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Graphic design of the cover: Massimo Delle Femmine, Istituto Superiore di Sanità
The photograph on the cover is a triple immunofluorescence staining of a human abdominal lymph node, showing CD3+ (green) CD4+ (red) T lymphocytes surrounding a secondary B cell follicle (nuclei are in blue). The image is provided by Barbara Serafini, Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy.
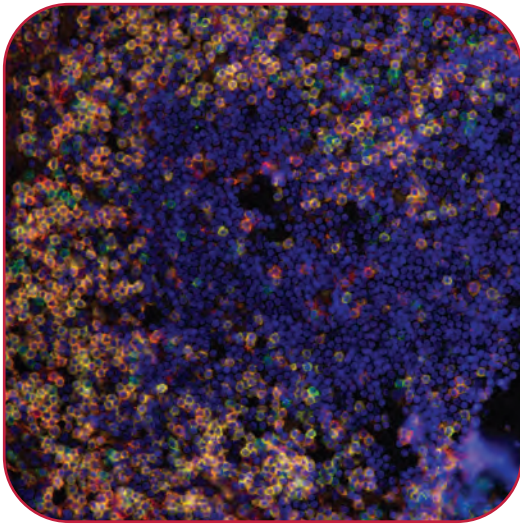
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Contents

EDITORIAL

The challenge of complexity in the Big Data era: how to ride the wave of high-dimensional data revolution

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A famous joke, reported in [1], clarifies some epistemological fault lines dividing different scientific traditions.

A very rich man, very fond of horse races, hired a top-class mathematician (e.g., Kurt Godel) and a top-class physicist (e.g., Albert Einstein) to build a model enabling him to exactly predict the winner of any horse race. After one year, both scientists returned to the rich man with their results. Godel said "Sir, I cannot say which is the specific horse who will win the race, but I discovered that the solution to the problem exists and it is unique".

The sponsor is not satisfied at all and asks Einstein if he can say something more practical and useful, Albert says "Why did you ask Kurt? You should know mathematicians have no sense of reality; on the contrary, I have the exact solution indicating the specific winner of the race. It applies only in the case of spherical horses but I am convinced this is not a problem and in any case it will be surely solved by inserting some minor adjustable parameters into the model".

Beside the delusion of the rich man, the joke reports two crucial limits of the classical mathematical and physical way of reasoning when dealing with biology: the lack of interest of both too abstract solutions and ideal cases to approximate real world. Notably, the rich man should be equally disappointed by a very long list of the "statistically significant features" differentiating frequent winners from less performant horses in the last two centuries proudly proposed to the sponsor by a famous geneticist with the help of a bioinformatics team.

We will go back in the following to this addition to the joke because it has to do with a new "player" of the game: the "machine intelligence" approach that mixes up the cards.

In summary, the joke reminds us we need an integration of different sciences to overcome the lack of real innovation [2] of nowadays research work. This process of integration is actually on the run [3] and we are already part of it. In this context, the revitalizing of the time-honoured science integration tradition of our Institute (where it is still possible to meet physicians and biologists involved in statistical epidemiology and multidimensional data analysis or physicists participating to neuroscience projects) is one of the reasons that fostered the creation of our group.

This is by no means an isolated initiative: the same urgent need generated many interdisciplinary groups in different Research Institutes all around the world. Just to name a few: the "Emergent Dynamical Systems Group" in New York (https://www.science.org/doi/full/10.1126/sciadv.aat1293) the "Complexity Science Institute" in Potsdam (https://www.pik-potsdam.de/en/institute/departments/complexity-science), the "Theoretical and Scientific Data Science Group" at SISSA in Trieste (https://datascience.sissa.it/).

Beside the peculiarities of these groups: some more focused on applicative studies, some more theoretically oriented, some very informal (like ours), some more academically structured, they all share the need of integration of different fields of enquiry to face the new challenges that cannot be faced by single scientific traditions.

To better focus the "state of the art" of the relations between biomedical and more quantitatively oriented traditions, we need to make a short digression toward a better understanding of the concept of complexity.

One of the fathers of information science, Warren Weaver, in his fundamental "Science and Complexity" 1948 paper [4], proposed a three-class partition of sci-

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ence into: 1) Organized Simplicity, 2) Disorganized Complexity, and 3) Organized Complexity.

The first class (Organized Simplicity) refers to the classical use of quantitative methods in science. Class 1 problems permit an extreme abstraction (e.g., a planet can be considered as a dimensionless "material point": we are into a "spherical horse" approach that perfectly works in many situations). This approach allows to generate differential equations predicting the behaviour of the investigated system because it relies on the stability in both space and time of the experimental (observational in the case of astronomy) results. The drastic reduction of the relevant properties to very few basic features like mass and distance, may generally allow for a straightforward prediction and explanation of what is going on and confirm or reject the proposed abstraction. However, a quantitative description in terms of differential equations does not necessarily imply a simple or even a real solution and therefore prediction and explanation could be limited and may require further abstractions. In any case, this was historically the main avenue of "hard sciences" and the reason why a great part of biomathematics redounds around Volterra-Lotka prey-predator models in which both the Godel and Einstein answers to the sponsor have important "real world" consequences.

The framework of Disorganized Complexity (class 2) allows for a still greater generalization power than class 1 by means of a very different style of reasoning. Here, the predictive (and explanatory) power stems from the generation of coarse grain macroscopic descriptors corresponding to gross averages on a transfinite number of atomic elements. Thermodynamics is one of the brightest examples of this statistical approach: emergent collective state variables like temperature or pressure fully describe the system without resorting of full knowledge of microscopic (noise-dominated) details, which can be considered homogeneous.

Class 1 approach asks for few involved elements interacting in a stable way, while class 2 style needs a very large number of identical particles with only negligible (or very stable and invariant) interactions among them. Biological systems only in very few cases do fulfil these constraints, so we step into Weaver's third class (Organized Complexity): the biomedical sciences kingdom.

Organized Complexity arises when many (even if not so many as in class 2) non-identical elements each other interact with time-varying correlation strength. Organized Complexity presents unique features like non-stationarity (this is why some apparently "Disorganized Complexity" situations when out of equilibrium enter the Class 3 domain) and structuring across different mutually interacting organization scales.

The above features generate an extreme context dependence of the results so giving rise to the "information crisis" biology is experiencing [5]. This is the "middle kingdom" where life sciences live that was recognized as the XXI century frontier of basic science [6].

It is worth stressing that the conscious adoption of class 3 style, asks for a deep recasting of both "number crunchers" and "test tube lovers" way of thinking. Quantitatively oriented scientists must accept that con-

tingencies can be more important that general laws. A graph with nodes (indicating players like protein species or genes) linked by edges (empirical correlations between nodes, mutual interactions) is not a proxy of a law of nature but only a specific configuration of the system that does not necessarily happen and could be substituted by an alternative one under different environmental pressures. On the other side, biology-oriented scientists must understand that the pure addition of finer details to an already complicated picture does not generate a most efficient explanation but only increases confusion and irrelevance [7].

As a matter of fact, the relation between "number crunchers" and "test tube lovers", often encompasses a mixing of the first two Weaver's classes: the "number cruncher" offers the "test tube lover" a "statistical significance" obtained by a "rigorous methodology" (Class 2), the "test tube lover" translates these results into a "plausible mechanism" mimicking Class 1 style. Diagrams made of boxes and lines connecting them, very frequent in biological papers and normally referred as "mechanism", for the neat prevalence of contextual information and constraints over general laws, only delineate one out of many possible descriptions of the system at hand. On the other side, the reaching of a "statistical significance" is nothing more than a suggestion of a potentially interesting case and not the seal of "scientific truth": these two epistemological biases concur to the actual reproducibility crisis affecting science [8].

The above sketched liaison between the "biologist" and the "mathematician" does not allow for any fruitful integration between different scientific traditions: the biologist looks at the "mathematical person" as a plumber to call when some hydraulic problem arises, while the "mathematician/plumber" looks condescendingly to whom he/she considers as a self-defining scientist lacking the very bases of quantitative thinking.

Before going ahead, it is worth stressing this is an ultra-simplified (and thus necessarily flawed) picture that does not take into account fields like ecological and environmental studies characterized by a more balanced relation between the two extreme approaches (it is not by chance that emerging fields like the study of microbiome are tailored upon ecological approaches [9]). Nevertheless, this duality is evident in the great majority of the work experiences of the members of our group.

Something changed with the pervasive use of very powerful and cheap computers: biomedical scientists (fascinated by user-friendly sophisticated software) decided that probably they had no more need of that arrogant plumber. They consequently began to use very refined mathematical tools without an adequate knowledge of their applicability and motivations; this in some way made things still worse but prepared the scene for taking seriously the organized complexity character of biological systems.

The deluge of data provoked by high throughput technologies (e.g., omics, neuroimages…) made the "plumber toolbox" anachronistic: the number of variables largely exceeded the number of independent observations putting upside down classical bio-statistical methods. The technological revolution (as often hap-

pens with revolutions) originated an apparently paradoxical phenomenon: the reviving of old traditions by their embedding into a novel perspective. The time-honoured (still alive in protected niches like phytosociology and psychometrics) tradition of multivariate data analysis became essential to get rid of the problems raised by transcriptomic, metabolomic, proteomic and microbiome studies. In the same time, the need of predicting complex outcomes with huge amount of heterogeneous information made machine learning approaches enter into the scene; these computational intensive methods, in order to be explainable (and thus usable in biomedical realms) pushed toward the necessary erosion of the epistemological barrier separating the home-owner and the plumber. Both of them must understand the dynamics of correlation structures (complex networks to use a fashionable term) at the basis of the studied phenomena. The classical separation of scientific enterprises into a linear sequence made of: "hypothesis setting" – "experimental methods" – "data analysis" – "hypothesis verification/falsification" is no more tenable. The mutated conditions introduced new issues spanning the entire research process. Just to name a few: 1) the necessity of standardization of data (along their entire "life cycle") to guarantee their efficient exploitation; 2) the chance of integrating heterogeneous data such as those provided by different "omics" 3) the benefits of appropriate visualization methods for multilevel and multidimensional data to facilitate information extraction and effective communication.

The introduction of machine intelligence went together with the need of "explainability", i.e., the need to conjugate the prediction of a relevant biomedical end-point with a coherent theoretical model validating the obtained result. This urgent need provoked a resurgence of interest in some historical pillars of scientific methodology like Bayesian and dimensionality reduction approaches.

Overall, the new reference frame fostered the resurgence of the quest for integration of different scientific traditions and prompted the collaboration of different kinds of number crunchers (engineers, physicists, statisticians, mathematicians, biophysicists, chemists) and test tube lovers (pharmacologists, biologists, physicians). The entanglement of "content" and "methodological" knowledge is the most promising epistemological novelty made necessary by the actual information crisis and consequent lack of efficacy [5] of scientific research, while the discarding of theory-oriented science in favour of a purely brute-force approach based on an acritical use of informatics tools is a deadly temptation to be avoided [10, 11].

Conflict of interest statement

The Authors declare that there are no conflicts of interest.

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The risk of post-traumatic stress disorder (PTSD) in Italian Highly Specialized Research Hospitals staff within two months of the pandemic declaration: an on-line survey

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Abstract

Background. The whole hospital system was stressed and at risk in the first phase of the pandemic. This study examined the prevalence of post-traumatic stress disorder (PTSD) in all hospital staff, medical and non-medical, within two months of the pandemic declaration. Survey concerned staff 4510 health workers of Italian Highly Specialized Research Hospitals.

Method. Subjects were asked to complete an on-line self-reported questionnaire, the PTSD Checklist 5 (PCL-5) and subjective perception of safety related to personal protective equipment (PPE).

Results. The sample included staff working in hospitals with or without COVID-19 patient admissions. Overall, 11.56% of the hospitals staff met the symptoms criteria for probable PTSD. The sample included 80.63% (3467) medical staff workers and 19.37% (833) non-medical staff workers. The 31.91% of participants worked in COVID-19 hospitals/wards. The prevalence of positive screening for PTSD symptoms in medical staff was 12.42% (426) and in non-medical staff, 8.59% (70). Among medical staff, anesthesiologists had a significant prevalence of PTSD (22.35%), followed by health care assistants/technicians (15.38%) and physicians (10.11%). Among non-medical staff, personnel involved in cleaning, catering, maintenance, security, and transportation, the symptoms of PTSD reached a rate of 12.24% and in administrative staff 8.47%. Risk factors associated with PTSD included working as an anesthesiologist, perceiving PPE as inadequate, and working in COVID-19 hospitals/wards.

Conclusions. In the present study, as in other studies, the prevalence of PTSD symptoms among hospital workers was significatively higher than the lifetime prevalence of PTSD in the general population, showing the pandemic's incredible impact.

INTRODUCTION

Italy was the first nation in Europe to implement lockdown measures to deal with the COVID-19 pandemic. The Italian National Health System has been overwhelmed, collapsed under the number of infected patients, with huge losses. The novel coronavirus SARS-CoV-2-causing COVID-19, first spread in China in late December 2019, was declared a pandemic by the World Health Organization on 11th March 2020. Italy has been one of the most affected countries and, in April 2020, became the epicenter of the spreading pandemic.

As well as other nations, frontline Italian health care workers experienced high rates of infection and death partly due to inadequate access to personal protective equipment (PPE) (i.e. gloves, face shields, gowns, and hand sanitizer) [1].

During the pandemic, the Italian government separated hospitals into COVID-19 and non-COVID-19 patient admissions.

Health care workers have been exposed to emotional

overload for the risk of infection, ethical issues related to decision-making, constant vigilance, reorganization of workspaces, and physical exhaustion. Moreover, they had to face a high number of deaths among patients, colleagues, and loved ones and often prolonged separation from the family [2, 3]. Non-medical staff also has been under pressure to follow the national and regional directives, which required continuous updating and operational decisions based on the local situation and timely decisions in unforeseen conditions. Hospital management had to make countless decisions (e.g., managing intensive care units, setting up new "COVID-19 blocks", postponing elective surgeries to prepare for a spike in coronavirus cases, and redeploying staff).

These unprecedented circumstances were likely to increase the risk of mental health disorders such as post-traumatic stress disorder (PTSD) [4, 7], the sentinel mental health consequence of natural disaster exposure. Many studies had shown that health care workers were likely to suffer from PTSD after participating in an emergency both for the COVID-19 pandemic and for the previous infectious outbreaks (severe acute respiratory syndrome, SARS; Middle East respiratory syndrome, MERS) [5-9]. PTSD individuals are more at risk of suicidal ideation, suicide attempt, and deaths by suicide, even considering that health care workers are previously at-risk occupations [6] because burnout (chronic work-related stress) is already present. Exposure to work-related stressors is expected to have long-term psychological consequences for many healthcare professionals [10].

Guidelines for post-traumatic stress disorder of the National Institute of Health and Care Excellence recommend "for people at high risk of developing PTSD after a major disaster, those responsible for coordinating the disaster plan should think about the routine use of a validated, brief screening instrument for PTSD at one month after the disaster (NG 116, 2018)".

The aim of the study was to rapidly identify the number of people at risk, to set appropriate strategies to provide evidence-based support or care [4].

In this study, we observed the prevalence of PTSD symptoms in health care professionals in close contact with patients (physician, nursing staff, health care assistants, and technicians) and non-medical staff (hospital administrators, administrative staff, maintenance estates workers) from twenty-four out of fifty-one Italian IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico, Italian Scientific Institute for Research, Hospitalization,and Healthcare) through an anonymous, on-line self-check tool. Although the risk of PTSD for healthcare professionals has been well documented [6-9], it is not for non-medical staff.

Hospital administration should take proactive steps to minimize the impact of COVID-19 in health care workers because early interventions may mitigate the mental health impact on health care workers and non-medical staff [11].

METHODS AND MATERIALS
Study setting and population
Hospital workers (health care professionals and non-medical workers) were asked to complete a self-reported questionnaire, the PTSD Checklist 5 (PCL-5). An on-line survey ran continuously from 14 April to 24 May 2020 to estimate the proportion of PTSD symptoms in hospital staff since one month after the pandemic declaration of COVID-19 (11 March 2020). A sample of 4510 participants was recruited from Italian IRCCS hospitals. IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico, literally "Scientific Institute for Research, Hospitalization, and Healthcare") are hospitals granted by The Italian Department of Health as a benchmark for the whole public health system for both the quality of patient care and the innovation skills. The study received ethical approval from the Human Research Ethics Board of IRCCS Neurological Institute Carlo Besta of Milan.

On-line survey
The survey consisted of demographics, the PCL-5 Checklist, and questions about the safety perception related to PPE.

DSM-5-based PTSD Checklist (PCL-5) is one of the most widely used self-report questionnaires for PTSD [12]. The PCL-5 has a variety of purposes, including screening individuals for PTSD. It takes approximately 5-10 minutes to complete. The PCL-5 evaluates the degree to which an individual has been bothered in the past month by DSM-5 PTSD symptoms. Criterion A was established a priori in the questionnaire by asking participants to report their responses to the COVID-19 pandemic. Checklist items are rated from 0 ("not at all") to 4 ("extremely"). Subscale severity scores are calculated by summing items in each of the four DSM-5 PTSD symptom clusters: Criterion B intrusions (Items 1-5), Criterion C avoidance (Items 6-7), Criterion D negative change in cognitions and mood (Items 8-14), Criterion E change in arousal and reactivity (Items 15-20), presence/absence of dissociative symptoms (depersonalization/derealization). A provisional PTSD diagnosis can be made by treating each item rated as 2 = "moderately" or higher as a symptom endorsed, then following the DSM-5 diagnostic rule, which requires at least: 1 B item (questions 1-5), 1 C item (questions 6-7), 2 D items (questions 8-14), 2 E items (questions 15-20). This symptom cluster scoring method is well established [13].

The safety perception related to PPE was measured with a 5-point Likert scale ranging from 0 ("very poor") to 4 ("excellent").

Procedure
The on-line survey was conducted from 14 April 2020 through 24 May 2020. The Authors drafted an invitation letter with a link to the survey (Microsoft FORMS application). The management of each hospital sent employees the survey invitation email (survey closed - personal e-mail with password). Participants clicked on the link to agree to take part to gain access to the questionnaire. The banner provided the only link to the survey. The survey did not use cookies and neither collected user internet protocol (IP) addresses nor stored them with the submitted data. Further, it did not record any identifying information of the responder, nor did it ask for any protected health information identifiers. The data collection link remained active for each IRCCS

for 15 days, starting from the first response received.

The first page of the survey provided the responder with essential information regarding the purpose of the study, a statement regarding the host institution and regulatory approval from the institution, and approximate completion time. Multiple checkboxes provided multiple checkboxes, rating scales, yes/no questions, or specific fill in the blank questions. No open-ended questions or queries that required free text entry were used. Our survey adheres to the Checklist for Reporting Results of Internet E-Surveys (CHERRIES) [14] this paper provides the first comprehensive review of its diagnostic utility. Eighteen diagnostic accuracy studies of the PCL are presented, followed by an examination of the potential roles of spectrum effects, bias, and prevalence in understanding the variation in sensitivity, specificity, and other operating characteristics across these studies. Two related issues as to the interchangeability of the PCL's three versions (civilian, military, and specific and UE Regulation 2016/679 concerning the processing of personal data.

Other data were collected about gender, age, educational level, hospital staff role (medical/non-medical), job qualification (physician in contact with patients or biological material; anesthesiologist; non-physician staff in contact with patients or biological material; health care assistants and technicians; researchers; administrative staff; other: cleaning, catering, maintenance, security, transport staff), affiliation to hospital/ward dedicated to COVID-19 patients (COVID-19 *vs* non-COVID-19 hospital/ward) and subjective perception of safety related to PPE.

Study outcomes

First, we analyzed PTSD prevalence in the overall sample to investigate the burden of trauma in health care professionals in close contact with patients and non-medical staff during the COVID-19 pandemic. Second, a subgroup analysis of the prevalence of PTSD by the socio-demographic variable as gender, age was conducted. Third, we sort prevalence of PTSD by hospital staff role (medical/non-medical) and by job qualification (physician in contact with patients or biological material; anesthesiologist; non-physician staff in contact with patients or biological material; health care assistants and technicians; researchers; administrative staff; other: cleaning, catering, maintenance, security, transport staff). Forth, we explore the prevalence of PTSD symptoms in hospital workers more exposed to COVID-19 at work (COVID-19 *vs* non-COVID-19 hospital/ward). Fifth, we assess the association of PTSD and a hospital/ward with COVID-19 patients or not and subjective perception of safety related to PPE. Sixth we examined a cluster prevalence of PTSD ("intrusions", "avoidance", "negative change in cognitions and mood", "change in arousal and reactivity"). Finally, to examine risk prediction of PTSD, we tested whether socio-demographic data (independent variables), COVID-19 *vs* non-COVID-19 hospital/ward, staff role, job qualification and PPE safety perception predicted PTSD in hospital workers (dependent variable) using bivariate and multivariate logistic regression analyses.

Statistical analyses

A sample size of about 3500 participants was estimated to be appropriate to meet the study's primary objective, which was to evaluate the prevalence of PTSD symptoms in hospital workers. Assuming that in previous infectious outbreaks (SARS and MERS), the prevalence of PTSD in health care professionals reported varied between 10% and 40% [15], based on Wald's approximate binomial method, the number of 3500 subjects would have guaranteed a margin of error not exceeding ± 2% (i.e., a 95% confidence interval, 95% CI, width equal to 4%).

Appropriate descriptive analysis was provided; in particular, the qualitative variables were summarized through the absolute and percentage frequency distributions. Regarding the primary endpoint, adequate point and interval estimates were provided for proportions, using the Wald approximate binomial method. The chi-square test and the multivariate logistic model were used to investigate the association between risk factors and PTSD. P-values <0.05 were considered statistically significant, and all tests were two-tailed. Statistical analyses were performed using STATA statistical software, version 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

RESULTS

Participants

Twenty-four out of fifty-one Italian IRCCS participated (*Figure 1*). The total number of hospital workers responders was 4510. Incomplete PCL-5 Checklist (n=17) or atypical timestamp (n=6) submission (inferior to 2 minutes) were excluded, leaving a total group of 4487 respondents (*Table 1*). Numbers may not add up due to missing data about gender, age, educational level, hospital staff role, job qualification, COVID-19 *vs* non-COVID-19 hospital/ward or subjective percep-
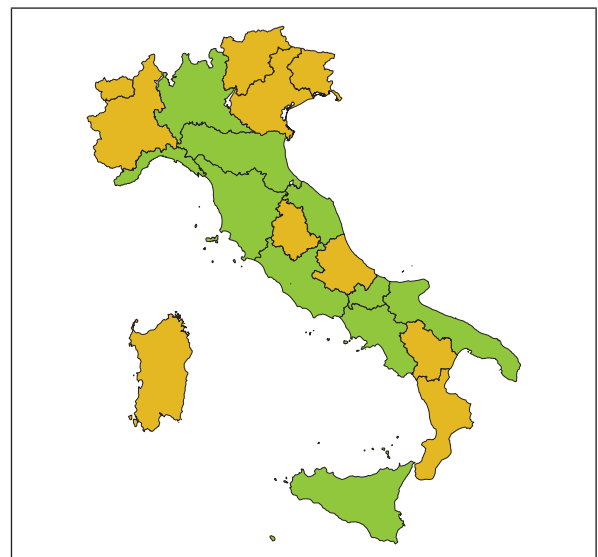
Figure 1
Regional location of participants IRCCS (green) in Italy.

Table 1
Socio-demographic characteristics of the surveys' participants

	N. total (weighted %)
Total N. subjects	4487
Gender	
Male	1266/4468 (28.33%)
Female	3202/4468 (71.67%)
Age	
18-30	479/4478 (10.70%)
31-50	2277/4478 (50.85%)
51-65	1656/4478 (36.98%)
66-75	62/4478 (1.38%)
> 75	4/4478 (0.09%)
Educational Level	
Middle School	99/4231 (2.34%)
High School	858/4231 (20.28%)
University	3274/4231 (77.38%)
Staff	
Medical staff	3467/4300 (80.63%)
Physicians in contact with patients and/or biological material	808/4300 (18.79%)
Anaesthesiologists	87/4300 (2.02%)
Non-physician staff in contact with patients and/or biological material	157/4300 (3.65%)
Health care assistants and technicians (nursing, physiotherapy, laboratory/radiology)	1742/4300 (40.51%)
Researchers	673/4300 (15.65%)
Non-medical staff	833/4300 (19.37%)
Administrative staff (hospital administration and management)	773/4300 (17.98%)
Other (cleaning, catering, maintenance, security, transport staff)	49/4300 (1.14%)
Volunteers	11/4300 (0.26%)
Workplace	
COVID-19 hospitals/wards	1423/4460 (31.91%)
Non-COVID-19 hospitals/wards	3037/4460 (68.09%)

tion of safety related to PPE. The majority of responders, 71.67% (3202), were females; 50.85% aged 31-50 years (2277), and the university educational level was the most representative (3274; 77.38%). Among the respondents, 80.63% (3467) were medical staff workers, and 19.37% (833) were non-medical staff workers. Healthcare assistants and technicians (nursing, physiotherapy, laboratory/radiology) were the most representative: 1742 (40.51%). The 31.91% (1423) of participants worked in COVID-19 hospitals/wards.

Our sample is representative of the general gender and staff organizational structure of the Italian hospital workers. Based on the entire staff of IRCCS hospitals, females represent 67.3%, medical staff workers represent 86.2% (physician 14.5%, non-physician staff

3.1%, health care assistants and technicians 60.4%, and researcher 8.1%) compared to non-medical staff [16].

Prevalence of PTSD symptoms

Overall, 511 out of 4429 (11.6%, 95% CI 10.6%-12.5%) of hospital workers met PTSD symptom criteria (BCDE) *(Table 2)*.

PTSD symptoms were more frequent in female (392, 12.41%) than male (117, 9.33%) participants (p-value 0.004). PTSD symptoms were significantly related to educational level, with a higher prevalence among participants with high school degree (126, 14.89%) compared to those with middle school diploma (11, 11.83%) and the university degree (349, 10.76%) (p-value 0.004).

The prevalence of positive screening for PTSD symptoms in medical staff was 12.42% (426) and in non-medical staff, 8.59% (70). Among medical staff, anesthesiologists (a category chosen a priori by authors for their specific role in the care of COVID-19) had the most significant percentage of PTSD symptoms (19, 22.35%), followed by health-care assistants and technicians (264, 15.38%) and physician in contact with patients or biological material (81, 10.11%) *(Figure 2)*.

The same trend was observed for COVID-19 and non-COVID 19 hospitals/wards, with a significant increase in PTSD cases in COVID-19 hospitals/wards.

Regarding the lack of PPE, 59.7% of responders with probable PTSD rated as inadequate PPE (range 0-1) with no significant difference between COVID-19 and non-COVID-19 hospitals/ward (p = 0.385).

Concerning symptom clusters, intrusive symptoms were considered relevant ("moderately" or higher) in 41.3% (1852 responders), avoidance in 37.0% (1653), changes in arousal in 36.3% (1628), a negative change in cognitions and mood in 33.7% (1510). Dissociative symptoms were present as derealization in 39.1% (1638) and depersonalization in 25.0% (1118) (data not shown).

Factors associated with PTSD symptoms

Results of the multivariate logistic regression analysis (see *Table 3*) showed that female were more likely to develop PTSD (adjusted odds ratio, OR, 1.43; 95% CI 1.13-1.81) as compared with the male.

Respondents with lower educational level were more likely to develop PTSD compared to participants with the university degree, OR was 1.62 (1.26-2.08) for participants with high school, and 1.23 (0.63-2.41) for participants with middle school diploma.

When examining job qualification, the major risk for PTSD symptoms was for anesthesiologist 3.14 (1.68-5.84), for cleaning, catering, maintenance, security, transport staff 1.94 (0.76-4.95) and medical staff (excluding anesthesiologist) 1.69 (1.32-2.16).

The probability of developing symptoms of PTSD was 2.03 (1.64-2.51) times higher for respondents working in COVID-19 hospitals/wards than respondents who worked in non-COVID-19 hospitals/wards.

The chances of developing PTSD were 2.22 (1.77-2.77) times higher among respondents who had poor or very poor PPE safety perception compared to fair safety perception.

