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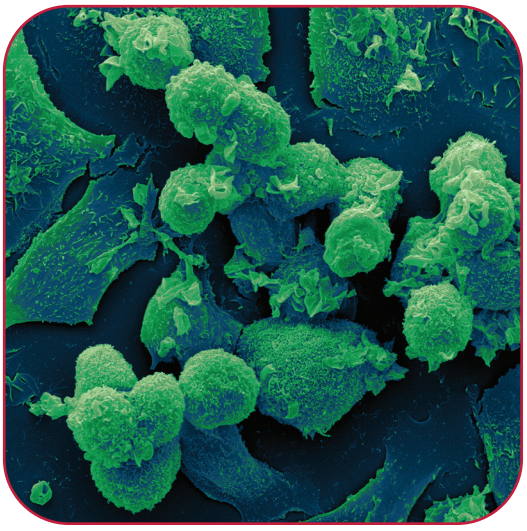

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LETTER
Legionella pneumophila and SARS-COV-2 co-infection: the importance of laboratory diagnosis

Key words
- *Legionella pneumophila*
- SARS-COV-2
- co-infections

To the Editor

From the onset of the pandemic in Italy, February 2020 to 15 February 2021, over 2.6 million confirmed SARS-COV-2 infections were reported with a case fatality rate of 3.4% [1]. Legionnaires' disease (LD) is a mandatory notifiable disease in Italy and according to the national LD surveillance, in the same period, 25 cases of *Legionella pneumophila* and SARS-COV-2 co-infection, requiring hospitalization, were reported, seven of which have died. All cases were confirmed by urinary antigen test (UAT), except two cases, classified as probable LD cases according to EU case definition [2]. Median age was 72.1 years (range 37-93); male/female ratio 2.1:1.

According to epidemiological investigation and surveillance data, among the 25 LD cases, eight had underlying diseases, e.g., chronic heart and kidney diseases, chronic obstructive pulmonary disease, obesity and lung microcitoma. As for the possible setting of infection, four LD infections were of nosocomial origin in patients hospitalized for COVID-19; two LD cases, which were part of a 13-case community cluster detected in October 2020, acquired COVID-19 while hospitalized for LD; one case was travel-associated and one was associated with an elderly nursing home. For the remaining 17 cases who were admitted to hospital with pneumonia symptoms and tested positive for both infections, the possible setting was unknown, so it was impossible to ascertain which of the two infections was acquired first.

In 2019, in Italy, 3199 LD cases were reported (incidence rate 52.9 cases/million), with a case fatality rate of 11.2% [3], while in 2020, preliminary data show a 35% decrease in the number of reported cases. Travel associated LD usually represent 10% of the total annual cases. The substantial decreasing trend in LD cases is, therefore, not justified by the decreased number of travel-associated LD cases, due to travel limitations in response to the COVID-19 pandemic. Vice versa, the lockdown relaxation and the reopening of buildings and other community facilities, after months of closure, could have allowed the growth of *Legionella* in plumbing systems and led to an increase in cases [4]. The significant decreased number of cases could rather be explained by under-diagnosis. Physicians may have repeatedly tested patients with pneumonia symptoms for SARS-CoV-2, neglecting to test them for *Legionella*.

From the ongoing COVID-19 pandemic we have learnt that LD and COVID-19 clinical manifestations may be indistinguishable and that the incubation period is similar in both diseases [5]. However, LD can be treated with targeted and effective antibiotics such as fluoroquinolones or macrolides, and, therefore, differential diagnosis is essential to prevent fatal or severe outcomes. UAT is a quick and relatively inexpensive test, based on easily collected specimens providing results even after starting antibiotic therapy. Testing hospitalized patients with severe pneumonia, especially vulnerable subgroups (e.g., elderly or those with co-morbidities), for *Legionella* and for SARS-COV-2 would allow to make both a differential diagnosis and the assessment of the impact of the two infections. The detection of LD or SARS-COV-2 co-infection is also extremely important from a public health perspective, as LD fatality rate is higher than COVID-19 fatality rate and LD prevention and control measures are available and can be effectively adopted.

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COMMENTARY

Challenges on the achievement of World Health Organization goals for HCV elimination in Italy: need for a Regional programmatic approach on screening and linkage to care

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Abstract
Italy has been one of the countries with the greatest burden of HCV in Western Europe and with the highest number of HCV liver-related deaths. In order to achieve HCV elimination by 2030 Italy, like many other countries, will need to succeed in tackling the undiagnosed individuals with active HCV infection. To this aim beginning in 2021, a nationwide action has been implemented, consisting of the performance of screening tests among key populations and birth cohorts (1969-1989), estimated to have a high prevalence of undiagnosed individuals. The realization of the proactive screening during the first two years will define the tracks for the whole optimized screening strategy, including also the screening of 1948-1968 birth cohorts, reported to be the best cost-effective strategy in achieving the HCV elimination targets by 2030 in Italy. Each Italian region needs to define the present and future steps to reach HCV elimination goal by 2030 guaranteeing the equity of care.

Key words
- hepatitis C
- infection
- WHO elimination goal

In 2015 Viral Hepatitis C (HCV) infection was acknowledged as a global health and development priority signed up to 17 Sustainable Development Goals. Viral hepatitis was included as a focus area in the health related goal – Goal 3.3. In response, the World Health Organisation (WHO) drafted the Global Viral Hepatitis Strategy which was then subsequently adopted in 2016 by all Member States. The world leaders will be pledging to "combat" viral hepatitis infection as a global health threat through ambitious targets which if reached will reduce the number of deaths by 65% and increase treatment rates up to 80% by the year 2030 [1]. Stakeholders from each country are compelled to define Nation Hepatitis Plan, including specific roadmaps to achieve the WHO elimination goals for chronic HCV infection.

Italy has been one of the countries with the greatest burden of HCV in Western Europe and with the highest number of HCV liver-related deaths [2, 3]. Thanks to health policies that permitted the universal use of direct acting antiviral drugs (DAAs) for all HCV diagnosed patients, using a dedicated fund for innovative non-oncological drugs, since 2017 Italy has been on track for HCV elimination. However, recent evaluations have reported that due to the declined number of the treated patients, starting since 2019 and continuing during the year 2020-2021, due to the COVID-19 pandemic [4, 5], Italy will not be able to achieve the HCV elimination goals. Up to now, more than 220,000 patients have been treated with DAAs, the remaining are estimated at least 280,000 individuals unaware of their HCV active infection [2, 6]. HCV elimination in Italy will be possible if immediate action is taken now. Italy has drafted a National Hepatitis Plan and the State, continuing its

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efforts to make the health system more sustainable, needs to strengthen its role in addressing and verifying regional health systems to guarantee the equity of care based on guiding principle that no one will be left behind. Italy is divided into twenty regions and under the Italian Constitution, each region is an autonomous entity with defined powers. Based on broad discretion in planning, organizing, and financing health care services within their territory each region needs to identify the objectives and strategies to define the present and future steps to reach HCV elimination goal by 2030. In particular, the regional roadmaps to HCV elimination need to address the following key points.

- In order to achieve HCV elimination by 2030 Italy, like many other countries, will need to succeed in tackling the undiagnosed individuals with active HCV infection. To this aim beginning in 2021, a nationwide action has been implemented, consisting of the performance of screening tests among key populations and birth cohorts (1969-1989), estimated to have a high prevalence of undiagnosed individuals [7, 8]. The active screening offer requires a regional governance that manages the complexity of the processes integrating a well-organized network between territory assistance and hospital with the goal of an effective HCV care cascade. The realization of the proactive screening during the first two years is important because it will define the tracks for the whole optimized screening strategy, including also the screening of 1948-1968 birth cohorts, reported to be the best cost-effective strategy in achieving in Italy the HCV elimination targets by 2030 [8].
- Emphasis will need to shift from treatment of diagnosed patients to screening of infected, but undiagnosed individuals according to optimized diagnostic and care pathways. A substantial loss of patients between each step from diagnosis to cure of infection has been observed in different populations [9, 10]. In the context of scarce healthcare resources, information on impact and real-world affordability of different diagnostic pathways, that guarantee substantial increases in diagnoses for entry into the treatment cascade is crucial. The traditional diagnostic algorithm approach is based on HCV Antibody (HCV-Ab) and HCV Ribonucleic Acid (RNA) assays which both have very high sensitivity and specificity, making false-positive and false-negative results rare occurrences. However, this traditional approach, that include several steps in the diagnostic process, often lead to incomplete diagnosis and miss of cure opportunity. Simple rapid test using saliva or capillary test have been widely used to detect the presence of HCVAb with particular regard in key populations and has shown to reduce time between the initial observation and treatment administration [11]. However the real challenge of the traditional or rapid antibody detection approaches is the need for two or more steps for the diagnosis of active infection which has been shown to complicate the patient journey and as consequence to increase the number of patients lost before receiving treatment. The cheap price of HCV Ab capillary tests is an attractive option for Regional stakeholders. However, this approach, though attractive for outreach first step of screening for special populations, subsequently requires referral for conventional phlebotomy confirmation of active infection and was the less cost-effective option out of different screening pathways analysed. Based on cost effectiveness data in the Italian setting, the most cost effective diagnostic approach for general population screening is "Reflex testing" which means that HCV-RNA test should be performed on the same serological specimen with a positive anti-HCV finding in individuals who have never been previously tested for HCV infection [Marcellusi et al EASL Annual meeting 2021 Poster presentation S652 (PO-1374)]. Different solutions may work better in specific clinical settings, but as also suggested by EASL and WHO indications [12, 13] the use of reflex RNA testing for Ab-positive samples, could be considered as preferential diagnostic approach for HCV screening of individuals who are unaware of an HCV infection in each region. It can support increases in diagnosis, the streamlining of diagnosis cascade and subsequent treatment of infected patients. It has been shown that HCV RNA reflex testing is the most cost effective diagnostic approach being also affordable within the dedicated budget for HCV screening in Italy [3, 7].
- In the Services for Dependencies the Screening Ministerial decree indicate the Point-of-Care and Rapid HCV RNA Diagnostic Tests for people who actively use drugs in order to ensure the direct diagnosis and potentially linkage to care of individuals at high risk of infection [3, 7]. This indication should be carefully taken into the consideration in each Italian region in order to avoid the ongoing HCV transmission in drug users who represent the population with the highest infection burden and also at high risk of lost during diagnosis and care cascade, if the diagnosis is delayed.
- Simultaneously with the approval of the Milleproroghe Law decree for HCV free of charge screening in key populations and specific birth cohorts, the dedicated fund for the innovative non-oncological drugs was expired. Although the new HCV screening policies addresses key points for HCV elimination and a specific fund for HCV screening has been released for each Italian region, the lack of a dedicated fund for the DAAs would stress the regional budget. Considering that more than 20% of treated patients in 2019 had cirrhosis or advanced liver fibrosis and a similar prevalence of the advanced disease has also been estimated for undiagnosed individuals, DAAs should be considered life-saving drugs. The investment in treating newly diagnosed patients was translated into a significant reduction of liver disease complications with great economic benefits [14]. The evidences produced by National Center for Global Health of Istituto Superiore di Sanità and Center for Economic Evaluations of Tor Vergata University of Rome demonstrated a high cost benefits of treating patients diagnosed by screening. For 10,000 standardized treated patients diagnosed through an active HCV screening, over a 20-year time horizon there are

7,769 avoided events of progression which are associated with € 838.73 million net savings accrued by the Italian NHS. The initial investment in treatment is recouped in the form of savings from disease complications avoided in 4.3 year [15]. Investing in the immediate DAA treatment of individuals with active infection means improving health and having an economic return for NHS in the short to medium term [3, 14]. These evidences are helpful for the ongoing central and regional decision-making process. Establishing an *ad hoc* fund for the DAA treatment for each Italian region binding resources both for case finding by active screening and treatment, within the National Plan for the Prevention and Treatment of Hepatitis C is of paramount importance to keep Italy on track to achieve the WHO elimination targets by 2030 [3].

- Patients' tailored therapy approach still remain a challenge. The Italian Medicines Agency (AIFA) have recently defined therapeutic equivalence between the pan genotypic DAAs. High efficacy of DAA treatment has been guaranteed up to date by a tailored and simplified therapy evaluated case by case considering specific clinical and socio-behavioural characteristics of treated population. The European Clinical practice guidelines for HCV infection emphasize the need to assess drug-drug interactions prior to starting the DAA therapy. "Prior to starting treatment with a DAA, a full and detailed drug history should be taken including all prescribed medications, over-the-counter drugs, herbal and vitamin preparations and any illicit drug use discussed and documented. The pre-treatment appointment can be used to rationalise prescribing. The pharmacokinetic profiles and how HCV drugs impact key drug-drug interactions and potential listing of drug-drug interactions, are reported in www.hep-druginteractions.org for a list of 800 co-medications" [12]. Based on the evidences of PITER cohort [16], which is one of the most representative Italian real life cohort which includes more than 10.000 patients with chronic HCV infection, enrolled about 60 Italian centers from all geographical macro areas, of patients with F0-F3 fibrosis stage, treated with different DAA regimens, 53-67% reported more than one comorbidity and 40-60 % of them reported more than one co-medication (33-37% of them more than 3 co-medications). As also reported by the PITER cohort over 70% of patients with F4/cirrhosis have reported comorbidities and co medications' use. In 10% of DAA treated patients, changes in the comedications have been required prior or during antiviral therapy. In addition, around 46% of patients treated with specific DAA regimens, careful/monitoring may be required or coadministration is not recommended (category 2 or 3 recommendations on potential DDI in their use with DAA regimens) [17]. Undiagnosed HCV infected individuals in Italy have different age group, disease severity, lifestyle and life's conditions. Drug users, yet to be diagnosed are estimated to be around 150,000 and in many of them different viral coinfections are also present [6]. Of patients from general population, around 100,000 are estimated to have a severe liver damage (F4/cirrhosis) for whom, an immediate linkage to care and a personalized DAA regimen with no or minimal drug-drug interactions is required [6].
- The COVID-19 pandemic will change the delivery of care forever and adjust our approaches to this pandemic, and to other future health demand accordingly. The possibility to eliminate an infection requires health systems to place greater focus on shifting from reactive to proactive care. This has been extensively focused worldwide in fighting SARS-CoV-2 infection. In the field of hepatitis, this includes the capacity to provide greater operational support where this is needed. With specific regard to the achievement of WHO hepatitis elimination goals, all measures that will be put in place during and after COVID-19 pandemic could be transferred in increasing diagnosis and linkage to care of people with hepatitis [14, 18, 19]. COVID 19 vaccination could be used to implement HCV screening, as it has been shown by successful initiatives conducted in different Italian regions.

Conflict of interest statement

No conflict of interest is declared by all the Authors regarding this paper.

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Mortality risk in a population of patients treated for gambling disorders: results of a follow-up study

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Abstract

Aims. To examine mortality risk and causes of death in a cohort of a population of patients treated for gambling disorders in northern Italy from 1992 to 2019.

Methods. Cohort study.

Results. Half of the patients were diagnosed with psychiatric disorders, substance use disorder or alcohol dependence. The excess mortality compared to the general population (SMR) was 1.16 (0.85-1.58), more elevated among females aged 40 to 59 and males aged 20 to 29. Females had higher SMRs for all cancers and suicide; males for malignant neoplasm of liver, of lung, of prostate, and of bladder.

Conclusions. Despite patients increasing, subjects who most turn to the services are the most serious ones, in older age, with comorbid mental disorders and with a compromised health status. This is reflected in the high risk of death for all cancers.

Key words

- gambling disorders
- mortality risk
- suicide
- cancer

INTRODUCTION

Pathological gambling (PG) is characterized by the presence of persistent and recurrent maladaptive gambling behaviour the person is unable to adequately control [1]. Problem gambling is a term used when an individual has developed some problems related to gambling but does not fulfil the PG diagnostic criteria [2].

First recognized in DSM-III [3], PG was classified as a disorder of impulse control in DSM-5 [4] and the disorder was moved to the chapter on substance-related and addictive disorders [5]. To reduce the stigma attached to the term pathological gambling, there is now a tendency to use the term gambling disorders (GD) [6].

There are strong associations between gambling participation and problem gambling [7]. Adult problem gambling prevalence rates worldwide in the past 12 months are mostly within a range of 0.5% and 3.0%, with three to four times as many people reporting subclinical problems and harm [8, 9] and is highly comorbid with other mental disorders [10], substance dependence [11], alcohol dependence [12], and cigarette smoking [13]. In Italy, the estimate of the prevalence of problem gamblers in the general population (15-64 years) was estimated from 0.5% to 2% in 2019 [14]. Furthermore, given the differences in the diagnostic criteria for PG and GDs (as defined in DSM-5), it is possible that future studies could yield slightly higher prevalence rates, as a GD diagnosis requires one less diagnostic criterion than PG [15].

A significant percentage of gamblers develops clinically relevant gambling problems [16], but only 10% seek treatment in clinic-based programs [17]. Many surveys found that approximately half of people classified as "life-time" pathological or problem gamblers did not report experiencing problems currently, implying that a substantial proportion cease having problems [18].

The increasing prevalence of gamblers in recent years [19-21], and the fact that a significant percentage of them do not seek treatment in clinic-based programmes, make it necessary to study morbidity in this population and mortality linked to the emergence of social and health problems. In particular, cohort studies are needed about overall mortality risk and causes of death.

GD are associated with several medical disorders,

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particularly tachycardia, angina, cirrhosis and other liver disease [22], arteriosclerosis and any heart condition [23], chronic medical conditions and obesity [24, 25]. Furthermore, it was reported that PGs suffer from physical symptoms, such as fatigue, headaches, gastric pain, nausea [26], heartburn, and backache [27].

As for suicide, an association between gambling problems and suicidal ideation [28], suicide attempts [29] and suicide completed was found [30, 31] and pathological gambling should be seen as a chronic condition with a similar risk for suicidal ideation and behaviour as with other mental illnesses [32].

As regards the risk of death, there is only one study on general mortality in patients with a GD diagnosis, in which the first cause of death was suicide, followed by all cancers and diseases of the circulatory system [33]. A mortality excess with respect to the general population of 1.8 (95% CI 1.4-2.2) was reported, higher among females and also among subjects aged 29 to 49, more elevated for suicide.

In Italy, PGs can seek treatment in Services Dedicated to Drug Addicts (SERD), in Community Mental Health Centres (CMHC) or in hospital wards. In these three different public settings, treatment is covered by the National Health Service and is voluntary cohorts.

The aim of this study was to examine mortality risk and causes of death in a cohort of patients treated for GD in residents in the Emilia-Romagna region (northern Italy), over a 27-year follow-up period (from 1992 to 2019). Our aims were to estimate overall crude mortality rates and excess mortality by age, gender, substance use disorders, alcohol dependence, and psychiatric diagnoses

METHODS

We selected subject resident in the Emilia-Romagna region, aged over 17 years, referred for the first time to hospitals, SERDs and CMHCs with an ICD-9 (312.31) (ICD: International Classification of Diseases) or ICD-10 (F 63.0) diagnosis of pathological gambling in the period from 01/01/1992 to 31/12/2018. The cases were selected from the IT systems of SERDs (9 units), CMHCs (11 units), and hospitals (10 facilities). Each person may have sought treatment in all these services and the information was collected at the first contact.

Data were retrospectively collected from the various digitalized archives: the PG diagnosis in SERDs could be made at the first contact or in the subsequent periods, in CMHCs and hospitals it could have been concomitant with other psychiatric diagnoses. The date of first access considered in this study refers to the first PG diagnosis.

To be noted that DSM diagnosis was not recorded in the digitalized archives and services did not use ICD-11 codes during the study period.

The cohort was made up of 826 subjects, 20% female, 12% non-native, with an average age at first admission of 47.6 years.

All the variables were drawn from the electronic databases: age, gender, country of birth, residence, diagnoses, services addressed, date of first contact, and of diagnostic assessments.

To identify any other mental disorder, drug dependence, or alcohol dependence in the entire period, the cohort was cross-checked with the electronic data available for all subjects referring, respectively, to the hospitals, the SERDs, and the CMHCs in the metropolitan area of Bologna, and ICD-9 CM psychiatric diagnoses at first admission were included.

Person years (PY) were calculated from the first documented episode to 31 December 2019 or up to the date of death. The patients lost at follow-up were included in person-years until the date they moved out of their last known stable place of residence.

Based on the ICD9 (until 2008) and ICD10 (from 2009) codes, mortality was verified at the registry offices of the municipality where the patients were living at the end of the study period (i.e. 31 December 2019) or at death; personal identifiers were used following the rules of privacy regulation.

In keeping with Italian privacy regulations, the study design was approved by the local research ethics committee (Cod. CE: 19131).

Continuous and categorical variables were analysed with Student's t and chi-squared test, respectively. Crude mortality rates (CMRs) per thousand PY and relative confidence intervals (CI) at 95% were calculated.

In order to compare the mortality of PGs with that of the general population, the standardized mortality ratios (SMR) and the exact 95% relative confidence intervals specific for cause and sex – using as standard the relative specific mortality rates of the population of the Emilia Romagna Region – have been calculated as well. The SMR, adjusted according to age and calendar year was calculated overall and for all specific causes represented.

A Poisson regression analysis was performed to analyse the combined effect of gender, age, any psychiatric diagnosis, substance use disorders, alcohol dependence and interval from first registration on mortality risk.

Data analyses were performed using the STATA 15.1 statistical software program.

RESULTS

Follow-up continued until 31 December 2019, or until the date of death for 99% of the subjects (eight subjects were lost to follow-up). There were 4,672 at-risk PY (885 female, 3,787 male) and 39 deaths, the first of which occurred in 2003.

The number of patients has been increasing over time and 75% of subjects accessed services after 2012. As regards the first service accessed, 88% were referred to a SERD, 9% to a CMHC, and 3% to an hospital. Overall, half of them had another diagnosis: 41% were diagnosed for any psychiatric disorder (mainly neurotic and somatoform syndromes, personality and behavior disorders, depression), 10% for substance use disorders, and 8% for alcohol dependence *(Table 1)*.

In this cohort, 4.7% (39 subjects) died. Twenty-six were male (3.9% of all males), and 13 were female (7.9% of all females). Cancers (62% of all deaths) and diseases of the circulatory system (13%) were the main causes of death. We highlight five deaths for neoplasm of liver (13%), three for neoplasm of bronchus and lung (8%), and two for intentional self-harm (5%).

Table 1
Problematic gambling: patients diagnosed from 1992 to 2018 in the metropolitan area of Bologna

		Total (826)		Males (661)		Females (165)		P
		N	%	N	%	N	%	
Country of birth	Natives	724	87.7	573	86.7	151	91.5	0.092
	Non-natives	102	12.3	88	13.3	14	8.5	
Age at first admission	Mean (± standard deviation)	47.6±13.8		45.9±13.3		54.7±13.4		<0.0001
Period of first access	% ≤2000	7	0.9	7	1.1	-	-	0.110
	% 2001/2006	52	6.3	44	6.7	8	4.9	
	% 2007/2012	146	17.7	108	16.2	38	23.0	
	% 2013/2018	621	75.1	502	76.0	119	72.1	
First access	Public service for addictions	726	87.9	587	88.8	139	84.2	0.015
	Hospital	26	3.1	15	2.3	11	6.7	
	Mental Health Service	74	9.0	59	8.9	15	9.1	
Other diagnoses	Substance use disorders	80	9.7	73	11.0	7	4.2	0.008
	Alcohol dependence	68	8.2	56	8.5	12	7.3	0.616
	Any psychiatric diagnosis	337	40.8	244	36.9	93	56.4	<0.0001
Psychiatric diagnosis	Schizophrenia and other functional psychosis	36	4.4	30	4.5	6	3.6	0.612
	Mania and bipolar affective disorders	37	4.5	29	4.4	8	4.9	0.798
	Depression	89	10.8	65	9.8	24	14.6	0.194
	Neurotic and somatoform syndromes	158	19.1	108	16.3	50	30.3	<0.0001
	Personality and behavior disorders	92	11.1	67	10.1	25	15.2	0.067
	Other psychic disorders	62	7.5	47	7.1	15	9.1	0.388

The CMR for 1,000 PY were 8.35, higher for females (14.7) and for the oldest patients (<30 years CMR 3.50, 95% CI 0.49-24.85; 30/59 years CMR 3.42, 95% CI 1.90-6.18; ≥60 years CMR 23.05, 95% CI 15.80-33.60).

As regards the causes of death, the CMR in both genders was higher for all cancers, followed by diseases of the circulatory system, and external causes of death, particularly suicide *(Table 2)*.

The excess mortality compared to the general population was 1.16 (0.85-1.58), and it was not statistically significant among males or among females. We highlight elevated and statistically significant SMRs for all cancers and among subjects aged less than 30 years *(Table 3)*.

Regarding gender, females had higher statistically significant SMRs for all cancers (malignant neoplasm of the pancreas) and suicide; males had higher statistically significant SMRs for malignant neoplasm of liver, of lung, of prostate, and of bladder *(Table 2)*.

Among males, SMRs were elevated and statistically significant among subjects aged less than 30; among females, SMRs were elevated and statistically significant among patients aged from 40 to 59 *(Table 3)*.

Furthermore, we highlighted elevated statistically significant SMRs for patients with a diagnosis of schizophrenia, and personality and behavior disorders.

The combined effect of gender, age, any psychiatric diagnosis, substance use disorders, alcohol dependence and interval from registration was obtained from a Poisson regression analysis. A higher risk of death was found for patients aged more than 60 (<45 years RR 1; 45-60 years RR 2.47, 95% CI 0.70-8.75; >60 years RR 13.14, 95% CI 3.80-45.45), more elevated in the year of first contact and decreasing after three years (<1 year RR 1; 1-3 years RR 0.42, 95% CI 0.14-1.26; >3 years RR 0.13, 95% CI 0.04-0.39).

DISCUSSION

This is one of the first cohort studies, characterized by a 27-year follow-up duration, showing all the causes of death in a population of subjects treated for GD.

