



**La vía de las lectinas en lupus y tuberculosis: polimorfismos de la MBL2 y MASP2, beneficiosos o perjudiciales/ The lectin pathway in lupus and tuberculosis: MBL2 and MASP2 polymorphisms, beneficial or harmful**

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## **1. Abbreviations**

AZA, Azathioprine

CC, Corticosteroids

HHC, household contacts

MASP, Mannan Binding Lectin Serine Peptidase

MBL, Mannose Binding Lectin

MMF, mycophenolate mophetil

MTB, Mycobacterium Tuberculosis

MTX, Methotrexate

na, non available

ns, non significant

SLE, Systemic Lupus Erythematosus

SNP, Single Nucleotid polymorphisms

TB, tuberculosis



## 2. Abstract

Introduction: MBL is a serum protein that activates de complement and mediates phagocytosis. MBL levels and MBL2 genotypes may impact upon host susceptibility to TB or SLE, but evidence to date has been conflicting. No studies have been reported analyzing MASP2 polymorphisms and MASP-2 levels in TB and only one in SLE.

Methods: MBL2 exon 1 and promoter genotyping and serum MBL concentrations were determined in a cohort of TB patients (79) an HHC (120) and in a cohort of SLE cases (39) and healthy controls (59). Moreover MASP-2 serum levels and 5 polymorphisms in the MASP2 gene (Thr73Met, Arg84Gln, Arg103Cys, Asp105Gly, Pro111Leuc) were analyzed in 49 TB patients and 50 HHC and in the same SLE cohort and healthy controls as before. Most of the participants were Caucasians.

Results: Diplotype *LXPA/HYPA* producer of high levels of MBL was significantly more frequent in HHC than in patients (16.8% vs 6.4%,  $p=0.03$ ). Significantly higher serum MBL levels were found in patients with active TB than in HHC (median MBL concentrations 3420ng/mL [10-28415] and 2600 ng/mL [5-20000] respectively,  $p=0.02$ ). This higher MBL levels were mostly related to the most prevalent *YA/YA* wild-type diplotype.

In the SLE study *LYQC/HYPD*, *LXPA/LYQC* and *LYPB/HYPD* were only found in the SLE patients, and all of them related to severe MBL deficiency (<100ng/mL). SLE patients showed a trend to more severe MBL deficiency compared to the control group (25.6% and 13.6% respectively,  $p=0.19$ ). There was a strong correlation between MBL2 Exon 1 and promoter genotype and MBL levels in the overall participants.

Asp105Gly MASP2 variant was the only one detected in the TB and in the SLE study. No relation was found between the presence of the allelic variant and the development of TB or SLE. No differences were observed in MASP-2 levels or moderate or severe

MASP-2 deficiency among TB patients and HHC. There was a tendency towards a higher frequency of severe MASP-2 deficiency in SLE patients compared to the control group (2/0).

Conclusions: the high MBL-producer diplotype *LXPA/HYPA* was significantly more frequent in HHC than in TB patients suggesting a protective role against the development of TB disease, which was not previously described. SLE patients had a trend towards more severe MBL deficiency than healthy controls and some of the low-MBL producer genotypes were only found in SLE patients suggesting that low MBL levels and MBL2 variants could be a risk factor for the development of SLE. Asp105Gly MASP2 variant was the only one detected among all the participants. Further studies are needed to understand the role of MBL and MASP in TB and SLE susceptibility.

### 3. Summary in Spanish

Introducción: MBL es una proteína sérica que activa el complemento y participa en la fagocitosis. Los niveles de MBL y los genotipos de MBL2 podrían interferir, dependiendo de la susceptibilidad del huésped, en el desarrollo de tuberculosis o lupus eritematoso sistémico, aunque los resultados son controvertidos. Aún no hay estudios publicados que analicen la relación entre los polimorfismos de MASP2 y los niveles de MASP-2 con tuberculosis y únicamente uno con lupus eritematoso sistémico.

Métodos: se determinaron los genotipos del exon 1 y del promotor del MBL2 y las concentraciones de MBL en una cohorte de pacientes con tuberculosis (79) y en contactos sanos (120), así como en una cohorte de pacientes con lupus (39) y controles sanos (59). Además se analizaron los niveles de MASP-2 y 5 polimorfismos del gen MASP2 (Thr73Met, Arg84Gln, Arg103Cys, Asp105Gly, Pro111Leuc) en 40 pacientes con tuberculosis y en 50 contactos sanos y en la misma cohorte de pacientes con lupus y sanos analizada previamente. Casi todos los participantes eran caucásicos.

Resultados: *LXPA/HYPA* productor de bajos niveles de MBL fue significativamente más frecuente en los contactos sanos comparado con los pacientes con tuberculosis (16.8% vs 6.4%,  $p=0.03$ ). Pacientes con tuberculosis activa comparado con controles sanos tuvieron significativamente niveles más altos de MBL (3420ng/mL [10-28415] and 2600 ng/mL [5-20000] respectivamente,  $p=0.02$ ). Estos niveles altos de MBL se relacionaron sobre todo con el diplotipo más prevalente *YA/YA*.

En el estudio de lupus *LYQC/HYPD*, *LXPA/LYQC* y *LYPB/HYPD* solo se detectaron en pacientes con lupus y todos ellos relacionados con niveles muy bajos de MBL. Pacientes con lupus mostraron una tendencia a presentar más déficit grave de MBL comparado con los controles sanos (25.6% y 13.6% respectivamente,  $p=0.19$ ). En los

dos estudios se obtuvo una clara relación entre los genotipos del exon 1 y del promotor de la MBL2 y los niveles de MBL.

La variante Asp105Gly del gen MASP2 fue la única que se encontró entre los participantes de los dos estudios. No se obtuvo relación entre la presencia de esta variante alélica y el desarrollo de tuberculosis o lupus. No se obtuvieron diferencias entre los niveles de MASP-2 o el déficit moderado o grave de MASP-2 y los pacientes con tuberculosis o contactos sanos. Sin embargo si se vió una tendencia a mayor déficit grave de MASP-2 en el grupo de pacientes con lupus comparado con los sanos (2/0).

Conclusiones: el diplotipo *LXPA/HYPA*, productor de altos niveles de MBL fue significativamente más frecuente en los controles sanos, comparado con los pacientes con tuberculosis sugiriendo una función protectora frente a la tuberculosis, no descrito previamente. Pacientes con lupus mostraron una tendencia a mayor déficit grave de MBL comparado con controles sanos y determinados genotipos productores de bajos niveles de MBL solo se encontraron en pacientes con lupus, por lo que podría ser un factor de riesgo para desarrollar lupus. La variante Asp105Gly del MASP2 fue la única detectada. Más estudios son necesarios para entender el papel de las MBL y de las MASP en la tuberculosis y en el lupus.

#### **4. Introduction**

In mammals, the innate immunity is permanently active to preserve the integrity of the host organism. Innate immunity constitutes the first line defence against pathogens. It is required to generate a specific adaptive immune response and participates in remodelling tissues, in angiogenesis and in clearance of apoptotic or necrotic cells. The innate immune system's key components are phagocytes and natural killer cells, natural antibody production, cytokines and other soluble factors, and the complement system. Complement activation can occur through the classical, the alternative or the lectin pathway, and mainly functions in pathogen opsonization, chemotaxis, activation of phagocytes, and direct pathogen lysis through the formation of the membrane attack complex [1].

