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ORIGINAL ARTICLE

Post-infectious bronchiolitis obliterans and mannose-binding lectin insufficiency in Argentinean children

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ABSTRACT

Background and objective: **Post-infectious bronchiolitis obliterans (PIBO) is a severe disorder following acute lower pulmonary infection in young children, especially caused by adenovirus. Mannose-binding lectin (MBL) deficiency arising from polymorphisms in the coding and non-coding region on the *MBL2* gene has been associated with more frequent and severe respiratory infections. Our aim was to evaluate the influence of MBL variants in the susceptibility and evolution of children with PIBO.**

Methods: **One hundred eleven children with PIBO diagnosis were studied. The coding *A*, *B*, *D* and *X* promoter variants of *MBL2* gene were assessed by PCR-RFLP. *B* and *D* alleles were pooled as *O*. The combined genotypes *A/A* and *YA/O* were grouped as sufficient MBL (s*MBL*), and *O/O* and *XA/O* as insufficient MBL (i*MBL*) groups. To evaluate the frequency of *MBL2* polymorphisms in the general population, we studied DNA samples from 127 healthy donors from the blood bank of the hospital (control group).**

Results: ***iMBL* variants were significantly more frequent in PIBO children compared with controls (21.6% vs 10.2%, *P* = 0.01). PIBO patients with i*MBL* required intensive care unit (*P* = 0.001) and mechanical assistance at the moment of viral injury (*P* = 0.001) more frequently than those with sMBL.**

Conclusions: **Insufficiency of *MBL* was more common in PIBO children than in healthy controls. This genetic condition was significantly associated with more severe initial disease, illustrating the relevance of innate immune defence factors prior to the maturation of the adaptative immune system.**

Key words: children, mannose-binding lectin polymorphism, post-infectious bronchiolitis obliterans.

Abbreviations: Ad, adenovirus; ALRI, acute lower respiratory infection; BO, bronchiolitis obliterans; FEV1, forced expiratory volume in first second; IL-6, interleukin-6; iMBL, insufficient MBL; MBL, mannose-binding lectin; PIBO, post-infectious bronchiolitis obliterans; RSV, respiratory syncytial virus; SD, standard deviation; sMBL, sufficient MBL; TNF-α, tumour necrosis factor-alpha.

SUMMARY AT A GLANCE

The influence of mannose-binding lectin (MBL) variants in the susceptibility and evolution of children with post-infectious bronchiolitis obliterans (PIBO) was evaluated. Polymorphisms resulting in MBL insufficiency were more frequent in children with PIBO than in controls. This condition was significantly associated with more severe initial disease illustrating the relevance of innate immune system.

INTRODUCTION

Post-infectious bronchiolitis obliterans (PIBO) is an infrequent and potentially severe disorder following acute lower pulmonary infection in young children. PIBO is the most common form of bronchiolitis obliterans (BO) reported in infancy. BO implies a chronic necrotizing and ultimately fibrosing process affecting the small airways, which results in progressive obliteration with persistent obstructive lung disease and has been associated with a high morbidity and mortality rate.[1–3]

There are many reports of PIBO secondary to influenza, parainfluenza, measles, respiratory syncytial virus (RSV), varicella and *Mycoplasma*. However, adenovirus (Ad) is by far the most common cause of PIBO.[1–3] The initial event, usually in a child under the age of 3, has a severe acute lower respiratory infection (ALRI) (bronchiolitis, pneumonia, multifocal pneumonia) leading to high oxygen requirements, admission to the intensive care unit and sometimes requires mechanical ventilatory support. Progression to PIBO may occur in 50% of cases, with a mortality of 15%. Children may have long-term ventilator or oxygen dependency at home.[1]

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PIBO cases have been reported in many different countries. However, there has been for around 30 years, a remarkable preponderance from South American countries (Chile, Argentina and southern Brazil). BO in Latin America (BOLAT) Initiative estimates that there are more than 700 cases of PIBO in the region.[1–6] Ethnic characteristics have been suggested as a predisposing factor for the development of PIBO. However, other host conditions like immunity have not been thoroughly evaluated yet.[7]