Table 2
Post-traumatic stress disorder (PTSD) prevalence in COVID-19 and non-COVID-19 hospitals

	PTSD prevalence					
	Overall	**p**	**COVID-19 hospitals/wards**	**p**	**Non-COVID-19 hospitals/wards**	**p**
	511 (11.56%)		246 (17.52%)		265 (8.82%)	0.000
Gender						
Male	117 (9.33%)	0.004	74 (15.38%)	0.120	43 (5.64%)	0.000
Female	392 (12.41%)		172 (18.72%)		219 (9.81%)	
Age						
18-30	62 (13.00%)	0.485	27 (20.45%)	0.708	35 (10.14%)	0.510
31-50	246 (10.91%)		127 (16.87%)		118 (7.90%)	
51-65	197 (12.11%)		90 (17.93%)		107 (9.57%)	
66-75	5 (8.47%)		1 (7.69%)		4 (8.89%)	
> 75	0 (0.00%)		0 (0.00%)		0 (0.00%)	
Educational level						
Middle School	11 (11.83%)	0.004	62 (23.13%)	0.011	64 (11.13%)	0.168
High School	126 (14.89%)		5 (21.74%)		6 (8.70%)	
University	349 (10.76%)		157 (15.48%)		64 (11.13%)	
Staff						
Medical staff	426 (12.42%)	0.000	217 (18.84%)	0.002	208 (9.16%)	0.014
Physicians in contact with patients and/ or biological material	81 (10.11%)		48 (14.41%)		33 (7.10%)	
Anaesthesiologists	19 (22.35%)		15 (24.59%)		4 (16.67%)	
Non-Physician staff in contact with patients and/or biological material	12 (7.64%)		5 (10·00%)		7 (6.54%)	
Health care assistants and technicians (nursing, physiotherapy, laboratory/ radiology)	264 (15.38%)		142 (22.02%)		122 (11.39%)	
Researchers	50 (7.46%)		7 (11.11%)		42 (6.95%)	
Non-medical staff	70 (8.59%)		26 (11.66%)		44 (7.50%)	
Administrative staff (Hospital administration and management)	64 (8.47%)		22 (11.00%)		42 (7.61%)	
Other (cleaning, catering, maintenance, security, transport staff)	6 (12.24%)		4 (18.18%)		2 (7.69%)	
Volunteers	0 (0.00%)		0 (0.00%)		0 (0.00%)	

p are p-values from chi-square tests.
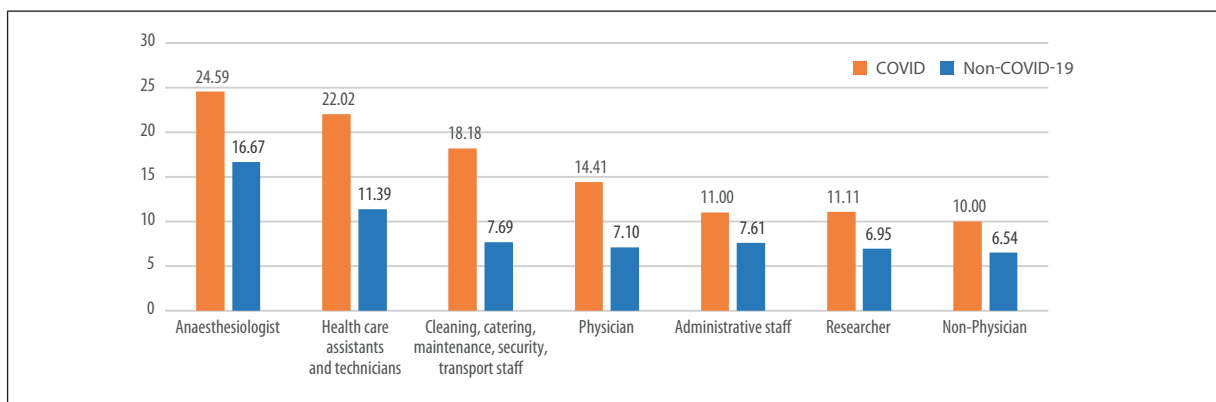
Figure 2
Percentage of respondents with post-traumatic stress disorder (PTSD) symptoms.

Table 3

Independent predictors of post-traumatic stress disorder (PTSD) from multivariate logistic regression analysis

		Odd ratio (95% CI)	p value
Gender	Male	1 (ref)	
	Female	1.430351	0.003
Age	18-30	1 (ref)	
	31-50	0.7964101	0.181
	>50	0.8399574	0.329
Educational level	University	1 (ref)	
	High School	1.616833	0.000
	Middle School	1.234976	0.537
Staff	Non-medical staff	1 (ref)	
	Medical staff	1.689937	0.000
	Anaesthesiologist	3.135096	0.000
	Other	1.939316	0.166
Hospitals/ wards	Non-COVID-19	1 (ref)	
	COVID-19	2.029542	0.000
PPE safety perception	Fair		
	Poor/very poor	2.216694	0.000
	Good/excellent	0 .896858	0.562

PPE: personal protective equipment.

DISCUSSION

Following National Institute for Health and Care Excellence's recommendations to rapidly evaluate the mental health impact of hospital workers, we decided to take advantage of the on-line survey. An on-line survey can be easily deployed and completed by participants, particularly when disseminated via e-mail with minimal costs. Mainly, the completion of survey questionnaires on-line could have been answered at the convenience of responders, at their own pace, as they were facing the pandemic.

In this study, the prevalence of PTSD among hospital workers was 11% when the lifetime prevalence of PTSD in Italy is about 2.4% in the general population [17], showing the significant impact of the pandemic [18,19].

The major risk of the female having PTSD symptoms, detected in our study, is in line with current research that observes that the lifetime risk for PTSD in women is twice that in men. Literature offers competing explanations for this observation that might reflect a combination of greater exposure and genetic vulnerability [20].

Working in COVID hospitals/wards is related to two times the probability of developing PTSD symptoms with respect to non-COVID hospitals/wards.

In the present study, anesthesiologists, core figures of health care emergency during COVID-19 spread, are those with higher rate (22%) with a chance three times more likely of developing PTSD symptoms with respect to other categories.

This study clearly evidence that the risk of developing PTSD disorder not only regards health care profession-

als in close contact with patients but also those who silently work backstage, such as administrative employees and all non-medical staff [21-23].

Since a critical symptom of PTSD involves avoiding environments related to the traumatic event, hospitals workers traumatized at work often have extreme difficulty returning to their place of employment with the negative consequences of absenteeism and decreased productivity [24].

Healthcare professionals' perception of inadequate PPE during the current pandemic has been a possible factor influencing our global results because most responders with probable PTSD rated as inadequate PPE. Protective factors can provide valuable insights into the variability of individuals' responses to COVID-19.

Interesting is the massive presence of dissociative reactions with a prevalence of derealization symptoms. Derealization is the feeling of being disconnected from one's surrounding environment and, in this case, probably triggered by fundamental changes in the way of life in this pandemic event (fear, lockdown, employment of army, etc.). Dissociative reactions are associated with more severe PTSD symptoms and decreased functioning [25].

Three major limitations regarding this study must be considered. Firstly, the gold standard for diagnosing PTSD is a structured clinical interview and the PCL-5 can be scored only to provide a provisional PTSD diagnosis. Secondly, we explicitly investigated the PTSD symptoms related to the outbreak establishing a priori criterion A (DSM-IV), asking participants to report their responses to the COVID-19 pandemic. It cannot be excluded that some subjects had a pre-existing post-traumatic stress disorder linked to a different traumatic experience since previous traumas have not been investigated. Thirdly, the assessment of PTSD is examined regardless of the time of assessment, which ranges from 1 year to a few years, sometimes decades after the disaster [26]. Only a few studies, such as the present, have documented PTSD one to six months after trauma [27, 28]. Therefore, PTSD studies are limited in comparability. The Clinical Neuropsychological Service of our Institute (IRCCS Carlo Besta Neurological Institute) has set up a prompt psychological intervention team based on existing resources and capacities in association with the survey. Further, we quickly shared the survey results with the other involved IRCCS.

CONCLUSIONS

Considering that an on-line survey inevitably produces distortions and limited generalizability, conventional methods were not feasible during the pandemic. To reduce the distortions: the main tool for PTSD screening was administered; scores with the most stringent method (to limit false positives) were calculated; available guidelines and the European privacy laws for the collection of data with on-line survey were applied. Finally, sample size is reliable and representative of the target population.

The timely collection of information from employees is the first step towards local solutions and facilitates support for the most vulnerable staff. Psychological interven-

tions can reduce the severity of PTSD when the intervention is targeted at those with early symptoms [29], and psychotherapy is generally recommended as a first-line treatment [30]. This maximizes the opportunity for staff to experience psychological growth from overcoming the challenges faced during the pandemic [4, 31, 32].

Authors' contributions

SHMJP and SP conceived and designed the study and wrote the manuscript. IT acquired and analyzed data.

All Authors interpreted the data, revised the manuscript for important intellectual content, and agreed with this article's results and conclusions.

Conflict of interest statement

The Authors declare no competing interests.

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ORIGINAL ARTICLES AND REVIEWS

First report of two Asian invasive mosquito species, *Aedes japonicus* and *Aedes koreicus*, in Piedmont, northwest Italy

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Abstract
Introduction. *Aedes japonicus japonicus* and *Aedes koreicus* are two invasive mosquitoes recently reported in various parts of Europe, including areas very close to Piedmont where, since 2012, specific tools have been implemented to allow the early detection of invasive mosquitoes, through the surveillance of the main points of entry.
Results. Thanks to the regional surveillance system, *Ae. j. japonicus* was intercepted in Piedmont for the first time in 2019, in the northernmost part of the region and now it is reported in six provinces. *Ae. koreicus* was intercepted for the first time in 2012 in three provinces.
Discussion and conclusion. The spread of these two invasive mosquitoes in Europe is still ongoing. Where *Aedes albopictus* is abundant, probably their presence goes undetected, therefore, it is crucial to begin surveillance early in the season. Due to their competence for several arboviruses and tolerance to the cold temperatures *Ae. j. japonicus* and *Ae. koreicus* represent a further concern for Public Health. A longer seasonal period for surveillance and response to mosquito-borne diseases, as well as a shift up of these activities to previously uncovered altitudes are indeed needed.

Key words
• invasive mosquitoes
• surveillance
• *Aedes*
• Piedmont
• Italy

INTRODUCTION

Several invasive mosquito species, such as *Aedes albopictus*, *Aedes atropalpus*, *Aedes japonicus japonicus*, and *Aedes koreicus*, were recently reported in various parts of Europe [1], and most of them are competent vectors for various arboviruses [2]. The recent cases of autochthonous transmission of dengue and chikungunya fever in southern Europe, which was attributed to the presence of *Ae. albopictus,* confirm invasive mosquito species as an actual threat to human health in Europe [3]. Therefore, national and regional plans have been made in many countries in the last decade to implement the surveillance of invasive mosquito species [2].

Ae. j. japonicus and *Ae. koreicus* are both invasive container mosquito species native to the far East.

Unlike *Ae. albopictus* and *Ae. aegypti*, *Ae. j. japonicus* is not considered an important vector of mosquito-borne diseases, mainly because there have been no confirmed reports of pathogen transmission through *Ae. j. japonicus* in the field [4] so far. Nevertheless, the species was experimentally proven to be a competent vector of several arboviruses, including chikungunya and dengue [5]. Field-collected *Ae. koreicus* samples were found infected

by the Japanese encephalitis virus [6] and *Dirofilaria repens* [7]. Furthermore, the species has shown to be a competent vector of chikungunya in the laboratory [8].

In Europe, *Ae. j. japonicus* was first reported in France, where it was eradicated [9], and in Belgium [10], where it appears not to have spread [1]. The species was definitively introduced in 2008 in Switzerland, where its range has expanded in all directions, and it has now been reported in at least 12 European counties [10]. In Italy *Ae. j. japonicus* was reported for the first time in 2015, in the north easternmost provinces of the country, close to the Austrian and Slovenian borders [11].

Ae. koreicus was first detected outside its native range in Belgium in 2008 [12]. Now, its populations have been identified in at least nine other European countries i.e., Austria, Germany, Hungary, Italy, The Netherlands, Russia, Slovenia, Switzerland, and Ukraine [10]. For the first time in Italy, it was reported in the northeast (in 2011) [13], then in Lombardy (in 2015) [14] and finally in Liguria, in 2017 [15]. In Piedmont, before our findings, *Ae. albopictus* was the only invasive mosquito species recorded [16]. Piedmont borders the Republic and Canton of Ticino, in the Swiss Confederation, and

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the province of Genoa, in the Liguria Region, where *Ae. j. japonicus and Ae. koreicus* have been reported [10, 15].

MATERIALS AND METHODS

For the early surveillance of invasive mosquito species that eventually spread to the Piedmont region, a set of specific tools has been set up in the whole region since 2012.

Particular attention was paid to identifying and monitoring the key potential points of entry, e.g., tyre companies, greenhouses, international airports, container terminals, transportation routes close to borders with neighbouring countries and Italian regions, etc. according the ECDC Guidelines for the surveillance of invasive mosquitoes in Europe [2].

The surveillance was mainly based on adult trapping and larval searching. BG-Sentinel traps (Biogents® AG, Regensburg, Germany) baited with BG-Lure (Biogents® AG) and carbon dioxide (CO_2) as attractants were chosen for trapping adults, due to their performance for *Aedes* container-inhabiting species [17]. Traps were placed fortnightly for about 24 h in a dozen of the most important points of entry. The CO_2 attractant gas was obtained by the sublimation of about 0.5 kg of dry ice put in an adiabatic container with a nozzle and placed 30 cm above the trap. The West Nile virus surveillance network, which used CDC-CO_2 traps, was also used to cover a larger area away from the points of entry.

Larval searching consisted of sampling immature mosquito stages in small man-made water containers e.g., catch basins, pot dishes, drums, and used tires. The samples were transferred into an insectary and reared to obtain adults that were far easier to identify than in their juvenile stages [2]. Dichotomic keys [18, 19], a magnifier (30x) for the overall observation and a stereomicroscope (Olympus SZX12) for the details, were employed to morphologically identify the specimens from the different sources.

Table 1

Positive sites for *Aedes japonicus japonicus* and *Aedes koreicus* in Piedmont, sorted by increasing distance from the closer Italian-Switzerland border crossing point (for *Ae. j. japonicus*) or the closer mountain road pass between Piedmont and Liguria (for *Ae. koreicus*)

Species	Site (province)	Distance	Year
Ae. j. japonicus	Cannobbio (VB)	4 km	2019
	Cannero Riviera (VB)	9 km	2019
	Oggebbio (VB)	12 km	2019
	Crodo (VB)	14 km	2019
	Suna (VB)	20 km	2019
	Pallanza (VB)	21 Km	2020
	Stresa (VB)	27 km	2019
	Gravellona Toce (VB)	28 km	2019
	Dormelletto (NO)	42 km	2020
	Castelletto Ticino (NO)	43 km	2020
	Gattinara (VC)	60 km	2021
	Lessona (BI)	69 km	2021
	Mombarone (AT)	132 km	2021
	San Mauro Torinese (TO)	133 km	2021
Ae. koreicus	Sezzadio (AL)	23 km	2021
	Rocchetta Tanaro (AT)	35 km	2021
	Mombarone (AT)	51 km	2021
	Rocca di Cavour (TO)	75 km	2021

AL: Alessandria province; AT: Asti province; BI: Biella province; NO: Novara province; TO: Metropolitan City of Turin; VB: Verbano-Cusio-Ossola province; VC: Vercelli province.

RESULTS

All the field-collected or reared mosquitoes from 2012 to 2018 belonged to *Ae. albopictus* or native species. On 18 April 2019, a dozen larvae and pupae were collected in a stone vase in the Suna cemetery (45°55'51.4"N;
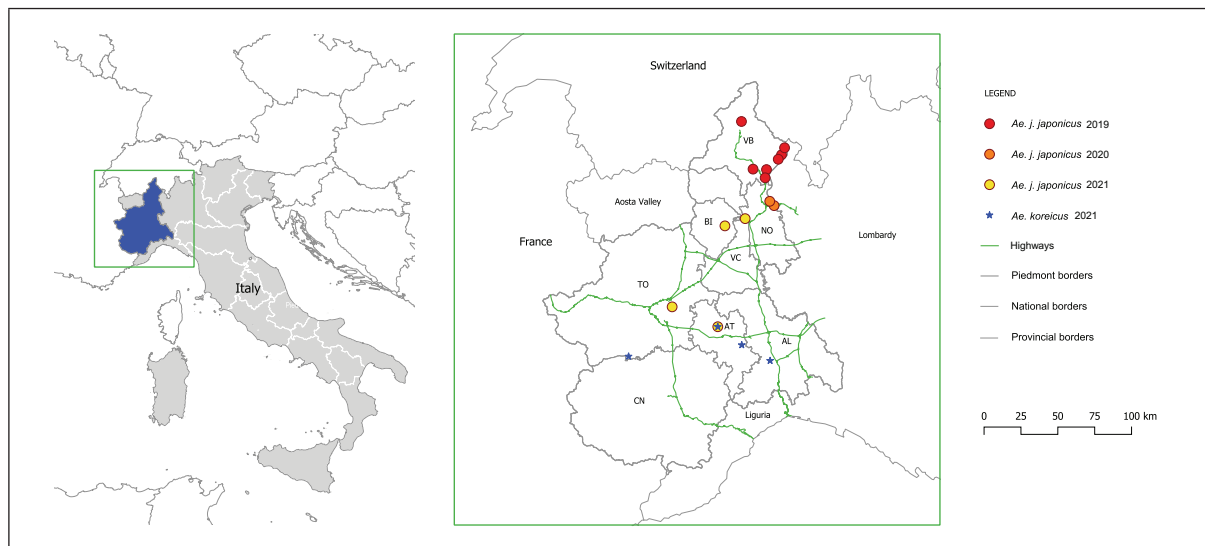
Figure 1
Positive sites for *Aedes japonicus japonicus* and *Aedes koreicus* in Piedmont, sorted by year.

8°32'51.0"E), in the municipality of Verbania, located along a road from Switzerland. The specimens were reared in the insectary, and a few days after, two adults (a male and a female) emerged: they were morphologically identified as *Ae. j. japonicus* and were the first proof that this species was now present in Piedmont and the whole of north-west Italy.

During the following months, *Ae. j. japonicus* was found in other six sites close to the Italian-Swiss border in the province of Verbano-Cusio-Ossola (VB). In 2020, the species was found at more than a 40 km radius from the nearest Italian-Swiss border crossing point (province of Novara, NO); while in 2021 at 60-70 km in the provinces of Vercelli (VC) and Biella (BI) and more than 130 km in the provinces of Asti (AT) and in the Metropolitan City of Turin (TO), which were probably introduced by passive transportation (*Table 1 and Figure 1*).

Ae. j. japonicus and *Ae. koreicus* adults emerged from the same bunch of larvae and pupae sampled in some man-made water containers in Mombarone (municipality and province of Asti - 44°58'38.4"N; 8°08'14.8"E) on 19 February 2021. It was the first time that the *Ae. koreicus* had been identified in Piedmont. During the rest of the season, larvae and pupae, or adults of *Ae. koreicus* were sampled in other 3 sites at a distance ranging from 23 to 75 km from the nearest mountain road pass between Piedmont and Liguria in the provinces of Alessandria (AL), Asti and Turin (*Table 1* and *Figure 1*).

Finally, between September 2011 and July 2022, *Ae. koreicus* was found in some locations in the provinces of Biella and Verbano-Cusio-Ossola (data not shown in the *Table* and *Figure*), leading us to formulate the hypothesis of at least a second entry point in the north of the region.

DISCUSSION AND CONCLUSION

The identification of *Ae. j. japonicus* and *Ae. koreicus* in Piedmont confirms their increasing expansion in Europe. Both species were found for the first time in Piedmont early in the season and at about 20 km from national or regional borders in territories where they had previously been reported. Due to these findings, both the species seem to behave quite discreetly,

at least in the first phases of the introduction process. It is probable that their presence goes undetected when considering specific periods of the year and areas with an abundant presence of *Ae. albopictus*; therefore, it is crucial to begin surveillance in early spring.

From a Public Health perspective, the introduction of two new mosquito species competent of transmitting pathogens to animals and humans may be additional challenge for the health system. In a territory such as Piedmont, which is now almost completely colonized by *Ae. albopictus*, at least at the lowest altitudes, these new introductions do not represent a serious threat to human and animal health. Nevertheless, the higher tolerance to the cold temperatures of *Ae. j. japonicus* [20] and *Ae. koreicus* [21] in comparison to *Ae. albopictus* will result in a longer seasonal period for surveillance and response to mosquito-borne diseases, as well as a shift up of these activities to previously uncovered altitudes.

A long-term surveillance and an early detection are needed to limit the further spread of these species and properly plan their control.

Authors' contributions

AM wrote the paper and identified the specimens; all the Authors were involved in the planning, the mosquito collection, and the laboratory rearing. All Authors read and approved the final version of the manuscript.

Conflict of interest statement

The Authors declare that they have no conflict of interest.

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ORIGINAL ARTICLES AND REVIEWS

Blackflies (*Simulium* spp.) attacks on humans and animals in Rome and surrounding areas (Central Italy)

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Abstract
Background. Due to their abundance in some environments, the blood-sucking habit and the involvement in the transmission of several diseases, blackflies of the genus Simulium (Diptera: Simuliidae) can be considered among the most annoying biting arthropods.
Methods. Following repeated attacks to humans and animals, entomological investigations were carried out in green areas of Rome and surroundings. Site and period of attacks were reported, together with the human and animal reactions to the bites.
Results. Four Simulium species have been identified through morphological or molecular analysis: *Simulium intermedium*, *Simulium lineatum*, *Simulium lundstromi* and *Simulium ornatum* (complex). Larval breeding sites were identified in small moats, where a high density of blackflies larvae and pupae was revealed.
Conclusions. Being able to thrive in highly polluted water, even in few mm depth watercourses, some blackfly species are widely distributed in the area and their bites caused mild to severe reactions to humans and animals.

INTRODUCTION

Blackflies (Diptera: Simuliidae) of the genus *Simulium* are biting midges infamous worldwide for their direct attacks to men and animals and for their role as vectors of pathogens of outstanding relevance in human and veterinary medicine. Biting activity of the females of this genus in man can elicit allergic reactions known as "black-fly fever" or simuliotoxicosis, characterised by itching, haemorrhage and oedema, at times needing medical intervention [1-5]. Similar allergic reactions can occur in bovines, and cattle mortalities due to *Simulium* spp. attacks have been recorded in many European countries and North America [6-8]. Furthermore, animals under attack from blackflies can be considerably irritated, with consequent reduction of feeding and mating activities and damage to production [9, 10]. Wounds provoked by blackflies can facilitate infection by secondary pathogens.

Simulium spp. are vectors of viruses, protozoa and helminths. From a veterinary point of view, the most relevant pathogens they transmit are avian protozoa of the genus *Leucocytozoon*, responsible for leucocytozoonosis, an infection of wild and domestic birds with a Holarctic distribution [11], and filarial nematodes of the genus *Onchocerca*, affecting cattle and equids [12,

13]. To the same genus, it belongs *Onchocerca volvulus*, the greatest public health problem involving blackflies, responsible for human onchocerciasis or river blindness in the tropics. Onchocerciasis is among the more relevant parasitosis affecting man worldwide, with an estimate 17 million people infected in Africa and tropical America [14].

Simulium sp. larvae breed in running water, from snow melting streams to large rivers close to the mouth [15, 16]. Environmental heterogeneity of Italy allows the presence of suitable habitats for a rich fauna of this genus, with 70 recorded species [17]. In our Country, attacks by blackflies to men and animals have been historically recorded in many Regions, with several species involved [18]. Nevertheless, studies regarding these flies are quite scanty in Italy, with few, old reports of attacks [18] and studies dealing with Simuliidae as indicators of river environmental quality [15]. These insects, despite their relevance, are rather unfamiliar to most people, hence problems related to *Simulium* sp. attacks to humans and animals go often unnoticed or unreported and the responsible of the problem unidentified.

A problem due to *Simulium* sp. attacks to people living near Rome was first brought to the attention of the Authors in 2013, when *S. intermedium* (*ornatum* group

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[19]), was responsible of repeated attacks to people living in a villa close to the village of Castel Gandolfo, 15 km southeast from Rome. From then on, many other cases have been brought to our attention, regarding both man and animals. We here describe in detail these cases and, when possible, the larval breeding sites.

MATERIALS AND METHODS
Study area and specimens collection

Samples were collected in the southeast of Rome urban area and around the Lake Albano (22 km southeast from Rome city centre) from 2013 to 2021. The six cases summarized hereafter concern people that contacted the Laboratory of Entomology of the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) and brought to our attention their problems related to human or animals attacked by unidentified insects. Following these reports, surveys were carried out to identify the species involved in the attacks and the putative larval breeding sites. Adult blackflies were sampled using an entomological net and an aspirator or collected using traps of the model Italian Mosquito Trap (IMT) (a modified CDC light trap) lured with 1 kg of dry ice (CO_2). Larvae were sampled from stones and water vegetation during watercourses inspection. Samples were stored in vials with 70% ethanol and 5% glycerol until molecular identification. When molecular identification was not possible, larvae and adult specimens were morphologically identified [20]. Blackflies species identification often relies on a holistic approach, where morphological characteristics of eggs, larvae, pupae, males and females are used. Hence, molecular analysis was performed whenever the morphological identification of black flies posed critical issues.

Molecular identification

Adult blackflies were treated with lysis buffer and genomic DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Molecular specific identification was conducted with barcode sequences obtained by amplification of a 710 bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI). The polymerase chain reactions (PCRs) were performed using the Folmer primers (LCO1490 and HCO2198) [21]. PCR product underwent an enzymatic clean-up ExoSAP-IT and amplicons were Sanger sequenced on a 3500 Series Genetic Analyzer with BigDye Terminator chemistry (Applied Biosystems, USA) using the same primers. Sequence data analysis and trimming was performed using the CLC DNA workbench software version 5.7.1. The resulting sequences were compared with the sequences deposited in GenBank using BLAST [22].

RESULTS

All the inspected sites resulted positive for the presence of *Simulium* sp. (*Figure 1*). In five cases (only exception: case 3) identification to species level was possible, highlighting the presence of *S. intermedium*, *S. lineatum*, *S. lundstromi* and *S. ornatum* (complex).

Cases description

1. Attacks to people (Castel Gandolfo, Rome; June 2013) – Attacks by blood-sucking midges occurred in a country house, 15 km southeast from Rome (350 m asl). People were attacked while in the garden of the villa in daytime. Bites involved mainly legs, provoking a considerable discomfort. During a survey, blackflies flying around the legs of the Authors and resting in the hedge bordering the garden were sampled. The specimens were morphologically identified as *S. intermedium*, within the *ornatum* group. In the proximity of the villa, no possible larval breeding site was identified, the closer stream being that described in the following case.

2. Attacks to dogs (Marino, Rome; 2018-2021) – In October 2018, the owner of a dog training field situated 1 km apart from the country house of case 1 (330 m asl) brought to IZSLT some specimens of small biting midges that were seriously threatening his work of dog trainer, due to massive attacks to the dogs in the training field. Some of the dogs were so seriously bitten and presented so severe consequences, even with a case of systemic shock, that owners refused to bring them again for training, thus impeding the working activity. The trainer referred about swarms attacking dogs simultaneously, mainly to legs and belly, in full daylight. The attacks went on also during winter, whenever sunshine warmed up the lawn. During a survey at the end of October 2019, it was possible to ascertain the presence of active blackflies in the lawn and swarms around nearby reeds. No larval breeding site was identified in the proximities of the training field. During subsequent surveys (March and April 2021), larvae were collected and pupae observed inspecting two sites along a nearby
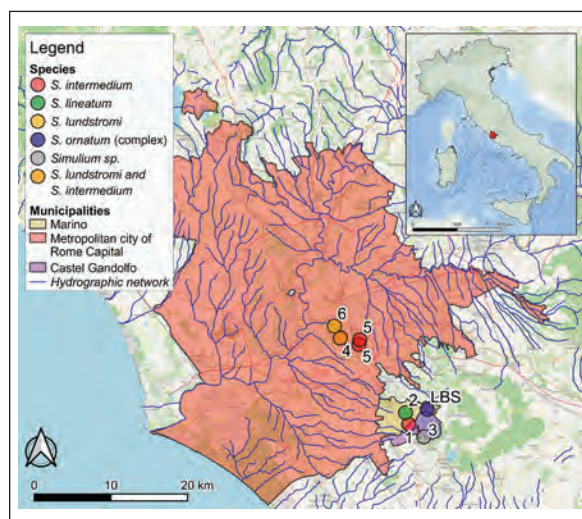
Figure 1
Map of the reported cases of *Simulium* spp. attacks to men and animals. Numbers indicate the cases on the Alban Hills (1-3) and within the Rome urban area (4-6). Blue lines indicate the hydrographic network downloaded from SINANET, the Environmental Information Systems developed and managed by ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale, www.sinanet.isprambiente.it/it). Blue circle indicates the larval breeding site (LBS) highlighted in Marino municipality (Pietrare moat).

small moat, described in *Larval breeding sites* section. Ten adult blackflies were collected with an entomological net and an aspirator while flying and alighting on one of the Authors in the dog-training field. Molecular analyses allowed identifying adults as *S. lineatum*, within the *equinum* group, and larvae from the nearby moat as *S. ornatum* (complex).

3. Attack to cattle (Castel Gandolfo, Rome; August 2019) – A veterinary managing a cattle dairy farm at the fringe of the village of Castel Gandolfo (426 m asl) referred of insects heavily bothering free-ranging young cattle and provoking open wounds, possibly favouring papillomavirus infection. All the cattle in fact presented lesions provoked by this virus, first appeared three years before, concurrently with the first attacks by these biting insects. From then on, insects were always present at the farm and papillomatous lesions, regarding mainly teats, became more frequent and serious. During a survey, many specimens of *Simulium* sp. were sampled while feeding on cattle. Biting activity almost exclusively concentrated along the ridge of the belly. Cattle resulted to be bothered by the presence of these insects and very brittle. During the survey it was not possible to identify any larval breeding site and no potential breeding sites were highlighted, neither studying satellite imagines of the zone. Unfortunately, DNA amplification did not yield any results due to bad conditions of the specimens sampled in this locality, neither it was possible to carry out additional surveys in this farm.

