High prevalence of genetically-determined mannose binding lectin deficiency in young children with invasive pneumococcal disease

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Abstract

Susceptibility to invasive pneumococcal disease (IPD) correlates with age, younger children being the group with the highest burden of disease. The relevance of the innate immune response and particularly the role of mannose-binding lectin (MBL) in combating IPD is not well known. This is a 2-year prospective study (February 2011 to March 2013) including patients with IPD who attended two hospitals from Catalonia, Spain. Variables including attack rate of pneumococcal serotype (high or low invasive potential serotypes) and genotypes associated with low serum MBL levels were recorded. One hundred and forty-seven patients were included in the study. One hundred and two (69.4%) patients were children or adolescents <18 years and 45 (30.6%) were adults. Overall, low-MBL genotypes (O/O; XA/O) were detected in 23 (15.6%) patients. Children <2 years showed a higher frequency of low-MBL genotypes compared with other patients (31.0% vs. 11.9%; $p = 0.031$). Further sub-analysis revealed a higher proportion of low-MBL genotypes in children <2 years with IPD caused by opportunistic or low-attack-rate serotypes when compared with older patients (46.2% vs. 13.2%; $p = 0.02$). However, no statistically significant differences between the two groups were observed when including patients infected with invasive or high-attack-rate serotypes (18.8% vs. 10.0%; $p = 0.59$). Our data suggest that young children with a genetically determined low-MBL production are at a higher risk of developing IPD, particularly that caused by opportunistic or low-attack-rate pneumococcal serotypes.

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Introduction

Invasive pneumococcal disease (IPD) is a serious health problem in children and adults, and causes almost one million childhood deaths worldwide every year [1]. *Streptococcus pneumoniae* usually colonizes the nasopharynx of healthy children but is less frequently found as a colonizer in adults. It is estimated that most children are colonized by pneumococcus

at least once during the first 2 years of life and nasopharyngeal colonization is the first step towards development of mucosal and invasive diseases [2]. Further spread of pneumococcus to the bloodstream and other normally sterile sites occurs less often. However, young children, young adults with immunosuppressive and chronic conditions and older adults are at higher risk of IPD. The complex interaction between impaired host factors and the presence of virulence determinants of the pneumococcus may be responsible for developing IPD [2].

The main virulence factor for pneumococcus is the polysaccharide capsule, with more than 94 serotypes that cause varying rates of carriage and IPD. Some of these serotypes have 'low attack rate' and are frequently detected in carriers. These so-called 'opportunistic serotypes' are more prevalent in children <2 years old, elderly people and patients with co-morbidities [3]. In contrast, serotypes with a 'high attack rate', also called 'high-invasive potential serotypes', are seldom detected in carriers and often cause IPD, particularly in older children and adults without co-morbidities [4,5]. Intriguingly, serotypes with a high attack rate, such as serotypes 1, 5 or 7F, have been associated with a less complicated course of disease and lower mortality rates than opportunistic serotypes [6,7],whereas serotypes with a low attack rate have been related to high mortality and more serious clinical manifestations, such as meningitis and sepsis [8].

Mannose-binding lectin (MBL) is a serum protein of the innate immune system, which recognizes pathogen structures, mainly of a carbohydrate nature. It can then promote opsonophagocytosis of a wide range of microorganisms and subsequent antibody-independent complement activation [9,10]. It is considered a pre-antibody that has a relevant defensive role in the first period of life, when an immature adaptive immune system still exists. The serum levels of MBL are genetically determined as a consequence of single nucleotide polymorphisms (SNPs) embedded into the promoter and the exon 1 of the human *MBL2* gene [11]. Homo- and heterozygous combinations of those SNPs give rise to different genotypes responsible for high (A/A, XA/A), intermediate (O/A, XA/XA) or low (O/O, XA/O) serum MBL levels [12]. Previous reports indicate that genetically-determined MBL deficiency is relatively frequent in all human populations analyzed (ranging from <15% in Caucasian populations to 20% in sub-Saharan African populations) [13,14]. This deficiency has been linked to increased susceptibility to infectious diseases, including those caused by pneumococcus [15]. Nonetheless, this hypothesis remains controversial because some studies have not observed a significant association of MBL deficiency with the development of IPD [16].

The aim of this study was to evaluate the prevalence of genotypes responsible for low serum MBL levels in patients with IPD according to age group and serotype attack rate characteristics. This information could be useful for designing strategies for prevention and personalized treatment of patients based on previous analysis of host–pathogen interactions.

Patients and Methods

Participant recruitment

This is a prospective study that includes all patients with IPD who attended two medical centres (Hospital Sant Joan de Déu and Hospital de Mataró) from 1 February 2011 to 1 March 2013. The Hospital Sant Joan de Déu (HSJD) is a 360-bed referral paediatric centre located in the metropolitan area of Barcelona, which annually captures around 17% of all hospitalizations (*c.* 200 000 children) from the population <18 years in Catalonia (Spain). The Hospital de Mataró (HM) is a public general hospital that covers a catchment area of 400 000 inhabitants from the Catalonian area of Maresme.

Only one episode (the first) per patient was included in the study sample. Patients with functional deficit of classical or alternative pathways of complement activation were excluded from the study, as well as patients with immunocompromised conditions (HIV infection, immunoglobulin deficit), cystic fibrosis, bronchiectasis or cerebrospinal leak.

Demographic and clinical variables including age, sex, ethnicity, IPD risk factors, pneumococcal vaccination status, pneumococcal serotypes and their invasiveness potential, MBL production levels, clinical diagnosis, course of disease, length of hospital stay (LOS) and admission to intensive care unit (ICU) were registered for each episode.

The study was performed following the guidelines of the Ethics Committees of Hospital Sant Joan de Déu and Hospital de Mataró.

Microbiological and immunological methods

Invasive pneumococcal disease was defined as the presence of clinical findings of infection (which were used for classification of disease) together with isolation of *Streptococcus pneumoniae* and/or DNA detection of the *pneumolysin* (*ply*) gene and an additional capsular gene of *S. pneumoniae* by real-time PCR in plasma, cerebrospinal fluid or any other sterile fluid. All pneumococcal isolates were identified by standard microbiological methods. DNA detection of the *pneumolysin* (*ply*) gene by real-time PCR in normal sterile fluids was performed according to a previously reported assay [17]. Serotyping of strains isolated by culture was carried out by a molecular