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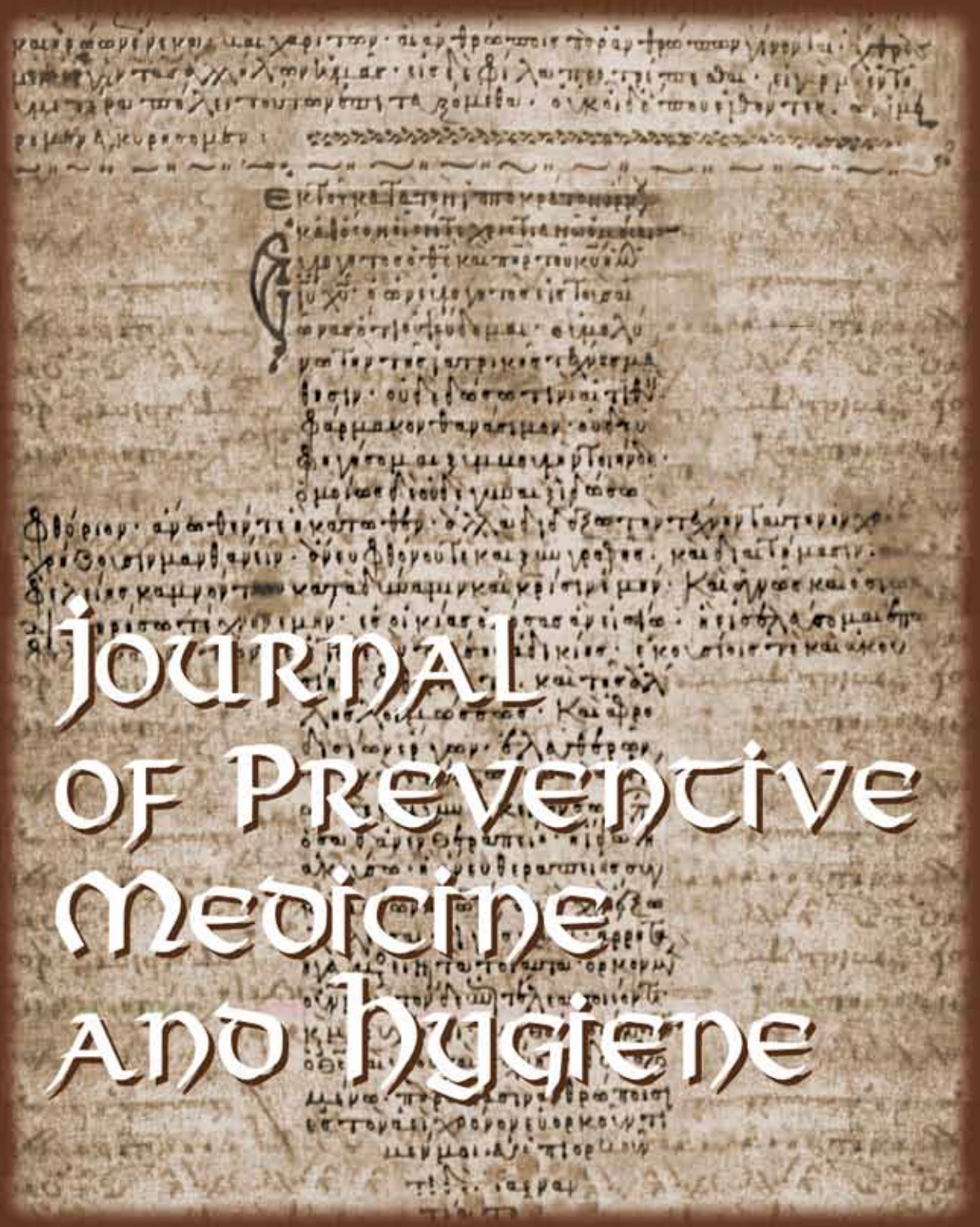

JOURNAL OF PREVENTIVE MEDICINE AND HYGIENE

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CONTENTS

Reviews

Estimates of the burden of meningococcal disease in Italy: implications for prevention and control
D. Martinelli, F. Fortunato, R. Prato E112

Epidemiology of Neisseria meningitidis infections: case distribution by age and relevance of carriage
G. Gabutti, A. Stefanati, P. Kuhdari E116

Meningococcal disease in childhood: epidemiology, clinical features and prevention
S. Bosis, A. Mayer, S. Esposito E121

Meningococcal serogroup B vaccine in Italy: state-of-art, organizational aspects and perspectives
C. Signorelli, V. Chiesa, A. Odone E125

Meningococcal B vaccination strategies and their practical application in Italy
R. Gasparini, D. Amicizia, P.L. Lai, D. Panatto E133

Meningococcal B vaccine and the vision of a meningitis free world
A. Bianchi, S. Fantoni, A. Prugnola E140

Methodological criticisms in the evaluation of Pneumococcal Conjugate Vaccine effectiveness
C. Trucchi, C. Paganino, F. Ansaldi E144

Review

Estimates of the burden of meningococcal disease in Italy: implications for prevention and control

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Key words

Meningococcal disease • Incidence • Serogroup B

Summary

Meningococcal disease is an acute, severe bacterial infection caused by Neisseria meningitidis. The most common presentations of invasive meningococcal infection (IMD) are meningitis and sepsis, less common pathologic presentations include focal infections. IMD can develop from initial symptoms to death within 24 hours. As many as 20% of survivors have permanent sequelae. Infants < 1 year of age have the highest incidence and adolescents the highest carriage prevalence.

In Italy, the incidence of IMD was 0.25 confirmed cases per 100,000 in 2011, but this may have been considerably underestimated due to under-detection and under-reporting. Recently, we estimated the impact of the MenC universal vaccination on the burden of meningococcal meningitis in Puglia by assessing the completeness of three registration sources (notifications, hospitalizations, and laboratory surveillance). The sensitivity of the three systems was 36.7% (95% CI: 17.5%-57.9%) and registrations lost nearly 28 cases/year in the period 2001-2013.

In the National Surveillance of Invasive Bacterial Diseases, serogroup B accounted for 64.9% of samples serotyped in 2011. Applying this percentage to the total number of hospitalizations for IMD registered in the same year (n = 256), we obtained an estimated 166 episodes attributable to serogroup B.

Our work highlights the importance of enhancing surveillance for meningococcal disease and strengthening vaccinations against all preventable serogroups.

Brief overview of meningococcal disease

Meningococcal disease is an acute, severe bacterial infection caused by *Neisseria meningitidis*. The bacteria are transmitted by droplet aerosol or secretions from the nasopharynx of colonized people (10% to 20% of adolescents and adults are asymptomatic transient carriers). Meningitis is the most common presentation of invasive meningococcal infection (IMD) and results from the spread of the bacteria through the bloodstream to the brain. Meningococcal sepsis (bloodstream infection or meningococcemia) may occur with or without meningitis (5% to 20% of IMDs). Less common pathologic presentations include pneumonia (5% to 15% of cases), arthritis (2%), otitis media (1%), and epiglottitis (less than 1%) [1, 2].

IMD is a feared, rapidly progressive childhood infection that can develop from initial symptoms (easily misdiagnosed) to death within 24 hours [1, 3]. In the first 8 hours, most children have only non-specific symptoms (irritability, loss of appetite, fever, nausea/vomiting, sore throat, coryza, general aches, leg pain, drowsiness, floppy muscle tone in infants < 1 year of age) that can often resemble those of common viral illnesses. Only about half these children are sent to hospital after the first consultation. Specific meningitis symptoms and signs of sepsis and shock (cold hands/feet, petechiae, purpuric rash, meningism, neck stiffness, photophobia, bulging fontanelle in infants < 1 year of age) are seen later, around 12-15 hours from the onset of the illness, due to the rapid replication of *Neisseria meningitidis* in the body. The last signs (such as confusion/delirium, unconsciousness, seizure, septic shock, multisystem failure, death) develop late, with a median onset of 15-24 hours. Intervention often does not occur until specific late-stage symptoms have already appeared (median time from onset to hospital admission = 19 hours) [3, 4]. Even when the disease is diagnosed early and adequate treatment is started, 5% to 10% of patients die, typically within 24 to 48 hours after the onset of symptoms [2]. Potentially lethal complications of fulminant meningococcal disease include increased intracranial pressure, uncal herniation (included during lumbar puncture), cerebral infarction, status epilepticus, cardiac arrest, metabolic acidosis, primary respiratory failure, multi-system failure, intractable shock, circulatory collapse, disseminated intravascular coagulation [4-6].

As many as 20% of survivors of IMD (all serogroups) have permanent sequelae, such as hearing loss, neurologic damage, or loss of a limb [1]. Most children survive serogroup B meningococcal disease without major sequelae. However, nearly one in ten experience major disabling deficits, including limb amputations, seizures and hearing loss, and more than a third have one or more deficits such as psychological disorders, digit amputations and unilateral hearing loss [7].

Infants younger than one year of age have the highest incidence (17.3-fold increase over average in Europe) [8]

due to a naive/immature immune system, waning of protective maternal antibody levels and exposure to young adult carriers in the household [9, 10]. Adolescents are the population with the highest carriage prevalence (1.8-5.3-fold increase over other age groups) [11]. Close and prolonged contact with a carrier, such as kissing, sneezing or coughing on someone, household crowding, or living in dormitories, sharing of drinks, cigarettes, and utensils, respiratory tract infection, both active and passive smoking, travelling to countries with epidemic or hyperendemic meningococcal infection are associated with an increased risk for the disease [12-14]. Most cases of meningococcal disease occur in previously healthy people without any warning [15].

Under-reporting of meningococcal disease incidence in Italy

IMD is rare in Italy where 0.25 confirmed cases per 100,000 population were observed in 2011, based on surveillance data submitted to The European Surveillance System [16]. Reported incidence, however, may be considerably underestimated due to underdiagnosis (under-ascertainment) and under-reporting affecting IMD surveillance, particularly in some regions [17].
Monitoring the incidence of meningococcal disease is essential to evaluate the impact of the implemented vaccination strategies with the meningococcal serogroup C conjugate vaccine (MenC) or the quadrivalent meningococcal conjugate vaccine (MenACWY), and to advise on the use of the new multicomponent serogroup B meningococcal (4CMenB) vaccine, recently introduced in some Italian regions and under discussion for introduction on a national scale. In a recent study, we estimated the impact of the MenC universal vaccination on the burden of meningococcal meningitis in Puglia by assessing the completeness (sensitivity) of three registration sources (notifications, hospitalizations, and laboratory surveillance) in the period 2001-2013. We found that only 213 cases of meningococcal meningitis out of an estimated 580 (95% CI: 368-1,216) total cases were recorded in at least one of the three sources, with an overall sensitivity of 36.7% (95% CI: 17.5%-57.9%). This means that the routine surveillance systems lost nearly 28 cases/year in the study period [18].

Incidence of meningococcal B disease in Italy

In order to estimate the potential impact of the new 4CMenB vaccine, the distribution of *N. meningitidis* serogroups detected by the National Surveillance of Invasive Bacterial Diseases (referred to as MIB, 2011 and 2013 data) was applied to the total number of both reported and hospitalized cases in each of the 21 Italian regions. Hospitalizations were extracted from the National Hospital Discharge Registry (HDR, 2011 data) [19], where IMD is identified by the ICD9-CM codes

Fig. 1. Number of invasive meningococcal disease cases and estimated distribution of cases attributable to serogroup B, by Italian region, 2013.

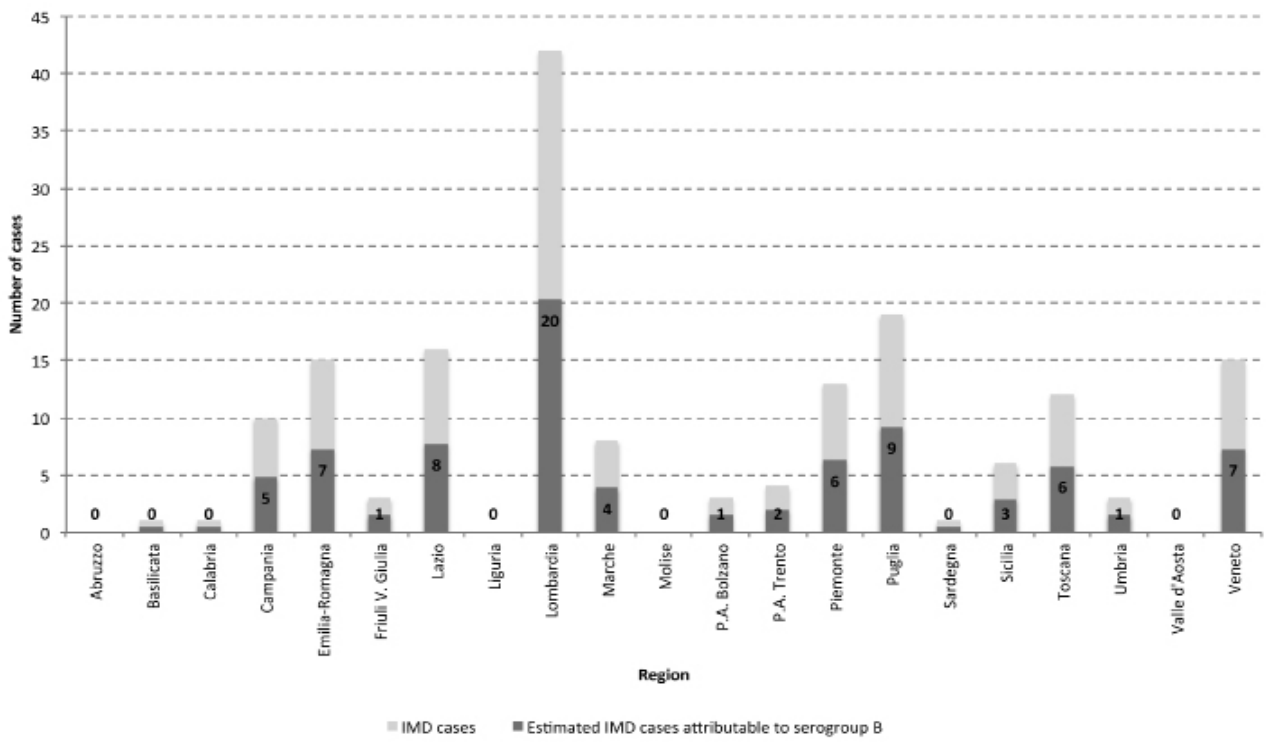
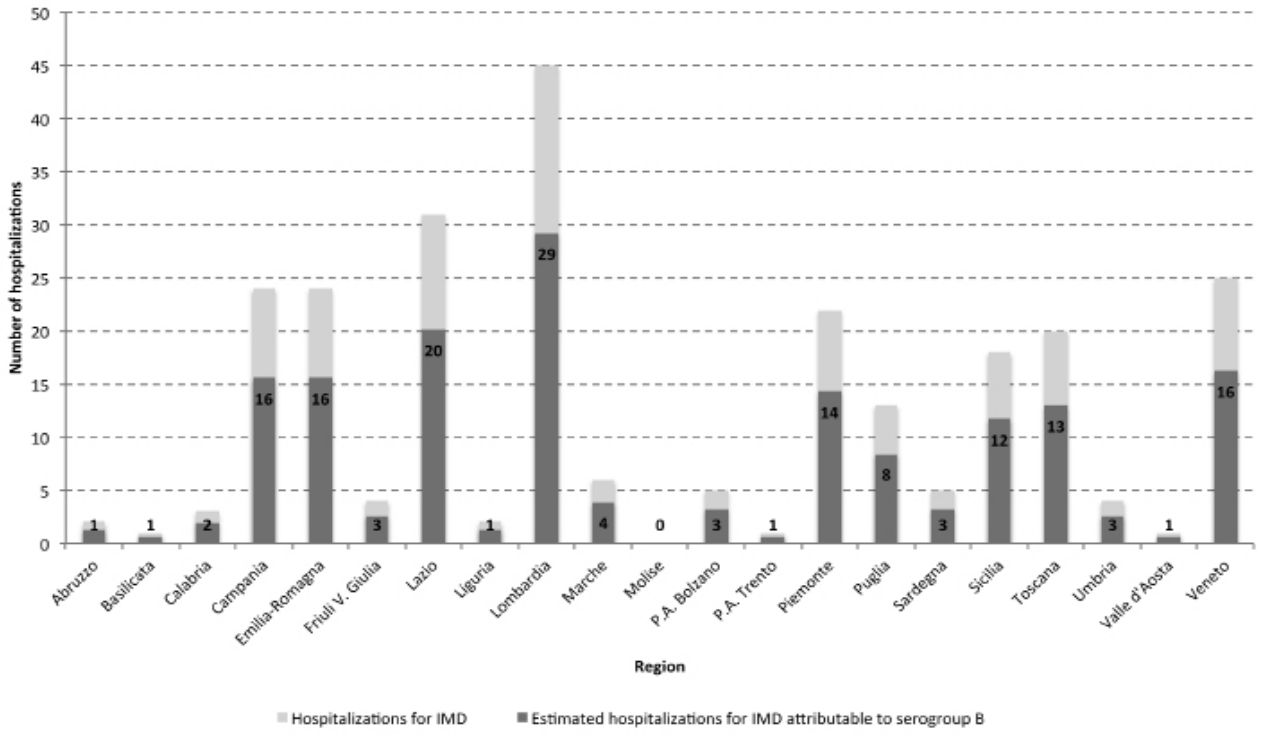


Fig. 2. Number of hospitalizations for invasive meningococcal disease and estimated distribution of cases attributable to serogroup B (% from the MIB surveillance), by Italian region, 2011.