4. Attack to golfers (Appia Antica, Rome; October 2019, July 2021) – The Local Health Authorities reported to the Authors that since three years people playing golf in a club within Rome urban area (40 m asl) were occasionally attacked by small insects, mainly at the ankle. The bites provoked serious reactions to two women, one of them, presenting systemic allergic reaction with glottis oedema, required medical intervention. The Almone River, known as producing blackflies [15], runs through the golf course, nearby the site where the attacks occurred. Golfers reported further attacks in the late afternoons of July 2021, especially on legs and ankles, even if wearing long trousers. Two specimens were caught with an IMT in July 2021 and identified as *S. intermedium* and *S. lundstromi* by molecular analysis.

5. Nuisance to people (Parco degli Acquedotti, Rome; autumn 2019, spring 2021) – Two kilometres away from the golf course of case 4 (60 m asl), nuisance to people caused by biting midges was reported in a green area, the Parco degli Acquedotti. During a survey in November 2019, adult *Simulium* sp. were sampled using an entomological net and an aspirator. A second survey, aimed at looking for breeding sites was carried out in April 2021. Blackfly larvae and pupae were found in a moat and a ditch, respectively inside and outside the Park. In late spring 2021, further attacks to people doing gymnastics were reported to the Authors. The collected larvae, from both watercourses, were identified by molecular analyses as *S. intermedium*.

6. Attacks to people (Caffarella Park, Rome; summer 2020) – Around 1 km away from the golf club of case 4 (20 m asl), a couple reported attacks at ankles and lower legs in a large grassy area of the Caffarella Park. They referred the presence of flying midges around them. They both complained by the attack but apart from small wheals with a haemorrhagic centre and an intense pruritic condition, no further reaction occurred. The attacks occurred from June to the end of July, 100 m from the Caffarella moat and 260 m from the Almone river. The event was reported to the Authors and a survey was carried out in March 2021 to identify the larval breeding site (see Larval breeding sites section). The collected larvae were identified by molecular analyses as *S. lundstromi*.

Larval breeding sites

Survey nearby cases 1, 2 and 3 – A larval breeding site of *S. ornatum* was identified along a moat running through the villages of Marino and Ciampino (southeast from Rome, 340 m asl) (*Table 1*). The moat was investigated looking for the breeding sites of the species responsible for the attacks to people, dogs and cattle, respectively of cases 1, 2 and 3. In two inspected sites along the watercourse, even where the water was only few millimetres deep, several larvae at different developmental

Table 1

Summary of the surveys data together with the results on species identification. Identification was carried out by means of molecular analysis (*) or morphological identification (**). Details of the larval breeding site (LBS) of Pietrare moat (Marino municipality) were here reported due to its proximity to three cases (cases 1-3)

Case N.	Coordinates	Species	Collected (c) or observed (o) life stage	Coverage (%)	Identity (%)
1	41.75009 N, 12.63610 E	*Simulium intermedium*	Adult** (c)	NA	NA
2	41.76364 N, 12.63099 E	*Simulium lineatum*	Adult* (c)	100	99.67
3	41.73533 N, 12.65963 E	*Simulium* sp.	Adult (o)	NA	NA
4	41.85269 N, 12.53391 E	*Simulium intermedium*	Adult* (c)	100	100.00
		Simulium lundstromi		100	96.42
5	41.84304 N, 12.55833 E	*Simulium intermedium*	Adult (o), larvae* (c)	100	99.84
5	41.84866 N, 12.55936 E	*Simulium intermedium*	Larvae* (c)	100	99.73
6	41.86461 N, 12.51960 E	*Simulium lundstromi*	Adult (o), larvae* (c), pupae (c)	98	97.12
LBS (nearby cases 1-3)	41.76744 N, 12.66524 E	*Simulium ornatum*	Larvae* (c) and pupae (o)	100	98.87

stages and pupae were observed, despite the high level of pollution due to urban sewages [23]. Larvae and pupae were attached to stones and construction debris, as well as to the rocks of the riverbed.

Survey nearby cases 4 and 6 – During a survey in March 2021, two streams were identified as larval breeding sites for *S. lundstromi* in the Caffarella Park: the Almone river and the Caffarella moat. Both watercourses are perennial and present medium to bad ecological conditions, considering both the Extended Biotic Index [24] and river functionality index [25], as reported by [26]. The survey was carried out nearby the site where the attacks to humans occurred in 2020. Larvae and pupae were collected from different substrates: stones and clay roof tiles on the riverbed and plant stems, mainly cane (*Arundo donax* L., 1753). *S. lundstromi* larvae were found in both watercourses, even where the moat was only few centimetres deep. Inspecting the underwater surface of few cane stems in the Almone river, up to 50 cm length of the stems were covered by *S. lundstromi* pupae, with a density of ~10/cm².

Survey nearby case 5 – The last survey, carried out inside and outside the Acquedotti Park, confirmed two watercourses as larval breeding sites of *S. intermedium* (*Table 1*), both tributaries of the Almone river [27]. Both watercourses are characterized by bad ecological conditions and polluted water, considering their Extended Biotic Index and the low diversity value of the macroinvertebrate community [23].

DISCUSSION

All identified species were previously reported from Rome urban area and surroundings [28]. The species belonging to the *ornatum* group are well known for their attacks to people in Italy [29, 30]. On the other hand, this is the first report of attacks to people by *S. lineatum*. Given the proximity of *S. lundstromi* larval breeding sites to the areas where attacks to people occurred in the Caffarella Park, this species is the putative responsible of the nuisance described in case 6. Nevertheless, an analysis of adult biting blackflies is still needed to confirm its involvement.

Simulium sp. attacks to people and animals in Rome and the surrounding area seem to have become more frequent in the last few years. Although Authors have been working for 20 years in a public health institution in Rome (IZSLT), point of reference for sanitary entomology for people and other health institutions, only since the last few years people experiencing problems related to blackflies contacted them. Indeed, since the first case in 2013, these requests became more frequent. These findings suggest that further ecological investigations could be useful to pinpoint potential imbalances in the aquatic community (e.g., reduction of larvae and pupae predators), which could have led to adults' proliferation. Blackflies are a neglected group of insects, unknown to most part of people and identifiable only by expert entomologists. Hence, presumably many similar cases remain unrecorded. People contacting Authors usually refer to "mosquitoes", "small biting insects", even "grass mites" as being the origin of their problems and only after surveys and accurate study of each case, Au-

thors were able to identify blackflies as responsible of the nuisance. Interestingly, all cases occurred in proximities of watercourses belonging to the same aquifer, part of the Alban Hills hydrogeological unit, and southeast from Rome urban area [23, 27, 31]. The high levels of underground circulation generate groups of peripheral water springs and perennial streams [32, 27], suitable habitats for several *Simulium* species. These watercourses have been subjected to several flow managements during centuries [32], maybe increasing habitat suitability [33]. *S. intermedium*, due to its high ecological plasticity [15, 34], in many cases replaced other *Simulium* species, when environment underwent anthropic changes, such as elimination of trees and hedges from streams margins, disappearance of free-ranging cattle and progressive pollution of waters [15, 18, 29, 35]. Indeed, this is often the dominant group of species in polluted, eutrophic streams, with basins dominated by agricultural or urban use and it has been already reported in literature as the dominant group in Lazio Region [15, 36]. *S. ornatum* (complex) resulted one the most frequent species in urban areas and in highly polluted waters [15], but it is also present in less disturbed watercourses, where can be associated with *S. lineatum* (*equinum* group). Our findings confirm the extreme ecological plasticity of this species group, capable to thrive even in highly polluted watercourses. Our results confirm the punctual distribution of *S. lundstromi* [28] and its preference for plain watercourses [20] and the wider distribution of *S. intermedium*, present in both plain and hilly sites. Among the species-groups and species present in Central Italy, *S. ornatum* group and *S. intermedium* in particular have been frequently reported as biting people, at time being very aggressive and causing considerable nuisance [29, 34, 37]. Peculiar biological trait of this group of species is the attack to the lower body parts of the victims, confirmed in our reports, where dogs and cattle were attacked to the belly and people mostly at legs and ankles. Blackflies control is a hard target to achieve and is possible, although difficult, only by means of microbial insecticide on larval stages, usually *Bacillus thuringiensis* var. *israeliensis* [38, 39]. Given the low effectiveness of larval breeding sites treatments, which need to be frequently repeated in lotic watercourses, it was very difficult for the Authors to give any suggestion to face the problem. In the case of the dog-training field, it was suggested to cut reeds along the border, given that this vegetation could act as a visual cue attracting flying *Simulium* individuals. It cannot be excluded that flying individuals, maybe originating from the Pietrare moat (1 km away), might have been attracted by the long row of reeds as indicator of the possible presence of a moat and, once landed on the reeds, started attacking dogs. In the case of the golf course, due to the limited number of players complaining for the nuisance, club manager considered not necessary any kind of intervention. In this case, Authors gave indications to the interested persons about individual protection measures, such as the use of repellents and of long trousers and sockets when playing on the course. Finally, in the Caffarella and in the Acquedotti Parks, where the larval breeding sites were identified, considering the limited number of people

complaining for blackflies attacks and the environmental value of the two parks, no control treatments were performed. Even if not demonstrable, very interesting is the possibility that blackflies bites were the point of entry of papillomavirus in cattle (case 3). In this case, even though indirectly, besides being a nuisance, blackflies would have provoked a real damage to the animals and to farm production. Indeed, papillomatous lesions were in some cases so severe to prevent the milking and some of these animals had to be removed from the herd.

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Authors' contributions

Conceptualization, FR, AM and CDL; entomological surveys, FR, DS, AM and CDL; molecular analysis, ELD and MI; writing-original draft preparation, FR, ELD, MI and CDL; writing-review and editing, FR and CDL. All Authors have read and approved the final manuscript.

Availability of data and materials

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

Conflict of interest statement

The Authors declare that they have no competing interests.

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The use of concept maps as an assessment tool in students' risk education about occupational safety and health

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Abstract
Given the relevance of students' risk education about occupational safety and health (OSH), the need for reliable assessment tools for learning evaluation is widely advocated. However, multiple-choice tests or inventories show several limitations, including the emphasis on basic definitions instead of organized conceptual systems and passive information transfer. Instead, concept maps may represent a non-traditional evaluation tool to inspect how students actively organize and represent the acquired knowledge in line with the theory of meaningful learning. A concept map specifically developed in the field of risk education and its quantitative scoring protocol are here proposed. Then, a case study with a pre- post-test design is presented, including 611 upper school students who undertook an OSH-related risk educational course. The findings are briefly discussed and help identify the possible information we can get from concept maps in terms of problem-based learning.

INTRODUCTION

The European Agency for Safety and Health at Work (EU-OSHA) has indicated young people aged 16-24 as a priority group for risk prevention initiatives [1]. Indeed, young workers tend to be more vulnerable to accidents, showing a risk of about 25-40% higher compared to other age groups [1]. This is supported by several studies reporting significant rates of occupational injuries and work-related illnesses among youths, especially when entering the workplace [2-4]. Such a vulnerability may depend on the types and features of jobs young people come in contact with, often requiring to perform harmful or physically demanding tasks [5]. Since early employment opportunities mostly refer to part-time and temporary work, further contextual factors may have a role, including reduced freedom of action, uncertain outcome, poor supervision, and low social support [5]. In this regard, previous research has shown that young workers tend to underestimate risks and deem accidents as part of the work routine [6, 7]. Besides, they are reluctant to share their concerns about work-related hazards as they could appear immature workers and displease their superiors [5]. Occupational safety and health (OSH) are, therefore, relevant in vocational training and workplace-based learning of pupils as they lack experience, have limited knowledge about risks,

and consider themselves as mainly responsible for safety at the workplace [1, 5]. Accordingly, close school-work cooperation is advocated to involve students in risk prevention and help them acquire consistent knowledge during their studies [1, 5, 8]. The positive impacts of educational interventions in terms of OSH-related knowledge in student populations have been demonstrated [9-11]. In program evaluation, beyond the specifically adopted training strategies and methodologies, student knowledge is generally assessed through multiple-choice tests or inventories. However, such a way to evaluate learning shows several limitations, including the emphasis on basic definitions instead of organized conceptual systems and passive information transfer [12, 13]. Indeed, typical objective tests mostly probe rote learning, based on mnemonic processes regarding computational procedures or propositional statements, with a scarce emotional commitment to integrate new with existing knowledge [14, 15]. Instead, concept maps may represent a non-traditional evaluation tool to inspect students' understanding, more able to grasp active learning processes and the use of acquired knowledge within specific contexts in line with the theory of meaningful learning [12, 16]. Besides, since concept maps are theoretically grounded on a constructivist perspective, they can be effective in identifying creative

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ideas or misconceptions held by students, with the advantage of being less time-consuming than clinical interviews or other qualitative methods [17, 18].

Concept maps are graphical tools for organizing and representing knowledge in a specific domain through nodes and labeled lines, which consist of several elements including concepts, propositions, hierarchical structures, cross-links, and examples [12, 16]. Concepts refer to important terms or central objects/events, graphically enclosed in circles or boxes, which are designated by a label (usually a word). Propositions represent statements regarding such objects/events, through a combination of two or more concepts connected by linking words (e.g., "causes!", "requires", "such as" or "contributes to"), as basic units of meaning. The hierarchical structure deals with the organization of concepts based on different levels of relevance and inclusiveness according to a top-down order, thus providing the context of the concept map. Cross-links define the relationships between concepts in different domains or segments of the concept map, allowing the visualization of the connections between same-level nodes. Then, examples of specific events or objects can also be considered to concretely describe and better clarify the underlying meaning of concepts.

THE DEVELOPMENT OF A CONCEPT MAP IN THE FIELD OF OCCUPATIONAL SAFETY AND HEALTH

Based on this premise, a concept map has been developed in the field of risk education [19] using as a focus question how OSH could be defined. The concept map followed an approach to OSH consisting of three different steps, i.e. hazard identification, risk assessment, and measures (i.e. actions, controls, and procedures) to minimize risk. Hazard identification involves the acknowledgment of the existence of a hazard, defined as any situation, substance, activity, event, or environment that could potentially cause injury or occupational disease. In this first step, it is important to make the description of the features of every single hazard available through an OSH-oriented organizational model allowing its identification, from homogeneous situations shared by workers. The second step involves both risk measurement and risk evaluation. Indeed, the risk is meant as the product of the likelihood of a hazard occurrence and the severity of its negative impact on health and safety. Therefore, risk assessment takes into consideration both the degree of exposure to hazardous situations and the potential to cause damage (e.g., injury or ill conditions), to understand whether a specific risk can or not fall within an acceptable threshold. Then, actions, controls, and procedures are enacted to minimize every single risk and their effectiveness is monitored. Such procedures involve prevention and protection measures concerning the reduction of hazard probability and severity, respectively. For instance, prevention may include OSH-related education and training whereas protection may refer to the use of special equipment at work depending on the risk specifically assessed.

At a first level, the developed concept map consists of the concepts "hazard", "risk" and "measures", which are linked to OSH through the propositions "identification", "assessment", and "determination", respectively. Indeed, they represent the main nodes of the logic framework about OSH, necessarily intended as sequential steps (as indicated by the dashed lines between such same-level nodes with the linking word "requires"). Indeed, whereas a hazard can be identified in absolute terms as a potential danger, risk definition involves an assessment procedure able to contextualize hazards within workplaces and processes, based on specific exposure and use conditions. Then, only in the last step, it is possible to determine consistent actions, controls, and procedures aimed at minimizing and monitoring the previously assessed risks. At a second level of the hierarchy, more specific and less inclusive concepts are introduced regarding both risk and measures. In detail, "severity" and "probability" represent the core features of the "risk" concept since it can be defined as the product of the damage a hazard could create (severity) and the chance that the harmful event actually occurs (probability). Whereas, "prevention" and "protection" contribute to better defining the concept of measures of risk mitigation. Two cross-links show that prevention aims at minimizing the chance of occurrence whereas protection aims at minimizing the severity of the damage, as previously discussed. Then, the third level of the hierarchy includes the concepts "injury" and "illness", which further specify the consequences of the damage at work. Injury refers to an accident event characterized by a violent and sudden cause, compromising safety and resulting in damage to health varying in severity from reversible injury to death. Instead, occupational illness is due to a not violent cause that acts slowly and progressively with negative outcomes on health and wellbeing.

Based on the criteria proposed by Novak and Gowin [12], concept map can be evaluated using the following quantitative scoring protocol *(Figure 1)*. Each valid proposition indicating a meaningful connection between two concepts is assigned one point. Propositions are illustrated by 9 labeled lines with the linking words "identification", "assessment", "determination", "combines", and "include(s)". Each concept correctly assigned to a valid hierarchical level showing reliable order of relevance and inclusiveness is given five points. The 6 levels of hierarchical subordination refer to "severity" and "probability" as specifications of risk, "protection" and "prevention" as specifications of measures, and "injury" and "illness" as specifications of severity. Each cross-link highlighting an original and worthwhile relationship between concepts in different segments of the hierarchical structure is assigned ten points. Cross-links are illustrated by 4 dashed lines with the linking words "require(s)" and "minimizes". Then pertinent and specific examples that well illustrate concepts are assigned one point each, with a maximum of one example per concept to ensure standardization of scores. Students are asked to write them in parentheses below each of the 9 concepts.

METHOD
Participants and procedures
In the wide ranges of studies about concept maps as an assessment tool, the present manuscript proposes
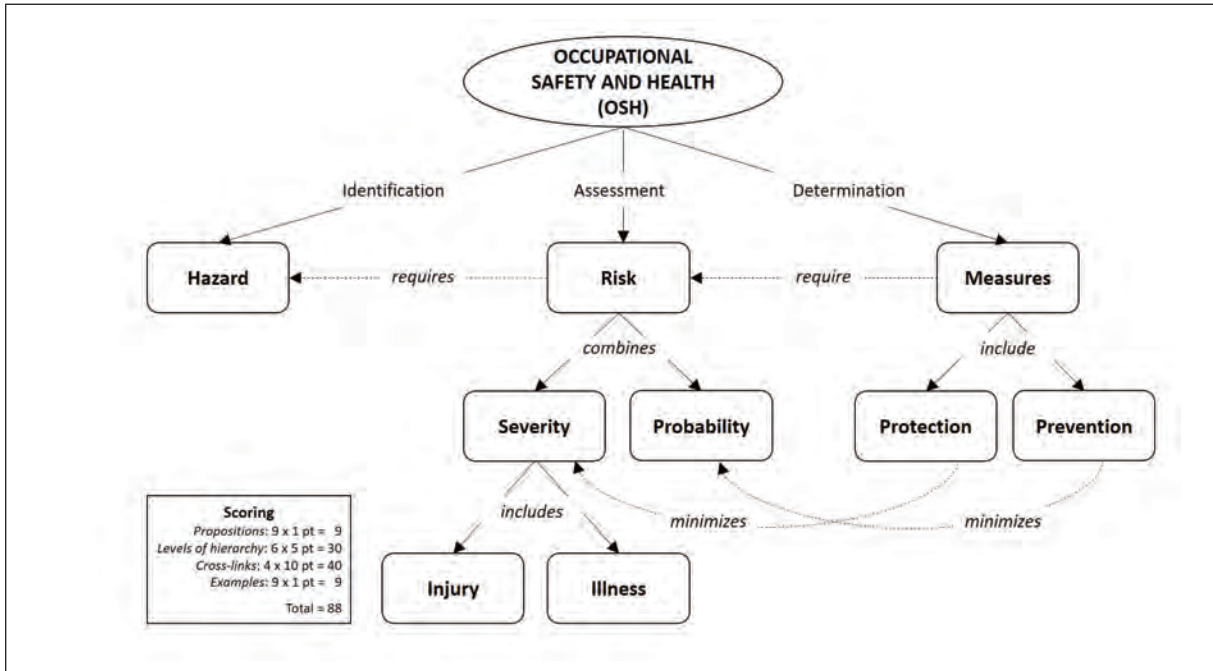
Figure 1
Concept map about OSH with the scoring system.

a small case study regarding a specific OSH-related risk educational course addressed to upper school students. According to the Italian Law no. 107/2015, OSH training is mandatory in all school-to-work transition programs, named PTCO (Percorsi per le Competenze Trasversali e l'Orientamento, i.e., education and training in soft life skills), offering students short working periods in companies or public institutions to acquire theoretical and practical skills. Specifically, the present case study involved 611 upper school students aged 16-18 years, who were female in 62.4% of cases, attending the OSH courses held by the National Institute of Health (Istituto Superiore di Sanità, ISS) from 2016 to 2020 [20]. The course, developed in line with the Italian regulations, consisted of a 6-hour face-to-face training about a variety of contents, including the main risk factors at the workplace (e.g., physical, biological, chemical, work-related stress, gender differences in work risks, first aid, and emergency management). After having received basic instruction on concept mapping, the developed concept map was used at the beginning and the completion of the training course to assess students' learning. Students were administered a white sheet showing the focus question at the top ("What is OSH?") and nine words, written on the side without a specific order, that represented the nodes of the concept map. Specifically, participants were asked to draw a concept map from the provided words, graphically illustrating propositions, levels of hierarchy, cross-links, and examples. The administration procedure lasted on average 30 min for both pretest and posttest assessment.

Data analysis

The concept maps drawn by the participants were scored by two trained raters based on the previously

discussed protocol and interrater agreement was established through the Intraclass Correlation Coefficient (ICC). The measure scores were computed as raw sums with ranges of 0-9 (propositions), 0-30 (levels of hierarchy), 0-40 (cross-links), 0-9 (examples), and 0-88 (total), respectively. Then, paired t-tests were conducted to show statistically significant differences between the pre- and post-test assessment.

RESULTS AND DISCUSSION

Reliability was 0.88 (95% CI, 0.81-0.95) for propositions, 0.92 (95% CI, 0.89-0.95) for the levels of hierarchy, 0.90 (95% CI, 0.85-0.95) for cross-links, and 0.81 (95% CI, 0.77-0.85) for the reported examples.

In *Figure 2*, a concept map made by a participant and its scoring are illustrated as an example. Overall, four propositions are correctly identified, which refer to "risk assessment", "determination of measures", as well as "prevention" and "protection" as further specifications of the concept of measures. Besides, regarding the levels of hierarchy, only prevention and protection are well-placed in the hierarchy, whereas no valid cross-link is shown. Then, five specific examples can be deemed pertinent as follows: rules of conduct as a possible organizational measure, contusion as a type of injury, stress as a work-related illness, hospitalization as a negative consequence on health in terms of severity of the damage, and lack of information as inherent to the concept of probability since it affects the degree of exposure to hazard situations. The overall score of the concept map – equal to 19 points out of the total of 88 – adequately reflects some misconceptions about OSH held by the student. First, risk assessment and taking control measures are not seen as sequential steps of the same process. As well, the preliminary action concerning hazard identification
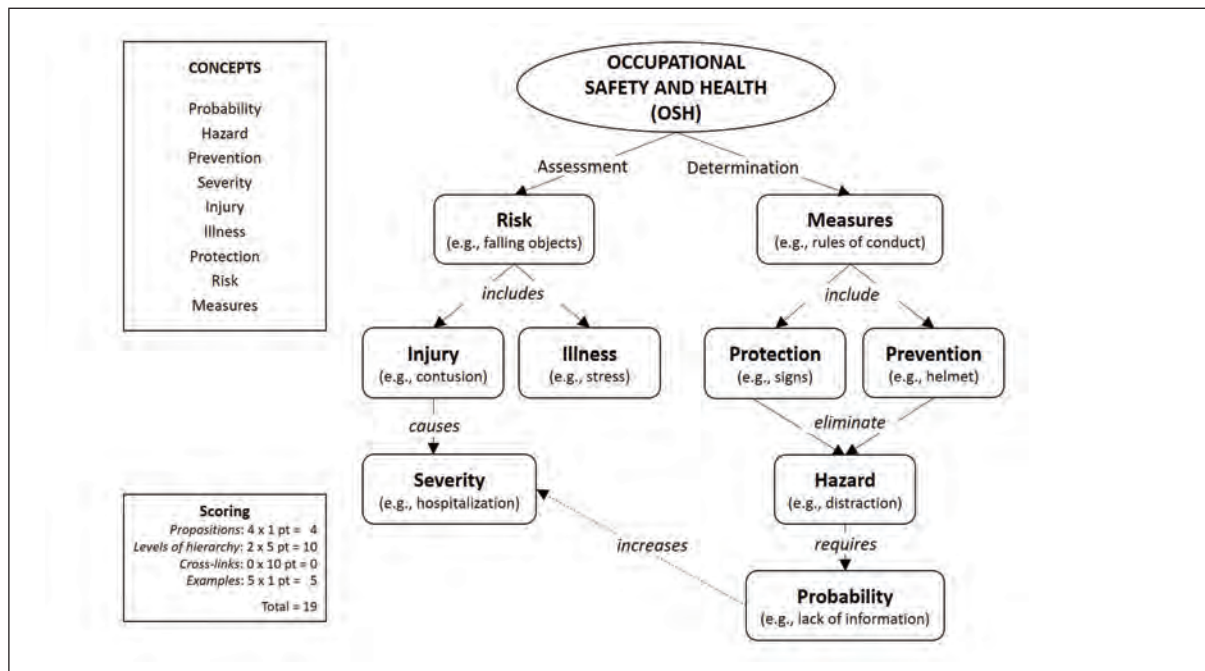
Figure 2
A concept map made by a participant and its scoring.

is omitted, probably because of the confusion between the concepts of hazard and risk. This is suggested by the specific examples proposed to illustrate them, i.e., "distraction" for hazard and "falling objects" for risk, and by the concept of hazard as something eliminable requiring the concept of probability, rather than a source of potential damage in absolute terms. Besides, injury and illness are not well understood as specifications of the concept of severity in the hierarchy structure, whereas only injury is connected with adverse consequences, thus suggesting fatalistic beliefs about OSH. In this regard, severity and probability are not identified as same-level nodes, but the extent of the damage is seen as depending on the likelihood of exposure to hazard situations. Then, based on the provided examples, protection and prevention measures appear as unclear and mostly interchangeable concepts, since warning "signs" should prevent risks and the "helmet" should protect from them, not the other way round.

Table 1 includes the means and SDs of the pre- and post-test assessment about the concept mapping scoring levels. Looking at the descriptive statistics of the pre-test assessment, the normalized values of the dimensions suggest that on average students have difficulties in relying on problem-based and creative reasoning, especially in identifying valid cross-links ($z=0.05$) and concrete examples ($z=0.19$).

Paired t-tests show statistically significant differences, indicating higher scores at the completion of the training course for all the four parameters, with a Cohen's d ranging from small to large. The categories "propositions" and "examples" show the largest effect size, suggesting a strengthened capacity to establish meaningful connections between the core concepts of OSH as well as concretely describe and better clarify the underlying meaning of concepts. Along with this, a moderate improvement emerges in the "levels of hierarchy", demonstrating meaningful progressive differentia-

Table 1
The paired t-test summary of concept mapping scoring levels in the pre-post test assessment (n=611)

Parameters (*raw score range*)	Pre-test		Post-test				
	M	SD	M	SD	t(610)	p	Cohen's D (95% CI)
Propositions (*0-9*)	1.58	1.93	4.11	2.38	27.05	<0.001	1.09 (0.99, 1.19)
Levels of hierarchy (*0-30*)	0.52	1.41	7.08	4.89	16.74	<0.001	0.68 (0.59, 0.76)
Cross-links (*0-40*)	0.03	0.57	2.11	5.86	8.74	<0.001	0.35 (0.27, 0.43)
Examples (*0-9*)	0.11	0.58	7.79	6.38	29.48	<0.001	1.19 (1.09, 1.30)
Total	2.24	2.93	21.09	12.73	30.35	<0.001	1.23 (1.12, 1.33)

M: mean; SD: standard deviation.

tion of the knowledge structure. Instead, despite being statistically significant, the effects on "cross-links" between concepts in different segments of the hierarchy are small-sized, thus revealing that the tendency to include the acquired knowledge in separate blocks is only partially affected.

CONCLUSIONS

This short communication aimed to propose the use of concept maps as an effective and feasible assessment tool in students' risk education about OSH. This is in order to overcome the main limitations of traditional evaluation methods based on passive learning transfer and promote innovative ideas to inspect how students may structure their acquired knowledge. As an example, a case study on different cohorts of upper school students undertaking a specific OSH training within school-to-work transition programs helped us to identify the possible information we can get from concept maps in terms of problem-based learning. Future research could be conducted to examine the concurrent validity of concept maps with self-report inventories or standardized measures. As well, longitudinal studies are needed to explore the extent to which concept mapping-related skills may explain student risk perception in the OSH field and predict their future risk-taking behaviors in real work settings.

Conflict of interest statement

The Authors do not report any potential conflict of interest.