MBL is an important component of the innate immune system. It has four distinct functions: activation of the lectin-pathway of the complement system, direct promotion of opsonophagocytosis independent of complement activation, modulation of the inflammatory response, and promotion of apoptosis. Because of its clinical role, MBL has been the focus of interest over the past 20 years, primarily through epidemiological studies. Susceptibility to and the course of different types of infections as well as autoimmune, metabolic and cardiovascular diseases may be influenced by genetically determined variations in MBL serum concentrations. [2]. Defects in MBL could entail a deficient clearance of apoptotic material, thus increasing exposure to autoantigens that drive the production of autoantibodies, and further tissue inflammation [2-4].

##### **a) MBL genetics**

MBL production is controlled by the MBL2 gene that lies in chromosome 10, and polymorphisms of the structural regions of the gene or its promoter have been

associated with relative or absolute serum MBL deficiencies [2]. Three mutations in exon 1 of the MBL2 gene are currently known: codon 52 (rs5030737; C>T; Arg>Cys), called D variant, codon 54 (rs1800450; G>A; Gly>Asp), called B variant, and codon 57 (rs1800451; G>A; Gly>Glu), called C variant. B, C and D variants are referred to collectively as O, while A is referred to as the wild-type. Individuals with the wild-type genotype (A/A) have generally high MBL levels; heterozygotes A/O have about 10% of wild-type serum concentrations of MBL, and homozygotes O/O have very low or absent MBL levels [5]. Other polymorphisms in the promoter 1 region modulating MBL levels have been identified at position -550 (rs11003125; G>C; polymorphism H/L) and -221 (rs7096206; C>G; polymorphism X/Y) [6], and a P/Q variant has been identified in a 5' untranslated region at position +4 (C>T, rs7095891) [7]. Because of a strong linkage disequilibrium between the polymorphisms which are present in the promoter and the structural variants in exon 1 of the MBL2 gene, only seven common haplotypes have been described (*HYP A*, *LYP A*, *LXP A*, *LYQ A*, *HYP D*, *LYP B*, and *LYQ C*) and there are therefore 28 possible diplotypes, the frequency of which varies among populations [6]. The combination of exons and promoter polymorphisms results in up to 1000-fold variations in MBL concentrations in different individuals. Among haplotypes carrying the wild-type A allele, *HYP A* and *LYQ A* are related to high MBL levels, *LYP A* is related to medium to low levels, and *LXP A* is related to low serum MBL levels [5, 7,8].

b) The impact of MBL levels on infections

Low serum MBL has been described as the world's most common immune deficiency. MBL deficiency typically manifests as recurrent infections of the respiratory tract in the paediatric population aged 3 months to 2 years. MBL deficiency in adults may be important during the initial stages of an infection before the specific immune response is activated, and this defect may become more relevant in association with an

additional immune defect such as systemic lupus erythematosus, chemotherapy-induced neutropenia, AIDS or cystic fibrosis [9]. An association between MBL deficiency and susceptibility to and severity of a wide range of infectious diseases (Mycoplasma infection, hepatitis B and C, HIV infection, schistosomiasis [10], and pneumonia [11]) has been described in adults, but results are controversial.

Ethnic differences have also been studied, and it has been observed that low MBL level-related genotypes are present in 10% of Caucasians and in up to 40% of Africans [7,12,13]. This has been related to the possibility that low MBL levels could protect against intracellular infections like malaria or tuberculosis [9,13,14]. This can be explained analyzing MBL activity. MBL binds to specific carbohydrate structures on various pathogens, including MTB. Together with the MASP, MBL activates the lectin pathway of the complement, opsonizing and facilitating the phagocytosis of microorganisms by macrophages. Thus, low MBL serum levels could confer resistance against mycobacteria by impairing opsonization. Some investigators have suggested that low MBL producer genotypes could be protective against developing TB disease [14-18], but other studies and a recent Chinese meta-analysis [19,20] have concluded that the exon1 mutant B allele carriers, who are low MBL producers, have an increased risk of TB.

#### c) The impact of MBL levels on SLE

The role of the MBL pathway in complement activation and in the clearance of apoptotic cells suggests that genetic variability in MBL may be involved in the pathogenesis of SLE. SLE is a multifactorial disease and susceptibility is related to genetic, hormonal, immunological, and environmental factors. A strong genetic link has been identified through the use of genome-wide association and family studies, and more than 30 SLE related loci have already been identified [21].

On the one hand, previous studies have shown an association between MBL deficiency and SLE, SLE severity, renal disease [2,22,23,], infections [24], thrombosis, and antiphospholipid syndrome [23], while others have found an association restricted to European-derived patients [8,25], and a meta-analysis showed that MBL variant alleles were a significant SLE risk factor [22]. On the other hand, a study in Spain did not find a significant association between SLE and MBL deficiency [26]. There have been few MBL and SLE studies in the Spanish population [1,23,26].

#### d) MASP-2 genetics

Complement activation of the lectin pathway is triggered by the binding of MBL to the surface carbohydrates of different microorganisms. This leads to the cleavage of C4 and C2, and the formation of the C3 convertase (C4b2a), the opsonization and phagocytosis of target microorganisms, and the formation of the membrane attack complexes. In human plasma, MBL is found in association with four structurally related proteins: MASP-1, MASP-2, MASP-3 (the MBL-associated proteases) and MAp-19. MASP-2 is believed to be the most important for complement activation and generation of the C4b2a [27, 28]. Polymorphisms of the MASP2 gene are known to cause impaired complement activation [29], due to the impossibility of forming a complex between MASP-2 and MBL [30].

An inherited MASP-2 deficiency was described for the first time in 2003. The patient was homozygous for the Asp105Gly mutation in exon 3 of the MASP2 gene in the CUB1 domain, which led to a reduction in MASP-2 concentrations and interrupted the lectin pathway of the complement activation. This patient had a history of infections and inflammatory diseases [30].

One study suggested a low clinical penetrance of the MASP-2 deficiency as there are healthy subjects with a MASP-2 deficiency without infections, autoimmune or



inflammatory disorders [15]. Despite its low penetrance, MASP-2 deficiency was included in the list of primary immunodeficiencies and it has been associated with SLE and infectious diseases [31].

Other different single nucleotide polymorphisms have been described in exon 3 of the MASP2 gene among individuals from Africa [28], Europe and Brazil [32]: Pro111Leu, Arg84Gln, Thr73Met and Arg103Cys [28,33], but none have been related to MASP-2 deficiency.

e) The impact of MASP-2 levels on infections

The impact of MASP-2 levels on the development of infections is controversial. Following allogeneic stem cell transplantation, a cohort of adult patients heterozygous for Asp105Gly MASP2 variant was found to have an increased risk of invasive aspergillosis, but not of bacterial or viral infections [33]. Other studies have analyzed the relationship between the same MASP2 variant and community-acquired pneumonia [11], pneumococcal bacteraemia in HIV-infected patients [35], renal transplant recipients with infectious complications [36] or patients with sepsis [33], finding no differences. No studies have been published in tuberculosis.

f) The impact of MASP-2 levels on SLE

MASP-2 data in inflammatory disorders are scarce and conflicting. A study related Asp105Gly with inflammatory diseases [30], but another study in a Spanish cohort did not find a relationship between this allelic variant and the development of SLE [15]. Recently, another study found lower MASP-2 levels in rheumatoid arthritis patients than in healthy controls [37].