036.x - *Meningococcal infection* as main or secondary diagnosis.

In 2013, a total of 172 cases were notified to the MIB surveillance (incidence rate of 0.29 per 100,000). Among the 116 (67.4%) strains typed, serogroup B accounted for 48.3% of isolates (56 cases, incidence rate of 0.09 per 100.000) [20]. Lombardia reported the highest notification rate (42 cases, incidence rate of 0.43 per 100,000) [20], thus the estimated number of cases that could be attributable to serogroup B was 20. Four regions (Abruzzo, Liguria, Molise, and Valle d'Aosta) reported zero cases (Fig. 1) [20].

A total of 256 hospitalizations for IMD were recorded in the HDR (hospitalization rate of 0.42 per 100,000) in 2011. Out of 22 day-hospitals, 13 reported main diagnosis coded as meningitis and six were coded as sequelae (i.e.: paralytic syndromes, late effects of cerebrovascular disease, disarticulation of elbow, etc). Applying the percentage of the typed B strains in 2011 retrieved from the MIB surveillance (76 cases, 64.9% of samples serotyped [20]) to the total number of hospitalizations for IMD, we obtained an estimated 166 episodes that could be attributable to serogroup B (hospitalization rate of 0.27 per 100,000). Lombardia confirmed the highest rate (45 discharges for IMD; 0.46 per 100,000), with an estimated number of 29 cases that could be attributable to serogroup B. Molise reported zero hospitalizations for IMD (Fig. 2).

Closing remarks

The incidence of invasive meningococcal disease is relatively low in Italy; however, it is a disease with a high fatality rate and high risk of complications [1, 2, 16, 20]. The assessment of the sensitivity of data sources available for monitoring the incidence of meningococcal meningitis showed that they are not sufficiently comprehensive in terms of the cases they contain [18]. Both under-reporting and under-ascertainment affect the Invasive Bacterial Diseases surveillance in some Italian regions [17], complicating efforts to understand their occurrence and burden, particularly when the planning and evaluation of vaccination programmes need timely, reliable incidence data.

Despite significant differences in reporting practices between regions, cases from serogroup B remain dominant in Italy, as the estimated number of discharge records for IMD that could be attributable to group B in our analysis shows.

Our work highlights the importance of enhancing surveillance for meningococcal disease and strengthening vaccination programmes against all preventable meningococcal serogroups.

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Review

Epidemiology of Neisseria meningitidis infections: case distribution by age and relevance of carriage

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Key words

Neisseria meningitidis • Age classes • Carriage

Summary

Notwithstanding different meningococcal serogroups have changed their distribution and their impact in different age classes over time, N. meningitidis' invasive diseases are a major public health issue worldwide, due to the related complications and severe sequelae. Nowadays, the highest rates of invasive disease are registered in children younger than 1 year of age, with a second lesser peak in adolescents and young adults (15-25 years of age). On the contrary, the prevalence of carriage is low in newborns and in school-age children, and increases during adolescence and young-adult age; then it decreases again in older age. N. meningitidis' infection prevalence has greatly decreased in Europe and North America thanks to the use of conjugate vaccines (MenC and MenACWY) as well as the incidence of invasive disease due to serogroup A in sub-saharian Africa after the introduction of MenAfriVac conjugate vaccine.

The great success of conjugate vaccines is related not only to the direct protection from disease but also to the impact on carriage; this latter allows an indirect protection of unimmunized subjects. For these reasons, the implementation of immunization with the new generation vaccines in the age classes most impacted by disease and carriage (first year of life, adolescence and young adulthood) could permit to achieve an extraordinary decrease of the incidence of meningococcal disease.

Introduction

Neisseria meningitidis (*N. meningitidis*) is an aerobic, Gram-negative diplococcus, exclusively hosted by man; it usually lives as a temporal commensal in the upper respiratory tract without causing any disease. Reasons for the transition from asymptomatic carriage to invasive disease have still not completely understood; anyway, some factors, such as genetic and capsular structure of pathogenic strains, are believed to play a relevant role [1, 2]. *N. meningitidis* is classified in 12 serogroups (accordingly to capsular polysaccharide structure) and in serotypes and sub-serotypes (accordingly to outer membrane proteins). The role played by each most epidemiological relevant serogroup (A, B, C, W-135, and Y) greatly changes in relation to time period and geographical area considered; anyway, notwithstanding the ample underestimate of its global epidemiological impact, meningococcus is a relevant public health issue worldwide [3, 4].

N. meningitidis is transmitted through respiratory droplets of infected subjects or, more often, of asymptomatic carriers. Usually humoral immune response is enough to prevent the spreading of the pathogen and the occurrence of invasive disease. Anyway, if the humoral response is not adequate, bacteriaemia occurs due to not yet completely understood mechanisms [5]. Once in the bloodstream, meningococci circumvent immunological response by several virulence factors (capsule, IgA protease, surface "blebs" containing LPS, that act as an endotoxin). Endotoxin induces a cascade of pro-inflammatory citokines with a subsequent endotelial damage, increase of vascular permeability, protrombotic condition with subsequent development of microthrombosis. Meningococcal disease is a quite rare event and meningitis is its most common feature (about 50% of cases) [6], followed by bacteriaemia (40% of cases). Fulminant disease occurs in 10-20% of cases and it is characterized by organ failure and disseminated intravascular clotting (e.g. Waterhouse-Friderichsen syndrome); in these cases, mortality could be equal to 50% [7]. Lethality of meningococcal infections could reach 10%, while permanent sequaelae occur in up to 20% of survivors. Permanent sequelae involve neurological damage, psychological disturbances, hearing loss, visual loss, cutaneous scarring and/or limb amputations [8].

Pathogenesis and Epidemiology

The global incidence of meningococcal disease greatly changes in relation to considered geographical areas; worldwide, 500,000-1,200,000 invasive meningococcal diseases occur each year, with 50,000-135,000 deaths [9,10].

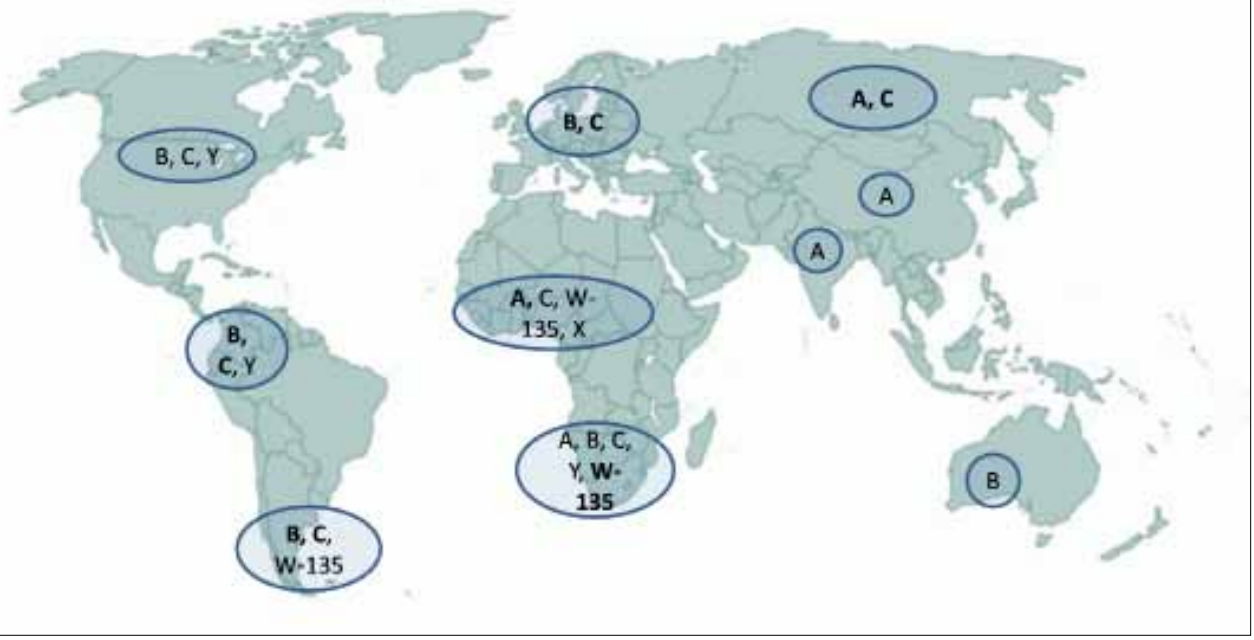
Nowadays, in Europe, North America and Australia incidence ranges between 0.3 and 3 cases per 100,000 inhabitants [11], while the same could reach 10-1,000

cases/100,000 in Africa during epidemics (in particular in the so-called sub-saharian "meningitis belt").
The epidemiology of meningococcal infections has significantly changed over the years in many regions of the world. Serogroup A has been the principal agent of invasive meningococcal disease in Europe before and during I and II World Wars, serogroup B has been prevalent since 1970 in Europe and since 1980 in South America; epidemic outbreaks due to W-135 and Y serogroups have emerged more recently during the XXIst century. Besides, a change in the age classes affected by invasive disease has occurred, with an increase of incidence of serogroup Y in elderly and a decrease of serogroup C in adolescents. The epidemiological trend of invasive disease has almost remained unchanged in Africa, where serogroup A is most prevalent; very recently, serogroups X and W-135 have had a relevant impact in terms of morbidity and mortality [12].
Disease caused by serogroup A in Africa has an annual incidence equal to 10-20 cases per 100,000 inhabitants; epidemic outbreaks, occurring during dry season, imply an attack rate greater than 1,000 cases per 100,000. Data from Latin America and Asia are limited. In Latin America, incidence ranges between 0.1/100,000 in Mexico to 2 cases/100,000 in Brasil, with a predominance of serogroups B and C [13]. In Asia, the epidemiological burden of meningococcal disease is not well defined. Serogroup A has been considered prevalent; anyway, all five serogroups (A, B, C, Y and W-135) have been reported, even if with a regional variation [14]. In Australia, meningococcal incidence is greater than 3 case/100,000. In most American and European countries a low level of endemicity is registered. In 2011 [15], 29 European countries (27 UE countries plus Norway and Iceland) have reported 3,808 confirmed cases of invasive disease; the global notification rate has been equal to 0.77/100,000 (range 0.09-1.99), serogroup B has been the most relevant (73.6% of cases), followed by serogroup C (14.4%) and Y (8.2%). Invasive disease incidence sustained by serogroup B in Europe accounted for 0.5 cases per 100,000 inhabitants. Italy reports the lowest incidence rate, equal to 0.25 case per 100,000 [16]. Figure 1 shows the distribution of main *N. meningitidis* serogroups in different geographical areas.

Age classes and categories at risk of developing meningococcal disease

There is an ample consensus that the categories at highest risk of developing meningococcal disease are newborns and children in the first year of life (as natural immunity against *N. meningitids* is particularly low), adolescents (due to their habits and behaviours that facilitate strict interpersonal contacts; besides, they have the highest carriage rate), travellers that stay for long time in endemic areas (Sub-saharian Africa, ect.), immunosuppressed subjects (functional or anatomic asplenia, thalassemia, sickle cell anemia, persistent complement deficiencies, organ transplant, cancer or high dosage corticosteroid therapy, diabetes, HIV infection, congenital immunodeficiencies), and elder subjects [17, 11].
The highest rate of disease is registered in children younger than 1 year of age, with a second lower peak in adolescents and young adults (15-25 years of age) [18]. Since 2008, European incidence rate has decreased form 0.95/100,000 to 0.68/100,000; higher rates have been registered in Lituania and UK (1.77 and 1.36, respectively). Newborns, 1-4year-old children and adolescents (15-25 years of age) are the most affected subjects in all

Fig. 1. Meningococcal serogroups in the world (reference 9, modified).



countries, irrespective of ongoing or not immunization programs against MenC.
The most relevant rate of invasive disease, in particular in children younger than 5 years of age, is related to serogroup B, followed by serogroup C. In 2012, the notification rate of MenB infection in children <1 year of age has been three-fold higher than the one registered in the age group 1-4 years (12.3 and 4.1 per 100,000, respectively). The highest rate of MenC cases has been registered among young adults and adults (25-44 years of age); serogroup Y has been mostly identified in ≥65 year-old subjects [19].
In Italy, Azzari e co-workers have confirmed, in a study conducted in the period 2006-2012 [20], that as in other European countries, meningococcal disease caused by serogroup B has a greater incidence during the first 5 years of life and that 70% of cases are registered during the first year of age, with a peak between 4 and 8 months.
As far as immunization with MenC conjugated vaccine has been implemented in Italian regions, the incidence of the disease related to this serogroup has progressively decreased. Nowadays, serogroup B is the most common serogroup causing invasive meningococcal disease, being involved in more than 80% of cases in patients <24 years of age. Figure 2 shows the distribution of MenB invasive disease in Italy in the period 2007-2012, and its peak of incidence in the first year of life [16].
In USA, the rate of invasive meningococcal disease has been equal to 0.14/100,000 in 2013; incidence mainly involved children younger than 5 years and subjects aged 18-35 years (in the two groups the incidence was almost equal: 1.7/100,000). Serogroup B impacted mostly in <1year-old babies (0.68/100,000), while serogroup C showed an higher incidence in the age class 1-4 years (0.41/100,000) [21].

Carriage

N. meningitidis is a human infective agent usually residing in the nasopharynx. Human upper respiratory tract is a stable ecological niche; anyway, meningococci can be habitual components of the microbial flora in buccal mucosa, anus, urethra, urogenital mucosa, and dental plaque. Carriage at pharingeal level involves 8-25% of subjects; this means hundreds of millions people in the world, adolescents being the most relevant group [22].
The relationship between asymptomatic carriage and development of invasive disease in not completely known, nor the timeframe necessary for the transition from one status to the other. Concerning this point, humoral immunity certainly plays a crucial role. In most cases the microorganism persists in the nasopharynx for days or weeks, and even months. Carriage is crucial not only in the transmission dynamics but even in the onset of invasive disease. As a matter of fact, the lack of bactericidal antibodies is a relevant risk factor for the transition to invasive disease. The repeated occurrence of carrier status, not only of *N. meningitidis* but also of *N. lactamica*, even not protective against subsequent new carriage, can elicit a cross-protection against invasive disease [23].
Strains in carriers are genetically and antigenically different from the ones isolated in subjects affected by invasive disease. Age is one of the most relevant factor related to meningococcal carriage.
Carrier status has been studied and even more in depth understood during last years, in particular after the implementation of immunization with MenC conjugate vaccine

Fig. 2. Estimated incidence of invasive MenB disease in Italy, 2007-2012 (data from reference 16).

and the achievement of relevant results in terms of epidemiological and immunological impact. Differently from natural infection, carriage has a low prevalence in the first years of life and in older age classes, and reaches its peak in adolescents and young adults. In the "meningitis belt" meningococci are, in respect to other geographical areas, more uniformly distributed irrespective from age [24].
Besides, more than age, other factors influence carriage such as male gender, concomitant viral or bacterial respiratory infections, active and passive smoke, low socioeconomic status. One of the most relevant risk factor is the number and the mixing pattern of social interactions; seasonality does not seem relevant [25].
Both in Europe and in North America, the highest rate of nasopharingeal carriage is registered in adolescents and in young adults; they act as the most relevant source of infection. Carriage prevalence increases from 4.5% in infancy to a peak of 23.7% in the age class 19-20 years, and then decreases to about 10% in adults [26, 27]. These data have been recently confirmed in a study performed in Italy evaluating the molecular and serological diversity of *N. meningitidis* carrier strains isolated from students aged 14 to 22 years [28].