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Self-Awareness Multilevel Assessment Scale (SAMAS): psychometric analysis of inter-rater reliability

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Abstract

Background. Severe acquired brain injury (sABI) frequently causes impairment in self-awareness (ISA), leading to reduced patients' compliance to treatment, worse functional outcome, and high caregiver distress. Self-awareness (SA) is a multilevel and complex function that, as such, requires a specific and effective assessment. To date, many tools are available to evaluate the declarative, but not emergent and anticipatory levels of awareness, therefore the Self-Awareness Multilevel Assessment Scale (SAMAS) was recently proposed. The new tool proved to be useful to assess SA at different levels across all domains of functioning (motor, cognitive, psycho-behavioural, etc.) because it measures not only the declarative SA, but also emergent and anticipatory levels of SA, thus overcoming some important limits of other current assessment methods.
Aim. This study evaluated the inter-rater reliability (IRR) of the SAMAS.
Methods. Four professionals blind to each other evaluated 12 patients with sABI. Each patient was rated by two professionals.
Results. Inter-rater reliability was moderate-to-excellent, adding evidence in support of the use of SAMAS to specifically diagnose ISA after sABI.
Conclusions. The SAMAS can help to better address neurorehabilitation, as it allows assessing ISA as early as possible, at all possible levels of awareness and functional domains.

INTRODUCTION

Self-awareness (SA) is frequently impaired after a severe acquired brain injury (sABI) [1-3]; therefore, in neurorehabilitation it is fundamental having an as early and as much accurate as possible assessment of SA after sABI. Impaired self-awareness (ISA) has been associated to dysexecutive syndrome [4, 5], apathy or anosodiaphoria [6-10], reduced subject's compliance to treatment, worse functional outcome [11-19], and caregiver distress [20].

However, some issues are still debated in the literature. In fact, ISA is a multifaceted concept, which to date remains not fully understood. Two main explanatory models have been proposed. Firstly, Crosson *et al.* [21] posited a pyramidal model consisting of three interdependent and hierarchical levels, namely a) intellectual awareness, i.e. the subject's ability to understand (mostly thanks to external feedbacks) and refer that a

function is impaired; b) emergent awareness, i.e. a subsequent ability to recognize problems when they happen; and c) anticipatory awareness, i.e. the final ability to anticipate that a problem will occur due to the deficit already known by the patient at the two previous levels [21]. More recently, Toglia and Kirk [22] proposed an alternative Dynamic Comprehensive Model of Awareness (DCMA) that, rather than as a series of hierarchical levels, posits the relationship between different aspects of metacognition and awareness as a dynamic process. The DCMA differentiates between: a) metacognitive awareness, i.e. knowledge of task characteristics and knowledge of one's own capabilities (similarly to the concept of intellectual SA of Crosson *et al.* model), and b) online awareness, which can be activated during a task and consists of self-monitoring and recognition of errors (similarly to emergent SA of Crosson *et al.* model), as well as of the person's appraisal of current

task demands (comparable to anticipatory SA of the same previous model). For the purpose of the present study, we will adopt the term "declarative" SA, referring to both intellectual and metacognitive levels of SA.

A further issue, closely related to the first two, is how to assess at best ISA after sABI. Indeed, many measures are already present in the literature, i.e., clinical observation [23-24]; structured and semi-structured interviews [15, 25]; comparison between patient's self-assessment and their performance on neuropsychological tests [26]; and comparison between patient's self-report and clinician/relative's report [11, 27, 28]. However, all these measures present with some important limits. For example, self-report questionnaires cannot be administered to patients who suffer relevant cognitive deficits, such as aphasia, severe memory deficits or reduced reasoning and judgment abilities. Moreover, questionnaires and interviews can assess mainly declarative awareness, even when they try to investigate anticipatory SA [15]. Indeed, the patients' report can be considered a mere declarative report if not supported by actual and effective behaviours, such as preventing difficulties in daily life, by adopting strategies to cope with them. Accordingly, a report of significant others is necessary to verify that patients are concretely self-aware at an anticipatory level (see [29] for details). Finally, as for emergent SA, the existing measures [25, 30-33] can assess SA only through some standardized tasks, rather than in a more comprehensive and ecological variety of situations directly related to the current patients' difficulties [34].

In a recent study [29] we tried to overcome the limits above mentioned by means of a new measure, namely the Self-Awareness Multilevel Assessment Scale (SAMAS) [29], which can be considered a versatile tool for the assessment of SA at different levels and across several possible domains of functioning (see below for details). Indeed, through the SAMAS, professionals in the neurorehabilitation setting can assess patients' SA at the emergent level, that is regarding their ability to self-monitor online their performance in all possible critical areas of functioning. Moreover (going beyond the merely declarative level), thanks to the contribution of other professionals in the rehabilitation team and of caregivers, the scale allows for investigating the real level of anticipatory SA [29].

The validation study of the SAMAS [29] used two external measures to assess the concurrent validity: a) a gold standard given by a blind clinical judgment of an expert neuropsychologist; b) the correlation between the SAMAS and two of the mainly adopted ISA measures, i.e., the Patient Competency Rating Scale (PCRS) [35] and the Self-Awareness Deficits Interview (SADI; [15]). Our results showed that the SAMAS can be conceived as a valid tool to assess SA since it significantly predicted all dimensions of SA. Indeed, the scale revealed to be able not only to assess declarative SA, but even to specifically and broadly assess both emergent and (actual) anticipatory levels of SA.

In line with our previous study, the aim of the present study was to assess the inter-rater reliability (IRR) of the SAMAS.

MATERIALS AND METHODS
Participants

We recruited twelve patients with severe ABI, consecutively admitted to the Post-Coma Unit of Fondazione Santa Lucia of Rome (Italy) from February 2020 to April 2021. The study was approved by the local Ethics Committee, and all participants and their caregivers were included in the study after providing their (or by one legal surrogate) informed consent.

The inclusion criteria for the patients were: a) age ≥16 years; b) diagnosis of severe ABI (i.e., Glasgow Coma Scale (GCS) score ≤8 in the acute phase); c) score at the level of Cognitive Functioning Scale (LCF) ≥6, with inclusion of the patient according to the judgment of the neuropsychologist involved in the study; d) capacity to undergo a formal psychological evaluation; e) availability of informed consent. Exclusion criteria were: a) history of drug and alcohol addiction; b) psychiatric diseases; c) repeated sABI and/or other neurological disorders.

Sociodemographic and clinical characteristic of patients were: 8 males and 4 females, with a mean age of 45.8 years (SD=14.8; range=46.0; median=48.5; IQR=16.5); mean educational level of 11.8 years (SD=3.6; range=10.0; median=12.5; IQR=5.0); time since injury: from 42 to 386 days, with a mean of 133.5 days (SD=101.0; range=344; median =102.0; IQR=119.0); aetiology of severe ABI was: traumatic brain injury (TBI) (n=5), haemorrhagic stroke (n=3), and ischemic stroke (n=4).

We also recruited one informal caregiver ("caregiver" from now on) for each patient. The inclusion criterion for caregivers was the absence of any current or previous severe neurological or psychiatric disorder.

Self-Awareness Multilevel Assessment Scale (SAMAS)

The SAMAS is a single and comprehensive tool conceived to be administered by a cognitive-behavioural therapist in neurorehabilitation. The SAMAS assesses the different levels of SA (i.e., declarative, emergent, and anticipatory) across all possible domains of functioning (i.e., motor, cognitive, psycho-behavioural, and others – such as phoniatric, dysphagic, etc).

As for the declarative SA, the SAMAS takes into account two aspects, i.e., the patient's recognition of a) the presence of current difficulties (e.g., paresis of a limb and/or memory deficits), and b) of the functional implications of such difficulties (e.g., the patient's impossibility to eat alone and/or to remember what he did in the past day). As for the emergent SA, the SAMAS assesses the patient's online recognition of difficulties in each functional domain (e.g., the patient's ability to realize the impossibility to stand up while trying to do it, and/or to realize difficulty in remembering something while trying to do it). Finally, the anticipatory level takes into account five aspects, i.e.: a) the patient's ability to recognize the problematic nature of a task with respect to his/her own deficits; b) the patient's ability to set realistic goals in relation to his own difficulties; c) the patient's expression of strategies to avoid having difficulties; d) the patient's effective use of such strategies; and e) the

patient's ability to generalize such strategies (when they are used) to all the contexts in which he/she acts.

The SAMAS provides three different scores, one for each level of SA. In particular, the scale includes two items for the declarative, one for the emergent, and five for the anticipatory level of SA. Since each item encompasses the above mentioned four domains (i.e., motor, cognitive, psycho-behavioural, and others), the item score can range from 0 to 8 – being each sub-score for each domain ranging from 0 ("good SA") to 2 ("relevant ISA") – (See [29] for details). In each level assessed by the SAMAS, 0 is assigned when patients show good SA; 1 if they need a cue by the therapist (moderate ISA); 2 when they show no SA despite such cue (severe ISA).

Of note, the SAMAS should be completed with the involvement of the patients' caregiver (by a clinical interview) and of the inter-professional neurorehabilitation team (when necessary), in order to accurately assess all the levels of SA and, in particular, emergent and actual anticipatory SA (see the Procedure section below for details).

Operationally, the examiner fills in the SAMAS form assigning 32 sub-scores (ranging from 0 to 2), for a maximum score of 8 for the declarative, 4 for the emergent, and 20 for the anticipatory level of SA. Regarding the evaluation of the declarative level of SA, each sub-score is established by the examiner based on his/her direct observation of the patient. As for the other two levels of SA, each sub-score is established by the examiner based on information that the same examiner gathers indirectly, that is through other professionals of the neuro-rehabilitation team and/or the caregivers.

Procedure

The study involved four professionals, i.e., two speech therapists expert in cognitive-behavioural neuroreha-

bilitation (GF and SL), and two researcher psychologists expert in clinical neuropsychology (MA and MC). After enrolment, each patient was randomly assigned to a couple composed of one speech therapist and one psychologist, so that each of the couples assessed three different patients according to the scheme reported in *Table 1*.

Therefore, each rater blindly assessed six out of the 12 patients enrolled. In particular, each patient was assessed by the two raters within the same week.

Table 2 shows the assessment scheme for the three levels of SA. In particular, for each patient the declarative and the anticipatory SA were assessed by the two raters separately and in three different sessions: 1) through a clinical interview with the patient; 2) by an interview with the physiotherapist and/or with other members of the neurorehabilitation team (to assess declarative SA by comparing what reported by the patient to what reported by the other therapists concerning the patient's functional impairments); 3) by a clinical interview to the patient's caregiver to assess the actual anticipatory SA. As for the emergent SA, instead, the two raters filled in the SAMAS at the same time in the presence of the patient, based on the need of assessing SA in relation to an (only) objective patient's behaviour, i.e., during the administration of a task related to the real patient's difficulties (e.g., based on what emerged by the patients' medical records, by the rehabilitation staff or by the neuropsychological report); of course, even in this case, each of the two raters filled in the SAMAS in blind.

Statistical analysis

The IRR was calculated as intra-class correlation coefficient (ICC) using a 2-way random effects, single measure (2,1) model, and measured separately for the global (8 items, comprised of 32 sub-scores), the declarative (2 items, 8 sub-scores), emergent (1 item, 4 sub-scores), and anticipatory (5 items, 20 sub-scores) SA levels. The IRR results are shown in *Table 3*.

RESULTS

The IRR results were all significant *(Table 3)* and indicated a good IRR for the overall scale (ICC=0.77). In detail, the IRR was good (ICC=0.76) for the declarative, excellent (ICC=0.92) for the emergent, and moderate (ICC=0.67) for the anticipatory levels. Since the anticipatory level was based also on information gathered from the caregiver, which potentially may have

Table 1
Assessment scheme of each patient for each of the four couples of raters

Couple speech therapist/ psychologist	Patients blindly assessed
Rater 1: GF - Rater 2: MA	"a", "b" and "c"
Rater 1: GF - Rater 2: MC	"d", "e" and "f"
Rater 1: SL - Rater 2: MA	"g", "h" and "i"
Rater 1: SL - Rater 2: MC	"j", "k" and "l"

GF and SL: Speech Therapists; MA and MC: Psychologists.

Table 2
Assessment scheme for the three levels of Self-awareness (SA)

Level of SA	Rater 1	Rater 2
Declarative	Patient	Patient
	Physiotherapist (and/or other members of the neurorehabilitation team)	Physiotherapist (and/or other members of the neurorehabilitation team)
Emergent	Patient	
Anticipatory	Patient	Patient
	Caregiver	Caregiver

Table 3
IRR separately for the SAMAS global score and the scores of the declarative, emergent, and anticipatory levels. The intercorrelation coefficient – ICC (2,1) – was based on a 2-way random effects model (absolute agreement). The last line reports the IRR for the global score computed excluding the anticipatory level

	Cronbach Alpha	ICC	CI 95%	Test F	p value
SAMAS global score	0.891	0.77	0.36-0.93	9.20	0.000
Declarative level	0.867	0.76	0.37-0.92	7.51	0.001
Emergent level	0.954	0.92	0.74-0.98	21.61	0.000
Anticipatory level	0.836	0.67	0.20-0.89	6.08	0.003
SAMAS global score without anticipatory score	0.916	0.84	0.55-0.95	11.87	<0.001

introduced variability due to different criteria from non-professional observers, the IRR for the global SA was also computed excluding the anticipatory SA subscores, resulting in a better ICC (0.84) *(see Table 3)*.

DISCUSSION

As it is well known, ISA is the first obstacle to neurorehabilitation and in many cases it long-lasts, thus dramatically hampering not only the quality of life of the patients with sABI, but even more that of their family system [14, 36-42]. Accordingly, an early and accurate diagnosis of ISA allows for better addressing the ISA treatment and reducing the possible primary and secondary implications of this disturbance.

The aim of the present study was to evaluate the IRR of the SAMAS, a new tool recently developed to assess declarative, anticipatory, and emergent dimensions of SA in persons with sABI. The SAMAS has already proved [29] to be able to assess specifically and broadly not only declarative but also emergent and (actual) anticipatory SA, thus overcoming the lack of tools in measuring the last two levels.

The present study revealed an overall moderate-to-excellent agreement between the blind raters for each of the four couples of professionals. Therefore, in line with the first study on the psychometric properties of the SAMAS [29], the present study showed good results in terms of IRR of the scale, thus increasing the strength of the scale.

Admittedly, as for anticipatory SA, the Cronbach's Alpha and ICC indexes were sensibly lower than those related to declarative and emergent SA. This result may be due to the higher complexity in assessing anticipatory SA with respect to the other SA levels. Indeed, as above mentioned, the assessment of this level requires not only an online assessment (i.e., in the presence of the patients, as for the assessment of declarative and emergent SA) but even an offline assessment aimed at verifying, by mean of other significant reports, what was previously stated by the patients at a merely declarative level (see [29] for details). This further investigation could likely enhance the variability of the data collected. In particular, the main witnesses involved in the assessment of the anticipatory SA are usually the informal caregivers, who in many cases can show psychological defence mechanisms (such as denial) [37], related to a poor acceptance of the dramatic functional changes that occurred in their loved-one. Therefore, in assess-

ing this level of SA, caution is needed through an accurate clinical observation of the caregivers themselves, to evaluate their viewpoint as an external confirmation of what is reported by the patients.

However, the present study confirmed the utility of the SAMAS as an innovative and comprehensive measure of SA that, if supported by careful interviews with other members of the neurorehabilitation team (e.g., occupational therapists, speech therapists, physiotherapists) and the informal caregivers, could help the neuropsychologist and the cognitive-behavioural therapists in assessing at best one of the main obstacles in rehabilitation, that is ISA.

We acknowledge that the present study presents with some limitations. First, the small sample size requires caution in considering data on the SAMAS IRR as definitive. Indeed, we considered our results preliminary and further studies with larger samples of patients are necessary to corroborate them. However, we would also underline that despite the small sample size, thanks to our inclusion criteria (i.e., LCF scores ≥6) this group of patients likely represents the overall population of patients with full recovery of consciousness, admitted to the Post-Coma Unit of Fondazione Santa Lucia.

Second, we tested the IRR of the SAMAS on four professionals, but each patient has not been evaluated by all the possible combinations of couples of raters, as in a fully crossed design. Nevertheless, to reduce such variability, we designed the data collection by ensuring that each of the four couples of professionals could rate the same patients at least in three different cases. A limit of the scale is that none of the studies conducted to date on the SAMAS assessed the intra-rater reliability. Therefore, the use of the scale requires caution, until further studies will confirm the psychometric qualities of the SAMAS. Finally, it must be reminded here that we administered an Italian version of the SAMAS. Further studies, possibly conducted in other countries on the same population of patients are needed to test the cross-cultural validation of the SAMAS.

Despite the limitations above, we would underline the usefulness of utilizing SAMAS to assess all levels of SA in neurorehabilitation, particularly with respect to the emergent and declarative SA.

CONCLUSIONS

Given the lack to date of specific tools capable of assessing emergent and anticipatory SA (which are as rel-

evant as the declarative level for a successful therapy), the SAMAS is the result of our attempt to help neurore-habilitation professionals quantitatively describing what they usually observe in the neurorehabilitation setting. Unfortunately, as above mentioned, the scale presents still with several limits and, accordingly, requires further validation studies, conducted on larger samples.

Nevertheless, the SAMAS can be considered a useful measure of SA, since it has been conceived within a ho-listic perspective, which implies a combined use of clini-cal observation, interviews, and scales, in order to ob-tain an early and accurate diagnosis of ISA [29], thanks to a multi-disciplinary and inter-professional teamwork. Indeed, we would point out here that the SAMAS re-quires to be completed within the context of accurate clinical observation, as well as that it also needs an ac-curate interview with the informal caregivers (to verify their compliance and credibility as external witnesses on what is reported by the patients) and the other mem-bers of the inter-professional neurorehabilitation team.

The present study confirmed that this scale can be utilized in neurorehabilitation as a useful measure to specifically assess SA, since to our knowledge it remains to date the only tool in the literature that also allows the assessment of emergent and actual anticipatory SA.

Author's contribution

Conflicts of interest statement

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Recognition of early warning signs and symptoms – the first steps on the road to Autism Spectrum Disorder diagnosis

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Abstract
Objective. To identify developmental symptoms reported at firsts doctor visits by parents of children later diagnosed with Autism Spectrum Disorder (ASD).
Design. Cohort study.
Setting. The study was conducted in the Department of Psychiatry Research of "Prof. Dr. Alexandru Obregia" Clinical Psychiatry Hospital from Bucharest between September 2019 and May 2021.
Patients. 105 cases: 82 boys and 23 girls, 100 children with autism, and 5 patients with Asperger's syndrome.
Intervention. ASD was diagnosed according to the DSM-5 criteria, ADOS-1 (Autism Diagnostic Observation Schedule, 1st Edition) and/or ADI-R (Autism Diagnostic Interview-Revised) tests scores; features reported by the parents for which they presented to the doctor for a diagnosis were taken into consideration.
Main outcome measures. The age at first presentation to the doctor; the most common early signs reported by the parents of children with ASD.
Results. The age at first presentation to the doctor in our group was between 9 months and 14 years. The most common early signs reported by parents were: delayed language development, deficits in understanding verbal instructions/indications, and hyperactivity and aggressivity. In the case of patients with Asperger's syndrome, the reported features were hyperactivity and aggressivity, learning difficulties, and social interaction problems. Regression and delay in language development occurred more often in boys than in girls.
Conclusions. Parents, as well as family doctors or paediatricians, should pay great attention to the children's behaviour, alongside their cognitive and language development. Early detection is essential for early intervention and our results can be used to develop training programs for parents and paediatricians for early recognition of ASD.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a heterogeneous group of disorders characterized by a specific combination of social relationships and communication deficiencies, repetitive behaviours, restricted interests, and/or atypical sensory behaviours, with onset in early childhood [1]. The global prevalence of autism is now less than 1%, but estimates are higher in high-income countries [2]. The latest Community Report on Autism from the CDC (Centers for Disease Control and Prevention) states that 1 in 44 (2.3%) 8-year-old children were identified with ASD in 2018, a considerably higher figure compared to previous data [3, 4]. Among the mental health disorders specific to the pediatric population, ASD have been and are among the most studied, the definition, criteria, and methods of

diagnosis changing very much over time. In Romania, so far, there is little data on children with ASD. From a historical perspective, the first depictions of autism spectrum traits in a group of children with disabilities have been mentioned by Rutter *et al.* which described "quasi-autistic models" in the well-known study on a group of institutionalized children in Romania [5, 6]. In another study that included children from institutions in Romania, Levin *et al.* demonstrated that early institutionalized education was associated with an increased risk of social communication difficulties and ASD [7]. Regarding the prevalence of ASD in Romania, so far, the research is limited [8]. In a study published in 2017, Budişteanu *et al.* identified 14.3% of children with ASD in a group of students aged 7-9 - 9,135 students from 122 regular schools and 95 special needs schools [9]. The increasing prevalence of Autism Spectrum Disorders (ASDs), together with more widespread media coverage on the subject has led to a greater number of parents paying more attention to their children's developmental milestones and expression of possible ASD symptoms and thus, presenting these concerns to their GPs or paediatricians earlier than before. Usually, ASD is diagnosed around the ages of 3-4 years [10]. Nevertheless, an increasing number of parents have started to present their concerns to their GP or paediatrician by the 18-months age of their children [11, 12]. The early recognition of the features specific for ASD is very important to apply an early intervention. In the diagnostic process, the role of the GP/paediatrician – the family's primary contact with the health system, is essential for a child who might be diagnosed with ASD. The diagnosis of autism is based on behaviours identified in the child at the time of presentation to the specialist. Although there is substantial heterogeneity between and within individuals throughout development, a set of basic diagnostic features of ASD (regarding social interaction, communication and restricted, repetitive behaviours) can be reliably identified by trained clinicians [13, 14]. Most importantly, early identification of ASD is essential for early access to targeted, evidence-based interventions that contribute to the improvement of long-term outcomes, with the paediatrician's early identification of these symptoms being the pivotal start of a hopefully-not-to-elaborate diagnostic process [15].

In this paper we will present our experience in the Department of Psychiatry Research at "Prof. Dr. Alexandru Obregia" Clinical Psychiatry Hospital in Bucharest, Romania, regarding children's developmental symptoms that concerned parents/caregivers report to their doctors which are actually early signs of ASD. Our intent is to provide useful information for GPs and paediatricians and raise awareness that certain developmental delays could be early ASD symptoms, in order to contribute to faster recognition of the disorder that cand lead to shorter diagnostic journeys for these families.

MATERIAL AND METHODS

The study was conducted in the Department of Psychiatry Research of "Prof. Dr. Alexandru Obregia" Clinical Psychiatry Hospital from Bucharest between September 2019 and May 2021 and the inclusion criteria were: children diagnosed with ASD according to the DSM-5 criteria, and together with the ADOS-1 (Autism Diagnostic Observation Schedule, 1st Edition) [13] and/or ADI-R (Autism Diagnostic Interview-Revised) scores, as well as the agreement of the legal representative to participate in the study, expressed by signing the informed consent. To identify the parents' concerns and the signs that they observed in their children at the beginning of their evaluation route, as well as their children's symptoms and history, we conducted structured clinical interviews with both parents or caregivers. In all children, we noted the gender and the age of the child, the age at diagnosis, and the symptoms reported by the parents for which they presented to the doctor for a diagnosis. Our purpose was to identify those symptoms which are the first that get the parents' attention when it comes to their child's development, be them a concern related to a possible ASD diagnosis or not. We wish to underline the fact that there are cases in which parents express worry towards symptoms that could be early ASD signs (such as delay in language development, deficits in understanding verbal instructions/indications, hyperactivity and aggressivity) but are sometimes overlooked in clinical settings and are not referred to a specialist for further evaluation, and thus an ASD diagnosis is sometimes missed or delayed, which consequently delays the start of the therapeutic intervention.

For statistical analysis we used IBM SPSS 22 statistical analysis program, JASP 0.14.1.0 and JAMOVI 1.6.21.0 software, Mann Whitney U Test and Fisher's Exact Test for mean difference. We used the confidence interval 95% CI and the statistical significance is accomplished for $p<0.05$. In addition, statistical significance is obtained when the p-value of the all tests is less than 0.05.

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of "Prof. Dr. Alexandru Obregia" Clinical Psychiatry Hospital (Protocol approval N. 33/26.11.2019).

The written informed consent was obtained from all subjects before participating in these studies.

RESULTS

The study included 105 patients (82 boys, 23 girls). The age interval of the children at the time of inclusion in the study was 9 months to 15 years (mean age=5.5 years/66.15 months, and a standard deviation (SD) = 3.63 years/43.62 months). The age at which the parents observed first manifestations and referred the child to a doctor for a diagnosis was between 9 months and 14 years, with a mean of 30 months and a standard deviation of 2.5 years/29 months.

In 95 cases the parents reported as the main reason for presenting to the doctor a delay in language development; in 49 children, deficits in understanding of orders; in 37 cases, hyperactivity and aggressivity; in 31 cases, lack of eye contact; in 23 children, repetitive behaviours/movements; and 20 children, lack of functional play (*Table 1*).

Table 1
Parents' expressed concerns in relation to diagnosis

Parents' expressed concerns	ASD	Asperger's Syndrome
Delay in language development		
Absent	5	5
Present	95	0
Deficits in understanding verbal instructions/indications		
Absent	51	5
Present	49	0
Hyperactivity and aggressivity		
Absent	63	4
Present	37	1
Lack of eye contact		
Absent	69	5
Present	31	0
Repetitive behaviours/movements		
Absent	77	5
Present	23	0
Lack of functional play		
Absent	80	5
Present	20	0
Does not point the finger		
Absent	95	5
Present	5	0
Regression		
Absent	87	4
Present	13	1
Learning difficulties		
Absent	98	3
Present	2	2
Difficulties in social interaction		
Absent	84	2
Present	16	3

ASD: Autism Spectrum Disorder.

Regarding the ASD type, 100 children had ASD and 5 patients were diagnosed with Asperger's Syndrome (technically no longer a diagnosis on its own, and called now a high-functioning type of ASD). If in the case of children with Asperger's Syndrome the most common early signs were hyperactivity and aggressivity, learning difficulties, and social interaction problems, in the case of children with ASD the most common early signs were delayed language development and deficits in understanding of orders (*Table 1*).

Thirty-eight patients with ASD were diagnosed within 9-24 months (30 boys and 8 girls), 50 patients with ASD were diagnosed within 25-48 months (36 boys and 14 girls), 11 patients with ASD were diagnosis between 49-72 months (10 boys and one girl), and one boy was diagnosed with ASD at the age of 73 months (*Table 2*).

Regarding the differences in the early signs in terms of age category at which the child with ASD was diagnosed, significant differences were found only in terms of delay in language development, deficits in eye contact, non-responsiveness, hyperactivity and aggression, learning difficulties and deficits in social interaction, p<0.05 (*Table 3*).

The deficits in understanding verbal instructions/indications (non-responsiveness) are reported by most parents of children with the age at diagnosis between 9 months and 2 years (26 parents, 20 boys and 6 girls), and the delay in language development is reported by the parents of the children with the age at diagnosis between 2 and 4 years (49 of the parents, 36 boys and 13 girls) (*Table 2*).

The lack of eye contact is a statistically significant early sign reported by the parents, it is reported in most children (16 parents out of 38, 12 boys and 4 girls) with age at diagnosis between 9 months and 2 years (*Table 3*).

Other statistically significant factors are hyperactivity and aggressivity, difficulties in social interaction and learning difficulties, although they were reported by most parents (22 patients, 5 and 2, respectively) in their child's 2-4 age range and 4-6 age (*Table 2 and 3*).

Lack of functional play and regression was reported by the parents in only 9 and 7 children, respectively, with age at diagnosis between 9 months and 2 years, but these factors are not statistically significant (*Table 2*).

The most common early signs for the age group 49-72 months were: delay in speech development, repetitive behaviours/movements, and difficulties in social interaction (*Table 2*). At this age, most social interactions occur, and deficits in verbal language and social behaviors are the easiest to identify.

For children with ASD older than 73 months, the most common early signs were deficits in understanding of orders, delayed language development, social interaction difficulties, and learning difficulties. The child goes to school and parents and teachers can see discrepancies between the typical development of a child and that of ASD.

There are statistically significant differences between boys and girls in terms of language delay and lack in sense game. In the case of boys, these signs were reported by the parents more often than in the case of girls (*Table 2 and 4*). Lack of eye contact, deficits in understanding of orders, and repetitive behaviour/movements were also reported by the parents more frequently in boys than in girls, but this difference was not statistically significant.