Our data suggest several interesting observations: the number of PGs has been increasing over time and half of them were diagnosed with psychiatric disorders, substance use disorder or alcohol dependence; all cancers are the most common cause of death, followed by diseases of the circulatory system; excess mortality compared to the general population is more elevated among females aged 40 to 59 and males aged 20 to 29.

Psychiatric comorbidity is the rule and not the exception for PGs [34], and mood/anxiety disorders, substance use disorders, impulse control disorders, and

Table 2
Crude Mortality Rates and Standardized Mortality Ratios for the most commons causes of death

	O/E	CMR	95% CI	SMR	95% CI
All causes	39/33.7	8.35	6.10-11.43	1.16	0.85-1.58
Infectious and parasitic diseases	2/1.12	0.43	0.11-1.71	1.79	0.45-7.15
Tumours/Neoplasms	24/11.6	5.14	3.44-7.66	2.07	1.38-3.08
Malignant neoplasm of stomach	2/0.11	0.43	0.11-1.71	17.41	4.35-69.60
Malignant neoplasm of liver and intrahepatic bile ducts	5/0.11	1.07	0.45-2.57	45.17	18.80-108.52
Malignant neoplasm of pancreas	2/0.15	0.43	0.11-1.71	13.22	3.31-52.87
Malignant neoplasm of bronchus and lung	3/0.42	0.64	0.21-1.99	7.07	2.28-21.91
Malignant neoplasm of prostate	3/0.09	0.64	0.21-1.99	35.43	10.46-100.57
Malignant neoplasm of bladder	2/0.07	0.43	0.11-1.71	29.93	7.49/119.67
Mental and behavioural disorders	2/1.5	0.43	0.11-1.71	1.37	0.34-5.49
Diseases of the circulatory system	5/10.2	1.07	0.45-2.57	0.49	0.20-1.18
External causes of morbidity and mortality	3/13.7	0.64	0.21-1.99	0.22	0.07-0.68
Intentional self-harm (suicide)	2/0.6	0.43	0.11-1.71	3.32	0.83-13.26
	O/E	**CMR**	**95% CI**	**SMR**	**95% CI**
Males	26/24.0	6.87	4.67-10.08	1.08	0.74-1.59
Infectious and parasitic diseases	1/0.8	0.26	0.04-1.87	1.28	0.18-9.06
Tumours/Neoplasms	14/8.8	3.70	2.19-6.24	1.59	0.94-2.68
Malignant neoplasm of liver and intrahepatic bile ducts	5/0.1	1.32	0.55-3.17	52.69	21.93-126.60
Malignant neoplasm of bronchus and lung	2/0.4	0.54	0.13-2.11	5.67	1.42-22.66
Malignant neoplasm of prostate	3/0.1	0.79	0.26-2.46	35.56	11.47-110.24
Malignant neoplasm of bladder	2/0.1	0.53	0.13-2.11	33.22	8.31-132.83
Mental and behavioural disorders	2/1.1	0.53	0.13-2.11	1.79	0.45-7.14
Diseases of the circulatory system	4/6.8	1.06	0.40-2.81	0.59	0.22-1.56
External causes of morbidity and mortality	2/9.8	0.53	0.13-2.11	0.20	0.05-0.81
Intentional self-harm (suicide)	1/0.6	0.26	0.04-1.87	1.79	0.25-12.69
	O/E	**CMR**	**95% CI**	**SMR**	**95% CI**
Females	13/9.7	14.70	8.53-25.31	1.34	0.78-2.30
Infectious and parasitic diseases	1/0.34	1.13	0.16-8.03	2.98	0.42-21.15
Tumours/Neoplasms	10/2.8	11.30	6.08-21.01	3.57	1.92-6.64
Malignant neoplasm of pancreas	2/0.0	2.26	0.57-9.04	47.48	11.88-189.86
Diseases of the circulatory system	1/3.4	1.13	0.16-8.03	0.30	0.04-2.12
External causes of morbidity and mortality	1/3.9	1.13	0.16-8.03	0.26	0.04-1.84
Intentional self-harm (suicide)	1/0.0	1.13	0.16-8.03	22.91	3.23-162.61

PY: person year; O: observed deaths; E: expected deaths; CMR: crude mortality ratio for 1000 PY; SMR: standardized mortality ratios; CI: confidence interval.

personality disorders are frequently comorbid with GD [10-12].

The CMRs for 1,000 PY were 8.35, more elevated among females and the oldest patients. Mortality rates were higher for all cancers, followed by diseases of the circulatory system and suicide. These data can be justified by the fact that they involve older subjects with high physical and psychological comorbidity. In fact, the multivariate analysis documents a higher risk of death in the year of first access to the service and for patients aged over 60.

SMRs for all causes of death were much lower than those reported by the literature regarding alcohol dependence [35], cocaine use [36] heroin use [37], and psychiatric outpatients [38]. Moreover, SMRs were lower than those reported by Karlsson and Håkansson [33], both among males and females, than for subjects aged 20 to 49.

Regarding psychiatric diagnosis, in our study SMRs were higher for patients with a diagnosis of schizophrenia and personality disorders. Notably, while SMRs of people with personality disorders is consistent with oth-

Table 3
Standardized Mortality Ratios for sex, age-group, first access service, substance disorders, alcohol dependence, psychiatric diagnosis

		Total		Males		Females	
		SMR	95% CI	SMR	95% CI	SMR	95% CI
Age-group	<30 years	7.71	1.09-54.70	7.93	1.12-56.27	-	-
	30/39 years	2.30	0.32-16.34	2.44	0.34-17.33	-	-
	40/49 years	2.22	0.83-5.91	1.80	0.58-5.59	7.21	1.02-51.18
	50/59 years	1.54	0.69-3.43	0.87	0.28-2.71	6.49	2.09-20.12
	≥ 60 years	0.98	0.67-1.43	0.98	0.62-1.56	0.99	0.51-1.90
Other diagnosis	Substance use disorders	1.48	0.48-4.60	1.68	0.54-5.21	-	-
	Alcohol dependence	1.75	0.66-4.67	2.59	0.97-6.91	-	-
	Psychiatric diagnosis	1.34	0.89-2.02	1.34	0.81-2.22	1.34	0.67-2.69
Psychiatric diagnosis	Schizophrenia and other functional psychosis	6.00	2.86-12.59	4.64	1.93-11.14	22.72	5.68-90.86
	Mania and bipolar affective disorders	1.99	0.50-7.95	2.33	0.58-9.32	-	-
	Depression	0.99	0.44-2.19	1.21	0.51-2.92	0.59	0.07-3.61
	Neurotic and somatoform syndromes	1.57	0.89-2.77	1.25	0.56-2.77	2.12	0.95-4.73
	Personality and behaviour disorders	2.72	1.30-5.71	2.20	0.92-5.29	6.66	1.67-26.64
	Other psychic disorders	1.72	0.77-3.82	1.35	0.34-5.42	1.99	0.75-5.29

SMR: standardized mortality ratios; CI: confidence interval.

er studies conducted on psychiatric outpatients, PGs with schizophrenia had higher SMRs [39].

Regarding mortality risk for diseases of the circulatory system, from various studies, it emerges that GD are associated with any heart condition, particularly arteriosclerosis [26], tachycardia, and angina [25].

Regarding suicide, mental and behavioral disorders, chronic pain, alcohol and drug abuse are the main risk factors [40, 41] and many studies have shown a high mortality risk among PCs [30-32]. Among the OCSE countries, Italy has one of the lowest suicide mortality rates [42], and crude mortality rate for suicide in 2016 in Italy (8.20 per 100,000 population) was much lower than Sweden (14.80 per 100,000) [43]. Nevertheless, mortality excess for suicide in our cohort was much lower than in the Swedish study and SMRs were statistically significant only for females, being suicide mortality risk very low for females in the general population.

Regarding mortality risk for all cancers, we highlight that GD are associated with liver disease [22], chronic medical conditions and obesity [24, 25], and PGs suffer from gastric pain, nausea [26], and heartburn [27]. Furthermore, particular lifestyles associated with GD, such as alcohol use and cigarette smoking, may have affected cancer mortality [44, 45]. Thus, changes in lifestyle (alcohol and tobacco use) must be a further relevant message for patients treated for GD.

Such as all researches based on large population, this study presents some limitations: the data used are those available from the first admission and data on the evolution of gambling habit are lacking. Moreover, data relating to alcohol use and tobacco smoking are lacking.

CONCLUSIONS

An elevated excess of mortality compared to the general population for older females and for younger males emerges from this study. Despite patients increasing, subjects who most turn to the services are the most serious ones, in older age, with comorbid mental disorders and with a compromised health status. This is reflected in the high risk of death for all cancers.

Moreover, suicide-related mortality is higher in females.

Further follow-up studies are necessary to confirm these evidences.

Author's contribution

Pavarin R designed the study, analyzed data and drafted the manuscript; Turino E, Marani S, Domenicali M and Caputo F collected the data and analyzed the literature; all Authors revised the manuscript for intellectual content.

Conflict of interest statement

All Authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLES AND REVIEWS

The evaluation of capacity in dementia: ethical constraints and best practice. A systematic review

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Abstract
The progressive ageing of a population leads to an increase in the number of people suffering from cognitive deterioration. This requires particular attention in terms of the necessity to assess these people's cognitive functions and their capacity to make decisions. The present systematic review analyses the clinical and ethical aspects of any assessment of capacity, with a specific focus on the capacity of the individual to give informed consent for medical treatment and also with regard to their testamentary capacity. The results indicate that the concepts of capacity, competence and decision-making need to be better clarified, ad-hoc devised tools are required and a multidisciplinary, clinical and legal approach to assessments of capacity needs to be adopted. This is crucial to guarantee that the two ethical principles of capacity assessment are adhered to: respect for an individual's autonomy and the protection of fragile individuals.

Key words
- decision-making
- assessment of capacity
- ethical issues
- clinical competence
- testamentary ability

INTRODUCTION

According to Art. 2 of the Italian Civil Code (CC), the *Ability, or capacity, to act,* or natural capacity, refers to an individual's power to perform acts that are legally valid and effective. In Italy, this capacity is acquired at the age of eighteen, with some exceptions (e.g., sixteen for an employment contract). The term ability to act implies that the person is responsible for his/her acts and this differs from *Legal capacity* which is acquired at the time of birth and represents the condition of being a bearer of rights and duties (art. 1, Italian CC). Consequently, whereas legal capacity is guaranteed and recognised for every human being, the ability to act may be uncertain under some clinical conditions, such as mental deterioration.

The assessment of patients' ability to act and their degree of awareness regarding the consequences of decisions represents a challenge in both neuropsychological and legal fields and ethical dilemmas may arise.

Nowadays, this is a particularly relevant and urgent issue due to the introduction in the Italian legal system of some regulations that allow individuals to delegate specific areas of decision to a "proxy" – that is, a *Support Administrator* or a *Trustee*. The figure of the Support Administrator (AdS) has been introduced in Italy (Law 6/2004, artt. 404 onwards) [1] with the aim of changing the approach to the protection of vulnerable subjects. The person is appointed by a tutelary judge with the aim of helping and safeguarding individuals who are no longer autonomous in terms of decision-making. The introduction of this figure is innovative in that the areas covered (for example decisions about asset management, daily purchases or medical treatments) are not based on a medical diagnosis but on the effective deficits and abilities of each individual. The figure of the *Trustee* has been established as part of a recent legislation on informed consent and advance treatment directives (Law 19/2017) [2]; this proxy is directly appointed by the patients themselves, and represents them in dealings with doctors and healthcare facilities. Both these regulations represent useful opportunities for people who are unable to take decisions or are expected to have difficulties in the future due to the diagnosis of a pathology. For the Support Administrator, it becomes crucial to determine the areas in which the proxy needs to make decisions. For this reason, the judge may ask for a clinical assessment.

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In its clinical meaning, the ability to act indicates an individual's capacity to perform tasks of varying degrees of complexity. This capacity relies on cognitive functions such as, for example, decision-making abilities. However, the capacity to act also depends on the congruency between each individual's ability and the contingencies involved (i.e., the specific situation in which a decision is taken). This implies that a clinical assessment should pursue the maximum coincidence between these factors.

It is worth noting that, although a clinician's opinion may have a relevant weight in the final decision of a judge, the outcome of this assessment is rather a legal matter [3, 4].

The assessment and determination of capacity are particularly sensitive fields of investigation [5, 6]. On one hand, a judgment of incapacity may lead to a significant reduction in a person's rights, while on the other hand, not recognising a decrease in capacity can expose a patient with dementia, as well as other people, to various risks (domestic accidents, failure to plan both simple and complex actions, necessity to request assistance, etc.). For this reason, any reductions in a person's rights must necessarily be offset by an evaluation of what is in the "best interest" of that person and his/her family.

As a result, any assessment of capacity needs to take into account the reasons underlying the request for an evaluation, the patient's environment, his/her affective, social and financial resources and any potential benefits resulting from the adoption of support measures, such as the appointment of a legal proxy.

Since the prevalence of dementia increases dramatically with age, capacity assessments – previously a marginal aspect of clinical and legal practice – have become a common issue. There are many areas involving capacity that clinicians may be called to examine (for medical and/or legal purposes) including the individual's capacity to consent to treatment, to act as a witness, to make a will, to manage their finances, to vote, to drive, to carry out a profession, and so on. In particular, in addition to the assessment of the ability to express their informed consent (essential for a patient following a medical therapy or participating in a clinical trial), clinicians are more and more required to assess the ability of elderly people to make a valid will. Indeed, Italian law states that some patrimonial acts may not be valid if performed by a person who is deemed incapable to act, although not prohibited from doing so (art. 428, CC).

This study was prompted by a document published in Italy by the "Ethics" sub-group within the National Dementia Plan (www.regioni.it/newsletter/n-3900/del-10-08-2020/raccomandazioni-per-la-governance-e- la-clinica-nel-settore-delle-demenze-21590/) [7], which some of the authors of this paper take part in as experts. The search for scientific sources was further extended with the aim of raising awareness concerning the ethical debate on respect in clinical and legal fields for the autonomy of patients with dementia. Then, we focused on the assessment of two specific capacities that have become more and more important: clinical and testamentary capacities.

METHODOLOGY

A search of the literature on the subject was carried out using PubMed, PCM and Cochrane Library databases. It took into consideration English language publications from January 1990 to June 2020. Over this period, there is an enormous quantity of papers (7,098) on both the ethical and methodological aspects of assessing the capacities of patients suffering from dementia indicating the relevance of this topic in scientific research.

In order to narrow down the research, a further search was run which focused only on systematic and major reviews. The following keywords were entered: [dementia] AND [decision-making] or [competence evaluation] or [informed consent] or [ethics] or [testamentary capacity] or [Testamentary capacity evaluation] AND [review] or [systematic review].

RESULTS

The second search yielded 1,821 articles. Further screening was then carried out following the process outlined in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [8] statement for systematic reviews and the outcome of this is shown in *Figure 1*. Forty-four articles met the eligibility criteria: 12 systematic reviews, 21 reviews and 2 brief reviews, 2 integrative reviews, 2 narrative reviews, 2 clinical reviews, 1 critical review, 1 methodology review and 1 literature review; in addition, there were 46 referenced papers, 6 web resources and 5 books, giving a total of 101 references. Among the 44 reviews included in the study, 12 mainly addressed the decision-making process, 13 informed consent, 6 ethical issues, 5 assessments of capacity and there were also 8 concerning financial and testamentary capacity. It is worth noting that this classification was not rigid as in reality a number of these studies addressed multiple aspects. The studies selected are summarised in the *Supplementary Material available online*.

In the following sections, the results are outlined starting with a definition of the main constructs (that is, capacity, competence and decision-making) that form the basis of any assessment of capacity. Two specific capacities and the assessment of these are then discussed: the capacity to give informed consent and testamentary capacity.

Competence, capacity and decision-making: three concepts in one

A long-standing, heated debate concerns the disambiguation of the concepts of "competence" and "capacity", but some confusion in the clinical field remains. In many countries, both terms are often used interchangeably, although in the scientific and legal literature on the subject, subtle differences are sometimes reported *(Table 1)*.

Both in medical and legal terms, *capacity* is primarily considered to be established by means of a clinical assessment. It has been variously defined as: i) the ability to learn, process and make decisions based on available information [9]; ii) the individual's capacity to decide or to perform daily life activities, such as working, driving,

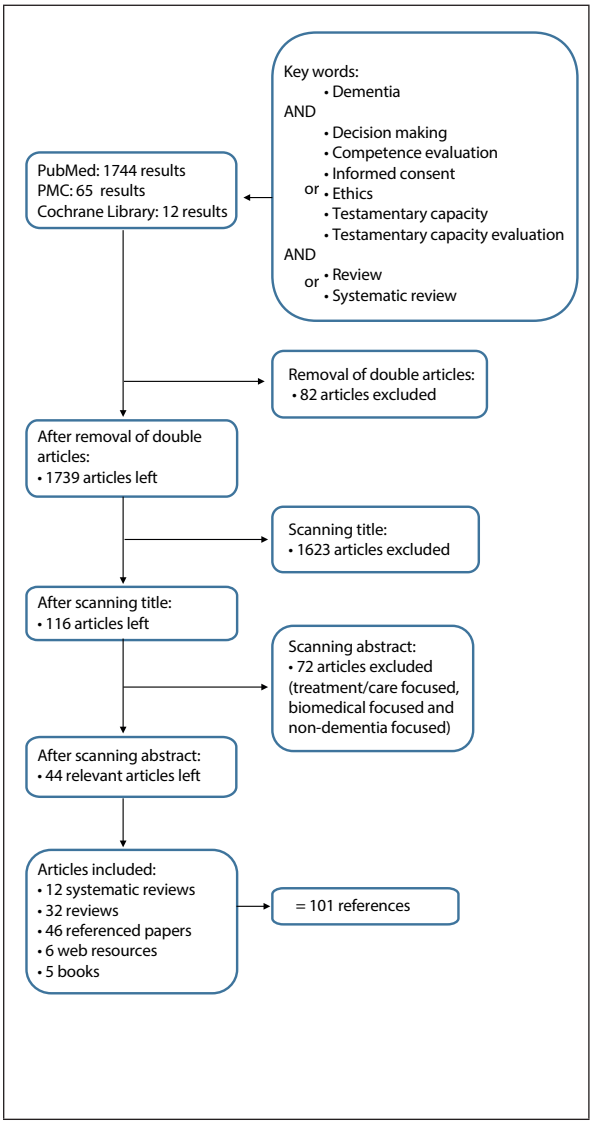

Figure 1
Flow chart of the systematic literature review process.

looking after relatives, making medical decisions, and entering into legal contracts [10] and iii) the functional determination of whether an individual has the ability to adequately make a context-specific decision [11].

In contrast, *competence* is generally considered to be established legally and is defined as: i) the legal determination of whether an impaired mental capacity limits a patient's ability to make legally relevant decisions or actions [12]; ii) a legal construct established and governed by the courts [10] and iii) a legal state, namely the degree of mental soundness necessary to make decisions about a specific issue or to carry out a specific act [13].

In Italy, capacity and competence are synonyms and are used interchangeably to indicate the ability of an individual to use personal, social and/or methodological skills in various different contexts [3].

For the purposes of the present study, and in an effort to overcome confusing terminology, we will refer to the term capacity as the equivalent of competence.

The notion of capacity is closely related to that of *decision-making*, namely, the process of selecting an appropriate action from a number of possible options. Decision-making involves many cognitive processes, including selecting one's goal and motivation, weighing the potential consequences of different options, and determining expected consequences [12]. The loss of decision-making capacities represents one of the most dramatic consequences of cognitive decline in patients with neurological or psychiatric disorders, and it progressively affects everyday life and medical and legal choices, thus potentially exposing patients to the risk of adverse events and financial abuse [17-19]. The prevalence of disorders in decision-making in patients suffering from cognitive decline is consistent, ranging from a percentage of 34% of hospitalised patients to 45% of psychiatric patients [20].

Multiple cognitive functions, in particular executive functions, contribute to the case of decision-making deficits in Alzheimer's disease (AD) [21-23]. Nevertheless, a recent systematic review [24] has shown that in people with dementia, factors other than cognitive impairment contribute to decision-making abilities, including varying degrees of freedom of choice and contextual factors (e.g. socio-economic and personal factors).

The role of emotions and motivation also needs to be considered, as these can help a patient to understand various situations and take appropriate decisions despite a deficit in their decision-making abilities. Emotions represent a source of knowledge that provides crucial information about the internal states of an individual and the responses to external events; these are essential in order to reach decisions that are consistent with the individuals' value system. This refers to the concept of "authenticity", that indicates a congruency between an individual's values (i.e., their beliefs, relationships and commitments) and their decisions. In contrast to "autonomy" – which mainly refers to situations in which an individual exercises their right to express self-determination – "authenticity" does not require an intact capacity of self-determination, but only that the decision is consistent with the individual's values [25]. In people suffering from dementia, the relationship between autonomy and authenticity is complex as a patient may be lacking in self-determination but conserve ethically relevant skills such as communicating a preference, maintaining relationships and certain levels of decision-making [25, 26], including the ability to appoint a proxy for specific areas of decision [2]. It is therefore worth remembering that cognition and emotions are closely interconnected in all the decision-making processes, as cognition involves affective values when reaching a choice, and that inadequate levels of emotional activation – both reduced or in excess – may raise doubts on the appropriateness of the decision.

A recent review [27] recommends a more active role in the decisional process for patients with dementia, and shows that in mild to moderate stages, patients would like to be involved in day-to-day decision-making, especially with regard to health, financial and end-of-life issues. Things are different for geriatric patients

Table 1
Definitions of capacity and competence

Authors	Capacity	Competence
Ganzini *et al.*, 2005 [14]	A clinical assessment of a patient's ability to make specific healthcare decisions: evaluated by physicians specific, not comprehensive	A legal term used to describe a person's overall, comprehensive ability to make decisions: decided by a court/judge permanent unless overturned by a court/judge
Resnick and Sorrentino, 2005 [13]	An individual's ability to make an informed decision	The degree of mental soundness necessary to make decisions about a specific issue or to carry out a specific act
Moberg and Kniele, 2006 [8]	An individual's capacity to decide or to perform activities of daily living	a legal construct established and governed by courts
Willner, 2011 [15]	The ability to make decisions	The ability to perform actions needed to put decisions into effects
Moye, Marson, Edelstein, 2013 [11]	*Clinical capacity*: the functional determination of whether an individual has the ability to adequately make a context-specific decision *Legal capacity*: the specific ability or abilities which are sufficient to carry out a specific action according to law.	
Stracciari, Bianchi, Sartori, 2014 [3]	Legal capacity or "legal competence": ability to make a decision, regardless of its reasonableness Clinical capacity or "clinical competence": set of skills that allow individuals to perform more or less complex actions.	
Darby *et al.* 2017 [12]	The functional determination of whether a patient has the ability to adequately make a specific decision, such as financial decisions, or perform a specific task, such as driving	The legal determination of whether an impaired mental capacity limits a patient's ability to make a legally relevant decision or action
Sabatino, 2018 [16]	Clinical capacity is specific to a particular health care decision	Also called legal capacity, this is a legal status. It cannot be determined by health care practitioners
Gossman *et al.*, 2019 [9]	The ability to learn, process, and make decisions based on information given	A legal term, stating that a court of law has decided whether a person can make his/her own decisions. A legal declaration of incompetence may be global, or it may be limited

who can no longer make decisions for themselves. For them, family members in the role of surrogate decision makers are usually preferred since the family is the primary social unit involved in safeguarding the patient's welfare and wishes [28].

Experimental studies on the neuronal bases relating to decision-making have often focused on this interface between cognitive and emotional abilities. Overlaps have been found between cognitive and emotional abilities networks in the frontal, temporal and parietal areas, such as the dorsolateral prefrontal cortex, the medial prefrontal cortex, the medial temporal structures and the precuneus [29]. A lack of equilibrium between these networks has also been suggested as a cognitive marker of a deficit in decision-making, and as an empirical criterion for an impairment in cognitive abilities relating to informed consent [30]. The neural correlates of decision-making have also been assessed in relation to the neuroanatomical changes that occur in several neurodegenerative diseases [22], showing an elevated correlation between executive functions (planning, anticipation, judgment, reasoning) and decision-making. On the whole, although the anatomical model for decision-making is still being studied, there is a broad consensus with regard to the involvement of an extended neuronal network, including frontostriatal and limbic loops, the orbitofrontal and anterior cingulate cortices, the parietal cortex, the striatum, the amygdala and the basal ganglia [31].

Accordingly, decision-making can be considered to be a multidimensional construct rather than a single function, and it depends on the integrity of attention, orientation, memory and executive functions to ensure inferential processes [32], and is closely related to the appropriateness and intensity of emotions expressed [33].

The assessment of capacity

Evaluating whether or not a patient is able to make decisions in the real world is sometimes a challenge for clinicians. The necessity for this type of evaluation arises in particular when the clinician is faced with a patient with behavioural or cognitive symptoms which are suggestive of a decline in his/her abilities. Although a number of different tools have been developed, the absence of a "gold standard" persists making assessments difficult [34].

Freedman, Stuss and Gordon [32] proposed some guidelines for assessing capacities which focus on the evaluation of neurobehavioral deficits rather than on the neuropathology of disorders. The authors argue that the ability to make competent decisions depends on the nature and severity of the cognitive impairment, rather than on its cause. Sturman [35] shared a similar point of view and assumed that mental illness represents a risk factor for – but does not automatically define – a condition of incapacity. Similarly, Johnson and Karlawish [36] claimed that a diagnosis of AD in the mild to moderate stage did not coincide with an automatic judgment of incapacity: clinicians need to look at the

severity of the general cognitive impairment of each individual patient and should specifically assess his/her decisional capacity.

