic/Financial
- Work related
- Family related
- Other, specify _____

13. Which of the following situations worries or upsets you most right now?
- Organ rejection and complications
- Concern for family members
- Adherence to therapeutic plan
- Economical/financial aspect
- Being unable to know anything about the donor or their family
- I am neither worried nor upset
- Other, specify_____

14. Rate the clarity of the information you received for managing the post-transplant period?
- Very clear
- Clear enough
- Sufficiently clear
- Not clear at all

15. Of the following feelings, which one/ones do you perceive more often?
- Fear
- Anxiety
- Excitement
- Gratitude
- Elation
- Other, specify _____

16. Among the following opportunities, which one/ones would you find more helpful now?
- Psychological support
- Economical support
- Continuous assistance
- Knowing the care path to follow and the reference persons
- Occupation/work
- I do not know
- Other, specify_____

17. Do you think that transplant volunteer associations play an important role in supporting the patients?
- Very important
- Quite important
- A little important
- I do not know
- Other, specify_____

18. As of right now, how is your health?
- Very good
- Good
- Decent
- Bad
- Terrible

19. Observations *(Tell us about any thought you might have or situation you might have encountered that was not included in the questionnaire)*

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Figure 2
Survey form for the transplanted patients.

of questions in each questionnaire to 32, making the integrations operational from February 1st, 2022. All this has transformed the initiative from a time-limited survey to a permanent monitoring tool, so much so that the questionnaires are still online. Consequently, being the situation an evolving one, any consideration formulated in this article might change over time.

Due to the dynamic nature of the responses, it was therefore necessary to establish a time limit within which to start an initial processing of the observed data. The present analysis is therefore based on the questionnaires received by April 30th, 2022 and only refers to the 19 initial questions, so far excluding those that were added afterwards.

RESULTS

From 10 April 2020 to 30 April 2022, 8,899 participants joined the research, i.e., 14% of all patients registered in the Italian Transplant Information System (SIT) waiting for transplant in the study period or followed up post-transplantation, out of which 5,289 transplanted patients and 3,610 waitlisted ones, with an average age of 44.9 years old, mostly males (54.8%). A mere three foreign patients (0.08%) enrolled on the Italian waiting listed took part, and 50 Italian patients transplanted abroad (0.94%). Nationality has not been therefore taken into account in subsequent analysis due to lack of worth in the analized population.

Kidney patients are the most represented both among waitlisted (60.1%) and transplant recipients (39.6%), making up 47.9% of the entire sample. This percentage rises to 51% including kidney-pancreas (or nephropathic patients). Hepatopathic (25.5%) and cardiopathic patients (14.6%) follow. Patients transplanted or awaiting lung (6.1%), pancreas and kidney-pancreas (4.2%), tissue (1.6%) and heart-lung transplantation (0, 04%) complete the series with lower percentages *(Table 1)*.

In general, a sense of discouragement prevails among waitlisted patients, especially due to the long waiting times (59.2%). Many of them even believe that they will not make it (30.9%) and only 19.7% report positive feelings of trust and hope. The fear of dying is common among those waiting for a lung or a heart, indicated by 60% of the former and 52.9% of the latter *(Table 2)*. 70% of the latter also indicate surgery as their greatest worry as far as transplantation is concerned, a percentage that increases to 77% among females *(Table 5)*. Time on the list is a particularly felt issue among patients expecting a pancreas (74.6%) or a kidney (65.2%). Both are also the most disheartened, since only 16.2% of the former and 19.4% of the latter picked "feeling hopeful" as a response *(Table 2)*. Among already transplanted patients, gratitude prevails (65.7%), although anxiety (44%) and fear (18.2%) indicate a distress common to 62.2% of patients. This is found in all participants regardless of the transplant they underwent. Liver transplant recipients are particularly grateful (70.7%), while pancreas transplant recipients (51.6%) are the most anxious. Conversely, "serenity" is poorly reported by all participants, indicated by a mere 2.8% *(Table 3)*. It should be noted that, for all multiple-choice questions, the number of answers is not equal to the number of respondents, since more options can be ticked. Therefore, the answers can apparently reach a higher-than-100% percentage.

The testimonies of those directly involved are also notably different when it comes to the waiting time. The answers analyzed so far show that 77.8% of transplant

Table 1
Patients responding to the survey on the waiting list or transplanted by organ

Organ/tissue	Waitlisted patients		Transplanted patients		Global case history	
	N.	Ratio (%)	N.	Ratio (%)	N.	Ratio (5)
Kidney	2.168	60.1	2,097*	39.6	4,265	47.9
Liver	563	15.6	1,702	32.2	2,265	25.5
Heart	463	12.8	836	15.8	1,299	14.6
Lung	190	5.3	352	6.7	542	6.1
Pancreas	67	1.9	31	0.6	98	1.1
Kidney-Pancreas	105	2.9	174	3.3	279	3.1
Heart-Lung	3	0.1	1	0.01	4	0.04
Tissue	51	1.4	96	1.8	147	1.6
Total cases	3,610	40.6	5,289	59.4	8,899	

* It includes 11 double kidney transplants.

Table 2
Answers to the question: "What is your most recurring mood or thought throughout the wait?"

Answer	Waitlisted patients											
	All (%)		Kidney (%)		Liver (%)		Heart (%)		Lung (%)		Pancreas (%)	
Wait length	59.2		65.2		49.9		45.4		45.3		74.6	
Anxiety/discouragement	44.5		53.9		27.7		25.1		35.8		65.7	
Fear of not making it	30.9		19.9		45.3		52.9		60		34.3	
Trust/hope	19.7		16.2		23.1		28.5		23.7		19.4	
Concern for family members	19.4		19.9		20.2		19.9		14.2		14.9	
Economic concerns	10.9		11.4		12.8		9.5		5.8		9	
Surrender	3.9		4.8		2.3		2.2		1.6		3	
Other	12.2		11.9		9.1		11.0		28.9		16.4	
Answer by gender	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**
Wait length	57.5	61	64.3	66.1	50.6	48.9	46	44.2	44.1	46.4	71.4	76.9
Anxiety/discouragement	42.1	47.1	49.6	57.7	25.1	31.4	26.2	23	36.8	34.7	64.3	66.7
Fear of not making it	31.8	30	19.4	20.4	44.6	46.3	52	54.5	60	60	32.1	35.9
Trust/hope	20.5	18.9	17.5	15.1	23.1	23.1	26.8	31.5	21.1	26.3	17.1	21.5
Concern for family members	19.1	19.6	18.3	21.4	21.6	18.3	19.5	20.6	18.9	9.5	14.3	15.4
Economic concerns	13.4	8.3	13.9	9.2	15	9.6	12.4	4.2	9.5	2.1	17.3	2.6
Surrender	2.9	4.9	3.4	5.9	2.1	2.6	2	2.4	3.2	0	0	5.1
Other	11.5	12.9	11.2	12.6	8.7	9.6	11.1	10.9	26.3	31.6	28.6	7.7

recipients report having received the transplant within 24 months of enrollment on the list. The percentage rises to 91.7% for liver transplant recipients, while 52.7% of patients currently on the list have been waiting for more than 24 months, with cases exceeding 5 years. The overall figure is probably influenced by the large number of renal patients, who are the majority of participants in the survey (60.1%). Among these, the number of those who have been on the waiting list for more than 24 months is over 60%. For the other types of transplant, there are no large differences in waiting times between those on the list and transplant recipients *(Table 4)*.

There are other situations, where the experiences of the two groups coincide or differ less. Anxiety, for example, is a condition common to many patients on the list (44.5%) and particularly frequent among those expecting a kidney (53.9%), but the same emotional state is also experienced by transplant recipients (44%). This data is also reflected by the answers to the next question of the questionnaire *Among the following opportunities, which one/ones would you find more helpful now?* In this case, transplant recipients ticked as their most desired form of help "continuous assistance" (33.7%), followed by "knowing the care path to follow" (32.1%) and "psychological support" (27.9%). The latter is also indicated

Table 3
Answers to the question: "Of the following feelings, which one/ones do you perceive more often?"

Answer	Transplanted patients											
	All (%)		Kidney (%)		Liver (%)		Heart (%)		Lung (%)		Pancreas (%)	
Gratitude	65.7		61.2		70.7		68.8		66.2		54.8	
Anxiety	44		47.7		38.7		44.3		46		51.6	
Fear	18.2		19.7		15		20.2		18.2		29	
Enthusiasm	11.7		11.2		9.7		12.9		17.3		19.4	
Uncertainty/insecurity	8.9		7.7		9.5		9.1		8.5		9.7	
Elation	4.6		4		5.2		5.4		4.8		3.2	
Peacefulness	2.8		2.5		3.3		3.2		1.4		0	
Other	6.6		6		6.8		6.8		9.1		3.2	
Answers by gender	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**
Gratitude	65.1	66.4	60.5	62	71.7	68.7	67.7	70	64.7	67.8	57.1	52.4
Anxiety	43.1	45.7	46.1	49.6	37	40.4	42	47.5	43.1	49	49.7	53.4
Fear	16.1	21.2	17.5	22.1	13	18.8	17.8	23.8	17.9	18.5	26.7	31.3
Enthusiasm	12.5	10.6	12.5	9.8	10.4	8.4	13.1	12.6	17.9	16.8	22.6	16.3
Uncertainty/insecurity	7.8	10.3	6.8	8.6	9.7	9.1	8.3	11.2	6.6	10.6	8.7	10.5
Elation	4.7	4.3	4.1	3.8	4.9	5.7	5.7	5	6.1	3.5	4.6	2.2
Peacefulness	2.2	3.6	1.7	3.4	2.5	4.7	2.2	4.7	1.7	1.2	0	0
Other	6.8	6.4	5.9	6.2	6.5	7.2	8.3	4.7	10.1	8.1	4.1	2.4

Table 4
Answers to the question: "Time spent on the waiting list"

	Waitlisted patients						Transplanted patients					
	All	Ki (%)	Li (%)	He (%)	Lu (%)	Pa (%)	All (%)	K (%)	Li (%)	He (%)	Lu (%)	Pa (%)
<24 months	52.7	39.9	82.8	71.3	66.4	31.3	77.7	62.7	91.7	87.7	80	80.7
>24 months	47.3	60.1	17.2	28.7	33.6	68.7	22.3	37.3	8.3	12.3	20	19.3

as a service that is often not offered by the system but as a need fulfilled through the patient's personal initiative. A necessity that, among waitlisted patients, is identified by a large number of participants (41.8%) and is considered most helpful; second only to the certainty of transplantation (81.3%). The same is observed for organ rejection which represents the main concern among transplant recipients (53.5%), particularly among kidney ones (69.7%). A concern which is shared by waitlisted patients, so much so that to the question *"What is your main concern about the transplant?"* 41.5% of the participants replied "organ rejection" and "complications", indicating them as their greatest source of anxiety, especially those who are waiting for a kidney (50.6%) *(Table 5)*.

Surprisingly, the second most frequent answer to the same question among transplant recipients is *being unable to know anything about the donor or their family members*, which was indicated by 1,646 patients, i.e., 31.1% of the participants *(Table 6)*. This data is also confirmed by the provided open answers and gains even more relevance if analyzed by type of transplant and by gender. Among liver transplant recipients, the regret for the lack of information about the donor and their family is indeed indicated by 37.7% of the participants and, among them, especially by females (41.5%). The same goes for heart transplant recipients, where it is indicated by 39.7% of patients, 45.5% of whom are females. The anguish caused by the lack of information about the donor and their family, is even greater, among recipients of these two types of transplant than the fear of organ rejection and it is reported by 38.3% of heart transplant recipients and 36.8% of liver transplant recipients.

Another common thought among participants of both surveys is the strong reference to the SARS-CoV-2 pandemic as a situation of additional concern for their condition. The data was obtained from the provided open answers, which highlighted an aspect initially neglected in the questionnaires, an opportunity seized by 26.4% of the participants (2,352 patients). Of these, 11.7% explicitly refers to the pandemic and the measures taken to curb it, as disturbing and worrying factors in the respective care pathways. Questions on the subject were not initially foreseen, because no one could have imagined such a rapid and lasting evolution of COVID-19.