DISCUSSIONS

In our study the mean age of diagnosis of ASD was 30 months, comparing with the age of around 24 months reported by the majority of previous studies [16-18]. As the age interval of our study group was 9 months to 15 years, we would argue that the 30 months mean age of diagnosis in the lot was influenced by the higher ages of the few older patients that we included in the study. A meta-analysis of studies published between 2012-2019

Table 2
Frequencies of early signs in children with ASD depending on the age of diagnosis and their gender

Diagnostic age			Delay in language development		
			Absent	Present	Total
9-24 months	Gender	Boy	1	29	30
		Girl	2	6	8
		Total	3	35	38
25-48 months	Gender	Boy	0	36	36
		Girl	1	13	14
		Total	1	49	50
49-72 months	Gender	Boy	0	10	10
		Girl	0	1	1
		Total		11	11
≥73 months	Gender	Boy	1	0	1
		Total	1	0	1
Total	Gender	Boy	2	75	77
		Girl	3	20	23
		Total	5	95	100

			Non responsiveness		
			Absent	Present	Total
9-24 months	Gender	Boy	10	20	30
		Girl	2	6	8
		Total	12	26	38
25-48 months	Gender	Boy	19	17	36
		Girl	9	5	14
		Total	28	22	50
49-72 months	Gender	Boy	9	1	10
		Girl	1	0	1
		Total	10	1	11
≥73 months	Gender	Boy	1	0	1
		Total	1	0	1
Total	Gender	Boy	39	38	77
		Girl	12	11	23
		Total	51	49	100

			Lack of sense game		
			Absent	Present	Total
9-24 months	Gender	Boy	22	8	30
		Girl	7	1	8
		Total	29	9	38
25-48 months	Gender	Boy	27	9	36
		Girl	13	1	14
		Total	40	10	50
49-72 months	Gender	Boy	9	1	10
		Girl	1	0	1
		Total	10	1	11
≥73 months	Gender	Boy	1	0	1

Table 2
Continued

	Total		1	0	1
Total	Gender	Boy	59	18	77
		Girl	21	2	23
		Total	80	20	100

			Stereotypes		
			Absent	Present	Total
9-24 months	Gender	Boy	25	5	30
		Girl	7	1	8
		Total	32	6	38
25-48 months	Gender	Boy	29	7	36
		Girl	9	5	14
		Total	38	12	50
49-72 months	Gender	Boy	6	4	10
		Girl	0	1	1
		Total	6	5	11
≥73 months	Gender	Boy	1		1
		Total	1		1
Total	Gender	Boy	61	16	77
		Girl	16	7	23
		Total	77	23	100

			Lack of imitation		
			Absent	Present	Total
9-24 months	Gender	Boy	28	2	30
		Girl	7	1	8
		Total	35	3	38
25-48 months	Gender	Boy	36	0	36
		Girl	14	0	14
		Total	50	0	50
49-72 months	Gender	Boy	10	0	10
		Girl	1	0	1
		Total	11	0	11
≥73 months	Gender	Boy	1	0	1
		Total	1	0	1
Total	Gender	Boy	75	2	77
		Girl	22	1	23
		Total	97	3	100

			Does not point the finger		
			Absent	Present	Total
9-24 months	Gender	Boy	28	2	30
		Girl	6	2	8
		Total	34	4	38
25-48 months	Gender	Boy	35	1	36
		Girl	14	0	14
		Total	49	1	50
49-72 months	Gender	Boy	10	0	10

Table 2
Continued

			Absent	Present	Total
		Girl	1	0	1
	Total		11	0	11
≥73 months	Gender	Boy	1	0	1
	Total		1	0	1
Total	Gender	Boy	74	3	77
		Girl	21	2	23
	Total		95	5	100

Lack of eye contact

			Absent	Present	Total
9-24 months	Gender	Boy	18	12	30
		Girl	4	4	8
	Total		22	16	38
25-48 months	Gender	Boy	25	11	36
		Girl	11	3	14
	Total		36	14	50
49-72 months	Gender	Boy	9	1	10
		Girl	1	0	1
	Total		10	1	11
≥73 months	Gender	Boy	1		1
	Total		1		1
Total	Gender	Boy	53	24	77
		Girl	16	7	23
	Total		69	31	100

Hyperactivity and aggression

			Absent	Present	Total
9-24 months	Gender	Boy	22	8	30
		Girl	7	1	8
	Total		29	9	38
25-48 months	Gender	Boy	21	15	36
		Girl	7	7	14
	Total		28	22	50
49-72 months	Gender	Boy	4	6	10
		Girl	1	0	1
	Total		5	6	11
≥73 months	Gender	Boy	1	0	1
	Total		1	0	1
Total	Gender	Boy	48	29	77
		Girl	15	8	23
	Total		63	37	100

Regress in language

			Absent	Present	Total
9-24 months	Gender	Boy	23	7	30
		Girl	8	0	8
	Total		31	7	38

Continues

Table 2
Continued

			Absent	Present	Total
25-48 months	Gender	Boy	32	4	36
		Girl	14	0	14
	Total		46	4	50
49-72 months	Gender	Boy	8	2	10
		Girl	1	0	1
	Total		9	2	11
≥73 months	Gender	Boy	1	0	1
	Total		1	0	1
Total	Gender	Boy	64	13	77
		Girl	23	0	23
	Total		87	13	100

Difficulties of social interaction

			Absent	Present	Total
9-24 months	Gender	Boy	26	4	30
		Girl	6	2	8
	Total		32	6	38
25-48 months	Gender	Boy	31	5	36
		Girl	14	0	14
	Total		45	5	50
49-72 months	Gender	Boy	7	3	10
		Girl	0	1	1
	Total		7	4	11
≥73 months	Gender	Boy		1	1
	Total			1	1
Total	Gender	Boy	64	13	77
		Girl	20	3	23
	Total		84	16	100

Learning difficulties

			Absent	Present	Total
9-24 months	Gender	Boy	30	0	30
		Girl	8	0	8
	Total		38	0	38
25-48 months	Gender	Boy	36	0	36
		Girl	14	0	14
	Total		50	0	50
49-72 months	Gender	Boy	9	1	10
		Girl	1	0	1
	Total		10	1	11
≥73 months	Gender	Boy	0	1	1
	Total		0	1	1
Total	Gender	Boy	75	2	77
		Girl	23	0	23
	Total		98	2	100

ASD: Autism Spectrum Disorder.

Table 3
Fisher's Exact Test for the difference between the age categories diagnosed with ASD and the early signs

	Value	Exact Sig. (2-sided)
F delay in language development	8.654	0.031
F non-responsiveness	14.364	0.010
F lack of sense game	1.38	0.677
F stereotypes	4.49	0.191
F lack of imitation	5.66	0.162
F does not point of finger	4.31	0.234
F lack of eye contact	6.91	0.018
F hyperactivity and aggression	5.98	0.041
F regress in language	3.27	0.337
F difficulties in social interaction	8.25	0.025
F learning difficulties	13.15	0.002

Table 4
Mann-Whitney U test for differences between gender in terms of early signs

	W	df	p
Delay in language development	978.000		0.041
Non responsiveness	899.000		0.902
Lack of sense game	1015.500		0.033
Stereotypes	800.000		0.340
Lack of imitation	870.000		0.678
Does not point the finger	843.000		0.362
Lack of eye contact	892.000		0.951
Hyperactivity and aggression	911.000		0.807
Regress in language	NaN[a]		
Difficulties of social interaction	919.500		0.666
Learning difficulties	NaN[b]		

W: Mann-Whitney U test.
[a]The variance in regress in language is equal to 0 after grouping on gender.
[b]The variance in learning difficulties is equal to 0 after grouping on gender.

reports 60.48 months mean age at ASD diagnosis, with a range of 30.90-234.57 months, underlining the prioritization of early symptoms description and detection [19].

The most common concern expressed by caregivers at first presentation to the family doctor's office was represented by speech delay, followed by deficits in understanding instructions/indications, hyperactivity and aggressivity. More specific ASD signs such as lack of eye contact, repetitive behaviours/movements, and lack of functional play were reported by some parents at first presentation, however not as frequent as language development delays. These data are similar with some previous studies, which indicate that parents of children who had been subsequently diagnosed with ASD most frequently express concerns regarding language delay and communication difficulties [20-24]; however, the reported social interaction difficulties identified in

other papers [20, 25, 26] were not expressed similarly frequent by the parents in our group. This brings to light the fact that, in certain cases, cardinal ASD symptoms such as social interaction deficits are overlooked by caregivers or even GPs, especially if more evident symptoms such as absent or delayed language are not present and thus the risk of delayed presentation to a specialized evaluation can occur.

Detecting early signs of autism in young children can be difficult. Some of these signs, such as slight delays in expressive language development, occasional repetitive movements or narrow symbolic play abilities can be observed in neurotypical children, as some developmental variations are physiological [27]. However, children with ASD express these behaviours more frequently and at higher intensities, with disruption of daily functionality.

Families' first contact with a specialist in childcare is the GP/paediatrician, these clinicians having a significant contribution to the child's progress and future prognosis, as they are the ones who subsequently refer patients to a specialized evaluation if they observe atypical features in their young patients. Our study's main purpose is to underline the fact that features such as the three most common concerns (speech delay, deficits in understanding instructions/indications, hyperactivity) that parents in our group expressed at first presentation at their family doctor's office, although not solely specific to ASD, should be taken into consideration in the GPs or paediatricians everyday clinical practice as an indicator for a possible neurodevelopmental disorder and thus the family should be directed to a specialized evaluation. Thus, if a diagnosis of ASD would be established, doing this sooner rather than later is of utmost importance for better prospects. Furthermore, as medical comorbidities are highly prevalent among children and adolescents with ASD, with 8-25% of these patients being diagnosed at some point in their lives, with a coexisting medical condition [28, 29], the presence of these comorbidities may delay, mask or complicate early diagnosis of ASD. Considering this, clinicians should keep in mind to check for ASD signs at regular visits, as well as when the child is brought for different somatic symptoms.

Early indicators of ASD are represented by: developmental deficits or delays in the areas of shared attention, symbolic play, affective behaviour, decrease/absence of name response, decrease/absence of imitative behaviour, verbal and nonverbal communication delay, motor development delay, repetitive behaviours, atypical sensory reactivity, inflexibility of visual attention and extreme variations of temper [30, 31]. At any age, the parents or caregivers should be concerned if they observe: loss of previously acquired skills, difficulties in maintaining eye contact, preference for spending time alone, persistent repetition of words or phrases (echolalia), difficulties in accepting minor changes in routine, restricted interests, repetitive behaviours/movements (hand-flapping, rocking back and forth, twirling), unusual and intense reactions to sounds, smells, tastes, textures, lights and/or colours [27, 30, 31]. However, in the group we analysed, these symptoms were not the ones which were the most reported

by parents at first presentation. As they were mainly concerned by expressive and receptive language difficulties, our study shows that these signs must not be ignored at usual check-ups in the GPs office and, if observed, the child should be directed to a mental health specialist for detailed evaluation. The reason why we want to stress this out is that in spite of intense scientific and public activity that involved ASD awareness and the development of ASD screening instruments, in our country, there is still a lot to be done in this area, as information does not seem to reach as many GPs and paediatricians as it should and ASD diagnosis is still delayed, as we observed in our study. One study that analysed the difficulties Romanian GPs have in identifying early signs of ASD was published by Rahbar *et al.* in 2021 and showed that although the physicians in their study group had basic information on ASD, a deficiency in knowledge about some aspects of ASD were identified as well and the authors indicate the need for "relevant supplementary educational activities" [32]. Our study could thus be a starting point of such educational activities, as it offers concrete evidence of what medical practitioners should pay attention to at routine check-ups, whether caregivers express concern or not, as subtle early ASD signs can be easily overlooked or considered part of normal development.

Previous studies do not point to a single behavioural sign or developmental trajectory that predicts all diagnostic forms of the autism spectrum. Given the heterogeneity of clinical expression of ASD, it is unlikely that a single behaviour will be found universally in all children or serve as a defining marker for later emerging ASD. Thus, assessment needs to be multidisciplinary, taking into account age-specific neurodevelopmental characteristics. According to the NICE Clinical Guideline, local autism teams led by a professional should be set up, in order to set the right strategy for the identification, evaluation and diagnosis of children with ASD, within 3 months after referral from the GP [33]. The SIGN Clinical Guideline underlines the importance of referral for professional assessment being done based on clinical characteristics identified in certain patients during child health monitoring, prioritizing this over screening instruments. At the same time, parents are encouraged to proceed for further evaluation if their concerns regarding their children's development are not properly addressed at routine health check-ups [34]. Available guidelines from The American Academy of Paediatrics, recommend that ASD screening with specific instruments be done in the GP/paediatrician's office at 6, 12, 18, 24 and 36 months of age [35]. Similar recommendations are available on a national level in Romania, where family doctors are indicated to make use of a screening instrument, developed under the National Programme for Early Identification of ASD, and evaluate children for possible autism signs at 12, 18, 24 and 36 months [36].

As per these recommendations by The American Academy of Pediatrics, GPs should use ASD screening tools habitually at primary care visits; however, recent studies have shown that these tools are infrequently used [31] and thus we underline the importance of the clinical evaluation and timely recognition of ASD symptoms by family doctors.

LIMITATIONS OF THE STUDY

We acknowledge that for this study the patient sample we selected might not be representative for all children diagnosed with ASD in Romania, as they were not randomly selected and, additionally, the sample size might not be considered significant. Instead, we collected data from patients who addressed our clinic in "Prof. Dr. Alexandru Obregia" Psychiatry Hospital, after referral from other services or by parents' choice. The results may also be influenced by the nonresponse bias for some questions. The data collecting method by clinical structures interview could have some limitations as well, taken into consideration the degree of subjectiveness of responders and the recall bias, as our study partially depended on caregiver-reported data.

CONCLUSION

ASD is a complex neurodevelopmental condition with an increasing frequency worldwide. The importance of the parents' expression of concerns regarding their children's development and the first and timely evaluation of these concerns in the GP'S office lies in the fact that early identification of possible ASD symptoms could lead to well-timed diagnosis and prompt intervention with better outcomes. Developmental symptoms described by the parents in our group at first presentation such as speech delay, deficits in understanding instructions/indications, hyperactivity should alarm the GP from the start as in some cases, although not specific to ASD, these symptoms could be early autistic traits. Our intention is to raise awareness among clinicians about the overlap of neurodevelopmental disorders-specific symptoms in early ages so that children that have such difficulties are directed to specialized evaluation. The need to raise awareness on this matter in our country raises from the fact that, although there are already numerous studies which describe ASD early signs recognition by caregivers, in Romania there are still GPs and/or pediatricians who don't consider symptoms such as language delay a significant developmental problem and thus refrain from recommending an evaluation by a mental health specialist and delay the confirmation of diagnoses such as ASD. We consider that a study on a Romanian group of children could have a greater informational impact and our results could be used to enrich training programs for family doctors or pediatricians for early recognition of ASD, such as their residency training during their rotations in mental health departments. Finally, as the medical and social burden associated with this diagnosis is constantly increasing, our aim is to contribute and act as starting point for future research in our country that should contribute to the development of comprehensive, multidisciplinary services, provided by general practitioners and pediatricians together with mental health specialists.

Authors' contributions

MB, FIL and LEA wrote the manuscript. MB, FIL, LEA, LM, AG, DI, and FR evaluated it; LM, AG, ID, ES, and FR contributed to acquisition, analysis, or interpretation of data; all Authors critically revised the manuscript for important intellectual content and approved the final version for submission.

Conflict of interest statement

The Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Original articles and reviews

The Italian fund for Alzheimer's and other dementias: strategies and objectives to face the dementia challenge

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Abstract
The Italian Fund for Alzheimer's and other dementias was approved and signed in December 2021. The Fund is financed with 15 million euros in three years. The main goal is to provide new strategies in the field of dementia with a Public Health perspective. The Fund includes eight main activities that will be monitored and supervised by the Italian National Institute of Health: 1) development of a guideline for the assessment, management and support for people with dementia and their families/carers; 2) updating of the Dementia National Plan (DNP); 3) implementation of the documents of the DNP; 4) conducting surveys dedicated to the Italian Dementia Services; 5) promotion of dementia prevention strategies; 6) training strategies for healthcare professionals, families and caregivers; 7) creation of a National Electronic Record for Dementia; 8) evaluation and monitoring of activities promoted by Regions and Autonomous Provinces in the field of dementia, together with the dementia National Permanent Table. These activities are outlined in detail in the present paper.

INTRODUCTION

Dementia represents a global challenge for public health [1]. Age is the main risk factor for dementia and it was estimated that nearly 14 million elderly individuals (age >65) live in Italy [2]. Around 3 million people are directly or indirectly involved in dementia care, and 1.2 million are the individuals with dementia [3]. In Italy, the social and healthcare costs for dementia care are estimated at around 10-12 billion euros per year [4].

Several international public health initiatives world-wide urged countries to develop and/or implement their national dementia plans to place structural, financial, and human resources for giving adequate support and care management for people with dementia [5].

While several research and public health initiatives are noteworthy in the field of dementia, relevant issues still negatively affect the management of dementia care in Italy. Indeed, strengthening interventions are needed to improve every aspect of dementia care management. In contrast, the different aspects of dementia management are still addressed separately, thus significantly

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limiting the possibility of actually implementing an integrated approach to dementia.

The Ministry of Health (MoH) in close cooperation with the Italian National Institute of Health (Istituto Superiore di Sanità, ISS), the Regions, and the three major National families and patients associations (www.alzheimer.it, www.alzheimer-aima.it, www.alzheim-erunitiitalia.it) approved the Italian Dementia National Plan (DNP) in 2014, also to address some of the mentioned issues, which were already under observation [6].

More recently, the law 30 December 2020 n. 178, article 1, paragraph 330, established the Alzheimer's and other dementias Fund, with funding of 5,000,000 euro annually for a three-year plan (2021-2023). The Fund's Decree, concerning specific regulations, was approved by the State-Regions Conference on December 2, 2021, and signed by the MoH and Ministry of Economy (MoE) on December 23, 2021 [7].

To guarantee the monitoring and implementation of the DNP and related documents produced by the Permanent National Table (NPT) for dementias coordinated by the MoH, the decree establishes the criteria and methods for the use of the Fund.

In application of the DNP and according to the law no. 178 of 2020, Regions and Autonomous Provinces (APs) will develop three-year action plans, to address specific critical areas in the diagnosis and care of patients with dementia through experimental and innovative solutions. Five main research areas of interest have been identified (*Table 1*). The ISS, as a component of the Permanent National Table for dementias, will not only support the definition of the three-year action plans of the Regions and APs but will also contribute to their monitoring and implementation. The eight main activities, indicated by the Plan, will be briefly described below and in the *Table 1S* available online as Supplementary Material.

ACTIVITY 1 - TO DEVELOP A GUIDELINE FOR THE ASSESSMENT, MANAGEMENT AND SUPPORT FOR PEOPLE WITH DEMENTIA AND THEIR FAMILIES/CARERS

The MoH, with the support of the ISS, provides the development of a guideline on the diagnosis and treatment of patients with dementia within the National Guidelines System (SNLG), based on the best scientific evidence of the physiopathology and treatment options and good clinical practices on dementia. All relevant National institutions will be involved as part-

ners or stakeholders together with patients and families associations and a number of scientific societies.

The guideline will offer a quality assessment of the most relevant and high-quality scientific literature and an evaluation of the benefits/risks assessment of pharmacological and non-pharmacological treatments for dementia. Based on the methodology proposed by the *NGD Methodological Manual* [8], an adaptation and update of the document NG97 published by the National Institute for Health and Care Excellence (NICE) from the UK in 2018 will be proposed.

ACTIVITY 2 - UPDATE OF THE ITALIAN DNP

The MoH, with the support of the ISS and the dementia NPT, will update the Italian DNP. The Italian DNP currently in use consists of four actions and 17 related objectives. It will be updated following recent scientific advances in dementia prevention, early diagnosis and the organization and provision of care services. It will be also updated taking into account the global action plan on the public health response to dementia 2017-2025 adopted by the 70th World Health Assembly, which represents the most recent and important international commitment to improve the lives of people with dementia, their caregivers, and their families.

In addition, various questions on dementia will be addressed, from a public health perspective. Some emerging aspects of dementia such as early onset dementia, dementia in migrants and cognitive deficits following COVID-19 will be part of the DNP update.

ACTIVITY 3 - IMPLEMENTATION OF THE DOCUMENTS OF THE DNP

The MoH, through the NPT, and with the technical support of the ISS, has produced the four following documents between 2017 and 2020 [9]:

a. *National Guidance on diagnostic and therapeutic care pathways for dementias*
b. *National Guidance on the use of information systems to characterize dementia*
c. *National Guidance for Building Dementia Friendly Communities*
d. *Recommendations for the governance and clinical care in the treatment of dementia*

Objective of this activity is to ensure that Regions and APs included in their local planning the recommendations coming from these documents, at the same time evaluating their actual implementations.

Table 1
Five areas of interest in the diagnosis and care of patients with dementia to implement research in public health with experimental or observational study designs in order to increase scientific knowledge

Improving the early diagnosis of Minor Neurocognitive Disorder/Mild Cognitive Impairment and developing a prediction model of conversion to dementia that would be useful for clinical practice
Timely diagnosis of the Major Neurocognitive Disorder
Experimenting, evaluating and disseminating telemedicine interventions to ensure continuity of care in different dementia care settings
Experimenting, evaluating and disseminating of tele-rehabilitation to provide rehabilitation models that guarantee a better participation, inclusion, and quality of life of patients with dementia and their families/caregivers
Experimenting, evaluating and disseminating of psycho-educational, psycho-social, and cognitive interventions in patients with dementia

ACTIVITY 4 - SURVEYS DEDICATED TO THE DEMENTIA SERVICES

Three surveys will be carried out in the main health facilities for the treatment and management of dementia located in Italy: Centres for Cognitive Disorders and Dementias (CCDDs), nursing homes (NHs), and dementia day centres (DDCs). Previously, between 2014 and 2018, three surveys were conducted to characterize dementia services in Italy, obtaining an online map of dementia facilities available on the "Dementia Observatory" website [10]. An update of the online map appears urgent, also considering new scenarios in the organization of health services due to the future impact of the possible approval of new drugs, at European and Italian level.

Representatives from all Regions and APs will be called to participate in updating all dementia service listings. A questionnaire will be addressed to all dementia services with the aim of developing structure, process, and outcome indicators to supervise the levels of assistance and care management in all Italian territories. The results from the new surveys will allow to have an update of all structural and human resources highlighting the disparities between different Regions and territories in the field of prevention, diagnosis, management and pharmacological, cognitive, and psychosocial treatments.

ACTIVITY 5 - PROMOTE PRIMARY AND SECONDARY PREVENTION STRATEGIES

Although age is the main and unmodifiable risk factor for dementia, a growing evidence on dementia prevention is now available. Seven potentially modifiable risk factors (low education attainment, diabetes, mid-life hypertension, mid-life obesity, smoking, depression and physical inactivity) were identified in 2014 by the *Lancet Commission* [11]. The number of Alzheimer's disease (cases attributable to potentially modifiable risk factors) has been estimated to be around a third of AD cases worldwide. The collaboration between the Dementia Observatory and the PASSI (Progressi delle Aziende Sanitarie per la Salute in Italia) surveillance system has yield to an overview of the Italian regional distribution on the seven modifiable risk factors, highlighting a huge variability; according to this data, 6.4% and 6.5% case of dementia and vascular dementia, respectively, would be avoidable by reducing of 20% the prevalence of all those factors [12]. Recently, the *Lancet Commission* updated to twelve the modifiable risk factors for dementia, by further including: air pollution, alcohol consumption, hearing impairment, traumatic brain injury, and low social contact [13].

The main goal of this activity is to provide policy makers with the strongest scientific evidence on primary and secondary prevention strategies for dementia. Furthermore, information campaigns must be designed and implemented to facilitate the inclusion of marginalized people and those with specific risk factors. Estimates of cases with dementia and MCI will be defined for each Region and APs. Finally, with the support of the PASSI surveillance system and the statistics of the National Institute of Statistics (ISTAT), the prevalence of the risk factors attributable to dementia in each territory will be assessed. Moreover, a review of the regional prevention plans will be performed for identifying which risk factors related to dementia are targeted. Together, all these activities will promote primary and secondary prevention strategies for dementia, integrating this information within regional plans, the National Plan for Prevention, and the National Plan for Chronic Diseases.

ACTIVITY 6 - EDUCATION AND TRAINING OF HEALTHCARE PROFESSIONALS AND FAMILY MEMBERS OF PATIENTS WITH DEMENTIA

The management of patients with dementia requires the integrated expertise and skills of several specialists in dementia care. In addition, other specialists such as nutritionists, physiotherapists, and social workers are crucial professionals equally involved. However, caregivers and family members still play a key role in managing their loved ones with dementia. In our country, partners and sons still represent the main caregivers of dementia patients. However, recent data show that caregivers are less represented by family members in favour of paid staff [14].

A new National survey on 10.000 caregivers will be conducted to investigate caregivers' conditions in caring for people with dementia and to establish a training path for family members of these patients. Subsequently, focus groups will be conducted to better understand economic and social needs of the families of patients with dementia after the COVID-19 pandemic.

The focus groups and the survey will involve all Italian Regions and will be aimed at allowing the development of appropriate training courses for healthcare professionals involved in the management of dementia and for the families of patients affected by dementia. In particular, three training courses will be carried out: for caregivers, for family members and for healthcare professionals involved in rehabilitation (occupational therapists, psychologists, physiotherapists).

ACTIVITY 7 - CREATION OF A NATIONAL ELECTRONIC RECORD FOR DEMENTIA

The design and implementation of an electronic medical record for data collection of patients with dementia as a part of a National Dementia Information System is the major objective of this activity.

In this project, the electronic medical record will be defined for all Regions and APs and its implementation will be promoted in current clinical practice. The source of these clinical data will be integrated with the current healthcare administrative databases working at regional level and connected with the New Health Information System (NHIS) of the MoH.

In Italy, there are few and different regional experiences in the use of electronic medical records. In Veneto the implementation of the electronic medical record in CCDDs (CaCeDem) has already been promoted while a pilot project on four CCDDs is underway in Lazio.

The National Electronic Record for Dementia and the use of data from NHIS are propaedeutic for a Na-

tional Dementia Information system. In this regard, an algorithm for the identification of cases of dementia by using four sources of data of the NHIS has been validated in four Italian regions [15]. Although a standardized and validated methodology is now easily accessible, this experience showed the opportunity to consider more sources of data of the NHIS, and to replicate the study on a population covering all the spectrum of dementia, form mild to severe forms. Therefore, a validation study is planned with this aims in two Italian Regions, in a primary healthcare setting.

ACTIVITY 8 - EVALUATION AND MONITORING OF THE REGIONAL PLANS/PROJECTS

The ISS Dementia Observatory, as a member of the National Permanent Table, will contribute to the evaluation and monitoring of the interventions provided in the three-year plan as required by article 3 of the Decree of the Ministry signed by the MoH and the MoE on December 23, 2021, and also reported in the technical annex. The evaluation and monitoring of the regional activities will be carried out through the acquisition of half-yearly reports. Each Regional Plan will be assessed and monitored according to the five areas of interest. In addition, the ISS will encourage the collaboration between regions and APs that will submit projects with similar and/or overlapping topics (*Table 1*).

FUTURE PERSPECTIVES

There is a need to implement public health initiatives regarding financial and structural support to services, as well as to plan additional human resources dedicated to dementia. With the aim of implementing and monitoring the Italian DNP, the Fund we have been presenting represents the first Public Health initiative, in Italy, that allocates funding of this magnitude (15 million euro) for dementia. The WHO Global plan on dementia has urged governments to develop national policies on dementia by 2025 [1] and with this specific approach, Italy aligns itself with the standard of other advanced countries. Obviously, since in Italy the costs of dementia have a huge economic impact on the health system, the funding currently allocated is not sufficient to carry out all the actions necessary to implement diagnostic tools and infrastructures, treatment options, and care pathways for patients with dementia. However, this initiative represents the first step towards the achievement of adequate resources capable of addressing the upcoming challenges on dementia. The management of patients with dementia requires a multidisciplinary and multi-professional approach, with continuous collaboration between several healthcare professionals as well as those working in many sectors other than health. This cooperative and integrated approach aims to improve the synergistic effect of the quality of care and, consequently, to improve the quality of life of patients with dementia and their families.

Conflict of interest statement

The Authors have no conflicts of interest to declare.

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Proportionate mortality among workers exposed to hardwood dust in Italy

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ORIGINAL ARTICLES AND REVIEWS

Abstract
Aims. The main aim of this study is to estimate the impact on workers' health caused by hardwood dust exposure in selected industrial sectors in Italy.
Methods. Proportionate mortality ratios (PMR) by cancer site were calculated by linking the information on occupational exposure to carcinogens from the Italian national registry (SIREP, 1996-2018) to the national mortality archives (ISTAT, 2005-2018), assuming a Poisson distribution of the data.
Results. An elevated proportion of deaths from sino-nasal cancer was found in male exposed workers (PMR=4.25, CI 95%=1.37-13.23). Increased proportions of deaths were also found from stomach cancer in both genders (PMR=3.14, CI 95%=1.41-7.00 women; PMR=1.64, CI 95%=1.22-2.19 men).
Conclusions. Although hardwood dust is a known human carcinogen, there are still many high-risk occupational exposure settings. Epidemiological surveillance and continuous monitoring of workers is highly recommended in order to ensure compliance with regulatory obligations and limit the burden of associated mortality causes.