## **5. Objectives**

The objective of this study is to analyze the relationship between MBL2 genotypes, including exon 1 and promoter polymorphisms, MBL serum levels, MASP2 polymorphisms and MASP-2 levels, and the susceptibility to develop:

- TB disease, in a cohort of TB patients and healthy house-hold contacts of these TB patients.

- SLE, SLE-specific clinical manifestations, and severe disease in a cohort of SLE patients and a healthy control group.

## **6. Patients, material and methods**

### **6.1. Patients**

#### a) Participants of the TB study

Spanish patients with active TB disease, attending the TB Unit in Son Llàtzer Hospital in Palma de Mallorca during two periods (January 2007-December 2008 and January 2010-December 2012) were included in the MBL study and only those from the second period were included for the MASP analysis. TB diagnosis was confirmed by the growth of MTB in culture or by clinical, radiological and histological findings with improvement with the anti-tuberculous therapy. Patients with pulmonary TB, extra-pulmonary TB (one extrapulmonary organ affected) and miliary TB (with lung and at least another viscera affected) were recruited. In addition, HHC-persons genetically related or not to the patient, who at the time of the diagnosis of tuberculosis, had cohabited with the patient for at least two months, evaluated at our unit, were included in the study; none of them had previously received isoniazid treatment nor had developed TB disease. A tuberculin-skin test was done to all the participants [38].

#### b) Participants of the SLE study

Patients visited between 2010 and 2012 at the Son Llàtzer Hospital in Palma de Mallorca with a diagnosis of SLE that fulfilled American Rheumatology SLE criteria [39] and a control group of healthy volunteer blood donors were included in the study. A code was assigned to each participant, and demographic, clinical and analytical data (cell blood count, complement, antiDNAantibodies and urinary sediment), as well as Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [40] at inclusion were entered into an anonymous database. A subgroup of Caucasian subjects was performed.

## 6.2. Material and methods

Demographical and epidemiological data were obtained from the medical records. The study was approved by the Ethics Committee of the Balearic Islands and all participants provided written informed consent.

### a) Blood sampling protocol

The blood was aseptically collected into plain and ethylenediaminetetraacetic acid tubes. For all samples, serum was separated immediately and transferred into cryovials and preserved at -80°C for further testing.

### b) MBL quantification

MBL serum concentrations were determined by enzyme-linked immunosorbent assay (ELISA) performed in microwells coated with a monoclonal antibody against the MBL carbohydrate-binding domain in a commercial kit (oligomerized mannan-binding lectin; AntibodyShop®, Gentofte, Denmark). MBL serum concentrations were expressed as ng/mL.

### c) MBL2 SNP genotyping

EDTA blood samples were used for genomic DNA isolation. Genomic DNA was extracted using the Maxwell® 16 Blood DNA Purification Kit (Promega, United States). Six SNP in the MBL2 gene (-550 G/C [rs11003125], -221 C/G [rs7096206], +4 C/T [rs7095891], codon 52 CGT/TGT [rs5030737], codon 54 GGC/GAC [rs1800450], and codon 57 GGA/GAA [rs1800451]) (GenBank accession numbers AF360991) were analysed. Genotyping was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP). Polymorphisms within the promoter and exon 1 of the MBL2 gene were analysed, using primer pairs spanning from the promoter region through exon 1. The primers used were as follows: MBL2-1F - GGGAATTCCTGCCAGAAAGTAGAG, MBL2-1R GGA-TCCTAAGGAGGGGTTTCATCT, MBL2-2F - ACTAGTCACGCAGTGTCAAGGA, and MBL2-2R - CAGGCAGTTTCTCTGGAAGGTA. In

short, 50 ng of genomic DNA were amplified in a 50  $\mu$ L volume of reaction mixture containing 1 $\times$  PCR buffer, 2.5 mM of MgCl<sub>2</sub>, 0.2 mM of dNTPs, 0.5 mM of each primer, and 1 U GoTaq® Hot Start Polymerase (Promega, United States) on a T1 Thermocycler (Biometra, Germany). Thermal cycling parameters for amplification of both regions were 95°C for 10 minutes (initial denaturation); 40 cycles of 30 seconds at 95°C (for denaturation), 30 seconds at 55°C (for annealing), 45 seconds at 72°C (for extension); and 10 minutes at 72°C (for final extension). PCR products were cleaned up by use of Exela Pure 96-Well UF Plate (EdgeBio, United States), and 1  $\mu$ L of the purified product was directly used as a template for sequencing, using the BigDye terminator v. 3.1 cycle sequencing kit (Applied Biosystems, United States) on an ABI 3730XL DNA sequencer, following the manufacturers' instructions. Polymorphisms were identified by assembling the sequences with respective reference sequences (NG\_008196.1), using Sequencer, version 5.0 (available at: <http://genecodes.com/>), and were reconfirmed visually from their respective electropherograms.

#### d) MASP-2 quantification

MASP-2 serum concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) performed in microwells coated with a monoclonal antibody against MASP-2 in commercial kits (oligomerized mannan-binding lectin; AntibodyShop®, Gentofte, Denmark and MASP2 ELISA kit, Cusabio, E-17966H), following the manufacturers' instructions. MASP serum concentration was expressed as ng/mL.

Low MASP-2 levels were defined as a concentration  $\leq 75$  ng/mL, intermediate levels as a concentration  $>75$  ng/mL and  $\leq 200$  ng/mL and high levels as a concentration  $> 200$  ng/mL [41].

#### e) MASP2 SNP genotyping

EDTA blood samples were used for genomic DNA isolation. Genomic DNA was extracted using the Maxwell® 16 Blood DNA Purification Kit (Promega, United States). For the MASP2 genotype, genotyping was performed by polymerase chain reaction with sequence-specific primers (PCR–SSP). Five SNP in the MASP2 gene (Thr73Met [rs61735601], Arg84Gln [rs61735600], Arg103Cys, Asp105Gly [rs72550870], Pro111Leu [rs56392418]) were analyzed. The primers used were as follows: MASP2-1F GCCTCTACTTCACCCACTTCGAC, MASP2-1R GCCTGGCCTAAGACAGAGTTACC. In short, 50 ng of genomic DNA were amplified in a 50 µL volume of reaction mixture containing 1× PCR buffer, 2.5 mM of MgCl<sub>2</sub>, 0.2 mM of dNTPs, 0.5 mM of each primer, and 1 U GoTaq® Hot Start Polymerase (Promega, United States) in a T1 Thermocycler (Biometra, Germany). Thermal cycling parameters for amplification of both regions were 95°C for 10 minutes (initial denaturation); 40 cycles of 30 seconds at 95°C (for denaturation), 30 seconds at 55°C (for annealing), 45 seconds at 72°C (for extension); and 10 minutes at 72°C (for final extension). PCR products were cleaned up by use of Exela Pure 96-Well UF Plate (EdgeBio, United States), and 1 µL of the purified product was directly used as a template for sequencing, using the BigDye terminator v. 3.1 cycle sequencing kit (Applied Biosystems, United States) on an ABI 3730XL DNA sequencer, according to the manufacturers' instructions. Polymorphisms were identified by assembling the sequences with respective reference sequences (NG\_007289.1), using Sequencer, version 5.0 (available at: <http://genecodes.com/>), and were reconfirmed visually from their respective electropherograms.

