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Seroprotection against tetanus in the Italian general population

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ABSTRACT

Background: Tetanus is a non-communicable disease, preventable with vaccination. Despite the implemented vaccination strategy, a certain number of tetanus cases per year continue to occur. The aim of the study was to evaluate the seroprevalence of anti-tetanus antibodies in the Italian population by age, sex and geographical area.
Methods: To determine the level of tetanus-specific antibodies, an immunoenzymatic assay was used.
Results: A total of 3,821 serum samples were collected in the years 2019–20 from healthy subjects aged 6–90 years residing in 13 Italian regions. Overall, 85 % of the tested subjects resulted positive. The rate of subjects protected against tetanus showed a gradual decrease from the younger age groups to the older ones (6–12 years: 93.6 %, 13–24 years: 91.8 %, 25–39 years: 91.0 %, 40–64 years: 78.2 %, ≥ 65 years: 45.3 %); this is particularly evident in the Southern regions and Islands. Moreover, the prevalence of subjects with low protection (<0.1 IU/ml) was significantly higher in the ≥ 65 age group (10.3 %). Males and females' prevalence showed a significant difference only in the oldest age group (M: 60.8 %, F: 30.4 %). In general, a higher prevalence was observed for Northern (90.8 %) and Central regions (87.3 %) than Southern regions and Islands (80.0 %).
Conclusion: These data, compared with epidemiological ones which showed a high number of cases in the elderly, confirmed that the population with lower protection has a greater risk of contracting the disease, demonstrating the need for adequate immunization through both primary vaccination and boosters for all ages and both sexes, in order to provide lifelong protection.

1. Introduction

Tetanus is a severe acute infectious disease caused by a potent neurotoxin produced by the bacterium *Clostridium tetani*. Its spores are widespread in the environment and enter the organism through abrasions and superficial or deep wounds. The disease affects the nervous system and is characterized by pervasive and painful spasms and muscle contractions which can also compromise breathing capacity and, therefore, endanger life [1].

Tetanus is preventable with vaccination and adequate wound care. However, since the etiological agent is ubiquitous in the environment and there is an animal reservoir, eradication is impossible. Therefore,

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efforts to reduce incidence aim to achieve control, rather than elimination/eradication [2]. Since this is a non-communicable disease, herd immunity has no role in protecting unvaccinated individuals. Furthermore, natural infection does not lead to immunity and people not adequately vaccinated is potentially at risk of infection.

In 2015, a total of 10,301 tetanus cases, including 3,551 neonatal cases, were reported through the WHO/UNICEF Joint Reporting Form, reflecting the low reporting sensitivity for tetanus cases and uncertainty about the true disease incidence [3]. Indeed, estimating the actual impact of tetanus is difficult because most cases occur in low- and middle-income countries, where surveillance systems are limited. To date, *C. tetani* infection remains a serious threat in these countries, mainly due to poor health conditions. Moreover, most of reported cases are birth-associated, occurring among insufficiently vaccinated mothers and their newborn infants, following unhygienic deliveries and abortions, and poor post-natal hygiene and cord care practices. In developed countries, by contrast, cases of tetanus have become quite rare, mainly due to the implemented vaccination programs [2].

In 2021, the average estimated vaccine coverage against tetanus for the European countries was 94 % (range 86–99 %). Over the last five years, a small decrease in the vaccine coverage has been observed in several countries, among which Italy [4].

In Italy, tetanus vaccination has been made compulsory since 1938 for the military personnel, since 1963 for children in the second year of life and for some professional categories considered more exposed to the risk of infection (agricultural workers, livestock breeders, etc.), and since 1968 for all newborns in the first year of life.

The current vaccination schedule includes five doses of vaccine: three doses in the first year of life, followed by a booster dose at 6 years of age and one at 12–18 years. Subsequently, to maintain immunity, further boosters are recommended every 10 years [5,6]. Indeed, vaccination confers lasting protection for at least 10 years; however, after this period of time, immunity decreases, exposing newly unprotected subjects to the risk of infection.

In the last years, the incidence of tetanus in Italy remained quite stable ranging from 0.05/100,000 population in 2017 (33 cases), to 0.06/100,000 population (36 cases) in 2018, and to 0.04/100,000 population (25 cases) in 2019. Only in 2020 the number of cases strongly decreased (10 cases, 0.02/100,000 population), probably due to the COVID-19 pandemic which had an overall effect on epidemiology and reporting for many notifiable diseases worldwide. In addition, the confinement strategies and control measures implemented during the pandemic probably played a role also on tetanus infection [4].