Vaccines and herd immunity

Knowledge on the epidemiology of *N. meningitidis*, the role of carriers and the invasive disease, has allowed to better understand both the relevance and the impact of immunization. There is an ample consensus on the point that immunization is the best and most efficacious preventive approach against meningococcal disease.
Since '70-'80s polysaccharide vaccines against serogroups A, C, Y and W-135 have been available. Later, conjugate vaccines has been developed; these vaccines, differently from polysaccharide ones, elicit a T-dependent immune response with the production of high affinity antibodies, immune memory and responsivity to subsequent doses. Conjugate vaccines are efficacious also in newborns, have an impact on carriage and induce herd immunity.
For all these reasons, the availability of conjugate vaccines and the implementation of immunization programs has allowed to achieve a great impact on the epidemiology of meningococcal disease. Since 2005, the evaluation of the results obtained after the immunization program adopted in UK has demonstrated that MenC conjugate vaccine directly protects immunized subjects, decreases carriage and blocks the spreading of the agent [24]. All these effects have amplified the impact of vaccination against serogroup C even in age classes not directly involved in the immunization program [29]. Another study performed in Africa in 2012 has showed a persistent decrease of carriage following the use of a MenA conjugate vaccine (MenAfriVac); carriage prevalence decreased from 0.39% in the pre-immunization period to 0,02% two years after the implementation of vaccination [30].
In addition to conjugate vaccines against serogroups A, C, Y and W-135, since January 2013 a new 4-components MenB vaccine (4cMenB) has been licensed in Europe. Read and co-workers in UK have recently evaluated the impact on carriage after immunization with Men ACWY-CRM conjugate (1 dose) and 4CMenB (2 doses; time interval between doses: 1 month) vaccines in university students aged 18-24 years [31]. The impact on carriage of both vaccines has been evaluated 1 month and during the 12 months following immunization. Concerning 4cMenB, since the 3rd month after administration a significant decrease of carriage of any meningococcal strain (18.2%), capsular groups BCWY (26.6%), capsular groups and serogroups CWY (29.6% and 28.5%, respectively) has been registered. A significant decrease of carriage rate has been registered also in subjects immunized with MenACWY-CRM compared to controls; the decrease was equal to 39% and 36.2% for serogroup Y and CWY, respectively. This study confirms that 4cMenB vaccine could impact on carriage not only for meningococcus B but also for other serogroups, as it does not contain capsular antigens but proteins shared with other nonB serogroups. Anyway, even if this study shows a first evidence of the impact on carriage of 4cMenB vaccine (as well as of MenACWY-CRM vaccine), its results should be considered with caution; the impact on carriage at individual level cannot be considered predictive of herd immunity. As a matter of fact, several other factors play a relevant role in the determinism of herd immunity, not only the ability of the vaccine to block or decrease the acquisition of the carriage status.

Conclusions

Notwithstanding the results achieved in the fight against disease caused by *N. meningitidis*, this etiological agent continues to be a relevant worldwide treat for health. Knowledge about age classes at highest risk and about relationship between nasopharingeal carriage and disease is fundamental in order to understand epidemiology and pathogenesis of meningococcal disease and to identify adequate immunization strategies.
Newborns and children <1year of age are at highest risk for the disease as their immune system is not completely developed and the maternal passive immunity tends to progressively fade out. The highest prevalence rate of carriage is registered in adolescents and young adults; in these age groups the efficacy of conjugate vaccines against carriage is equal to 75% [32].
The incidence of meningococcal disease has decreased during the last decade thanks to the immunization programs with conjugate vaccines against serogroups A, C, Y, W-135. More recently the new 4CMenB vaccine has been introduced with the aim to decrease the incidence of the disease sustained by serogroup B.
All these vaccines are safe, well tolerated and highly efficacious against the most relevant invasive serogroups; they elicit a long-lasting immune response in all age groups and induce herd immunity.
For all these reasons, the implementation of immunization programs against meningococcal disease should be a public health priority.

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Review

Meningococcal disease in childhood: epidemiology, clinical features and prevention

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Key words

Children • Invasive meningococcal disease • Meningococcal disease • Pediatric age • *Neisseria meningitidis*

Summary

Invasive meningococcal disease (IMD) represents a public health problem and a leading cause of morbidity and mortality worldwide. IMD can occur as an endemic disease with sporadic cases or epidemics with outbreaks. Neisseria meningitis strains are divided into 13 serogroups, but only five (A, B, C, W-135, and Y) are responsible for most IMD across the world. All age groups are at risk for IMD, but infants and adolescents are particularly vulnerable. The most common clinical manifestations of IMD are meningitis and septicemia, although in some cases both clinical pictures are present. The clinical pattern can differ according to age; in young children, the clinical manifestations may be more insidious and the diagnosis may be more difficult compared to older children or adolescents. Death occurs in 6-10% of cases and sequelae in 4.3-11.2% of cases. Early recognition of children with meningococcal infection is important in order to initiate systemic antibiotic therapy, although vaccination remains the best strategy to control meningococcal disease. Recently, different meningococcal vaccines have been introduced worldwide, resulting in a reduction in the overall burden of the disease. The goal of the next few years should be to increase vaccination coverage against meningococcal diseases, continue to monitor IMD and develop a unique vaccine able to cover all of the main meningococcal strains.

Introduction

Neisseria meningitidis is an anaerobic Gram-negative diplococcus that is responsible for invasive meningococcal disease (IMD), which represents a public health problem and a leading cause of morbidity and mortality worldwide [1, 2]. Globally, the incidence of IMD is 500,000 cases every year, although the incidence varies from <1 per 100,000 per year in North America and Europe to 10-1,000 per 100,000 per year in the "meningitis belt" of sub-Saharan Africa [3, 4]. Death occurs in 6-10% of cases and sequelae in 4.3-11.2% of cases [5].
Meningococcal carriage, which represents the first step of disease transmission, varies with age and setting. It is known that *N. meningitidis* colonizes the nasopharynx in up to 5-10% of adults who are asymptomatic. A recent study demonstrated that the carriage prevalence increases throughout childhood from 4.5% in infants to a peak of 23.7% in 19 year old subjects, then decreases to 7.8% in 50 year old adults [6]. This overview summarizes new data on meningococcal disease in children and the possibilities of its prevention.

Epidemiology

N. meningitidis strains are divided into 13 serogroups on the basis of the immunochemistry of their capsular polysaccharides; however, only five serogroups (A, B, C, W-135, Y, and X) are responsible for most IMD cases around the world [4]. IMD can occur as an endemic disease with sporadic cases or epidemics with outbreaks. All age groups are at risk for IMD, but infants and adolescents are particularly vulnerable due to the disappearance of maternal antibodies early in life and the high rate of nasopharyngeal colonization [2, 3]. Some settings, such as schools, university dormitories and barracks, are at high risk for *N. meningitidis* transmission. Moreover, low socioeconomic status, minority ethnicity, immune deficiencies and asplenia predispose individuals to meningococcal infection [2, 3].
The serogroups causing IMD vary geographically, mostly likely due to differences in population immunity and environmental factors. Meningococcus serogroup A (MenA) occurs in Africa and some areas of Asia, whereas serogroups B (MenB), C (MenC) and Y (MenY) are predominant in the other continents, including Europe and North America [2, 4]. In the 13 countries included in the African "meningitis belt", MenA was responsible for the majority of cases in 2007-2009, while meningococcus serogroup W135 (MenW135) predominated in 2010 and 2011 [4]. Although MenC is rare in Africa, in 2013 and 2014 two outbreaks due to a novel strain of MenC were reported in Nigeria and Kebbi, respectively [7]. Moreover, during 2006-2010 outbreaks of MenX were described in Niger, Togo, Kenya, Uganda, and Burkina Faso [8]. In Europe, MenB is the main cause of IMD, followed by MenC and MenY [4]. In countries with established MenC vaccination programs, the incidence of MenC disease has significantly declined [2, 4]. In comparison with the US, IMD caused by MenY is rare in

Europe. However, an increase in this serogroup has been reported in recent years, particularly in the Nordic European countries [9].

Clinical manifestation and sequelae

The most common clinical manifestations of meningococcal infection are meningitis and septicemia, although in some cases both clinical pictures are present [3, 10-12]. However, signs and symptoms at the onset of the disease, such as coryza and sore throat, may resemble those of common respiratory viral infections [11]. The incubation period varies from 1 to 14 days, although it usually lasts less than 2 days [11].
The clinical pattern can differ according to age, and it is not infrequent for children to be initially misdiagnosed. The clinical manifestations may be more insidious in young children, with non-specific signs; thus, diagnosis may be more difficult than in older children or adolescents. Irritability and lethargy are common features at this age. In some cases, seizures with focal onset may occur at the beginning of the disease. Moreover, neck stiffness is rare in children younger than 2 years of age [3, 10-12]. Bulging anterior fontanalle may occur in infants <18 months of age. In general, infants exhibit a more rapid progression of the disease compared to older children [12]. Similar to adults, in older children the most common symptoms are fever, nausea, vomiting, photophobia, headache, agitation, decreased level of consciousness and neck stiffness. However, seizure and focal neurological signs are less common [3, 10-12]. Septic shock is more common in children and progresses rapidly, with multiple organ failure and death occurring within 24 hours [3, 10-12]. Often, non-specific symptoms such as fever, drowsiness, nausea and vomiting, irritability and poor feeding are present within 4-6 hours from the onset of the disease [3, 10-12]. One of the most common symptoms associated with sepsis is a rapidly progressive hemorrhagic rash that usually starts on the lower extremities, although mucous membranes and sclera may be involved [3, 10-12]. Skin lesions include macules, maculopapules, urticaria, petechiae, purpura and ecchymoses. The purpuric rash may progress to purpura fulminans, a cutaneous manifestation of disseminated intravascular coagulation. These cases are often associated with septic shock and skin necrosis, ischemia, or infarction of digits or limbs that usually require amputation [3, 10-12]. Three clinical signs of early sepsis have been identified in children and adolescents: leg pain, cold hands and feet, and abnormal skin color. These features may suggest that the vital signs are compromised [11]. Some authors reported chronic meningococcemia that consisted of recurrent attacks of fever, arthralgia and/or arthritis associated with a rash and headache [3, 11].
IMD is a life-threatening disease with a very high rate of severe sequelae among survivors. In a recent article, Sadargani et al. evaluated the outcomes of IMD in 868 subjects (52% adults and 48% children) in Canada between 2002 and 2011. The mortality was lower in children than in adults, but 21% of children (particularly those < 5 years of age) had at least one complication compared with 15% of adults. The highest complication rates occurred in children with septic shock without meningitis, and the most common sequelae were hearing loss, deafness, seizure, amputation and skin scarring [13]. In a study that evaluated the outcome of IMD in 181 children < 15 years of age, the case fatality rate was 11.6% and at least one long-term sequelae was reported in 33% of patients: learning academic difficulties (22.6%), hearing impairment (7%), and neurological (12.2%), behavioral (14.8%) and motor (10.4%) deficits [14].
The duration of illness prior to admission and the presence of seizures, focal neurological deficits, depressed level of consciousness and low levels of cerebrospinal fluid glucose may be associated with a high risk of sequelae [11]. Wang at al. demonstrated that sequelae occurred in all children <1 years of age with IMD and a history of prematurity [15].

Treatment

Due to the severity of meningococcal disease including its high case fatality rate and possibility of sequelae, early clinical and laboratory diagnosis is very important. Lumbar puncture must be performed in all suspected cases with clinical signs and symptoms of IMD. In most cases, cerebrospinal fluid (CSF) reveals high opening pressure, pleocytosis, high protein levels and low glucose levels [10]. *N. meningitidis* should be detected in the CSF or blood by Gram staining, standard culture and/or polymerase chain reaction (PCR) [10, 11]. These examinations must be performed very quickly to avoid delays in the administration of therapy. Sometimes standard cultures may result in false negatives due to prior administration of oral antibiotic treatment that reduces the sensitivity of the exam. In these cases, PCR could be useful [10]. Lumbar puncture can be hazardous in patients with prolonged seizures, immunocompromised patients, in the presence of signs of space-occupying lesions and in patients with severe impairment of consciousness and shock [10].
Prompt and adequate intravenous antibiotic treatment is essential to stop the proliferation and kill *N. meningitidis*. Local antibiotic resistance should guide the choice of the antibiotic. In most cases, an intravenous third-generation cephalosporin (e.g., 100 mg/kg/day ceftriaxone administered i.v. in one daily dose or 100 mg/kg/day cefotaxime administered i.v. divided into three daily doses) should be used as the first choice of treatment. If these antibiotics are not available, intravenous penicillin should be started [10, 11]. If available, the result of antibiotic sensitivities could guide the continuation of the antibiotic therapy. The recommended duration of treatment is 7-10 days, although recent studies demonstrated that CSF sterilization may occur within 3-4 days [10, 11].
Prophylaxis is indicated and recommended only for close contacts of the index case (i.e., subjects who remained with the index case for more than 4 hours during the 7 previous days). In these cases, oral rifampicin

or ciprofloxacin are the drugs of choice according to age [11].

Meningococcal vaccines

Vaccination remains the best strategy to prevent meningococcal disease due to the high fatality rate and the significant sequelae that can result from the infection despite prompt antibiotic treatment. Recently, new meningococcal vaccines have been introduced worldwide, resulting in a reduction in the overall burden of the disease.

Since the introduction of the meningococcal serogroup C conjugate vaccine, the incidence of MenC disease has significantly declined [16]. Commercially available monovalent conjugate vaccines include Menjugate (MCC-CRM197), Meningitec (MCC-CRM197) and NeisVac (MCC-TT). Quadrivalent vaccines containing the meningococcal ACWY serogroups conjugated with different proteins have also been produced. Those available in the market are Menactra (ACWY-DT) licensed for children aged 2-55 years, Menveo (ACWY-CRM197) licensed in Europe and the US for children ≥2 years of age and Nimenrix (ACWY-TT) licensed in Europe for infants ≥12 months of age. These conjugated vaccines have proven to be safe and effective in protecting individuals against meningitis; moreover, these vaccines have demonstrated herd immunity by interrupting carriage transmission [17-19]. The quadrivalent polysaccharide vaccines Menomune and Mencevax have also proven to be safe and elicit bactericidal antibodies in older children and adults; however, they are not used in routine vaccination programs due to their poor immunogenicity in young children, short-term protection and inability to produce herd immunity [16, 18]. Unfortunately, quadrivalent ACYW meningococcal vaccines (conjugated or polysaccharide) are not widely used in developing countries due to high costs. An effective and safe conjugate vaccine against MenA (MenAfrivac) first licensed in India in 2009 was introduced into the African "meningitis belt" in 2010 for subjects between 1-29 years of age; this vaccine is being implemented in these regions with the goal of protecting the entire population at risk by the year 2016 [18].