INTRODUCTION

Wood dust is generally classified into two categories: dust from "softwood" which is a wood cheap to produce and easy to work, such as pine; and dust from "hardwood" which is hard heavy wood mostly from deciduous trees as oak, beech, birch, mahogany and others. The International Agency for Research on Cancer (IARC) provides an evaluation of carcinogenicity for wood dust as a whole (both soft and hard), while the European Union (EU) has classified only hardwood dust as a carcinogen (directive no. 1999/38/EC) [1]. The association between sino-nasal cancer and exposure to wood dust is well-known, particularly for adenocarcinoma and exposure to hardwood dust [1]. Sino-nasal cancer is a relatively rare tumour worldwide, with a ratio of mortality rate to incidence rate varying from 0.21 to 0.48 in men and 0.12 to 0.69 in women [2]. Based on literature data, however, other occupational cancers may contribute to the disease burden in workers exposed to wood dust [3]. In particular, many studies have investigated the association of wood dust (both soft and hard) exposure and lung cancer risk, suggesting a possible role for the exposure to hardwood dust [4-6]. Another study observed an effect on laryngeal cancer risk for exposure to wood dust, particularly strong for hardwood (OR=2.6, 95% 1.3-5.2) [7]. Workers exposed in Italy to wood dust (both soft and hard) were first estimated by the WoodEx project

at about 351,000 in the period 2000-2003 [8]. Subsequently, on the basis of the Italian Information System on Occupational Exposure to Carcinogens (SIREP) at 31 December 2011, and only for some specific sectors, workers potentially exposed to hardwood dust in Italy were estimated at around 117,000 [9].

The present study intends to estimate the possible impact of hardwood dust exposure on the health of workers by studying the proportionate mortality in a national registry-based occupational cohort. The analysis was only possible for hardwood dust as the notification of exposed workers is mandatory merely for this type of woods. Indeed, the Italian law specifically classifies hardwood dust as a carcinogen according to EU directive no. 1999/38/EC.

METHODS

Data on exposed workers were collected from the occupational exposure databank (SIREP), a relational database gathering information on exposures to carcinogens in the workplaces. The SIREP system has been fully described elsewhere [10]. In brief, according to the Italian regulation on health surveillance at workplace (Italian Law Decree n. 81/2008), data on exposures to occupational carcinogens have to be collected by employers and regularly sent (every three years) to the SIREP system. Employers are required to report the carcinogens used

or produced during industrial process, data on exposed employees and the exposure levels. The workers reported to the SIREP system in the period from 1996 to 2018 as exposed to hardwood dust were selected. The underlying cause of death of exposed workers was retrieved from the mortality archives of the Italian National Institute for Statistics (ISTAT) for the period 2005-2018 [11]. The number of deaths retrieved was 8,345,104, of which 52% for women. The record linkage was performed using an anonymous worker identification code. Approval from an Ethics Committee was not required as the record linkage was requested by a public institution using an anonymized code. Gender specific proportionate mortality ratios (PMRs), standardized by 5-year age ("20-24", "25-29", …, "85 and over") and 3-year calendar period (except for the last period of only 2-years), were evaluated in order to estimate the relative risk of death from a specific cause among workers exposed to hardwood dust. The 10th International Classification of Diseases (ICD) codes were used to classify the cause of death, and proportional mortality of the whole Italian population was taken as the standard. A Poisson distribution of the data was assumed for calculating the PMRs and 95% confidence intervals (CIs). Sub-analyses by industry, job profession, and cumulative exposure were performed, and the results for the most significant cancer sites found in the main analysis *(see Table 1)* were reported in *Figures 1*, *2* and *Table 2* respectively. Occupational groups were coded using the international standard classification of occupations (ISCO-88) at the

Table 1

Proportionate mortality ratios (PMRs) for hardwood dust exposed workers by gender and cause of death (2005-2018)

Gender	Cause of death (ICD X code)	Observed*	Expected**	PMR	95%	CI
Women	All causes of death	78	78.0	1.00	-	-
	Malignant neoplasm (C00-C97)	56	45.5	1.23	0.95	1.60
	Malignant neoplasm of stomach (C16)	6	1.9	3.14†	1.41	7.00
	Malignant neoplasm of colon (C18)	4	3.0	1.33	0.50	3.55
	Malignant neoplasm of bronchus and lung (C34)	16	6.6	2.42†	1.48	3.95
	Malignant neoplasm of breast (C50)	4	11.6	0.35	0.13	0.92
	Malignant neoplasm of ovary (C56)	3	2.96	1.01	0.32	3.14
	Diseases of the respiratory diseases (J00-J99)	3	2.1	1.44	0.46	4.46
Men	All causes of death	1,157	1,157.0	1.00	-	-
	Infectious and parasitic diseases (A00-B99)	23	36.5	0.63	0.42	0.95
	Malignant neoplasm (C00-C97)	545	462.8	1.18†	1.08	1.28
	Malignant neoplasm of nasopharynx (C11)	3	1.73	1.73	0.56	5.39
	Malignant neoplasm of other and ill-defined sites in the lip, oral cavity and pharynx (C14)	6	1.9	3.24†	1.45	7.22
	Malignant neoplasm of oesophagus (C15)	14	9.6	1.46	0.86	2.47
	Malignant neoplasm of stomach (C16)	45	27.5	1.64†	1.22	2.19
	Malignant neoplasm of colon (C18)	35	33.9	1.03	0.74	1.44
	Malignant neoplasm of rectum (C20)	7	9.3	0.75	0.36	1.58
	Malignant neoplasm of liver and intrahepatic bile ducts (C22)	43	35.3	1.22	0.90	1.64
	Malignant neoplasm of other and unspecified parts of biliary tract (C24)	10	4.7	2.13†	1.14	3.96
	Malignant neoplasm of pancreas (C25)	35	32.5	1.08	0.77	1.50
	Malignant neoplasm of other and ill-defined digestive organs (C26)	12	4.7	2.55†	1.45	4.50
	Malignant neoplasm of nasal cavity and of accessory sinuses (C30-C31)	3	0.7	4.25†	1.37	13.23
	Malignant neoplasm of larynx (C32)	4	8.5	0.47	0.18	1.26
	Malignant neoplasm of bronchus and lung (C34)	129	121.6	1.06	0.89	1.26
	Malignant melanoma of skin (C43)	13	9.7	1.34	0.78	2.31
	Mesothelioma (C45)	10	5.4	1.86†	1.00	3.47
	Malignant neoplasm of other connective and soft tissue (C49)	10	3.8	2.64†	1.42	4.92
	Malignant neoplasm of prostate (C61)	25	12.4	2.01†	1.36	2.98
	Malignant neoplasm of kidney, except renal pelvis (C64)	15	12.6	1.19	0.72	1.97
	Malignant neoplasm of bladder (C67)	13	12.6	1.03	0.60	1.77

Table 1
Continued

Malignant neoplasm of other and unspecified urinary organs (C68)	3	2.1	1.43	0.46	4.43
Malignant neoplasm of brain (C71)	26	22.0	1.18	0.81	1.74
Malignant neoplasm, without specification of site (C80)	17	11.8	1.44	0.89	2.32
Other and unspecified types of non-Hodgkin lymphoma (C85)	13	11.4	1.14	0.66	1.96
Multiple myeloma and malignant plasma cell neoplasms (C90)	7	7.0	0.99	0.47	2.09
Lymphoid leukaemia (C91)	4	4.4	0.90	0.34	2.41
Myeloid leukaemia (C92)	7	9.1	0.77	0.37	1.62
Malignant neoplasms of independent (primary) multiple sites (C97)	4	3.7	1.07	0.40	2.86
Neoplasms of uncertain or unknown behaviour (D37-D48)	18	12.6	1.43	0.90	2.27
Diabetes mellitus (E10-E14)	24	29.2	0.82	0.55	1.23
Obesity and other hyperalimentation (E65-E68)	6	5.3	1.12	0.50	2.50
Mental and behavioural disorders due to psychoactive substance use (F10-F19)	6	5.6	1.07	0.48	2.39
Diseases of the nervous system (G00-G99)	26	33.5	0.78	0.53	1.14
Hypertensive diseases (I10-I15)	13	21.2	0.61	0.36	1.06
Ischaemic heart diseases (I20-I25)	107	116.9	0.92	0.76	1.11
Acute myocardial infarction (I21)	84	70.1	1.20	0.97	1.48
Chronic ischaemic heart disease (I25)	20	40.4	0.49	0.32	0.77
Other forms of hearth diseases (I30-I51)	50	61.5	0.81	0.62	1.07
Cerebrovascular diseases (I60-I69)	31	44.4	0.70	0.49	0.99
Diseases of arteries, veins and lymph nodes (I70-I89)	12	13.9	0.86	0.49	1.52
Diseases of the respiratory system (J00-J99)	26	40.6	0.64	0.44	0.94
Chronic liver diseases (K70-K74)	34	40.3	0.84	0.60	1.18
Alcoholic liver disease (K70)	5	10.9	0.46	0.19	1.10
Toxic liver disease (K71)	9	4.2	2.13†	1.11	4.10
Fibrosis and cirrhosis of liver (K74)	19	23.3	0.82	0.52	1.28
Ill-defined and unknown causes of mortality (R95-R99)	17	19.6	0.87	0.54	1.40
All external causes of death (S-T)	164	136.6	1.20†	1.03	1.40

Only causes with more than two cases are shown; *Observed deaths in the cohort of SIREP exposed workers; **Expected deaths in the cohort of SIREP exposed workers based on Italian general population deaths; †=p<0.05.

lowest group level (four-digit code), and economic activity sectors were classified using the international statistical classification of economic activities (NACE rev. 1) at the division level (two-digit code). Person-years at risk were assessed from the year of first exposure to the date of death. The year of first recorded exposure was to 1947, the last was 2016. Cumulative exposure, in milligrams per cubic meter-year (mg/m³-years), was calculated for each cohort member having measurement data. Categorical cumulative exposure was modelled on the basis of the quartile distribution (≤4.5, 4.5 to ≤25.6, >25.6 mg/m³-years). Co-exposures of selected workers were ascertained, and mean latency for the major cancer sites was calculated. The data were collected routinely as an institutional activity and were analysed anonymously using R software v.3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The cohort of workers exposed to hardwood dust was selected from the SIREP database. The subjects linked to the ISTAT mortality archives were 77,311, of which 68,578 men and 8,733 women. A total of 1,235 deaths among exposed workers (1,157 men and 78 women) were identified in the period 2005-2018. Even if the ISTAT mortality archive covers the whole Italian population, uncertainty persists on the vital status of the unmatched subjects due to possible errors in the anonymized code linking the two databases. The mean age at death of the study population was 56.3 years with a standard deviation of 12.0, 54.1 (9.4) in women and 56.4 (12.1) in men. Cohort members having measurement data were 1,100, 94% of whom men. There was an elevated proportion of bronchus and lung cancer deaths in the group of female exposed workers (PMR=2.42, CI 95%=1.48-3.95) compared to the general population. The highest increase in PMR was found for malignant neoplasm of other and ill-defined sites in the lip, oral cavity and pharynx in men (PMR=3.24, CI 95%=1.45-7.22). As expected, a significant excess mortality from malignant neoplasm of the nasal cavity and accessory sinuses was found (four-fold greater than in the gen-
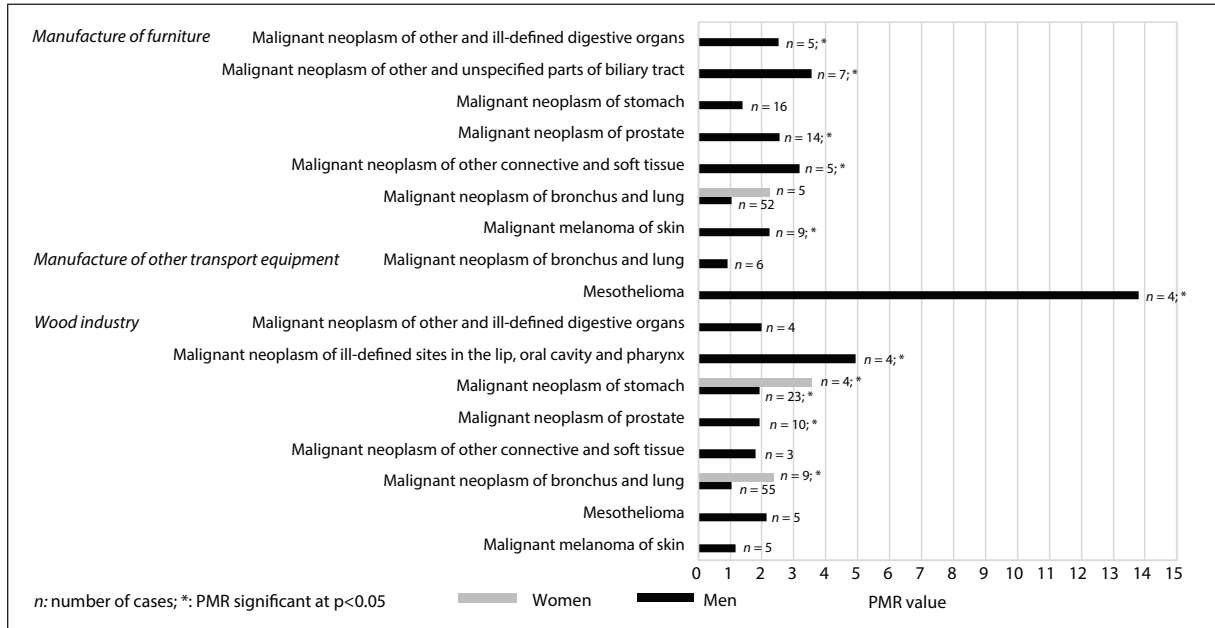
Figure 1
Proportionate mortality ratios (PMRs) for hardwood dust exposed workers by activity sector and gender at selected cancer sites (2005-2018).
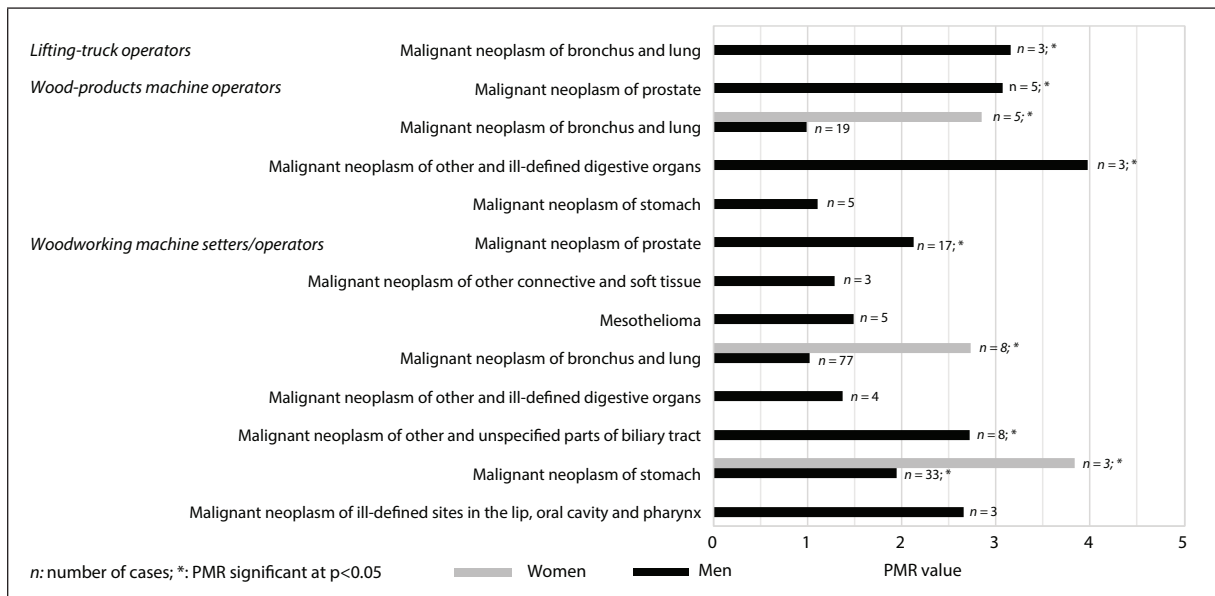

Figure 2
Proportionate mortality ratios (PMRs) for hardwood dust exposed workers by occupational group and gender at selected cancer sites (2005-2018).

eral male population). Among malignant neoplasms, increased proportions of deaths were also found from stomach cancer (in both genders), malignant neoplasm of other and unspecified parts of biliary tract, malignant neoplasm of other and ill-defined digestive organs, and malignant neoplasm of other connective and soft tissue. *Table 1* shows the PMRs for causes of death with more than 2 cases. The significant excess of lung cancer mortality in women was also confirmed in the sub-analysis by economic activity for the wood industry sector (n=9, PMR=2.35, CI 95%=1.22-4.52), as well

as the increased risk for gastric cancer in both genders (n=4, PMR=3.55, CI 95%=1.33-9.47 in women; n=23, PMR=1.93, CI 95%=1.28-2.91 in men). An increased mortality risk was detected in the manufacture of furniture for malignant neoplasms of other and unspecified parts of biliary tract (n=7, PMR=3.55, CI 95%=1.69-7.46 in men) and of connective tissue and other soft tissues (n=5, PMR=3.19, CI 95%=1.33-7.67 in men), whereas an excess for mesothelioma was found in the manufacture of other transport equipment in men (n=4, PMR=13.80, CI 95%=5.17-36.77). *Figure 1* displays the

Table 2
Proportionate mortality ratios (PMRs) for hardwood dust exposed workers by gender, cause of death and cumulative exposure at selected cancer sites (2005-2018)

Gender	Cause of death (ICD X code)	Cumulative exposure (mg/m³-years)	PMR	95%	CI
Women	Malignant neoplasm of bronchus and lung (C34)	≤4.5	0.59	0.08	4.17
		4.5-25.6	3.86†	2.08	7.18
		>25.6	1.31	0.33	5.23
Men	Malignant neoplasm of other and ill-defined sites in the lip, oral cavity and pharynx (C14)	≤4.5	4.93†	1.23	19.7
		4.5-25.6	2.39	0.60	9.55
		>25.6	2.40	0.34	17.1
	Malignant neoplasm of stomach (C16)	≤4.5	1.71	0.92	3.17
		4.5-25.6	1.55	0.99	2.42
		>25.6	1.72	0.95	3.11
	Malignant neoplasm of other and unspecified parts of biliary tract (C24)	≤4.5	2.09	0.52	8.36
		4.5-25.6	1.94	0.73	5.17
		>25.6	2.57	0.83	7.96
	Malignant neoplasm of other and ill-defined digestive organs (C26)	≤4.5	1.02	0.14	7.22
		4.5-25.6	2.39	0.99	5.74
		>25.6	1.82	0.45	7.27
	Malignant neoplasm of bronchus and lung (C34)	≤4.5	1.09	0.75	1.59
		4.5-25.6	0.86	0.64	1.15
		>25.6	1.32	0.96	1.80
	Malignant melanoma of skin (C43)	≤4.5	0.89	0.22	3.54
		4.5-25.6	1.57	0.75	3.29
		>25.6	1.49	0.48	4.63
	Mesothelioma (C45)	≤4.5	0.93	0.13	6.61
		4.5-25.6	2.14	0.89	5.14
		>25.6	2.21	0.71	6.84
	Malignant neoplasm of other connective and soft tissue (C49)	≤4.5	2.24	0.56	8.95
		4.5-25.6	2.30	0.86	6.14
		>25.6	5.30†	1.99	14.1
	Malignant neoplasm of prostate (C61)	≤4.5	1.72	0.65	4.59
		4.5-25.6	2.10†	1.17	3.80
		>25.6	2.65†	1.38	5.10

Only causes with complete data by cumulative exposure categories are shown; †=p<0.05.

results for sub-analysis by economic activity and gender at selected cancer sites, for causes of death with more than 2 cases. As regards the sub-analysis by occupational group, male woodworking machine setters/operators resulted to be at excess risk of mortality from sino-nasal cancer (n=2, PMR=5.93, CI 95%=1.48-23.82). An excess risk for gastric cancer was also found in this occupational group in both genders (n=33, PMR=1.94, CI 95%=1.38-7.73 in men; n=3, PMR=3.83, CI 95%=1.24-11.88). *Figure 2* illustrates the results of the sub-analysis by occupational group and gender at selected cancer sites, for causes of death with more than 2 cases. Lastly, cumulative exposure seems not to be associated with any excess of mortality risk across categories and tumours sites, with a few exceptions *(see Table 2)*. Co-exposed workers resulted to be 29 (3 women), of which

12 co-exposed to formaldehyde mainly in the sector of wood industry (80%). The average latency from the date of first exposure was 14 years for lung cancer in females, 12 and 18 years for stomach cancer in females and males respectively, 14 years for sino-nasal cancer in males.

DISCUSSION

In the present study, we performed a proportional mortality analysis of a cohort of workers exposed to hardwood dust in Italy in the period 1996-2018. The methodological approach used, applied on data of an exposure surveillance system compulsory by law, has allowed to detect significant excesses of mortality risk for sino-nasal and other site cancers (e.g., stomach cancer, malignant neoplasm of other connective and soft tis-

sue, etc.). To estimate the relative mortality risk among workers exposed to hardwood dust, PMR analysis was used according to the characteristics of the cohort, as already applied in other similar studies [12-14]. This decision was made as data on the entire population at risk were not known. The healthy worker effect could have influenced the study results and must be taken into account in their interpretation [15]. This effect particularly influences the PMR estimate since, if one or more causes of death are lower (e.g., some cardiovascular diseases as in our study), systematically the other causes, such as tumours, will be higher. These higher values, however, do not always correspond to a real risk. The particular characteristics of our occupational sample, based on a wide number of firms with a heterogeneous workforce, have probably limited the size of this effect on the estimates. A limit of the PMR method is that, in small population studies, the results might be overestimated by up to 20%, depending on the sample size ratio [16]. Although PMR analysis does not provide a true estimate of risk, it has the advantage of being a quick and efficient method of detecting unusual or elevated causes of death. Moreover, the misclassification of the death cause due to inaccurate reports may have been a source of bias in our study. A possible extra-Poisson variation (overdispersion) might have occurred due to the presence of an excessive number of zeros in the data, although the Pearson's chi-square ratio with its degrees of freedom (χ^2/df) in the applied models was mostly low (typically less than 2), suggesting that no statistical intervention was required [17].

Despite the low number of sino-nasal cancer deaths found in the study population, possibly explained by the relatively low fatality of this tumour site (representing less than 1% of all cancer in Italy), the findings confirm an increased risk in workers exposed to hardwood dust [2-3, 18-19]. Meta-analyses of published studies have demonstrated a clear causal association between sino-nasal cancer incidence or mortality and exposure to wood or leather dust, supporting the need of a systematic surveillance system for these diseases in exposed workers cohorts and in the general population [20-21]. Nasopharyngeal cancer, another cancer site specifically addressed by IARC in its Monograph on wood dust, presents a more than expected number of deaths but without reaching statistical significance. Currently, the IARC classification of wood dust carcinogenicity for this type of cancer is characterized by limited evidence [1]. Moreover, in our analysis, an excess of mortality from lung cancer was found, even if limited to female exposed workers. A number of previous studies support a causal relationship between occupational exposure to wood dust and occurrence of lung cancer even after controlling for smoking, suggesting also a differential effect for hardwood and softwood dusts [4]. The worker exposure has been defined on the basis of data from a national exposure surveillance system which are collected and reported under the responsibility of employers, thus minimizing the misclassification of exposure and covering most of the industrial sectors involved. The increased risk of lung cancer in our study was found mainly in the same sectors (wood

industry) and occupations (woodworking machine setters/operators) as for sino-nasal and gastric cancers. Evidences of an association between wood dust exposure and gastric cancer are still scarce, but our findings uphold the opportunity of further studies on this issue [22-24]. A clear confounding effect of asbestos exposure is recognizable for mesothelioma mortality in the sector of manufacture of transport equipment [25], whilst the excess mortality for cancers of biliary tract and of connective tissue in workers exposed to hardwood dust, emerged from our analysis in the manufacture of furniture, needs further investigations in order to exclude possible unrecorded co-exposures. In the last decades a decreasing trend in occurrence of sino-nasal cancer was documented, mainly attributed to increased implementation of prevention and protection measures at workplace [2, 26]. Our study shows a still measurable excess risk of cancer among Italian workers exposed to hardwood dust. The lack of a risk trend in the cumulative exposure analysis may be due, on the one hand, to the lack of measurement data (not all cohort members have measurements) and, on the other hand, to the greater number of measurements in more recent years, at which is hypothesized a greater attention by companies to the implementation of more restrictive control measures, to adapt to increasingly stringent regulations. The low mean age at death of the cohort of exposed workers could be due to the young age of the epidemiological surveillance system (fully operational only since 2008 after the enactment of the law that provided for the procedure rules of exposure data transmission), and the relatively short follow-up study period. It is plausible that at the next follow up, the number of subjects linked between SIREP exposure data and ISTAT mortality archives will increase. Despite knowing about the risk of sino-nasal cancer from furniture manufacturing studies, there is still evidence that hardwood dust is not still sufficiently controlled at workplace. Beyond the long latency of this and other cancers possibly related to hardwood dust, it is noteworthy that, based on the still relevant number of preventable tumours estimated, the European Commission has recently lowered from 5 to 2 mg/m^3 (provisionally 3 mg/m^3, until January 17, 2023) the limit value for occupational exposure to this carcinogen. The implementation of the European Directive n. 2017/2398 should further support technical interventions and good working practices, and increase the use of personal protective equipment in the sectors involved, with the real goal of reducing the level of workers' exposure and preventing the onset of cancer.

In conclusion, prevention actions against occupational carcinogens are a priority for safeguarding the health of workers, and the analysis of exposure risks is useful for the implementation of OSH policies, including the development of occupational exposure limit values. In this study, the record linkage between data from an exposure surveillance system and health statistics provided valuable insight into the occupational cancer risk associated with hardwood dust exposure, suggesting greater efforts to ensure compliance with regulatory obligations, even by increasing the effectiveness of la-

bour inspections, and claiming the need for future researches.

Conflict of interest statement

The Authors declare they have no competing financial interests. The Authors declare they have read and approved the paper.

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Where we are in the fight against Antimicrobial Resistance and Healthcare-Associated Infections. The opinion of the stakeholders of the European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI)

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Abstract
During the second European Joint Action on Antimicrobial Resistance (AMR) and Healthcare-Associated Infections (HCI) (EU-JAMRAI) annual meeting, the Evaluation Team elicited stakeholders' opinions regarding the implementation of the National Strategies and Action Plans to fight AMR and HCI, the One Health integration and the EU-JAMRAI support to the national and EU authorities in two Focus Groups. This qualitative exercise contributed to identify criticalities and possible improvements in aspects such as political priorities, legislation/legal requirements, human and financial resources, and supervision in many health sectors to ensure effective implementation of the action plans. Stakeholders pointed out at the different speed of EU member states, in particular concerning the One Health integration in the plans. Finally, the Stakeholders strongly asked the EU-JAMRAI to reinforce the integration and dissemination of the best practices and results, to help policymakers at national and European levels in defining and implementing harmonized policies and actions against AMR and HCI.

INTRODUCTION

Antimicrobial resistance (AMR) is one of the most serious threats challenging modern medicine which have required international cooperation [1, 2]. The present crisis is linked not only to the emerging and spread of bacteria that are resistant to first and second-line antibiotics but also to the lack of new, effective antibiotics in the pipeline of research and development of the pharmaceutical industry [3]. Hence, the therapeutic options for infections due to antibiotic-resistant bacteria are very limited or non-existent jeopardizing the management of patients in surgery, oncology, intensive care, and other critical conditions. According to estimates of the European Center for Disease Prevention and Control (ECDC), based on 2015 AMR surveillance data, every year more than 600,000 antibiotic-resistant infections occur in European Union (EU) and Economic European Area (EEA) countries leading to more than 33,000 deaths and 870,000 disability-adjusted life years. The burden for the EU and EEA coun-

tries was highest in infants and in people aged 65 years or older and was on the rise from 2007 [4]. Up to 1.1 billion Euros are expected to be spent yearly between 2015 and 2050 due to AMR across EU and EEA countries. This corresponds to about 1.8 Euros per capita per year on average, with about 4.1-4.8 Euros per capita in countries where AMR is highest, that is Italy, Malta, Luxembourg and Greece [5].

Besides this dramatic development, AMR is a complex phenomenon, including but not limited to, human medicine, veterinary medicine, animal husbandry, agriculture and the environment [6]. For this reason, international institutions unified the efforts to tackle this problem and launched several coordinated and comprehensive initiatives. In 2015 World Health Organization (WHO) endorsed a Global Action Plan (GAP) with a "One Health" (OH) approach, a multisectoral plan that includes human and animal health as well as the environment and asked member countries to elaborate a National Action Plan (NAP) following this principle [7]. "One Health" is defined as "the collaborative effort of multiple health science professions, together with their related disciplines and institutions-working locally, nationally, and globally-to attain optimal health for people, domestic animals, wildlife, plants, and our environment" [6]. It has been estimated that as many as 75% of human infectious diseases that have emerged or re- emerged in recent decades are zoonotic; that is, they originated in animals [8]. The origins of "One Health" are centuries old and are based on the mutual dependency of humans and animals and the recognition that they share not only the same environment, but also many infectious diseases [6, 9]. One of the major areas in the application of the OH approach is the AMR. Several international organizations have made important One Health contributions to the containment of antimicrobial resistance. Since the early 1990s, WHO has undertaken several expert, multidisciplinary, multisectoral consultations and advisory groups, compiled considerable objective evidence of and scientific opinion about the human health impacts of antimicrobial use in animals, and formulated wide-ranging recommendations applicable to all stakeholders (e.g., regulatory authorities, pharmaceutical industry, animal production industry, veterinarians, farmers, public health, consumers) [10].