The aim of this study was to evaluate the seroprevalence of anti-tetanus antibodies in a representative sample of the Italian population stratified by age, gender, and geographical area, as well as the impact of already implemented vaccination in order to identify any group with a lower level of protection.

2. Materials and methods

2.1. Study design and participants

The study was part of a multicenter seroepidemiological survey in the Italian population, promoted by the Italian Institute of Health (ISS), which aimed at evaluating protection against the main vaccine-preventable infectious diseases in the general population [7–9].

Specifically, serum samples taken from subjects living in thirteen regions (Northern Italy: Autonomous Province of Bolzano, Emilia-Romagna, Liguria, Piedmont and Veneto; Central Italy: Tuscany and Marche; Southern Italy and Islands: Basilicata, Calabria, Campania, Apulia, Sardinia and Sicily) were analyzed.

The samples were collected in the period June 2019-May 2020 from subjects who presented at participating Centers' laboratories for routine blood tests. Eligible people were subjects between 6 and 90 years of age, without any immune-depressive condition or any acute infection, who have not recently undergone blood transfusion. Subjects who consented to participate in the study were informed in advance about the anonymous use of their serum for the purpose of this research. All individuals who provided serum samples gave verbal informed consent: consent for minors was provided by parents. The residual sera were anonymized and the only available demographic data included age, gender and geographical area of residence. After collection, the anonymized serum samples were stored at − 20 °C.

The number of sera needed for the study was calculated on the basis of antibody prevalence estimates in the different age groups made during the seroepidemiological studies conducted as part of the European ESEN (European Sero-Epidemiological Network) project [10], whose sampling was carried out for the national seroprevalence studies conducted in 1996, 2003–2004, and 2013–2014.

2.2. Detection of anti-tetanus antibodies

All collected sera were analyzed at the Laboratory of Hygiene of the Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy. The VaccZyme Anti-Tetanus Toxoid IgG enzyme immunoassay (The Binding Site Group Ltd, Birmingham, UK) was used to determine the levels of specific IgG antibodies against tetanus toxoid (T.Tox). The intensity of the colorimetric reaction was determined by the Labtech Microplate Reader LT4000 (Labtech International LTD, United Kingdom) at 450 nm. The absorbance of samples was converted into antibody concentration using an algorithm provided by the manufacturer. Antibody concentration < 0.01 IU/ml was considered negative, while values ≥ 0.01 were considered positive, indicating subjects protected against the disease. In addition, the 0.1 IU/ml value was considered the minimum level for a long-lasting protection [11]; therefore, positive subjects were divided in subjects with high level ($\geq$0.1 IU/ml) and low level ($<$0.1 IU/ml) of protection. In accordance with what was declared by the manufacturer, the sensitivity and specificity of the test were equal to 93.06 % and 91.82 %, respectively.

2.3. Statistical analysis

All the personal data of the participants (age, sex, and geographical area) and the results of the immunoenzymatic investigations were entered in a Microsoft Excel database. The prevalence of positive and negative samples in the immunoenzymatic analysis was calculated. Any differences in the prevalence of anti-tetanus toxoid antibodies in the study population by age group, sex or geographical area were assessed using the chi-square test and the results were considered significant at p-value < 0.05.

2.4. Ethical aspects

The study was approved by the Ethical Committee of the Istituto Superiore di Sanità (ISS) and performed accordingly to the protocol previously adopted in other seroepidemiological studies conducted in Italy and in Europe (European Sero-Epidemiological Network: ESEN).

The samples collection and management were conducted in compliance with the current legislation on the protection of personal data (Resolution 1 March 2012: General authorization for the processing of personal data for scientific research purposes. Resolution n.85, in compliance with the laws and regulations in force, the Codes of ethics and good conduct issued in the health sector, as well as the Provisions issued by the Guarantor for the protection of personal data on the subject and the EU Regulation 2016/679 "General Data Protection Regulation").

Each subject was coded with a specific code of the participating Center (which identifies the province in which the Center operates in each Region) followed by a number in progressive order without any reference that would allow tracing back to the patient's name or initials.

The blood samples collected, once the serous component was

separated, were destroyed in accordance with current safety regulations.