The necessity for an extensive cognitive assessment may be decided after the administration of short screening measures, and, among these, there is a broad consensus that the use of MMSE is useful. The cut-off scores considered to indicate an individual's capacity range from <19 (i.e. it is probable that the patient's ability to give consent is reduced) to ≥23 (i.e. it is probable that the patient's ability to give consent is adequate), depending on the risk/benefit ratio; a cut-off of 25 has been suggested to discriminate competent and incompetent individuals, with a sensitivity between 91% and 100% [37-39]. Marson's model [6] provides a score ≥20 for capacity, that however needs to be supported by non-pathological results involving tests for verbal fluency, attentional/executive capacities and logical memory. Of course, given their inherent limitations, screening tests should not be used alone to assess capacity [40].

Some authors [41, 42] have suggested that capacity assessments should be divided into at least three steps: a) a general cognitive level, b) specific cognitive abilities and c) an ecological survey. Sullivan [43] emphasised the fact that capacity is "decision specific" and suggested the necessity of planning assessments using this key concept as a base; her two-stage approach recommends an assessment of basic cognitive skills (e.g. orientation, reasoning/judgment, general knowledge and memory), followed by the administration of tasks focusing on specific skills. These skills would be assessed by means of *ad-hoc* devised tests or structured interviews.

In their extensive review, Moberg and Kniele [10] also recommend the use of multiple approaches for an ethical evaluation of capacity. They advise that since there is no single tool capable of evaluating ability, clinicians must integrate various approaches and standardised measures in order to adequately cover the various skills and attitudes pertaining to everyday life. Observation of a patient in the context of his/her day-to-day life would be the best approach, but this is often impossible for practical reasons. Thus, considering the absence of specific, standardised tools and the difficulties associated with directly observing an individual's abilities in their daily life, the authors recommended following a number of steps: i) a detailed interview with both the patient and caregiver(s); ii) a neuropsychological examination carried out by means of validated tools; iii) an evaluation of functional skills and iv) a check of the legal reference standards.

Furthermore, whenever possible, the examiner should identify and advise the adoption of supportive strategies (e.g. a prosthetic environment) to improve the patient's abilities. In fact, the principle of showing respect for each single individual is, according to them, fundamental in an ethical approach of capacity assessment that needs to be shaped and tailored to each patient.

In Italy, Stracciari, Bianchi and Sartori [3] proposed an all-inclusive three-stage approach to the evaluation of capacity. The first step, called "Evaluation", involves the administration of interviews to the patient and his/her family members, an examination of the patient's cognitive functions and an analysis of their functional skills in a daily life context. The second step involves the "Interpretation" of the data collected in the light of legal standards and requirements, taking into account the potential consequences of the patient's decisions. A final step entitled "Rehabilitation" involves the planning of interventions aimed at cognitive reactivation and support (that is, a prosthetic environment). A recent document published in Italy as part of the National Dementia Plan [7] also recommended an accurate neurocognitive assessment and provided a two-level evaluation algorithm depending on the severity of the disease, as well as a list of useful tests.

The picture that emerges from the literature shows that there is a need to follow a complex procedure when assessing capacity, one that requires multiple professional competences and a multidimensional approach. Two main principles would guide this procedure: i) a balance between demonstrating respect for the patient's autonomy (i.e. self-determination and the freedom of choice) and patient safety and ii) taking into account not only the patient's disabilities but also any residual abilities (for example, coping strategies).

Informed consent

The term *Informed consent* refers to clinical capacity, that is, an individual's free and voluntary choice to participate in a course of treatment or a research project. This, therefore, represents a central requirement of ethical research involving human participants [44]. The importance of an individual's expression of will came to be seen as fundamental after the Second World War and originated as part of the Nuremberg Code (1946) that established that participation in research needs to be voluntary, with the freedom for any person to choose participation after adequate understanding of the experimental procedures involved. This means that the person involved in a course of treatment should have the legal capacity to give consent and that whenever a clinical condition induces doubts, the patient's ability to give consent needs to be assessed.

Informed consent has been extensively studied with reference to many pathologies and deficits in this capacity have been reported in learning disabilities, as well as in psychiatric and neurological disorders [45]. In the mild stage of dementia, patients with a good insight into their condition are often sufficiently competent to make decisions regarding their treatment and wish to be involved to the extent that their abilities allow [36]. However, capacity to consent may fluctuate over time, depending on various factors, both medical (e.g. drug therapy) and clinical (hydration status, pain, etc.), which mean that the risk of misunderstandings may arise [46].

Over the last few decades, the issue of informed consent has played a major role in the debate concerning bioethics. There is a need to guarantee the individual's right to self-determination, even in presence of reduced cognitive capacity. The Mental Capacity Act was brought into force in England and Wales in 2005 with the aim of empowering and protecting people who may not be able to make some decisions for themselves [47],

and established that patients must be adequately supported in expressing any residual decision-making skills. These skills rely on certain functional abilities, as summarised by four functions: Understanding, Evaluation, Reasoning, and Expression of a choice [48].

The concept of informed consent therefore stems from the legal concern that patients should have adequate information to make informed decisions regarding medical treatments. However, information alone is not enough for an informed choice: the individual's freedom of choice needs to be guaranteed and his/her ability to use this information to make a rational choice preserved [44]. All of the ethical and legal issues concerning the assessment of this capacity stem from this latter point. Any assessment should therefore reflect the best balance between two extremes: on the one hand, the principle of protecting the patient from choices which may be potentially dangerous for his/her health, and on the other hand, the principle of inviolability of individual choice. This dilemma represents a point which is crucial to the debate on bioethics, as emphasised by some authors who have suggested that not adhering to a principle which safeguards patients with a reduced capacity to act may lead to the risk of "abandoning patients to their rights" [49].

The guidelines and standard procedures relating to obtaining informed consent have long been based on the simple assumption that consent is presumed if a patient does not provide explicit dissent. However, this assumption represents a meagre protection for patients who lack competence [36]. Indeed, this first principle has evolved over time into the current concepts of self-determination and autonomy as well as of a kind of therapeutic alliance involving mutual respect. However, despite this change in perspective, these principles still suffer from a lack of standardisation and adequacy with regard to the instruments available [50]. To date, informed consent has often been considered merely a formality, that is, a document to be signed by the patient to protect institutions and clinicians (rather than the patients themselves) in case there are accidents during the medical procedures. Furthermore, the consent form itself is invariably too complex, and is often incomprehensible (not only to those with a lower standard of education) [50] as language that is unfamiliar to the patient is used. Adopting clear, non-technical language and short consent forms which are appropriate to the patient's language skills is thus to be recommended [51]. A recent study in Italy [52] shows that the use of simplified texts considerably increases the possibility of patients with AD understanding the contents of the documents and thus being able to more easily express their preferences.

In fact, informed consent is not a one-time event but an interactive process, in particular for patients suffering from mental deterioration. The above-mentioned document in the Italian National Dementia Plan [7] states that information need not be fully transmitted at the beginning of the therapeutic relationship, but gradually provided as the clinical sessions progress in a way that is consistent with the progression of the individual's situation with regard to their medical condition, thereby integrating new information and advice. In this way, any request for informed consent regarding specific procedures becomes the result of a relationship built on trust based on the principles of protection and auto-determination.

As previously reported, four key elements in the consent procedure relating to treatment for medical (or research) purposes are recognised in the most widespread models of clinical competence [48, 53]: a) *Understanding*: the ability to understand information and the potential risks/benefits of a course of treatment (or of the lack of treatment); (b) *Appreciation*: the ability to apply the information received to one's own condition; (c) *Reasoning*: ensuring that the patient's decision reflects a consequential and comparative process, indicating a rational reasoning process based on the information; and (d) *Choice*: referring to an individual's ability to communicate a decision.

People suffering from dementia may be competent to make decisions regarding simple courses of treatment but incompetent when a choice requires them to weigh and balance the risks and benefits, or when the outcome is uncertain. Thus, thresholds for capacity vary according to the complexity and uncertainty of the decision in question: the higher the risk and uncertainty, the higher the threshold [54]. Some legal standards have been proposed based on the fact that there are various degrees of capacity that patients may reveal when faced with a specific decision. These standards can be easily applied to other competencies to consent, and to decision-making capacities in general *(Table 2)* [55, 56]

An additional ability [LS2] – *making a reasonable*

Table 2
Legal Standards [LS] for capacity, according to the complexity and uncertainty of the decision: the higher the risk and uncertainty, the higher the threshold. [LS2] is not accepted for judging the capacity since the reference to "what is a reasonable choice" is arbitrary

Legal standard	
LS1 (advanced stage)	*Evidencing a treatment choice*: this standard focuses on the presence or absence of a decision alone
LS3 (moderate stage)	*Appreciating consequences of treatment choice*: this standard emphasises the patient's awareness about the emotional impact and future consequences of their decision regarding treatment
LS4 (mild to moderate stage)	*Providing rational reasons for treatment choice*: capacity to use logical processes to compare the benefits and risks of various options
LS5 (mild stage)	*Understanding treatments, situation, and choice*: this standard requires memory for sequences of information about the treatment and their comprehension

choice regarding treatment when the alternative is unreasonable – is not accepted as a legal standard for judging the capacity to consent since the reference to "what is a reasonable choice" is arbitrary [57].

The assessment of an individual's capacity with regard to informed consent

Any assessment of capacity to consent to a course of treatment is challenging in cognitively impaired patients, but also crucial in order to respect the two principles of protection and auto-determination. Variable competence rates in elderly people are reported [58], with 54% of full competence among AD patients, 44% in nursing home residents and 68% in patients with learning disorders; however, the same study indicates that clinical judgment alone is reliable in only 42% of cases, with a prevalence of false negative over false positive results.

To date, the assessment tools available fall into two categories: traditional psychometric tests and *ad hoc* structured interviews/questionnaires. Both of these methods have advantages and limitations, so a combination of the two used in tandem is a good solution. Furthermore, it is important to investigate the role of emotions in the decision-making process, through both structured tools and in-depth psychological observations in clinical settings.

Several reviews on cognitive tools have been published over the last decade, but only a few of these have been systematic *(see Supplementary Material available online)*. A possible reason for the scarcity of contributions in this area is exemplified by the fact that a study by Hein and colleagues was initially (2014) published on the Cochrane Library website but was later withdrawn with the reason given that: "there is currently no clear diagnostic gold standard to compare the reviewed diagnostic tests making it impossible to assess the sensitivity and specificity of measures" (Cochrane Database of Systematic Reviews, 2015).

In effect, we found only two systematic reviews on this topic: the first [34] concerns assessments of capacity according to legal requirements in the UK, and the second [59] focuses on various different instruments that measure decision-making capacities in a medical setting. Both studies focused on two critical features: i) the fact that despite a wide range of instruments, there is no gold standard for the assessment of capacity, and this hampers any evaluation of the various different approaches and ii) none of the instruments available provides a clinical cut-off score that physicians can use to determine whether a patient has sufficient decision-making capabilities. However, even though this last factor may be considered to be a limitation, it is worth nothing that not having a pre-defined cut-off is very much in line with the idea that decision-making is not an all-or-nothing process. An integration between the opinion of a healthcare professional and the results of a structured assessment process would therefore constitute the most robust approach [34].

The association between informed consent and various specific cognitive domains has been widely studied over the last twenty years [60-62]. Extensive intra-individual variability in performance has proven to be predictive of a higher risk of deficits in decision-making capacities [63]. More specifically, studies assessing people's ability to give their consent to treatment have suggested that measures related to executive functions are the main predictive factors [64]. However, to date, there has not been a clear pattern of association between specific cognitive skills and decision-making, and the lack of a gold standard hampers the validation of specific instruments [65]. In an attempt to define the predictors of a person's ability to consent, Marson [6] examined the correlational studies between the Capacity to Consent to Treatment Instrument (CCTI) and Legal Standards (LS). Deficits in semantic memory, conceptualisation and verbal recall appear to be associated with reduced Understanding and Choice in mild to moderate stages of Alzheimer's disease (LS5). Deficits in executive functions are associated with reduced capacity for Appreciation and Reasoning in mild to moderate stages of dementia (LS4) and the identification of the consequences of the choice made in moderate stages (LS3). Finally, deficits in receptive language and semantic memory (naming) are associated with a reduced ability to communicate a simple choice in advanced stages of dementia (LS1).

Among structured interviews and/or questionnaires which assess clinical competence, Dunn, *et al*. [66] indicated the MacArthur Competence Assessment Tool for Treatment (MacCAT-T) and for Clinical Research (MacCAT-CR) as the best choices for measuring capacity to consent to treatment and research, due to their comprehensiveness and supporting psychometric data. Sessums, *et al* [58], however, reported the Aid to Capacity Evaluation (ACE) as being the best instrument to assist physicians in the assessment of medical decision-making capacities. This tool is available on the University of Toronto website and should always be associated with an MMSE lower than 24. However, this combination is not sufficient to determine capacity in patients with focal neurological disorders, and a comprehensive cognitive evaluation is always required [67].

The CCTI is similar to the ACE but has mainly been validated with AD patients. It shows a good correlation with several neuropsychological tasks [4], including phonemic and semantic fluency tests. In their model, Marson, *et al* [4] concluded that the integrity of frontal functions is a critical aspect in the assessment of decisional capacity, whereas memory defects are considered to be an "operational" deficit which may be compensated for by reminders [68].

While the standardisation of materials and procedures is quite simple for the cognitive tests that are used in clinical practice, any adaptation of interviews structured in order to cover specific situations may compromise the internal consistency of the test. In fact, standardisation is a key attribute for all psychometric tools, and any changes in content can potentially lead to a bias on inter-rater and test-retest reliability [69]. In principle, interviews may be useful in terms of guiding the assessment and supporting an experienced clinical judgment, but they cannot replace this latter [60, 70]. For example, the MacCAT-T takes into account the

interactive and contextual nature of capacity and thus intentionally does not provide cut-off scores and relies on the collection of additional information such as interviews with family members, behavioural and ecological observations and an evaluation of the patient's value system.

In conclusion, despite the fact that there are many instruments and tools, a comprehensive assessment of capacity is often difficult and requires time. Moreover, the majority of instruments require further testing [70]. The MacCAT-T and the CCTI are currently the most widely used interviews and the latter seems nowadays to be the only one with normative values [4]. However, research on this topic is still ongoing, as confirmed by a number of reports of new tools, such as, for example, the University of California Brief Assessment of Capacity to Consent (UBACC). This is considered to be particularly promising as a result of both its simplicity and its applicability in clinical practice [45], but it is currently only applied to patients with psychiatric disorders.

Unfortunately, none of the above-mentioned instruments are available in Italian.

An emerging area of clinical and legal interest: testamentary capacity

In western societies, requests for assessments of testamentary capacity have been increasing since many populations are progressively ageing and illnesses related to dementia are more prevalent. In addition, relationships have become more and more complex in terms of financial aspects and family structures now often characterised by divorce, second marriages, de facto unions with the individuals within a family often living at great geographical distances from one another. All of this makes it difficult to resolve conflicts that arise regarding wills and inheritance and thus there is a greater necessity to ascertain the testamentary capacities of the individual concerned.

Testamentary capacity (TC) may be defined as the ability of a person (testator/testatrix) to make his or her own will in a clear and valid way [71, 72]. While from a legal point of view TC is subject to variations from country to country in accordance with the relative civic codes, from a neuropsychological perspective it is based on two general functions, an individual's capacity to understand relevant facts and to appreciate the reasonably foreseeable consequence of a decision.

As with other capacities, TC is considered to be present until proven otherwise, and a diagnosis of illness does not *per se* mean a deficit in TC [73, 74]. It is important to note that TC does not necessarily imply the ability to comprehend or manage complex financial transactions for instance [73, 75], but rather refers to a minimum level of the mental capacity required to make a will [75]. However, some cognitive abilities are in effect needed in order to create a will: individuals should be aware that they are making a plan to dispose of their estate after their death, to recognise the natural beneficiaries and to know the nature and extent of their estate. When there are doubts about whether these abilities are compromised, additional information is necessary and an assessment to establish the presence of capacity needs to be carried out. This is particularly important in those situations where cognitive abilities are apparently preserved, and deficits are hidden by adequate social interactions.

A seminal contribution to the doctrine regarding TC was given by the Banks *versus* Goodfellow sentence, the criteria of which have been recently revised by Shulman and colleagues [76, 77]. According to their interpretation, the authors suggest that the testator must be able to understand not only the act of making a will but also its potential effects. He/she needs to know the nature and extent of his/her property and to be able to clearly communicate the distribution of this property (particularly if the current wishes are different from those previously expressed). In addition, the testator must be capable of evaluating the claims of those who might be expected to benefit from the estate and express the rationale behind his/her choices. Finally, the testator needs to be free of mental disorders. However, any such symptoms will only invalidate the will when they clearly influence the disposition of the estate [73].

In any situation where these abilities are in doubt, a specific assessment is required. As TC is a function relating to both legal and medical fields, a collaborative approach is necessary to carry out an evaluation. Currently, any request for an assessment is usually advanced by a judge or a solicitor, often because there are disputes among the people involved in the inheritance or because there is the potential for a controversy in the future. However, when assisting a patient with dementia and his/her family, we must take into account that the patient's clinician may understand when and if it is appropriate to discuss making a will with the patient.

Unfortunately, to date there are no standardised tools for the clinical assessment of TC [77] and studies focusing on instruments which have been specially designed for the investigation of TC in elderly people or people with dementia are meagre. As a consequence, clinicians are required to achieve a general picture of an individual's capacity by means of integrating psychometric measures and other complex information relating to the testator's daily life and social relationships.

In general, assessments may be based on Retrospective or Contemporary evaluations.

Retrospective evaluations are requested when the testator is deceased and his/her mental state at the moment of drafting the will is being questioned. This process has been described as a sort of neuropsychological "autopsy", that is, an evaluation of the testamentary capacity of a person who has deceased and thus for whom an objective assessment is not available [78]. In this case, only collateral information and pre-mortem documentation are available in order for an opinion on the testator's capacity to be formed. Useful information comes from the results of prior medical and nursing home records (with lists of the individual's medications) and neuropsychological assessments, in addition to copies of other wills (when present), academic records, work performance records and financial transactions [72]. Medical reports should be collected in a systematic way following a "chronological" approach which

makes it possible to track the progress of the testator's cognitive abilities. The date of the will is used as a reference point [79]. Another relevant source of information is the testator's personal correspondence and anything they have written, both of which may reveal the quality of the testator's interpersonal relationships and their intentions. Any other relevant legal documents can also be requested from people who were closely associated with the person in question, such as family members, close friends and medical staff (for details, see [79]).

Contemporary evaluation is, however, recommended where possible for obvious reasons. Unlike retrospective evaluations, in this type of assessment, it is possible for the examiner to garner a cognitive picture of the individual at the moment when the will was drafted. Furthermore, contemporary assessments represent a means of avoiding any subsequent inquiry into the requisite decisional capacity of the person after their death thus preventing potential litigation, expense, and any negative impact on family relationships [77].

As in the case of clinical competence, the tools employed for the assessment of TC include cognitive tests, interviews or questionnaires, and a variety of instruments for functional assessment. General tests of cognitive ability are recommended [80, 81] since these furnish indications regarding an individual's cognitive profile which will assist the examiner in the identification of strengths and weakness and in terms of deciding which cognitive functions need to be assessed in depth. In general, executive functions are assessed in order to have data regarding the testator's capacity for the planning and reasoning required for the distribution of his/her estate. Ability to calculate, working memory and cognitive estimation are tested with the aim of ascertaining the testator's awareness of the current values of any assets he/she wishes to bequeath. Tests relating to semantic and autobiographical memory allow the examiner to not only establish whether the testator understands the nature and extent of any properties to be disposed of, but also whether he/she recalls the nature of his/her relationships and is able to evaluate any potential claims from people who might expect to benefit from the estate. Finally, language competence is tested in order to ascertain whether the individual is capable of understanding the text of a will and of communicating his/her personal wishes in a clear and rational way [82, 76, 77, 74, 79].

In the literature on the subject, there are some semi-structured interviews that investigate financial capacities (e.g., Hopemont Capacity Assessment Interview (HCAI); the Financial Capacity Instrument (FCI) [83, 53], but these are not specific to TC. The Testamentary Capacity Instrument (TCI) [82] is more specific and involves a list of questions to be administered orally or in writing. The questions focus on the four legal criteria introduced by Banks *versus* Goodfellow (1870): "he ought to be capable of making his Will with (i) an understanding of the nature of the business in which he is engaged, (ii) a recollection of the property he means to dispose of, and (iii) of the persons who are the object of his bounty, and (iv) the manner in which it is to be distributed between them". Finally, any functional assessment should comprise an evaluation of the daily activities the person engages in including those which are associated with the management of finances and properties.

Whatever instruments are chosen for an assessment, it is crucial that the examination follows a systematic approach which provides evidence both of any individual weakness and any residual abilities and competencies. Furthermore, certain ethical aspects should be considered.

First of all, the testator needs to be informed about the specific, legal nature of the assessment and he/she must be informed that personal questions may be addressed to him/her that may be related to private issues. Whenever possible, assent for a cognitive evaluation should be requested, even if this could result in an impasse (i.e. when the patients themselves are asked for their consent to be assessed on their abilities to make a will). The person carrying out the evaluation should also clearly understand the main questions to be asked depending on whether, for example, there is a doubt regarding the testator's ability to estimate his/her property or comprehend financial issues or he/she has difficulty recognising his/her relatives.

Another aspect that is of value from an ethical point of view relates to the context of the assessment. In particular, this should be conducted in the absence of anyone who might benefit from the will [73, 84]. This in some way guarantees a reduction of any external influences on the testator's wishes. The risk of undue influence is particularly high in the case of vulnerable people or in cases where the testator's wishes have changed over time. In these cases, it may be useful to identify the timeline of these changes and to understand the circumstances in which these happened in the context of the testator's relationships. For example, a manipulator may be identified in a person who acts in order to isolate the client from their usual support networks, encourage mistrust in others whilst winning over the client with gifts and acts of kindness, thus placing the individual in a position where they feel they are obliged to change their financial arrangements in favour of the potential manipulator [74].

Last but not least, TC assessment requires sufficient time. An ethical approach to evaluation may involve the necessity of collecting information from various different sources and in various different contexts (e.g. not only in a clinical setting but also at the testator's home). This may take more time and multiple sessions of assessment may be necessary. Understanding the current capacities of an individual can often be a complex task and every effort must be made to realistically investigate any remaining capacity.

DISCUSSION

The purpose of the present review was to collect and synthesise current knowledge concerning two domains of capacity which are of particular interest for clinical purposes: the capacity to give informed consent and testamentary capacity. A multidisciplinary perspective that integrates ethical, medical-legal issues and clinical assessment was adopted. The aim was also to provide clinicians with a theoretical and empirical overview that might be useful in their clinical practice. Some consid-

erations emerged from our revision of the scientific literature on the subject covering the last 30 years.

Firstly, to date there have been no tests designed to measure capacity: all of the tools available have been adapted from those used for clinical diagnostics, and they investigate specific functions rather than abilities, with the result that the scores do not provide any relevant information concerning any compensatory, adaptive strategies implemented by the patient to face the demands of daily life [3]. Similarly, there are no standards for assessing capacity, probably due to the fact that patients vary greatly from one to another even though the diagnoses and levels of medical care are similar [65]. There is also the question of the specificity of the various different contexts relative to each individual (e.g. the risk/benefit ratio). This means that a case-by-case tailor made approach is required.

An issue in Italy regards the lack of translations and validation for structured interviews (e.g., informed consent, driving skills, testamentary skills, etc), that are widespread in English-speaking countries where these have been developed and validated. Although interviews should not be considered as indicators of ability but integrated into a neuropsychological examination, the absence of translations and validations constitutes a serious limitation for the Italian health system [7].

Secondly, the lack of a gold standard is now the greatest challenge since the complexity of assessments of capacity cannot be reduced to simple scores for cognitive tests or questionnaires but is a complex endeavour involving cross-disciplinary knowledge involving, for instance, ethics, law, neuropsychology and neuroscience [59]. Cognitive and emotional abilities, personal values and experiences are also important factors that influence decision-making. None of the instruments currently available are sufficiently flexible or broad in scope for individual and contextual factors to be taken into consideration, and thus in-depth investigations and consideration of every patient's narratives are essential.

In light of this, the following ethical recommendations for the neuropsychological evaluation of capacity are particularly important [3, 11, 85]:

i) the use of several tools and various different approaches to the evaluation of the patient's daily life functioning skills;

ii) respect for his/her residual autonomy to whatever degree it is present and

iii) a tailored approach to his/her emotional, functional and cognitive responses, as well as to clinical and socio-demographic conditions.

Thirdly, an issue which we consider needs to be addressed emerged from the present review. This concerns the lack of emphasis on the concept of awareness within any assessment of capacity. Anosognosia (i.e., a lack of awareness) is a multifaceted syndrome that may affect patients with dementia. Awareness has been defined as "a reasonable or realistic perception or appraisal of a given aspect of one's situation, functioning, performance, or of the resulting implications, which may be expressed explicitly or implicitly" [86]. In cases of mental deterioration, anosognosia may involve some cognitive functions and not others, and often concerns impairments in daily activities [87, 88]. Although not directly correlated with capacity, in AD awareness needs to be specifically investigated [89-91]. In fact, an inability to recognise one own's symptoms may lead patients to make inadequate decisions [92].

It is noteworthy that awareness shares some brain networks with decision-making. Recent studies indicate a role of the medial structures in anosognosia, in particular the right temporal medial cortex, including the hippocampus [93] There are also disconnections within medial subsystems of the default mode network, subserving autobiographic memory and emotional states [94, 95]. We thus consider that an evaluation of capacity in a patient with dementia should not ignore tests regarding the awareness of self and of one's own symptoms and disease.

Lastly, it is worth considering two ethical issues. The first arises from a reflection on the subject of autonomy proposed by Reichlin in *Ethics and neuroscience* (p. 112) [96]: "autonomy (in dementia) cannot be conceived of as being based on the full decision-making freedom to make decisions, by a rational individual, who is fully informed and capable of pursuing a proper life plan. This representation, which is disproportionate in any individual made weak and vulnerable by a disease, appears to be altogether inapplicable to patients suffering from dementia".