Since 2016 the Tripartite WHO, the Food and Agriculture Organization (FAO) and the World Organization for Animal Health (OIE) have monitored the progress of the countries on AMR policies and the implementation of the NAP [11].

In June 2017, the European Commission adopted the "EU One Health Action Plan against AMR "and proposed the creation of a "One Health" network in Europe [12, 13]. It is essential that OH teams work together from the early phase of a research program in order to gain a comprehensive view of the problems, generate new hypotheses, and innovate approaches based on transdisciplinary methods [13].

In parallel with the problem of AMR, the issue of Healthcare Associated Infections (HAI) cannot be considered separately from AMR. In fact, both control of AMR and infection prevention and control strategies for HAI are based on the same pillars: i) prudent use of antibiotics ii) appropriate tools for monitoring and surveillance and iii) accurate diagnostic tests to decide on the right therapy.

In 2017, the 3rd Health Programme of the European Union and the participating countries co-funded the European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI) with 3-year duration [14].

Within the second Annual Meeting of EU-JAMRAI, held on 16-17 September 2019 at the Italian National Institute of Health (ISS), Rome, Italy, the 2nd Stakeholder Forum took place to discuss the EU-JAMRAI progress and the emerging issue related to AMR and HAI. In this context, the aim of this study was to analyze the Stakeholders´ opinion about the implementation of NAP for Antimicrobial Resistance and Healthcare-Associated Infections, the OH approach of these plans and the EU-JAMRAI role in supporting the actions at country level.

MATERIALS AND METHODS
Study design
The qualitative research method was used since it is considered the most appropriate to identify and explore the phenomenon under study from the participants´ perspective. It was guided by a constructivist approach and elements of grounded theory which provide the methodology to develop a rich theoretical understanding of the people's perception [15]. This is a social scientific vision influenced by social constructivism and symbolic interactionism, and whose objective is an interpretative understanding of the participants´ multiple perspectives and meanings. The study design was defined taking into account the stakeholders' background and representativeness.

The focus group (FG) method was chosen because of its effectiveness in exploring the meaning of relevant concepts taking place in the healthcare context [16]. Two FGs were defined, the first to explore AMR and HAI and the second AMR and antimicrobial use in humans and animals.

To facilitate the engagement of the stakeholders in the discussion and to ensure that all the relevant topics were explored, for each FG an open-ended questions guide *(Supplementary Material available online)* was prepared to explore dimensions of the study related to the implementation of OH National Strategies and NAPs for AMR, Prevention and control of HAI, Antimicrobials use in humans and animals, and Implementation of antimicrobial stewardship.

The list of questions for the participants to explore each dimension is shown in *Table 1*.

Sample and setting
The recruitment process took place at the 2nd Stakeholder Forum that took place at ISS in September 2019. The European stakeholders involved in EU-JAMRAI represent the scenario. From the list of those who registered for the meeting a purposive sampling (deliberate selection to maximize variability and mini-

Table 1
List of questions asked to the participants of the focus groups

Dimension 1. Implementation of One Health National Strategies and National Action Plans for AMR
1. According to your knowledge, what are the weaknesses that can jeopardize the effectiveness of the national strategies to contain or reduce AMR?
2. According to your knowledge, what difficulties do you envisage in carrying out the implementation of One Health National Strategies and National Action Plans?
3. Concerning the support to One Health National Strategies and National Action Plans for AMR, what advice/suggestions would you give to the EU-JAMRAI project team that can be considered in the next year of activities?
Dimension 2. Prevention and Implementation of HAI
1. According to your knowledge, what are the weaknesses that can jeopardize the effectiveness of the national strategies to contain or reduce HAI and related AMR?
2. According to your knowledge, what difficulties do you envisage in carrying out improvement actions in the prevention of HAI and their implementation?
3. Concerning the prevention of HAI and their implementation, what advice/suggestions would you give to the E-JAMRAI project team that can be considered in the next year of activities?
Dimension 3. Antimicrobials use in humans and animals
1. According to your knowledge, what are the weaknesses of the present monitoring and surveillance of antibiotic use in humans/ in animals?
2. What strengths do you identify regarding the present monitoring and surveillance of antibiotic use in humans/in animals?
3. Concerning the appropriate use of antimicrobials in humans/animals, what advice/suggestions would you give to the EU-JAMRAI project team that can be considered in the next year of activities?

mize bias response) was used to invite the participants to the FGs. Nine stakeholders accepted the invitation and attended the two FGs. The demographics for the theoretical sample are reported in *Table 2*.

The FGs were carried out in two separate rooms of ISS and lasted about 60 minutes. Each FG was conducted by two members of the WP3 Evaluation Team of EU-JAMRAI, who had no previous contact with the participants and were experts in the management of qualitative data collection techniques.

Data collection and analysis

Data analysis took place during autumn 2019 and spring 2020. Following the recommendations of Strauss and Corbin [16], the analysis process followed the open, axial and integration coding. In the open coding, a preliminary code closely fitting with the data was given to each semantic unit of data (line-by-line coding). In the axial coding, the data were grouped aiming to obtain a clearer and more complete explanation about the phenomenon. The grouping was defined by a systematized analytical process of comparison and connection among the different categories [15]. In the integration phase, the categories and subcategories were compared, analyzed and refined, and a core category of this grounded theory related to the Stakeholder's perception about the criticalities for an appropriate implementation of National Strategies and NAPs, the OH integration into the NAPs, and the support from the EU-JAMRAI emerged. During data analysis, memos were used to document ideas about the coding process and insights into the relationship among codes, concepts and categories [16, 17].

The latest were the areas of knowledge from which, and by theoretical saturation, the central category emerged: identifying the criticalities of the OH National Strategies and NAPs for AMR and EU-JAMRAI contribution from the stakeholders' perception. Regarding criteria for rigor, the recommendations of Guba and Lincoln were followed to set strategies to ensure validity and reliability [17]. In order to ensure credibility, the results were illustrated with textual fragments from the transcripts of the

Table 2
Demographics and methods of data collection for the theoretical sample

Topic	Method of data collection	Gender	Age	Organisation
1. Implementation of One Health National Strategies and National Action Plans for AMR and Policies for Prevention of Health-Care-Associated Infections and their Implementation 2. Appropriate use of antimicrobials in human and animals	Focus Group	3 Females 6 Males	Range 22-65 years	• Institutional and research organizations • Civil society-healthcare professionals • Joint Programming Initiative on Antimicrobial Resistance Virtual Research Institute (JPIAMR-VRI)

stakeholder's discussion. In addition to the recording, the evaluation team members, responsible for data analysis, kept a reflective diary of the transcriptions to ensure reliability of the results. Finally, the use of the Maxqda 10 Software (VERBI Software, Berlin, Germany), as operational support throughout the investigation process, allowed ensuring the auditability of the project. The research followed the Consolidated Criteria for Reporting Qualitative Research (COREQ) guidelines [18].

Ethics, data protection and confidentiality

The attendees agreed to participate voluntarily, after receiving verbal and written information about the FG and their contribution to it. Written informed consent was obtained from each participant and included permission for the voice recording and verbatim transcription of the FG discussions and the use of the collected information for the purpose of the analysis and reporting, guaranteeing confidentiality. Participants were informed of their rights, and that they could leave the FG at any time if they wished [19].

RESULTS

The analysis of the data indicated three main levels/ categories of drivers that have a direct negative impact on the implementation of OH National Strategy into NAP, namely: 1) macro level-political and governmental actions; 2) medium level-community hospital organization and infrastructures; 3) micro level-health professionals and population awareness and engagement. A fourth category, 4) synergies to ensure the success of the implementation, lists some specific actions identified by the stakeholders to improve the NAPs (as described in the chapters below).

The main categories emerged from the study were further divided into subcategories and the outline of the results is presented in *Figure 1*.

POLITICAL AND GOVERNMENTAL ACTIONS
Lack of effective central actions by the governments

The participants complained about a limited support for the HAI and AMR programs with need to improve political commitment and actions by the central governments of the countries. The stakeholders perceived that central governments should actively support the initiatives needed to control and prevent HAI and AMR; otherwise, the NAP implementation should not be realistic.

"(…) there is a lack of central actions against this problem; it is not a problem that can be solved by a
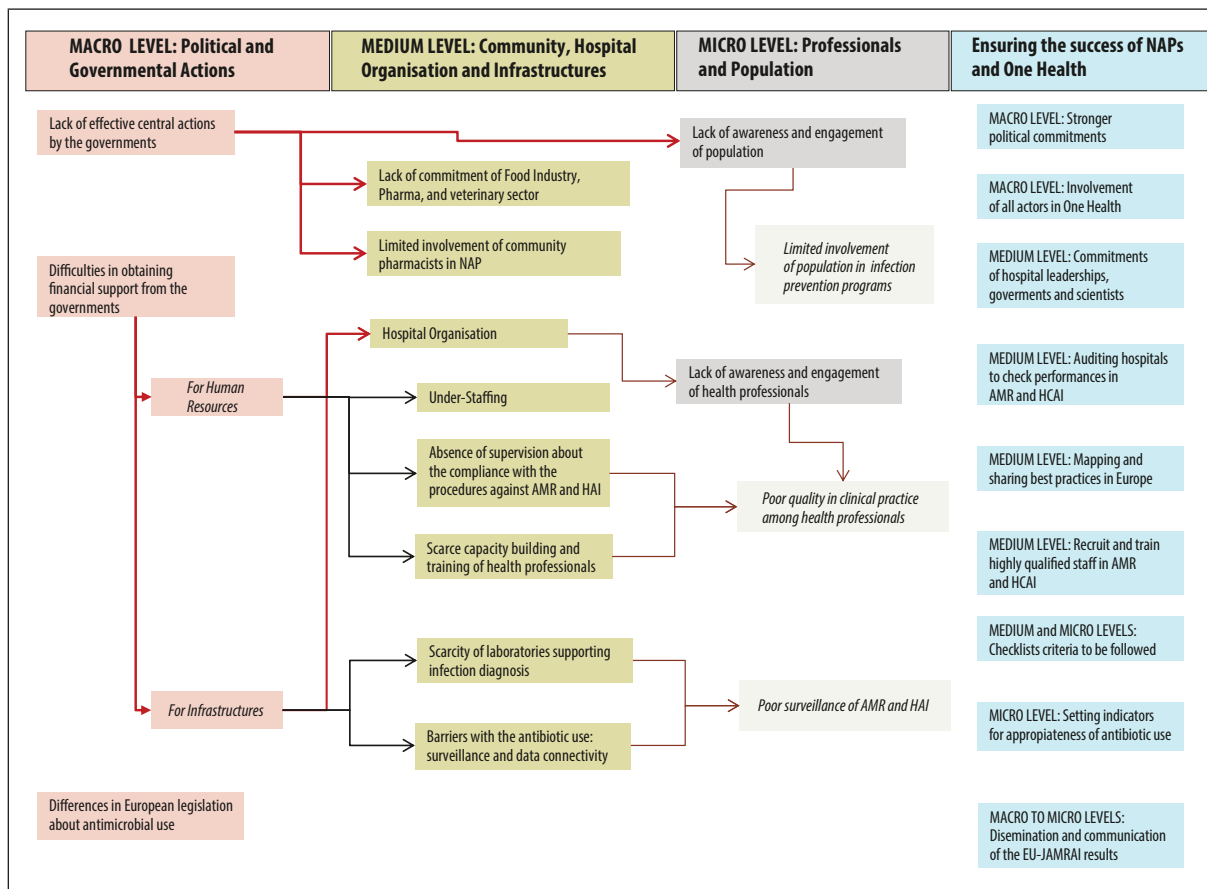

Figure 1
Description and interaction of the three main levels/categories of drivers with direct negative impact and some specific actions identified by the stakeholders on the implementation of One Health National Strategy into the National action plans against antimicrobial resistance and healthcare associated infections.

single hospital; wide national programs should be carried out." (FG1-Stakeholder -3).

"(…) and they should decide to implement national programs focused on strong political actions to reduce this problem. This is not only a problem of the single worker, it is a problem of the hospitals, regions and countries (…)" (FG1-Stakeholder -4).

Difficulties in obtaining financial support from the governments

The lack of specific financial support to hospitals by the governments would have a negative impact on the OH Strategy not providing enough resources and infrastructures needed for the appropriate implementation of NAP for AMR. Some stakeholders stressed that the lack of financing which should have been aimed at providing resources and infrastructures to support infection prevention negatively impacted on both human and animal health. The participants believed that such limitations contribute to jeopardize the process of the NAP implementation.

"The lack of financing, let's say, in order to provide the needed tools and structures to prevent those HAI" (FG1-Stakeholder -1).

Differences in European legislation about antimicrobial use

Stakeholders highlighted the importance of developing an appropriate legislation about the use of antibiotics. In human medicine, there is insufficient legislation regulating antibiotic use, as opposed to the veterinary sector. As a result of that, the veterinary sector has achieved great progress, especially because the use of antibiotics was really massive in some countries. One of the key points that should be considered at country level is the implementation of the legislation.

"(…) in veterinary, immense progress, very impressive", (FG2-Stakeholder-3).

"(…) all of the things we have identified in the last years are in the legislation (…) let's first implement the legislation (….) so we can start properly in January 2022" (FG2-Stakeholder-1).

All the critical issues concerning AMR, antimicrobials and their appropriate use in the veterinary sectors are regulated by the new Veterinary Medicine Regulation (Regulation EU 2019/6) that will come into force in January 2022. The priority antibiotics indicated by WHO are of restricted use in the veterinary fields.

"In animals, one of the key points is the implementation of the legislation. All the critical issues concerning AMR, antimicrobials and their appropriate use in the veterinary sectors are put in the legislation (FG2-Stakeholder-1)."

"(…) all of the things we have identified in the last years are in the legislation which has been adopted last year [note from the authors: Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC that will came into force January 1st, 2022], so we are in a phase where there is a lot of work to do because of the new requirements (FG2-Stakeholder-1).

COMMUNITY, HOSPITAL ORGANIZATION AND INFRASTRUCTURES
Community
Insufficient commitment of Food Industry, Pharma, and veterinary sector

Participants believed that there exists a scarcity of commitment from sectors related to health, such as the agriculture and farming sector and the Food and Pharmaceutical Industries. This limited engagement hampers an effective fight against AMR within the OH context.

"And the final thing is lack of engagement of other sectors; we are not talking only about the health sector, we are talking about food industry, we are talking about pharma sector, veterinary sector and those that are related to health, because AMR is related to multiple other sectors so horizontal approach is being used more." (FG1-Stakeholder-5).

Limited involvement of community pharmacists in NAP

Stakeholders pointed out the poor involvement of the pharmacists in writing and implementing the guidelines for the appropriate use of antibiotics in the NAPs in most European countries, as opposed to the health professionals. Stakeholders believed that since the great majority of antimicrobials in human medicine is sold in the community, community pharmacists should be more integrated in the NAP strategies for appropriate antimicrobial use.

"(…) when it comes to guidelines on appropriate use or antimicrobial stewardship for healthcare professionals, we, as community pharmacists, see that in most EU countries community pharmacists are not structurally involved in NAPs, guidelines or strategies (…)" (FG2–Stakeholder-4).

Hospital organisation
Inadequate supervision about the compliance with the procedures against AMR and HAI

Participants stressed that hospital managers should make health professionals aware of the existence of protocols to prevent and control AMR and HAI, as well as to supervise the compliance to the protocols inside hospitals.

"(…) the directors at the hospital should alert that there is a procedure that professionals should follow, and this information has to be posted. That's probably something that could affect, because if you leave the organization without the piece of paper it doesn't work (…)" (FG1-Stakeholder-3).

Need to improve capacity building and training of health professionals

The participants considered important to promote the development of capacities in hospital organizations, to support the training and career development in HAI and AMR control. The stakeholders highlighted the training needs for health professionals to acquire awareness and responsibility about the OH Strategy, to define and achieve common goals on HAI and AMR prevention and control. This aspect is of paramount importance to address infection control in hospital setting.

"For me, I focus more on capacity building of different players, let's say (…) it could make that professionals are more effective, are more responsible and are more aware of the problem. (…)" (FG1-Stakeholder-2).

Under-staffing

Under-staffing has been considered a barrier to successfully implement infection control programs in hospital settings. The main reasons that were mentioned by participants were: scarce hospital policies to provide dedicated staff to this program, and a shortage of qualified professionals to address the infection control programs in clinical practice.

"Besides, the problem of lack of staff, under-staffing, and also the problem of education, having proper, let's say, personal working or dedicated to ensure the prevention of those infections". (FG1-Stakeholder-2).

Infrastructures

Scarcity of laboratories supporting infection diagnosis

According to the participants, most antibiotic prescriptions are based on empirical knowledge or personal experience, without the support of diagnostic tests or microbiology laboratories. Such circumstances prevent the practitioner from making an accurate diagnosis of the infection, which in turn will affect the appropriateness of prevention measures and antibiotic treatment.

"I am always surprised that doctors for humans very often prescribe what we consider critical antibiotics without any sensitivity testing. It is not allowed in my profession". (FG2-Stakeholder-3).

"(…) And also the problem of the logistic, or not adequate logistics and materials and structures in order to ensure appropriate prevention of those infections". (FG1-Stakeholder-4).

Barriers with the antibiotic use:
surveillance and data connectivity

The stakeholders perceived that a scarcity of computerized data recording system limit the development of adequate surveillance and monitoring of antibiotic use in both medical and veterinary sectors. Although such system is present in some European countries, its implementation in all EU countries is complex and not achievable in a short time, due to the system complexity and contextual differences among countries.

"(…) we (Europe) are one but countries go at different speeds (…)" "It is good to have computer data (for prescriptions) (…), but there are countries where they keep records in a booklet or not even that". "(…) It is different if you have a good system for all EU" (FG2-Stakeholder-2).

"(Lack of) Infrastructures for appropriate surveillance and monitoring are key barriers for comparable results across EU countries" (FG2-Stakeholder-2).

Some participants highlighted that in both human and animal medicine, monitoring the prescription could give information also on the impact of switching from one antibiotic to another in terms of outcome and appropriateness. A correct antibiotic stewardship should be based on the knowledge of the antibiotic resistance at the local level (hospital or farm).

"What happens, what are the consequences if you change prescription or you switch from one antibiotic to another, for instance for UTI" (FG2- Stakeholder -1).

"I need to know what is happening [regarding antimicrobial resistance] in my area for the species I am treating". (FG2-Stakeholder-4).

The stakeholders perceived that a central database would be crucial in order to ensure data availability and usability. Monitoring of the use of antibiotics should not be evaluated separately from other information, such as AMR surveillance. It is important to link the use of the antimicrobials with the level of AMR and the outcomes of patients with infections due to AMR pathogens.

"(…) concerning AMR and antimicrobial usage surveillance, a thing that would help us a lot will be more granular data, for example patient-level usage linked to the outcome and linked to laboratory data (…)". (FG2-Stakeholder-1).

Health professionals and population
Dearth of awareness and engagement of health professionals and population

Most participants asserted that there is poor awareness and engagement in both professionals and general population due to limited knowledge about antibiotic resistance. This leads to insufficient collaboration and participation in health programs aimed at fighting AMR and HAI and, consequently, less success of these programs in the hospital and community settings.

"When we are working in those Action Plans, usually the lack of engagement or knowledge of professionals can contribute a lot in the non-success of these programs at the hospital level or in the clinical level in general". (FG1-Stakeholder-5).

"(…) Lack of awareness and engagement of the general population, if you want to implement a program it should not be only vertically; one thing to take in consideration is the collaboration of patients". (FG1-Stakeholder-2).

Some stakeholders pointed out that the scarcity of knowledge related to HAI among health professionals contributes to inadequate quality in clinical practice, making it difficult to manage the infections in the hospital.

"In my opinion, I think that lack of knowledge about HAI in healthcare professionals, is a challenge to focus on, there is bad practices inside hospitals, maybe it can contribute to these infections". (FG1-Stakeholder-3).

ENSURING THE SUCCESS OF THE OH INTO NAP IMPLEMENTATION

Stakeholders asserted the importance of the political level to support the implementation of OH Strategy in NAPs against AMR. They pointed out at the differences among MSs in terms of speed, infrastructures, resources and capacity in the implementation of the recommendations and the action plans, and these differences result in severe limitations for the implementation of integrated surveillance that involves both medical and veterinary sectors.

"One health approach is becoming more and more considered in the EU. Initiatives in MSs are ongoing

to make better use of data from the different sectors and try to integrate analyses to help understanding the phenomenon of AMR, but comparability and availability of data across sectors is still an issue". (FG2-Stakeholder-2).

According to the participants, the focus of the EU-JAMRAI should be on the following aspects: i) strengthening the political commitment and the governmental engagement, to ensure support and resources to the national and international initiatives against AMR and HAI, ii) committing hospital leaderships, governments and scientists to define common criteria and checklists to address the criticalities at hospital level; iii) making audit within hospitals, iv) mapping existing best practices in European countries in order to facilitate their sharing; v) checklist criteria to be followed, vi) highly qualified staff in AMR and HCAI, vii) involving all actors in OH Strategy, viii) defining recognized general standards for appropriateness of antibiotic use and setting indicators for its evaluation, ix) disseminating the EU-JAMRAI results and communication at high national levels.

"We need also a strong political commitment, because we a notice this initiative really not connected with political level, not just policy but political level (…)". (FG1-Stakeholder-5).

"(…) to share a checklist with a list of criteria to be followed and respected by every hospital". (FG1-Stakeholder-4).

"(…) to make audit within the hospitals…"(FG1.Stakeholder -5).

"What I would suggest is to have a clear idea on the practices from all the European Countries participating to the JAMRAI about practices and procedures to prevent HAI, education and training of the staff involved, so we can share best practice" (FG1- Stakeholder - 1).

"The need for a nation-wide program, not a single program based on hospitals. I want to see taking account of veterinarians, people, microbiologists, pharmacies, and industries (…)". (FG2- Stakeholder 2).

"(…) appropriate use can only be measured by own standards". (FG2-Stakeholder-2).

"(…) to reinforce the JAMRAI… to really work a lot on the dissemination of the results … so many interesting things have been done (…) but really the results should be communicated to everyone." (FG1-Stakeholder-1).

DISCUSSION

The debate generated inside the FGs provided valuable information derived from direct experiences, beliefs, perceptions and attitudes of the involved stakeholders in relation to the topics under study.

Although the stakeholders FGs consisted of a small number of participants, they proved to be very helpful in pointing out areas for improvements of the NAPs, considering different levels of intervention: government, hospitals and individuals. To the best of our knowledge, in the literature there are very few studies that provide enough qualitative research data on AMR at EU level [20-22]. Moreover, having stakeholders involved from different sectors and backgrounds, enriched the discussion with examples and comparisons across sectors on

how the different issues related to the NAPs and the fight against AMR and HCI have been addressed.

At governmental level, the main barriers reported during the discussion were insufficient central actions and dedicated resources to combat HAI and AMR. Although national efforts have grown steadily over the last two decades, what has been done so far does not match the recommended scale of actions, and progress with developing NAPs has been limited in many countries. At national level, the public health system organization can be a barrier, in case of shared powers between the central (Ministry) and the local authorities (Regions) and high differences in the regional/local capacities; hence the actions can result disjointed. Similar observations were found in several other studies [2, 23, 24]. As expected, both focus groups considered the insufficiency of financial, human and IT resources is a serious constrain. In particular, shortage of specialists in HAI and AMR, lack of engagement of health professionals were pointed out. Specific staff related issues, like scarce capacity building, staff turnover and new staff training, work overload and lack of incentives were also identified as barriers in a qualitative study conducted at a hospital in India [24]. Concerning IT, the limited capacity of collection and integration on a routine basis of AMR surveillance and antimicrobials use data was considered a limitation to the assessment, implementation and monitoring of the actions taken by national government. Data availability including diagnostic and molecular data, links between people and systems, which include organization, information technology and systems was pointed out also by other authors as critical issue [3, 25, 26]. Other studies pointed out the need of interactions between people and systems, which include organization, technology and systems [24]. All these aspects have been also shown in our research.

Between the medical and veterinary sectors, the stakeholders pointed out at the difference in legal provisions provided by the EU Commission that require AMR surveillance and antibiotics prudent use in animal health and food safety enforced at national level but nothing similar is so far requested for the medical sector. This difference weakens the power of the central governments to push forward and implement the actions detailed in the NAPs, in particular in the medical sector, since they cannot refer to any strong and harmonized international legal basis.

Another criticality pointed out by the focus group was the poor OH component of the NAPs, and the need to strengthen this approach to ensure integration and synergies among sectors. In particular, the commitment of the national authorities, the involvement of the main stakeholders and the development of reference criteria and standards were identified as main gaps.

The limitation of the FG results was mainly related to the representativeness of the stakeholders. Although all of them were representative of EU or international organizations or associations, some were also national experts or professionals, and it is possible that their opinions were sometimes driven by their national/local experience. This could have resulted in describing some specific situations, but the overall value of the exercise

was not reduced, and most of the comments were generalizable.

The information collected were analyzed to provide recommendations on how to improve capacity at local and national level. Policy briefs that addressed the unmet needs of resources, awareness, training, and reference standards at EU level to ensure proper national capacity were released by the experts. However, the One Health vision and approach, although promoted by EU-JAMRAI, was only partly addressed, due to the complexity and the heterogenicity of the situations found at national and local level.

CONCLUSIONS

The FGs proved to be helpful identifying the main criticalities and the improvements needed to the NAPs. The main criticalities discussed were in human and financial resources, political priority, legislation/legal requirements, and supervision in many health sectors. The FGs strongly asked the JA to improve integration, dissemination and communication of the best practices and results, to help policies and actions at national, hospital and community level.

Authors' contributions

JAS and EF equivalently contributed to the manuscript; JAS with the analyses of the data and the methodology; EF with the participation in the focus groups, data collection and data extraction from the recordings. Both wrote and revised the drafts. MPLA promoted and supervised the work. HH and MA contributed in data collection and revised the drafts.

AP and LB provided information on the general aspects of the topic AP for public health and LB for animal health aspects, both contributed writing part of the introduction and discussion and revised the drafts.

Conflict of interest statement

None declared.

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Case series of *Chlorophyllum molybdites* intoxication in Sicily: an "alien" mushroom species in Europe

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Abstract
Introduction. Mushroom poisoning is an important cause of intoxication worldwide. The toxic mechanism remains frequently unknown and the diffusion of non-endemic species may cause the emergence of new syndromes. An example is the widespread of *Chlorophyllum molybdites* in Sicily.
Case series. Pavia Poison Centre was recently involved in the management of 10 intoxications caused by the ingestion of *Chlorophyllum molybdites*, which was not considered part of the Italian mycological species. The clinical syndrome was characterized by severe gastrointestinal symptoms. In paediatric or vulnerable patients, it may bring to hypovolemic shock that necessitate intensive support. The possibly confusion with amatoxins-containing mushrooms may complicate the management.
Conclusions. *Chlorophyllum molybdites* is widespread on the oriental coast of Sicily and it could be confused with "parasol mushrooms". Cooperation between emergency physicians, clinical toxicologist and mycologist, supported by improving of laboratory tests, is essential for the appropriate clinical management. Climate changes and migration flows can interfere with the diffusion of new species and the development of novel syndromes.

INTRODUCTION

Mushroom poisoning is an important cause of intoxication worldwide. The real annual global fatality is unknown and underreported, but is estimated that the consumption of wild mushrooms causes every year an approximated number of 100 death/year in Europe alone [1].

New poisoning syndromes continue to emerge and being characterized from a clinical point of view. The toxic mechanism of many mushrooms species remains unknown. In some cases, mushrooms considered before as edible are now listed as potentially toxic, so it may result difficult to distinguish between edible and poisonous species; moreover, their toxicity may be different considering various factors such as the stage of growth, quantities ingested, cooking procedures or environmental conditions. Valid examples are the rhabdomyolysis following the ingestion of large quantities of *Tricholoma equestre* [2] or gastroenteric syndromes from *Armillaria mellea* consumed without the correct caution of boiling and throwing the cooking water away [3]. Another important bias is that the same species could be considered edible in some part of the world and non-edible in others, this because the same mushroom may develop different toxin content and consequently causes or not clinical manifestations. A representative member of this class is *Chlorophyllum molybdites* [4].

Chlorophyllum molybdites is a mushroom that can cause severe, short-onset gastrointestinal syndrome, also known as "Morganic syndrome". It may be confused with edible species, such as *Macrolepiota procera* (parasol mushroom) due to its aspect or *Agaricus guadalupensis*, because their habitat of growth (lawns and grassy fields) [5] *(Figure 1)*. In some cases, the

Figure 1
Macroscopic comparison between *Chlorophyllum molybdites* (A, B) and *Macrolepiota procera* (C, D).

symptoms, in particular watery diarrhoea, may persist over 12-24 hours, which may hide the onset of a long latency syndrome mainly due to amatoxin containing mushrooms. From a clinical point of view, the similarity between *Macrolepiota procera* and *Chlorophyllum molybdites* is particularly important because the edibility of the former mushroom brings the consumer, even an expert one, to understate the importance of the mycological recognition to avoid eating a non-edible mushroom. Moreover, a naïve collector could mistake and pick up toadstool containing amatoxins, with the subsequent risk of severe hepatotoxic damages if not correctly framed and treated.