### **6.3. Statistical analysis**

First, a descriptive analysis of the different populations of the study was performed. Qualitative data are expressed as percentages. Quantitative data are expressed as mean (+/- SD) or median and range.

Means are compared by t-test (or Mann Whitney U-test if the sample does not have a normal distribution). Diplotype frequencies were compared between cases and controls by  $X^2$  test or Fisher's exact test when necessary. P values with Yates correction and odds ratio (OR) with 95% confidence interval were calculated. Statistical significance was taken as a p-value less than 0.05. The Pearson correlation coefficient was calculated to measure the correlation between MBL and MASP-2 levels. The analysis was carried out with SPSS 13.0 and EPIDAT 3.1 software.

## 7. Results

### 7.1. MBL in TB

Seventy-nine patients with the diagnosis of active TB and 120 HHC were recruited. Due to technical problems, MBL levels were available in 73/79 TB patients and 104/120 HHC and complete genotyping results were obtained in 76/79 patients and in 106/120 HHC.

Median age was 45 (18-84) years in the TB patients and also 45 (18-94) years in the HHC. Fifty-nine TB patients (74.7%) were men. Fifty-eight (73.4%) had pulmonary TB and 21 (26.6%) extrapulmonary or miliary TB. Eighty-four (70%) of the HHC had positive TST test.

#### a) MBL2 exon 1 genotype frequencies

All the structural genetic variants of the MBL gene were within the range of Hardy-Weinberg equilibrium. The most frequent allele found in both groups was the wild type allele A/A. The complete MBL2 genotype frequencies are described in table 1.

#### b) MBL2 complete (exon 1 and promoter) diplotypes

The results are shown in table 2. *HYP A* was the most frequent haplotype (91/182 subjects), followed by *LXPA* (83/182 subjects) and *LYQA* (70/182 subjects). *HYA* haplotype was present in 36/76 (47.4%) in the TB cases and in 54/106 (50.9%) HHC, finding no statistical significant difference. *LYQA/HYP A* was the most frequent diplotype among TB patients and median MBL levels among these patients were high (6644ng/mL (1660-20000)). *LXPA/HYP A* was significantly more frequent in HHC (table 2) and it was also associated with high MBL levels, 3600n/mL (1000-12800). *LYQA/HYP A* was present in 12/57 (21.5%) pulmonary TB cases, 1/8 (12.5%) miliary TB and in none of the cases of extrapulmonary TB.



c) MBL levels

Comparing MBL levels in TB patients and HHC it was significantly higher in TB patients (3460ng/ml [10-28415] and 2570 ng/ml [5-20.000] respectively,  $p=0.002$ ). Patients with pulmonary TB had significantly higher MBL levels compared to patients with extrapulmonary TB (3980 ng/mL [20-28415] and 1100 ng/mL [10-8000] respectively,  $p=0.02$ ). On the other hand, MBL levels did not differ significantly, when comparing pulmonary and miliary TB (miliary TB 2197 ng/mL [55-12800],  $p=0.3$ ).

d) MBL2 genotype and MBL levels

There was a strong correlation between MBL2 Exon 1 and promoter genotype and MBL levels (Figure 1). For simplification purposes, only X/Y polymorphism from the promoter region was considered. Numerical results are described in table 3.

e) HIV infection

Eighteen of 79 TB patients (22.8%) were infected with HIV and 3 of 120 (2.5%) among the HHC. MBL levels in HIV TB patients and non HIV patients did not differ significantly (2353.5 ng/mL [20-28415] and 3800 ng/mL [10-20000] respectively,  $p=0.87$ ). MBL2 genotyping could be done only in 16 patients and there were no significant differences in complete diplotype depending on HIV status.

## 7.2. MBL in SLE

Thirty-nine SLE cases and 59 healthy controls were included. There were 37 (94.9%) women in the SLE group and 55 (93.2%) in the control group. Gender distribution was the same in both groups. All the controls were Caucasians (58 from Spain, one from Germany). Among the cases, there were 35 Caucasians (28 Spanish, one Irish, one Polish, three Argentinian, one German and 1 Brazilian), three Nigerian and one Bolivian.

A descriptive analysis of SLE disease activity of the SLE patients was done (Table 4).

MBL levels could not be determined in one of the cases due to technical problems.

a) MBL2 Exon 1 frequencies and allele B

All the structural genetic variants of the MBL gene were within the range of Hardy-Weinberg equilibrium.

There were no statistical significant differences between MBL 2 genotypes in SLE patients compared to controls (Table 5). Allele B was analysed in SLE patients compared to controls, 16 [(33.30%)] and 14 [(23.70%)] respectively, but no statistical significant result was found.

Considering exclusively Caucasian subjects, the wild-type allele was more frequent in the control group compared to SLE patients (41 [69.5%] vs. 18 [51.4%] respectively) but without reaching statistical significance  $p=0.1$ . Allele B was more frequent in the SLE group compared to the controls (13 [37.1%] and 14 [23.7%] respectively), but without reaching statistical significance ( $p=0.25$ ).

b) MBL 2 complete (exon 1 and promoter) diplotypes

MBL 2 diplotype frequencies for both SLE and control subjects are shown in Table 6.

*HYP A* and *LXPA* were the most frequent haplotypes (44/98 subjects respectively), followed by *LYQA*, in 40/98 subjects. *LYQA/HYP A* was the most frequent diplotype in SLE cases and in controls (6/39 [15.4%] and 12/39 [20.2%] respectively) with a median MBL level of 4160 ng/mL (2520-5040) in cases and 3570 ng/mL (1660-10194) in controls. The following diplotypes were only detected in patients with SLE:

*LYQC/HYPD* (one case), *LXPA/LYQC* (one case) and *LYPB/HYPD* (two cases), and all of them had very low MBL levels (20, 20, 10 and 2 ng/mL respectively).

c) MBL levels

Median MBL level in SLE patients and controls was 1675ng/mL (2-9726) and 2320 ng/mL (4-11534) respectively, showing no statistically significant difference (p=0.62). The relationship between gender and MBL levels is shown in table 7.

Very low MBL levels ( $\leq 100$  ng/mL) were observed in 18 subjects; 10 cases (25.6%) and 8 controls (13.6%) respectively (p=0.19). Levels  $> 1000$  ng/mL were found in 22 (57, 9%) SLE patients and 41 (69.5%) healthy controls (p=0.34).

In the Caucasian subjects, severe MBL deficiency was found in 9 cases (25.7%) and 8 controls (13.6%) respectively, finding no statistically significant difference (p=0.2).

Levels  $> 1000$  ng/mL were found in 19 cases (54.3%) and 41 controls (69.5%) (p=0.27).