Considering the high incidence of MenB disease in developed countries, the production of a vaccine effective against this serotype was a priority. However, vaccines against MenB were difficult to produce. Because the external polysaccharide capsule of MenB resembles the adhesion molecules on the surface of neural cells, conjugate vaccines were not protective against MenB disease and moreover could induce an autoimmune response [5, 20]. The first vaccine that showed a partial efficacy against MenB disease was produced in Cuba, New Zealand and Norway, but it was strain specific and therefore was used only to control the epidemics [5]. For these reasons, this vaccine could not be considered effective against the different MenB strains that cause epidemics worldwide. After several attempts, a multicomponent MenB vaccine (4CMenB, Bexsero) that covered different strains was produced using reverse vaccinology [5, 21]. 4CMenB was composed of four components: the first component was the factor H binding protein (fHbp) fused with the GNA 2091 protein, the second was Neisseria adhesion A (NadA), the third was the Neisseria heparin binding protein (NHba) fused with the GNA 1030 protein, and the forth component was OMV NZ98/254, which has several antigen components (the major component is PorA). The three antigens fHbp, NadA and NHba evoke serum bactericidal antibodies, while the two antigens GNA 2091 and GNA 1030 improve the immunogenicity of the major antigens when fused with them [5, 20]. In January 2013, 4CMenB was licensed in the European Union and thereafter in Australia and Canada for use in subjects older than 2 months of age [5, 20]. Considering the low incidence of IMD, it is quite difficult to evaluate the impact of any meningococcal vaccine through randomized controlled clinical studies. Although serological methods could be used to evaluate the protection of other meningococcal vaccines, these methods could not be used for 4CMenB due to the high number of genetically different MenB strains that cause IMD. To overcome this problem, the vaccine's manufacturer used a new method called the meningococcal antigen typing system (MATS) that uses a unique vaccine antigen-specific ELISA capable of detecting qualitative and quantitative differences in the fHbp, NHba, and NadA antigens; then, the results are combined with PorA typing information [5]. The results from the analysis of 1,052 MenB strains isolated from different countries in Europe showed that 4CMenB could protect against 68%-88% of MenB strains [5]. A bivalent fHbp recombinant vaccine (also known as LP2086; Trumenba) has been developed since 2006 and has now been approved by the US Food and Drug Administration for use in 10- to 25-year olds [26]. This vaccine appeared to be safe in a phase 3 study in approximately 5,600 healthy individuals aged 10 through 25 years and was immunogenic and safe when co-administered with routine meningococcal A, C, Y, and W and tetanus, diphtheria and pertussis (Tdap) vaccines in a phase 2 study in more than 2,600 healthy individuals aged 10 through 12 years [26]. Studies on this vaccine are ongoing in Europe, and approval from the European Medicines Agency is expected in 2017.

In the future, optimal preventive strategies necessitate a unique vaccine against MenA, B, C, W, and Y. A recent phase 2 study assessed the immunogenicity, safety and reactogenicity in healthy adolescents of an investigational formulation of a meningococcal ABCWY vaccine consisting of recombinant proteins (rMenB) and OMV components of a licensed serogroup B vaccine combined with components of a licensed quadrivalent meningococcal conjugate vaccine (MenACWY-CRM) [23]. The authors showed that the investigational MenABCWY formulation containing OMV components elicited a high response against all strains with an acceptable safety profile [23].

Due to the emergence of MenX in Africa in the last decades, it is extremely important to develop a new vaccine that is effective against this serotype. No licensed vaccine

is available at present, but interesting data were reported in a recent study conducted in mice that evaluated the immunogenicity of the MenX outer membrane vesicles (X-OMV) or MenX polysaccharide (X-PS) combined with a bivalent AW-OMV vaccine previously demonstrated to be immunogenic in mice [24]. The authors demonstrated that a high antibody response was induced after two doses of X-OMV alone or combined with AW-OMV. In contrast, X-PS was not immunogenic alone but was immunogenic in combination with AW-OMV.

Conclusions

IMD represents a severe and life-threatening problem for the worldwide community. Early recognition of children with meningococcal infection is mandatory in order to immediately start systemic antibiotic therapy and avoid death or long-term sequelae. Vaccination represents the best strategy to prevent meningococcal disease. Recently, the introduction of new conjugate vaccines that are effective in very young children has significantly reduced the incidence of IMD in many countries. Moreover, the availability of new vaccines against MenB will permit a further increase in preventive possibilities against IMD. The goal of the next few years should be to increase vaccination coverage against meningococcal diseases, continue to monitor IMD and develop a unique vaccine able to cover all of the main meningococcal strains.

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REVIEW

Meningococcal serogroup B vaccine in Italy: state-of-art, organizational aspects and perspectives

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Key words

Meningococcal serogroup B vaccine • Meningococcal diseases • Regional immunization schedules

Summary

Neisseria meningitidis causes severe invasive meningococcal diseases (IMDs) in humans including meningitis and septicemia, responsible for serious clinical conditions and leading to life-long disabilities and death. Serogroup B dominates IMDs burden in Italy, accounting for over 60% of total cases. On January 2013 the European Medicine Agency (EMA) licensed the first serogroup B meningococcal (MenB) vaccine in Europe. A number of European countries and Regions have introduced the new MenB vaccine in their immunization schedule, including Italy. In this paper we present the state of art, related critical issues and future perspectives of MenB vaccine introduction in Italy, in the context of the most recent available epidemiological data. In particular, we systematically assess the ongoing processes in the 8 Italian regions and one autonomous province that have already introduced MenB vaccine. With the new 2014-2018 National Vaccine Prevention Plan including active MenB vaccine offer about to be adopted, it is of fundamental importance to gather further evidence on MenB vaccine clinical effectiveness, duration of protection and cost-effectiveness. Italian regions are called to organize and manage MenB immunization programs. Careful consideration will need to be devoted on timing, doses, and co-administration with other vaccines but also to economic assessments and strengthened communication to the general public. Our data will help to plan, implement and evaluate MenB immunization programmes in other Italian and international settings.

Background

Neisseria meningitidis (meningococcus) causes severe invasive meningococcal diseases (IMDs) in humans including meningitis and septicemia, responsible for serious clinical conditions and leading to life-long disabilities and death [1]. It is estimated that between 10% and 14% of cases of IMD are fatal, and that up to 30% of survivors suffer from long-term sequelae [2, 3]. There are 13 identified sereotypes of meningococcus, with six (A, B, C, X, W-135 and Y) being responsible for over 90% of severe meningitis and septicemia cases [4]. The distribution of meningococcus sereotypes is setting–specific. Serogroup A is mainly distributed in the Sub-Saharan African countries of the *meningitis belt* where it causes around 85% of IMDs. Serogroup X, previously a rare cause of sporadic meningitis, has been responsible for outbreaks between 2006 and 2010 in the African region [5, 6]. The meningitis belt is the area where the highest burden of IMDs occurs with 14317 cases and 1304 deaths reported in 2014 [7].

In Europe, the USA and other industrialised regions, serogroups B and C are the major cause of IMDs [8]. In Europe good surveillance data is available in most countries and implementation of meningococcal immunization programs have largely contributed to decreasing endemic rates [9]. The most recent available surveillance data refers to 2012 with 3,463 reported IMDs, this corresponding to an incidence of 0.68 cases per 100,000 population, higher in children under one year of age (11.4/100,000) and between one and four years of age (3.7/100,000). The overall case fatality rate (CFR) was 7.9% and meningitis was the clinical presentation in 43% of cases [8].

Meningococcal disease is a vaccine preventable disease. There are several registered vaccines: a meningococcal A conjugate vaccine, C conjugate vaccines, tetravalent A, C, Y and W conjugate vaccines and meningococcal polysaccharide vaccines [10]. In Europe, serogroup C conjugate vaccination (MCC) implementation has had a major impact on the declining incidence of serogroup C meningitis [11]. In 2012, serogroups B and C were responsible for, respectively, 68% and 17% of confirmed IMDs cases in the EU [8]. Since the introduction of serogroup C conjugate vaccination (MCC) Meningococcus B had emerged as a relatively important cause of IMD in Europe, this due to the lack of preventative measures for this serogroup [12]. On 14 January 2013 the European Medicine Agency (EMA) licensed the first serogroup B meningococcal vaccine in Europe. A number of European countries and Regions have introduced the new MenB vaccine in their immunization schedule, including Italy. In the United States the first Meningococcal Group B vaccine was licensed by the Food and Drug administration in 2014 Vaccine [13].

General aim of the present study is to describe the introduction of the MenB vaccine in Italy, presenting the state of art, related critical issues and future perspec-

tives. In particular, specific objectives are: i) to describe the technical documents, decisions and polices taken at the national level and ii) to systematically assess the ongoing processes in the 8 Italian regions and one autonomous province that have already introduced the MenB vaccine in the Regional immunization schedules.

The new Meningococcal B vaccine

As introduced above, a new vaccine for the prevention of serogroup B meningococcal (MenB) disease is now available in Europe (4CMenB) [14, 15]. In the past, the antigenic diversity of the meningococcal surface proteins had been the main limitation in the design of broadly protective meningococcal vaccines, as well as the fact that the polysaccharide of serogroup B fails to stimulate the production of antibodies (phenomena of immune tolerance) MenB vaccine was developed through the 'reverse vaccinology' technique, which has been applied for the first time to develop the MenB vaccine [16] and allows the production of vaccines from genome-derived antigens. In fact, it applies bioinformatic tools to comprehensively screening of pathogens' genome data for surface-expressed proteins, in order to select candidate vaccine antigens. Proteins likely to be used as vaccine antigens are identified and further tested for immunogenicity on animal models [17]. In the case of MenB, the genome sequence of the virulent MenB strain MC58 was analysed (i.e The MenB genome was sequenced). Nearly 600 open reading frames were selected; from these, 350 candidate antigens were expressed in *Escherichia coli*, purified and used to immunize mice. Subsequent screening of the mice sera revealed 91 surface-exposed proteins that induced bactericidal antibodies in vivo [18]. This step-by-step elimination, based on the ability to induce broad protection in infant rat or mouse models, led to the identification of the antigens now included in the 4CMenB vaccine formulation. In other words, the reverse vaccinology strategy identified a set of proteins that had the characteristic for being effective vaccines' antigens: accessible to the immune system, immunogenic, inducing a protective response, present in all strains, and with minimal sequence variation [18, 19].
The 4CMenB contains the following four components [19]:

- The Neisserial adhesion protein (NadA);
- The Neisseria Heparin Binding Antigen (NHBA) fused with the Neisserial Antigens GNA1030;
- The factor H binding protein (fHbp) fused with the Neisserial Antigens GNA2091;
- Outer membrane vesicles (OMV)

4CMenB is indicated for active immunisation of individuals from 2 months of age and older, against invasive meningococcal disease caused by Neisseria meningitidis group B. It was licensed in Europe (European Medicine Agency - EMA, 2013) [20], in Australia (Therapeutic Goods Administration - TGA, 2013), in USA (Food and Drug Administration - FDA, 2015) in Canada (2013) and Chile [14, 15]. In the United States, also another MenB vaccine was licensed by FDA in 2014 (rLP2086 vaccine) [21].
Clinical trials have been carried out in the context of the vaccine registration process and some data are available on 4CMenB immunogenicity and safety. No studies have been conducted so far to test clinical efficacy and vaccine efficacy has been inferred by demonstrating the induction of serum bactericidal antibody response to vaccine antigens [20, 22-25].

MenB vaccine introduction in other countries: an update

As the new MenB vaccine has been licensed in several countries and scientific evidence is accumulating on its efficacy and safety, a number of countries are in the process of evaluating the introduction of MenB vaccine in their immunization schedule (Tab. I).
To our knowledge, the United Kingdom is the only country where universal 4CMenB vaccination has been recommended. The Joint Committee on Vaccination and Immunization (JCVI) published a document in March 2014 recommending to offer to offer the MenB vaccine to children at 2, 4 and 12 months (2+1 doses schedule). As the JCVI states, this recommendation depends on securing a cost-effective price for the vaccine [26].
In France, Germany, Spain, the USA as well as the UK, MenB vaccine is recommended on an individual basis to high-risk subjects and during outbreaks [26-29].
Other countries including Canada, Ireland and Belgium National Immunization Committees have not yet produced recommendations and are waiting to gather relevant epidemiological and economic data to support their decisions.
National Immunization Committees in Spain and Germany have recently concluded that there is not enough available data on vaccine efficacy and economic analysis to support the introduction of universal MenB immunization in national immunization schedules, this also considering that MenB meningitis incidence is decreasing in those countries [28, 29].
Setting-specific predictive models on the epidemiological impact of MenB vaccine introduction and cost-effectives analysis have been carried out in France, this model allowed to evaluate different vaccine strategies [27].
In decentralized health systems – as we will outline for Italy – some regions but not others have introduced MenB vaccine in their immunization schedule, including Saxony in Germany and Quebec in Canada [30].

The Italian setting

Epidemiology of Meningococcal diseases in Italy

To describe IMDs epidemiology in Italy, two sources of data are used: i) the National Surveillance System of Invasive Bacterial Diseases (MIB), coordinated by the

Tab. I. Recommendations and positions of selected European countries on MenB Vaccine.

Country	Reccomendations	MenBIMDIncidence (by age of group)	Country	Reccomendations	MenBIMDIncidence (by age of group)
Belgium	Currently not reccomended. The Superior Health Council is currently assessing the available evidente on the topic	Relative to 2011	Total: 0.8/100,000	<1 year: 10.8/100,000	1-4 years: 4.3/100,000
France	Not routinely recommended for children and adolescente. Recommended for high-risk subjects and durino outbreaks	Relative to 2011	Total: 0.6/100,000	<1 year: 8.4/100,000	1-4 years: 2.8/100,000
Germany	Currently not recommended. It may be recommended for people at increased risk of IMD, but the decision to vaccinate shold be based on individual considerations of risk/benefit	Relative to 2012	Total: 0.3/100,000	<1 year: 5.9/100,000	1-4 years: 1.7/100,000
Ireland	Ongoing evaluation. Cost effectiveness study in progress	Relative to 2012	Total: 1.3/100,000	<1 year: 23.5/100,000	1-4 years: 17.9/100,000
Portugal	Assessment in progress	Relative to 2011	Total: 0.3/100,000	<1 year: 17.8/100,000	1-4 years: 2.7/100,000
United Kindgdom	Reccommended conditional on the vaccine being available at low cost. the vaccine should also be offered to the same high-risk groups who are offered the ACWY vaccine.	Relative to 2011	Total: 1.3/100,000	<1 year: 25.3/100,000	1-4 years: 8.6/100,000
Spain	Currently not recommended. Health authorities may consider whether to use the vaccine in case of outbreaks and for immunocompromised patients.	Relative to 2011	Total: 0.7/100,000	<1 year: 13.1/100,000	1-4 years: 4.4/100,000
Canada	Currently not recommended	Relative to 2012	Total: 0.23/100,000	<1 year: 6.2/100,000	1-4 years: 0.4 to 1.4/100,000
United States	Currently it is recommended for individuale identified as being at greater risk of contracting IMD durino outbreaks	Relative to 2012	Total: 0.06/100,000	<1 year: 1.24/100,000	1-4 years: 013/100,000

Translated and adapted from: Istituto Superiore di Sanità (ISS): *Vaccinazione anti-meningococco B: dati ed evidenze disponibili per l'introduzione in nuovi nati e adolescenti.* 2015 [33].