3. Results

3.1. Study population

Overall, in the years 2019–2020, 3821 serum samples were collected from subjects aged between 6 and 90 years in 13 Italian regions: 1496 (39.2 %) came from the regions of Northern Italy (Autonomous Province of Bolzano, Emilia Romagna, Liguria, Piedmont, Veneto), 411 (10.8 %) from the Central Italy (Marche, Tuscany), 1914 (50.0 %) from Southern and Insular Italy (Basilicata, Calabria, Campania, Puglia, Sardinia, Sicily).

Of the total, 1820 (47.6 %) subjects were males, 2001 (52.4 %) were females.

The subjects were divided into 5 age groups (Table 1): 675 (17.7 %) were aged between 6 and 12 years, 1055 (27.6 %) between 13 and 24 years, 1154 (30.2 %) between 25 and 39 years, 531 (13.9 %) between 40 and 64 years, 406 (10.6 %) equal or above 65 years. Male and female subjects were equally distributed ($p > 0.05$) in each age group.

3.2. Seroprevalence

Overall, 85.0 % of the tested subjects resulted positive, showing an antibody titer $\geq$ 0.01 IU/ml. The mean concentration of antibody anti-T. Tox in the analyzed samples was 0.63 ± 2.10 IU/ml (95 % IC = 0.53–0.72).

In general, the rate of subjects protected against tetanus showed a slight decreasing trend up to 25–39 age group (93.6 % in the 6–12 age group, 91.8 % in the 13–24 age group, 91.0 % in the 25–39 age group) (Fig. 1). The seroprevalence drastically decreased in the older age groups ($p < 0.001$), 78.2 % in the 40–64 age group, up to 45.3 % in the $\geq$ 65 age group which recorded the lowest prevalence.

The prevalence of subjects with low protection (<0.1 IU/ml) was significantly higher ($p < 0.001$) in the $\geq$ 65 age group (10.3 %), while in the other age groups that prevalence varies between 5.2 % (25–39 years) and 7.9 % (40–64 years).

Considering the gender, the youngest age group did not show significantly difference between males and females. On the contrary, the difference appeared particularly evident ($p < 0.001$) in the oldest age group (M: 60.8 %, F: 30.4 %) (Table 2).

Considering the geographical areas, significant different values of prevalence ($p < 0.001$) were recorded in the Northern (90.8 %), Central (87.3 %), and Southern Italy and Islands (80.0 %).

The analysis of the seroprevalence by age (Fig. 2), showed higher values for Northern and Central regions, in comparison to Southern regions and Islands for all age groups except for 6–12 years group in which the seroprevalence in Northern Italy (89.7 %) was lower ($p < 0.001$) than in Central (97.4 %) and in Southern Italy and Islands (96.7 %). In particular, the percentage of positive subjects in Southern Italy gradually decreased from the 6–12 age group (96.7 %) to $\geq$ 65 age group (28.1 %). In the latter age group, the lowest value was observed also in Northern (70.5 %) and Central (45.3 %) regions.

Table 1
Distribution of studied samples by gender and age group.

Age groups (years)	Males (N = 1820) N (%)	Females (N = 2001) N (%)	Total (N = 3821) N (%)
6–12	341 (18.7)	334 (16.7)	675 (17.7)
13–24	510 (28.0)	545 (27.2)	1055 (27.6)
25–39	521 (28.6)	633 (31.6)	1154 (30.2)
40–64	249 (13.7)	282 (14.1)	531 (13.9)
≥65	199 (10.9)	207 (10.3)	406 (10.6)

4. Discussion

In Italy, tetanus is a mandatory notifiable disease, for which notification is required within 12 h, even just for suspicion of illness. On the basis of notifications recorded in previous decades, after years in which at least a hundred cases per year were regularly reported, tetanus seemed to show a decreasing trend. At the same time, the lethality also decreased, from 64 % in the 1970 s to 40 % in the 1990 s. From the 1980 s onwards, no cases of tetanus have occurred in newborns, children and young people under the age of 20 years. However, despite the mandatory vaccination, a certain number of tetanus cases per year continue to occur especially in people over 65 of age which accounted more than 80 % of all cases [12]. In 2000, 98 cases of tetanus (0.16/100,000 inhabitants) were reported, while in following years, the incidence of tetanus in Italy decreased to 0.10 cases per 100,000 inhabitants, with higher values in the Central and Northern Italy than in the Southern and the Islands [13].