Mannose-binding lectin (MBL) is a circulating plasma protein that plays a pivotal role in innate immunity. It is a liver-derived serum protein that binds to mannose and N-acetyl glucosamine residues commonly found in bacteria and viruses.[8,9]

Low levels of circulating MBL has been associated with more frequent and severe infections, especially in children between 6 and 17 months of age before specific immune protection is established by the adaptive immune system.[10–13]

MBL2-deficient variants have been reported in association with respiratory infections in childhood, increased pneumococcal infections in adults and in the immunocompromised, and more severe lung disease in children with cystic fibrosis or primary ciliary dyskinesia.[10,14–18] To our knowledge, there are no studies conducted to evaluate *MBL2* genotypes in children with the diagnosis of PIBO. Therefore, the purposes of the present study were to compare the frequencies of genetic variants related to MBL insufficiency between children with PIBO and healthy controls and to determine whether the presence of structural and promoter *MBL2* variants, which give rise to low MBL levels, influences the development of PIBO and might modify the course of this disease.

METHODS

A population-based case series study was conducted over a 3-year period (2011–2013). The study was performed on a white Hispanic population of Argentina and included 111 children with PIBO diagnosis, and stored genomic DNA obtained from 127 healthy unrelated blood donors (controls) taken at random from Hospital de Pediatría 'Dr. Juan P. Garrahan'.

Frequencies of polymorphisms related to MBL insufficiency were compared between the PIBO children and controls. Clinical characteristics and pulmonary function were evaluated within the PIBO group by *MBL2* variants.

The study included all children with PIBO diagnosis followed up in the Pulmonology Department at the Hospital Garrahan. PIBO diagnosis was made according to previous reports[1–4]: briefly (i) previously documented ALRI with persistence of airway obstruction; (ii) hyperinflation, atelectasis, bronchiectasis and/or 'mosaic oligohemia' on HRCT scans; (iii) persistent obstructive pattern on pulmonary function test.[1–3] Other respiratory diseases (bronchopulmonary dysplasia, cystic fibrosis, immunodeficiencies, aspiration syndromes, congenital heart disease) were excluded. Nasopharyngeal aspirates were studied in children admitted to the hospital in the acute stage of the disease by rapid detection of viral antigen with indirect immunofluorescence (Light Diagnostics-Chemicon International, Inc., Temecula, CA, USA).

Patients were seen in the Pulmonology Department outpatient settings every 2 months. Information on age at the viral infection, sex, previous infections, initial clinical presentation, length of initial hospital stay, initial intensive care and mechanical assistance requirements, oxygen requirements, severity and mortality were compared between groups. Oxygen requirements at discharge, hospitalizations during the first and second year of the disease and pulmonary function were also analysed. Spirometry was performed in children older than 6 years, at least 3 weeks after the most recent ALRI, and the last study was considered for the analysis. Studies were performed with the standardization of the American Thoracic Society by a trained technician who was blinded to the genetic variants of the MBL2 gene of the subjects.

The parameters considered were forced vital capacity, forced expiratory volume in first second (FEV1) and forced middle maximum flow. To assess the bronchodilator response, 200 μg of albuterol was administered using a metered dose inhaler and a paediatric chamber. A positive response was considered with an increase of >12% of predicted FEV1.

The ethics committee of the hospital approved the study. Written informed consent was obtained from the parents or legal guardians and from the blood donors.

Genotyping analysis

Genomic DNA was extracted from peripheral blood mononuclear cells using the purification kit QIAamp DNA Blood Mini Kit (Qiagen; Technolab Sa, Buenos Aires, Capital Federal, Argentina). Genotyping for MBL2 structural (*A*, *B*, *C* and *D*) and promoter (X/Y) variants was performed by restriction fragment length polymorphism polymerase chain reaction assays as described elsewhere.[13,16]