Table 5
Answers to the question: "What is your main concern about the transplantation?" (Question addressed to waitlisted patients)

Answer	Waitlisted patients											
	All (%)		Kidney (%)		Liver (%)		Heart (%)		Lung (%)		Pancreas (%)	
Organ rejection and complications	41.5		50.6		23.1		21		30		44.8	
Surgery	27.8		10.1		44.6		70		67.9		28.4	
Nothing	27.5		32.9		26.8		11.2		17.4		26.9	
Quality of organ	20.6		19		24		17.3		30		23.9	
Post-transplant therapies	10.7		10.5		13.7		9.1		7.4		11.9	
Everything	8.3		10.1		7.1		3.2		4.2		6	
Other (specify)	6.2		5.1		5.3		7.8		12.1		10.4	
Answer by gender	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**
Organ rejection and complications	40	43.1	50.7	50.5	21.6	25.3	22.1	19.9	29.5	30.5	43.5	46.2
Surgery	29.2	26.3	8.9	11.1	42.2	47.1	66.1	77	70.6	65.2	30.3	26.5
Nothing	28	27	32.7	33.1	28.2	25.4	11.6	10.4	16.8	17.9	26.8	26.9
Quality of organ	20.1	21	20.3	17.9	22.1	26.1	16.4	18.4	29.5	30.5	22.9	24.8
Post-transplant therapies	10.1	11.4	9.1	11.7	13.8	13.5	9.4	8.5	9.5	5.3	10.7	12.8
Everything	7.9	8.7	9.8	10.2	7.2	7	3	3.6	6.3	2.1	3.6	7.7
Other (specify)	6.6	5.8	5.2	5.1	5.1	5.7	9.1	5.5	12.6	11.6	14.3	7.7

Table 6
Answers to the question: "Which of the following situations worries or upsets you most right now?" (Question addressed to transplanted patients)

Answer	All (%)		Kidney (%)		Liver (%)		Heart (%)		Lung (%)		Pancreas (%)	
Organ rejection and complications	53.5		69.7		36.8		38.3		67		61.3	
Not knowing about the donor and their family	31.1		24.7		37.7		39.7		24.1		29	
I am not worried/upset	21		16.8		26.1		23.1		19.3		25.8	
Concerns about family members	17.6		15		19.6		23.8		14.5		9.7	
Adhering to the therapeutic program	12.8		11.3		14		15.2		11.6		19.4	
Economic/financial aspect	7.5		6.2		9.5		8		4.3		9.7	
Other	6.8		6.2		6.1		6		13.4		3.2	
Answer by gender	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**	**F (%)**
Organ rejection and complications	50.8	56.3	64.5	75.7	34.4	41.3	39.6	36.4	62.6	71.7	53.3	68.8
Not knowing about the donor and their family	29.2	33.8	21.9	27.9	35.6	41.5	35.8	45.5	22.3	26	40	18.8
I am not worried/upset	24.6	16.3	20.4	12.7	29.4	20	23.6	22.3	24	14.5	26.7	25
Concerns about family members	17.4	18	14.2	15.8	19.9	19.2	22.6	25.5	12.8	16.2	13.3	6.3
Adhering to the therapeutic program	12	14	10	12.8	13.2	15.6	15.2	15.2	11.2	12.1	26.7	12.5
Economic/financial aspect	9	5.4	6.8	5	10.6	7.6	11.1	3.5	6.1	2.3	13.3	6.3
Other	6	8	5.7	6.9	5.1	7.9	6.1	5.9	10.1	16.8	0	6.3

However, we anticipate that from the answers received after the introduction of specific questions, i.e., from February 2022, the references of patients to impact of the pandemic have increased. In fact, to the question: *Do you think that the state of emergency due to the coronavirus pandemic (SARS-CoV-2) has influenced your treatment path?* 64.8% of waitlisted patients and 33.4% of transplanted patients answered YES. The data, although preliminary, hints to the relevance of the pandemic for these groups, even if it needs to be stratified and analyzed by different variables, for its impact to be fully understood.

DISCUSSION AND CONCLUSIONS

The themes analyzed in the study are various and all of great interest. Some, in particular, have caught our attention.

Firstly, patients' experiences, either waitlisted or transplanted, reveal a daily reality made up of shared, emotional suffering and discomfort. The reported states of mind have some deeply different traits but coincide for many others. Anxiety is one of these: it is reported by 44.2% of survey respondents from both groups, who admit to feeling constantly anxious, whether they are awaiting or already underwent transplant; this emotional state is probably influenced by the patient's feeling of uncertainty, e.g., about the transplant date or the possibility of organ rejection.

On the other hand, for waitlisted patients, the waiting period is undoubtedly difficult for several reasons: first of all, a lot of anguish is caused by the lack of a defined timing, waiting for a transplant without knowing if and when it will happen also comes with the fear that it may not take place on time or that it may not work. All this generates conflicting emotions between the desire to receive the call for the transplant and the understandable fear for the outcome of the surgery. These contrasting emotions are shown in the free answers provided by survey respondents, by the repeated references that patients make to the fear of not hearing the phone ringing (37%), or to the fear of not waking up after surgery (12%), or that the surgery may not be conclusive (11%). This is particularly frequent among patients awaiting life-saving transplants such as heart or lung.

For those who have survived the wait unscathed and received the transplant, the concern shifts to the risk of organ rejection. The state of anxiety, therefore, lingers on and is renewed with every checkup or small alarm sign. Not everyone, however, is equally anxious. Some even appear nonchalant in the face of those same situations that for others, are a source of great concern. The latter are also those who indicate psychological support as one of the greatest needs both among waitlisted patients and transplant recipients. Essentially, the state changes and so do fears, but not the want of support. This denotes a condition of persistent emotional frailty, under which it is more difficult to accept or tolerate any event beyond one's control [5]. In addition, this condition should be taken into strong consideration because, as shown in other studies, this state of frailty may expose patients to a greater risk of depression [6]. These requests for help should be answered systematically with structured and integrated support to the treatment path and not merely if advanced by the patients themselves. The fact that many of them report that psychological support is more often than not met only on request, represents one of the system's critical issue that should be corrected.

Another interesting issue is the sorrow expressed by a third of the respondents regarding the impossibility of knowing the identity of the donor or of having information about them or their family. This is still a controversial and debated topic, despite the reference law on post-mortem organ and tissue donation in our country, being clearly founded on the principle of anonymity, as well as those of gratuity of the treatment and freedom of choice [7]. Most probably, during pre-transplant information talks, patients should be duly informed about legal provisions on anonymity of donor and recipient and verify this concept has been fully understood and accepted.

However, the evolution of both society and the Transplant Network has reopened the debate regarding the need for the current legislation to be revised precisely in the light of the changes that have taken place. The National Bioethics Committee itself has opened to the possibility that the principle of anonymity be reconsidered under certain conditions [8]. In the light of this, a legislative proposal was also presented to the Chamber of Deputies which incorporates all the suggestions of the National Bioethics Committee and, therefore, it cannot be excluded that in the near future, in Italy, contacts between donors and recipients may be allowed [9]. Documenting the position reported by over 31% of the survey participants represents an important element of knowledge and it might provide a pivotal contribution to the discussion.

What is unexpected is that the yearning for information, frequently expressed by the donor's relatives, is shared by many transplant recipients who believe the current limitation to be of social nature, both for those who just wish to leave a flower on the donor's resting place and for those who are truly tormented by the idea of not being able to personally express their gratitude to the family.

All this denotes a need for gratitude, which, although understandable, remains a delicate issue that can lead to dependence and open up scenarios of subjection that can result in a condition of fragility. It will therefore be necessary to thoroughly weigh the possible benefits of an emotionally strong relationship between the donor's family and the recipient, which comes with the high risk of both parties' expectations being disappointed, at some point. The rule that protects anonymity, even if it may seem excessively restrictive, represents a form of emotional protection both for the recipients and for the donor's family members. In the event of being able to meet, certainly neither party can be left alone, even when this will is clearly expressed by both. It is likely that the mediation of a third party, indicated by the National Bioethics Committee as one of the possible conditions, can represent an intermediate solution capable of better and more appropriately manage relationships that can prove to be very complex [10, 11].

Moving on, the wide response to the surveys (63.1% of patients who accessed the online questionnaires, equal to 14% of patients registered in SIT) testifies to their desire to interact with the system and to the isolation and difficulties exacerbated by the pandemic. However, the many comments freely formulated, whether they be of appreciation for the initiative, or questions or about critical issues, imply that we are faced with patients in need of expressing their doubts, uncertainties and of asking for explanations. All this draws attention to the importance and value of the doctor-patient relationship. Healthcare professionals should be more aware

that human qualities such as availability, ability to listen and dedication are not secondary to professional skills and, most importantly, that a greater communicative effort on their part can foster the empathic bond with the patient and improve their assistance [12].

The last aspect that emerges is the pandemic and its possible interference in the path of care of patients. According to part of the participants, the state of emergency caused by COVID-19 interfered in their care path not only as far as the risk of being infected by the virus is concerned, but also in terms of making access to treatments and care for waitlisted patients more complicated, as well as routine checkups for transplant recipients. In particular, from the answers to the specific questions introduced later in the questionnaires, 4 out of 10 patients report complications in their respective care pathways; of these, just over 2 out of 10 had difficulty in keeping in touch with specialists and reference centers for their pathology; 2 out of 10 have been struggling with practical problems such as abruptly canceled scheduled visits and exams. Among transplant recipients, the greatest difficulty relates to the reduction in checkups (55%); while among waitlisted patients to accessing diagnostic services in the area (51.8%).

From these preliminary data we can therefore conclude that the pandemic has created some concern. The hardships reported by patients make us believe that the problems can be attributable both to the state of suffering of health structures overwhelmed by the emergency, and to an attitude of caution or renouncement of patients for fear of the virus. This is a reasonable outcome, considering that these situations are also highlighted in other studies [13]. On the other hand, the feeling of being overwhelmed by the COVID-19 emergency is shared by everyone and had consequences in every field, throughout the globe. The death toll (over 6 million) and the number of infections (over 500 million) demonstrate its devastating effect [14]. It is therefore plausible that the transplant system may have been affected as well.

However, the opposite could be just as valid: the Italian transplant system has successfully withstood the emergency impact, since 6 out of 10 patients report that they have not been affected by the pandemic.

In reality, there is much left to be understood about COVID-19's effects so far and over time. Among others, our remarks are based on data freely reported by the survey participants which, although substantial in number, do not represent the entire sample of patients tracked by SIT, that is 16,822 waitlisted and 45,812 followed-up transplanted patients. At the end of the research, the results will necessarily be stratified and evaluated according to geographical area of residence of the patients and of the transplant, as well as age, type of transplant, gender and, last but not least, vaccination status.

So, even if the peak phase of the health emergency seems to be behind us and the latest variant of the virus under control, we can only make a preliminary assessment of the consequences for the Italian transplant system.

In conclusion, we can say that what we have observed so far is a multi-faceted capital of knowledge, deserving further and specific insights. The deep sufferings emerged are what researchers define as "criticalities". These hardships, regardless of the question asked, mainly revolve around the exhausting wait and fear of organ rejection. Two distinct topics that seem to be the common thread of the patients' anxieties and needs, even if with substantial differences between transplant programs. The psychological distress probably arises from this, also due to the lack of precise indication to the professional figures to rely on. It is one of the system's shortfalls, which unfortunately foresees different approaches and solutions throughout the national territory. Strengthening the local psychological care services is a fundamental action in order to reach an effective, consistent and integrated level of care of both waitlisted and transplanted patients, given that psychological balance is an integral part of the individual's well-being. However, although this may meet patients' needs, the underlying problem is still a structural one and it is caused by the gap between supply and demand for organs that generates long waits from which anxieties and discomfort arise. Measures to support patients are, therefore, welcome but more must be done both on finding potential deceased donors, for example by consolidating the DCD (Donation after Circulatory Death) donation program (NHBD), and by providing greater support for living donation which remains an additional resource of great importance.

Prospective activities, that are planned to be implemented as output of this survey are: a) the organization of a public Webinair, during which patients and transplant network stakeholders would have the opportunity to discuss the survey outcomes and identify proper measures to be taken; b) performing a deeper analysis of collected infos, in order to select most frequent critical issues, most affected geographical areas and kind of transplant, so as to be able to prioritize interventions by Regional Transplant Centres and hospitals; c) third, analysing the impact – on patients' attitude – of organizational measures put in place by Regional authorities to support donation and transplant activities, comparing this data with the trend of utilized donors, carried-out transplant and waitlist waiting time.

Our goal so far was to intercept and report the wants of patients, but we now have to take care of them. It would therefore be very important to submit these testimonials to qualified professionals who know how to interpret their meanings and promote adequate solutions. On the other hand, the identification and interpretation of needs is a preliminary activity of fundamental importance for setting up each service or intervention program [15]. This may represent the starting point for proposing monothematic researches capable of leading us to a fuller satisfaction of needs, on the one hand, and on the other to a better organization of the transplant system, a most articulated and complex one.

Acknowledgments

The Authors wish to thank Maria Francesca Arrivi for her administrative support to the implementation of the study.

Conflict of interest statement

No conflict of interest to declare.

Received on 31 May 2023.
Accepted on 26 September 2023.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 295-303
DOI: 10.4415/ANN_23_04_09

The rare malformation holoprosencephaly: pathogenesis, association with pregestational diabetes and the possible link with food pollutants

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Abstract

Background. Holoprosencephaly is a rare (1/16,000 livebirths) and severe brain malformation occurring during early embryogenesis. The malformation originates from absent or incomplete forebrain division and is associated with altered embryonic patterning.