If this is the case, autonomy cannot be the only value involved in the physician-patient relationship. It must also be accompanied by the concept of "best interest", which includes not only respect for the individual's previous wishes, but should also take into account the care required for his/her current well-being and quality of life. The second issue relates to the impact of ethical principles on clinical practice [97]. The care of dementia is deeply intertwined with ethical aspects, in all clinical and personal interactions, and general ethical principles such as "respect for patient autonomy" and "beneficence" should be at the basis of all medical decisions at every step (e.g. the communication of the diagnosis, information about the clinical course of the disease, drug therapy, etc.).

In conclusion, we consider that our findings will be useful for both clinicians and law practitioners when an analysis of protective measures and the patient's need for guardianship is being carried out. Limiting the role of guardianship to complex decisions may be enough to protect the majority of patients suffering from mild forms of AD. Less restrictive legal options, such as supporting in decision-making, might be applied for simpler decisions.

Limits

Our paper is structured as a systematic review. It focuses on reviews rather than on original articles, a choice which was necessitated by the multidisciplinary approach adopted and the long-time window considered (30 years). Unfortunately, quantitative findings on this topic are few. The field of systematic reviews on ethical issues lacks broadly consented standards, such as those available for systematic reviews on clinical research [98].

Moreover, the study does not consider the symptomatic predementia phase – that is, mild cognitive impairment (MCI). Along with the ascertaining of capacity in patients with dementia, some studies have focused on MCI, showing that some of these patients exhibit problems with making competent decisions, in particular regarding treatments and research protocols [40]. However, the diagnostic criteria for MCI state that cognitive changes should be sufficiently mild as not to compromise social and occupational functioning [99]. Furthermore, a reversion to normality has been described as a common outcome in this condition [100]. Addressing the issue of competence in MCI thus deserves a specific investigation and is beyond the scope of this study, which originated from the National Dementia Plan [7].

The main limitation of our study is probably due to the "cultural" bias present in the review of the literature. Indeed, the studies reported refer to various cultural contexts and legislative contexts and this makes it difficult to make comparisons. On the other hand, all of these studies came from Europe and North America and thus we cannot exclude the possibility that a rethink of the constructs of capacity and competence might be necessary when investing different cultural contexts.

Another limitation is that it was extremely difficult to find a solution to the issue regarding the confusion in terminology involving the various different shades of meaning that the authors give to the concepts of autonomy in competence and self-determination. We consider that only thorough integration between the many different professionals (e.g., legislators, judges, clinicians and neuropsychologists) will overcome this problem.

Finally, the interaction between cognitive and emotional dimensions in relation to the definition of capacity was not specifically discussed in this review as the literature on the subject is meagre. Further studies are necessary to investigate this relationship since it is fundamental in order to respect individuals in their uniqueness.

CONCLUSIONS

The evaluation of capacity in patients with dementia has several crucial aspects. On the one hand, these are linked to the patient's fragility which requires special care from people who are in contact with him/her (e.g., judges, caregivers and clinicians). Unfortunately, the urge to exclude the patient from the decisional process is common in real life, but his/her participation should instead be enhanced and validated. On the other hand, capacity is not a dichotomous condition to be merely defined as "present/absent". It always refers to a specific decision, in a given context and at a given time [101].

This involves an all-encompassing tailored approach to the assessment of capacity, and a continuous effort to help the patient to express his/her opinion when applying standardised procedures in a clinical setting. Clinicians should therefore be well trained in order to develop such skills, and to bear constantly in mind the importance of demonstrating respect for the autonomy and dignity of the patient.

The implementation of a series of tools to aid assessments of capacity and the validation of *ad hoc* questionnaires are strongly recommended in order for the current limitations in clinical practice to be overcome.

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The Authors certify that neither financial nor non-financial involvements that might represent a conflict of interest affect their work.

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ORIGINAL ARTICLES AND REVIEWS

Community waterborne outbreak linked to a firefighting response during the COVID-19 emergency

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Abstract

Background. On 6 March 2020, a big fire in a village forced the firefighters to draw water simultaneously from many sources, including the Adige river. From 9 March, an increasing number of inhabitants reported gastrointestinal symptoms. We describe the outbreak and the challenges linked to the concurrent COVID-19 spread.

Methods. Residents with enteric symptoms and their relatives were interviewed and samples from some of the patients and public water pipelines were tested for enteric pathogens with microbiological and molecular methods.

Results. By 20 March, 182 people reported symptoms and 131 met the case definition. Norovirus GI/GII and other pathogens were found in human and water samples.

Conclusions. Contamination of the public water network with sewage-contaminated river water through the firefighters pressurized water tank was the suspected source of the outbreak. The investigation was partly hampered due to the SARS-CoV-2 emergency. Control measures included avoiding tap water, alternative water supplies and chlorination of public water.

Key words

- gastroenteritis
- norovirus
- waterborne diseases
- community outbreak
- COVID-19

BACKGROUND

Waterborne diseases include many different types of infections that are transmitted through contaminated water and viruses, bacteria, protozoa, and helminths can be involved.

Contaminated drinking water may cause large community outbreaks with up to thousands of cases. Raw water contamination, treatment deficiencies, and distribution network failure are among the most common causes of water contamination [1]. In addition to problems with or failure of the water distribution system, heavy rainfalls and floods associated to seasonal trends and climate change can cause water contamination, with runoffs from wastewater treatment plants, and areas with intense agricultural activities [2].

The source of the contamination is most commonly wastewater which may harbor a large number of diverse pathogenic microbes [3].

In Europe waterborne outbreaks are commonly reported, and the most frequently involved pathogens are norovirus, hepatitis A virus, *Campylobacter*, *Salmonella*, pathogenic *Escherichia coli*, *Shigella*, *Cryptosporidium*, but for a proportion of waterborne outbreaks the agent remains unknown [4].

Noroviruses are a leading cause of sporadic cases and outbreaks of acute gastroenteritis. They account for approximately one-fifth of all acute gastroenteritis contributing substantially to the global burden of acute gastroenteritis across all settings and age groups [5].

Norovirus is highly contagious, and the infectious dose can be very low, in the range of 10-100 viral particles [6]. Although less common, waterborne outbreaks of norovirus are reported worldwide and often in association with groundwater contamination and poor chlorination [1, 7, 8].

On 6 March 2020 there was a big fire in a speck factory (typical smoked and spiced ham from South Tyrol, Italy) in the village of Postal (n = 1828 residents; Bolzano Province, Italy).

Firefighters used the hydrants connected to the pub-

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lic water network, but soon they faced water shortage and placed eleven "hydrosub" units in the Adige river, located at about 750 meters from the place of the fire (*Figure 1*). During the operations, firefighters also connected five private wells in the vicinity of the place of the fire.

Car pumps collected water from all the sources and mixed it in a pressurized water tank. The fire was extinguished on 8 March after 2.5 days, but the operations of the firefighters ended on 10 March. Twenty-six teams of firefighters in different shifts were involved in the fire operations, for a total of about 800 firefighters involved. Most of them were volunteers from the village and the neighborhoods.

On 9 March some inhabitants of the village reported to the Mayor changes in the appearance, taste, odor, and color of the tap water, and some consulted the local general practitioners (GPs) for gastroenteric symptoms starting from the evening of 6 March. The largest part of the inhabitants of Postal are used to drink tap water, considered safe and of high quality.

Tap water in the village of Postal, is supplied by the municipality that takes care of collecting, monitoring and distributing drinking water. The water source is ground water collected by a deep well (about 50 meters) located in the south west of the village (*Figure 1*). From the well the ground water is pumped to an elevated tank (*Figure 1*) placed in the eastern side of the village. The houses in the southern part of the village are supplied directly by the well, while those in the northern part receive the water from the tank, through gravity.

On 9 and 10 March the personnel of the local health department collected some samples of the tap water and on 10 March the residents were advised not to consume or use tap water (consumption was allowed only to wash and cook after boiling) and alternative water supplies (bottled potable water) were provided to the population for drinking. The water avoidance notice was extended to 18 March 2020. On 10 March the Local Health Unit started an epidemiological investigation after the reporting of gastrointestinal symptoms

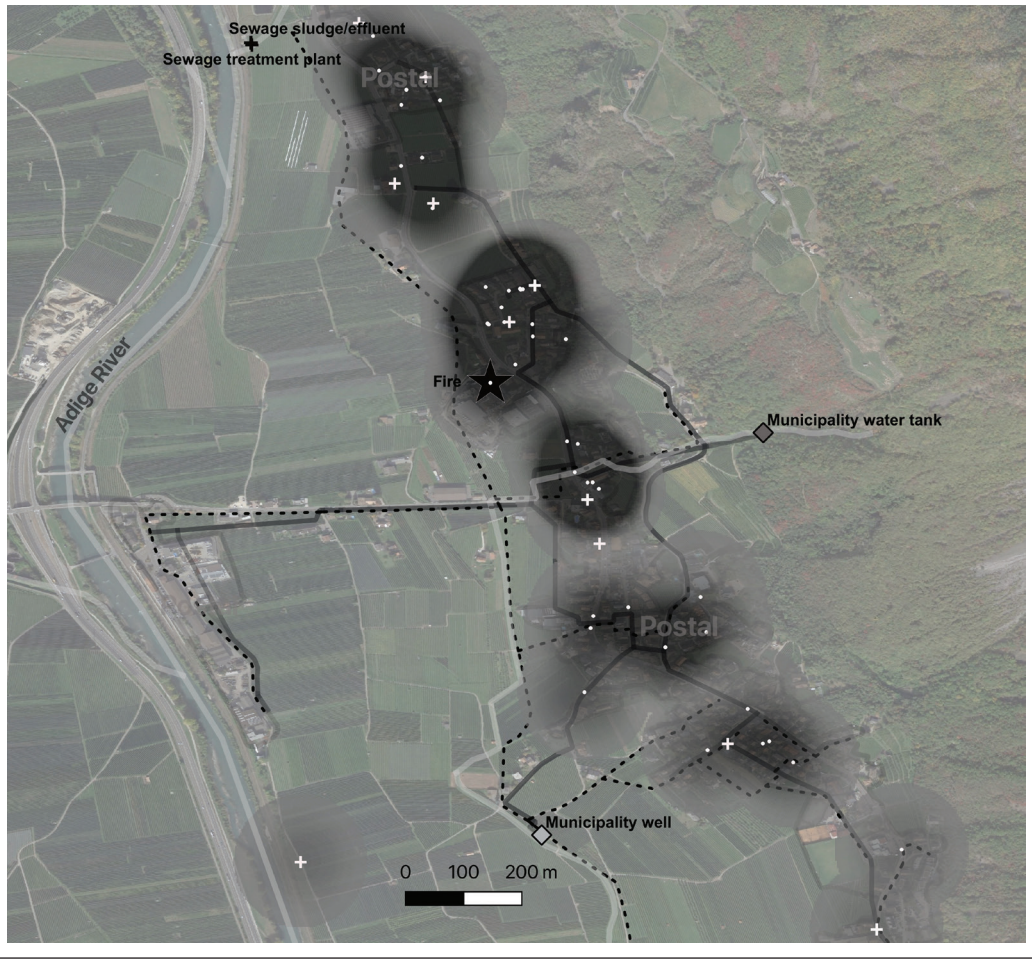

Figure 1
Map of the Village of Postal, North Italy, and density map of the cases of gastroenteritis. The black star is the place of the fire, the small white dots are the residence of the cases (n = 131), the solid dark gray line is the public water system. The density of cases is presented as dark areas; the darker area means higher number of cases. The white crosses are the places from where water samples were taken, the light gray diamond is the ground water deep well and the dark gray diamond is the water tank.

by 50 people, to confirm the outbreak, identify the size and the source.

This outbreak occurred during the beginning of the SARS-CoV2 epidemic in Italy. On 9 March the Italian Government declared COVID-19 a national public health emergency and on 11 March Italy's Prime Minister scaled up the emergency response with extreme measures of personal distancing, including mobility restrictions, banning of mass gatherings, closure of schools and work activities, isolation and quarantine (lockdown). On the same day, the World Health Organization (WHO) declared the SARS-CoV-2 pandemic.

This article describes the epidemiological and microbiological investigations of an acute gastroenteritis outbreak associated with potentially contaminated water during a firefighting response in a small village in Northern Italy. Moreover, we discuss the impact caused by the COVID-19 pandemic on the outbreak investigation and control measures.

METHODS

Epidemiologic investigation

Residents of Postal (Bolzano Province, Italy) who consulted the GPs or contacted the local health unit declaring symptoms referable to a gastroenteritis with onset from 6 March 2020 onwards and their relatives were interviewed by telephone by nurses of the local Public Health Department. A suspected case was defined as a person who had experienced diarrhea (three or more watery stools per day) OR vomiting (three or more times per day) from 6 to 20 March 2020. A confirmed case was a suspected case with a laboratory confirmation of the infection.

Data on clinical symptoms, the time of symptom onset, food consumption and other exposures were collected using the national official questionnaire for the investigation of acute gastroenteritis episodes.

The firefighters who responded to the fire were asked about gastrointestinal or respiratory symptoms (to detect a possible COVID-19 case) up to 14 days after the fire. All sick persons were interviewed.

Microbiological investigation

Stool samples and rectal swabs obtained from the patients were tested for a list of enteric pathogens with both microbiological and molecular methods.

The specimens were sent to the accredited diagnostic laboratory (UNI EN ISO 15189:2013) of the Local Health Unit in Bolzano, and cultured for the detection of *Salmonella, Shigella* and *Campylobacter*, using standard microbiological methods. A commercial multiplex RT–PCR assay was also used for simultaneous detection of *Campylobacter* (*jejuni, coli, upsaliensis*), *Clostridium difficile, Plesiomonas shigelloides, Salmonella* spp., *Yersinia enterocolitica, Vibrio* (*parahaemolyticus, vulnificus, cholerae*), *Shigella* spp., Enterohaemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga-like producing *E. coli* (STEC, stx1/stx2), *E. coli* O157, Enteroaggregative *E. coli* (EAEC), Enteroinvasive *Shigella/E. coli* (EIEC), *Cryptosporidium* spp., *Cyclospora cayetanensis, Entamoeba histolytica, Giardia intestinalis*, Adenovirus F40/41, Astrovirus, Norovirus GI/GII (NoVs), Rotavirus A, Sapovirus (I, II, IV, V) (FilmArray™ GI panel - BioFire Diagnostics, Salt Lake City, UT).

Environmental investigation

In *Figure 1*, the map of the village of Postal is presented, with the place of the fire, the main watercourses, the public water system and the sewage system.

An environmental investigation at the place of the fire, with identification of the water sources used by the firefighters to extinguish the fire was performed by a team composed by local health authority staff, firefighters' representatives and the Mayor.

The investigation included an examination of the well construction log, current well and tank, hydrants and public water network, monitoring records, and potential sources of contamination.

Routine sampling activity of the drinking water was complemented with additional sampling performed to check water quality and presence of contaminants almost daily from 9 to 19 March. Additional samples of 1-Litre of water were collected from the public well, water reservoirs, public fountains and taps along the distribution system (*Figure 1*).

On the 10 March, the filter of the household water filtering system of 2 cases was also sampled.

Bacteriological (revived 36 °C, revived 22 °C, coliforms, faecal Enterococci and *E. coli*), physical and chemical parameters were analysed. The presence of coliform bacteria was daily monitored at several sites of the water system before and after the chlorination treatment.

The analyses of the tap water were performed by the Provincial Agency for the Environment and Climate Protection.

Statistical analysis

Descriptive statistics were calculated for the cases using STATA software version 16.1 (StataCorp 4905 Lakeway Drive College Station, Texas 77845 USA).

The geographical mapping of the cases and the water system, the calculation of the distances matrix between the place of the fire and the cases, and the cases density heatmap were performed using QGIS version 3.10 [9].

RESULTS

Between 6 and 20 March, 2020, 201 people were interviewed by the local health staff, 182 declared symptoms referable to acute gastroenteritis and 131 met the case definition.

Sixty-six (50.4%) were males and sixty-five (49.6%) were females. The median age was 39 years (range 2 to 87 years, interquartile range [IQR] = 20-57 years). Symptom onset was acute, the first confirmed cases of gastroenteritis had their symptoms starting on 6 March, few hours after the firefighters' intervention.

The cases of gastroenteritis followed a steep increment from 6 to 9 March, and the peak occurred on 9 March, with 46 cases (third day after the fire; *Figure 2*). Common symptoms (*Table 1*) were diarrhea (71.76%), vomiting (62.60%), nausea (59.54%), abdominal pain (53.44%), head and joint ache (48.85%), and fever

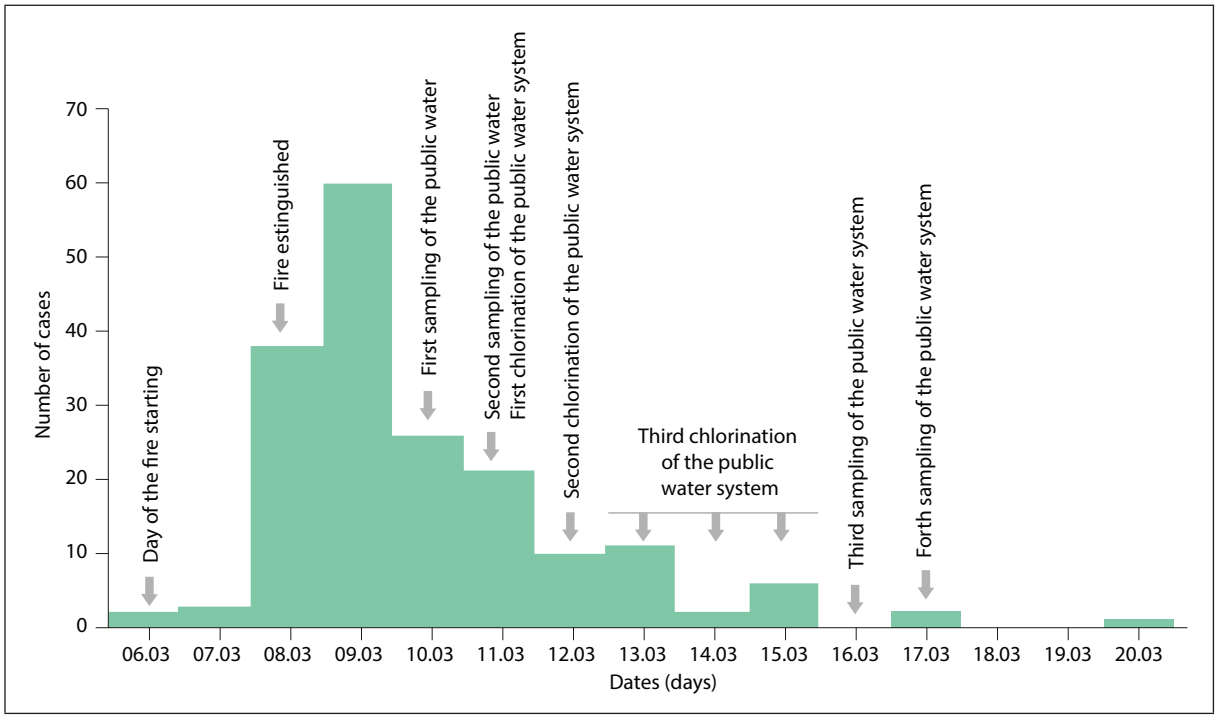

Figure 2
Distribution of the number of cases of gastroenteritis occurred in Postal, North Italy from 6 to 20 March 2020 by date of the onset of the symptoms. The events and the intervention measures put in place by the health authorities are reported by date of occurrence.

(34.35%). The mean incubation period was 72 hours (3 days), and it ranged from 8 hours to 14 days. The median duration of symptoms was 3 days (range 1 to 13 days). One case needed access to an emergency department but was not hospitalized. All the respondents declared usual drinking of tap water in the days before the onset of clinical signs.

The median distance between the place of the fire and the residence was 459.33 meters (IQR=238.72-773.56 meters). It was calculated on 130 cases, because for one case the geographical information was not available. The highest density of cases occurred in the area of the village located at about 300 meters North-East of the fire, followed by two other areas, one at about 650 meters North, and another at about 350 meters South of the fire (*Figure 1*). According to the water system flow, the hot spots North of the fireplace were downstream, while the hotspot South was upstream of the fire.

Due to the National lockdown imposed by the Government, the Local Health Unit and Laboratories reduced their activities. As a consequence, only 18 samples (rectal swab plus stool sample) were collected from the 201 suspected cases.

Among the 18 samples analyzed, twelve were positive for NoVs (66.6%). The molecular characterization showed the presence of two different genogroups: genogroup I (GI) and genogroup II (GII). Seven (38.8%) samples were co-infected (2 norovirus + rotavirus; 2 norovirus + EPEC; 1 norovirus + rotavirus + sapovirus; 1 norovirus + rotavirus + sapovirus + EPEC + EAEC; 1 norovirus + rotavirus + EPEC + EAEC;) and 1 was positive only for rotavirus. Five samples were negative.

The two water samples collected on 10 March and five out of nine water samples taken on 11 March in different points of the water system (*Figure 1*) revealed high concentrations of *E. coli* (>300 colony forming units [CFU]/100mL), Enterococci (>120 CFU/100mL), total coliforms (>300 CFU/100mL), while the chemical parameters were below the limits. The negative samples were those collected at the municipality well and in the southern part of the village.

Norovirus was detected on the filter collected on 10 March in the household water filtering system of two cases.

On 10 March the residents were advised not to consume or use tap water and bottled potable water was

Table 1
Frequency of the symptoms presented by the 131 cases of gastroenteritis in Postal, North Italy

Symptom	Number of cases (131 total)	Frequency (%)
Diarrhea (≥3 loose stools in 24 hrs)	94	71.76
Vomiting (≥3 times in 24 hrs)	82	62.60
Nausea	78	59.54
Abdominal pain	70	53.44
Head/joint ache	64	48.85
Fever (≥37.5 °C)	45	34.35

provided to the population for drinking. The chlorine treatment of the water system started on 11 March and was repeated four times, the last one ended on 15 March. Chlorine concentration of the water supply was then maintained through the whole surveillance period at 0.2 mg/L, corresponding to the currently recommended concentration for waters intended for human consumption in Italy. Twelve out of fourteen water samples taken on 16 and 17 March were negative, and only two samples revealed 1 CFU/100mL, while the five samples collected on 19 March resulted negative. The water avoidance notice was extended up to 18 March 2020.

DISCUSSION

We described an outbreak of gastroenteritis in a small village in Northern Italy that occurred in a close temporal and spatial relationship with a firefighting. We suspected the consumption of water contaminated by river water during fire extinguishing as the source of the outbreak. The outbreak involved at least 131 cases but up to 201 people declared symptoms referable to gastroenteritis in the period from 6 to 20 March 2020.

To our knowledge, this is the second community waterborne outbreak of gastroenteritis associated with firefighting [10].

The sudden onset of symptoms and clinical picture of the illness was suggestive of viral infection, in particular norovirus [11]. Stool samples collected from patients confirmed norovirus genogroups GI and GII. Moreover, norovirus was also detected in the household water filtering system of two cases. Unfortunately, this latter sample was not sequenced more comprehensively to determine Norovirus genotypes.

Norovirus constitutes one of the most frequently identified causative agent of waterborne outbreaks associated with tap water contamination in many countries [1, 12]. In Italy, norovirus outbreaks linked with contaminated drinking water from municipal supplies have already been identified in community settings and touristic resorts [7, 13, 14].

Microbiological investigations identified also other pathogens in the stool samples of patients, and this can explain the 3 days mean incubation period, since the incubation period of several gastrointestinal pathogens can vary from hours to weeks. In waterborne outbreaks multiple aetiologies are common [1], and in outbreaks due to surface water contaminated with sewage, a large number of different pathogens, including bacteria, viruses and parasites, may be involved [15]. Due to the limitations of the laboratory capacities for testing other than SARS-CoV-2 suspected samples, we tested only eighteen stool samples that allowed the confirmation of the Norovirus aetiology and the possible involvement of other pathogens.

Considering the dynamic of the event, we hypothesized an incident during the firefighting on 6 March 2020 that caused the contamination of the public water system, including the elevated tank but not the deep well. This hypothesis is substantiated by the highest density of cases in proximity of the fire and downstream, probably due to the entry of the contaminated water in one or more of the public water network points connected to the firefighters' hydrants (given also the lack of backflow valves in some municipal hydrants). An investigation into the actions taken by firefighters pointed towards the use of contaminated river water drawn right near the sewage pipelines, that from the pressurized water tank used by firefighters to extinguish the fire may have entered into the municipality water system. Infiltrations into the water system or breakages were ruled out by verifying water pipe installations integrity in the area around the fire. However, the cases were also found in different areas of the village, and this may have been caused by contaminated water rising along the pipes up to the municipality tank due to its high pressure, and from here flowing back by gravity into the system.

The actions taken by local health authorities included the provision of an alternative drinking water source and chlorination of the drinking water network. Furthermore, additional hygiene education to reduce the risk of waterborne infections was also provided to all citizens.

Chlorination ability to improve water safety can vary as a function of source water characteristics (e.g. turbidity, pH and temperature), and chlorine characteristics (e.g. concentration and dose) [3, 16].

Moreover, norovirus is resistant to chlorine disinfection when free chlorine levels are inadequate [17] The characteristics of the contamination (river water with sediments and sewage) and the relatively small size of the tank comparing with the pipeline system may have hampered the efficacy of the chlorination. These factors may explain the sporadic microbial findings in water samples after the first chlorination and the need to repeat the treatment.

This study presents some limitations, partly due to the concern of people related to the SARS-CoV-2 emergency and to the lockdown measures implemented by the Italian government. First, we faced a severe limitation in the laboratory testing capacity due to the sudden request of SARS-CoV-2 diagnosis for a large number of suspected cases; second, it was not possible to plan any analytical study to identify individual factors related to the risk of infection, as the local health staff was almost completely dedicated to the SARS-CoV-2 emergency; third, we probably underestimated the number of cases because the local health authorities advised the citizens to limit the visits to hospitals and laboratories to urgent issues, reducing the opportunity to identify those who had mild symptoms of acute gastroenteritis.