As in the overall group, it was not possible to find a statistically significant difference in median MBL levels by comparing cases and controls.

d) MBL 2 genotype and MBL levels

There was a correlation between exon 1 and promoter MBL 2 genotype and MBL levels (Figure 2 and table 8).

In the Caucasian subgroup there was no statistically significant difference. Analysing together cases and controls, wild-type genotype *YA/YA* showed very high MBL levels ( $> 1000$  ng/mL) except for one patient, who had a moderate deficiency, and genotype *O/O* showed very low levels ( $< 100$  ng/mL). The same results were found in the subgroup of Caucasian participants.

e) MBL levels and SLE activity, SLE manifestations, reactant phase proteins, and complement (CH50, C3, C4) levels.

SLE clinical manifestations depending on MBL2 polymorphisms are shown in Table 9.

There was no correlation between SLE clinical manifestations and exon 1 variant alleles (*A/O* or *O/O*), nor could we find a correlation between MBL levels and reactant phase proteins (erythrocyte sedimentation rate or C- reactive protein [CRP]). Complement deficiencies (C3, C4 and CH50) were analysed together and one by one to search for a relationship with MBL genotypes (*A/A* versus *A/O* or *O/O*) and no statistically significant difference was found.

f) MBL levels and drugs

Thirty (76.9%) SLE patients were receiving immunosuppressive drugs. The different treatments are represented in Figure 3. There was no relationship between MBL levels and the use of immunosuppressive drugs.

### 7.3. MASP in TB

Forty-nine TB patients and 50 HHC were included. Mean age was 48.6 (14.9) in TB patients and 47.9 (14.4) in HHC, finding no statistical significant difference. There were significantly more males among cases than controls, 40 (81.6%) and 22 (44%) respectively,  $p = 0.0003$ , OR 5.66, CI 2.27-14.1. Toxic consumption (tobacco and heavy alcohol) was detected in 10 (20.4%) patients and 12 (24%) contacts, finding no statistical significant difference. Seven-teen (34.7%) TB patients and 5 (10%) HHC were infected with HIV,  $p = 0.0031$ . Four (8.2%) patients and 6 (12%) HHC were diabetic finding no statistical significant difference. Only 2 (4.1%) TB patients had a hematologic or solid neoplasia and none of the HHC. Pulmonary TB was diagnosed in 39 (79.6%) patients, in 8 (16.3%) patients miliary TB and in 2 (4.1%) lymphadenitis. MASP2 genotype could not be determined in 6 participants (3 patients and 3 controls).

a) MASP2 polymorphisms

No statistical significant differences were found in MASP2 genotype between TB patients and HHC. The allelic variant Asp105Gly was found in two participants. The first participant was a patient with TB and HIV infection, 262.6 ng/mL was the MASP-2 level and 4000 ng/mL the MBL levels. The second one was a healthy HHC, with 129.8ng/ml as MASP-2 level and 100ng/ml as MBL level. The allelic frequency was 1.07. The other allelic variants (Thr73Met, Arg84Gln, Arg103Cys, Asp105Gly or Pro111Leu) were not found in any of the participants.

b) MASP-2 levels

MASP-2 levels were determined in all the participants. Globally, median MASP-2 levels were 235.09 ng/mL [67. 1- 532.9] and median MBL levels (in this part) were 2260.0 ng/mL [4.0-9400.0]. There was a weak negative correlation between MASP and MBL levels ( $r: -0.213$ ). No gender-related statistical significances were observed in MASP-2 levels. Mean MASP-2 levels in TB patients was 231.1ng/ml (83.6) and 270.3 (101.1) in HHC,  $p=0.063$ . Excluding HIV-infected patients, there was a trend towards lower MASP-2 levels in TB patients compared to HHC: 226 ng/mL (86.8) and 270.7 ng/mL (104.3) respectively,  $p=0.053$ . Mean MASP-2 levels in pulmonary TB were 236.5ng/ml (83.8) and 248.1 (87.3) in miliary TB, without reaching statistical significance ( $p=0.72$ ). Normal MASP-2 levels were found in 66 participants (66.67%) and moderate MASP-2 deficiency in 33 (33.33%). MASP-2 levels related to TB disease, HIV status or MBL deficiency are shown in table 10. A severe MASP-2 deficiency ( $<75\text{ng/mL}$ ) was found in 1 (2.04%) patient with TB and high MBL levels (3800ng/mL).

#### 7.4. MASP in SLE

Thirty-nine SLE cases and 59 healthy controls were included, the same as in the MBL study. There were 37 (94.9%) women in the SLE group and 55 (93.2%) in the control group.

##### a) MASP2 polymorphisms

The allelic variant Asp105Gly in the MASP2 gene was found in 5 controls (8.47%), namely Spanish Caucasians, and was absent in the SLE group. All of them were heterozygous (Table 11). Thus, it was detected among Caucasians with an allelic frequency of 2.66%.

As there was no mutated allele in the SLE group, its relationship with the severity of the disease could not be analyzed.

The allelic variants Thr73Met, Arg84Gln, Arg103Cys and Pro111Leu were absent in the overall group of subjects.

##### b) MASP-2 levels

Overall mean MASP-2 level was 287.94 ng/mL (SD 183.60). Mean MASP-2 level in SLE patients was 333.12 ng/mL (SD 265.71) and 258.08 ng/mL (SD 88.55) in controls ( $p = 0.08$ ). No statistically significant difference was found when MASP-2 levels were compared between subjects heterozygous for Asp105Gly and those with the wild-type genotype (220 ng/mL [SD 44.6] and 291 ng/mL [SD 187.6] respectively,  $p = 0.3$ ).

MASP-2 levels above 200ng/mL were found in 69 (70.4%) participants, 27 (69.2%) cases and 42 (71.2%) controls ( $p = 0.96$ ). Twenty seven (27.6%) participants had moderate MASP-2 levels, 10 (25.6%) SLE patients and 17 (28.8%) healthy controls

( $p=0.9$ ). Two participants (2.04%) were found to have very low MASP-2 levels, both SLE patients.

When comparing MASP-2 level according to ethnicity, mean MASP-2 in Caucasians was 284.64 ng/ml (SD 185.89) and 365.42 ng/ml (SD 101.77) in non Caucasians,  $p= 0.21$ .

## 8. Discussion

In our study we evaluated MBL levels and a complete MBL2 genotypic profile including promoter polymorphisms and MASP-2 levels and MASP2 polymorphisms in Spanish TB patients and in HHC, exposed to MTB but who have not developed the TB disease and in SLE patients and healthy controls. Globally *LXPA* and *HYP A* were the most frequent haplotypes in both parts of the study. The diplotype *LXPA/HYP A* was significantly more frequent in HHC than in TB patients, suggesting a protective role against the development of TB disease. Dipoypes that were only found in SLE patients (*LYQC/HYPD*, *LXPA/LYQC* and *LYPB/HYP*) were associated with very severe MBL deficiency, suggesting a higher risk to develop SLE.