Italian Institute of Health [31] and ii) the National hospital discharge records administrative register (SDO).
Surveillance IMDs data in Italy is available from the MIB system for the periods 1994-2006, 2007-2010 and 2011-2015 [32]. The most recent MIB data were updated in March 2015 [32]. In 2013 in Italy 172 cases of invasive meningococcal disease were reported, this corresponding to an incidence rate of 0.29 cases per 100,000, slightly higher as compared to previous years (0,23/100,000 in 2012 and 0,25/100,000 in 2011). IMD incidence in Italy is among the lowest in Europe [8].
IMDs' incidence is higher in the age group 0-4 years (1.75/100,000 in 2013) and in particular in the first year of life (4.01/100,000 in 2013), this mirroring IMDs epidemiology of most high-income countries. Nearly 50% of IMDs cases are sepsis or meningitis/sepsis, the percentage being higher children under 5 years of age (76% in 2013). A relatively stable trend is reported in 2011-2013 in all regions apart from 4 regions (Apulia, Lombardy, Marche, Tuscany) where number of cases slightly increased over the years and the Veneto region where it decreased. IMDs' Mortality data is derived from national mortality data and available for the period 2003-2010 where 122 IMDs deaths were reported [33].
In Italy Meningococcus B is the most common notified serogroup (46, 50 and 63% of the total of strains typed in 2011, 2012 and 2013), followed by meningococcus C (33, 17 and 17% of the strains typed in 2011, 2012 and 2013) and meningococcus Y (16, 17 and 13% of the strains typed in 2011, 2012 and 2013), this distribution remaining constant over the years. Of concern, the percentage of notified infection for which serogroup info

is not available, is high (33% in 2013) and increased as compared to 2011 (23%) and 2012 (21%). Although with fluctuations in incidence, serogroup B IMDs distribution over time (1994-2013) has been constantly greater as compared to serogroup C IMDs (except in the years 2004 and 2005), this being in line with the data from other European countries [8] from the literature, presents clear fluctuations of incidence over time. The number of cases of serogroup B between 1994 and 2012 has always been greater than the number of cases by serogroup C, except that in the years 2004 and 2005.
Comparing hospital discharge records data with National IMDs' surveillance system register allow to assess the latter's detection rate, which is estimated to slightly underestimate IMDs' burden in Italy [33]. In addition, the percentage of IMDs with no serotyping data, remains high at 20%. Furthermore, in 2013 the proportion of typified cases (67%) is lower than 2012 (79%) and 2011 (77%).
Combining data on IMD cases derived from hospital discharge records with the serogroup distribution reported by the IMD surveillance system, it is estimated that serogroup B IMD incidence in the period 2007-2012 was 0,23/100,000, higher in the first year of life (3.44/100,000 in 2013) and in the 1-4 years age group (1.07/100,000 in 2013). In particular, 133 serogroup B IMDs were reported in 2007, of which 43 in children <5 years and 19 in children <1 year. The average impact of serogroup B IMDs in Italy in 2007-2012 is estimated to be 5,194 DALY per year, with an average mortality impact of 4,817 years of life lost per year and average sequelae impact of 376 years with disabilities per year [33].

National-level immunization polices and guidance documents

In Italy, The National Vaccine Prevention Plan (PNPV) is the guidance document issued by the Ministry of Health that establishes immunization recommendations at the national level and sets national coverage targets with the overall aim of harmonizing immunization strategies among Italian regions. The 2012-2014 PNPV, published in February 2012 does not include recommendations on 4CmenB [34].
In 2014 it was published the second edition of the "Lifetime immunization schedule" [35], a joint proposal for an immunization schedule issued by the four Italian scientific societies, historically involved in the study of vaccines and vaccination policies, namely: the Italian Society of Hygiene, Preventive Medicine and Public Health (SItI), the Italian Society of Paediatrics (SIP), the Italian Federation of Family Paediatricians (FIMP), and the Italian Federation of General Practitioners (FIMMG). The 2014 "Lifetime immunization schedule" recommends the introduction of universal 4CmenB immunization with the 3+1 doses schedule at 3°-4°-6° months of age with a booster dose after 13 months of age. The "Lifetime immunization schedule" recommends also the use of MenB vaccine on an individual basis in at-risk subjects as specified in the vaccine's summary of product characteristics [20] and raises awareness on the importance of routinely vaccinating also adolescents against meningococcal disease [35].
While recommending the introduction of universal MenB immunization in Italy with a 3+1 doses schedule, the 2014 "Lifetime immunization schedule" leaves to regional health authorities the final decision on the best setting-specific immunization schedule to adopt. As for now the decision to include MenB vaccine in the immunization schedule is taken at the regional-level in a context where several regions are coping with deficit-reduction plans.

Introduction of Meningococcal B vaccine in Italian Regional immunization schedules

Currently there are three types of meningitis vaccine available in Italy: the meningococcal polysaccharide tetravalent (A, C, Y and W-135) vaccine, the meningococcal conjugate tetravalent (A, C, Y and W-135) vaccine and the Monovalent serogroup C conjugate vaccine. Since 4CmenB was licensed by EMA and became available in Italy, eight Italian regions and one autonomous province have introduced it in their regional immunization schedules as active immunization offer – free of charge for all children under one year of age. The eight regions are Apulia, Basilicata, Calabria, Friuli Venezia Giulia, Liguria, Sicily, Tuscany and Veneto [36]. Of them, five have implemented the 3+1 doses MenB immunization schedule and two the 2+1 doses one. The Autonomous Province of Bolzano has also implemented the 2+1 schedule [36, 37]. The MenB vaccine schedules in different Italian regions are schematized in Table II.
In particular, the region Apulia has been the first region to include the 3+1 doses universal MenB vaccine offer in the regional immunization schedule, legally formalized in January 2014, added to the updated edition of the regional lifetime immunization schedule and implemented starting with the 2014 birth cohort [38].
Similarly, the Basilicata and Tuscany regions adopted in February and October 2014, respectively, the 3+1 doses universal MenB vaccine [39-41] and implemented the new immunization programme starting from the 2014 birth cohort. More recently, in January 2015, the regions Sicily and Liguria adopted the 2014 "Lifetime immunization schedule" and introduced the MenB vaccine within the regional immunization schedules [42-44].
Two regions, Veneto and Friuli Venezia Giulia have introduced MenB vaccine starting with the 2015 birth cohort and recommending the 2+1 doses schedule [45-47].
The Autonomous Province of Bolzano has introduced the MenB vaccine in the immunization schedule with the 2+1 dose approach for subjects between 3 and 15 months of age [36, 37].
Also Calabria recently decided to introduce the vaccine against meningococcus B: since May 2015 the vaccine was included in the vaccination schedule with the scheme 3 + 1 doses, but timing of the third dose it is not clearly specified [48].

Tab. II. MenB Vaccine schedules implemented in different Italian regions [36-48].

Region	3° Month	4° Month	5° Month	6° Month	7° Month	8° Month	9° Month	13° Month	14° Month	15° Month
	SCHEDULE 2 DOSES+ 1 BOOSTER									
Autonomous Province of Bolzano	Still to be decided (2 doses+1 booster)									
Friuli Venezia Giulia					1° MenB dose		2° MenB dose			MenB booster dose
Veneto					1° MenB dose		2° MenB dose			MenB booster dose
	SCHEDULE 3 DOSES + 1 BOOSTER									
Apulia	1° MenB dose 76° day (after 15 days from the adminis-tratiin of hexavalent + PCV13)	2° MenB dose 106° day (afrte 1 month from the 1° dose of MenB vaccine)		3° MenB dose 151° day (after month from the 2° dose of MenB vac-cine)						MenB booster dose (in co-ad-minis-tration with MenC)
Basilicata	1° MenB dose 75°-90° day		2° MenB dose 135°-150° day		3° MenB dose 181°-210° day				MenB booster dose after the 13° month	
Calabria	1° MenB dose 76° day	2° MenB dose 106° day		3° MenB dose						MenB booster dose
Liguria	1° MenB dose 76° day	2° MenB dose 106° day		3° MenB dose 151° day						MenB booster dose
Sicily		1° MenB dose (after 1 month from the admin-istration of hexa-valent, PCV13 and Rota)		2° MenB dose (after 1 month from the admin-istration of hexa-valent, PCV13 and Rota	3° MenB dose at 7° or 8° month (after 1 month from the adminis-tration of 2° MenB dose)	3° MenB dose at 7° or 8° month (after 1 month from the adminis-tration of 2° MenB dose)				MenB booste dose at 15°-18° month (after 1 month from the admin-istra-tion of MRRV)
Tuscany	1° MenB dose 76° day (15 day after the adminis-tration of hexavalent + pneu-mo)	2° MenB dose 106° day (1 month after the 1° MenB vaccine)		3° MenB dose 151° day (after 1 month from the 2 dose of hexa-valent + pneumo)				MenB booster dose		

Data derived from regional immunization schedules [36-48]

Discussion

The introduction of MenB vaccine in Italian regional immunization schedules rises several issues around: 1) the organization and management of immunization programmes; 2) health education and communication to the general population and, in particular, parents; 3) economic sustainability in a context of deprived resources for the national health system.

Organization and management of immunization programmes

In the 2012-2014 National Vaccine Prevention Plan (PNPV) some general principles are outlined: a) the need of concentrating as much as possible the number of immunization sessions; b) the importance of avoiding that vaccines' co-administrations increase the risk of adverse effects; c) the urge of avoiding more than 2 injections in the same immunization session and d) the importance of guarantying the economic sustainability of immunization offers [49]. In this context, it is easy to understand the organisational problems related to the introduction of the MenB vaccine in children's immunization schedule. In fact, not only it requires to perform 4 doses in a limited and early-in-life period (3-4 doses during the first year of age), but also there is some evidence that MenB co-administration with other vaccines increase the risk of moderate to high fever [20].

As mentioned, the 2014 "Lifetime immunization schedule" recommends the introduction of universal 4CmenB immunization with the 3+1 doses schedule at 3°-4°-6° months of age with a booster dose after 13 months of age [35]. This schedule, although has the unavoidable disadvantage of adding three additional immunization sessions in the first year of age, has the following advantages [35]:

- administration of the first 3 doses in a short time;
- no changes to the current schedule for the other children routine vaccinations;
- administration of no more than two vaccines in a single session, minimizing the possibility of adverse events (fever);
- it allows to separately monitor any adverse events of the new vaccine;
- it makes it easier for parents to remember the next appointment.

When formulating a vaccine schedules some factors are to be taken into consideration, this including the age-specific distribution of diseases, age group with the highest disease incidence, the disease's clinical symptoms and complications, the vaccine's indicated doses and duration of protection [31]. In light of this reasoning the 2014 "Lifetime immunization schedule" also presents the MenB 2+1 doses immunization schedule with vaccine administration at 7°-9° months of age with a booster dose at two years of age [35]. The 2+1 doses immunization schedule had the advantage – as compared to the 3+1 doses immunization schedule – of adding only two additional vaccine sessions in the first year of age. However, if the 2+1 schedule is not combined with the effect of herd immunity and – ultimately – decreasing MenB incidence, it would fail to prevent IMDs in the first months of age which is when the highest burden of IMDs is concentrated [35].

Health education and communication

Challenges related to motivating parents to have their children vaccinated against MenB is a threat to the success of MenB immunization programmes. Low compliance to 4CMenB vaccination by parents might be associated on one hand by the introduction of additional immunization sessions in the first year of age and on the other by lack of information [50] around the new MenB vaccine. The Italian Center for Social Studies and Policies (CENSIS) has recently conducted a survey on a representative sample of 1,100 Italian parents (550 mothers and 550 fathers aged 22 to 55 years) with children from 0 to 12 years (target population for MenB vaccine) to assess parents' knowledge and attitudes towards IMDs and available vaccines, in particular MenB vaccine [51]. As emerges from the survey, 85.5% of parents report to be aware of meningococcal disease; among these 30% consider themselves to be adequately informed, especially parents with an high level of education. The main sources of information about meningitis and meningococcal vaccination are paediatricians (48.8% and 33.9%, respectively). However, 33% of respondents report not to have received proper health education on the topic. In addition, although one third of parents consider to have accessed to all needed information, almost half (45%) wants to be more informed. The majority of respondents (95%) believe that immunization campaigns targeting the general populations are necessary to educate and raise awareness on the benefits of MenB vaccination and to allow parents to take informed decisions. When specifically focusing on the new MenB vaccine, almost 70% of parents is not aware of its existence, this percentage being higher among parents with lower education. When asked about MenB vaccine population target, 23% report not to know it. Of crucial importance, half of the respondents do not know if MenB vaccine is available in their region of residence. More than half of the parents report their intention to vaccinate their children against meningococcus B, and if we add to this percentage 37.6% being 'undecided', the percentage of Italian parents not opposed to this new vaccine rises to about 90% [51].

Economic issues

The introduction of new vaccines needs to follow transparent criteria of efficacy, safety, economic sustainability and public health prioritization [52]. Immunization schedules are proposed by experts in the field of clinical medicine, epidemiology and public health on the basis of the available scientific evidence and are then implemented by policy makers also taking into consideration resources allocation and financial sustainability [53]. In context of deprived resources for the Nation Health system, economic sustainability of new immunization programmes should be carefully assessed. Taking into

consideration both the fact that serogroup B IMD incidence is relatively low and that 4CmenB does not protect against all circulating strains (around 87% [33], the estimated cost per IMD case prevented is very high [54]. This consideration has stimulated a lively debate in the scientific community and among health authorities. As for now, Italian setting-specific cost-effectiveness and cost-benefit analysis on the introduction on 4CMenB are still scant [55].

Conclusions

The new National Vaccine Prevention Plan (PNPV) has been drafted in close consultation with Italian scientific societies and is about to be approved. It will likely include MenB immunization with an offer active and free of charge. This is a relevant step towards a comprehensive immunization offer and a significant sign at the national level. The implementation of MenB immunization programmes, the organizational details such as the calling methods and the organization of the vaccination services are still to be discussed and will be planned at the level of individual regions considering the overall resources needed, the workforce of health services and the availability of different healthcare professionals groups (family paediatricians).

Compared to other vaccine-preventable diseases, the IMDs incidence in Italy is low; however, IMDs are associated with a high lethality rate and high risk of complications. Serogroup B accounts for the vast majority of meningococcal infections in Italy 4CMenB vaccine has good immunogenicity profile against invasive meningococcal disease B. Further evidence are needed and are currently being collected on the vaccine clinical effectiveness, duration of protection and cost-effectiveness. 4CMenB is under additional monitoring for the next five years and this will allow to collect detailed data on suspected adverse reactions. We present updated data on the eight Italian regions that have already implemented 4CMenB for the 2014 and 2015 birth cohorts taking into consideration IMDs burden in Italy. Our data will help to plan, implement and evaluate 4CMenB immunization programmes in other Italian setting. This will require careful consideration on timing, doses, and co-administration with other vaccines but also further economic assessments and strengthened efforts by institutions and scientific societies to promote health education and good communications among the population [56-59].

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REVIEW

Meningococcal B vaccination strategies and their practical application in Italy

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Key words

Meningococcal B vaccine • Vaccination • Italy

Summary

Immunisation against meningococcal meningitis has a long history, which has passed through several phases: the studies by Flexner, extraction of the polysaccharide capsule, the development of monovalent and multivalent conjugate vaccines, the outer membrane vesicle vaccines up to the development of effective and safe vaccines for meningococcal B invasive disease through the application of the techniques of molecular biology and reverse vaccinology.

The new available vaccines are Bexsero® and Trumenba®. Bexsero® has been approved and is available in Europe, the USA, Canada, Australia and Chile, and is currently under review in Brazil for the prevention of MenB invasive disease in subjects ≥ 2 months.

Trumemba® is currently approved only in the USA, for use in adolescents and young adults.

At present, the greatest obstacle to the extensive use of these vaccines in industrialised countries is the high cost and the need administer multiple doses in infants. However, in some European countries and in some Italian Regions, strategies (free and active call) to fight the disease through vaccination (Bexsero®) are already in place.

Introduction

Conjugate vaccines against *Haemophilus influentiae* type b, *Streptococcus pneumoniae* and *Meningococcus* C have dramatically reduced cases of bacterial meningitis in the industrialised nations. However, meningitis type B continues to be a threat to children and adolescents worldwide.

Unlike other serogroups, *Neisseria meningitidis* B (MenB) disease cannot be prevented by polysaccharide vaccines. The reason for this lies in the chemical structure of the MenB capsule, which contains units are identical to some human polysaccharides (human foetal neural cells) and, therefore, determine immunological tolerance [1]. Consequently, research into an effective MenB vaccine has focused on subcapsular antigens, outer membrane vesicles (OMVs). OMVs were successfully used to control specific outbreaks. OMVs are proteoliposomes that contain several different molecular components out of which the porin protein, PorA, is the principle antigenic source of bactericidal antibodies. The limitations of these vaccines are that effectiveness tends to be limited to strains containing the same PorA protein (serosubtype-specific), limiting its use to strain-specific outbreaks and they often elicited a scant immune response in young children [2].

In the last years, the vaccine industry has overcome this difficulty, and a MenB multicomponent vaccine – 4CMenB – Bexsero® – has been developed. This vaccine has been licensed in Europe and other developed countries for the prevention of MenB invasive disease in subjects ≥2 months. In addition, a vaccine containing two variants of factor H binding protein (fhbp) of the complement has been approved by the Food and Drug Administration in the USA (Trumemba®) for use in individuals 10 through 25 years of age.