Another limitation was the incomplete microbiological investigation of the water, that only included the microbiological parameters according to the Council Directive 1998/83/EC. Fecal or sewage contamination of drinking water is known to result in mixed bacterial and viral infections [18, 19], but the lack of an unified method to encompass the collection and analysis of a water sample for all pathogenic microorganisms of interest make difficult the microbiological screening of the water samples [15, 20].

Finally, we probably missed some cases, in particular among firefighters who were not resident in Postal and

were not interviewed nor sampled. The large number of firefighters involved in the fire made particularly difficult the identification of those potentially exposed and infected.

Amidst the chaotic situation due to the newly implemented lockdown measures, the ongoing gastroenteritis outbreak and the risk of infection and spread of the COVID-19 in the small community, the operations of the local health authorities became extremely complex. However, thanks to the support from the authorities and people's strict adherence to authorities' advices, the situation was maintained under control and more serious consequences were avoided.

Several improvements can be recommended, taking into account the outbreak dynamic. Operational guidelines and training for the firefighter volunteers on the use of the hydrosub units and the health risks related to contaminated water during fire operations should be advised. Improvements in the water system sanitization procedures to increase the efficacy of the chlorination interventions and reduce the time required to effective sanitization of the water are also advised, as the implementation of reflux valves in hydrants. A more thorough microbiological investigation of the water in case of suspected waterborne outbreak should be considered, in particular when the involvement of multiple pathogens cannot be excluded.

In conclusion, we described a waterborne community outbreak potentially related to a fire incident in a small village in Northern Italy occurred during the beginning of the SARS-CoV2 emergency, and we commented on the special difficulties encountered during the investigation due to this concomitant event.

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Conflict of interest statement

None declared.

Authors' contributions

SN coordinated the epidemiological investigation, collected the data from the different sources, undertook the search and interpreted data for the work. FB coordinated the environmental investigation and collected the data from the field. AS provided environmental data and contributed to the environmental investigation. RO provided clinical information on cases and revised the manuscript. LB made data analyses and mapping. SN and LB wrote the manuscript. DR supervised the investigation and revised the manuscript. All authors read and approved the final manuscript.

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Dioxins and PCBs contamination in milk and dairy products from Province of Taranto (Puglia Region, Southern Italy): a six years spatio-temporal monitoring study

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Abstract

Introduction. Taranto Province (Puglia Region, Southern Italy) is of particular Public Health relevance due to the presence of industrial sources of dioxins and PCBs. The aim of this study was to analyze the spatio-temporal distribution of these pollutants in milk and cheese produced from 2013 to 2018.

Materials and methods. Raw milk and dairy products were sampled in the farms located within 20 km from the industrial area.

Results. 1005 milk samples were collected. Median (IQR) concentrations were: dioxins 0.21 (0.21) pg WHO-TEQ/g fat; dioxins+DL-PCBs 0.83 (0.71) pg WHO-TEQ/g fat; NDL-PCBs 1.92 (1.56) ng/g fat. Overall, only 6 (0.6%) samples were found to be non-compliant for at least one pollutants group. Temporal analysis showed a decreasing trend in dioxins and PCBs concentrations over the observed years and higher values in the first trimester. Spatial analysis showed higher levels of PCBs in areas closest to the industrial pole. 70 dairy products samples were collected. Median pollutants concentrations were far below the EU limits and no exceedances were observed.

Conclusions. The extremely low number of exceedances appeared as an encouraging result and supported the validity of the Public Health measures adopted by the Department of Prevention of Taranto.

Key words

- dioxins
- PCBs
- environmental contamination
- Taranto
- milk
- dairy products

INTRODUCTION

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

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

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

Taranto, a coastal city in the South of Italy (Ionian Sea, Puglia Region), is of particular relevance in this context due to the type of industrial settlements accounting for known potential sources of PCDD/Fs and PCBs (the most important steel plant in Europe, an oil refinery, a cement works, thermoelectric plants, waste

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incinerators, discharges and military harbours) and to the environmental contamination present in different matrices, including soil [10-13].

In this regard, in the Province of Taranto several farms account for a significant production of different foods of animal origin, including milk and dairy products. Here because, since 2008, the Department of Prevention of the Local Health Authority of Taranto has carried out an extraordinary monitoring plan in order to assess PCDD/Fs and PCBs contamination in different food matrices produced in the farms adjacent to the industrial area of Taranto.

The aim of this study was to analyze the spatio-temporal distribution of PCDD/Fs, DL-PCBs and NDL-PCBs values and EU limit [14] exceedances in raw milk and dairy products collected between January 2013 and December 2018 from the farms located within a radius of 20 km from the industrial area of Taranto, in order to guarantee the healthiness of the product placed on the market, identify critical seasons for food contamination, and verify and develop effective public health strategies to protect the health of consumers together with the production chain of the territory.

MATERIALS AND METHODS

Sampling

We included in this study all the raw milk and dairy products samples collected by the staff of the Department of Prevention of Local Health Authority of Taranto between January 2013 and December 2018 from the monitored farms of Province of Taranto. The extraordinary monitoring plan activities included inspections and georeferenced samplings in farms located within a radius of 20 km from the industrial area of Taranto. All distances from the industrial area were measured from the reference center of the steel plant (40.505647, 17.210601). Raw milk sampling was carried out at the time of milking in farms within the study area and preferably from animals raised outdoors and fed on pasture. Dairy products sampling was carried out in dairies that produced milk-based products using raw milk from farms within the study area. The samples were sent for chemical analysis to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (National Reference Laboratory for Halogenated POPs in food and feed).

Chemical analysis

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

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

All standard solutions for PCDD/Fs, DL-PCBs and NDL-PCBs were supplied by Wellington Laboratories (Guelph, Ontario, Canada).

Analytical methodology. Samples were tested by validated and accredited methods (EN ISO/IEC 17025) routinely used for PCDD/F and PCB analysis in food; these methods have successfully been tested in a number of inter-laboratory studies.

The 17 PCDD/Fs, the 12 DL-PCBs and the 6 NDL-PCBs [14] were determined through methods based on US EPA (1994) Method 1613 B for PCDD/Fs and US EPA (2008) Method 1668 B for PCBs. Both methods are based on isotopic dilution and high resolution mass spectrometry (HRMS) detection. In order to adapt the analytical procedures to the matrix under examination, variations have been made in the extraction and purification phases of the sample.

All the samples under examination were homogenized and a representative amount of raw milk (100-200 g) and dairy products (10-30 g) reconstituted with water, was taken. Before extraction, samples were fortified with a mixture of the internal standards containing: 17 PCDD/Fs $^{13}C_{12}$-labeled (0.2-0.4 ng); 12 DL-PCBs $^{13}C_{12}$-labeled (1.0 ng); 6 NDL-PCBs $^{13}C_{12}$-labeled (1.0 ng).

Samples were mixed with ethyl alcohol and ammonia solution and then fat was extracted by a mixture of diethyl ether and petroleum ether 1:1 (v/v). Lipid content was determined gravimetrically for all samples after evaporation of the solvent. The extract fat was dissolved in hexane and a liquid-liquid partitioning process was performed to exclude the lipid component using concentrated sulfuric acid, 20% aqueous potassium hydroxide, and saturated aqueous sodium chloride. It was then purified on an automated Power-Prep™ system (Fluid Management System Massachusetts, USA) using multilayer silica, activated carbon and alumina columns. The two eluates containing PCDD/Fs and PCBs, were concentrated by evaporation under nitrogen stream and dissolved in the corresponding recovery standards solutions ($^{13}C_{12}$-labeled PCDD/Fs and PCBs different from the previous ones).

The instrumental analysis was performed using high resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS), using GC Trace Series 2000 coupled to a MAT 95 XL (Thermo Fisher Scientific, USA) and a Trace Series 1310 GC, coupled to a DFS (Thermo Fisher Scientific, USA). PCDD/F congeners were separated on a DB-5 MS capillary column 60 m × 0.25 mm × 0.10 µm (J&W Scientific, California, USA) while the chromatographic separation of DL-PCBs and NDL-PCBs was carried out on HT8-PCB capillary column 60 m × 0.25 mm × 0.25 µm (SGE Analytical science, Melbourne, Australia). The acquisition of the masses was performed in Single Ion Monitoring (SIM) mode at a resolution of 10,000.

Toxic equivalents (TEQs) for PCDD/Fs and DL-PCBs were calculated using the World Health Organization Toxic Equivalency Factors (WHO-TEFs) [9]. TEQ concentrations were determined multiplying the analytical result of each congener by the corresponding WHO TEF, while for NDL-PCBs, the result was reported as the sum of the 6 indicator congeners.

According to the European legislation [14], all reported sum concentrations and TEQ values were expressed in "upper bound" terms (not detects posed equal to the LOQ – limit of quantification) and the contamination levels were expressed on fat basis.

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

Statistical analysis

Statistical analysis was performed using R version 3.6.2 (released on 2019-12-12). Statistical significance α was fixed to 0.05.

In order to account for non-normality, evaluated through Shapiro-Wilk test, numerical variables (means of the measured values of the pollutants concentrations) [14, 15] were reported as range, IQR, median and mean and compared through Kruskal Wallis rank sum test. Comparisons were carried out between years and trimesters.

Categorical variables (means of the measured values of the pollutants concentrations minus the associated expanded uncertainty that are above the established EU maximum level) [14, 15] were reported as absolute and relative frequencies and compared through Fisher Exact Test (Fisher-Freeman-Halton Exact Test for contingency tables larger than 2×2). Comparisons were carried out between years and trimesters.

Maps were created with Microsoft Excel version 2002 (Build 12527.20194).

In order to assess the correlations between the pollutants (values) non-normally distributed and the distance from the industrial area as well as within each combination of these pollutants, Spearman rank correlation coefficients ρ were calculated. P-values were computed via the asymptotic t approximation.

RESULTS

1005 raw milk samples were collected between 2013 and 2018 from the monitored farms of Province of Taranto: 359 bovine, 324 sheep, 320 goat, 1 equine and 1 buffalo milk samples. Results of overall pollutants values and exceedances were reported in *Table 1*. Overall, only 6 (0.6%) samples were found to be non-compliant for at least one pollutants group: 2 bovine (2013), 1 sheep (2014) and 3 goat (2017) milk samples.

Temporal trends of milk pollutant values over the observed years and trimesters were reported in *Figure 1*.

Kruskal Wallis rank sum test between years showed a significant difference for dioxins ($p < 0.0001$), dioxins+DL-PCBs ($p < 0.0001$) and NDL-PCBs ($p < 0.0001$), with a decreasing trend over the years.

Kruskal Wallis rank sum test between trimesters showed a significant difference for dioxins ($p < 0.0001$), dioxins+DL-PCBs ($p < 0.0001$) and NDL-PCBs ($p < 0.0001$), with higher values in the first trimester.

Milk pollutants exceedances (%) distribution between years were (p from Fisher Exact test):

- for dioxins >2.5 pg WHO-TEQ/g fat: 1 (33.3%) in 2014 and 2 (66.7%) in 2017 ($p = 0.2636$);
- for dioxins + DL-PCBs >5.5 pg WHO-TEQ/g fat: 2 (33.3%) in 2013, 1 (16.7%) in 2014 and 3 (50.0%) in 2017 ($p = 0.2067$);
- for NDL-PCBs >40 ng/g fat: 1 (100.0%) in 2013 ($p = 0.5871$).

Milk pollutants exceedances (%) distribution between trimesters were (p from Fisher Exact test):

- for dioxins >2.5 pg WHO-TEQ/g fat: 1 (33.3%) in II and 2 (66.7%) in III ($p = 0.0398$);
- for dioxins + DL-PCBs >5.5 pg WHO-TEQ/g fat: 1 (16.7%) in I, 2 (33.3%) in II, 2 (33.3%) in III, 1 (16.7%) in IV ($p = 0.2919$);
- for NDL-PCBs >40 ng/g fat: 1 (100%) in IV ($p > 0.9999$).

Spatial distribution of milk pollutants values in the monitored farms of the Province of Taranto was shown in *Figure 2*.

Results of Spearman rank correlation ρ for milk pollutants were reported in *Table 2*.

Distance from the industrial area of Taranto showed significant ($p < 0.0001$) negative correlation with dioxins+DL-PCBs ($\rho = -0.19$) and NDL-PCBs ($\rho = -0.29$), while no significant correlation was found with dioxin ($p = 0.2717$).

All pairwise combinations of milk pollutants values

Table 1
Pollutants values in raw milk and dairy products (Taranto, 2013-18)

Milk (n = 1005)	Min	1st Qt.	Median	Mean	3rd Qt.	Max	EU limit	N. exceedances (%)
Dioxins (pg/g)	0.00	0.13	0.21	0.28	0.34	4.65	2.50	3 (0.3%)
Dioxins + DLPCBs (pg/g)	0.01	0.56	0.83	1.10	1.27	23.28	5.50	6 (0.6%)
NDLPCBs (ng/g)	0.03	1.30	1.92	2.53	2.86	57.32	40.00	1 (0.1%)
Dairy products (n = 70)	**Min**	**1st Qt.**	**Median**	**Mean**	**3rd Qt.**	**Max**	**EU limit**	**N. exceedances (%)**
Dioxins (pg/g)	0.04	0.10	0.17	0.21	0.26	0.63	2.50	0 (0.0%)
Dioxins+DLPCBs (pg/g)	0.25	0.43	0.62	0.75	0.85	2.96	5.50	0 (0.0%)
NDLPCBs (ng/g)	0.79	1.09	1.43	1.83	2.06	7.02	40.00	0 (0.0%)

Dioxins = sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs = sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs = sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs = sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants values = mean of the measured values, fat basis; pollutants exceedances = mean of the measured values minus the expanded uncertainty of the mean, fat basis [14, 15].

Figure 1
Temporal trends of raw milk pollutants values over the observed years and trimesters (Taranto, 2013-18).
Dioxins = sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs = sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs = sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs = sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants values = mean of the measured values, fat basis [14, 15].

showed significant correlation ($p < 0.0001$): the lowest correlation was found between dioxins and NDL-PCBs ($\rho = 0.53$).

Finally, 70 dairy products samples were collected between 2013 and 2018 from dairies that produced milk-based products using raw milk from the monitored farms of Province of Taranto: 41 bovine, 20 sheep and 9 goat cheese samples. Results of overall pollutants values and exceedances were reported in *Table 1*. No exceedances were observed.

DISCUSSION

Since milk and cheese production represents an important cultural and economic heritage for the population of the Province of Taranto, the Department of Prevention of the Local Health Authority have long been engaged on the dual front of protecting consumers and safeguarding primary production.

Median PCDD/Fs and PCBs concentrations in milk were far below the EU limits and very few exceedanc-

Figure 2
Spatial distribution of raw milk pollutants values (Taranto, 2013-18).
Dioxins = sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs = sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins + DLPCBs = sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs = sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants values = mean of the measured values, fat basis [14, 15].

Table 2
Spearman rank correlation ρ for raw milk pollutants values (Taranto, 2013-18)

Milk (n = 1005)			
Pollutant	**Distance**	**ρ**	**p**
Dioxins (pg/g)	Distance (km)	-0.03	0.2717
Dioxins + DLPCBs (pg/g)	Distance (km)	-0.19	<0.0001
NDLPCBs (ng/g)	Distance (km)	-0.29	<0.0001
Poll. 1	**Poll. 2**	**ρ**	**p**
Dioxins (pg/g)	Dioxins + DLPCBs (pg/g)	0.78	<0.0001
Dioxins (pg/g)	NDLPCBs (ng/g)	0.53	<0.0001
Dioxins + DLPCBs (pg/g)	NDLPCBs (ng/g)	0.77	<0.0001

Dioxins = sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs = sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins + DLPCBs = sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs = sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants values = mean of the measured values, fat basis [14, 15].

es were observed: overall, only 6 (0.6%) samples were found to be non-compliant for at least one pollutants group. In farms with non-compliant results, accurate epidemiological investigations were carried out to identify the potential hazards and the subsequent analyzes for dioxins and PCBs showed compliance results.

Temporal analysis showed a decreasing trend in dioxins and PCBs concentrations in raw milk over the observed years, with higher values in the first trimester and, mainly for NDL-PCBs, in the fourth trimester. The seasonal concentrations pattern we observed could be linked or to cyclical variations of animal physiological state or to a difference in animal pollutant concentrations exposures between indoor and outdoor environments among different seasons. Whatever the causes of this seasonal fluctuation, these findings could be useful in orienting the timing of sampling in Taranto or in any other areas affected by halogenated POPs contamination, focusing on the periods with presumably highest risk.

As far as spatial analysis of milk pollutants concentrations is concerned, despite areas closest to the industrial pole showed, as expected, higher levels of PCBs, maps and correlation analysis didn't show a clear distribution for dioxins, whose concentrations did not appear to be related to the distance from the pole. Moreover, our results didn't show strong correlation between dioxins and PCBs, suggesting a complex contamination scenario with partially unrelated polluting sources. All the more reason, precisely in such a varied and partly unpredictable scenario, the vigilant and constant control of the Prevention Department plays a pivotal role in intercepting those few possible unsafe products before they are placed on the market. On the other hand, the possibility to identify an inverse relationship between distance from the industrial area and PCBs concentrations in milk provides us with a useful tool to guide sampling in farms exposed to a greater risk of contamination and could be of great Public Health importance in Taranto as well as in any site contaminated by halogenated POPs, in the light of the fact that there is sufficient evidence in humans for the carcinogenicity (Group 1) of polychlorinated biphenyls (PCBs): in particular, PCBs cause malignant melanoma and positive associations have been observed for non-Hodgkin lymphoma and cancer of the breast [16]. Our results regarding the possible association between the levels of PCBs in raw milk and the emissions of the industrial pole seem consistent with the 2009 ARPA (Agenzia Regionale per la Prevenzione e la Protezione Ambientale) Puglia wind-selective environmental monitoring campaigns for organic micropollutants, in which the downwind/upwind concentration ratios in 4 sites located 0.5 km (two sites), 3.5 km and 6 km from the industrial area showed a clear directional origin of PCDD/Fs and PCBs [17]. Moreover, this is in line with the findings of the 2012 ISS (Istituto Superiore di Sanità – Italian National Institute of Health) exploratory biomonitoring study in which the blood levels of dioxins and PCBs among livestock farms workers of the Taranto Province appeared to be strongly associated with the distance of the farm from the industrial site [18].

Finally, also median PCDD/Fs and PCBs concentrations in dairy products were far below the EU limits and no exceedances were observed. In conclusion, despite the environmental pressures, the low median dioxins and PCBs concentrations and the extremely low number of exceedances over such a long period and so many samplings appear as an encouraging result regarding concerns about the safety of milk and cheese produced in the Province of Taranto. These findings offer a Public Health analysis and control model that is potentially applicable to other areas contaminated by halogenated POPs. The model's risk assessment is based on the distance from the principal polluting sources, even if the partial unpredictability of the contamination scenario makes it necessary to monitor even the most distant farms. In such a complex context, the constant control of the Prevention Department makes it possible to block the food at risk, protecting the health of consumers together with the production chain of the territory.

Conflict of interest statement

None to declare.

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The Italian National Faecal Microbiota Transplantation Program: a coordinated effort against *Clostridioides difficile* infection

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Abstract
Clostridioides (previously *Clostridium*) *difficile* infection (CDI) is a common cause of antibiotic-associated diarrhea, whose symptoms range from mild diarrhea to life-threatening pseudomembranous colitis. CDI is characterized by significant recurrence rate following initial resolution and recurrent *C. difficile* infection (rCDI) represents an onerous burden for the healthcare systems. Conventional antibiotic-based approaches are generally used for the treatment of rCDI but the effective therapy remains elusive. Recently, the faecal microbiota transplantation (FMT) has emerged as an alternative therapeutic strategy against rCDI, with high treatment success rate. In 2018, the Italian National FMT Program was launched, with the aim to provide high quality standards in FMT application to adults with rCDI not responding to antibiotic therapy. Here, we sketch out the key characteristics and the progress of the Italian National FMT Program during the COVID-19 pandemic.

Key words
- faecal microbiota transplantation
- *C. difficile* infection
- antibiotic resistance
- COVID-19
- quality assurance

CLOSTRIDIOIDES DIFFICILE INFECTION AND FAECAL MICROBIOTA TRANSPLANTATION

The faecal microbiota transplantation (FMT) consists in transferring of a faecal sample from a healthy donor into the gastrointestinal tract of a patient, in order to modulate the gut microbiota. The gut microbiota includes bacteria, archaea and eukarya colonizing the gastrointestinal tract, and its composition is shaped by environmental factors, such as diet, and also by host immune system and genetics [1]. In terms of susceptibility to infectious diseases, the microbiota imbalance (dysbiosis) has a remarkable impact on the colonization resistance, that is the resistance provided by the microbiota against enteric pathogens [2]. Antibiotic therapy is a common cause of dysbiosis reducing bacterial diversity and abundance, and is considered a risk factor for the host infection by different pathogenic microorganisms enriched following the microbiota perturbation. A well-known example is *Clostridioides difficile* infection (CDI) characterized primarily by diffuse diarrhea and marked by a significant rate of relapse following antibiotic treatment [3-4]. Antibiotics deplete gut microbiota resulting in decreased microbiota signaling and diminished local and systemic immune responses to CDI [2]. The clinical symptoms of CDI range from mild diarrhea to fulminant pseudomembranous colitis associated with toxic megacolon, colonic perforation and multiorgan failure [4]. In 2017, there were an estimated 223,900 CDI cases in hospitalized patients and 12,800 deaths in the United States [5]; in 2016, CDI surveillance in Europe reported a total of 7711 cases, 5756 of which (74.6%) were healthcare-associated and 611/7711 (7.9%) cases were classified as recurrent infections (rCDI) [6]. Besides the substantial effect on patient quality of life, the management of rCDI, defined as a relapse of CDI symptoms within 2-8 weeks of successful treatment of the initial episode [7], is also a

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relevant burden for the healthcare systems; it has been reported in fact that the risk of developing a first rCDI is 25%, with a 40% probability of a second recurrence episode and an increased rate of re-hospitalization [8]. Conventional antibiotic-based approaches are generally used for the treatment of rCDI but the effective therapy remains a challenge. The antibiotic treatment options for patients include standard course or tapered and pulsed regimen of vancomycin, vancomycin followed by rifaximin or a standard course of fidaxomicin [7]. Recently, FMT has emerged as an alternative and viable strategy against rCDI and both the European Society for Microbiology and Infectious Diseases and the American College of Gastroenterology recommended FMT for patients with multiple recurrences of CDI who failed therapy with the appropriate antibiotic agents [9, 10]. High treatment success rates have been reported and FMT has been well tolerated by patients with few reports of adverse events [10].

Considering that the role of FMT in clinical practice is evolving, it is relevant to establish standardized procedures that meet specific requirements of quality, safety and efficacy. In 2017, the European Consensus guidance document defined indications and methodology for the use of FMT in CDI treatment [11] and, in 2019 an international consensus conference on stool banking for FMT has been published with the aim to provide a guidance on the general organization of a stool bank including issues for donor recruitment/screening, preparation and storage of faeces as well as for release faecal suspensions to clinical centers [12]. It is worth mentioning that a common approach for the faecal microbiota regulation does not exist in Europe and Member States are free to decide on the most suitable framework either by establishing a specific regulatory framework at national level or by applying one of the existing legislative frameworks, such as national requirements for tissue and cell transplantation [13]. However, many European countries have no regulation on FMT at all; globally, the faecal microbiota classification is a demanding task because the underlying mechanism of action and the active component are still to be completely understood and FMT therapy does not fit entirely in any regulatory framework. In Italy, the faecal microbiota has been classified as human cell/tissue product and regulated according to DL 191/2007 and DL 16/2010, in compliance with Directives 2006/17/EC and 2006/86/EC implementing Directive 2004/23/EC on the quality and safety of tissues and cells [14, 15]; currently, the FMT regulatory application is falling within the remit of Italian National Transplant Center (CNT), which was appointed as the coordinator of the Italian National FMT Program launched in 2018 by the Italian Ministry of Health.

THE ITALIAN NATIONAL FMT PROGRAM: KEY CHARACTERISTICS AND STATE OF THE ART

The Italian National FMT Program was established with the aim to provide high quality standards in FMT application to adults with rCDI not responding to antibiotic therapy, ensuring best clinical practice for the patient care. The FMT Program was addressed to all public health structures and Regional hospitals, placed on the national territory, with appropriate expertise and facilities, including a gastroenterology unit, endoscopy service, clinical ward and outpatient clinic, a processing laboratory (biosafety level 2), as well as a microbiology testing laboratory and either an infectious diseases service or expert advice.

The FMT Program document provides a technical and operational guidance on how to set up the FMT process and CNT is in charge of evaluating if clinical centers fulfill the advised criteria needed for implementing a FMT service. Among the others, the guidance includes specific requirements relating to donor selection, a crucial issue to assure a safe FMT treatment. In particular, in agreement with the recommendations of the European FMT Working group [11], both related and unrelated donors may be enrolled and the donor selection/recruitment consists of three different steps: 1) a written medical interview to exclude history and risk factors; 2) blood and stool testing at most 4 weeks before donation to check the donor for any potentially transmittable disease; 3) questionnaire and stool testing on the day of donation. The screening panel for donor stool testing was established with the purpose to detect not only common enteric pathogens and faecal parasites but also multi drug-resistant organisms, including meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococci, extended-spectrum β-lactamase producing and carbapenemase-producing Enterobacteriaceae, in order to avoid the transmission of microorganisms that could lead to serious or life-threatening infections as it has already been reported [16]. Noteworthy, due to the ongoing COVID-19 pandemic the standard donor screening protocol was strengthened with additional measures to minimize the risk of COVID-19 infection [17]. In particular, according to the indications from an international FMT expert panel [18], CNT recommended that potential donors should be asked for: known diagnosis of laboratory confirmed SARS-CoV-2 infection; appearance of specific symptoms associated to COVID-19 including fever, cough, fatigue, muscle pain, dyspnea and headache; close exposure to subjects with suspected or proven infection, within the previous 30 days. Moreover, RT-PCR assay for SARS-CoV-2 on nasopharyngeal swab specimen and stool sample was strongly advocated, in accordance with the opinion of worldwide FMT experts [18, 19]. Recently, CNT indications for the screening of stool donors vaccinated for SARS-CoV-2 have been released [20].