There were no statistically significant differences in MASP 2 levels or in the presence of the allelic variant between TB patients and HHC or SLE patients and the control group. When HIV infection was excluded, a trend towards lower MASP-2 levels was observed in TB patients compared to HHC. A tendency towards a higher frequency of severe MASP-2 deficiency among SLE patients compared to the control group (2/0) was found. No other allelic variants studied could be found among the cohort.

### a) MBL in TB

According to other studies [42,43], *LXPA* and *HYP A* were the most frequent haplotypes. The biological meaning of the finding that the high MBL producer genotype *LXPA/HYP A* was significantly more frequent among HHC than in TB patients, described for the first time in literature, cannot be related to differences in serum MBL because the *HYP A* haplotype has been related to high serum MBL levels [5]. One explanation could be a linkage disequilibrium among this alleles and other genes in chromosome 10, such as *MRC1*, that encodes for mannose receptor C-Type lectin, that have been suggested to be associated with altered susceptibility to mycobacteria [44].



A previous study associated HYPA haplotype also with protection against another infection as schistosomiasis in Nigeria [10]. In one study developed in Spain [43], no diplotype was associated with TB; however HYPD haplotype was more frequent in the control group compared to TB patients.

Analyzing MBL levels and according to other studies [16,45,46], we found significantly higher serum MBL levels in TB patients, especially those with pulmonary TB, compared to HHC. It is mainly related to the most prevalent diplotype, wild-type YA/YA. It has been previously described, that high MBL levels are mostly related to the wild-type MBL2 genotype A/A or YA/YA [17, 47], but we could not demonstrate that the wild-type genotype was more frequent in patients than in HHC. On the contrary, we could not find that low MBL2 producer genotypes were more frequent among HHC conferring TB protection. Some studies suggest that low MBL producer genotypes could confer protection against TB [14-18], however data are contradictory; a Chinese article [19] and a recent Chinese meta-analysis [20] find that Exon 1 mutant Allele B carriers, related to low MBL levels, have an increased risk of developing TB. According to this results another study with an Italian population described, that the wild-type haplotype HYA (a high producer haplotype), could be protective against pulmonary TB [48]. Moreover, this haplotype *HYA* has been associated with reduced risk of bacterial, viral or fungal infection in allogenic hemopoietic stem cell transplantation [49]. However, in our study HYA haplotype was not more frequent among the HHC. As explained, there is controversy and no consistent association between MBL2 genotype and TB infection can be determined, what has been confirmed in a recent meta-analysis [45]. This analysis was limited by the heterogeneity in the designs of the included studies and most of it did no report a complete MBL2 genotyping.

Pulmonary TB has been related in some studies with higher MBL levels compared to extrapulmonary TB [16], different to our study, where no statistical significant differences were found. High MBL producer genotype was only found in pulmonary TB cases and not in the extrapulmonary TB patients, thus, the relationship cannot be excluded. Moreover, pulmonary TB has been related to higher expression of acute phase reactants and proinflammatory cytokines as interferon gamma compared to extrapulmonary TB [50]. Thus, it could be interesting to determine MBL levels in the TB patients at the end of the treatment to analyze its role as an acute-phase reactant.

In HIV positive TB patients a significantly higher frequency of YA/YA diplotype associated with very high MBL levels has been described [51] suggesting that MBL deficiency could be a protector factor against TB, as in other studies [52]. However, maybe related to the small sample of HIV patients in our study, we could not find any relation between HIV status and MBL levels or MBL2 genotype.

#### b) MBL in SLE

As in the TB study *HYP A* and *LXP A* were the most frequent haplotypes, which is in agreement with previous studies in the general European population, including a study in Caucasians (Spain) [1,42,43]. In the same way, the wild-type genotype *A/A* was the most frequent genotype in both, the general population and the SLE patients [2]. Several studies have suggested that genotypes related to very severe MBL deficiency are a risk factor for developing SLE in different ethnic populations [22,53]; however, the majority of previous studies did not report a complete MBL2 genotyping, promoter allelic variants were rarely analysed [54-56] and results are contradictory. An association between allele B in codon 54 of exon 1 and SLE has been extensively described [22,54,57]. In a recent study in Indian SLE patients, it was found that

genotypes producing low MBL levels, especially the presence of allele B, but also with allele X at position -221, were significantly more frequent in SLE patients compared to healthy participants [54]. A meta-analysis confirmed identical results and added allele L at position -221 as a risk factor for developing SLE [22]. In our study we also found in Caucasians a tendency to a higher incidence of allele B in the SLE group compared to the controls, although without reaching a statistical significance. Other studies conducted in the Canary Islands, China, and Hungary found no statistically significant differences for allele frequencies in exon 1 and promoter MBL2 genotype comparing SLE patients and a control group, but a trend towards a higher frequency of low MBL producer genotypes in SLE patients was generally found [26,56,58].

MBL serum levels largely depend on MBL2 exon 1 and promoter genotype, but there is no exact correlation between MBL2 genotypes and MBL levels probably because of other factors such as hormones or acute inflammation that modulate MBL production [1]. As said before, MBL has been considered an acute phase reactant, but studies are conflicting [1,53,59] and one showed that MBL did not act as an acute phase reactant in pneumonia [60] for example. Likewise, our study did not find any relationship between MBL levels and acute phase reactants such as CRP and globular sedimentation rate.

MBL has been considered a modulator of disease activity in SLE. On the one hand, a higher prevalence of cardiovascular, renal or severe disease was observed in patients carrying low-MBL producer genotypes compared to those carrying high MBL producer genotypes [23,24]. On the other hand, some studies have concluded that high MBL levels were associated with SLE nephritis and other SLE manifestations [59, 61]. In our study, most of our patients had a low SLEDAI score (between 0-2) and we did not find any significant relationship between MBL levels and disease severity, nor

between any allelic variants in exon 1 and clinical manifestations. Homozygosity for MBL variant alleles have been associated with infections in SLE patients [62,63], but we could not evaluate infections in this transversal study. MBL is associated with complement activation through the lectin pathway and therefore low complement levels in patients with high MBL could be expected, as it has been described in SLE in an Indian study [59], but results are contradictory [23,62]. In our study, we did not find any association between the complement system and MBL levels.

#### c) MASP in TB

The Asp105Gly MASP2 variant frequency in our study was 2.02%, similar to that previously reported among Spanish populations [28,32], but lower than the 3.6% reported in a Danish cohort [41]. The other allelic variants analyzed were not found in any of the participants, variants described previously in an African but not Spanish population [28]. The relation between Asp105Gly and infections is controversial. One study found an increased risk for aspergillosis in stem cell transplant recipients [34], however, other studies did not find an increased frequency of Asp105Gly in adult patients with community-acquired pneumonia [11], and pneumococcal bacteremia in HIV infected patients [35] or sepsis [34]. Other SNP of the MASP2 gene have also been studied, determining it as a risk factor for leprosy [64] or hepatitis C infection [65]. This is the first study analyzing the relationship between MASP2 polymorphism and TB.

Analyzing MASP-2 levels, there was no relation with TB disease. Only one patient had MASP levels below 75ng/mL, a Spanish TB patient, suggesting a possible relation with TB disease.

#### d) MASP in SLE

MASP-2 deficiency has been described previously in a homozygous patient for Asp105Gly mutation with a history of infections and inflammatory disease [30], suggesting a possible association between this mutation and autoimmune disease.