Objectives. A narrative review to identify and assess the evidence on non-genetic risk factors.

Results. Genes involved include sonic hedgehog, Zinc finger protein, SIX homeobox 3. Pregestational diabetes, with periconceptional hyperglycaemia, is the main non-genetic risk factor; increased oxidative stress in neuroectoderm, in particular neural crest cells, appears as the main mechanism. Several widespread pollutants, including inorganic arsenic, PFAS and PCBs, may increase the risk of pregestational diabetes by altering metabolic factors, including lipids and insulin. A scenario "widespread exposures-rare outcomes in susceptible subjects" suggests that exposure to dietary pollutants may increase the risk of pregestational diabetes, hence of holoprosencephaly in susceptible embryos.

Conclusions. This complex pathway is plausible and worth being investigated; moreover, it highlights the importance of assessing risk factors, and the associated uncertainties, in order to support primary prevention strategies for multifactorial malformations.

Key words

- malformation
- multifactorial
- endocrine disruptors
- risk factors
- pathogenesis

INTRODUCTION

Obesity, type 2 diabetes (T2D) and other metabolic diseases clustered in the "metabolic syndrome" are major health problems worldwide [1]; in addition, obesity and T2D represent important risk factors for other human diseases, including congenital anomalies (CA). Indeed, the European recommendations for the CA primary prevention rank obesity and pre-gestational T2D among the main risk factors to be targeted by community actions [2]. Noticeably, gestational diabetes, i.e., glucose intolerance usually occurring at the second-third trimester of pregnancy, is also an important risk factor for several adverse pregnancy outcomes, such as prenatal overgrowth, stillbirth and prematurity [3]; however, it is not a main risk factor for CA as it occurs after completion of organogenesis. The modes of action by which pre-gestational T2D can increase the CA risk include increased oxidative stress and perturbed glucose metabolism [4, 5]. Meanwhile, beyond being a general risk factor, epidemiological evidence indicates a role in enhancing the risk of specific malformations. One case is represented by holoprosencephaly, a severe early disturbance of brain and craniofacial development. Holoprosencephaly is rare in livebirths and it causes early death and/or different degrees of severe disability [6, 7].

The role of dietary patterns as risk factor for the various manifestations of the metabolic syndrome is recognized since decades; yet, growing evidence highlight a role for chemical pollutants as well. So-called "western type" dietary styles, matched with insufficient physical exercise and the aging of population, can interact with specific environmental pollutants able to alter glucose and lipid metabolic pathways. The contribution by specific, widespread pollutants to actual burden of meta-

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bolic syndrome manifestations such as T2D cannot be overlooked, because of both relevant modes of action and targets and multiple exposure routes, including diet. While exposure can occur along the whole lifespan, the prenatal programming appears as the most susceptible lifestage [1].

Hence a biologically plausible link between holoprosencephaly, T2D and specific widespread pollutants is worth being explored. Under this respect, the conceptual framework of adverse outcome pathways (AOP) might help assessing the interconnections between molecular markers, specific congenital anomalies and risk factors [8]. The growing data-set on AOP and related key events are being used to evaluate the biological plausibility of associations between exposures and some adverse developmental outcomes, e.g., pesticides and developmental neurotoxicity [9]. Meanwhile, AOP have been rarely applied to CA: a recent example is the assessment of a food contaminant, the mycotoxin fumonisin B1, as risk factor for neural tube defects [10]. While no AOP does exist for holoprosencephaly, so far, elements of the AOP approach might support discussing the relevance of some molecular and cellular events to holoprosencephaly and its risk factors. The present work intends to review and discuss the biological basis of a potential relationship between holoprosencephaly, T2D and selected food pollutants.

HOLOPROSENCEPHALY. PATHOGENESIS AND RISK FACTORS

Holoprosencephaly (HPE) is a complex brain malformation with a wide spectrum of associated craniofacial defects (sometimes very severe, such as cyclopia), all characterized by midline defects: the pathogenesis stems from incomplete cleavage of the prosencephalon, occurring between the 18th and the 28th day of gestation in humans [5]. Whereas the pathogenesis is common, the HPE includes a range of phenotypes whose increasing severity depends on the degree of cleavage impairment: lobar, semi-lobar and alobar HPE. The malformation is rare in newborns, with estimated incidence 1/16,000 live births; however due to high mortality in utero, the incidence is much higher in conceptuses, 1/250. In livebirths, HPE mainly leads to early mortality or severe disability; milder forms with minor anomalies and symptoms are also reported. Interestingly, HPE patients frequently present endocrine defects, often more than one in a single individual, including diabetes insipidus, and adrenal hypoplasia [11].

HPE has a well-established embryogenetic origin, yet the molecular basis of the disease is still uncertain in most cases. The main genes involved include Sonic hedgehog (SHH) [12]. Zinc finger protein (ZIC2), SIX homeobox 3 (SIX3), as well as other genes involved in the embryonic patterning processes [11]. Noticeably, the sonic hedgehog pathways involved in HPE are also essential in pituitary formation; as a consequence, HPE patients frequently show pituitary deficiency and diabetes insipidus [13].

In a putative AOP perspective, the above genes, as well as others with similar functions, may be target of HPE-relevant molecular initiating events, i.e., may represent critical starting points for the pathway(s) leading to the adverse phenotype. SHH knock-out mouse embryos develop a sever HPE phenotype, with fusion of the telencephalic vesicle and optic cup, deriving from the loss of the ventral midline portion of the neural tube [14]. ZIC2 is critical for the organization of the anterior notochord, in particular for providing maintenance signal to the prechordal plate; in its turn, this structure promotes, through SHH, the formation of the two hemispheres in the developing forebrain [15, 16]. The transcription factor SIX3 is essential for the development of the forebrain by establishing the anterior-posterior identity of brain vesicles and has a key role in pathways leading to retinal and lens morphogenesis [17, 18]. Noticeably, ZIC2 and SIX3 also inhibit Wnt signalling; in vertebrate embryos, formation of anterior neural structures requires suppression of Wnt signals emanating from the paraxial mesoderm and midbrain territory [16, 19, 20]. Overall, these critical genes point out the main embryonic districts targets of early events in putative pathway(s) leading to HPE, namely: the anterior notochord and the dependent forebrain induction, as well as a direct impairment of forebrain patterning. Inhibition of Wnt signalling immediately consequent to SIX3 or ZIC-2 dysregulation might feature as an early key event in the stream eventually leading to HPE [16, 19, 20]. As shown by animal models, the subsequent cascade of events leading to the craniofacial alterations typical of HPE would involve the pathways orchestrated by Bone morphogenetic protein, Fibroblast growth factor, and Nodal signalling [21].

Altered lipid and cholesterol metabolism may also play a role in HPE pathogenesis. The malformation has been associated with mutations of TG interacting factors (Tgifs) 1 and 2, which are homeodomain proteins; loss of Tgif function can disrupt the SHH signalling pathway [22-24]. Tgfs 1 and 2 interact with the ligand binding domain of retinoid X receptor α; in its turn, this is a main heterodimeric partner of other nuclear receptors that are key regulators of lipid and cholesterol pathways, liver X (LXRs) and peroxisome proliferator-activated receptors (PPARs). Indeed, Tgif1 and Tgif2 may act as transcriptional regulators of pathways activated by lipids [25]. Unfortunately, to our best knowledge, no further data have elucidated the connection between the risk of HPE and the perturbation of lipid and/or cholesterol metabolism.

HPE is largely recognized as a multifactorial malformation spectrum, where environmental factors interplay with gene defects/mutations. HPE has been defined as a polygenic perturbation of anterior midline formation, with environmental factors, as well as embryonic stochasticity, influencing the phenotypic outcome [26]. Maternal pre-gestational T2D is consistently identified as a major risk factor in humans [11, 12, 27-29]. The largest case-control study on birth defects carried out in the USA, the National Birth Defects Prevention Study, reported a higher rate of pre-gestational diabetes in mothers of case infants (775/31,007, 2.5%) compared to controls (71/11,447, 0.6%); gestational diabetes during the index pregnancy showed also a slight increase (5.3% *vs* 4.7% in controls). Pregestational diabetes had a

strong impact on the risk of several malformations, HPE showing the second highest increase (adjusted odds ratio, 13.1; 95% confidence interval, 7.0-24.5) [27].

Interestingly the highest increase was observed for another rare malformation, sacral agenesis, a malformation of distal body axis, also associated with sonic hedgehog pathway [30]. Conversely, gestational diabetes was not associated with an increased HPE risk (adjusted odds ratio 1.2, 95% confidence interval, 0.6-2.4); hence, the findings clearly identify the critical role of the glycaemic level before pregnancy through to the early phase of organogenesis [27]. The role of the glycaemic level itself, rather than of the type of diabetes, has been highlighted by a recent study, nested on the same dataset, that found no substantial differences between the increased risks associated with pregestational T2D and pregestational type 1 diabetes. The pathogenesis of type 1 diabetes is characterized by reduced insulin production, rather than weakened insulin balance and control as in T2D; meanwhile, both diabetes types result in periconceptional hyperglycaemia when occurring in women at fertile age [31]. The association between pregestational diabetes and HPE has been confirmed by a worldwide systematic analysis of 59 population-based studies (1990-2021), overall involving more than 80 million participants. In this data-set, pregestational and gestational diabetes showed almost the same prevalence, 3.0% and 2.9% respectively: while pregestational diabetes increased the risk for a number of malformations, HPE showed the highest relative risk (18.18, 95% confidence intervals 4.03-82.06). Conversely, the increase associated with gestational diabetes was significantly smaller in regard of overall malformations: in particular, no increased risk was observed for HPE [29]. The specific increase of risk due to pregestational diabetes may partly reflect the early developmental window for HPE induction: an altered glycaemic status and associated metabolic disruption would need to be already present at organogenesis start. In fact, based on available evidence, gestational diabetes does not elicit a major risk for HPE. Meanwhile some other factors might have contributed to the findings reported in [29]. As the authors point out, the included studies show heterogeneity due to the lack of consensus and uniformity in the screening and diagnostic criteria for both pre- and gestational diabetes. An overestimation of the risk for total birth defects still observed for gestational diabetes might derive from the presence of unrecognized pregestational diabetes discovered only during pregnancy; on the hand, the inclusion of gestational diabetes cases occurring after the early pregnancy – the vulnerable window for birth defects – might lead to an underestimation of the risk. In addition, most studies included live births only; information on stillbirths and terminations of pregnancy for birth defects could provide a more accurate estimate of the impact by the different forms of diabetes on HPE risk [29].

A recent case control study compared 92 HPE cases with 56 patients of a rare genetic syndrome, the Williams-Beuren syndrome, with manifestations very different from HBE and no known environmental risk factors [28]. The results confirmed the importance of maternal pregestational diabetes, present in the 9.2% of cases and 0% of controls (p=0.02). In addition, the findings suggest the potential relevance of dietary/lifestyle factors (higher consumption of alcohol, cheese and espresso coffee). The actual role of these dietary factors, e.g., flagging associated nutritional imbalances and/or exposures to toxicants, needs to be further investigated.

Another group of risk factors is the use of consumer products and biocides such as personal insect repellents, insecticides and acaricides for pets, and household pest control products [28]. While this data definitely deserve attention, more accurate exposure data will be needed to evaluate the association (including precise definition of chemicals and possibly biomarkers of exposure).

Veterinary medicine offers a unique example of HPE due to an exogenous dietary factor. Outbreaks of cyclopia were observed in lambs from sheep kept on mountain pastures in western USA. The alkaloid-containing forage grass corn lily (*Veratrum californicum*) was the feed ingredient involved; the main teratogenic agent was the steroidal alkaloid cyclopamine, an inhibitor of the SHH signal transduction pathway and an antagonist of the transmembrane receptor Smoothened [32, 33]; in its turn, the receptor is a key transducer in the SHH pathway [34].

In humans, no known natural or man-made toxicants have been firmly associated till now with HPE; pregestational diabetes is by far the major risk factor identified. As recognized by the World Health Organization, diet – in particular the excess of sugar and saturated fats as well as the excess caloric intake with low physical activity – is a major trigger for the onset and severity of T2D, which is by far the most common form [35].

The pathways involved in the HPE pathogenesis are summarized in *Table 1*.

PREGESTATIONAL DIABETES AND HOLOPROSENCEPHALY: MECHANISMS INVOLVED

The increased risk of malformations in general, and specifically of HPE, by pregestational diabetes may involve several pathways. One might postulate that embryos with mutations (or epigenetic alterations) of genes relevant to HPE onset will be highly vulnerable to such mechanisms.