Regarding the stool handling, another critical step of FMT procedure, laboratories are required to use standardized analytical techniques and the protocol for the preparation of both fresh and frozen faecal material is fully detailed in the Italian FMT guidance. Currently, the use of frozen faecal material is the preferred option to reduce the potential risk of SARS-CoV-2 transmission associated with FMT, since the freezing allows to quarantine the stool sample until screening results are available. Overall, the participants to the Program are required to adhere to standard operating procedures for the processing and to apply qualitative and quantitative

quality-control tests for the release of the final product to be delivered to the patient (via colonoscopy or retention enema or alternatively into the upper gastrointestinal tract). Finally, in order to ensure the traceability of the entire FMT process from the donor to the recipient, the product flow data need to be recorded and appropriate documentation relating to each step of the procedure must be prepared. In this respect, it is worth mentioning that the development and the implementation of the quality management and full traceability systems are key elements taken into account by CNT during the authorization process, which consists of both documentation review and on-site inspections.

To date, 18 health structures requested for the participation in the Italian National FMT Program, 11 from the Northern, 6 from the Middle and 1 from the Southern Italy, with an evident decreasing North-to-South gradient. Concerning the assessment of the minimum requirements, only 9 out of 18 FMT centers were audited, as the other applicants submitted incomplete or inadequate documentation in order to perform on site inspections. Reference guidelines and suggestions on how to deal with relevant issues are provided to the centers, with the aim to improve documentation and facilitate the implementation of corrective actions to overcome any critical deficiencies.

Out of the 9 inspected centers, 4 passed successfully each of the evaluation steps and were authorized to participate in the National Program, whereas 5 are still implementing the appropriate post-inspections corrective measures. It should be noted that the COVID-19 outbreak can certainly explain the slow progress in implementing the Program: approximately two thirds of the applicants come from Italian regions primarily impacted by the coronavirus spread (Lombardia, Piemonte, Emilia-Romagna, Liguria, Toscana) and, in addition, the clinical microbiology laboratories have undergone a progressive adaptation to meet growing demand for SARS-CoV-2 testing, with a consequent considerable reduction of all non-COVID-19 related testing activities. Furthermore, hospitals interested in participating in this Program are now playing a crucial role as COVID-19 vaccine hubs, and they are very committed to this task.

Currently, the FMT center within Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome is the only one to carry out microbiota transplants, as the service has been able to adapt its operational workflow during COVID-19 pandemic, in order to continue offering FMT treatment to patients with rCDI [21]. With regard to the surveillance of FMT safety and efficacy, the CNT has developed a client-server application (MySQL database) to register the procedures carried out within the Italian National FMT Program and the users can easily enter data relating to donor, patient, stool sample processing, route of delivery, and transplant outcomes including any serious adverse events and/or reactions occurring during the follow-up period (8 weeks). This system ensures transplants traceability and represents the nationwide collection of faecal microbiota transplants. The main characteristics of all data collected prospectively from June 2020 up to March 2021 are shown in *Table 1*. In particular, 31 patients (male=17, female=14) were transplanted with frozen faeces from 6 donors (male=2, female=4; unrelated=5, related=1). The median age was 70 and 50 for the patients and donors, respectively, and stool samples from the same donor served, on average, 6 patients. At 31 March 2021, 24 patients completed the follow up with a transplant success rate of 100%, while the follow up is ongoing in the remaining 7 patients. Noteworthy, no serious adverse events and/or reactions were notified.

Table 1
Characteristics of treated patients, donors, transplants and outcomes

	N
Patients	31
Male/female	17/14
Median age (min-max)	70 (20-94)
Vancomycin/fidaxomicin treatment pre-FMT	31/0
Donors	6
Male/female	2/4
Median age (min-max)	50 (37-60)
Unrelated/related	5/1
Transplants	
Frozen/fresh material	31/0
Colonoscopy/enema/nasogastric tube	31/0/0
Single/sequential infusions in patients with completed follow-up	18/6
Outcomes	
Follow-up at 8 weeks completed/ongoing	24/7
Successful/failed outcome	24/0
Serious adverse events/serious adverse reactions	0/0

CONCLUSIONS

The establishment of the Italian National FMT Program allowed to evaluate if the Italian centers comply with specific quality and safety standards for FMT application in rCDI. Furthermore, the Program provides a surveillance system for collecting and analyzing several information on FMT treatment, including the number of screened donors and performed transplants, stool manipulation, infusion procedure, outcomes and follow-up data, monitoring any deviations from the standard procedures. On the basis of data available at this stage, FMT approach results in a well-tolerated and efficacious treatment for adults with rCDI refractory to antibiotic therapy. On the other hand, as the sample is restricted, further data are needed to confirm these results and additional FMT centers should contribute to the progress of the Program. In order to encourage participation in the Italian National FMT Program, dissemination and training activities were carried out, such as webinars and CME (Continuing Medical Education) courses involving the whole regional transplant

network. Regrettably, the current pandemic has largely impacted on healthcare systems and imposed the reduction of medical procedures and other services COVID-19 unrelated. However, it needs to point out that Lewandowski *et al.* recently observed a significant higher incidence of CDI in hospitalized patients with COVID-19 [22], raising concerns about a potential cause-effect relationship between SARS-CoV-2 infection and CDI occurrence.

Overall, the Italian National FMT Program represents a structured model in order to standardize and harmonize the clinical FMT procedures nationwide, so guaranteeing patients access to safe, high-quality and effective service. Once a significant amount of data is achieved, the next step will aim to set up the minimum organizational, structural and technological requirements to develop a stool bank and FMT specific clinical pathways, in order to provide an equitable, timely and cost-effective access to FMT treatment, according to the national regulatory frameworks for human cell/tissue products [14, 15]. Finally, the Italian National FMT Program may serve as a template to implement additional joint activities for other FMT potential applications in non-CDI settings, including other gastrointestinal diseases as well as metabolic diseases, neuropsychiatric and immunologic disorders [23]. In this context, it should be noted that, with the exception of CDI, FMT approach is currently considered an experimental treatment and shouldn't be performed without CNT approval, in according to the national legislative framework for the experimental transplantation [24].

Conflict of interest statement

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

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Hepatitis B (HBV) reactivation in patients receiving biologic therapy for chronic inflammatory diseases in clinical practice

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Abstract

Introduction and aim. Biologic treatment – particularly with the anti-TNF molecules – is frequently used in clinical practice to treat the severe form for both chronic rheumatic diseases and inflammatory bowel diseases. The immunosuppression induced by biologic therapies increases the risk of infections, including tuberculosis, as well as hepatitis B virus (HBV) reactivation may occur in inactive carriers or occult HBV infection (OBI) subjects during biologic therapy. This study aimed to update data on HBV prevalence and reactivation in patients receiving biologic therapy for either chronic rheumatic diseases or IBD, and to describe their management in clinical practice.

Materials and methods. This study was performed in 6 Italian centers (3 Rheumatology Units and 3 Gastroenterology Units). Clinical, biochemical and virological data, as well as follow up information, were recorded and analyzed.

Results. 984 patients were considered, including 817 with rheumatic disease and 167 with IBD. A total of 43 showed HBV infection (38 OBI and 5 carriers) accounting for a prevalence of 4%. Among OBI patients, 1 (2.6%) case of HBV reactivation occurred in a male patient with Crohn disease. Among the 5 HBV carriers, two patients (1 with spondyloarthritis and 1 with rheumatoid arthritis) did not received HBV antiviral therapy, and both experienced flare of hepatitis at 47 and 49 months following biologic therapy starting.

Discussion. Data of our study highlight that guidelines on management of HBV patients treated with biologic therapies should be still implemented in clinical practice when considering that, although infrequent, HBV reactivation could be potentially life-threatening.

Key words

- hepatitis virus B reactivation
- ulcerative colitis
- Crohn disease
- rheumatoid arthritis
- spondyloarthritis
- biologic therapy

INTRODUCTION

Biologic drugs are considered a cornerstone therapy for both chronic rheumatic diseases and inflammatory bowel diseases (IBD). Therefore, biologic treatment – particularly with the anti-TNF molecules – is frequently used in clinical practice to treat the severe form of these diseases. Unfortunately, the immunosuppression induced by biologic therapies increases the risk of infections, including tuberculosis. Moreover, hepatitis B virus (HBV) reactivation may occur in inactive carriers or occult HBV infection (OBI) subjects during biologic therapy [1-6]. In both cases, HBV reactivation may cause a severe form of hepatitis, which may remain subclinical or evolve in an acute liver failure and death

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[6]. Risk of reactivation does correlate with HBV-DNA levels pre therapy; by immunosuppressive therapeutic agents used and on the duration of treatment [7]. Usually, the normal immune function restoration developed after immunosuppressive treatment interruption cause immune-mediated liver inflammation and consequent hepatitis [6, 7] and hepatitis reactivation usually occur between 12- and 18-months from discontinuation [8].

Viral reactivation occurred in 39% out of 89 HBV carriers and in 5% of 168 patients with OBI, some cases develop a fulminant hepatitis and death [9]. Antiviral therapy is considered recommended for HBV carriers and a tight control advised in those with OBI in ongoing biologic therapy [8, 10-12]. However, the behavior of physicians on management of these patients in clinical practice has been reported to be at times different from recommendations of guidelines, as well as vaccination policies in these categories of patients are also not well defined.

This study aimed to update data on HBV prevalence and reactivation in patients receiving biologic therapy for either chronic rheumatic diseases or IBD, and to describe their management in clinical practice.

METHODS

This study was performed in 6 Italian centers (3 Rheumatology Units and 3 Gastroenterology Units). Diagnosis of chronic rheumatic diseases and IBD were established according to criteria recommended by international guidelines [9-12]. All patients were assuming biologic therapy.

HBV patients are stratified into the following categories according to serological, virologic and biochemical variables: a) occult HBV infection (OBI) [HBsAg-negativity, antibodies to the core antigen (anti-HBc) positivity and very low (<200 international units [IU]/ml), or absent HBV-DNA levels], with alanine aminotransferase (ALT) persistently within normal range, unless other potential hepatotoxic agents or conditions (i.e. obesity, alcohol, drugs) may increase this latter test; 2) overt carriers (HBsAg-positivity). This group of subjects is further subdivided into chronic HBV infection (the former inactive carrier), characterized by normal or minimally altered ALT value and HBV-DNA persistently below 2,000 IU/ml, and chronic HBV hepatitis (the former active carrier), based on the persistence of ALT elevation for at least 6 months and HBV-DNA higher than 2,000 IU/ml [6]. In overt HBsAg carriers, definition of reactivation is based on a ≥1 log10HBV-DNA increase as compared to the value before immunosuppression, or the *de novo* HBV-DNA detection in a previously negative subject [6]. Reactivation in OBI is defined by the seroreversion, consisting in HBsAg re-expression. This occurrence represents a relevant virologic and clinical event, associated with re-appearance of active viral replication [12].

This was a retrospective study on data available for clinical practice in which clinical records of all patients in ongoing biologic therapy were reviewed. Data were anonymously collected in each center and were cumulatively gathered in an electronic database for analysis. Patients were not required to give informed consent to the study because the analysis used anonymous data that were obtained after each patient agreed to being followed up and to collect clinical records by institutions. No experimental procedures, novel devices or experimental drugs were used, as well as no founds were received. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

RESULTS

Data of 984 patients were considered, including 817 with rheumatic disease (83%) and 167 with IBD (17%). A total of 43 patients had HBV infection (38 OBI and 5 carriers) accounting for a prevalence of 4%. In detail, the prevalence was 3% (95% CI: 1.3-6.8) in IBD and 4.7% (95% CI: 3.4-6.4) in rheumatic disease patients. Clinical characteristics of these patients are summarized in *Table 1*.

Among OBI patients, 1 (2.6%) case of HBV reactivation occurred in a male patient with Crohn disease *(Table 2)*. Biologic therapy was stopped, and antiviral therapy with tenofovir performed until normalization of liver enzymes and undetectable HBV-DNA was achieved. The patient is actually on therapy with both adalimumab and entecavir without experience further HBV flare-up. In this group of patients, a prophylactic therapy with lamivudine was introduced in a patient with spondyloarthritis before starting biologic therapy with sekukinumab.

Among the 5 HBV carriers, two patients (1 male with spondyloarthritis and 1 female with rheumatoid arthritis) did not received HBV antiviral therapy, and both experienced flare of hepatitis at 47 and 49 months after biologic therapy initiation *(Table 3)*. Biologic therapy was therefore stopped and antiviral therapy with entecavir started, achieving remission of hepatitis. Then, biologic and entecavir co-therapy was continued without further hepatitis flare-up. In the other 3 patients, antiviral therapy was performed with lamivudine in two cases and with entecavir in the remaining patient. Two out of these patients were receiving a combo therapy with abatacept and methotrexate. None of these patients experienced HBV reactivation or side-effects.

DISCUSSION

Table 1
Clinical characteristics of patients with HBV

Parameter	Number
Sex (M/F)	20/23
Age (Years ± SD)	58.4 ± 14.2
Diagnosis (UC/CD/RA/SPA)	2/2/15/24
Therapy (infliximab/adalimumab/abatacept/enbrel/etanercept/others	6/6/9/7/8/7
Combo therapy with methotrexate (No/Yes)	24/19
HBV Status (OBI/Carrier)	38/5
Time of biologic therapy exposure (months SD)	50.5 ± 39

UC: ulcerative colitis; CD: Crohn disease; RA: rheumatoid arthritis; SPA: spondyloarthritis; OBI: occult b infection.

Table 2
Characteristics of patient with reactivation of occult HBV infection

Parameter	Status
Sex	Male
Age	52 years
Diagnosis	Crohn's disease
Treatment	Adalimumab
HBV status before starting biologic therapy	HBsAg negative HBcAb positive HBsAb: 6 UI/ml HBV DNA: <10 UI/ml
Time of biologic therapy exposure before reactivation	41 months
Time from therapy discontinuation	13 months
HBV status at reactivation	HBsAg positive HBcAb positive HBsAb negative HBV-DNA: 980 UI/ml
HBV treatment	Tenofovir
HBV status after 6 months from starting antiviral treatment	HBsAg positive HBcAb positive HBsAb negative HBV-DNA: <10 UI/ml

Table 3
Characteristics of HBV carriers who experienced flare of hepatitis

Parameter	Status
Sex (M/F)	1/1
Age; years	73/56
Diagnosis	RA/SPA
Treatment*	Abatacept
HBV Status before starting biologic therapy*	HBsAg positive HBcAb positive HBsAb negative HBV DNA >10 UI/ml
Time of biologic therapy exposure before reactivation	49/41 months
Time from therapy discontinuation	14/15 months
HBV treatment*	Entecavir

*Similar in both patients. RA: rheumatoid arthritis, SPA: spondyloarthritis.

HBV reactivation in patients receiving immunosuppressive biologic therapy has been reported in HBV carriers and, with less extent, in those with OBI [2-8]. Recent guidelines [9-12] advice to perform a complete HBV status screening before starting biologic therapy. In detail, patients with OBI should be, in the majority of cases, only monitored without anti-HBV prophylaxis, whilst HBV carriers should receive anti-HBV therapy before immunosuppressive therapy starting. In these case, anti-HBV drugs with a high resistance barrier, such as entecavir or tenofovir, should be preferred over low-barrier agents as lamivudine.

This study analyzes data of a large cohort of patients receiving biologic therapy, in whom a complete HBV status screening and a close follow-up were performed. We found that the overall prevalence of HBV infection was 4% in these patients. These high prevalence rates could be explained for different reasons: 1) the present is a population intensively screened for HBV infection and 2) the cohort studied come from different countries, including regions at high HBV infection prevalence. HBV reactivation occurred in 2.6% OBI patients and was successfully treated with antiviral therapy. This finding confirms that a close monitoring in OBI patients treated with biological therapy is mandatory [2-8]. Of note, our patient was HBsAb positive with a titer of 6 UI/ml. Few recent observations reported that HBV reactivation did not occur in those OBI patients with an anti-HBs titer >100 IU/mL [13]. Therefore, attention needs to be reserved for those patients with low anti-HBs titer. On the other hand, although the presence of anti-HBs might not prevent HBV reactivation, anti-HBs titer may be useful for surveillance given that the loss of anti- HBs may be a predictor of HBV reactivation [13]. Surprisingly, lamivudine prophylaxis was performed in 1 patient of this group, despite such an approach was not suggested by guidelines [9-12]. Conversely, among the HBV carriers, 2 out of 5 patients did not receive antiviral prophylactic therapy, and both experienced hepatitis. Moreover, in the 3 patients who were on antiviral therapy, only 1 received a high resistance barrier antiviral drug as suggested by guidelines [9-12], whilst the remaining 2 patients were on lamivudine therapy. All these observations highlight that the behavior of physicians on management of these patients in clinical practice remains occasionally different from recommendations of guidelines. All patients, candidates to immunosuppressive therapy should be tested for HBV infection before starting treatment. In our opinion this should considered a tricky point needing of further implementation, considering that often HBV infection is unknown by patient, and many physicians are not fully aware the need of preventing HBV reactivation in patients undergoing immunosuppressive therapy [14].

Guidelines recommend that HBV-negative patients before starting immunosuppressive biologic therapy should be managed with HBV vaccination [9, 15], whereas HBV vaccination of subjects with isolated anti-HBc positivity remains, although rationale, still debated [16]. HBsAg-positive carriers must be treated with antivirals according to their categories, while pOBI, considering their lower risk of reactivation [17-21], should be monitored during and after treatment with immunosuppressive drugs (especially those under biologic treatments), in order to promptly detect a reactivation. A different management should be considered if drugs at high risk of reactivation such as rituximab in rheumatological setting are adopted. In this condition the behavior should be like the one adopted in hematological setting [12, 17-21]. Treatment for both rheumatic disease and inflammatory bowel disease is generally lifelong, but HBV status (HBVDNA and HBsAg) should be

continued in OBI if, in case of remission of the disease, immunosuppressive treatments are stopped [6]. Adherence to guidelines remains an extremely important issue. As this study shows, the different management by clinicians of the same type of patient determines sometimes different outcomes. Therefore, sensitization actions are necessary for a homogeneous management of HBV infection in gastro/rheumatological patients as well as possibly further studies.

In conclusion, data of our study highlight that guidelines on management of HBV patients treated with biologic therapies should be still implemented in order to improve the management of these patients. In fact, it should strongly consider that, although infrequent, HBV reactivation could be potentially fatal and life-threatening. Patients suffering of HBV infection should be promptly identified and referred to specialist for a full evaluation of liver disease. Moreover, a close collaboration between rheumatologist/gastroenterologist and hepatologist should be considered mandatory and therefore strongly encouraged in order to obtain the better and safe management of HBV infection. Finally, the role of vaccination of seronegative patients is increasing and is considered highly recommended.

Authors contribution

Ridola L: conception and design, drafting of the article, critical revision of the article for important intellectual content, final approval of the article; Zullo A: drafting of the article, critical revision of the article for important intellectual content, final approval of the article; Laganà B, Lorenzetti R, Migliore A, Pica R, Picchianti Diamanti A, Gigliucci G, Scolieri P: acquisition of data; Bruzzese V: critical revision of the article for important intellectual content, final approval of the article.

Conflict of interest statement

None.

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Medication prescriptions before, during and after pregnancy in Italy: a population-based study

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Abstract

Background. Monitoring medicine prescriptions in pregnancy is an aspect of extreme interest in term of public health.

Methods. A retrospective prevalence study using administrative healthcare databases was performed in order to evaluate medication prescriptions in Italy. A cohort of 274,938 pregnant women (15-49 years) residing in three Italian regions (Emilia-Romagna, Lazio, Puglia), who delivered in 2014-2017, were enrolled. The prevalence of medication use was estimated as the proportion of pregnant women with any medication prescription in each of the following five trimesters: 1 before pregnancy (pre-T), 3 during pregnancy (1st TP, 2nd TP, 3rd TP) and 1 after pregnancy (post-T).

Results. About 80% of enrolled pregnant women received at least one prescription during pregnancy, 36.5% before pregnancy and 50.7% in the post-partum. The most prescribed medicine was folic acid (42%), mostly used in 1st TP (35%). Progesterone use was concentrated in 1st TP (19%) and increased as the number of previous abortions. Pregnancy use of antidiabetics, antihypertensives, and thyroid preparations were 24.1‰, 21.5‰, 101.8‰, respectively.

Conclusions. At the national level, this study confirmed the prescriptive trend observed in other European studies, but a regional variability for all medication groups was found. Further studies are needed in order to identify determinants of medication prescriptions during pregnancy in Italy.

Key words
- medicines
- pregnancy
- drug utilization
- prescription appropriateness
- regional variability

INTRODUCTION

The use of prescription medications during pregnancy is a common event worldwide. The prevalence of pregnant women who used at least one medication is ranging from 27 to 99% in developed countries, with a substantial inter-region variability in the type of medication used depending on the setting [1-4].

There are significant knowledge gaps surrounding the safety, dosage and long-term effects of medications in pregnancy because pregnant women are generally excluded from pre-authorization clinical trials of the majority of pharmacological treatments for conditions that may occur concurrently with pregnancy [5-7].

Pharmacological treatments commonly used in pregnancy are thus often untested in pregnant woman, not optimized in dose, with minimal information on pharmacokinetics and safety profile (especially on long-term outcomes), and prescribed off-label without an adequate information to judge their risks and benefits. This denies women appropriate drug therapies and an inadequate maternal treatment of disease can jeopardize both the mother's and the child's wellbeing [8, 9].

In the absence of available and clear data that can inform treatment choices, the risk-benefit profile of the use of medicines in pregnancy is assessed mostly through post-authorization studies. Currently, observational and/or descriptive studies are often performed on medication use in pregnancy in order to close the knowledge gap in this context [10].

In Italy a prevalence of about 75% of women exposed to at least one prescription medication during pregnancy [11] was reported. However, no recent national

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data about the prevalence of medication use during pregnancy were available in Italy [12] and the information on regional variability in prescription patterns are scarce [13-17]. To fill this important knowledge gap and to better inform clinical practice in Italy, we performed a population-based study aiming to evaluate the maternal medicine prescriptions before, during and after pregnancy across three Italian regions and to investigate the inter-regional variability.

METHODS

A retrospective population study using administrative healthcare databases was performed on a cohort of women aged between 15 and 49 years, who had a delivery (live births and stillbirths) in a Birth Unit from October 1st, 2014 to September 30th, 2017 and who were resident at the time of delivery in one of the following Italian regions: Emilia-Romagna (ER), a region in the North of Italy, Lazio (L), a region of Central Italy, and Puglia (P) in the South of Italy, whose total female residing population of childbearing age (15-49 years) in 2015 was 3.2 million. In the case of more than one delivery during the study period, only the first delivery was included in the study. All pregnant women were identified through the Regional Child-Birth Registry (CeDAP, *Certificato di Assistenza al Parto*) database, after linking the Regional Health Information Systems, containing data on prescriptions of all medicines reimbursed by the National Healthcare Service (NHS) in Italy. Only women covered by the Italian National Healthcare Service at least in one of the five identified trimesters were selected. Voluntary abortions and miscarriages were not included in the study, as this information were not recorded in the CeDAP database [18]. The data from the two different health care databases were combined using a deterministic record-linkage procedure based on anonymized personal identification codes, which is a procedure in line with privacy legislation [19].

The start date of pregnancy was estimated by subtracting the gestational age at delivery (defined as number of weeks of amenorrhea, as reported in the CeDAP database*7 days) from the date of delivery. On the basis of these information the following five trimesters were identified:

- the pre-pregnancy trimester (pre-T) defined as 91 days before the start date of pregnancy;
- the I trimester of pregnancy (1st TP) defined as the period between 0 (the start date of pregnancy) and the day 91 following the start date of pregnancy;
- the II trimester of pregnancy (2nd TP) defined as the period between the day 92 and the day 189 from the start date of pregnancy (or date of delivery if the delivery occurred during the 2nd TP, which is within 27 weeks of gestation);
- the III trimester of pregnancy (3rd TP) defined as the period between the day 190 from the start of pregnancy and the date of delivery;
- the post-pregnancy trimester (post-T) defined as the day 91 following the date of delivery.

For each woman enrolled in the study, the sociodemographic characteristics (e.g., age, nationality, education, occupational status), the clinical information related to pregnancy (e.g., gestational age and parity) and pregnancy history of the pregnant women (e.g., previous deliveries, previous cesarean sections, previous abortions) retrieved from the CeDAP database were collected.

The pharmaceutical prescriptions retrieved from the regional Health Information Systems provided in the identified five trimesters were linked. The medicines were classified according to the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC coding) classification system [20]. The use of some pharmacotherapy categories was investigated: vitamin B12 and folic acid (B03B), progestins (G03D) as medications indicated in pregnancy, antidiabetic drugs (A10), antihypertensive drugs (C02) and preparations for thyroid therapy (H03) as medications indicated for the treatment of chronic diseases. Medication dispensing records with missing information (name of medication, dispensing date) were excluded from the analysis.

The prevalence of overall medication use was estimated as the proportion of pregnant women with any medication prescription in each trimester considered. The prevalence of a specific medicine use was defined as the number of pregnant women receiving a specific medicine prescription per 100 pregnant women in the time frame of interest.

A woman was considered to be a prevalent user if she had received at least one prescription of the relevant medicine during the 3 months before the start date of pregnancy, while those who received a prescription during the pregnancy period were considered as new (incident) users.

RESULTS

A total of 337,437 pregnancies (24% of all deliveries occurred in Italy) were selected and a population of 274,938 pregnant women (81.5% of all selected pregnancies) were identified and included in the analysis (*Table 1*).