In 2021 the European Centre for Disease Prevention and Control (ECDC) reported 50 cases of tetanus in whole Europe, with higher frequency in warmer months associated with higher levels of outdoor activity. The highest notification rate was reported by Italy (0.05 cases/100,000 population), which accounted for 54 % (27 cases) of all European cases. Italy was the country with the highest number of reported cases also between 2013 and 2018, with 43.5 % (n = 267) of all cases (n = 614) recorded by 26 EU member states and between 2017 and 2021 Italy accounted for 40 % (n = 131) of all reported cases (n = 329) [4,14]. This high prevalence may be partly due to the different methods of case detection, since Italy uses a national case definition different from the EU case definition, where clinical cases are considered “confirmed” due to the specificity of the clinical presentation; while in the EU case definition, clinical cases are classified as “confirmed” only after laboratory testing. However, it is likely that the inadequacy of vaccination coverage, despite the increasing trend, also plays a role in this epidemiological picture.

In general, reported cases of tetanus in industrialized countries have significantly decreased following the introduction of vaccination. It is crucial to prevent the disease since it provides circulating and ready-to-use anti-tetanus antibodies which, in case of infection, are able to block the toxin before it reaches the central nervous system crossing the blood brain barrier [15]. However, an efficient vaccination strategy requires surveillance tools capable of monitoring its effectiveness over time and of early identifying any corrective interventions to guarantee the achievement and maintenance of the set coverage objectives. Seroepidemiological studies represent a particularly useful tool for having a reliable picture of the general immunization and, therefore, for evaluating the impact of universal vaccination over the years, as well as helping to identify any susceptible subject group in specific age class or geographical areas. Furthermore, the search for antibodies in serum samples collected from healthy subjects during routine laboratory testing represents a simple, inexpensive and reliable means for collecting epidemiological data at a national level.

In the present study, the results of serological investigations conducted on serum samples taken from 6 to 90-years-old subjects residing in thirteen Italian regions showed that 85.0 % of people were protected against tetanus. A previous seroepidemiological study conducted between 2003 and 2004 found that 70.7 % of 3,604 Italian individuals, ranging in age from 0 to 95 years, were protected [16]. This allowed us to state that, althoug tetanus vaccination (included in the hexavalent vaccine) is mandatory in the pediatric age and boosters in young adults, adults and elderly are recommended every ten years, the coverage rates are still sub-optimal as observed elsewhere [17]. Our study also highlighted a particularly high level of immunization against tetanus toxoid among the youngest age groups which gradually decreased in older ones. In particular, in the 6–12 age group the prevalence of immunized subjects was 93.6 %, and remained high also in the 13–24 and 25–39 age groups (91.8 % and 91.0 %, respectively). This was observed in other

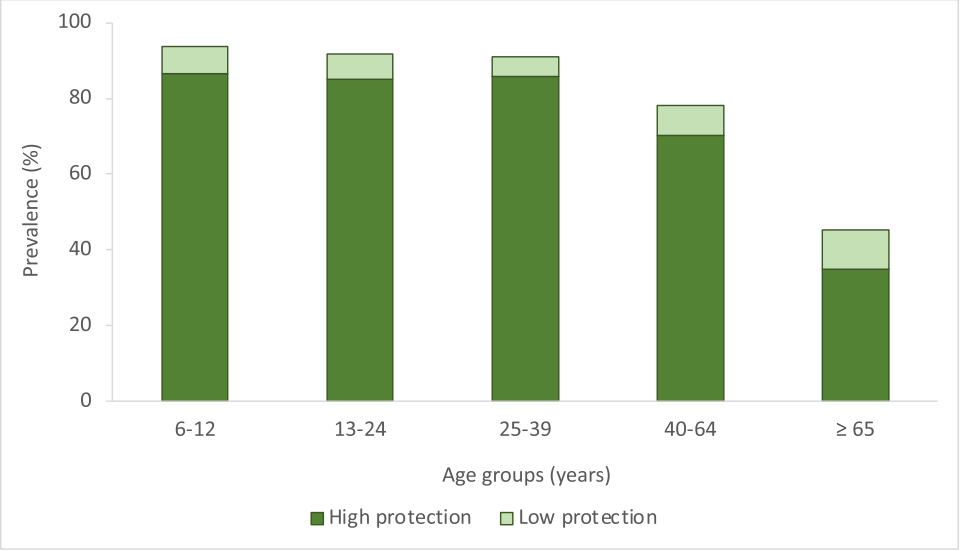

Fig. 1. Seroprevalence (%) of subjects protected against tetanus in the Italian population, divided in high (>0.1 IU/ml) and low (<0.1 IU/ml) protection groups.