The hyperglycaemia characteristic of diabetes increases oxidative stress in maternal-embryonic environment. In turn oxidative stress can cause genotoxic damage in cranial neural crest cells, a compartment particularly active in the early embryonic development, with proliferation, differentiation, apoptotic and migration processes. Neural crest cells are pivotal for craniofacial development, which show a wide spectrum of anomalies in HPE, up to cyclopia; stochasticity of damage might be involved in the observed phenotypic variability [36]. The oxidative damage in neural crest cells, associated with pregestational diabetes, may result in craniofacial defects also by targeting the ribosomal RNA, hence the, post-trascription translational process [36, 37].

Two additional risk factors for oxidative stress in the spectrum of changes associated with T2D need being mentioned. One is represented by glycated products: persistent hyperglycemia elicits a widespread non-enzymatic glycation reaction with cell components, such

Table 1
Summary of main genes and pathways involved in the pathogenesis of holoprosencephaly

Gene	Pathway/function	References
Zinc finger protein (ZIC2)	Organization of the anterior notochord, providing maintenance signal to the prechordal plate that promotes, through SHH, the forebrain patterning. With SIX3, suppression of Wnt signals from the paraxial mesoderm and midbrain territory	[11, 15, 16, 19, 20]
SIX homeobox 3 (SIX3)	Organization of the anterior- posterior identity of brain vesicles; initiation of retinal and lens morphogenesis. With ZIC2, suppression of Wnt signals from the paraxial mesoderm and midbrain territory	[11, 16-20]
Sonic hedgehog (SHH)	Patterning of telencephalic vesicle and optic cup from ventral midline portion of the neural tube; pituitary formation	[11, 13, 14]
Bone morphogenetic protein	Craniofacial patterning	[21]
Fibroblast growth factor	Craniofacial patterning	[21]
TG interacting factors (Tgifs) 1 and 2	Homeodomain proteins: regulation of SHH signalling; interaction with the ligand binding domain of retinoid X receptor α; transcriptional regulators of (LXR and PPAR-regulated) lipid pathways	[22-25]

LXR: liver X receptor; PPAR: peroxisome proliferator-activated receptor.

as proteins, lipids and nucleic acids. The by-products, named advanced glycated end products, activate a number of pathways that enhance oxidative stress [38]. Another important factor for enhanced, systemic oxidative stress is the low-grade but persistent inflammatory status of adipose tissue when T2D is associated with obesity as it frequently occurs; remarkably, pathways activated by glycation products are important also for obesity-related inflammation [39].

One component of the multi-faceted effect of increased oxidative stress is the perturbation of the cholesterol homeostasis, regulated via LXR activation, with increased cellular cholesterol efflux [40]. This effect was observed in human fetoplacental endothelial cells, representing a later pregnancy phase compared to developmental window for HPE, nevertheless, there are no reasons to discount a perturbation of cholesterol homeostasis in the early post-implantation embryo, too. Noticeably, mutations of Tgifs 1 and 2, transcriptional regulators of lipid pathways, mainly LXR and PPAR-regulated, are associated with HPE [22-25].

Overall, the available scientific evidences indicate that pathways leading from pregestational diabetes to the increased risk of HPE all pass through the glycemia-induced oxidative stress. In its turn, oxidative stress may damage the DNA and/or RNA of cellular populations involved in the anterior midline formation and craniofacial development: notochord, prosencephalon, cranial neural crest. Additionally, oxidative stress can disrupt the metabolome by altering the lipid and cholesterol pathways. Several factors may influence the onset and severity of HPE, a clinically multi-faceted CA: the amount of insult, favouring genetic predispositions as well as the induction of changes in the relevant genes/pathways, likely has a stochastic component.

DIETARY SUBSTANCES AND HOLOPROSENCEPHALY-RELEVANT PATHWAYS

Cannabinoids inhibit hedgehog signalling; the cannabinoid Δ9-tetrahydrocannabinol (THC) induced HPE in Cdon mutant mice, a strain with a subclinical deficit in hedgehog signalling; this effect is not observed in other mouse strains without the signalling defect. THC can exert this effect directly, even in cells devoid of cannabinoid receptor-type 1, the typical THC receptor [41].

THC, a well-known psychoactive drug, is also an undesirable substance of dietary relevance, due to its presence, albeit at low levels, in hemp used as human food as well as animal feed, whence it can pass to milk and dairy products [42, 43]. The existence of a teratogenic risk, if any, in human consumers would depend from intake levels and the threshold in genetically-susceptible individuals. Nevertheless, these data flag that even natural substances, present, e.g., in supplements and novel foods may induce mechanisms relevant to HPE.

From a risk assessment perspective, the most interesting aspect is that several pollutants widespread in food commodities may represent indirect risk factors for HPE by acting as metabolic disruptors, thus enhancing the risk of T2D (as well as obesity and other metabolic disorders), mainly by acting on endocrine-regulated pathways [1, 44]. Several such substances are persistent chemicals accumulating in food chains and representing important dietary contaminants. A recent systematic review and meta-analysis of available human studies (both cohorts and case controls) estimated the relationship between the risk of gestational diabetes mellitus and persistent, bioaccumulating pollutants such as the lipophilic polychlorinated biphenyls (PCBs) and poly-brominated diphenyl ethers (PBDEs) and per- and polyfluoroalkyl substances (PFAS), that persist in the body mainly by binding to serum proteins. All three families of persistent pollutants were associated with a moderate but statistically significant increases of the risk with average odds ratios ranging 1.32-1.06 [45]. Similar results were obtained also for phthalates, which are widespread, lipophilic but relatively non-persistent chemicals mainly released in foods from food contact materials [46]. Phthalates may increase the risk of diabetes through activation of PPARs, thus dysregulating

both the lipid and glucose homoeostasis and the development and progression of pancreatic β cells [46].

A significantly increased risk for gestational diabetes of a similar magnitude order as in [43] was observed by a recent meta-analysis focused on PFAS. In this case the responsible of the significant association was PFOA, one of the most widespread and most investigated PFAS [47]. As already specified, gestational diabetes is not associated with HPE [27]. Nevertheless, the results reported in [45-47] indicate a potential for unbalancing insulin homeostasis by several main food contaminants, prompting to further investigation on the contribution of toxicant exposures to pregestational diabetes and its impact as teratogenic factor.

Noticeably PFAS are also activators of PPAR-alpha [48]. A thorough *in vitro* study on the human hepatocyte cell line HEP-G2 was carried out on PFOA, a PFAS involved in major contamination hotspots worldwide. The results show that PFAS can increase the risk of glucose intolerance acting at hepatic level. PFOA, at the concentration of 0.1 ng/mL reduced glycogen synthesis: impaired membrane translocation of the glucose transporter Glut-4 upon insulin stimulation and early uncoupling of insulin receptor activation from downstream were considered early events involved in low grade chronic inflammation-related insulin resistance [49]. PFAS exposure can also worsen the clinical phenotype in organisms already affected by diabetes, as shown by dose-related changes in lipidomics and pancreatic insulitis in non-obese diabetic (NOD) mice caused by the exposure to the PFAS perfluoroundecanoic acid in drinking water. NOD mice represent a spontaneous animal model of immune diabetes, related to human diabetes type 1 [50]. Nevertheless, the possible relationship between PFAS and pregestational diabetes is not straightforward. Artificial intelligence was used to build an AOP-based investigation on the associations between PFAS and adverse metabolic outcomes. With a remarkable burden of uncertainties, the association is more plausible with dyslipidaemia and obesity rather than with insulin resistance [51]. This finding does not rule out the possibility that PFAS may aggravate a preexisting hyperglycaemic status or may increase the risk of non-insulin-dependent diabetes [52]; indeed, since the critical aspect appears to be the pregestational hyperglycaemia, type 1 diabetes is a risk factor for HPE comparable to the more frequent and later-onset insulin-resistant T2D [29, 31].

The two main long-chain PFAS, PFOS and PFOA, are being globally phased-out as new entries in the Persistent Organic Pollutant group [53]. These chemicals will remain as "legacy contaminants" in ecosystems and agrifood chains: pervasive presence is associated to their persistence as well to their widespread industrial uses (e.g., textiles) due to their tensioactive and hydrorepellent properties. In its 2020 evaluation the EFSA concluded that, in the European Union, fish, fruits and eggs – and the related products – are the main contributors to the dietary intake and that the exposure levels of parts of the general population are of concern [54]. Preliminary studies on fluorinated compounds proposed as alternatives indicate that at least some of them, such as the 6-chlorinated perfluoroalkyl ether sulfonic acids, have the potential to alter glucose homeostasis markers [55].

Another main example of persistent pollutants associated with diabetes is represented by polychlorinated biphenyls (PCB), a widespread group of chemicals widely employed for industrial uses till their ban in the 80s, due to their persistence and bioaccumulation, PCB still feature as significant food pollutants, even decades after ban. PCBs are lipophilic and contaminate mainly foods of animal origin, such as fatty fishes followed by eggs, milk and dairy products and meat [56]. Several human studies associate PCB serum levels with abnormal glucose metabolism and the risk of diabetes. A recent, large (>4,000 subjects) cohort study carried out in China evidenced the positive relationships of serum PCBs concentrations with fasting plasma glucose values and the risk of diabetes. While no association was found with an oxidative DNA damage biomarker (urinary 8-hydroxy-2'-deoxyguanosine), a clear, significant association was found between PCB exposure and the lipid peroxidation biomarker urinary 8-isoprostane. The highly persistent PCB 153 showed the strongest association with glucose disturbance and lipid peroxidation among the seven main congeners tested [57]. Interestingly, PCB congeners may trigger different toxicity pathways, and PCB 153 belongs to a congener cluster mainly modulating genes involved in steroid and lipid synthesis [58].

Inorganic arsenic, an important pollutant worldwide, is another major dietary contaminant associated with T2D. The main environmental sources are either man-made and geochemical; the main contributors to dietary intake are rice and rice-based products as well as dinking and household water (e.g., used for cooking) in contaminated hotpots. Conversely, organic arsenic metabolites (arsenobetaine, arsenosugars, arsenolipids) accumulate in fish and seafood, but they are generally considered to be of low toxicity [59]. Arsenic appears to disrupt directly insulin-regulated pathways by eliciting several, confluent early events: beta-cell dysfunction (though enhanced apoptosis and inhibited proliferation), impaired glucose tolerance by the reduction of glucose-stimulated insulin secretion, both directly and by increasing oxidative stress through disrupted mitochondrial function; downregulated insulin synthesis by decreasing insulin mRNA transcription [60]. Arsenic also alters the microRNA profile of pancreatic beta cells, especially targeting miR-29a. An *in vitro* study on INS-1 832/13 beta cells showed that genes downregulated by inorganic Arsenic(III) were enriched in insulin secretion and T2D pathways. On the other hand, such effects were not seen with the methylation product methyl arsenite, which downregulated genes enriched in cell cycle and critical beta cell maintenance factors. Overall, post-transcriptional control in pancreatic beta cells may be a main target of inorganic arsenic [61]. In humans, there is evidence that populations living in areas with high environmental arsenic have a greater risk of T2D [62]. On the other hand, it is less clear whether current exposure levels in industrialized world areas, with more severe regulations toward pollutants, are associated with an enhanced risk; however, growing

evidence [63-65] show that higher levels of biomarkers of exposure to arsenic are associated with increased T2D-relevant biomarkers, including altered cholesterol levels [65]. While arsenic likely interacts with several other risk factors, e.g., genetic polymorphisms [63], the available data indicate that the potential link between arsenic exposure, pregestational diabetes and the risk of HPE, and other malformations, deserves attention.

CONCLUSIONS

No studies till now have investigated the possible link-mediated through the enhanced risk of pregestational diabetes between a rare and severe malformation of early nervous system patterning, such as HPE, and widespread food pollutants, such as inorganic arsenic, PFAS and PCBs. Up-to-date scientific evidence lend support to the biological plausibility of such link, indicating that further investigation is worthwhile. Several widespread food contaminants alter metabolic pathways relevant to insulin and/or lipid regulation and increase the risk of pre-gestational diabetes, which is a recognized major risk factor for HPE. The main pathway by which pre-gestational diabetes increases the risk of HPE appears to be increased oxidative stress in neuroectoderm, in particular neural crest cells. The onset of HPE requires a genetic predisposition, including stochastic mutations in genes involved in embryonic patterning. Thus, the available evidence point to a plausible working hypothesis, based on a multifactorial pathway; the widespread exposure to dietary pollutants increases the risk of gestational diabetes which leads to an increased HPE risk in susceptible embryos. This hypothesis identifies a scenario "widespread exposures-rare outcomes in susceptible subjects", which presents a number of difficult issues. At the moment, limited investigations have been carried on the complexity of factors and pathways leading to rare malformations: a recent example is the use of the AOP approach in order to assess the biological plausibility of the link between exposure to a food pollutant, the mycotoxin fumonisin B1, and neural tube defects [10]. More insights are expected from the growing use of integrated transcriptomic/proteomic/metabolomics analysis in epidemiological studies, including those on the relationships between type 2 diabetes and environmental chemical exposures [66]. In the case of HPE, the use of integrated omics may help clarify the role of maternal and/or environmental factors and related biomarkers, e.g., the relevance of enhanced glycation products [38, 39]. Definitely, the issue of complexity of rare multifactorial conditions implies a number of uncertainties, which have to be tackled using interdisciplinary approach and structured uncertainty analysis [67]. Meanwhile, understanding pathogenetic pathways and associated risk factors paves the way to evidence-based primary prevention strategies [2]. In conclusion, the available data point out to a potential link between widespread pollutants and HPE, through enhanced diabetes risk, that is worth being further explored.