However a Spanish study analyzing Asp105Gly in SLE and healthy subjects revealed no difference in allelic frequency between both groups [15], in agreement with our findings. Furthermore, another study in patients with different autoimmune disease namely psoriasis, did not find a relation between the presence of a mutated Asp105Gly allele and the development of the disease [66].

Asp105Gly allelic frequency varies between populations. Two studies in Spanish healthy populations showed Asp105Gly allelic frequencies of 1.44% [28] and 2.4% [15] respectively, and 2.02% in our TB group, which are slightly lower than the frequency we have found in our small sample of Caucasians with and without SLE (2.66%). We found no Asp105Gly homozygous individual. Similarly, in a large study in Spain, no patients with a SLE diagnosis and only 2/868 healthy controls were found to be homozygous carriers [15].

The allelic variants Thr73Met, Arg84Gln, Arg103Cys, and Pro111Leu were also absent in our SLE population as explained in the TB group.

Mean MASP-2 level was lower than in a healthy Danish population [67]. Two individuals in our study had MASP-2 levels below 75ng/mL, which was not found in any of the subjects in the Danish study [32]. This could be explained by the inclusion in our study of SLE patients, in whom MASP-2 deficiency was more frequent compared to the control group, suggesting an association between MASP-2 deficiency and SLE. No correlation was observed between MASP-2 deficiency and the presence of a mutated

Asp105Gly allele. This association was described in the first case of MASP-2 deficiency described [27, 30], but was not reported in all studies [28].

## 9. Limitations

The small sample size in both parts of the study (the TB and the SLE) is an important limitation, which may affect the statistical significance of the results. The heterogeneous group of the TB patients, including patients with extrapulmonary TB and HIV infection could also interfere in the results.

In the SLE part, most of the participants had a low SLEDAI score, and treatments were heterogeneous. Moreover, SLE participants were of a multiethnic origin, whereas the controls were exclusively Caucasians. This is why we created the subgroup of Caucasian subjects. However, the results showed the same tendency in both analyses.

MBL levels were only measured at the time of inclusion of the study and were not evaluated at the end of the treatment or during an SLE flare. This would have allowed to determine their role as an acute phase reactant

MASP-2 levels can also change over time (15-20% over one year) [67]. In this study, MASP-2 concentration was measured only once, which could also be a limitation

Other new MASP2 allelic variants that have not been determined in this study [32,35] may be related to MASP-2 deficiency. Moreover other factors related to the lectin pathway such as Map-19 (a shorter spliced product of the MASP2 gene) or the activity of the MBL/MASP complex is lacking [41].

## **10. Strengths**

Nevertheless, our study has some important strengths: a complete MBL2 genotyping of exon 1 and promoter was done, an analysis which is not commonly found in published studies; what's more, very few studies analysing a Caucasian population are found in literature.

It is also important to notice that 5 different MASP2 polymorphisms were analyzed, although only one was found.

To date, this is the first study analyzing the relationship between MASP2 polymorphisms and MASP-2 in TB and the second cohort including a SLE group and a healthy control group.



## 11. Conclusions

1. The high MBL-producer diplotype *LXPA/HYPA* was significantly more frequent in HHC than in TB patients, suggesting that this diplotype has a protective role against the development of TB disease that has not been described previously.

2. *LYQC/HYPD*, *LXPA/LYQC*, and *LYPB/HYPD* diplotypes, which are associated with very low MBL levels were only found in SLE patients, and there was a tendency towards a higher incidence of allele B in the SLE group compared to the controls. Thus, MBL levels and some genetic variants could be a risk factor for developing SLE.

3. Asp105Gly was the only MASP2 variant found in our studies, and no relationship with the development of TB or SLE was found.

4. MASP-2 deficiency was more frequent, although not statistically significant, among the SLE group, suggesting a relationship with the disease.

5. The precise consequences of MBL2 and MASP2 polymorphisms, and MBL and MASP-2 deficiency in relation to the development of TB, SLE or disease progression, as well as the role of MBL as an acute phase reactant remain unclear..

## 12. Future directions

Further studies are needed, including large longitudinal cohort studies with a complete genotype profile of the protein (MBL2 exon 1 and promoter) and MBL levels. Studies of other components of the lectin pathway, including ficolins, as well as functional assessment of the lectin pathway are needed. Moreover, the use of MBL with a therapeutic aim, has been analyzed in case reports. Two MBL deficient patients received MBL infusions and one of the participants, who had been suffering of repeated infections, remained healthy for more than 3 years [68]. Indeed some phase I [69,70] and phase II [71-72] studies have been also published, with safety results, but with doubts about efficacy. Moreover, there is one Ib clinical trial still not finished. It is an interventional, randomized, safety clinical trial, including MBL deficient patients with multiple myeloma, treated with melphalan-based high-dose chemotherapy followed by autologous hematopoietic stem cell transplant. Patients are randomized to 0.5 mg/kg, 1.0 mg/kg, or no recombinant human MBL. The aim of this study is to analyze safety and tolerability of intravenous administration of MBL, however the study has been completed, but results are still not available.

Thus, more studies need to be developed to analyze the beneficial or harmful effect of MBL and MASP-2 levels and MBL2 and MASP2 polymorphisms. Determining the beneficial effect of low MBL and MASP-2 levels would provide important insights into the treatment and prevention of severe diseases. Moreover, functional assessment of the lectin pathway may be far more useful and clinically relevant than MBL level and/or genotype alone.

### 13. Tables

Table 1. MBL2 genotype frequencies in TB patients and HHC

MBL2 genotype	TB patients n (%)	HHC n (%)	p	OR	95% CI
<i>A/A</i>	48 (63.2)	71 (67)	0.71	0.84	0.45-1.56
<i>A/O</i>	24 (31.6)	34 (32.1)	0.93	0.97	0.51-1.84
<i>O/O</i>	4 (5.3)	1 (0.9)	0.19	5.83	0.63-53.2
<b>Total</b>	76	106			