Table 2
Seroprevalence (%) by age of subjects protected against tetanus among male and female subjects.

Age groups (years)	Males %	Females %	p-value
6–12	93.0	94.3	0.473
13–24	92.4	91.2	0.494
25–39	94.8	87.8	<0.001
40–64	80.3	76.2	0.256
≥65	60.8	30.4	<0.001

studies in Italy and Europe, where the proportion of individuals with protective antibody levels decreased with increasing age [16,17].

The increased general protection and the high seroprevalence in younger groups underline the good effect produced by the vaccination strategy adopted regarding the achievement of a high share of protected subjects among the population. Indeed, in 2021 (birth cohort 2019) the national vaccination coverage was 94.0 %, with a higher rate in the Central Italy (95.1 %) and lower in Southern (92.7 %) and Northern Italy (92.4 %) [18].

In older age groups, the majority of people resulted not or weakly protected against tetanus as the prevalence of subjects with anti-T.Tox antibodies decreased up to minimum levels (45.3 %) in the age group ≥ 65 years simultaneously with the increase in the prevalence of subjects with a low level of protection (10.3 %).

This is likely to be due to both inadequate vaccination coverage and waning immunity. In light of past vaccination strategies, most people ≥ 65 years of age probably never received a series of primary vaccinations including that against tetanus. In addition, many people may have been successfully vaccinated in the past but have not received the booster in

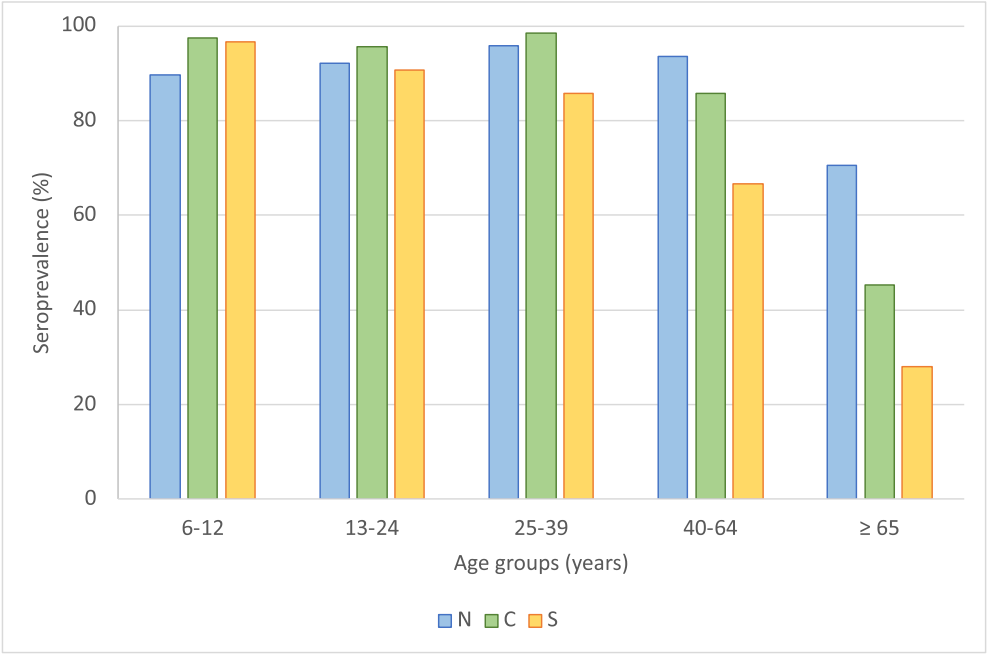

Fig. 2. Seroprevalence (%) by age and geographic area of subjects protected against tetanus in the Italian population. N: Northern Italy; C: Central Italy; S: Southern Italy and Islands.

subsequent decades. It may be the reason why cases continue to occur particularly in elderly, unvaccinated or incompletely vaccinated [19,20], as confirmed in the latest ECDC report on tetanus, which highlighted the highest incidence in the > 65 age group (78.0 %) [4].