Conflict of interest statement

The Authors declare that there are no conflicts of interest.

Received on 11 July 2023.
Accepted on 2 October 2023.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 304-312
DOI: 10.4415/ANN_23_04_10

Diagnostic assessment, therapeutic care and education pathways in persons with autism spectrum disorder in transition from childhood to adulthood: the Italian National Ev.A Longitudinal Project

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Abstract

Introduction. The transition from childhood to adulthood is one of the main critical points in the network of services for taking care of people with autism spectrum disorder (ASD). Within the framework of the national research programs on autism, an exploratory longitudinal multicentre study was conducted. This research program, called "Ev.A Project (Developmental and Adult Age)", was proposed by the Italian National Institute of Health (Istituto Superiore di Sanità, ISS) and the aim was the development and testing of a diagnostic, therapeutic, assistance and educational pathway (PDTAE) for autism.

Aim. The present study aimed to evaluate two impact outcomes of the care protocol: the response obtained by the ASD person, and the perception of the change in the family context.

Methods. Participants underwent an initial clinical evaluation and then after one year. Over the course of the year, participants undertook a program of intervention. The measures of adaptive functioning, need for support, psychiatric symptomatology and family quality of life were used for the outcome assessment. Linear mixed models were constructed for each measure to estimate the explanatory/predictive behavior of the intensity of the interventions, adjusted for the participant's level of symptom severity.

Results. The results estimate a main effect of Intervention Group ($b=-27.22$, $p<0.001$) and severity level ($b=-41.87$, $p<0.001$) on the adaptive functioning of the ASD person, but no effect on performance on the dimension of Family Quality of Life ($b=0.523$, $p=0.455$).

Conclusions. The most significant predictor of the impact on the ASD person is the activation of the service network, which must take into account the level of severity of the presented symptoms.

Key words
- autism
- transition age
- diagnostic assessment
- therapeutic care
- health services

INTRODUCTION

Autism spectrum disorder (ASD) represents an atypical and heterogeneous developmental condition that emerges early in life [1] but it has a life-long impact on an individual's development and adaptation [2]. Although there is an increasing competence of public mental health services in the early diagnosis and in structuring interventions to improve the treatment of the ASD person, the transition from developmental age to adulthood represents one of the main critical

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issues of the service network in caring for people with ASD.

The term "transition" generally refers to a planned and targeted process that addresses the needs of ASD young people (from the age of 16-18 years) holistically in the passage from a child to an adult-oriented health care system [3]. The number of ASD services decreases from childhood to adulthood and continues to decline into late adulthood, while the use of drugs and hospitalisation services increases [2, 4, 5]. Moreover, the transition from developmental age to adulthood represents a period characterised by significant changes in neurobiological and behavioral development [3] in which adolescents and young adults experience so-called "developmental challenges", such as the transition to secondary education, the conclusion of schooling, the access to employment, the acquisition of personal autonomy and the development of friendships and intimate relationships. These changes that occur during development are also made difficult by social interaction deficits [6], which become more evident with reaching adulthood, an age in which a person should achieve more independence.

Although the literature focusing on transition is still scarce, some studies emphasize that, at this stage, services should set a central objective to support adolescents with ASD in learning to "function as adults" [7, 8]. This aspect is of relevance as people with ASD show persistent impairments as well as regressions in functional and adaptive abilities in adulthood and high rates of mental health problems [5, 8, 9]. Moreover, they show a lower rate of independent living and employment opportunities [3, 10] and impairments in social cognition skills necessary for good social interactions, friendships and relationships [7, 11]. These aspects also appear to be associated with the severity of autism [12]. Generally, terms such as "high" and "low" functioning are used to define the symptom profile of persons with ASD. However, research supports the attribution of ASD severity by means of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), made based on the "intensity of support needs", differentiating into 3 severity levels (Level 1, 2 and 3) and considering impairments for social communication and restricted/repetitive behavior [1]. Analysing the support needed emphasises the impact of person-environment interaction, which is relevant for people with ASD who may be affected by the environmental context [13]. The severity level based on the intensity of support needs provides a picture of the ASD person's functioning, taking into account variables such as behavioral problems of cognitive and adaptive functioning, comorbidities and variables related to the context and environment. In fact, a worse transition outcome has a broader impact and is not only experienced by the person with ASD but also by their relatives who provide continuous support from adolescence to adulthood. Several studies show that caregivers of adults with ASD have more depressive symptoms, worse health, and lower functional capacity, leading to a negative impact on quality of life [14]. Based on these considerations, a successful transition should also be based on how relatives perceive this process and its outcomes.

There is a growing recognition of the need for expertise and resources to provide accurate clinical assessment appropriate for adulthood, as well as appropriate intervention services for persons with ASD of all ages from a developmental perspective [3]. Currently, in Italy, the National Institute of Health (Istituto Superiore di Sanità, Italy) is working on national guidelines concerning the diagnosis and treatment of adults with ASD by existing legislation (national law on autism n. 134 of August 18, 2015, guidelines approved by the 2018 State-Regions Conference). Furthermore, guidelines are also available in the international context, such as in the USA, the UK and New Zealand, to improve access and involvement in interventions and services for persons with autism [15-18]. It is interesting to note that the different guidelines share the evidence that the transition care should start as early as adolescence and continue beyond the day of leaving the pediatric services and the involvement of all figures responsible for the well-being of the person with ASD.

Based on these premises, the present study was conducted as part of a larger multicentre project, namely the Ev.A Project (from developmental age to adulthood) proposed by the Italian National Institute of Health, commissioned and founded by the Ministry of Health, within the implementing framework of the national law on autism n. 134 of August 18th, 2015, and officially joined by the regions of Piedmont, Valle d'Aosta, Tuscany, Abruzzo, and the Autonomous Provinces of Trento and Bolzano. The general purpose of the Ev.A Project was the development and testing of a diagnostic, therapeutic, assistance and educational pathway (PDTAE) for autism, from the age of development to adulthood and the relative procedures and preparatory actions for its implementation. In particular, the PDTAE addressed the efficient and effective planning and management of the transition phase between adolescence and adulthood in ASDs.

Specifically, the present study aimed to assess the outcome by taking into account two levels of impact of the caring protocol: 1) the impact on the perception of the response obtained by the ASD person and 2) the impact on the perception of the change in the family context. Therefore, the study was conducted to assess the possible impact of the activation of the service network on young ASDs and the family context. For this purpose, the participants were divided into two groups based on the interventions activated during the trial, and measures of adaptive functioning, need for support, psychiatric symptomatology, and family quality of life were used for the outcome assessment.

METHODS

Participants and procedure

139 individuals with ASD and aged between 16 and 25 years participated in the study. The diagnosis of ASD was formulated by experienced clinicians according to the criteria of the DSM-5 [1], using the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) [19] and the Childhood Autism Rating Scale, Second Edition – Standard Version (CARS 2-ST) [20] for ASD individuals with communication

difficulties and intellectual disability. All participants with ASD were classified as Level 1, Level 2, or Level 3 according to DSM-5 criteria. An exploratory, pre-post treatment longitudinal study was conducted in which participants underwent an initial clinical evaluation (T0) and then after one year (T1). Over the year, the participants undertook a therapeutic and intervention pathway in collaboration with different services and institutions (health, social welfare, education, and training) involved in the clinical management of the patient in the transition phase.

At the beginning of the project (T0), participants underwent a cognitive assessment by Wechsler Adult Intelligence Scale, Fourth Edition, Italian version (WAIS-IV) [21, 22], and Leiter International Performance Scale, Third Edition (Leiter-3) [23], based on the ASD participants' language abilities. The inclusion criterion was the availability of pre (T0) and post (T1) observations and several missing values in the pre- and post-measurements not exceeding 10%. Thus, the final sample consisted of 112 subjects with a mean age of 20.05 years (SD=2.71) of which 89 were male and 23 female (*Table 1*).

All participants or their legal tutors (parents or others), when necessary, gave informed consent to participate in the study. The study was conducted according to the Declaration of Helsinki and the rules of good clinical practice and ethics in public mental health services.

Therapeutic and intervention pathway

In the Ev.A Project, an individualised life plan was developed for each person involved based on the diagnostic process and support needs.

The treatment and intervention pathways in the PDTAE included:

- *medical-psychiatric interventions*: consultations and follow-up visits to monitor any psychiatric comorbidities and any pharmacological therapies;
- *psychological-psychotherapeutic interventions*: psychoeducational counseling of family members, teachers, and other caregivers; supportive interviews (acceptance/awareness of the diagnosis), individual cognitive-behavioral psychotherapies for the treatment of anxiety and/or depressive symptoms, social skills training groups; neuropsychological skills enhancement;
- *educational interventions*: for persons with intellectual disability, it concerns the cognitive enhancement and promotion of personal and domestic autonomy, and basic social skills through the use of structured educational strategies; for persons with good cognitive functioning or mild intellectual disability, it concerns the enhancement of emotional-social and communicative pragmatic and self-regulation skills;
- *social welfare and social inclusion interventions*: coordinated by the social worker, with the aim of providing the person with an experience of social inclusion, within everyday social contexts and typical functioning peer groups, by accompaniment of the person by a trained worker (e.g., "adult buddy"); in the school setting, provide for the involvement of trained peers (peer-mediated education);
- *employment interventions*: training courses aimed at occupational guidance, employability assessment, and professionalization;
- *parent-training and sibling programs*: they aim to create a space for emotional sharing, to make parents as aware as possible of their adolescent or young adult child's difficulties and abilities. They provide information on psycho-educational aspects and the implementation of intervention techniques. Siblings can also present different forms of psychological distress, so through group meetings with other siblings it is possible to share experiences and emotions in a protective and pleasant context.

The *Table 1S* available as *Supplementary Material* shows the percentages of activated interventions for the total sample.

Table 1
Mean scores of clinical and cognitive data of ASD (autism spectrum disorder) participants

	Mean (SD)
Chronological age	20.05 (2.71)
Gender (F; M)	23; 89
ADOS-2 MODULE 4	
SA	12.22 (4.04)
RRB	2.06 (1.69)
Total	12.22 (4.04)
CARS 2-ST	33.39 (6.54)
Total IQ	
Verbal IQ	83.78 (24.79)
Non verbal IQ	71.57 (27.77)

SA: social affect; RRB: repetitive and restricted behaviors; Total: total ADOS-2 score.

Outcome measures for ASD person

Adaptive functioning

The Adaptive Behaviour Assessment System-II (ABAS-II) [24] and the Vineland Adaptive Behavior Scale II (VABS-II) [25] were used to assess the adaptive functioning of participants with ASD. Both measures are interviews with caregivers and assess the person's adaptive behavior, i.e., the activities habitually performed to meet expectations of autonomy and social responsibility. The scales investigate 4 domains respectively: communication/conceptual (expressive and receptive language skills, school skills), daily life (self-care, life at home, community) social (socializing, leisure), and motor skills. Only three domains were used in the study (communication/conceptual, daily life, and social). Both measures provide a standard score (M=100, SD=15) and a higher score indicates better functioning. In the course of the trial, the participating regions used both measures. For the study, as the measures assess the same construct (adaptive behavior) and domains, providing a standard score of equal magnitude, they were considered as a single index of adaptive functioning in the analysis.

Support intensity scale

The Support Intensity Scale (SIS; Thompson *et al.*) [26] is a semi-structured interview with caregivers useful for assessing the support needs of persons with intellectual disabilities in medical, behavioral, and living activities. The scale consists of 49 items on life activity support needs and is divided into six subscales: home life activities, community life activities (e.g., going shopping) learning activities, work activities (e.g., learning and using specific work skills), health and safety activities (e.g., taking medication) and social activities (e.g., socializing within the family).

The items are scored on a 5-point scale about frequency (0=none or less than once a month; 1=monthly; 2=weekly; 3=daily; 4=hourly), daily support time (0=none; 1=less than 30 minutes; 2=between 30 minutes and 2 hours; 3=between 2 and 4 hours; 4=4 hours or more) and type of support (0=none; 1=monitoring; 2=verbal or gestural prompting; 3=partial physical assistance; 4=full physical assistance). The subscale scores are obtained by summing the ratings of frequency, daily care time, and type of care and transformed to obtain the standard scale scores. Finally, the standard scale scores can be converted into a Support Needs Index. A higher score indicates a greater need for support.