The 38% of all pregnant women were residing in Lazio, the 32% in Emilia-Romagna, and the remaining 30% in Puglia. About 33.7% of women were aged between 30 and 34 years old, while the 9.4% of women were at least 40 years old, the 7.7% of which was 45 years old and over. The 15.9% of the pregnant women was of a foreign nationality, with a higher percentage in Emilia-Romagna (29.1%). Almost 125,000 women (45.3% of the selected study population) already had a previous delivery (33.4% of which were cesarean sections). Furthermore, the 22.1% of pregnant women suffered at least one previous abortion (6.2% of these had two abortions and over). The majority of the pregnancies (92.1%) ended at term, while the 7% was a preterm pregnancy. The 1.9% of the deliveries were multiple (≥2 babies).

In all 221,066 pregnant women (80.4% of the selected study population) received at least one drug prescription during pregnancy: 81,807 (79.0%) women were residing in Lazio, 70,414 (79.6%) women in Emilia-Romagna, 68,845 women (83.0%) in Puglia. Furthermore, the 36.5% and 50.7% of all women received at

Table 1
Study cohort characteristics (n=274,899)

		L		ER		P		Overall	
		All pregnant women		All pregnant women		All pregnant women		All pregnant women	
		103,556		88,440		82,942		274,938	
		n	%	n	%	n	%	n	%
Age group	≤24	7,169	6.9	7,576	8.6	9,404	11.3	**24,149**	**8.8**
	25-29	19,266	18.6	19,459	22.0	18,647	22.5	**57,372**	**20.9**
	30-34	34,783	33.6	29,761	33.6	28,178	34.0	**92,722**	**33.7**
	35-39	30,684	29.6	23,588	26.7	20,585	24.8	**74,857**	**27.2**
	≥40	11,654	11.3	8,056	9.1	6,128	7.4	**25,838**	**9.4**
	≥45	*1,038*	*8.9*	*564*	*7.0*	*396*	*6.0*	***1,998***	***7.7***
Level of education	none/elementary school (≤ 5 years)	8,761	8.5	2,106	2.4	1,213	1.5	**12,080**	**4.4**
	middle school (8 years)	21,566	20.8	18,925	21.4	25,673	31.0	**66,164**	**24.1**
	high school (12-13 years)	47,561	45.9	38,220	43.2	36,161	43.6	**121,942**	**44.4**
	bachelor degree/post-degree (>13 years)	25,602	24.7	29,189	33.0	19,895	24.0	**74,686**	**27.2**
	Missing	*66*	*0.1*	*0*	*0*	*0*	*0*	***66***	***0.0***
Nationality	Italian	89,780	86.7	62,828	70.9	78,572	94.7	**231,180**	**84.1**
	Foreign	13,776	13.3	25,612	29.1	4,370	5.3	**43,758**	**15.9**
Gestational age	preterm delivery (<37 weeks)	7,726	7.5	5,815	6.6	5,738	6.9	**19,279**	**7.0**
	term delivery (37-41 weeks)	94,643	91.4	81,482	93.2	76,960	93.1	**253,085**	**92.1**
	post-term delivery (>41 weeks)	1,187	1.1	1,143	0.2	244	0.0	**2,574**	**0.9**
Previous deliveries	No	61,655	59.5	45,732	51.7	42,975	51.8	**150,362**	**54.7**
	Yes	41,901	40.5	42,708	48.3	39,967	48.2	**124,576**	**45.3**
	Cesarean section	*15,448*	*36.9*	*10,206*	*23.9*	*15,921*	*39.8*	***41,575***	***33.4***
Previous abortions	0	79,575	76.8	66,261	74.9	68,308	82.4	**214,144**	**77.9**
	1	17,434	16.8	15,764	17.8	10,479	12.6	**43,677**	**15.9**
	2+	6,547	6.3	6,415	7.3	4,155	5.0	**17,117**	**6.2**
Parity	1	101,404	97.9	86,845	98.2	81,424	98.2	**269,673**	**98.1**
	2+	2,152	2.1	1,595	1.8	1,518	1.8	**5,265**	**1.9**

L: Lazio; ER: Emilia-Romagna; P: Puglia.

least one drug prescription in the pre-T and in post-T respectively. The number of women receiving at least one drug prescription showed an increasing trend with increasing age in all the pregnancy trimesters and in the considered pre-T and post-T, even if in lower percentage for all age groups (*Table 1S, available online as Supplementary material*). The medicines belonging to the category of drugs for blood and hematopoietic organs (ATC B) were the most prescribed medications during pregnancy, with a peak of prevalence of 39.2% in the 1st TP, followed by antimicrobials for systemic use (ATC J) with a peak of 22.4% in the 2nd TP and by medicines belonging to the category of drugs for genitourinary system and sex hormones (ATC G) with a peak of prevalence of 19.8% in the 1st TP (*Figure 1*).

About 10% of the overall pregnant women included in the study population was prescribed exclusively with vitamins, minerals, and antianemic preparations purchased also as over the counter (OTC) medications.

Among the top 30 medicines most prescribed during pregnancy, 9 drugs were antimicrobials for systemic use (J), 6 were drugs for blood and blood-forming organs (B), 4 were drugs for alimentary tract and metabolism (A), 3 were drugs for genitourinary system and sex hormones (G) and 3 were drugs for to systemic hormonal preparations, excluding sex hormones and insulins (H) (*Table 2*).

The most prescribed medication during pregnancy was folic acid with an overall percentage of 41.9% of women receiving at least one prescription, ranging from the maximum of 35.0% in the 1st TP to the minimum of 11.4% in the 3rd TP; only the 6% of women received

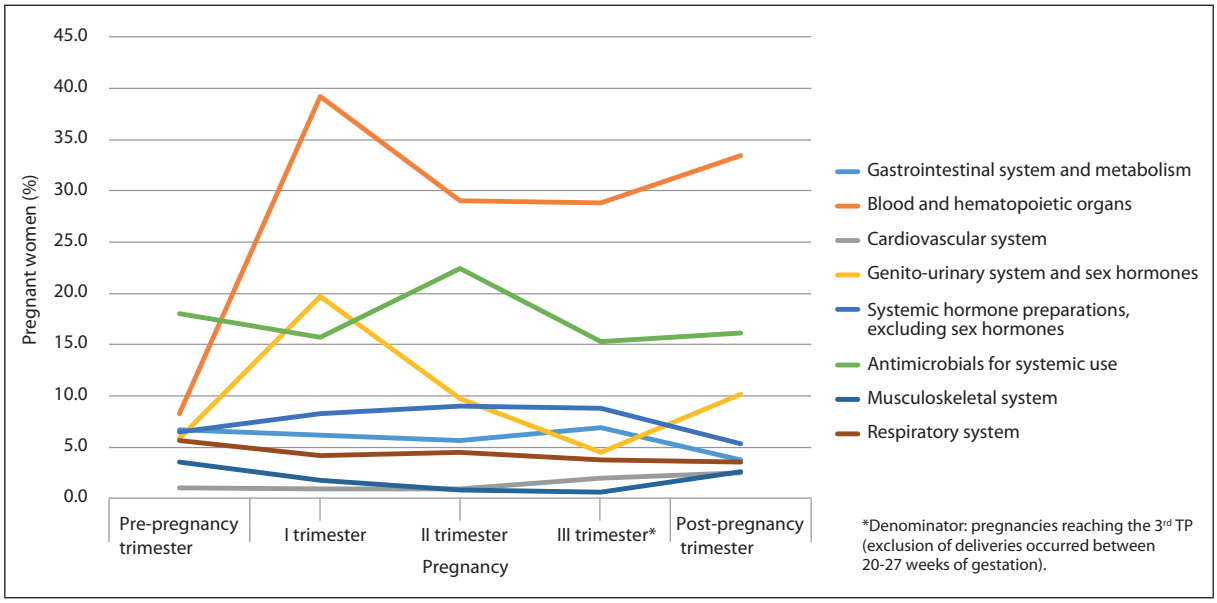

Figure 1
Medication prescription trends per ATC (I level) before, during and after pregnancy.

at least one prescription in the pre-T. Some regional variability was found in 1st TP (ER-39.4%; A-35.6%; L-30.7%) and in pre-T (ER-7.1%; L-6.7%; A-4.7%), when the use of folic acid was most recommended [21-23].

The antimicrobial for systemic use were the second most prescribed medication group during pregnancy, with a peak (22.4%) of prescription rate observed in the 2nd TP increasing with maternal age: 17.8% in women under 35 years of age, 27.9% in women between 35 and 39 years, and 33.0% in women 40 years old and over (*Figure 2*). Azithromycin and amoxicillin/clavulanic acid were the most prescribed agents (*Table 2*).

The progesterone and hydroxyprogesterone, medicines both mainly prescribed for the treatment of woman at risk of miscarriage or preterm delivery, were the second and eleventh most prescribed drugs during pregnancy in the selected study population, with an overall percentage of women receiving a prescription during pregnancy of 22.3% and 5.8%, respectively (*Table 2*). Particularly, the users of progesterone were mainly concentrated in 1st TP with the 18.2% of pregnant women receiving at least one prescription, while the percentage progressively decreased in 2nd TP (7.3%) and in 3rd TP (2.6%). The prescription of progestins during the 1st TP showed an increasing trend in relation to the number of previous abortions, ranging from 18.3% in women with no previous abortion to 28.2% for women with 2 abortions and over, with a different range within each considered Italian region: Δ=4% in Puglia (from 28.2% to 32.2%), Δ=10% in Emilia-Romagna (from 10.1% to 20.1%) and Δ=17% in Lazio (from 16.6% to 33.5%) (*Figure 3*).

The overall distribution of pregnant women with at least one prescription of medications indicated for the treatment of chronic diseases (antidiabetics, antihypertensives and preparations for thyroid therapy) during pregnancy was shown in *Table 3*. The mean prevalence for antidiabetics was 24.1 per 1,000 pregnant women, ranging from a maximum of 26.5 in Lazio to 20.5 in Puglia, 21.5 per 1,000 for anti-hypertensives, ranging from the maximum of 33.6 in Lazio to 11.9 in Puglia, 101.8 per 1,000 for thyroid preparations, with a range from 122.8 in Emilia-Romagna to 71.1 in Puglia.

Comparing to the number of prevalent users, expression of a preconception chronic treatment, Emilia-Romagna had the highest percentage of new users in pregnancy of both antidiabetic drugs and thyroid preparations, respectively 83.5% and 63.4%, while Puglia had the lowest percentage, respectively 68.1% and 44.5%. The Lazio had the highest percentage of new users in pregnancy of anti-hypertensive drugs (81.5%), followed by Puglia (68.1%) and Emilia-Romagna (63.6%).

Nifedipine (calcium channel blocker) and methyldopa (centrally acting antiadrenergic agent) were the most prescribed medications in the category of antihypertensives, ranking among the top 30 most prescribed medications, in line with the choice of antihypertensives compatible with pregnancy [24-26], while the most prescribed medicine among preparations for thyroid therapy was levothyroxine sodium, a thyroid preparation indicated in the treatment of hypothyroidism, which was the sixth most prescribed drugs in pregnancy with a prevalence of 10% in pregnancy. No antidiabetic drug was found among the top 30 most prescribed medications during pregnancy (*Table 2*).

DISCUSSION

Between 2014 and 2017 in Italy an overall of 80.4% of women received at least one medication prescription during pregnancy period, ranging from the 83% of Puglia region to the 79,0% of Lazio region.

The prevalence of overall medication prescription throughout the study period varied by trimester and by cohort demographic characteristics, showing the same clear change pattern across all the trimesters in all the

Table 2
The most prescribed 30 medications during pregnancy: analysis by pregnancy trimester

	ATC	Drug	Pregnancy		1st TP		2nd TP		3rd TP*	
			n	%	n	%	n	%	n	%
1	B03BB01	folic acid	115,086	41.9	96,103	35.0	51,974	18.9	30,895	11.2
2	G03DA04	progesterone	61,274	22.3	49,944	18.2	20,081	7.3	7,246	2.6
3	B03AA07	ferrous sulfate	59,498	21.6	7,138	2.6	27,524	10.0	42,426	15.4
4	J01FA10	azithromycin	36,413	13.2	10,729	3.9	23,049	8.4	4,679	1.7
5	J01CR02	amoxicillin/clavulanic acid	33,689	12.3	11,270	4.1	13,418	4.9	12,910	4.7
6	H03AA01	levothyroxine sodium	27,614	10.0	15,807	5.7	20,585	7.5	18,200	6.6
7	J01XX01	fosfomycin	25,892	9.4	7,645	2.8	12,292	4.5	9,234	3.4
8	J01CA04	amoxicillin	20,279	7.4	5,706	2.1	9,610	3.5	6,797	2.5
9	R03BA01	beclometasone	18,790	6.8	5,789	2.1	8,054	2.9	6,647	2.4
10	B01AB05	enoxaparin	16,151	5.9	5,715	2.1	7,181	2.6	13,497	4.9
11	G03DA03	hydroxyprogesterone	15,809	5.8	7,157	2.6	9,029	3.3	5,419	2.0
12	B01AC06	acetylsalicylic acid	10,876	4.0	7,560	2.7	6,965	2.5	2,998	1.1
13	H02AB01	betamethasone	8,217	3.0	2,149	0.8	2,028	0.7	4,546	1.7
14	A02BX13	alginic acid	7,877	2.9	2,940	1.1	3,544	1.3	3,824	1.4
15	H02AB07	prednisone	6,874	2.5	5,290	1.9	2,423	0.9	1,625	0.6
16	J01DD08	cefixime	6,418	2.3	2,155	0.8	2,421	0.9	2,254	0.8
17	J01CA01	ampicillin	5,252	1.9	967	0.4	2,179	0.8	2,376	0.9
18	G03CA03	estradiol	5,046	1.8	5,032	1.8	292	0.1	27	0.0
19	A02AD02	magaldrate	4,979	1.8	2,148	0.8	1,738	0.6	1,824	0.7
20	B01AB06	nadroparin	4,559	1.7	2,120	0.8	2,222	0.8	3,417	1.2
21	J01FA09	clarithromycin	3,958	1.4	1,823	0.7	1,094	0.4	1,184	0.4
22	A11CC05	colecalciferol	3,890	1.4	1,819	0.7	1,728	0.6	1,690	0.6
23	M01AE03	ketoprofen	3,477	1.3	1,959	0.7	1,073	0.4	879	0.3
24	R03AC02	salbutamol	3,439	1.3	1,444	0.5	1,476	0.5	1,137	0.4
25	B03AA01	ferrous glycine sulfate	3,231	1.2	363	0.1	1,626	0.6	2,173	0.8
26	J06BB01	anti-D (rh) immunoglobulin	3,178	1.2	220	0.1	1,016	0.4	2,104	0.8
27	C08CA05	nifedipine	2,701	1.0	340	0.1	837	0.3	2,326	0.8
28	A02BC02	pantoprazole	2,601	0.9	1,756	0.6	693	0.3	701	0.3
29	C02AB01	methyldopa (levorotatory)	2,496	0.9	539	0.2	937	0.3	2,137	0.8
30	J02AC01	fluconazole	2,433	0.9	1,437	0.5	653	0.2	427	0.2

ATC: Anatomical Therapeutic Chemical Classification system.
TP: trimester during pregnancy.
*Denominator: pregnancies reaching the 3rd TP (exclusion of deliveries occurred between 20-27 weeks of gestation).

regions considered. In general, we found a more intense medication use during the 1st TP (59.1%) in all regions (Puglia: 63.7%, Emilia-Romagna: 59.6%, Lazio: 55.0%) and an increase in overall prescription rate with the maternal age until a maximum of 86.9% in pregnant women of 40 years and over (Puglia: 89.3%, Lazio: 86.9%, Emilia-Romagna: 85.1%).

The peak of prevalence in the 1st TP was mainly due to a potentially higher demand for medication treatment indicated in early pregnancy (such as iron preparations, folic acid, vitamins and progestins). The prevalence of folic acid use was very low during the preconception period (6.2%) and highest after pregnancy confirmation within the 1st TP (35.0%), probably because women do not plan their pregnancy or do not request a preconception medical visit [27] (*Figure 2*).

Although the real consumption of folic acid in this study was probably underestimated because of non-reimbursed OTC medications and vitamin supplements [28], a noteworthy low prescription rate and regional variability was found in both pre-T and 1st TP periods, showing a national and regional clinical practice far from the recommendations of national and international clinical guidelines on the prevention of neural tube defects, that recommend a daily supplementation with 0.4 mg of folic acid in women who is planning to

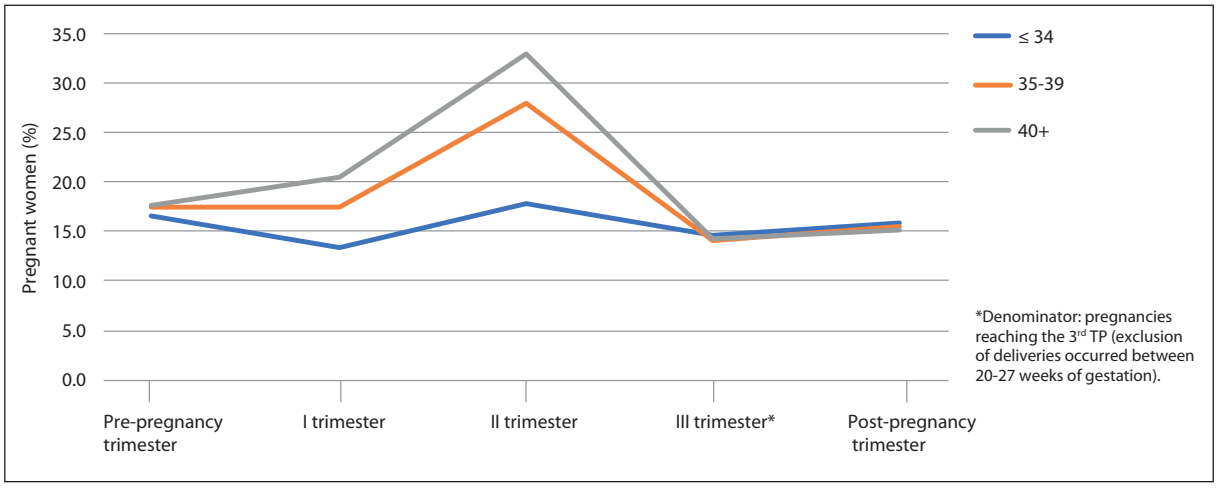

Figure 2
Prescription trends of antibiotics for systemic use before, during and after pregnancy.

become pregnant at least one month before the conception and until 12 weeks of gestation, in order to reduce the risk of neural tube defects and other congenital anomalies in their infants [21-23].

As regard to progestins, a proportion of 22.3% of women was exposed to progesterone during pregnancy, mainly concentrated in the 1st TP (18.2%), probably in an attempt to prevent an early pregnancy loss (*Figure 3*). The prescriptive trends were in line with the number of previous abortions reported in the maternal pregnancy history for all regions, even if different variations were found within each region. In particular, a high prescription prevalence of progestins were observed in Puglia region, which has almost 30% of women receiving one or more prescriptions regardless of the number of previous maternal abortions.

The use of progestins, in particular in the prevention of non-recurrent miscarriage, is worthy of attention because the appropriateness of the clinical use in terms of efficacy is still widely debated. In 2009 the World Health Organization (WHO) recommended not to prescribe progestogens for preventing miscarriages. In 2015 the American College of Obstetricians and Gynecologists (ACOG) Guideline stated that conclusive evidence supporting progestins use to avoid early pregnancy loss is lacking and that women who have experienced at least three prior pregnancy losses may benefit from progesterone therapy in the first trimester [29]. This recommendation is supported by the results of recent large randomized clinical trials, which reported no evidence of efficacy for these medications [30-32]. On the other hand, recent systematic reviews, concerning the efficacy of progesterone use in the treatment of miscarriage and in the prevention of preterm birth, suggest a probable efficacy, even in the early stages of pregnancy [33-36]. The use of progestins remains controversial and conclusive evidence supporting their use in lowering the risk of miscarriages is still lacking. The

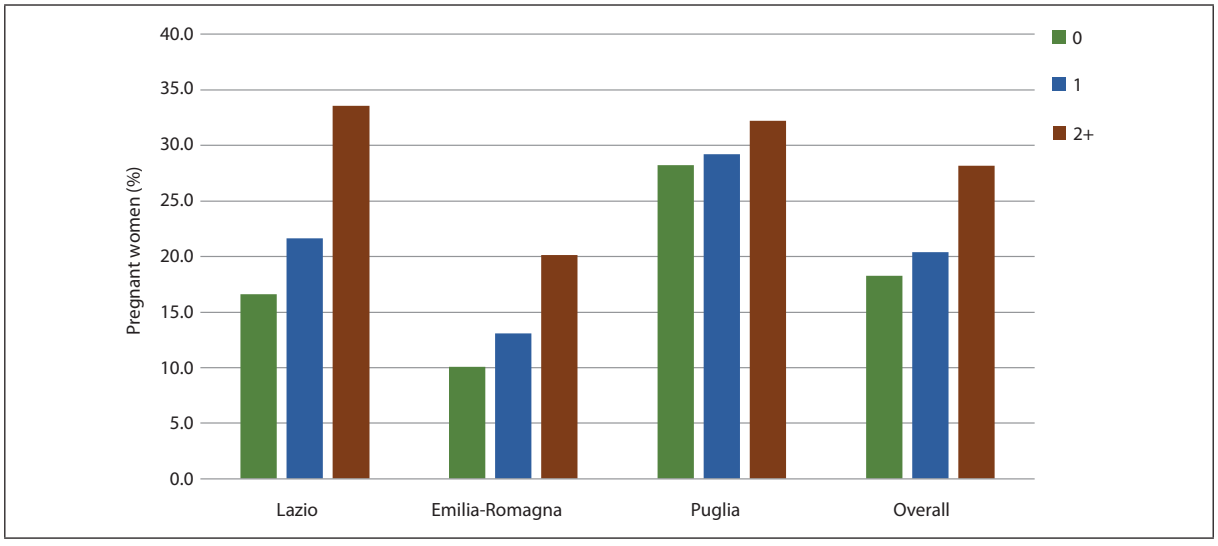

Figure 3
Progestin prescription trends during first trimester of pregnancy per region and number of previous abortions.

persistence of this inappropriate prescriptive habit in Italy makes the monitoring of medication prescriptions in this therapeutic area necessary in order to avoid inappropriate use.

In our study antimicrobials for systemic use were the second most prescribed medications during pregnancy (peak of 22,4% in the 2nd TP), even if the observed rate is lower than other European countries (27-40%) [37-40]. Given the growing problem of bacterial resistance, the use of antibiotics during pregnancy would require more attention in terms of appropriate use [41]. Therefore, there is an urgent need to understand the motivation for the peak of antibiotic prescription rate in the 2nd TP.

As regard medications for chronic diseases, regional differences were found in prescription of antidiabetics, antihypertensives and thyroid preparations, with the highest regional variability observed for antihypertensive therapy (Lazio: 33.6 per 1,000 pregnant women; Emilia-Romagna: 16.4; Puglia: 11.9) (*Table 3*).

A decrease in the rate of prevalent users of antidiabetic and antihypertensive drugs during pregnancy (in particular in the II and III trimesters) was observed in all regions. Furthermore, nearly half of the women suspended the antihypertensive therapy before pregnancy and this could be a reason of concern for the health consequences for mothers and foetus. On the contrary, new users in pregnancy increased, covering 60-80% of women who received prescriptions of these drugs during pregnancy.

The increase proportion of the new users in pregnancy for antidiabetic or antihypertensive therapy, even with regional differences, may indicate an increasing number of women firstly diagnosed at their first prenatal medical visit with a chronic disease (such as diabetes mellitus or hypertension) or an incidence of some pregnancy-related acute problems (such as gestational diabetes, hypertension or preeclampsia) [42, 43], while the proportion of new user of thyroid preparations seems to suggest an increasing number of pregnant women being diagnosed with subclinical hypothyroidism in pregnancy, requiring a thyroid hormone replacement therapy, as recommended by clinical national and international guidelines on management of thyroid disease during pregnancy and the postpartum [44, 45] (*Table 2S, available online as Supplementary material*).

Generally, the overall rate of medication prescriptions, as well as the prescription patterns, observed in our large cohort of Italian pregnant women are generally comparable with medication prescriptive trends observed in other European population-based studies [46] reporting rates of 69.2-79.1% in the Netherlands, 85.2-96% in Germany and 89.9% in French population [47-49].

The strength of our study is the availability of the medication prescription data in pregnancy from three different Italian regions, which are representative of all geographical areas (Emilia-Romagna region for the North of Italy, Lazio region for the Centre, and Puglia region for the South). Previous population-based studies conducted in Italy were limited to a single Italian region [13-16]. To our knowledge, this is the largest and most representative population-based study illustrating the medication prescription during pregnancy in Italy.

A limitation of the study is that our data were referred only to prescription of medicines reimbursed by the Italian National Health Service, excluding over-the-counter (OTC) and non-reimbursed medications (i.e. vitamin supplements), that may lead to an underestimation of medication use among the target population. On the other hand, the medication use in the real world could be over-estimated, in case the medicine is dispensed but not actually taken by the pregnant woman. Although several studies based on administrative data consider drug dispensation as a good "*proxy*" measure for medication use [50-51], it cannot be considered a direct measure of real maternal drug exposure rate.