Polymorphisms of *MBL2* gene were evaluated in patients and controls. The structural variant alleles (*B*, *C*, and *D*) were grouped in one category (allele *0*) to avoid small groups and combined with the promoter variants (*X/Y*). Six haplotype pairs were identified: *YA/YA*, *YA/YO*, *XA/YA*, *YO/YO*, *XA/YO* and *XA/YO*. To define MBL sufficiency, the haplotypes were divided into two groups: sufficient MBL group (sMBL) and insufficient MBL group (iMBL). The sMBL group has medium and higher MBL serum levels (variants *A/A* and *YA*/O (*YA/YA*, *YA/XA*, *XA/XA*)); the iMBL group has undetectable MBL serum levels (variants *O/O* and *XA/O*).[8–10,12,15]

Statistical analysis

Standard descriptive statistics were used to describe the baseline characteristics of the population. Both the Mann–Whitney *U*-test and the Kruskal–Wallis test were used to compare continuous data between carriers of different *MBL2* genotypes. Chi-square and Fisher's exact tests were used for categorical data. A difference was considered statistically significant if the *P*-value was ≤0.05. Data were analysed using a Stata 10.0 software package (Stat Corp., College Station, TX, USA).

Table 1 Genotypic and allelic frequencies of structural and promoter variants of *MBL2* gene

MBL2 genotypes*	PIBO patients (n = 111)	Healthy controls (n = 127)
Structural alleles	*n* (%)	*n* (%)
A/A	65 (58.6)	79 (62.2)
All A/0	37 (33.3)	42 (33.1)
A/B	28 (25.2)	36 (28.3)
A/D	9 (8.2)	6 (4.7)
All 0/0	9 (8.2)	6 (4.7)
B/B	8 (7.3)	4(3.2)
B/D	1 (0.9)	2 (1.6)
Allelic frequencies		
A	0.75	0.79
0	0.25	0.21
B	0.2	0.18
D	0.05	0.03
Promoter		
Y/Y	72 (64.9)	96 (75.6)
X/Y	31 (27.9)	27 (21.26)
X/X	8 (7.2)	4 (3.15)
Allelic frequencies		
Y	0.79	0.86
X	0.21	0.14

*Genotypes were found to be in Hardy–Weinberg equilibrium.
PIBO, post-infectious bronchiolitis obliterans.

Table 2 Haplotype frequencies of *MBL2* gene in PIBO patients and healthy controls

Haplotype variants	PIBO patients (n = 111)	Healthy controls (n = 127)
Sufficient	*n* (%)	*n* (%)
YA/YA	42 (37.8)	55 (43.4)
YA/XA	15 (13.5)	20 (15.8)
YA/YB	15 (13.5)	31 (24.4)
YA/YD	7 (6.3)	4 (3.1)
XA/XA	8 (7.2)	4 (3.1)
Insufficient		
XA/YB	14 (12.6)	5 (3.9)
XA/YD	2 (1.8)	2 (1.6)
YB/YB	7 (6.3)	4 (3.1)
YB/YD	1 (0.9)	2 (1.6)

PIBO, post-infectious bronchiolitis obliterans.

RESULTS

MBL polymorphisms were studied in a total of 111 Argentinean unrelated children with diagnosis of PIBO. To determine the frequency of MBL2 structural and promoter variant (*A*, *B*, *C*, and *X/Y*), we studied 127 Argentinean blood donors.

All of the alleles except *C* were found. Table 1 summarizes the *MBL2* genotypic and allelic frequencies.

In the group of patients with PIBO, 58.5% of children (n = 65) were homozygous *A/A* (the normal genotypic variant), 8.2% (n = 9) were *O/O* and 33.3% (n = 37) were heterozygous *A/O* genotype. Within compound *O/O*, variant *B/B*, which produces undetectable levels of MBL, was two times more frequent in the PIBO group than in the healthy controls (7.3% vs 3.1%, respectively) (Table 1). When combining the structural alleles with promoter alleles, 21.6% (n = 24) of children in the PIBO group were iMBL (*XA* +*O/O*), while 10.2% (n = 13) were observed in the healthy controls (P = 0.01). The number of PIBO patients and controls with each variant combination is shown in Table 2.