Other studies observed that, among elderly, women registered the highest number of cases of tetanus in developed counties [19–21], in Europe [4], and in Italy [16].

As a matter of fact, the present study recorded lower levels of protection in women than men, especially in the ≥ 65 age group (30.4 % vs 60.8 %, $p < 0.001$). This is likely due to the fewer opportunities of older women to be vaccinated during their lifetime compared to men who, instead, may have received the vaccine during military service or for work purposes [16].

Considering the incidence data in the different geographical areas, from the 1970 s to the 1990 s there was an almost uniform reduction in incidence across Italy. In fact, the incidence rate per 100,000 inhabitants standardized by age (reference population: 1991 ISTAT census) decreased from 0.64 to 0.19 in the North, from 0.62 to 0.25 in the Center and from 0.37 to 0.11 in the South. In all decades the incidence rate of tetanus in the South is lower than the national average [22].

In general, we found a higher prevalence of protected subjects in Northern (90.8 %) and Central (87.3 %) than in Southern Italy (80.0 %). This trend was also highlighted in the study of Filia et al. [16], which reported 75.1 % of immune subjects in Northern Italy, 68.1 % in Central Italy, and 67.0 % in Southern Italy in the years 2003–2004. Moreover, differences were more evident in subjects over 45 years of age than the younger ones, as underlined also in the present survey. In our study, although the seroprevalence of subjects in the 6–12 and 13–24 age groups was comparable across the various geographical areas, there was a more pronounced decline in seroprevalence among the older age groups in Southern regions and Islands than in other geographical areas. These differences could be attributable to a higher rate of vaccination in Northern Italy for working purposes in the past decades, as confirmed by the higher occupation rate in this area [23].

5. Conclusion

The results presented in this study illustrated the decrease of seroprotection in older age groups and in women. These data, compared with epidemiological ones, confirm that the population with lower protection has a greater risk of contracting the disease, demonstrating the need for adequate immunization through both primary vaccination and boosters for all ages and both sexes, in order to provide lifelong protection. Moreover, being a non-contagious disease, achieving high vaccination coverage does not allow for an indirect population protection (herd immunity); therefore, it is essential to maintain individual protection through booster vaccinations.

Booster responses can still be elicited after intervals of 25–30 years, demonstrating the persistence of immunological memory. Serological survey data suggest that booster doses in adolescents and adults are critical in maintaining high antibody levels, which can persist for decades [24].

Moreover, the results of this study confirmed that the adoption of mandatory tetanus vaccination in childhood contributed to a significant increase in immunized subjects and a drastic reduction in incidence. It is essential to maintain a high proportion of protected subjects by introducing appropriate strategies that allow individual protection to be maintained through booster vaccinations.

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Declaration of Competing Interest

All Authors declare no conflicts of interest directly related to this manuscript. Outside this paper, Giovanni Gabutti reports having received personal fees for advisory board membership and consultancy from Emergent BioSolutions, the GSK group of companies, Merck Sharp & Dohme, Pfizer, Sanofi Pasteur Italy, Moderna and Seqirus, as well as personal fees for lectures from Merck Sharp & Dohme, Pfizer, Novavax, Viatris and Seqirus.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use generative AI and AI-assisted technologies in the writing process of the present work.

CRediT authorship contribution statement

Francesco Bagordo: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Tiziana Grassi:** Writing – original draft, Data curation. **Maria Cristina Rota:** Supervision, Methodology, Conceptualization. **Paolo Castiglia:** Writing – review & editing. **Tatjana Baldovin:** Writing – review & editing. **Giorgia Della Polla:** Writing – review & editing. **Alessandra Panico:** Writing – original draft, Investigation. **Matilde Ogliastro:** Data curation. **Serena Marchi:** Data curation. **Costanza Vicentini:** Writing – review & editing. **Palmira Immordino:** Writing – review & editing. **Marta Savio:** Investigation. **Giovanni Gabutti:** Writing – original draft, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Giovanni Gabutti reports a relationship with Emergent BioSolutions, the GSK group of companies, Merck Sharp & Dohme, Pfizer, Sanofi Pasteur Italy, Novavax, Viatris, Moderna and Seqirus that includes: board membership, consulting or advisory, and speaking and lecture fees. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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