Psychiatric symptomatology

The Brief Psychiatric Rating Scale Version 4.0 (BPRS 4.0) [27, 28] is a semi-structured interview containing 24 items, translated into Italian by Morosini and Casacchia [29] that assesses 24 psychiatric symptoms. The presence and severity of psychiatric symptoms are assessed on a Likert scale with a range from 1 (no symptoms) to 7 (extremely severe symptoms). The final score ranges from a minimum of 24 to a maximum of 168, with lower scores indicating less psychopathological severity.

Outcome measure in family

Family quality of life was assessed through the administration of the Italian translation of the Family Quality of Life Survey - 2006 (FQoLS) [30], named Strumento d'Indagine della Qualità di vita della Famiglia (SIQF). It is a questionnaire composed of 9 domains covering specific areas of family life, such as health, economic well-being, family relationships, support from others, support from services, the influence of values, career, time for oneself, fun, and integration in the community. Each domain contains two sections: section A, which contains questions that gather some general information and provide context, and section B, which contains questions on six key concepts: importance, opportunity, initiative, achievement, stability, and satisfaction. The items in section B are rated on a 5-point Likert-type scale from 1 to 5, where 1 expresses "not at all" and 5 "very much". The overall QoL scores are calculated by weighting the satisfaction scores with the importance scores [31]; thus, the maximum QoL score results from the maximum importance value and the maximum satisfaction value; the minimum score, on the other hand, results from the maximum imbalance, i.e., the highest importance value and the lowest satisfaction value in a range between -10 and +10.

Statistical analysis

For the analysis and given the sample size, treatment intensity was categorized using the maximum value of the frequency distribution of activated treatments during the study (i.e., at least 3 activated interventions). In this way, two groups were formed: Group 1 (n=49; 40 males and 9 females) with 3 or more activated interventions and Group 2 (n=63; 49 males and 14 females) with less than 3 activated interventions.

Firstly, an exploratory univariate analysis (t-test paired samples) was used to explore whether there were any differences in performance on the measures considered between T0 and T1 for the two groups.

Secondly, linear mixed models (SIS) were constructed given the longitudinal nature of the experimental design, taking into account confounding variables (number or intensity of intervention and level of severity). For each model, the probability of introducing a random slope along with the random intercept was analyzed to estimate the random effects; the fitting of the model was assessed using the Wald Test. To assess the impact of the caring protocol, we included the symptom severity of the ASD participants in the analysis in addition to the intensity of the intervention. Examining the influence of the level of severity on the basis of the required support according to the DSM-5 has implications for clinical and research practice in treatment planning, activation of necessary services and the prognosis of the ASD person. So, the models estimated the explanatory/predictive behavior of the intensity of the interventions on the outcome measures considered, adjusted for the participant's level of symptom severity (DSM-5). The effect of the interaction between the intensity of the activated intervention and the level of severity was also estimated (see *Table 2S* available online as *Supplementary Materials)*.

Analyses were conducted using STATA (version 17.0).

RESULTS

Differences in outcome measures between time points

About measures directed at the ASD person, t-test paired samples revealed only a significant reduction in SIS scores in Group 1 ($t(48)=2.26$, $p=0.026$). No significant differences emerged in Group 2. Regarding the family context, the results also show no significant differences in the SIQF within the two groups. Results of t-test paired samples are reported in *Table 2*.

Outcomes linear mixed models

Adaptive functioning

About the impact on the ASD person, the results obtained from the LMMs show ($\chi^2_{df=5} = 50.49$, $p<0.001$) a main effect of treatment intensity ($\beta=-27.22$, $p<0.001$) and severity level on adaptive functioning, in particular, level 3 appears to have a significant influence ($\beta=-41.87$, $p<0.001$).

The adjusted multivariate analysis supports the conclusion that belonging to the group with a lower intervention intensity (<3), presents a worsening of adaptive abilities compared to the group with a higher intervention intensity; this change is also explained by the Level of severity ranging from 2 to 3, compared to Level 1.

Table 2
Mean scores and comparison between the two time points (T0 and T1) of outcome measures in the two groups

	Group 1, three or more interventions (n=49)				Group 2, less than three interventions (n=63)			
	Mean (SD) T0	Mean (SD) T1	t	*p*	Mean (SD) T0	Mean (SD) T1	t	*p*
Adaptive index	64.79 (28.22)	68.60 (31.49)	-1.82	0.075	49.95 (23.93)	50.79 (25.86)	-0.498	0.621
Comunication skills	68.53 (24.63)	70.87 (25.67)	-1.36	0.179	55.84 (23.69)	57 (24.44)	-0.643	0.621
Daily life skills	71.09 (27.66)	72.74 (27.96)	-0.992	0.372	67.51 (22.02)	67.90 (22.72)	-0.297	0.768
Socialization skills	62.60 (21.98)	64.87 (23.6)	-1.72	0.091	49.46 (22.86)	48.80 (24.14)	0.358	0.721
SIS	87.21 (18.94)	83.69 (19.16)	2.26	**0.028**	87.87 (15.19)	85.97 (15.42)	1.58	0.119
BPRS	49.48 (15.65)	47.21 (17.22)	1.81	0.076	47.07 (16.86)	44.92 (17.21)	1.47	0.147
SIQF	1.76 (2.66)	2.22 (2.33)	-1.14	0.260	1.69 (2.27)	1.76 (2.52)	-0.310	0.758

Significant differences (p<0.05) are reported in bold. Group 1: group with more than 3 interventions activated; Group 2: group with less than 3 activated interventions. SIS: Support Intensity Scale; BPRS: Brief Psychiatric Rating Scale; SIQF: Strumento d'Indagine della Qualità di vita della Famiglia (Family Quality of Life Survey).

Analyzing the components of the adaptive index communication/conceptual skills, practical/everyday life skills, and social skills), the results show that belonging to the group with lower intervention intensity significantly predicts a worsening of communication/conceptual skills (β=-22.05, p=0.001), daily life skills (β=-13.64, p=0.021) and social skills (β=-22.34, p=0.001) compared to belonging to the group with higher intervention intensity (see *Table 3*).

There is also a main effect of severity level, in particular, presenting a severity Level 3 significantly predicts lower scores in communication/conceptual skills (β=-35.78, p<0.001), daily life skills (β=-46.94, p<0.001) and social skills (β=-20.30, p=0.007) compared to severity Levels 2 and 1. However, these changes do not seem to be explained by the interaction between severity level and intervention intensity (*Table 3*).

Psychiatric symptomatology

The LMM results show a main effect of severity level on the possible change in psychiatric symptomatology over the course of the trial. Specifically, the results show that Level 3 influences the increase in score on the BPRS compared to Level 1 symptomatology (β=15.17, p=0.004). It would appear, on the other hand, that membership in the intervention group does not significantly influence the possible change in symptoms (β=0.569, p=0.907). The interaction between the intervention group and the level of severity also did not appear to be significant (*Table 3*).

Support index

A main effect of both intervention group membership and severity Level 3 (β=32.611, p<0.000) on the need for support is shown. Specifically, the results show that belonging to the group with the lowest intervention intensity (β=10.364, p=0.007) requires more support. It would also appear that presenting a severity Level 2 (β=22.719, p<0.000) or 3 (β=32.611, p<0.000) influences the increase in the support index in comparison to participants with Level 1. The results also show a significant influence of the interaction between the intervention group and severity level; participants with Level 2 and belonging to the group with lower intervention intensity would show a decrease in the score on the SIS scale (β=-15.51, p=0.003) (*Table 3*).

Outcome LMM in quality of life of family

The fitting of the model does not reach statistical significance ($\chi^2_{df=5}$=4.36; p=0.498) and the predictors are not statistically significant (see *Table 3*). The model does not provide evidence for a predictive role in the perceived quality of the family about the intervention, the level of severity as well as about any interaction between severity and the intervention administered.

DISCUSSION

Awareness of the importance of establishing pathways for clinical assessments and providing appropriate intervention services for adults with ASD has increased in recent years; however, the transition from childhood to adulthood represents one of the main critical points in the network of care services for people with ASD.

This study examines the impact of a large multicentre project, namely the Ev.A Project, on the outcome of the ASD person and family context. Specifically, the general aim of the research program was to develop a protocol of evaluative and diagnostic instruments focused on the general functioning of the ASD person in order to estimate the impact of the activation of rehabilitation services both on the individual and on the quality of life of the family context.

During the project's experimental phase, different interventions for participants with ASD and their families were planned and implemented in cooperation with territorial institutions, such as health, social welfare, education, and training services. Our analyses examined the outcome of individual participants with ASD and the family context, that were divided into two groups based on the number of interventions activated. In addition, we estimated the explanatory/predictive behavior of the intensity of the interventions on the measures considered, adjusted for the participant's level of symptom severity, and estimated the effect of the interaction between the intensity of the activated intervention and the level of severity.

Table 3
Outcome LMM (linear mixed model) in ASD (autism spectrum disorder) person and in quality of life of family

	Adaptive index			Comunication skills			Daily skills			Social skills		
	β	SE	p	β	SE	p	β	SE	p	β	SE	p
Predictors												
Intervention group	-27.22	7.35	**<0.001**	-22.05	6.71	**0.001**	-13.64	5.92	0.021	-22.34	6.94	**0.001**
Severity level	*Reference*											
Level 2	-23.97	7.75	**0.002**	-18.91	7.10	**0.008**	-25.59	6.24	<0.001	-11.06	7.32	0.131
Level 3	-41.87	7.98	**<0.001**	-35.78	7.31	**<0.001**	-46.94	6.43	<0.001	-20.30	7.54	**0.007**
Group Intervention*severity level												
Group 2 x Level 2	14.25	10.11	0.159	11.03	9.27	0.234	13.54	8.15	0.097	10.90	9.55	0.254
Group 2 x Level 3	18.20	11.14	0.102	14.51	10.23	0.156	11.61	9.00	0.197	14.40	10.54	0.172
Wald Chi² Test	$\chi^2_{df=5}$ = **50.49; p<0.001**			$\chi^2_{df=5}$ = **43.32; p<0.001**			$\chi^2_{df=5}$ = **86.42; p<0.001**			$\chi^2_{df=5}$ = **19.42; p=0.001**		

	SIS			BPRS			SIQF		
	β	SE	p	β	SE	p	β	SE	p
Predictors									
Intervention group	10.36	3.84	**0.007**	.56	4.89	0.907	0.523	0.700	0.455
Severity level									
Level 2	22.71	4.05	**<0.001**	15.27	5.09	**0.003**	.857	0.738	0.245
Level 3	32,61	4.17	**<0.001**	15.17	5.25	**0.004**	1.20	0.759	0.112
Group Intervention*severity level									
Group 2 x Level 2	-15.51	5.29	**0.003**	-7.12	6.59	0.280	-0.788	0.957	0.410
Group 2 x Level 3	-8.92	5,82	0.126	3.00	7.27	0.679	1.866	1.05	0.076
Wald Chi² Test	$\chi^2_{df=5}$ = **100.09; p<0.001**			$\chi^2_{df=5}$ = **25.14; p<0.001**			$\chi^2_{df=5}$ = 4.36; p=0.498		

*Interaction between the intensity of the activated intervention and the level of severity; significant differences (p<0.05) are reported in bold.
SIS: Support Intensity Scale; BPRS: Brief Psychiatric Rating Scale; SIQF: Strumento d'Indagine della Qualità di vita della Famiglia (Family Quality of Life Survey).

Our results showed that both the activation of rehabilitation services and the level of severity predicted the support the individual needs in daily life. In fact, the activation of more interventions and, consequently, a more comprehensive approach would seem to improve the person's autonomy. However, it must be considered that the type and intensity of support the ASD person needs varies according to the level of severity as defined by the DSM-5 [1]. Furthermore, the interaction between the intervention group and severity level was found to be significantly predictive of the intensity of the support needs, showing that ASD persons with severity Level 2 who received fewer interventions would have a lower need for support in daily life. Although this result could be considered counterintuitive, it could be traced back to the clinical characteristics of Level 2. Compared to Level 1, which is generally attributed to the so-called "high functioning", and Level 3, which includes the most severe degree of autistic symptomatology, ASD persons with Level 2 generally exhibit emerging abilities that need to be acquired, as well as greater variability in clinical presentation. We believe that this result can be interpreted as an incentive to intensify intervention since the activation of some services already shows a trend toward a perceived improvement in the family's need to support the person.