To evaluate the influence of *MBL2* genotypes on the development and course of the PIBO group, 111 children were studied. Mean age of children at the time of the study was 10 years (standard deviation (SD) 4.9). Mean age at initial injury was 12.2 months (SD 11). Median time of hospitalization at acute stage of disease was 40 days (interquartile rank 17–90). Ad was isolated from the nasopharyngeal aspirates in 78.3% of cases (n = 87), RSV in six children (5.4%) and influenza in four (3.6%). No aetiologic agent was identified (negative virologic test or not performed) in 12.6% of children (n = 14). There were no differences in the distribution of *MBL2* variants between children with and without viral isolation.

According to the *MBL2* genotypes, children were stratified in iMBL group (n = 24) and sMBL group (n = 86). A comparison of clinical characteristics and course between groups is shown in Table 3. Patients with iMBL developed more severe initial disease. Almost all of them (96%) required intensive care at the moment of viral injury, while 53.4% of children in the sMBL group needed intensive care (P = 0.0001). Mechanical assistance support was required in 84% of cases in the iMBL group compared with 42% of patients in the sMBL group (P = 0.007). No differences were observed in mortality between groups. Pulmonary function was evaluated in 68% (n = 17) of patients with iMBL and in 77.9% (n = 67) of sMBL children. Spirometries were similar between subjects carrying different *MBL2* genotypes (Table 3). However, when patients were grouped considering only homozygous alleles, a significantly lower FEV1 was observed in children with iMBL compared with the sMBL group (43.6% vs 51.4%, respectively; P = 0.05).

There were more children with oxygen requirements at discharge in the iMBL than sMBL group (72% (n = 18) vs 63.9% (n = 55)) and fewer patients were able to withdraw it (48% (n = 12) vs 63.9% (n = 55), respectively). During the first year of follow-up, hospitalizations were more frequent in children in the iMBL group compared with the sMBL group (96% (n = 24) vs 72% (n = 62)). Nevertheless, no statistically significant differences were observed among these analysed variables.

DISCUSSION

In this study, evaluating the distribution of *MBL2* polymorphisms in children with PIBO, significantly more children carrying iMBL genetic variants were observed within the PIBO group compared with healthy controls. Among PIBO iMBL group, more patients required intensive care unit support and mechanical ventilation.

Table 3 Characteristics of children with PIBO by MBL genotypes (*n* = 111)

Analysed variables†	Insufficient MBL group (*n* = 25)	Sufficient MBL group (*n* = 86)	*P*-value
Age at viral infection (months)‡	9 (4–12)	11.4 (5–15)	0.09
Sex (male)	17 (68)	53 (61.6)	0.5
Previous infections§	20 (80)	69 (80.2)	0.7
Initial presentation			
Bronchiolitis	14 (56)	49 (56.9)	0.5
Pneumonia	4 (16)	13 (15.1)	0.6
Multifocal pneumonia	7 (28)	24 (27.9)	0.6
Sepsis	1 (4)	4 (4.6)	0.7
Adenovirus isolation	19 (76)	68 (79)	0.5
Initial intensive care requirement	24 (96)	46 (53.4)	0.0001
Mechanical assistance requirement	21 (84)	36 (41.8)	0.007
Days of mechanical assistance‡	10 (7–17)	10 (7–20)	0.2
Days of hospitalization‡	31.5 (23–80)	40.5 (15–90)	0.5
Mortality	0 (0)	1 (1.1)	0.3
Pulmonary function¶	(*n* = 17)	(*n* = 67)	
FVC	79% (54–90)	73% (65–87)	0.4
FEV1	51.1% (41.5–60)	47.1% (42.5–51.6)	0.4
FEV1/FVC	60	59	0.8
FMMF	18.5% (12–32)	22% (16–32)	0.6

†Values are expressed as number of cases and percentages.
‡Values are expressed as median and interquartile rank (IQR).
§Otitis and pneumonia.
¶Values are expressed as median and IQR. FEV1, forced expiratory volume in first second; FMMF, forced middle maximum flow; FVC, forced vital capacity; MBL, mannose-binding lectin; PIBO, post-infectious bronchiolitis obliterans.