Finally, our administrative databases do not provide information on drug use in pregnancies ended in a miscarriage or induced abortion, as well as no information on therapeutic indications for drug prescribing were

Table 3
New and prevalent users in pregnancy of antidiabetics, antihypertensives and thyroid preparations by region

During pregnancy	Antidiabetics drugs (24.1‰)			Antihypertensive drugs (21.5‰)			Thyroid preparations (101.8‰)		
n per 1,000 pregnant women									
	L	**ER**	**P**	**L**	**ER**	**P**	**L**	**ER**	**P**
New users	19.4	20.7	14.0	27.3	10.4	8.1	62.9	77.8	31.6
Prevalent users	7.2	4.1	6.6	6.2	6.0	3.8	45.5	45.0	39.5
Total	26.5	24.7	20.5	33.6	16.4	11.9	108.4	122.8	71.1
% prevalent and new users									
	L	**ER**	**P**	**L**	**ER**	**P**	**L**	**ER**	**P**
New users	73.0	83.5	68.1	81.5	63.6	68.1	58.0	63.4	44.5
Prevalent users	27.0	16.5	31.9	18.5	36.4	31.9	42.0	36.6	55.5
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

L: Lazio; ER: Emilia-Romagna; P: Puglia.

available, consequently we were not able to investigate the medication use patterns more in depth. On the other hand, the population-based approach together with a large cohort of pregnant women enrolled, which is geographically representative of the whole Italian context could be considered a very strong point for this study.

CONCLUSIONS

This study showed that pregnancy medication use is very common in Italy. Eight in 10 pregnant women was prescribed with at least one medication, with an increasing trend in the 1st TP, therefore recommending cautious in drug prescribing for women in the childbearing age. The evaluation and monitoring of medicine prescriptions in the preconception period, during pregnancy and after childbirth are aspects of extreme interest in term of public health.

Given the limited information on medication use during pregnancy in Italy, this study could be periodically replicated, even involving more and different regions of the country, with the objectives of monitoring some critical aspects of drug prescribing in pregnancy, as well as to detect and investigate the observed regional variability in the prescription patterns, in order to identify all the determinants of the drug prescriptions, also in terms of appropriateness of use [52].

Authors' contributions

All the authors contributed to the study conception and design. VB, FRP, AP, VS and PS performed data collection and analysis. VB coordinated the analysis and prepared the final version of tables and figures included in the manuscript. FF drafted the first version of the manuscript. RB contributed to interpretation of data. All authors read, commented, revised and approved the final version of the manuscript.

Conflict of interest

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Book Reviews, Notes and Comments

Edited by
Federica Napolitani Cheyne

PRAECURRIT FATUM!
Arrivare prima del destino
Marcantonio Lucidi,
Alessandro Orlandi (Eds)
Roma: La Lepre Edizioni;
2021
ISBN 978-88-99389-83-3
€ 16,00

[Praecurrit fatum! Anticipating the destiny]

The substantive "Fatum" (fate, destiny) is what Latin grammars call a "vox media" (literally, "middle noun"), that is, a "neutral" term which, based on the specific context, can have two opposing meanings.

Thereby, the book's very title reminds the reader of a fundamental truth. Humankind's fate can be ill or prosperous: it all depends on the actions and efforts collectively deployed by society. Can intellectuals analyze and subsequently isolate enough elements to predict the fate of our increasingly precarious and geographically limited wellbeing? The book attempts to provide a positive answer. It marks the beginning of a rather original collaborative editorial project: bringing together diverse competencies, spanning from theoretical physics to ethology, from history and economics to molecular biology, and music. The envisioned outcome is a "lab of ideas and proposals".

For the sake of synthesis, I will not delve into the essays covering social psychology, contemporary history, economics, and social sciences. Nonetheless, even a layperson cannot help but notice the enriching and well-condensed perspective with which intricate topics, such as the role of social media, quantum computing, international fiscal policies, the changing labor market, European history, and the future of democracy, are illustrated in this collection of short essays.

Before COVID-19, the World Health Organization (WHO) listed climate change as the most urgent threat to global health. It is still imperative that governments and the private sector should not disregard this crucial challenge now. In a dedicated book section, Enrico Alleva, evolutionary behavioral scientist, and oceanographer and Intergovernmental Panel on Climate Change (IPCC) member Vincenzo Artale discuss climate change thoroughly, with an original joined dissertation. They provide detailed technical explanations of the assumptions behind climate models, often clarified by incisive metaphors. For instance, it is intriguing to picture the various interactions happening inside a chaotic system, like climate, as a Billiards game where the ball's trajectories vary amply, depending on the initial impulse. They also offer meaningful insights from an evolutionary biology viewpoint. The challenging definition and boundaries of the so-called "Anthropocene", the continuous mapping of the disproportionate impact of *Homo sapiens* on the Earth's biodiversity (including viral spreading in population outbreaks of both animals and plants), and the usefulness and limitations of the Big Data and the Citizen Science approaches, are among the explored topics. The essay ends with a call to action, fueled by the awareness that COVID-19 has dramatically unveiled the entanglement between environmental degradation and spillover risk.

The short but incisive essay titled "RNA and biology's future" by molecular biologist Piero Benedetti is of the utmost interest for a biomedical readership. He provides a brief historical overview of genetics and its evolutions from the Human Genome Project onwards. In particular, the complex theoretical milestones of the "RNA world" hypothesis are laid out, along with the most updated evidence of translational research arising from the new CRISPR-Cas9 genome editing technique. In a matter of years, genomics will easily treat hitherto incurable hematologic disorders linked to single-gene abnormalities, and several chronic diseases will be fought by innovatively acting on the microbiota. The rising discipline of "personalized medicine", the author notes, is promising but raises ethical ambiguities, which must be identified.

Finally, celebrated musician Nicola Piovani and journalist Marcantonio Lucidi ask themselves whether modern Artifical Intelligence (AI) has created such evolved products that deserve to be acknowledged as "artificial souls". Paraphrasing the famous essay by Walter Benjamin, the authors seem to shed an overall pessimistic light on arts' originality at the time of its algorithmic reproduction. In medicine, entire diagnostic fields (e.g., pathology and radiology) already feature massive amounts of computer-assisted inputs and guidance. Will more traditional disciplines such as psychiatry and internal medicine follow? Recent evidence already shows that machine-learning analysis can handle the differential diagnosis between major depression and anxiety almost perfectly.

In conclusion, *Praecurrit fatum* represents a thought-provoking and intellectually challenging collection of selected essays. It overtly cultivates the ambitious project to rebuild a form of "modern humanism" by part-

nering up intellectuals who can give society a unified cultural lens to read the most critical phenomena of our time. It may represent a stimulating reading for biomedical scientists of a variety of disciplines and social scientists alike.

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ALLATTAMENTO.
Mi hanno detto che…
Anna Maria Altobelli,
Valentina Della Bella (Eds)
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CE.S.VOL. Umbria; 2019
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[Breastfeeding. They said...]

Breast milk is an irreplaceable asset: it is organic, ecological, zero kilometre, free, practical, always available and, at the right temperature, easily digestible. Breastfeeding is not only physical nourishment, but also a fundamental emotional and psychological bond between the mother and the offspring [1]: it promotes the mother-child bond, satisfying and strengthening their mutual need to survive and live together. Maternal milk has been species-specifically evolved for the newborns, is endowed by all the "optimal " ingredients to nourish physiologically them, has the power to strengthen their immune system by protecting from infections and diseases, while promoting both the health of the mother and of the offspring in the short and long term [2].

Quite recently, in 2019, the Italian Ministry of Health launched a sensitization campaign to promote breast milk feeding. This campaign is finalized to raise awareness among women about such a practice and the spontaneity of this behavioural and physiological action, powerfully diffusing the message that every woman should feel free to breastfeed publically or at work, always and always, its spot vividly claiming: "That's natural!"

The Italian National Institute of Health (Istituto Superiore di Sanità, ISS) has been of course involved in most of these activities aimed at promoting breastfeeding. In particular, in protecting vulnerable and fragile populations, human milk, for its lipophilic capability to concentrate environmental toxicants, represents a very useful medium to evaluate human body contamination [3, 4], as in the case of caesium contamination following the Chernobyl accident [5] or of exposure to persistent pollutants [6, 7]. Nutritional value [8] and long-term ameliorative effects on neuropsychological development in infancy [9]. Presently, the ISS institutional website presents a whole array of public health counselling and socio-sanitary promotion initiatives aimed at both health professionals and the general public.

Breastfeeding itself was a focus of public, sometimes "political" debate. A long time ago, in the '70, Nobel laureate Daniel Bovet, who for decades ran a laboratory at ISS (from 1947 to 1964), participated in a long and at times harsh international debate on health problems possibly attributable in poor countries to the free distribution of milk powder Nestlè. In 1981, the 34th World Health Assembly (WHA) adopted Resolution WHA34.22 which included the International Code of Marketing of Breast-milk Substitutes. In the recent history of global public health this episode perhaps remains a matter of forgotten discussion.

The present mini-guide is a simple, fluid, and somehow innovative contribution possibly useful to "explore", while educating, the specific theme of breastfeeding. The real aim of this short volume is not to improve in a few, sometimes naive statements, the available scientific knowledge about such a delicate and still not rarely debated issue, but simply to offer some different perspectives to raise awareness and to inform correctly about this apparently familiar topic. More importantly, the contemporary fragmentation or even pulverization of the social tissue, jointly mixed with the widespread gravitational force attracting rural communities toward immense metropolitan areas, makes such an editorial effort worth mentioning. Without an appropriate social setting, e.g., the peculiar case of teen mothers in deprived urban areas or favelas, substantial and consistent difficulties arise in caring for their newborns.

In a concise form, the authors give voice to mothers living in central Italy (Umbria region) in order to highlight some putative incorrectness of current popular myths and prejudices that revolve around breastfeeding traditions. To avoid them, they propose a list of practical suggestions in order to help particularly primiparous mothers to face a few inaccuracies disseminated in their social milieu.

The essay is composed by a first section dedicated to the beliefs of relatives and friends regarding breastfeeding, a second entrusted to some thoughts of health personnel, and a third concerning the ideas of pediatricians. Although far from being scientifically grounded, the authors attempt to overturn some current beliefs about this issue. They try with a rather superficial overview to explain for example any rigid duration of the breastfeeding period, the possibility to eat any kind of food without major consequences on the milk composition or the importance of excluding any other nutritional input food besides maternal milk to the neonate, infant or early children. It appears surprising that in Central Italy such ideas are still circulating.

It is worth mentioning that this booklet is enriched with photos representing the parent(s)-child dyadic union to even more emphasize the spontaneity and the universality of the breastfeeding phase. Despite its "natural" occurrence during the human history [10], it is however possible that the cultural anthropology of breastfeeding practice deserves continuous maintenance and adjustment.

Finally, the present booklet may testify the recent and increasing effort by a variety of complementary, even non-strictly socio-sanitary, institutions (Agenzia Regionale Protezione Ambiente - Umbria, under informal collaboration with USL Umbria2 consultorio Narni-Amelia, family pediatricians belonging to local USL Umbria2, "Associazione lattemiele", and Auser, voluntary and social promotion association, the latter committed to promoting the active aging of the elderly while enhancing their role in society) to ensure a truly territorial and locally-based, multi-faceted material.

Possibly, the fact that author Valentina Della Bella, graduated some time ago with a thesis on water health quality assessed by monitoring of freshwater microinvertebrates at the ISS Department of Environment and Health may have played a not minor role in such a vivid public health editorial strategy.

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Publications from International Organizations on Public Health

Edited by
Annarita Barbaro

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)

FAO's work on climate change - Fisheries and aquaculture 2020. Rome: Food and Agriculture Organization of the United Nations 2021; 79 p. ISBN 978-92-5-133989-3. This publication presents FAO's work on climate change and fisheries and aquaculture. It includes examples of FAO's support to countries so that they are better able to adapt to the impact of climate change in the fisheries and aquaculture sectors. It also brings together FAO's most up-to-date knowledge on climate change, including a portfolio of adaptation tools and measures used to support countries' climate commitments and action plans.

The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets. Rome: Food and Agriculture Organization of the United Nations 2020; 320 p. ISBN 978-92-5-132901-6. The report shows that the number of people affected by hunger globally has been slowly on the rise since 2014 and that the burden of malnutrition in all its forms continues to be a challenge. There has been some progress for child stunting, low birthweight and exclusive breastfeeding, but at a pace that is still too slow. Childhood overweight is not improving and adult obesity is on the rise in all regions. The report complements the usual assessment of food security and nutrition with projections of what the world may look like in 2030, if trends of the last decade continue. Projections show that the world is not on track to achieve Zero Hunger by 2030 and, despite some progress, most indicators are also not on track to meet global nutrition targets. The food security and nutritional status of the most vulnerable population groups is likely to deteriorate further due to the health and socioeconomic impacts of the COVID-19 pandemic. The report puts a spotlight on diet quality as a critical link between food security and nutrition and also introduces new analysis of the cost and affordability of healthy diets around the world, by region and in different development contexts. It presents valuations of the health and climate-change costs associated with current food consumption patterns, as well as the potential cost savings if food consumption patterns were to shift towards healthy diets that include sustainability considerations. The report then concludes with a discussion of the policies and strategies to transform food systems to ensure affordable healthy diets, as part of the required efforts to end both hunger and all forms of malnutrition.

Microbiological Risk Assessment – Guidance for food. Microbiological Risk Assessment Series No. 36. Rome: Food and Agriculture Organization of the United Nations – World Health Organization 2021; 288 p. ISBN 978-92-5-134518-4. Microbiological Risk Assessment – Guidance for food (MRA 36) provides a structured framework for assessing the risk of microbiological hazards in food. It updates three previous FAO and WHO guidance documents (MRA 3, MRA 7, and MRA 17) and brings them into a single volume, providing an overall umbrella for microbiological risk assessment. In doing so it captures the growth and experience in this field, which continues to evolve in line with science and risk management demands. This document provides guidance on undertaking risk assessment of all microbial hazards which may adversely affect human health in foods along a food supply chain. This document is also intended to provide practical guidance on a structured framework for carrying out risk assessment of microbiological hazards in foods, focussing on the four components including hazard identification, hazard characterization, exposure assessment and risk characterization. These guidelines therefore represent the best practice at the time of their preparation, and it is hoped that they will help stimulate further developments and disseminate the current knowledge. The overarching objectives of these guidelines are to help the reader to: identify the key issues and features of a microbiological risk, recognize the properties of a best-practice risk assessment, avoid some common pitfalls of risk assessment, and perform risk assessments that are responsive to the needs of risk managers.

UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION (UNESCO)

UNESCO Science Report: the race against time for smarter development. Paris: UNESCO Publishing 2021; 739 p. ISBN 978-92-3-100450-6. The UNESCO Science Report – the race against time for smarter development – focuses on the global shift towards economies that are greener, knowledge-based and make the best use of digital technologies. This seventh edition in the series arrives at a crucial juncture, as countries approach the halfway mark for delivering on their Sustainable Development Goals. The report finds that sustainability science is not yet mainstream in academic publishing at the global level and that it is developing countries which are publishing most, proportionately, on related topics. This trend, combined with greater

government support for start-ups and small businesses in many countries, suggests that the current knowledge gap could narrow in the coming years, as long as the challenge of chronic underfunding can be overcome as four out of five countries still spend less than 1% of GDP on research and development. The UNESCO Science Report series targets policy-makers, academics, the intergovernmental and non-governmental communities, the media and other groups interested in understanding how science governance is shaping countries' development agendas.

JOINT UNITED NATIONS PROGRAMME ON HIV/AIDS (UNAIDS)

Preventing HIV infections at the time of a new pandemic. A synthesis report on programme disruptions and adaptations during the COVID-19 pandemic in 2020. Geneva: Joint United Nations Programme on HIV/AIDS 2021; 54 p. This report is primarily directed to key partners and decision-makers in the global HIV and COVID-19 response. The lessons from successful HIV responses in countries and communities are identified and shared with a view to promote and sustain resilience strategies and programme improvements – even under the circumstances required to prevent the ongoing transmission of SARS-CoV-2 and address its consequences. The report is intended to serve as a basis for decision-making in the next year or two, as countries step up their efforts to control both epidemics. Relevant and illustrative experiences from all countries from the Global South served by UNAIDS have been considered, if available since efforts to collect data comprehensively and systematically on this topic have not yet been put into place. An attempt has been made, however, to include country experiences across all regions and all main types of HIV epidemics.

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD)

Brick by Brick: Building Better Housing Policies. Paris: OECD Publishing 2021; 164 p. ISBN 9789264739871 (PDF) ISBN 9789264551183 (Epub). This report brings together evidence, international experience and policy insights for the design of housing policies. Emphasis is placed on three broad aspects: inclusiveness, efficiency and sustainability. Inclusive access to housing has become increasingly challenging in many OECD countries due to a large extent to rising housing costs, which reflects the failure of housing supply to meet demand, particularly in jobs-rich urban areas. Geographical constraints play a role, but in many cities regulations, including on land-use and zoning provisions, also constrain supply. At the same time, some regulations on tenant-landlord relations can discourage the development of rental markets, pushing up rents. Moreover, the transition to a low-carbon economy poses challenges for a sector that accounts for 17% of CO2 emissions and 37% of fine particulate matter emissions globally. Almost two-thirds of countries worldwide still lack mandatory building energy codes. Frontloading efforts is critical as dwellings have a very long lifespan. The report lays out evidence-based options for concerted policy action to address these challenges, while recognising complementarities and trade-offs among the different objectives of housing policies. The report is part of the OECD Housing Toolkit, which includes an interactive online dashboard of housing indicators and country snapshots.

Strengthening Climate Resilience. Guidance for Governments and Development Co-operation. Paris: OECD Publishing 2021; 187 p. ISBN 9789264415133 (PDF) ISBN 9789264388758 (Epub). This guidance provides a tool governments and development co-operation can draw on in their efforts to strengthen the resilience of human and natural systems to the impacts of climate change. It highlights three aspirations to consider when planning and implementing action to build climate resilience (country ownership; inclusiveness; and environmental and social sustainability). The guidance also outlines four mechanisms (governance; sector-level approaches; finance; and monitoring, evaluation and learning) and three enablers (data and information; capacity; and technologies) in support of climate resilience, proposing concrete actions in the form of checklists.

INTERNATIONAL LABOUR ORGANIZATION (ILO)

World Employment and Social Outlook: Trends 2021. Geneva: International Labour Office 2021; 164 p. ISBN 978-92-2-031958-1 (print) ISBN 978-92-2-031959-8 (web PDF). This year's World Employment and Social Outlook: Trends examines global and regional trends in employment, unemployment, labour force participation and productivity, as well as dimensions of job quality such as employment status, informal employment and working poverty. It also provides extensive analysis of the crisis's varied impact on enterprises and workers. The report forecasts that employment recovery, though strong, will be insufficient to close the gaps. Workers whose labour market position was disadvantageous prior to the crisis – women, young people, migrants, informal workers and workers in lower-skilled occupations – suffered disproportionately. The report proposes a human-centred recovery strategy to avoid scarring of global labour markets for the years to come.

WORLD HEALTH ORGANIZATION (WHO)

Estimating the burden of foodborne diseases: A practical handbook for countries. A guide for planning, implementing and reporting country-level burden of foodborne disease. Geneva: World Health Organization 2021; 72 p. ISBN 978-92-4-

001226-4 (electronic version) ISBN 978-92-4-001227-1 (print version). This handbook provides detailed guidance on assessing the burden of diseases caused by microbiological agents commonly transmitted through foods. It is particularly intended for use at national level, and gives a complete picture of the requirements, enabling factors, challenges and opportunities involved, and the steps in the process. It also aims to foster harmonization of methodologies for estimating foodborne disease burden across countries. The goal of a national burden of foodborne disease study is to rank and prioritize foodborne diseases based on their overall public health impact in the population. The objectives of such a study are to: estimate the burden of disease for selected foodborne hazards, develop a framework for routine updating of estimates and evaluation of trends, and provide a baseline against which food safety interventions can be evaluated.

Helping Adolescents Thrive Toolkit. Strategies to promote and protect adolescent mental health and reduce self-harm and other risk behaviours. Geneva: World Health Organization and the United Nations Children's Fund (UNICEF) 2021; 172 p. ISBN (WHO) 978-92-4-002555-4 (electronic version) ISBN (WHO) 978-92-4-002556-1 (print version) ISBN (UNICEF) 978-92-806-5221-5. The Helping Adolescents Thrive (HAT) programme, jointly conceived by the World Health Organization and UNICEF, focuses on the promotion of mental well-being among adolescents and the prevention of mental health conditions. The Helping Adolescents Thrive toolkit, the latest material of the programme to be released, provides programmatic guidance for people working in the health, social services, education and justice sectors on how to implement mental health promotive and preventive interventions that are appropriate to local needs and the contexts where adolescents live. The toolkit covers the legal foundations required for such programmes to succeed, the features of environments that are conducive to the well-being of adolescents, what support should be provided to parents and other caregivers, and psychosocial interventions that work. The complementary Teacher's Guide and Comic Book can be used in schools as part of mental health promotion programmes to facilitate understanding and discussion of mental health issues.

Ethics and governance of artificial intelligence for health. Geneva: World Health Organization 2021; 150 p. ISBN 978-92-4-002920-0 (electronic version) ISBN 978-92-4-002921-7 (print version). The WHO guidance on Ethics & Governance of Artificial Intelligence for Health is the product of eighteen months of deliberation amongst leading experts in ethics, digital technology, law, human rights, as well as experts from Ministries of Health. While new technologies that use artificial intelligence hold great promise to improve diagnosis, treatment, health research and drug development and to support governments carrying out public health functions, including surveillance and outbreak response, such technologies, according to the report, must put ethics and human rights at the heart of its design, deployment, and use. The report identifies the ethical challenges and risks with the use of artificial intelligence of health, six consensus principles to ensure artificial intelligence (AI) works to the public benefit of all countries. It also contains a set of recommendations that can ensure the governance of artificial intelligence for health maximizes the promise of the technology and holds all stakeholders – in the public and private sector – accountable and responsive to the healthcare workers who will rely on these technologies and the communities and individuals whose health will be affected by its use.

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- key words up to a maximum number of five (MeSH headings, whenever possible. Refer to: www.nlm.nih.gov/mesh/meshhome.html).

Tables and figures should be kept to a minimum and be presented only if necessary.
Authors should deal responsibly and effectively with security issues that might be raised by their papers (see: Statement on Scientific Publication and Security *Science* 2003;299:1149).
This journal has adopted the SAGER reporting Guidelines for Sex and Gender Equity in Research.
These guidelines apply to original research articles and review papers. Authors should use the terms sex and gender carefully in order to avoid confusing both terms. Where subjects can also be differentiated by gender (shaped by social and cultural circumstances), the research should be conducted similarly at this additional level of distinction. Where the subjects of research comprise organisms capable of differentiation by sex, the research should be designed and conducted in a way that can reveal sex-related differences in the results, even if these were not initially expected.
Please consult the guidelines (https://researchintegrityjournal.biomedcentral.com/articles/10.1186/s41073-016-0007-6).
Authors are also encouraged to use fair, accurate and respectful language, but preferences can change and vary across groups and individuals and can also evolve overtime. The following guidelines may help in use of a correct terminology in the area of HIV: https://www.cdc.gov/stophivtogether/library/stop-hiv-stigma/fact-sheets/cdc-lsht-stigma-factsheet-language-guide.pdf
https://www.hptn.org/resources/HIVLanguageGuide
https://unesdoc.unesco.org/ark:/48223/pf0000144725
The name of the bioresource (and identifier, if available) which provided samples/data useful for the conduct of the study should be reported in extense, either in the Material and methods section or in the Acknowledgements.

LENGTH OF THE TEXT

To provide a text that meets the requirements of our publication:

- the *letter* to the Editor should be about 450 words; it does not need an abstract;
- the *editorial* should be no longer than 1000 words; editorials are submitted on invitation. Please contact the editorial office in advance if you wish to submit an editorial;
- the *commentary,* 2000 words; the commentary is an opinion piece or reflection on recent papers previously published on *Annali ISS* or elsewhere; an abstract is required; please contact in advance the editorial office;
- the *brief note*, 3000 words, including about 15 references, one table and one figure;
- the *article,* 6000 words, including about 40 references, three tables and two figures;
- the *review* should be no longer than 10 000 words, including no more than 100 references up to a maximum of four tables and three figures.

FORMATTING GUIDELINES

Text

- Use Times New Roman font, 10 point, single spaced;
- do not use the automated features of your application (endnotes, headers, footers, especially for references);
- avoid using bold characters to emphasise words or sentences within the text;
- indicate clearly titles of chapters and subchapters avoiding numbering.

Tables and figures

They should be understandable also without reference to the text and should be numbered in Arabic numerals in a consecutive and independent way according to their citation within the paper.

Tables should be presented on a separate sheet and preceeded by a title. Each column within the table should have a heading. Abbreviations should be reported in full in the legend.

Figures should be loaded as separate files. The following file formats are acceptable: JPEG, TIFF or EPS. Vectorial images (graphs, flow charts, schemes, and other non bitmap material) should be in Excel, Adobe Illustrator, Microsoft Power Point so as to allow the editorial formatting of the material.

Figures are redrawn into the *Annali* style by our in-house illustrators.

Photographs must have a minimum resolution of 300 dpi.

Captions should be presented on a separate sheet and contain a sufficient explanation of their object. They should be concise but comprehensive.

REFERENCES

All references in the text must be numbered in square brackets, *i.e.* [1, 2, 3-6], and mentioned at the end of the article in the order in which they are quoted. They should conform to the "Recommendations for the Conduct, Reporting, Editing, and Publications of Scholarly Work in Medical Journals" (www.icmje.org), according to the following examples.

Titles of periodicals should be abbreviated in accordance with the Medline abbreviation of the US National Library of Medicine (www.nlm.nih.gov/bsd/aim.html). Online journal articles can be cited using, in addition to the complete citation, the DOI number.

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.

The authors should check that each reference cited in the text appears in the reference list and viceversa. References should not include works submitted for publication but not yet accepted or unpublished results, etc. These can be mentioned in the text in parentheses.

CONVENTIONS

All Latin or foreign words should be in italics. The authors should use internationally accepted abbreviations. All abbreviations should be spelled out in full the first time they occur in the text, followed by the shortened term in parentheses; afterwards use the abbreviation only.

Avoid abbreviations in the title of the manuscript.

For writing symbols, quantities and units of measurements refer to the International Systems of Units (SI) and the ISO standards.

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