These advances have prompted some authors to wonder whether we are witnessing "the beginning of the end for invasive MenB disease" [1].

In order to eliminate an infectious disease, the first essential requirement is undoubtedly the availability of safe and effective vaccines. However, strategic planning of the application of the vaccines which have become available is equally important.

This overview examines the policies of vaccination with new meningococcal B vaccines, particularly 4CMenB, in Italy.

Natural history of MenB infections

Meningococci have their natural and unique survival niche in humans. This fundamental biological fact implies that *N. meningitis* has acquired several mechanisms for cohabitation with the human organism [3]. Only in particular conditions of frailty of the human host or in certain environmental situations is the microorganism able to manifest its aggressiveness, leading to meningococcal diseases and even death [4, 5].

From sero-epidemiological studies [6, 7] we have learnt much about the biology of this microorganism. In the blood of infants, bactericidal antibodies against Neisseriae are present as a result of the passage of maternal antibodies through the placenta during pregnancy. However, as this protection wanes early, infants are soon exposed to the risk of infection. Indeed, the percentage of infants and children who show bactericidal antibodies grows until the age of about four years. Subsequently, antibody titres decline until adolescence, before increasing again. This pattern is in line with the fact that the two main peaks of morbidity of the disease are seen in subjects under 4 years of age (particularly in infants under 1 year) and in young adults. These epidemiological observations are of primary importance in understanding the spread of the microorganism in the environment around the infant and adolescent. The greatest risk to the infant is engendered by premature contact with virulent strains of *N. meningitidis*, as has been shown by mathematical modelling [8]. Indeed, the risk of MenB invasive disease has been calculated to be 400 times higher in such cases than in the case of non-early contact.
The situation changes during adolescence, when a more promiscuous social life (kissing, sexual contact, frequenting recreational premises such as pubs, etc) exposes subjects to more frequent contact with the microorganism. Indeed, it is precisely in these subjects that *Neisseriae* find their ideal niches for survival, as demonstrated by studies on carriers [9, 10].

Epidemiology of MenB infections

The distribution of the various serogroups of meningococcal pathogens fluctuates considerable. However, serogroup B currently predominates over the other serogroups in Europe, Australia, Canada and Japan. One reason for this predominance is attributable to extensive vaccination with conjugate vaccine against meningococcal serogroup C [11-16]. Of a total of 3463 confirmed cases of invasive meningococcal disease (IMD) reported in 28 EU/EEA countries in 2012, 2078 were caused by serogroup B; this predominance of serogroup B was most pronounced in infants (83% of cases, 8.9 per 100,000) and 1-4-year-olds (9% of cases, 2.9 per 100,000) [17].
The Italian surveillance system of invasive bacterial infections detected 991 cases of invasive meningococcal disease from 2007 to 2012, with an average of 165 cases per year. Information on typing is available for 764/991 cases (77.1%). Serogroup B was the most frequent (455 cases), constituting 59.6% of the cases typed, followed by serogroup C (220 cases) and serogroup Y (59 cases). During the reporting period, a decrease was observed in cases of meningococcal B (from 81 cases in 2007 to 52 in 2012) and C (from 43 cases in 2007 to 34 in 2012), while cases of serogroup Y gradually increased (from 3 in 2007 to 17 in 2012). Furthermore, on analysing the distribution of serogroups by age-group, it was observed that serogroup B was the most frequently isolated in the younger age-groups. Indeed, considering the 762 cases for which age information was available, serogroup B accounted for 81.1% (77/95) of all cases occurring in the first year of life, 66.2% (92/139) of cases in 1-4-year-olds and 70.1% (54/77) in 5-9-year-olds; in the other age-groups it accounted for about 50% of cases [18]. However, as demonstrated by Azzari et al. [19] by means of real-time PCR, in those countries (such as in Italy) [20] where only positive-isolate samples are counted as meningococcal cases, the incidence is largely underestimated. Furthermore, it is well known that culture-based methods have even lower sensitivity than molecular methods when the patient has been treated with antibiotics [21]. In addition, Azzari et al. found in their study that the case fatality rate was 13.2%, which is higher than the 5% rate recently reported in MenB in patients of any age [19, 22].

History of meningococcal vaccines against MenB

In the early 1900s, several attempts at using inactivated vaccines containing whole bacterial cells were made [23-27]. However, both these studies and subsequent clinical trials revealed that whole inactivated vaccines were excessively reactogenic. Later vaccines obtained from meningococcal culture filtrate also yielded contradictory results [28, 29]. The first successful meningococcal vaccines were obtained as a result of studies by Gotschlich et al., who were able to extract and purify high-molecular-weight meningococcal polysaccharides at the Walter Reed Army Institute [30, 31]. However, unlike the polysaccharides of serogroups A and C, the polysaccharide of serogroup B did not raise the production of antibodies on account of phenomena of immune tolerance. Thus, studies to prepare a vaccine against meningococcal B shifted to subcapsular antigens. The first and simplest approach was to use the meningococcal outer membrane vesicles containing membrane proteins (OMVs) and lipopolysaccharide (LPS). Four different formulations of these vaccines were used in Cuba, Chile, Norway, New Zealand and France [32].
However, as subcapsular proteins are very variable, these vaccines proved to be of limited use in containing clonal epidemics. Therefore, in order to identify antigens for development of universal MenB vaccine, studies were oriented towards the determination of the entire genome of a pathogenic strain of *N. meningitidis* type B (MC 58 strain) [33]. Thus, thanks to remarkable advances in bioinformatics and molecular biology, along with the knowledge acquired over the entire genome of MenB, a new science was born – "reverse vaccinology". Indeed, the computer-assisted screening of the genome of the microorganism enabled the proteins that were the best candidates for a vaccine against MenB to be identified [34]. It was thus possible to identify about 600 open reading frames that were believed to express surface or exported proteins of MenB. Starting from these 600 proteins, it was possible to express 350 in *E. coli*, which, after being purified, were able to elicit bactericidal an-

tibodies in mice. Finally, through successive studies on rat and mouse models, the best components were found: Neisserial heparin binding antigen (NHBA), factor H binding protein (fHbp), Neisseria adhesion A (NadA) [32]. The new universal MenB vaccine (4CMenB – Bexsero®) also contains the OMVs of the New Zealand strain NZ98/254.
Contemporarily, other researchers developed another new-generation MenB vaccine (rLP2086 – Trumemba®), a preparation containing two representative variants of subfamilies A and B of fHbp [35-39].
Another approach pursued by scientists in order to develop a vaccine for meningitis B was to improve vaccines containing antigens of the outer membrane. This approach is based on the ability of genetically modified MenB to express different subtypes of porin A. The latest development of this vaccine contains 9 subtypes of PorA [40].

Availability of new MenB vaccines

Bexsero® has been approved and is available in Europe, the USA, Canada, Australia and Chile, and is currently under review in Brazil [41]. Trumemba® is currently approved only in the USA, for use in adolescents and young adults [42].

MenB vaccination policies

Vaccination strategies should be considered in terms of both collective prevention and individual prevention.
The natural history of meningococcal infections and invasive disease epidemiological trends clearly suggest that: a) it is necessary to protect infants as early as possible; b) it is important to vaccinate adolescents and young adults, who are a risk group, as they constitute the reservoir of the microorganism and can transmit the pathogen to infant siblings. Moreover, in the individual perspective, it is important to reach vulnerable subjects.
The best policy would be to vaccinate all subjects from 0 to 18 years of age through an extensive campaign, as suggested by the results achieved in the UK with the conjugate vaccine for meningococcus C [43]. When this is not economically sustainable, it is very important to study the conditions which regulate the spread of the disease. Mathematical models with simplified algorithms can provide the key to obtaining the maximum yield with the minimum of resources. The first question concerns how many subjects the sick person is able to infect [44]. Naturally, this will depend on the characteristics of diffusivity of the pathogen, the number of subjects with whom the patient comes into contact, the number of susceptible, partially susceptible or protected individuals, and the period of time during which the subject is able to spread the disease. It is logical to imagine that, if a large number of subjects are protected, for example through vaccination, the pathogen will have difficulty spreading in the healthy population. If the proportion of vaccinees is high enough, it may be assumed that the patient can infect only a small number of people; if this number falls below 1, there is hope that the disease can be eliminated, as it will be impossible for the microrganism to circulate among humans. The mathematical modellers call this proportion the critical percentage of vaccination coverage. The higher the critical percentage is, the harder it will be to eliminate the disease. Now, in the case of meningitis, this critical percentage is estimated to be not very high. Indeed, to calculate the critical percentage of coverage, it is necessary to know the value of the basic reproductive number (R_0), which has been estimated to be approximately 1.36-1.4 [45] for meningococcal type C. It may be even lower for meningococcal type B [46]. If, however, we imagine that the value of R_0 is between 1.26-1.4 for MenB, we can calculate [47] that the critical percentage of coverage ranges from 26.5 to 28.6%. Furthermore, during our recent study [9], we did not find the carriage state for meningococcus C among young adults in a setting where a coverage rate for the conjugate vaccine against *N. meningitidis* of serogroup C is 87% among children and 49% among adolescents [48].
It is also important to consider that herd immunity against *N. meningitis C* has been substantially achieved through vaccination with the conjugate vaccine. Indeed, Trotter et al. found that, although the protection provided by the vaccine is, theoretically limited in British infants immunised with conjugate vaccine this protection persisted over time. This was also in agreement with the decrease in meningococcal C carriers among young British adults after the 1999-2000 vaccination campaign [49, 50]. It could therefore be surmised that herd immunity can be effectively induced by MenB vaccine, too.
Recently, the World Health Organization (WHO) drew up some criteria for the introduction of a new vaccine. The basic criteria concern: disease burden, efficacy, safety and quality of the vaccine, comparison with other interventions against the disease, economic and financial issues, fiscal impact, financial sustainability, vaccine presentation, supply availability, and programmatic strength [51].
The guidelines defined by the WHO are in line with the criteria of Health Technology Assessment (HTA), which can obviously be applied to vaccines, too. Indeed, HTA is a method of multidisciplinary assessment that deals with analysing the technical, scientific, economic, ethical, legal, social and organizational issues arising from the application of new technologies [52]. Thus, in order to insert a new vaccine into the vaccination schedule (free and active offer by National Health Service), it is necessary to conduct an HTA study [53]. Indeed, vaccines are to be regarded as any other medical technology [52]. In HTA evaluations, cost-effectiveness studies assume great importance. In the specific case of Bexsero®, these have yielded contrasting results and there is still uncertainty as to whether MenB vaccination by means of the 4CmenB vaccine should be introduced in developed countries. Indeed, on evaluating the introduction of Bexsero® in England,

Christensen et al. concluded that vaccination would be cost-effective from the National Health Service (NHS) perspective at a cost of £9-£17 per dose [54]. Subsequently, however, after re-evaluating the cost-effectiveness of universal vaccination with Bexsero® in England could be cost-effective with a low vaccine price [55]. By contrast, the results of a study conducted in Italy by Capri et al. demonstrated the cost-effectiveness of vaccination at a cost of € 60 per dose, from the societal perspective [56]. However, a study conducted by Tirani et al. in Italy concluded that, from the NHS perspective, the immunisation programme was unlikely to be cost-effective [57].

We recently carried out a cost-effectiveness study of this issue (article submitted to Human Vaccines & Immunotherapeutics). Our results confirmed that, especially from the societal perspective, the vaccination of Italian infants is cost-effective; the study considered various scenarios and also took into account the fact that cases occurring in Italy are underestimated [19].

It is important to consider that economic studies on vaccinations can have some limitations and they often adopt conservative estimates as not considering the underestimation of cases of illness and considering a short-term duration of protection. It is certain that 4CmenB stimulates the immune memory as it is made from MenB surface proteins. Indeed, it is well known that the protein antigens are much more immunogenic in comparison with the polysaccharidic ones even if the latter are conjugated.

Fig. 1. Italian Regions where MenB vaccine is offer free vaccination (February 2015).

The offer of MenB vaccination against in Italy

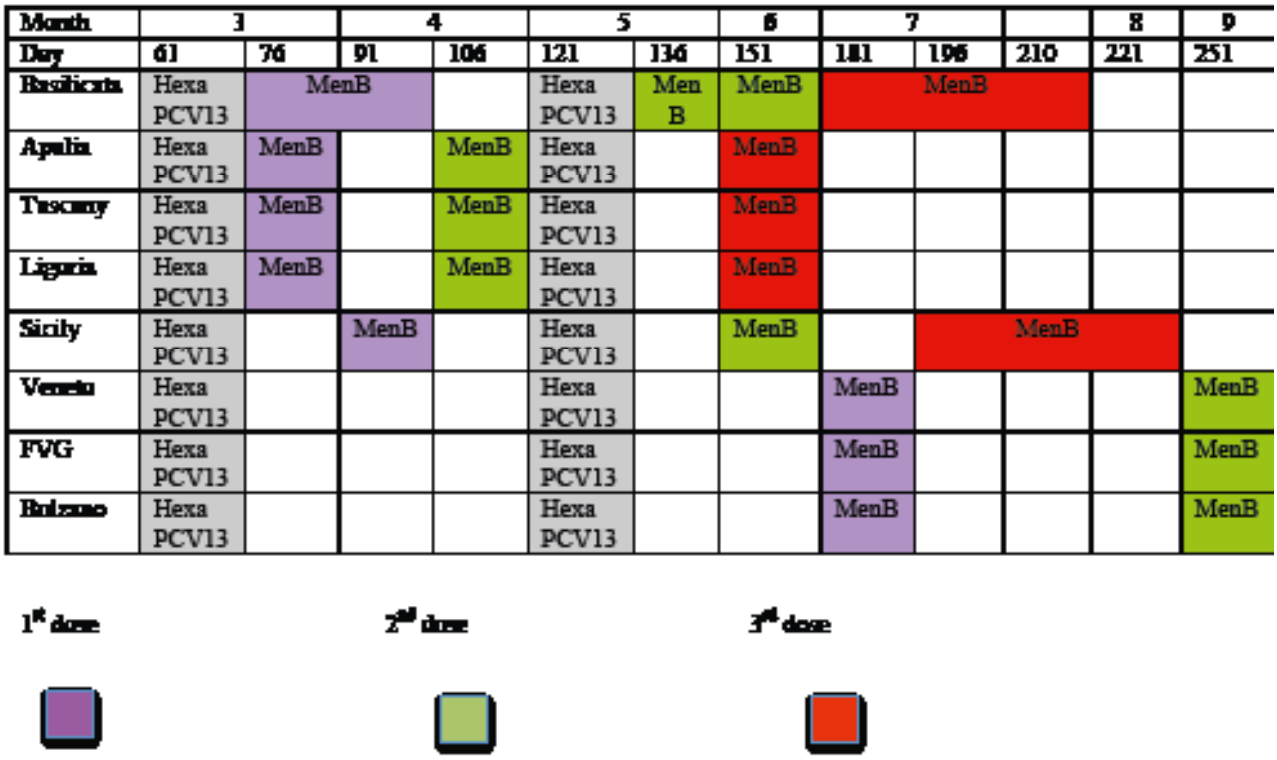
Eight Italian Regions and one Autonomous Province currently offer free vaccination for MenB to certain groups of people (Fig. 1). While Piedmont and Emilia Romagna offer it only to subjects at risk [58, 59], seven Regions and one autonomous Province offer it actively and free of charge for infants. Basilicata was the first Region to insert it into the childhood vaccination calendar [60]. Subsequently, Puglia, Veneto, Friuli Venezia Giulia, Tuscany, Liguria, Sicilia and the Autonomous Province of Bolzano included it in their vaccination schedules [61-67].

The above-mentioned seven Regions and the Autonomous Province of Bolzano, following the possible vaccine schedules (Tab. I), display slight variations in the age at which vaccination is administered. Indeed, as can be seen in figure 2, most of the Regions start vaccination early, as most cases of MenB invasive disease occur within the first year of life; only Veneto and Friuli Venezia Giulia schedule vaccination to begin at 7 months. With regard to the booster dose, most of the Regions schedule this at the 15th month, while Basilicata and Tuscany provide a booster at the13th month. For the moment, there is no plan to offer active and free vaccination for teenagers.