The level of severity and intensity of the intervention also appears to influence the adaptive behavior of the ASD person. As we expected, the group with fewer interventions would present lower abilities in all domains of adaptive behavior, i.e., in communication, daily life and socialisation abilities. Moreover, people with Level 3 would show almost twice as much impairment as Level 2 in adaptive functioning, particularly if we take social skills into account. Adaptive behavior is defined as the extent to which a person is able to be self-sufficient in real-life situations, including the functional use of communication, socialisation, daily living and motor skills [8, 25]. Our findings are in line with evidence in which continuity of services and interventions appear to be crucial in ASD persons, in whom regression of adaptive abilities in the transition phase is not uncommon [8, 32]. The acquisition of adaptive skills in young adults with autism is largely dependent on their inclusion in intensive and routine rehabilitation programs and the stabilisation of daily life routines [8]. This would be of particular importance for ASD persons with Level 3 severity, in which according to DSM-5 criteria, they present severe deficits in communication and social interaction, as well as resistance to change and repetitive behavior that significantly interferes with general functioning. This may be especially true for social skills, as

individuals with more severe early social deficits present more severe current adaptive deficits [33, 34]. In our sample, the most activated interventions, i.e., educational (70.5%) and social assistance and inclusion (41.1% and 50.9% respectively) are precisely those that aim to promote social skills, personal and domestic autonomy and social inclusion. For the latter aspect, the accompaniment of trained persons as a "buddy" for persons with ASD in the experience of everyday contexts is becoming increasingly helpful.

In the assessment of psychiatric symptomatology evaluated by BPRS, in which scores are taken from direct observation of the person, the results show that the increase in psychiatric symptomatology is associated with the severity of the level, independently of the intensity of the intervention. Although psychiatric symptoms are common in young ASDs, at a significantly higher rate than in non-autistic populations [5, 35], this result confirms the importance of monitoring psychiatric symptomatology even in lower-functioning ASDs, as it would appear that both lower IQ and adaptive functioning are risk factors in obtaining higher scores on psychiatric scales [36].

Our analysis shows that psychological-psychotherapeutic interventions account for 63.4% and psychiatric and pharmacological interventions for 61.6% and 15.2% respectively. Pharmacological and psychiatric interventions seem to be more frequent in Level 2 and 3 participants and less so in Level 1 (7.9%), while psychological-psychotherapeutic interventions are, as one would expect, more frequent for Level 1 and 2 people (84.2% and 66.6% respectively) and less so for Level 3 (31.03%). We also analysed the perceived quality of life in the family context. Our results show that there are no perceived changes in this dimension, and it does not seem to be influenced either by the intensity of the support from the intervention or by the level of severity presented by the ASD person.

During the trial, parent-training programs were activated for 53.6%, while programs including siblings were activated for only 9.8%. Parents in our sample perceive the quality of life as problematic (*Table 2*) [37]. This result is in line with existing literature, showing that families often perceive an imbalance between the degree of importance and satisfaction in specific areas of family life, such as health, economic well-being, family relationships, support received from the social context and services, as well as personal values, career, time for oneself and community and leisure activities [14, 38]. Parents are usually directly responsible for the ASD person and experience negative transition outcomes, face ongoing challenges that may affect their family life and require support to achieve a better quality of life [14, 38]. Many families require transition planning to adulthood with increased support for social skills, life skills training and employment support. Currently, the literature regarding the transition phase of ASD persons is scarce, but it points out that a greater understanding of risk and protective factors at the level of families is needed to develop and plan a functional transition [14]. Although we are aware that 12 months is a short timeframe in which to perceive changes in an intervention, quality of life assessment in clinical practice would also make it possible to identify needs, plan interventions and mobilise resources in the most useful way to increase satisfaction in the most important areas of people's lives.

To date, few evidence-based services exist to support people with ASD in the transition phase and the literature on this is developing slowly [18, 39]. Despite this, intervention programs for people with ASD in transition age that focus on adaptive skills, social skills, and self-determination, as well as programs that specifically target work and university employment are also proposed in the international landscape [18, 39, 40]. To our knowledge, the Ev.A Project represents one of the first studies in assessing the impact of care protocol on people with ASD and caregivers in the transition phase including all levels of severity of the condition. Furthermore, our results seem to support and be in line with the international scenario. In fact, many of the available documents (UK, USA, Australia) recommend planning and coordination of the transition at least from the end of adolescence (12-14 years), that each young ASD has a "named worker", involvement of family members, sharing of information with the general practitioner, creation of a 'transition team' involving children and adult services [18].

In conclusion, our results suggest that one of the most significant predictors of the impact on the ASD person is the activation of the service network, which must take into account the level of severity of the presented symptoms and support the idea that intervention planning should be based on evidence. However, the study is not without limitations. Except for the BPRS, the outcome measures used for the assessment of adaptive behavior (ABAS-II and VABS-II) and the need for support (SIS) represent indirect measures of the ASD person, as they represent information reported by the parents.

With regard to our sample, there is a predominance of the male gender, however, this is characteristic of the clinical condition [8]. In addition, the participants came to the care services consecutively, so it was not possible to carry out a gender balance. The PDTAEs were activated by the territorial structures and services of each region that participated in the Ev.A Project through organisational practices already in use (e.g., the transition to the Multidisciplinary Disability Assessment Unit). Consequently, each participant is subject to more than one intervention within the PDTAE. So, no analysis was conducted to assess the effect of the type of intervention proposed to the participants, as the effect (improvement or worsening) could be associated with one intervention rather than another.

Future studies should take these aspects into account controlling for the variables just mentioned. This would make it possible to analyse possible gender differences and the effectiveness of the type of intervention on the outcome of the ASD person. A strength of the present project is that it included all three levels of severity, considering that many existing studies do not include persons with intellectual disabilities. We believe that our results provide an important contribu-

tion to the scenario of the care and management of people with ASD, highlighting how the activation of the network of territorial services represents the main predictor in the improvement of the symptomatology and functional abilities of the ASD person. Communication between child and adult services is in fact one of the main components in the planning and coordination of the transition phase, but it does not disregard direct work with people with ASD, carers, and their support network.

Authors' contributions

MV, MM, GMA, and RK conceived the study, designed the research protocol, and drafted the manuscript; FM and ILD analyzed the data and gave a substantial contribution to the manuscript; GR and MLS coordinated the project data collection protocol and gave a substantial contribution to the manuscript.

Acknowledgments

Project Ev.A (Ev.A dall'età Evolutiva all'età Adulta: percorsi diagnostici e terapeutici assistenziali ed educativi in fase di transizione - Scientific co-ordinator: Giuseppe Maurizio Arduino).

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Funding

The Ev.A Project received funding following the Decree of Ministry of Health December 30th 2016.

Conflict of interest statement

The Authors declare no conflict of interest.

Received on 23 May 2023.
Accepted on 26 October 2023.

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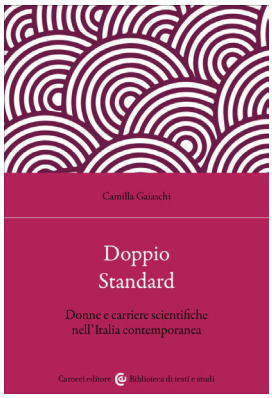
Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 313-314
DOI: 10.4415/ANN_23_04_11

Book Reviews, Notes and Comments

Edited by
Federica Napolitani Cheyne



DOPPIO STANDARD
Donne e carriere scientifiche nell'Italia contemporanea
Camilla Gaiaschi
Roma: Carocci Editore; 2022.
272 p.
30,00 €

[Double standard, women and scientific careers in contemporary Italy]

The European Commission constantly monitors the status of gender equality in research and scientific innovation. In the recent report *She Figures 2021*, it has been once again pointed out that, notwithstanding a positive trend, women continue to be underrepresented in all areas of research, compared to their male counterparts. The data clearly indicate that although on the undergraduate and master's degree level female students outnumber their male colleagues, a gender balance is achieved at the doctoral level. Nevertheless, women are less represented in the population of employed scientists and engineers at the European level (41.3 percent). These data clearly highlight how, despite the adoption of a new European strategy targeting gender equality, more efforts are needed to increase women's participation in scientific careers. It is worth noting that education and gender equality are also an integral part of the 2030 Agenda for Sustainable Development adopted by the United Nations General Assembly in 2015, as distinct Sustainable Development Goals (SDGs) but also as catalysts for the achievement of all others. This agenda considers it crucial to understand and reduce the specific obstacles that keep young women away from science subjects, promoting their careers.

Among the many reasons that have been introduced to explain the gender inequalities in science, in general, and in STEMM (Science, Technology, Engineering, Mathematics and Medicine) in particular, is the notion that women and men do not play according to the same rules: women are judged more harshly than their peers because of an implicit bias, an unconscious conditioning that, even keeping all other things equal, leads us to view a female person as less capable of factually pursuing a scientific career or holding leadership roles than a male colleague, a bias that leads women to work harder to achieve the same results or held the same positions as their male colleagues.

This implicit bias is the "double standard" described by sociologist Camilla Gaiaschi in her interesting essay entitled precisely *Double Standard, Women and Scientific Careers in Contemporary Italy* (Published by Carocci). The book stems from an academic interest in the reasons that lay behind the different career trajectories that characterize women and men who start a scientific career, using both quantitative and qualitative observations. The original and particularly interesting aspect of this assay is that, unlike most available texts, which report aggregate data at the European level, it focuses on the Italian situation, illustrating its peculiarities in terms of scientific careers and work-life balance, broadening the focus also to the analysis of careers in medicine. Through her analytical lens, the author not only confirms the dual track, a premise of the entire work, but also points out the "paradoxes" of gender differences, that is, "unexpected or accidental spaces" in which some distortions are smoothed out, giving reason for the complexity of cultural and structural constraints that hold back women's race for success.

While the premise of the book is an analysis of the "double standard of evaluation" in scientific careers, as we get deeper into the work, which is very dense with data and citations from numerous sources, it becomes clear that the evaluation "bias" alone is not sufficient to explain why women still struggle to build careers in science and medicine. Through careful analysis, the author highlights the complexity of variables involved in changing career trajectories. The multiplicity of "reasons for inequalities" takes the synthetic form of a multilevel map that is nothing but a metaphor for the web that harnesses every woman every day, made up of individual and contextual elements that, like quicksand, limit the potential for achievement at work and in life in general. One example: accumulated work experience represents a measure of a person's human capital. Because women have, on average, more family responsibilities, they more frequently choose to work part-time than men, especially in those situations or countries where there is little employment in general. This difference in the accumulation of hours worked has important negative effects on women's professional growth, as well as on their pay.

The paradox described by the author here is that countries with generous work-life balance policies and high female employment are also those with high pay gaps and high horizontal gender segregation. Italy, although among the less advanced countries in Europe in terms of female employment, is paradoxically among the countries with the smallest pay gaps. As the author well describes, the trade-off between segregation and women's employment is by no means a

foregone conclusion since when you gain in women's employment, you can go backwards in terms of gender equality.

A further employment paradox is that the lack of life-work balance policies means that a country like Italy has more ordinary female professors than Germany and Belgium as a result of female self-selection in high-skilled professions. This effect can also be explained considering the peculiar Italian situation of the last decade, characterized by a high male outflow from the academy due to the retirements of professors, which has accelerated women's access to top academic positions, accidentally oiling career mechanisms that are often slow, unpredictable and discontinuous.

Interesting and original is the author's inclusion of the analysis of careers in the medical field where we see another paradox, namely that in this discipline the glass ceiling has some cracks, if, however, one manages to get to the intermediate career levels.

If some women are just as likely as their male colleagues to make it to the top, the explanation lies in the fact that here, too, they have gone through a self-selection, for example, by limiting the number of children or organizing family life through a dense network of support. The goal has no zero costs and, in any case, would seem to concern a small number of women.

It is a truth, however, that notwithstanding some positive data, women behaviors often hold back their careers. Amongst the many examples brought by the author, it is useful to highlight here the phenomenon of lack of self-promotion: women often ask less frequently than men for career advancement or raises. This behavior can be explained by an underestimation of one's own abilities but also by the realization that if women in a certain company struggle to advance their careers, one will be less likely to go knocking on the door of the manager(s) to ask for career advancement.

In the face of this evidence showing the existence of gender bias in academia and the workplace what can be the solutions? The answers are in the folds of the pages throughout the chapters of the book. Interestingly, it is mentioned that, as far as boards of directors are concerned, our country has made a great leap forward thanks to the Golfo-Mosca law, passed in 2011, by which gender quotas were introduced on boards of directors and boards of statutory auditors of listed companies. This is undoubtedly an important positive action that must, however, be accompanied by many others. Among these, building networks that support women's scientific careers to identify their talents and make them competitive, for example, when they want to access funding that requires an extended research team. In fact, the author reports that, while at the individual level (see Marie Sklodowska Curie and ERC research grants), women are competitive on par with their male colleagues, they have a harder time accessing funding where large research partnerships need to be created.

Gender equality is a society-wide issue. If we are to address the many urgent global challenges, we need all the talents at play, and women in this regard are an important assett. The road ahead to break the glass ceiling is still long and bumpy. Recognizing the barriers that separate us from that goal, means climbing one more rung of the ladder that can get us to break that ceiling, which is what Camilla Gaiaschi's book achieves.