A correlation between *MBL2* polymorphisms and MBL serum levels has been well described.[9,12] In a homozygous or a heterozygous combination, the *0* variant is commonly associated with low or undetectable MBL levels, while the heterozygous occurrence results in moderately reduced levels. Within *0* components, the *B* variant has the most dominant effect on decreasing serum MBL. More than one-third of the population has genotypes associated with reduced MBL levels, with very low levels expected in 12%.[9] In our population, 21.6% of patients with PIBO had iMBL, whereas in the control group 10.2% of the subjects had iMBL.

MBL is active against respiratory viruses such as RSV and influenza.[19–21] Previous reports suggest that iMBL leads to severe viral respiratory infection. In children with iMBL the subsequent development of persistent pulmonary symptoms was described in association with RSV infection.[21]

The initial PIBO event occurs in early years of life and is strongly associated with severe Ad infection. The severity of the acute illness seems to correlate with the development of PIBO in young children.[1,2] In our series although similar distribution of Ad was observed in children carrying all *MBL2* genotypes, patients with iMBL presented a more serious disease after the infection. Almost all of them required intensive care with ventilatory support at the time of acute infection, evidencing that not only Ad but also a personal genetic condition might influence the harshness of the initial infection.

Previous reports suggest that severe inflammation after severe pulmonary infection leads to PIBO. Argentinean patients with severe Ad pneumonia have been shown to have immune complexes containing Ad antigen in the lung, as well as increased serum levels of interleukin-6 (IL-6), IL-8 and tumour necrosis factor-alpha (TNF-a).[2] Even though factors such as infection, ischaemia and reperfusion likely play a role in the molecular and cellular changes observed in PIBO, the host immunologic response might also contribute to the severity of the initial pulmonary infection as well as the subsequent development of BO.[2]

Children with PIBO evidenced a typical pattern of severe airway obstruction on spirometry without reversibility. All patients showed a fixed and markedly reduced forced flows. No differences on pulmonary function parameters were observed between children of both MBL groups. However, when patients were grouped considering only homozygous genotypes, patients with iMBL had a significantly lower FEV1. This finding might be explained by the severity of the initial viral infection. Most reports have shown that pulmonary function of patients followed for a long time had the same fixed obstructive disease through the years.[3,22] The aggressiveness of the initial viral injury itself as well as the treatments required to solve it seem to produce a more severe damage in the airways of iMBL children, which become constant over time.

With few exceptions, all MBL genotypes occur worldwide, but the frequency varies between populations.[23] The *B* allele is quite frequent in Caucasians (0.13), in Inuits from east Greenland (0.12) and in the Chinese population (0.11), but it is surprisingly frequent in some South American Indian groups, such as in the Argentinean Chiriguano population (0.42).[24] In our sample population, we found a similar *B* allele frequency as published in other reports from Argen-

tina (0.20), and all of the *MBL2* variants except for the *C* allele were observed.[13] Furthermore, genotype *B*/*B* was significantly more frequent in patients from our study compared with a cohort of children from Greenland ($P = 0.0001$).[10]

The absence of the *C* allele and the frequency of *B* alleles were expected because our population, considered to be white Hispanic, is mainly composed of descendants from whites from Southern European countries, with almost no African admixture.[13,25,26]

The ethnic background of patients has also been linked with the risk for developing PIBO. Some studies have described a greater occurrence of Ad infection in Amerindian populations from Northern America and from Argentina, which may be related to a genetic predisposition to develop the disease.[3,7] The present study evaluated children of different geographical origins within Argentina but did not assess ethnicity. However, the ethnic-specific condition of this Argentinean population related to the frequency of MBL variants would account for the findings observed in this report.

In summary, we found that polymorphisms resulting in MBL insufficiency were more frequent in children with PIBO than in healthy controls. This condition was significantly associated with more severe initial disease, illustrating the relevance of innate immune defence factors prior to the maturation of the adaptive immune system.

Ethnic-specific conditions might contribute to explaining these findings.

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