Tab. I. Authorized vaccination schedules of Bexsero® (Novartis Vaccines and Diagnostics Limited. Bexsero® Prescribing information. Document available at: http://www.bexsero.co.uk/healthcare-professional/pdfs/Bexsero%20PI.pdf. Accessed on 1st July 2015)

Age of administration	Primary immunisation	Time interval between doses	Booster dose
From 2 to 5 months	3 doses (0.5 ml)	At least 1 month	One dose from 12 to 23 months after primary immunisation
From 6 to 11 months	2 doses (0.5 ml)	At least 2 months	One dose in the 2nd year of life (at least 2 months after primary immunisation)
From 12 to 23 months	2 doses (0.5 ml)	At least 2 months	One dose (from 12 to 23 months after primary immunisation)
From 2 to 10 months	2 doses (0.5 ml)	At least 2 months	
From 11 months	2 doses (0.5 ml)	At least 2 months	

Fig. 2. Meningococcal B vaccination schedules of the Italian Regions that offer active, free vaccination for infants (primary immunisation).



Legend: Hexa: Hexavalent vaccine (diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B, and Haemophilus influentiae b); PCV13: Pneumococcal 13-valent conjugate vaccine; FVG: Friuli Venezia Giulia; MenB: Meningococcal B vaccine).

Conclusions

Strategically, infants constitute the first class of subjects to be vaccinated, and the Regions which offer free vaccination are rightly oriented in this direction. Indeed, the incidence rate of meningococcal meningitis in Italian infants under 1 year of age is 3.7 per 100,000, i.e. more than 10 times higher than the overall rate of invasive meningococcal diseases observed in Italy. Furthermore, serogroup B is more frequently detected among infants aged under 1 year, accounting for 65% of the total [68]. Moreover, both in order to better protect (indirectly) new-borns and to achieve the best herd immunity, it would be very useful to vaccinate young adults. It is likely that a similar multicohort strategy, even with relatively low coverage rates, could prevent the circulation of MenB.

Finally, the vaccination plans of the Italian Regions that offer vaccination for infants are appropriate to epidemiological reality, although Veneto and Friuli Venezia Giulia should bring forward the time of vaccination.

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REVIEW

Meningococcal B vaccine and the vision of a meningitis free world

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Key words

Meningococcal B vaccine • MATS • Cross protection

Summary

A century of traditional vaccinology lost the fight against meningococcus serogroup B (MenB). However, thanks to an innovative genome-based approach, the first broadly effective MenB vaccine, Bexsero® (GSK Vaccines), was developed and has been licensed for use in various age groups by the European Commission and other regulatory authorities. Genes encoding for the main meningococcus B antigens were identified and screened in order to achieve a broadly protective vaccine, taking into account the fact that meningococcus B has many different subtypes whose membrane proteins may be different. Since the antigens selected for Bexsero® are also harbored by meningococci belonging to other serogroups there may be the potential for Bexsero® to offer a certain level of protection against non-B serogroups. Therefore preliminary studies were carried out to investigate the potential of the vaccine to also provide a degree of cross protection against non-B serogroups. Here we review the potential for Bexsero® to offer a certain level of protection against the diversity of meningococcus type B subtypes and its potential ability to offer some cross protection from non-B serogroups. Lastly, we describe the future perspectives in pentavalent meningococcal vaccine (ABCWY) development which hopefully will result in a vaccine able to help prevent Invasive Meningococcal Diseases (IMD) from the majority of currently circulating meningococcal strains.

Introduction

Two centuries of meningococcal infection have 'pushed' the scientific and medical community to search for vaccines in order to harness the ingenious and almost infinite and dynamic survival/infective strategies of meningococci: from live-attenuated vaccines (1900) to subunit polysaccharide vaccines (1970) to glyconjugated vaccines (1990) to OMV vaccines, to quadrivalent vaccines ACWY (2003) and most recently to reverse vaccinology and the licensure of Bexsero® in the EU for individuals above the age of 2 months on 14 January 2013.

At the beginning of the twentieth century, the majority of bacterial meningitis in children was caused by *Haemophilus influenzae* type b, pneumococcus, and meningococcus. Available vaccines for the first two bacteria have led to a dramatic incidence reduction of the disease leaving *N. meningitidis* as the major cause of bacterial meningitis worldwide [1-3].

Considering the panel of meningococcal vaccines now available, the prevention of serogroup B-related IMD placed unique challenges in the development of a MenB vaccine: firstly, the inability to use the Men B capsule because its structure resembles a self-antigen and secondly the high variability of the antigenic membrane protein mix [3, 4].

Glycoconjugate vaccines against serogroups A, C, W, and Y exploit the antigenicity of the capsular polysaccharides that characterize each one of the four serogroups: however, for MenB this approach was not feasible due to the similarity of the capsule to a self-antigen; if used in a vaccine the capsular polysaccharide would be very poorly immunogenic [3].

An alternative approach was devised when Outer Membrane Vesicles (OMVs) were successfully used to control specific outbreaks. OMVs are proteoliposomes that contain several different molecular moieties, out of which the porin protein, PorA, is the principle antigenic source of bactericidal antibodies. The limitations of these vaccines are well recognized: effectiveness tends to be limited to strains containing the same PorA protein (serosubtype-specific), limiting its use to strain-specific outbreaks [5]. Thus, advancements toward MenB IMD protection using OMPs (Outer Membrane Proteins) were limited, since multivalent OMPs-based vaccines may not be effective in preventing the majority of endemic disease. Indeed data from Tondella et al. showed that a large number of serosubtypes (20 or more) might have to be included in a multivalent OMPs-based vaccine to target 80% of sporadic disease caused by meningococcus B in US [6].

Therefore, due to the inability to use either the capsule or the OMVs, MenB vaccine development was significantly impaired. The challenge was to develop a MenB vaccine demonstrated to have acceptable safety and immunogenicity profiles in all age groups, particularly in infants, who represent the major group at risk of IMD, and able to induce immunity against the majority of circulating serosubtypes [4]. An innovative genome based approach was then devised, so to identify genes encoding for the main Men B antigens. These antigens were screened to select molecules possessing a good immunogenicity and being

surface exposed. Once the candidate antigens were finally selected, a four component formulation was tested in clinical trials, showing a good immunogenicity and safety profile for the developmental vaccine [7].
The four main components of Bexsero® have a major role for the virulence of *N. meningitidis*, from adherence to colonization of the nasopharynx, to survival in blood stream and cerebrospinal fluid. The first component, factor H binding protein (fHbp), fused with GNA2091 protein, binds human factor H on its surface; once the bacteria is "covered" with factor H it can evade the host immune response by mimicking a self-antigen. The second component, NadA, is a major adhesion protein involved in colonization, invasion, and induction of pro-inflammatory cytokines. The third component, NHBA, is a heparin-binding protein that increases resistance against the bactericidal activity of human serum and is virtually present in all strains. NHBA is fused with protein GNA1030. The fourth antigen, OMV NZ98/254, has several antigenic components the major of which is PorA, and has successfully demonstrated tolerability and effectiveness when used to help control the New Zealand serogroup B outbreak [8].
The resulting vaccine, Bexsero® was the first broad-coverage MenB vaccine based on recombinant proteins approved for use in individuals 2 months of age and above by the European Commission in January 2013, and was approved for use in individuals from 10 to 25 years of age by the US FDA in January 2015. Bexsero® has also been approved for use in individuals of varying ages in Australia, Canada, Brazil and Uruguay among other countries [9, 10].
A further analysis was needed in order to predict the ability of the vaccine to be broadly protective against a variety of Men B subtypes: therefore a specific Meningococcal Antigen Typing System was devised (MATS) [9].
According to conventional genotyping and other preliminary studies the antigens contained in Bexsero® are not only pan genomic, i.e. present in the majority of circulating serogroup B strains, but in addition are evolutionarily conserved in the meningococcal population leading to the fact that Bexsero® may also offer a certain level of protection against non-B serogroups meningococci [8, 11-13].

The Meningococcal Antigen Typing System: MATS

Since the 1960s immunogenicity of meningococcal vaccines has been evaluated by means of complement-mediated killing of bacteria in the serum bactericidal antibody assay with human complement (hSBA) [14-17]. However, since protein antigens may vary in their presence, sequence and level of expression, evaluating the effectiveness of protein-based vaccines such as Bexsero® would require testing many different meningococcal subtype strains directly in hSBA, an impractical undertaking especially when the tests are done for infants, because serum volumes are very limited [5]. Due to frequent recombination in the MenB subtypes genotyping-based methods such as multilocus sequence typing are not suitable either [18, 19]. An alternative means of measuring the presence of surface-based antigens was needed and the Meningococcal Antigen Typing System (MATS) was developed to meet that need [9].
MATS evaluates the degree to which circulating serogroup B strains express each of the vaccine antigens, fHbp, NadA, NHBA, and PorA1.4, and helps determine the probability that strains will be killed in hSBA by antibodies obtained from individuals immunized with Bexsero® [20, 21]. Positive results in this type of test (MATS) are obtained if antigens are: (1) expressed to a sufficient degree; and (2) similar enough in terms of structure and sequence to the antigens in the vaccine so that the antibodies generated by Bexsero® will kill the bacteria. Good expression of at least one Bexsero® antigen is sufficient for a strain to be killed. MATS has been validated and standardized and is used by national reference laboratories around the globe to estimate the predictive effectiveness of Bexsero®. MATS has already been used to estimate strain coverage in the following countries: Australia, Brazil, Canada, Czech Republic, England and Wales, France, Germany, Greece, Italy, Norway, Spain, and the United States, with predicted coverage ranging from 66% in Canada (95% CI, 43–78%) to 91% in US (95% CI, 72–96%) [8]. In particular Vogel and colleagues assessed all MenB isolates from 5 European countries that were submitted to reference laboratories over a full epidemiological year from July 2007 to June 2008 [20]. Overall, 1052 MenB strains were collected in England and Wales (n = 535), France (n = 200), Germany (n = 222), Italy (n = 54), and Norway (n = 41). The predicted Bexsero® coverage in individual countries ranged from 73% (95% CI, 57-87%) in England and Wales to 87% (95% CI, 70-93%) in Italy. Importantly, 50% of all strains and 64% of covered strains could be targeted by antibodies against more than one Bexsero® antigen, thus ensuring redundancy: this is an important factor to help reduce the risk of the emergence of escape mutants not covered by the vaccine [9].
Although very important to evaluate potential effectiveness generated by Bexsero®, MATS is an *in vitro* test which was designed to provide a proxy of the ability of Bexsero® to help protect against the diversity of MenB subtypes [9]. Of course the results obtained can only be used as an indicator of vaccine effectiveness, whereas the true vaccine effectiveness will only be available after extensive use of the vaccine, i.e. in vaccination campaigns. However, if countries intend to use currently available MATS data, they should be aware that the current estimation obtained by MATS may be an underestimation of the true ability of Bexsero® to help protect against circulating Men B subtypes for several reasons [5]. In fact MATS does not account for the activity of antibodies from non-PorA OMV antigens nor it can take into account synergistic effects among the multiple vaccine components [21] or differential expression of antigens when expressed *in vivo* rather than *in vitro*: for example NadA expression is repressed under in vitro growth conditions used in both MATS and hSBA and NHBA expression is temperature-

regulated and is reduced at 37°C, the temperature at which MATS is performed [22, 23].
Recently, a new study aiming at experimentally validating the accuracy of MATS predictions tested strains isolated from England and Wales between 2007 and 2008 in the hSBA assay with pooled sera from infant and adolescent vaccinees, and compared these results with MATS. The results showed that 66% of the strains predicted not covered by MATS were killed in the hSBA assay (false negatives). Only one of the 28 strains predicted positive by MATS was resistant to killing in the hSBA assay. The authors concluded that MATS is a conservative predictor of the strain coverage of Bexsero® in infants and adolescents [24]. The same conclusion was reached in a second study conducted in Spain in which pooled sera from adolescents and infants have been tested by hSBA assay against 10 meningococcal group B strains that were negative or that had very low levels of the 3 antigens by MATS. It was found that all strains were killed by sera from adolescents and that 5 out of 10 strains were also killed, although at a low titer, by sera from infants [25].
In the future MATS could be useful in post implementation programs to monitor the effectiveness and coverage of Bexsero® over time [9].

Potential coverage of Bexsero® vaccine on non-B meningococci

Bexsero® main antigens are not exclusive to serogroup B because the genes encoding for the antigens fHbp, NHBA and NadA can be present and expressed also in the other serogroups suggesting that the immunization with Bexsero® could potentially offer a certain level of protection also against non-B strains [12]. Some investigations have been carried out in order to explore the potential impact of MenB vaccination against non serogroup B disease in different geographic areas (Australia, Europe and Brazil) [12, 13, 26].
In a study designed to estimate Bexsero® coverage in Australia, 108 meningococcal non-B isolates (serogroups C [n = 50], W [n = 27], Y [n = 30] and X [n = 1]) were tested using MATS. Of the non-B strains tested, 56% (39-76%) exceeded thresholds for at least one Bexsero® antigen [C, 64% (46-86%); W, 63% (41-93%); Y, 37% (27-43%)]. These preliminary results using MATS with non-B strains indicate that non-B strains circulating in Australia express significant levels of Bexsero® antigens [26]. However it should be noted that the MATS thresholds established for fHbp, NHBA and NadA were derived on serogroup B strains and their use to predict non-B strain coverage has not yet been validated [9].
Due to this, the ability of pooled sera from infants and adolescents immunized with Bexsero® to kill meningococcal C, W and Y strains isolated in Europe and Brazil has been recently described. In this study, a subpanel of 147 non-B meningococci isolates, representative of the genetic diversity of non B strains isolated in UK, Germany, France and Brazil, was collected and tested in serum bactericidal assay using human complement. The results showed that sera of subjects immunized with Bexsero are able to induce complement mediated killing of MenC, MenW and MenY in a range from 45% to 90%, suggesting that Bexsero® could potentially have an impact on meningococcal disease caused by non B serogroups [13].
It is noteworthy that the first investigation about the potential ability of Bexsero® to cover non-B serogroups meningococci was a pilot evaluation on the possibility of controlling the emerging *Neisseria meningitidis* capsular group X causing some recent outbreaks in Africa [27].
These preliminary results can represent an indication that Bexsero® may potentially have an impact on prevention of the meningococcal disease caused by non B serogroups [12, 26, 27].
The UK Joint Committee on Vaccination (JCVI) has stated that "the multicomponent MenB vaccine Bexsero® would likely provide some protection against other serogroups of meningococci, including serogroup C" thus suggesting the possibility to remove the dose of meningococcal C vaccine at 3 months of age in the immunizations infants calendar, after the introduction of MenB vaccine as a universal vaccination for infants in the UK schedule [28, 29].

Future perspective

Even if the circulation of *N. meningitidis* serogroups is typically dynamic and diverse in its geographic distribution, 5 serogroups are accountable for the majority of invasive meningococcal disease: A, B, C, Y and W135.
Since the year 2000 many European states, as well as Canada, have experienced substantial declines in the incidence of serogroups C disease after the extended use of glycoconjugate polysaccharide monovalent serogroup C vaccine [30, 31].
Most recently also tetravalent glycoconjugate vaccines including serogroups A,C,W,Y are available to help prevent the disease in Europe, North and Latin America and Asia and lastly the first broadly effective MenB vaccine, Bexsero®, has been licensed in the EU, USA, Canada, Australia, Brazil and Uruguay among other countries for various age groups [8, 10].
Efforts are currently ongoing to develop a combination meningococcal vaccine including antigens against the 5 meningococcal serogroups: A, B, C, Y and W135 [32, 33].
Investigational formulations of a meningococcal ABCWY vaccine, containing oligosaccharides from meningococcal serogroups ACWY conjugated to a CRM197 carrier protein, as well as MenB vaccine components, have been administered in healthy adolescents in clinical trials in the USA and Latin America [32, 33]. Studies results showed that the investigational MenABCWY formulations are able to elicit a robust immune response against ACWY serogroups and serogroup B test strains with an acceptable reactogenicity and safety profile. If confirmed and approved by regulatory agencies, this approach could lead to the availability of a pentavalent vaccine capable of helping to prevent invasive meningococcal diseases from the majority of circulating strains [32, 33].