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Ann Ist Super Sanità 2023 | Vol. 59, No. 4: 315-317
DOI: 10.4415/ANN_23_04_12

Publications from International Organizations on Public Health

Edited by
Annarita Barbaro

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)

The state of food security and nutrition in the world 2023. Urbanization, agrifood systems transformation and healthy diets across the rural–urban continuum. Rome: Food and Agriculture Organization of the United Nations 2023; 316 p. ISBN 978-92-5-137226-5. This report provides an update on global progress towards the targets of ending hunger (SDG Target 2.1) and all forms of malnutrition (SDG Target 2.2) and estimates on the number of people who are unable to afford a healthy diet. Since its 2017 edition, this report has repeatedly highlighted that the intensification and interaction of conflict, climate extremes and economic slowdowns and downturns, combined with highly unaffordable nutritious foods and growing inequality, are pushing us off track to meet the SDG 2 targets. However, other important megatrends must also be factored into the analysis to fully understand the challenges and opportunities for meeting the SDG 2 targets. One such megatrend, and the focus of this year's report, is urbanization. New evidence shows that food purchases in some countries are no longer high only among urban households but also among rural households. Consumption of highly processed foods is also increasing in peri-urban and rural areas of some countries. These changes are affecting people's food security and nutrition in ways that differ depending on where they live across the rural–urban continuum. The report provides recommendations on the policies, investments and actions needed to address the challenges of agrifood systems transformation under urbanization and to enable opportunities for ensuring access to affordable healthy diets for everyone.

Food Outlook – Biannual report on global food markets. June 2023. Rome: Food and Agriculture Organization of the United Nations 2023; 160 p. ISBN 978-92-5-137230-2. Issued twice a year, the June issue of the Food Outlook offers market summaries of FAO's reviews of market supply and demand trends for the world's major foodstuffs, namely cereals, oil crops, sugar, meat, dairy and fish. It also looks at trends in food import bills, Ocean freight rates, international food prices and futures markets. The Markets and Trade Division of FAO, as part of the Global Information and Early Warning System (GIEWS), produces and publishes the Food Outlook to enhance transparency in global food markets.

The impact of disasters on agriculture and food security 2023 – Avoiding and reducing losses through investment in resilience. Rome: Food and Agriculture Organization of the United Nations 2023; 168 p. ISBN 978-92-5-138194-6. This FAO flagship report provides a timely and comprehensive overview of how disasters are affecting agriculture and food security around the world. Built on previous work of the FAO on this topic, the report estimates losses caused by disasters on agricultural production over the past three decades and delves into the diverse threats and impacts affecting the crops, livestock, forestry, and fisheries and aquaculture subsectors. It analyses the complex interplay of underlying risks, such as climate change, pandemics, epidemics and armed conflicts, and how they drive disaster risk in agriculture and agrifood systems at large. This report provides also examples of actions and strategies for investing in resilience and proactively addressing risks in agriculture. It demonstrates ways to mainstream disaster risk into agricultural practices and policies and calls for a deeper understanding of the context in which these solutions are implemented.

INTERNATIONAL SCIENCE COUNCIL (ISC)

Flipping the science model: a roadmap to science missions for sustainability. Paris: International Science Council 2023; 16 p. Both natural and social sciences have made significant contributions to the understanding of the challenges and issues affecting our societies and planet. Notwithstanding that, it is now clear that new approaches are urgently needed if science is to be effectively used to make rapid progress. Following the release of the report Unleashing Science, coordinated by the ISC, the Council established the Global Commission on Science Missions for Sustainability in 2021 to explore how these recommendations might be condensed to practice. This report summarizes the conclusions reached after extensive consultation with experts, detailed in the accompanying report "A model for implementing mission science for sustainability". As part of the proposed shift in how to tackle the 2030 Agenda and its SDGs with greater expediency, the ISC Global Commission is calling for science in support of progress towards the SDGs to be undertaken and supported differently. By better integrating science with other perspectives, it is possible to achieve what the 2030 Agenda set out to do: creating the conditions for a fairer and more sustainable world, while living within planetary boundaries. According to the report, it will be required to supplement and rebalance the current scientific model, by incentivizing collaboration and outcomes between scientists, and of scientists, with other

stakeholders, especially civil society, on large-scale sustainability challenges. Furthermore, the current model should shift from intense competition and fragmented science, both in terms of disciplines and funding, to building collaborative science communities.

A model for implementing mission science for sustainability. Paris: International Science Council 2023; 36 p. This report offers a model proposed by the Technical Advisory Group (TAG), established to assist with the ISC Global Commission on Science Missions for Sustainability's work. The strategy proposed by the TAG flips the more traditional science model, allowing the agenda and priorities to be determined by regional communities and stakeholder needs, and engaging science in service to society in which science communities collaboratively design, produce, integrate, implement and evaluate potential pathways to achieve sustainability outcomes. It also aims to break down silos and radically increase regional capacity to understand and address nexus issues. To achieve the above-mentioned goals, the TAG proposes the establishment of a globally funded and empowered network of regional Sustainability Hubs. Each Hub will serve as a boundary-spanning platform for mobilization, coordination and alignment of diverse relevant actors and existing initiatives to address regionally identified complex nexus sustainability challenges.

UNITED NATIONS ENVIRONMENTAL PROGRAMME (UNEP)

One Health Joint Action Plan of Action (2022-2026): working together for the health of humans, animals, plants and the environment. Geneva: Food and Agriculture Organization of the United Nations, United Nations Environment Programme, World Organisation for Animal Health and the World Health Organization 2022; 86 p. The One Health Joint Plan of Action (2022–2026) (OH JPA) has been developed by the Food and Agriculture Organization of the United Nations (FAO), the United Nations Environment Programme (UNEP), the World Organisation for Animal Health (WOAH, founded as OIE), and the World Health Organization (WHO) to respond to international requests to prevent future pandemics and to promote health sustainably through the One Health approach. The scope of the OH JPA is guided by the imperative for an inclusive One Health approach to addressing the health threats of humans, animals and plants in an integrated manner, while promoting environment and biodiversity protection and acknowledging the broader systems benefits of cross-sectoral collaboration to achieve collective outcomes. Specifically, the OH JPA addresses the risks and consequences of emerging zoonotic diseases with epidemic and pandemic potential, endemic infectious diseases of zoonotic and vector-borne origin, food and water safety hazards, antimicrobial resistance (AMR) and the health of the environment.

Strategic framework for collaboration on antimicrobial resistance – together for One Health. Geneva: World Health Organization, Food and Agriculture Organization of the United Nations and World Organization for Animal Health 2022; 22 p. This Strategic Framework presents the background and context for the collaboration between the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and the World Health Organization (WHO) ("the Tripartite") with the United Nations Environment Programme (UNEP) on antimicrobial resistance (AMR), describes the comparative advantage and catalytic role of the four organizations in the One Health response to AMR in support of efforts by their Members, civil society, the private sector and other stakeholders, and proposes a theory of change including the goals, objectives, desired impact at country level, intermediate outcomes and related Tripartite and UNEP functions.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

EFSA (European Food Safety Authority), Gkrintzali G, Georgiev G, Garcia Matas R, Maggiore A, Merten C, Rortais A, Giarnecchia R, Robinson T and Bottex B. **Technical report on EFSA's activities on emerging risks in 2021**. EFSA supporting publication 2023; 20(9): EN-8233, 41 pp. The current technical report summarises the activities of all groups involved in the emerging risk identification (ERI) procedure and the issues identified by EFSA in the course of 2021. It also describes the methodologies being developed and the collaborative activities. In total, 18 potential emerging issues were discussed in 2021 and eight were concluded to be emerging risks. The potential issues were classified according to the hazard and/or driver identified. In more than half of the issues discussed in 2021, a change in consumer trends was identified as a driver. A better understanding of these trends is particularly relevant for EFSA to improve preparedness.

EFSA (European Food Safety Authority). **Targeted risk assessment for maximum residue levels for nicotine in spices**. EFSA Journal 2023;21(10):1-12. EFSA received a mandate from the European Commission to perform a targeted risk assessment of maximum residue levels (MRLs) for nicotine in spices. EFSA performed the acute (short-term) and chronic (long-term) dietary risk assessment considering the nicotine exposure via residues in spices at the level equal to the temporary maximum residue level (tMRL) of 0.3 mg/kg as proposed by the European Commission based on the new monitoring data provided. Based on the risk assessment results, EFSA concluded that the tMRL for the group of spices of 0.3 mg/kg is unlikely to pose a risk to consumer health.

WORLD HEALTH ORGANIZATION (WHO)

WHO Guidelines for malaria. 16 October 2023. Geneva: World Health Organization 2023; 451 p. This WHO Guidelines for malaria supersedes two previous WHO publications: the Guidelines for the treatment of malaria, third edition and the Guidelines for malaria vector control. Recommendations on malaria will continue to be reviewed and, where appropriate, updated based on the latest available evidence. With each update, a new PDF version of the consolidated guidelines will be available for download on the WHO website.

Safety evaluation of certain food additives: prepared by the ninety-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva: World Health Organization and Food and Agriculture Organization of the United Nations 2023 (WHO Food Additives Series, No. 86); 192 p. ISBN (WHO) 978-92-4-006843-8 (electronic version) ISBN (WHO) 978-92-4-006844-5 (print version) ISBN (FAO) 978-92-5-137758-1. The monographs contained in this volume were prepared at the Ninety-fifth meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met virtually on 6-17 and 22 June 2022. These monographs summarize the data on specific food additives reviewed by the Committee and are based on evaluations of original studies and the dossiers provided by the sponsor(s) of the compound, of the relevant published scientific literature and of data submitted by Codex members.

WHO operational handbook on tuberculosis. Module 1: prevention - infection prevention and control. Geneva: World Health Organization 2023; 116 p. ISBN 978-92-4-007815-4 (electronic version) ISBN 978-92-4-007816-1 (print version). The WHO operational handbook on tuberculosis. Module 1: prevention – infection prevention and control provides practical advice on how to implement the WHO recommendations on tuberculosis (TB) infection prevention and control, within the clinical and programmatic management of TB, using a public health approach. It is part of a modular series of practical guides meant for the implementers of various aspects of the programmatic management TB. The handbook emphasizes the importance of building integrated, well-coordinated, multisectoral actions across all levels of health care and other settings where there is a high risk of tuberculosis transmission. It shares best practices and experiences and provides checklists and job aids to support the implementation and monitoring of actions to cut transmissions, and promotes an implementation hierarchy of interventions across all settings as an integrated package. The target audience for the handbook includes policy-makers at national and subnational level; programme managers for TB, HIV and noncommunicable disease programmes; managers and clinicians at inpatient and outpatient health care facilities; managers at various congregate settings; occupational health officials; engineers; medical practitioners; frontline health care workers; and other key stakeholders in the public and private sectors.

Acknowledgements to Referees

Annali ISS would like to thank the following individuals for their contributions as referees for the articles published in the four issues of 2023. Our success as a scientific journal depends on the quality of our peer-reviewers. We are grateful to all of them.

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The name of the bioresource (and identifier, if available) which provided samples/data useful for the conduct of the study should be reported in extense, either in the Material and methods section or in the Acknowledgements.

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• Use Times New Roman font, 10 point, single spaced;
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Tables should be presented on a separate sheet and preceeded by a title. Each column within the table should have a heading. Abbreviations should be reported in full in the legend.
Figures should be loaded as separate files. The following file formats are acceptable: JPEG, TIFF or EPS. Vectorial images (graphs, flow charts, schemes, and other non bitmap material) should be in Excel, Adobe Illustrator, Microsoft Power Point so as to allow the editorial formatting of the material.
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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

Books and chapters in a book

Godlee F, Jefferson T. Peer review in health sciences. London: BMJ Books; 1999.
Van Weely S, Leufkens HGM. Background paper: orphan diseases. In: Kaplan W, Laing R (Eds). Priority medicines for Europe and the world – a public health approach to innovation. Geneva: World Health Organization; 2004.

Proceedings

Fadda A, Giacomozzi C, Macellari V. Comparative measurements to validate a new telemetric pressure insoles system. In: 2. International Symposium on measurement, analysis and modelling of human functions. 1. Mediterranean Conference on measurement. Workshop on evaluation check of traceability. Proceedings. Genova: June 14-16, 2004. p. 425-7.

Technical reports

Della Seta M, Di Benedetto C, Leone L, Pizzarelli S, Siegmund U. ETHICSWEB technical guides. Manual for the creation of standards and guidelines for sharing information about knowledge organization systems on ethics and science. Roma: Istituto Superiore di Sanità; 2011. (Rapporti ISTISAN, 11/32).

Legislation

Italia. Decreto legislativo 29 ottobre, n. 419. Riordinamento del sistema degli enti pubblici nazionali, a norma degli articoli 11 e 14 della legge 15 marzo 1997, n. 59. Gazzetta Ufficiale – Serie Generale n. 268, 15 ottobre 1999.
US Social Security Administration. Evidentiary require-

ments for making findings about medical equivalence. Final rules. Fed Reg. 2006 Mar 1;71(40):10419-33.
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