To date, the syndrome related to *Chlorophyllum molybdites* has been reported in medical and botanical literature since the beginning of 20th century in tropical and subtropical areas, in particular USA, Tahiti, Philippines, New Guinea, Australia, India, Africa, West Indies, South America (where in some countries was considered edible), Japan, China and Israel. In Europe this species is not considered part of the endemic mushroom flora; it was first identified in Scotland and The Netherlands at the beginning of 1990's, in association with exotic ornamental plants [4]. In Italy, *Chlorophyllum molybdites* was first spotted in 2005, on the Ionian coast of Sicily by mycologist, but during the last two years it became widespread. The mycologists of Azienda Sanitaria Provinciale (ASP Catania, Sicily, Italy) involved for territorial competency, due to the huge amount of intoxications in which *Chlorophyllum molybdites* was recognized as responsible of the characteristic clinical syndrome, sent a specific warning to the Italian Ministry of Health, that developed and spread an alert on October 26th 2021 [6, 7].

We describe ten cases of *Chlorophyllum molybdites* in-

toxication registered in the last 2 years (2020-2021) by Pavia Poison Centre (Pavia-PC).

CASE SERIES

Ten patients were studied, 2 females and 8 males (age ranging from 10 to 58 years old). Demographic data, clinical manifestation and treatment details are summarized in *Table 1*.

All the patients had in common the consumption of mushrooms considered as *Macrolepiota procera*, prepared with different cooking procedures or consumed raw, but after a latency between 30 minutes and 7 hours, they developed severe gastrointestinal syndrome, characterized by abdominal cramps, vomiting, and diarrhoea. All of them were taken to the local Emergency Department (ED), where they received fluids and gastrointestinal decontamination.

In particular, three patients (a family cluster of 2 adults and a child) were treated with the Pavia Protocol in the suspect of intoxication from mushroom containing amatoxins, because of the persistence of gastrointestinal syndrome after 12-24 hours since ingestion and in a case of late onset of symptoms (more than 6 hours). The treatment consists in gastric lavage, activated charcoal (AC), 30 g in adults and 10 g in child, followed by multiple doses activated charcoal (MDAC) 5 g every 2 hours, N-acetyl cysteine (NAC: 150 mg/kg in 90 minutes, then 300 mg/kg/day – i.v.), cathartics and fluids to force diuresis [8]. All the patients reached fully recovery and they were discharged from hospital within two days.

DISCUSSION

Chlorophyllum molybdites, also known as *Lepiota morganii* and commonly named "false parasol" or "green-

spored lepiota", is a mushroom that belongs to the *Agaricaceae* family, considered non-edible in many countries. Mature specimens are recognized by green spores and gills, the flesh is firm and white, the cap measures 7-30 cm, is broad, convex, knobbed or flat usually white and brown spotted, the stalk is 10-25 cm long and 10-25 mm thick. Younger specimens are usually white and the cap is button shaped. Colonies assume a ring-configuration and characteristically found in meadows and lawns, typically at the end of summer and autumn [4].

Currently the toxic mechanism remains unknown. In the past, authors agreed to consider at the base of the *Chlorophyllum molybdites* toxicity a polymeric protein with a molecular weight greater than 400 kDa and a monomer size between 40-60 kDa; specifically, the experiment from Eilers and Nelson evidenced that this polymer was thermolabile and degraded from pepsin and acid [9]. In 2012 Yamada *et al*. discovered a small protein, homologue of metalloendopeptidase, named "Molybdophyllysin" (23 kDa), that may correspond to the monomer reported by Eilers and Nelson, that seems to be responsible of the toxicity in animal model, in particular it was evidenced lethal in mice. It is able to develop its proteolytic activity in a pH range between 4.0-11.0 and maintain thermostability after heating for 10 minutes at various temperatures between 30-70 °C (86-158 °F) [10]. In the majority of the cases followed by Pavia-PC, the gastrointestinal symptoms broke out after a meal with cooked mushrooms, prepared with different cooking times, so it is likely that a thermo-

stable toxin is responsible of the human gastroenteric syndrome.

Often the mycologists have to recognize mushrooms from cooked residues or the spores in digestive samples. Recently, Wang and co-authors described a loop-mediated isothermal amplification (LAMP) assay to help in the visual identification of *Chlorophyllum molybdites*: a set of specific primers were designed and tested against the target and other 43 different mushroom species to define specificity; LAMP method is able to identify the presence of traces of *Chlorophyllum molybdites* in boiled and digested samples with high specificity and sensibility [11].

From a clinical point of view, *Chlorophyllum molybdites* is classified as a gastrointestinal irritant mushroom [1]. The primary symptoms seen are gastrointestinal discomfort, characterized by abdominal pain, cramps, nausea, vomiting, and severe diarrhoea (initially watery, then may become bloody), accompanied by profuse sweating. In isolated cases, autonomic nervous system effects can cause altered perception, dilated/pinpoint pupils, blurred vision, dizziness, lacrimation, salivation, hypotension and tachycardia. ECG abnormalities and T-wave inversion have occurred. Hematologic abnormalities can also be present with bleeding and a condition similar to disseminated intravascular coagulation (DIC). Prolonged dehydration following gastrointestinal symptoms may lead to acute renal insufficiency and electrolyte imbalance [1, 4]. The clinical manifestation may cause also hypovolemic shock, requiring intensive support with fluids resuscitation, endotracheal intuba-

Table 1
Summary of case series from Pavia Poison Centre, in which *Chlorophyllum molybdites* was identified

Age Sex	Cooking method	Latency a: meal - symptoms b: symptoms - hospital	GI symptoms	FL	GL	AC	MDAC	WBI	NAC	Hospital stay
28, M	*Risotto*	a: 2 hours b: 6 hours	+	✓						5 h *Self-discharged*
52, M	Grilled	a: 2 hours b: 6 hours	++	✓	✓	✓		✓		1 day
50, M	Raw	a: 2.5 hours b: 6 hours	++	✓	✓	✓		✓		2 days
56, M	Pan cooked	a: 5 hours b: 9 hours	+++	✓		✓		✓		2 days
58, M	Pan cooked	a: 5 hours b: 9 hours	++	✓		✓		✓		2 days
47, F*	Pasta sauce, baked	a: 4 hours b: 7 hours	+++	✓	✓	✓	✓	✓	✓	2 days
10, M*	Pasta sauce, baked	a: 4 hours b: 7 hours	+++	✓	✓	✓	✓	✓	✓	2 days
47, M*	Pasta sauce, baked	a: 7 hours b: 7 hours (long latency)	+++	✓	✓	✓	✓	✓	✓	2 days
42, M	Pasta sauce	a: 3 hours b: 8 hours	++	✓	✓	✓		✓		1 day
38, F	Pasta sauce	a: 3 hours b: 8 hours	++	✓	✓	✓		✓		1 day

*: family cluster.
GI symptoms: abdominal pain, nausea, vomiting, diarrhoea; +: mild; ++: moderate; +++: severe.
FL: fluids; GL: gastric lavage; AC: activated charcoal; MDAC: multiple doses activated charcoal; WBI: whole bowel irrigation; NAC: N-acetyl cysteine.

tion and dopamine drip, especially in paediatric population [12]. The onset of symptoms typically varies within 30 minutes and 2 hours. In some cases, symptoms may not appear for up to 4 hours, which may hide a delayed onset syndrome. Generally, symptoms resolve spontaneously in 6-24 hours, only with supportive care [1, 4, 5].

The cases in which Pavia-PC was involved, had in common the consumption of mushrooms, considered *Macrolepiota procera*, but after a variable latency (30 minutes - 7 hours) they developed severe gastrointestinal syndrome, characterized by abdominal cramps, vomiting, and diarrhoea. All of them were taken to the local Emergency Department, where they received fluids and gastrointestinal decontamination. Three patients underwent the Pavia Protocol in the suspect of ingestion of mushroom containing amatoxins: a prompt mycologist identification of *Chlorophyllum molybdites* permitted the hospital discharge in day 2. A complete recovery followed. The correct identification of the mushroom involved is crucial to evaluate the severity of the clinical hallmark and subsequently to apply the appropriate medical treatment. As general advice, mycological identification before the consumption of picked mushrooms should be always carried out, even if they are collected by expert picker, to avoid the ingestion of non-edible species. Additional problem in the management of suspected mushroom poisoning for long latency was the fact that in whole Sicily, but also in other Italian regions, it is difficult to determine urinary alfa-amanitin because, currently, only a few laboratories are able to test it. This test is essential to confirm intoxication but result more useful to exclude the potential intoxication due to lesional toxins such as alfa-amanitin.

CONCLUSIONS

Chlorophyllum molybdites is an "alien" widespread mushroom in Eastern Sicily, that may even disseminate in Europe. It could be confused with both edible and poisonous species. It is responsible of a severe gastrointestinal syndrome with abdominal pain, nausea, vomit and diarrhoea; secondary dehydration could cause renal failure, electrolytes imbalance and hypovolemic shock in paediatric and fragile patients. It is important to consider the presence of *Chlorophyllum molybdites*, especially when patient referred the ingestion of single "parasol mushroom", to frame and treat the gastrointestinal syndrome in the right way. Cooperation between ED physician, clinical toxicologist and mycologist is fundamental for this purpose.

It should be important to improve the availability of laboratory tests over the national territory to permit to diagnose the presence of amatoxins and distinguish even in early stages, where is necessary to extend therapy over the symptomatic treatment.

Climate changes may seriously influence the spread of non-endemic species, currently considered tropical or subtropical, also in Mediterranean areas of Europe, a valid example of this is what is happening in Sicily with *Chlorophyllum molybdites*. In addition, migration flows may introduce different food customs. So, in front of a mushroom intoxication physicians should always contact mycological department to reach the correct identification and not consider only the old known illnesses, but also be alert on these emerging syndromes.

At the same time, it is fundamental to made aware the population about the risk correlated to the consumption of unknown mushrooms, improving local information about the kind of species diffused on the territory and the mycological services available.

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Book Reviews, Notes and Comments

Edited by
Federica Napolitani Cheyne

L'INVENZIONE DELLA NATURA
Le avventure di Alexander Von Humboldt, l'eroe perduto della scienza
Andrea Wulf
Roma: Luiss University Press;
2017.
544 p.
ISBN 978-88-6105-262-8
€ 20.00

[The invention of nature. Alexander von Humboldt's new world]

L'INVENZIONE DELLA NATURA SELVAGGIA
Storia di un'idea dal XVIII secolo a oggi
Franco Brevini
Torino: Bollati Boringhieri;
2013.
438 p.
ISBN 978-88-339-2355-0
€ 28.00.

[The invention of wild nature. History of an idea from the XVIII century to today]

Biomedical issues such as wellness "wellbeing", "psychosocial stability" and psychophysical resilience are fruitfully emerging and progressively penetrating in the biomedical literature. Strictly biomedical issues, such as the recognized role of Volatile Organic Compounds (VOCs) are populating a number of high-standard scientific journals [1]. Overall, the perspective considers the human brain (and the human mind) as a product of a Darwinian evolution process, therefore necessitating a constant, regular and well-balanced contact and immersion within a natural environment. Those environments, representing the ecological niche in which *Homo sapiens* evolved from pre-human ancestors, need to be defined and characterized. In other words, which are (qualitatively and quantitatively) the stimulus sets constituting a "natural environment" for the human mind? Are contemporary urban environments insufficient to provide the expected degree of natural stimulation?

How and when full (or partial) immersion within a natural environment is relevant for people affected by mental suffering, patients having to cope with frank, overt mental pathologies, babies, children and adolescents passing through delicate and fragile ontogenetic stages? Also, other subjects not endowed by sufficient resilience mechanism are potential targets in such an analytic framework.

However, the definition of a "natural environment" had a very long and even tortuous history. Philosophical perspectives and historical reconstruction may both help experts, scientists and a part of the biomedical readers dealing with human mental suffering [2]. These two books represent valid and well-written volumes, among the classical references to enter these delicate matters, going into the long-lasting debate on nature\ culture determinants of human behaviour and psychophysical wellbeing.

Wulf's book *The invention of nature. Alexander von Humboldt's new world* (the author is a professional historian and writer, and this volume represents his most prestigious success) is focused on the pivotal figure of Alexander von Humboldt, an adventurous explorer described from some of his contemporaries devoted to him as "the most celebrated man after Napoleon", as reported in the Prologue. This brilliant introduction acts as initial framework for the entire book. One of the explanatory avenues, a basic framework of the volume, exploits the relationship between Humboldt and other cultural pilasters, in order to arrange specific chapters on selected themes: Simon Bolivar & Humboldt (Revolution and nature), Charles Darwin & Humboldt (Evolution and nature), Henry David Thoreau & Humboldt (Poetics, science and nature), George Perkins Marsh & Humboldt (Man and nature), Ernst Haeckel & Humboldt (Heart ecology and nature) and finally John Muir & Humboldt (Protection and nature). A suggestion for readership is chapter XVIII (The Cosmos of Humboldt).

The second volume in this review, *The invention of wild nature. History of an idea from the XVIII century to today* (in Italian) signed by Brevini, a recognized expert of dialectal poetry, also a very passionate and experienced mountaineer, enlists a series of relevant paragraphs: Domination of nature or participation?; The industrial revolution and the concept of wilderness; An agreeable kind of horror; The esthetics of sublime; The Alps and the Arctic lands; Life in the woods and the myth of the Wild; Wild nature and the mass culture.

Some other paragraphs do represent somehow peppered contributions of a never-ending debate on nature/ culture, such as those on environmental ethics (a newborn discipline), primitivism and modernity (of course, given the format of the book, it may inevitably result in a rather superficial scrutiny) and "Grizzly man", the latter under a zoo-anthropological perspective, inevitably opening a window on the human "fixed action

patterns" and the ethological framework investigating human behavioural patterns. The book provides an exhaustive and well selected reference list.

Overall, for a portion of the biomedical readership interested in understanding the epistemological transformation of the term "natural", these books may result of some utility. This applies mainly to those whose research and/or social lives deal with (human and animal) mental suffering. A recent book review, also provides some hints in the same perspective [3]. The use and abuse of the term "green" and "natural" presently seem to be in need of a better definition, which of course includes boundaries and limitations.

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LO SPLENDORE CASUALE DELLE MEDUSE

Judith Schalansky
Traduzione di
Flavia Pantanella
Original title: *The giraffe's neck*
Milano: Nottetempo Ed; 2013
258 p.
ISBN 978-88-7452-4198
€ 16,50

[*The random splendor of jellyfish*]

The book softly proposes three main pathways to the reader.

The first is addressed to educators in the biomedical field since it proposes a magnificent pondering on the ultimate sense of the technical and personal skills required by a biologist teacher, Professor Inge Lohmark (a character of the book), willing to provide a solid education to her adolescents pupils. She is a total failure, with her rigidity and anger towards her young students. She proposes a spoiled narrative of biological and biomedical topics; she looks at her class as a kind of ever-competing young males and females, despite the fact that she nearly hates the most talented ones. At the very end of the book she is almost expelled by her decadent school. The book testifies the silent battle between Prof. Lohmark and her pedagogical opposite Prof. Schwanneke, arts teacher, who approaches the students in a totally different way. In the school corridor, the two women "fight" on how to position artistic manufacts and posters with scientific schemes. Eventually, on the same wall, a Monet's painting is positioned close to a Haeckel's jellyfish illustration (wherein the Italian title of the book).

The second plan is the most succeeded interpretation which has to do with her inflexible Darwinian reading of any living event. With a strict sociobiological approach she attempts to interpret the individual personality, the social skills, the diverse effects of the various socio-economical environments to explain the different pathways of each of her pupils. Moreover, all the natural scenarios, the main cultural and socio-economical constraints of the area where the school is located are interpreted as a stereotyped selection for the survival of the fittest. Not coincidentally, this gymnasium is entitled to Charles Darwin himself. These parts, which permeate the entire volume, are very instructive about a few current socio-economical and ecological styles of analysis, yet soaked by a profound reductionist thought.

A third, historical reflection emerging from this nice and brilliant book, regards the quite recent changes occurred in Germany. The school, in the former teaching institution belongs to the East Germany. Despite the profound changes occurred in the meanwhile, Professor Lohmark is still looking for analogies somehow embedded into the mind of mature professors and overall in the architecture of the building. It is in fact a classical Eastern-European construction.

The Italian translation of this volume has been awarded the Literary prize "Giuseppe Acerbi" in 2021, among others. The translator Flavia Pantanella made a really magnificent job, dealing with a particularly difficult narrative style, which intertwines short phrases with diverse perspective inputs: a kind of mixture between biology, psychology, ecology, human and animal sociology, and so on.

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Publications from International Organizations on Public Health

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)

Framework for Action on Biodiversity for Food and Agriculture. Rome: Food and Agriculture Organization of the United Nations 2022; 56 p. ISBN 978-92-5-135643-2. Biodiversity for food and agriculture comprises all the components of biodiversity that contribute to crop and livestock production, forestry, fisheries and aquaculture – both wild and domesticated, and at genetic, species and ecosystem levels. It is vital to food security and nutrition, livelihoods and the supply of a range of ecosystem services. The Framework for Action on Biodiversity for Food and Agriculture was endorsed by the FAO Council in 2021. It contains more than 50 individual actions grouped into three strategic priority areas: characterization, assessment and monitoring; management (sustainable use and conservation); and institutional frameworks. It was developed in response to the country-driven report on The State of the World's Biodiversity for Food and Agriculture.

Schindler C, Heral E, Drinkwater E, Timoshyna A, Muir G, Walter S, Leaman DJ. and Schippmann, U. Wildcheck – **Assessing the risks and opportunities of trade in wild plant ingredients**. Rome: Food and Agriculture Organization of the United Nations 2022; 140 p. ISBN 978-92-5-135965-5. Thousands of consumer products around the world contain ingredients obtained from wild plants. Wild harvest accounts for some or all the harvest of the great majority of plant species in trade (between 60-90 percent). Wild-harvested plants often come from the most biodiverse ecosystems on earth and many have been used traditionally or by local communities for generations. While these products have global markets and provide critical sources of income, they can also have deep ties to particular cultures and places. Demand for wild plant ingredients is growing rapidly, having grown by over 75 percent in value over the past two decades. Thousands of harvested species are at risk mainly from a combination of overharvest and habitat loss: of the 21 percent of medicinal and aromatic plant species whose threat status has been assessed, 9 percent are considered threatened with extinction. Despite their ubiquity, importance, and the threats facing them, wild plant ingredients are often obscured from consumers and escape companies' due diligence due to a lack of awareness and traceability. Best practice standards exist but have yet to capture a significant portion of the market. This report aims to address these challenges by making information on a selection of "flagship" wild

plant ingredients, the Wild Dozen, readily available and easy to understand. By offering this information without obligation to a specific prescription for follow-up action (e.g., through certification or policy change), it is hoped that a wide range of users will access the report as a first step towards responsible sourcing. Along with a broader update on the state of wild plants trade, the report provides a "profile" on each of the Wild Dozen species, summarising key facts on production and trade. Each profile contains a traffic-light risk rating on biological and social factors, along with an overview of opportunities for responsible sourcing. The information is aimed at industry, consumers, policy-makers, investors, and practitioners, concluding with a summary of what these various stakeholders can do to contribute to a sectoral shift towards responsible sourcing of wild plant ingredients.

Food Outlook – Biannual Report on Global Food Markets. Rome: Food and Agriculture Organization of the United Nations 2022; 174 p. ISBN 978-92-5-136028-6. In view of the soaring input prices, concerns about the weather, and increased market uncertainties stemming from the war in Ukraine, FAO's latest forecasts point to a likely tightening of food markets in 2022. Meanwhile, the global food import bill is on course to hit a new record high of USD 1.8 trillion, an all-time high, almost entirely on account of higher prices. Issued twice a year, Food Outlook offers FAO's reviews of market supply and demand trends for the world's major foodstuffs, including cereals, oil crops, sugar, meat and dairy and fish. It also looks at trends in futures markets and shipping costs for food commodities. The new edition also contains two special chapters examining the role of rising prices for agricultural inputs, such as fuel and fertilizers, and the risks the war in Ukraine poses for global food commodity markets. Food Outlook is published by the Markets and Trade Division of FAO as part of the Global Information and Early Warning System (GIEWS).

UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION (UNESCO)

The United Nations World Water Development Report 2022 groundwater: making the invisible visible. Paris: UNESCO Publishing 2022; 225 p. ISBN 978-92-3-100507-7. Accounting for approximately 99% of all liquid freshwater on Earth, groundwater has the potential to provide societies with tremendous social,

economic and environmental benefits and opportunities. Groundwater already provides half of the volume of water withdrawn for domestic use by the global population, including the drinking water for the vast majority of the rural population who do not get their water delivered to them via public or private supply systems, and around 25% of all water withdrawn for irrigation. However, this natural resource is often poorly understood, and consequently undervalued, mismanaged and even abused. Groundwater is central to the fight against poverty, to food and water security, to the creation of decent jobs, to socio-economic development, and to the resilience of societies and economies to climate change. Reliance on groundwater will only increase, mainly due to growing water demand by all sectors combined with increasing variation in rainfall patterns. The report describes the challenges and opportunities associated with the development, management and governance of groundwater across the world. It aims to establish a clear understanding of the role that groundwater plays in daily life, of its interactions with people, and of the opportunities for optimizing its use in order to ensure the long-term sustainability of this largely available yet fragile resource.

JOINT UNITED NATIONS PROGRAMME ON HIV/AIDS (UNAIDS)

Integration of mental health and HIV interventions. Key considerations. Geneva: Joint United Nations Programme on HIV/AIDS and the World Health Organization 2022; 92 p. ISBN (WHO) 978-92-4-004317-6 (electronic version) ISBN (WHO) 978-92-4-004318-3 (print version) ISBN (UNAIDS) 978-92-9253-092-1. This publication emphasizes the importance of integrating HIV prevention, testing, treatment and care; mental health services and care for people living with HIV and key and other vulnerable populations, including linkages to social protection services. It provides a compilation of tools, best practices and guidelines that facilitate the integration of interventions and services to address the interlinked issues of mental health and HIV. Although focus is on integration of mental health with HIV services, the considerations may be relevant to other services, including HIV comorbidities such as tuberculosis, viral hepatitis and sexually transmitted infections. This publication is primarily intended for national and local policy-makers; global, regional, country and local programme implementers; organizations working in and providers of health, HIV, mental health and other relevant services; civil society; and community-based and community-led organizations and advocates. It brings together and refers to existing HIV and mental health, psychosocial support and other service provision guidelines, recommendations and tools, including the World Health Organization (WHO) Mental Health Gap Action Programme (mhGAP) mhGAP intervention guide and mhGAP operations manual; WHO, United Nations Office on Drugs and Crime (UNODC) and other guidelines and

tools related to substance use; and WHO consolidated guidelines, tools and resources on HIV testing, prevention, treatment and care.

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD)

Healthy Eating and Active Lifestyles: Best Practices in Public Health. Paris: OECD Publishing 2022; 367 p. ISBN 9789264942134 (EPUB) ISBN 9789264430495 (HTML) ISBN 9789264472921 (PDF). This report is part of the OECD's work on promoting best practices in public health in OECD and EU27 countries. It aims to help countries improve their response to high rates of overweight by examining the potential to scale-up and transfer best practice interventions. Interventions included in the report range from those targeting individual behaviour, such as lifestyle counselling programmes, to those that change the environment in which people live, such as community-based programmes. Examinations involved an assessment of the intervention against validated best practice criteria outlined in the OECD Guidebook on Best Practices in Public Health. The set of criteria includes effectiveness, efficiency, equity, the quality of the evidence-base, and the extent of coverage, as well as an assessment of the intervention's potential to be transferred to another region. Drawing upon key findings from an examination of selected interventions, this report outlines five ways policy makers can improve their response to high rates of overweight and obesity: Create comprehensive policy packages that include interventions covering a range of settings – such as schools, primary care and the community – as well as population groups; target the needs of disadvantaged groups such as those with a lower socio-economic status; boost participation in weight reduction programmes using several strategies; adequately resource transfer and scale-up and provide incentives that strengthen evidence-based research.

INTERNATIONAL LABOUR ORGANIZATION (ILO)

The future of diversity. Geneva: International Labour Organization 2021; 285 p. ISBN 978-92-2-031962-8 (print) ISBN 978-92-2-031963-5 (web PDF). In this collection, scholars and practitioners reflect on the most appropriate interventions to create a more inclusive labour market for all. They explore the economic case for diversity and diversity management strategies, finding that diversity and inclusion must go hand in hand. The book also sheds light on the policy dilemma between respecting individuals and countering structural inequalities, which often requires categorization into groups. The authors remind us that there is diversity within diversity: not everyone receiving the same label has the same needs. The book covers a range of issues including gender equality and mainstreaming, migration and ethnic diversity, racism, violence against

LGBTI people and age discrimination. It is thus a rich source of inspiration for anyone wishing to move towards greater justice in the labour market.

WORLD HEALTH ORGANIZATION (WHO)

WHO guidance on the ethical conduct of controlled human infection studies. Geneva: World Health Organization 2021; 106 p. ISBN 978-92-4-003781-6 (electronic version) ISBN 978-92-4-003782-3 (print version). This guidance has been developed in response to requests to the World Health Organization (WHO) for guidance on ethical questions associated with controlled human infection studies (CHIS), especially in the context of growing interest in conducting CHIS in endemic settings. This document aims to provide guidance to scientists, research ethics committees, funders, policy-makers, and regulators in deliberations regarding the design, conduct and governance of controlled human infection studies. This guidance aims to inform well-considered and contextualized decisions about the ethical acceptability of proposed CHIS, including priorities for engagement and social science research to support deliberation and practice, and requirements for oversight and governance. In addressing ethical issues that should be considered during the planning, design, conduct and governance of CHIS, this guidance takes the position that CHIS are not, in themselves, an exceptional and morally distinct form of research, but instead fall within the continuum of health-related research conducted with human participants. When applying this guidance, stakeholders involved in CHIS are encouraged to develop approaches that take into account their own local social, cultural, and political contexts. In addition to the guidance itself, included in the annexes are essential information on consent (Annex 1), a checklist for ethics committees (Annex 2) and eight case studies (Annexes 4 to 11), all designed to further assist with the implementation of this guidance.

World mental health report: transforming mental health for all. Geneva: World Health Organization 2022; 296 p. ISBN 978-92-4-004933-8 (electronic version) ISBN 978-92-4-004934-5 (print version). The World mental health report: transforming mental health for all is designed to inspire and inform better mental health for everyone everywhere. Drawing on the latest evidence available, showcasing examples of good practice from around the world, and voicing people's lived experience, it highlights why and where change is most needed and how it can best be achieved. It calls on all stakeholders to work together to deepen the value and commitment given to mental health, reshape the environments that influence mental health, and strengthen the systems that care for mental health. While promoting a multisectoral approach, this report is especially written for decision-makers in the health sector. This includes ministries of health and other partners in the health sector who are generally tasked with developing mental health policy and delivering mental health systems and services.

Global status report on blood safety and availability 2021. Geneva: World Health Organization 2022; 172 p. ISBN 978-92-4-005168-3 (electronic version) ISBN 978-92-4-005169-0 (print version). The Global Database on Blood Safety (GDBS) reports important data from many countries on a range of indicators covering the transfusion chain from donor to recipient, as a basis for more informed discussion on the provision and governance of blood transfusion services. This report provides the status and progress of countries in developing nationally-coordinated and well-managed national blood transfusion services. It reports on global and regional trends in blood collection and transfusion during the period 2008-2018, and provides an update on the status of WHO Member States in making quality-assured plasma available for fractionation of plasma-derived medicinal products (PDMPs) to meet the treatment needs of patients.

Global report on infection prevention and control. Geneva: World Health Organization 2022; 148 p. ISBN 978-92-4-005116-4 (electronic version) ISBN 978-92-4-005117-1 (print version). The WHO global report on infection prevention and control (IPC) provides a global situation analysis of how IPC programmes are being implemented in countries around the world, according to evidence from the scientific literature and various reports, and new data from WHO studies. It also highlights the harm to patients and health workers caused by health care-associated infections and antimicrobial resistance, addresses the impact and cost-effectiveness of IPC programmes and the strategies and resources available to countries to improve them. Primarily, this document targets those in charge of making decisions and formulating policies in the field of IPC at the national, subnational and facility levels. This includes policy-makers, senior managers, administrators who are managing health budgets, and IPC focal points at national level (Ministry of Health, public health institutes, etc.), and subnational and health care facility levels.

Instructions to Authors

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Articles in journal

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

Books and chapters in a book

Godlee F, Jefferson T. Peer review in health sciences. London: BMJ Books; 1999.

Van Weely S, Leufkens HGM. Background paper: orphan diseases. In: Kaplan W, Laing R (Eds). Priority medicines for Europe and the world – a public health approach to innovation. Geneva: World Health Organization; 2004.

Proceedings

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

Technical reports

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

Legislation

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

US Social Security Administration. Evidentiary requirements for making findings about medical equivalence. Final rules. Fed Reg. 2006 Mar 1;71(40):10419-33.

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