With the availability of the existing glyconjugates, the protein-based multi-component MenB vaccine and the potential pentavalent combination aiming at offering protection to human populations against the five major serogroups (A, B, C, Y, and W-135), the world might reach the milestone of being for the first time ever capable of preventing the majority of meningococcal meningitis, adding a new chapter in medical history [3].

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Review

Methodological criticisms in the evaluation of Pneumococcal Conjugate Vaccine effectiveness

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Key words

Conjugate vaccine • Effectiveness • *Streptococcus pneumoniae*

Summary

Globally, lower respiratory tract infections (LRTIs), including community-acquired pneumonia (CAP), cause considerable of morbidity and mortality in adults, especially in the elderly. In addition to age, underlying medical conditions are associated with an increased risk of CAP. From an aetiological point of view, Streptococcus pneumoniae is the leading cause of adult CAP throughout the world. Two types of vaccine are available for the prevention of pneumococcal diseases: the pneumococcal polysaccharide vaccine (PPV23) and the pneumococcal conjugate vaccine (PCV7, PCV10 and PCV13). An accurate understanding of the LRTIs burden and the types of subjects at risk of CAP, allow to find an appropriately targeted immunization strategy and provide baseline data to evaluate pneumococcal vaccine effectiveness. Given the high variability in available estimates of LRTIs burden and associated risk factors, the objective of the study was to discuss the methodological criticism in its evaluation, in the light of the gradual introduction of PCV13 immunization strategy targeted to elderly and risk groups in middle-high income countries.

Introduction

Globally, LRTIs, including CAP, are a major cause of morbidity and mortality in adults in developed countries, leading to high hospitalizations rates, especially in the elderly [1-4].

According to recent estimates, LRTIs are the fourth most common cause of death, exceeded only by ischaemic heart disease, strokes and chronic obstructive pulmonary disease (COPD), and 1.9 million adults aged ≥15 years die from LRTIs every year worldwide. The 2010 Global Burden of Disease Study reported also that LRTIs, are the second most frequent reason for years of life lost [5].

Among Europe, CAP is the leading cause of death due to infection [4], with almost 90% of deaths due to pneumonia occurring in subjects > 65 years-old [6]. Pneumonia has also a substantial burden on healthcare resources and society, with associated annual costs in Europe estimated at approximately €10 billion, mostly due to hospitalization and lost working days [7].

Studies have shown that the risks of CAP and CAP-related deaths increase with age and are highest among the elderly [2, 3], indicating that the burden of pneumonia is growing in this era of global population aging [8-11]. The "oldest old" (≥ 85 years) are at particularly high risk of infections, due to comorbidities and waning immune function [12]. Moreover, in these subjects CAP can have serious consequences and aggravate underlying comorbidities [13].

In addition to age >65 years, other risk factors for CAP are recognized, such as chronic cardiovascular or respiratory diseases, cerebrovascular diseases, epilepsy, dementia, dysphagia, chronic liver or renal diseases, lifestyle factors (smoking, alcohol consumption, being underweight, regular contact with children and dental hygiene), and immunosuppressive conditions [14, 15].

From an aetiological point of view, *Streptococcus pneumoniae* is the leading cause of adult CAP throughout the world [3, 16, 17], and has been estimated to be the cause of 30-50% cases of CAP requiring hospitalization in adults in developed countries [18]. Nevertheless, in high-income countries it has been decreasing as a consequence of the wide use of antibiotics and of the introduction of pneumococcal vaccines [19].

From the clinical and public health perspectives, estimates of the overall health care burden and aetiological patterns of CAP are crucial for effective disease control programs [1, 2]; however, available estimates largely vary, so that its true burden remains unclear.

The objective of the present study is to discuss the methodological criticism in the evaluation of the burden of LRTIs, including pneumonia, and of the pneumococcal vaccine effectiveness, in the light of the gradual introduction of PCV13 immunization strategy targeted to elderly and risk groups in middle-high income countries.

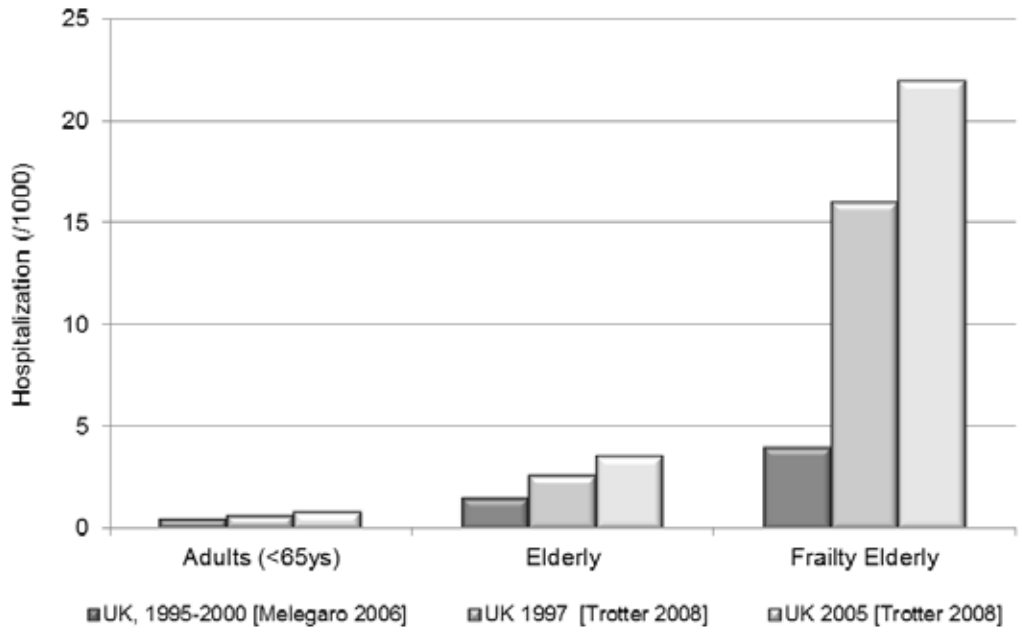
Knowledge gap for PCV introduction in adults: criticisms in the definition of the burden of pneumonia and LRTIs

Although LRTIs, including pneumonia, are common diseases, the real burden and their related risk factors remain unclear, even in high-income countries [20].
Available incidence estimates largely vary, making the comparison of LRTIs and CAP incidence obtained from different studies difficult (Fig. 1) [15].
Epidemiological studies conducted in the second half of 2000 among adults have reported hospitalization rate of about 1.1 and 2.8 per 1,000 year in the UK and in Germany, respectively [21, 22]. The overall incidence estimated in hospitalized adult patients for CAP who lived in two countries in Ohio, USA, was 2.6 per 1,000 inhabitants year [23]. Furthermore, a study conducted in Denmark between 1993 and 2008 reported rate of hospitalization for pneumonia lower than 4 per 1,000 in adults aged >50 years [24].
Several factors explain the variation of available CAP estimates and they are deepen below.
First, the performance of surveillance system in terms of specificity and sensitivity in capturing LRTI cases is suboptimal. This limit could be overcome using a syndromic surveillance system that combines high sensitivity in identifying suspected cases obtained by scanning the chief complaint field for the word strings assigned to the single syndrome and automatic review of ED acceptance data folders and high specificity as a result of critical revision of each reported case according to the operative case definition [15].
Second, the definition of pneumonia differs among studies [25]: some studies used chest X-ray findings to determine pneumonia [9, 11, 26], whereas others used clinically defined criteria or simply relied on reported cases at the sentinel sites [25, 27]. Additionally, the diagnosis of pneumonia is not standardized in clinical settings [20]. Furthermore some studies have reported incidences of CAP including both outpatients and hospitalized patients [11, 29], while other studies evaluated hospitalized cases only [21, 30], introducing a selective bias towards severe patients. Lastly, mild cases must be overlooked in countries in which access to health care is limited, affecting the incidence estimates by the health care-seeking pattern [20].
Third, the heterogeneity of study design and difference in the underlying risk profile and age categorizations of the populations studied [12, 31-33] produce different estimates [20]. Furthermore, some studies of regional and socio-economic variations in LRTIs incidence have not age-stratified further after 65 years, but this group include very different subjects, both people working full-time and those that require round-the clock care [12].
Last but not the least, available incidence estimates also vary from setting to setting, reflecting national differences in health systems and medical practice [3, 11, 12, 21, 28-30, 34-35].

The pneumococcal immunization strategies in adults

Currently, two type of vaccines are available to prevent pneumococcal-related disease in adults: a polysaccharide vaccine and pneumococcal conjugate vaccines [36]. During 1970s the PPV-23 was introduced in high-income countries for the prevention of pneumococcal

Fig 1. Differences in available LRTIs incidence estimates by age group [30, 75].



diseases caused by the 23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F) in adults and children aged ≥2 years [37].
Furthermore, in many countries the PPV23 has been recommended for high-risk groups, including the elderly [38, 39]. However, there is little evidence that it is effective in adults with chronic diseases and in the elderly [40,41].
Although available systematic reviews and meta-analyses demonstrate that PPV23 confer protection against invasive pneumococcal disease (IPDs) [38, 41], its duration is limited [42, 43], and its effectiveness against pneumococcal pneumonia is still controversial, particularly for the elderly [40, 41].
The first pneumococcal conjugate vaccine (PCV7) was licensed in 2000 for protection against IPDs, including sepsis, meningitis, and non-invasive diseases, such as pneumonia and acute otitis media (AOM), caused by the seven serotypes contained in the vaccine (4, 6B, 9V, 14, 18C, 19F and 23F) in infants and children aged from 2 months to 5 years [44].
In 2009 PCV10 (serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) was approved for protection against IPDs, pneumonia and AOM in infants and children aged from 6 weeks up to 5 years [45].
Today a PCV13 vaccine, including six additional serotypes to PCV7 (1, 3, 5, 6A, 7F and 19A) is available for the prevention of IPDs and pneumococcal pneumonia in adults aged ≥18 years and the elderly, and for the prevention of IPDs, pneumonia and AOM in infants and children aged between 6 weeks and 17 years [46].
Since 2006, the WHO has recommended that PCV be included in all routine childhood immunization programs [47]. Of the European region member states, 49% had introduced PCV by 2012.
In countries with high immunization coverage the benefits of childhood immunization have been observed over time also in unvaccinated children and adults, as a result of the "herd immunity" effect [48-51].
However, despite extensive childhood immunization plan, the burden in the elderly and high risk groups remains high [15, 52].
Since the indication of PCV13 use has been extended to adults ≥ 50 years-old in 2011, its gradual inclusion in adults immunization plan has been observed in high-income countries, in addition to childhood immunization programs [53].
Nevertheless, pneumococcal immunization strategies vary with regards to age groups and risk groups to be immunized, the type vaccine (PPV and/or PCV) and the eligibility for reimbursement [15, 53].
Based on available epidemiological evidence, the best pneumococcal immunization strategy to reduce the burden of LRTIs should be age- and risk-based. In fact, although "at-risk strategy" has many disadvantages (i.e. difficult access to health services, involvement of different healthcare professionals, difficult to achieve high levels of vaccine uptake), it should be greatly implemented and coupled by age-based strategy [15].
The majority of the Western European countries has implemented this coupled strategy [53-54], however the number of identified risk groups and the age group eligible for vaccination varies in the different countries [36, 53].

PCV13 effectiveness estimation in prevention of LRTIs in elderly and risk groups

Today, the estimation of PCV13 effectiveness in prevention of LRTIs in elderly and risk groups is of particular scientific interest due to its more recent introduction than PPV23, but it shows many methodological issues.
RCTs, such as the recently published CAPITA study [55], provide the most definitive data about the efficacy of PCV13 vaccine, but performing such trials is extremely difficult [56] and expensive and entails many ethical issues. In fact, pneumococcal vaccine is recommended in the elderly, those with chronic conditions and immunosuppressed subjects, making placebo-controlled trials unethical in these groups [57]. Furthermore, pneumococcal pneumonia is a relatively uncommon outcome, so RCTs of PCV13 must consider large populations to have adequate statistical power [56].
Existing observational methods for evaluating vaccine effectiveness, such as cohort and case-control studies, are cheaper and logistically easier, but they implies the risk of introduction of biases that may interfere with vaccine effectiveness estimates [56]. Routinely collected administrative data don't provide adequately accurate databases to estimate vaccine effectiveness. Furthermore many biases (some of which are difficult to detect) pose challenges in distinguishing vaccine-related effects from other potential confounders that may affect the same outcomes. They include differences in susceptibility to infection and differences in health care utilization in vaccinated and unvaccinated populations. In particular, vaccinated group usually include healthy subjects that have social interactions and then are exposed to LRTIs. Conversely, they have a lower risk of developing complications and serious outcome, such as deaths, than unvaccinated subjects. As demonstrated in the study published by Weycker D et al. in 2010, the annual incidence of non-bacteremic pneumococcal pneumonia requiring inpatient care is 17 and 10 folds higher in high risk subjects in 64-74 years and 75-84 years, respectively [58]. Then, the evaluation of vaccinated and unvaccinated groups should take into account the differences in LRTIs outcomes.
Otherwise proxy indicators such as antibody response are not applicable, in particular to evaluate the effectiveness against non-invasive diseases. Enzyme-linked immunosorbent assay (ELISA) can be used to measure antipneumococcal IgG antibodies [59], giving reproducible results. However, there is no consensus regarding the protective antibody levels in adults [56]. Furthermore, older adults develop antibodies characterized by reduced function [60] and ELISA cannot distinguish between functional and nonfunctional antibodies [61].

Opsonophagocytic killing (OPK) activity [56] has been shown to correlate with immune protection in animal studies [60] and have also been shown to correlate with protection better than ELISA for AOM in children [62]. However, no available studies correlates OPK assay results with protection in adults [56].

Finally, it is hard to find the correct clinical and laboratory endpoint to accurately estimate the incidence of pneumonia pneumococcal-related. The choice of clinical pneumonia as an endpoint is therefore biased in favour of high sensitivity, at the expense of specificity. Indeed, a large proportion of the cases that meet the case definitions for clinical pneumonia have a low positive predictive value and are, therefore, not pneumonia [63]. Conversely, radiologically-confirmed pneumonia is a relatively more specific measure of CAP and so evaluating vaccine efficacy on this outcome measure is a better indicator. Furthermore, the level of vaccine-induced pneumococcal antibody in adults that correlates with protection against clinical disease, including IPDs or pneumococcal pneumonia, has not been established [64]. Furthermore, classical microbiological assays, such as Gram-staining and culture from sputum and/or blood, understimates the burden of pneumococcal pneumonia and the results are delayed. The isolation of *Streptococcus pneumoniae* from blood allows a specific aetiological diagnosis but with a detection rate of 10%-20% [36]. Urinary antigen tests for *Streptococcus pneumoniae* have been developed to overcome the limits of culture-based tests, and are characterized by high specificity and sensitivity in adults [65-68] and can help monitor changes in overall burden of pneumococcal CAP [69] but they should be developed for a broader research use and a wider range of pneumococcal serotypes, before their widespread use [64]; thus, documenting *Streptococcus pneumoniae*-specific impact is quite challenging [56].

Molecular methods represent another non-culture-based diagnostic approach that allows to rapidly and accurately quantify the bacterial load [36]. These methods are more sensitive than blood culture and may be a useful tool for the assessment of the severity of pneumococcal pneumonia [70].

Finally, molecular methods, in addition to conventional laboratory methods, are the best strategy to detect pneumococcal pneumonia [71-74].

Conclusions

Available evidence show that the burden of LRTIs, including pneumonia, in adults is relevant and strongly age- and risk factors-related [15]. Nevertheless the estimation of LRTIs and their prevalence in risk groups largely vary among published studies.

Considering the availability of effective vaccine in prevention of pneumococcal pneumonia, i.e. PCV13, an accurate understanding of the LRTIs burden and the types of subjects at risk of CAP, allow to find an appropriately targeted immunization strategy that optimize the vaccine effect and provide baseline data to evaluate pneumococcal vaccine effectiveness [14, 15].

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