Table 2. Frequencies of complete diplotypes in TB patients and controls

MBL2 diplotypes	TB patients n (%)	HHC n (%)	p	OR	95% CI
<i>LYQA/HYPA</i>	13 (17.1)	15 (14.1)	0,73	1.25	0.55-2.81
<i>LYQA/LYPB</i>	2 (2.6)	5 (4.7)	0.73	0.54	0.1-2.86
<i>LYQA/LYPA</i>	1 (1.3)	1 (0.9)	0.62	1.4	0.08-22.73
<i>LYQA/LYQA</i>	2 (2.6)	7 (4.7)	0.38	0.38	0.08-1.89
<i>LYQA/HYPD</i>	1 (1.3)	2 (1.9)	0.76	0.69	0.06-7.78
<i>LYQA/LYQC</i>	1 (1.3)	0			
<i>LYQC/HYPD</i>	1 (1.3)	0			
<i>LXPA/HYPA</i>	5 (6.6)	20 (18.9)	0.03	0.3	0.1-0.84
<i>LXPA/LYQA</i>	10 (13.1)	10 (9.4)	0.59	1.44	0.57-3.65
<i>LXPA/LXPA</i>	8 (10.5)	6 (5.7)	0.35	1.96	0.65-5.9
<i>LXPA/LYPB</i>	5 (6.6)	10 (9.4)	0.67	0.67	0.22-2.06
<i>LXPA/HYPD</i>	2 (2.6)	2 (1.9)	0.86	1.4	0.19-10.2
<i>LXPA/LYPA</i>	2 (2.6)	3 (2.85)	0.7	0.93	0.15-5.69
<i>HYPA/HYPA</i>	4 (5.3)	8 (7.5)	0.75	0.68	0.2-2.35
<i>LYPB/HYPA</i>	8 (10.5)	6 (5.7)	0.35	1.96	0.65-5.9
<i>LYPB/LYPB</i>	2 (2.6)	1 (0.9)	0.77	2.84	0.25-31.87
<i>LYPB/HYPD</i>	1 (1.3)	0			
<i>LYPA/HYPA</i>	3 (3.9)	2 (1.9)	0.7	2.14	0.35-13.11
<i>LYPA/LYPB</i>	2 (2.6)	2 (1.9)	0.86	1.4	0.19-10.2
<i>LYPA//HYPD</i>	0	2 (1.9)			
<i>HYPD/HYPA</i>	2 (2.6)	3 (2.8)	0.93	0.93	1.51-5.69
<i>HYPA/LYQC</i>	1 (1.3)	1 (0.9)	0.62		
<b>Total</b>	76	106			

Table 3. Relation between mean MBL levels and MBL2 genotype (exon1 and promoter) in TB patients and HHC

Mean MBL levels ng/mL (SD)			
Genotype	Patients	HHC	P
<i>YA/YA</i>	8387.75 (5945)	5555.03 (3289.62)	0.027
<i>YA/XA</i>	5691.87 (4221.58)	3710.37 (2309.7)	0.55
<i>XA/XA</i>	2310 (3199.86)	1200 (978.3)	0.53
<i>YA/O</i>	1107.88 (1017.66)	951.18 (668.53)	0.6
<i>XA/O</i>	31.25 (20.97)	100	0.06
<i>O/O</i> *	*	*	*

\* Cannot be computed because one of the groups is empty

Table 4. Activity index and clinical manifestations in SLE patients

MBL genotype	SLE n (%)	Control n (%)	P
<i>A/A</i>	21 (53.8)	41 (69.5)	ns
<i>A/O</i>	14 (35.9)	17 (28.8)	ns
<i>O/O</i>	4 (10.3)	1 (1.7)	ns
<b>Total</b>	<b>39</b>	<b>59</b>	<b>na</b>

Table 5. Frequency of MBL 2 genotypes in SLE patients and in controls

n (%)	
<b>SLEDAI</b>	
<b>0-2 (inactive)</b>	27 (69.2)
<b>3-4 (mild)</b>	6 (15.4)
<b>5-7 (moderate)</b>	2 (5.1)
<b>&gt;8 (severe)</b>	4 (10.3)
<b>Clinical manifestations</b>	
<b>Arthritis</b>	20 (51.3)
<b>Nephritis</b>	11 (28.2)
<b>Serositis</b>	14 (35.9)
<b>Neuropsychiatric</b>	7 (17.9)
<b>Hematologic</b>	13 (33.3)

Table 6. Frequencies of complete genotypes in SLE patients and controls

<b>MBL genotype</b>	<b>SLE n (%)</b>	<b>Control n (%)</b>	<b>P</b>
<i>LYQA/HYPA</i>	6 (15.4)	12 (20.2)	ns
<i>LYQA/LYPB</i>	2 (5.1)	5 (8.5)	ns
<i>LYQA/LYPA</i>	1 (2.6)	1 (1.7)	ns
<i>LYQA/LYQA</i>	2 (5.1)	4 (6.8)	ns
<i>LYQC/HYPD</i>	1 (2.6)		na
<i>LXPA/HYPA</i>	2 (5.1)	9 (15.3)	ns
<i>LXPA/LYQA</i>	1 (2.6)	6 (10.2)	ns
<i>LXPA/LXPA</i>	3 (7.7)	4 (6.8)	ns
<i>LXPA/LYPB</i>	4 (10.3)	5 (8.5)	ns
<i>LXPA/HYPD</i>	0	1 (1.7)	na
<i>LXPA/LYPA</i>	4 (10.3)	4 (6.8)	ns
<i>LXPA/LYQC</i>	1 (2.6)	0	ns
<i>HYPA/HYPA</i>	3 (7.7)	1 (1.7)	ns
<i>LYPB/HYPA</i>	4 (10.3)	1 (1.7)	ns
<i>LYPB/LYPB</i>	1 (2.6)	1 (1.7)	ns
<i>LYPB/HYPD</i>	2 (5.1)	0	ns
<i>LYPA/HYPA</i>	2 (5.1)	2 (3.4)	ns
<i>HYPD/HYPA</i>	0	2 (3.4)	na
<b>Total</b>	<b>39</b>	<b>59</b>	<b>na</b>

Table 7. MBL levels and gender

	n		p
	Mean MBL levels (ng/mL) ( $\pm$ SD)		
	Male	Female	
<b>SLE</b>	2	36	<b>0.8</b>
	1989.5 (2670.74)	2333.25 (2393.94)	
Controls	4	55	0.65
	3107.5 (1157.39)	2517.35 (2537.66)	

Table 8. MBL 2 genotype and mean MBL levels

MBL 2 genotype	Mean MBL levels (ng/mL)( $\pm$ SD)		p
	SLE	Control	
<i>YA/YA</i>	4374.43 (2036.35)	4157.86 (2355.86)	ns
<i>YA/XA</i>	2636.71 (2249.57)	2855.65 (2511.58)	ns
<i>XA/XA</i>	1770 (636.4)	1676.6 (928.33)	ns
<i>YA/O</i>	711.67 (263.77)	661 (685.91)	ns
<i>XA/O</i>	30.6 (23.33)	98.5 (107.28)	ns
<i>O/O</i>	33 (45.27)	100	ns

Table 9. Exon 1 MBL 2 polymorphisms and SLE clinical manifestations

Clinical manifestations	<i>A/A</i> n (%)	<i>A/O and O/O</i> n (%)	p
<b>Cutaneous</b>	10 (28.57)	16 (76.19)	ns
<b>Arthritis</b>	9 (25.71)	11 (52.38)	ns
<b>Nephritis</b>	6 (17.14)	5 (23.81)	ns
<b>Serositis</b>	2 (5.71)	12 (57.14)	ns
<b>Neuropsychiatric</b>	4 (11.43)	3 (14.29)	ns
<b>Hematologic</b>	4 (11.43)	9 (42.86)	ns

Table 10. Characteristics of MASP-2 deficiency and non-deficient cases

	<b>MASP-2 &lt;200mg/mL n=33</b>	<b>MASP-2&gt;200ng/mL n=66</b>	<b>p</b>
<b>TB patient/HHC</b>	18/15	31/55	ns
<b>HIV satus +/-</b>	5/28	17/49	ns
<b>MBL deficiency (&lt;500ng/mL)</b>	4 (out of 32 patients)	15 (out of 61 patients)	ns

Table 11. MASP2 Asp105Gly genotypes and alleles

<b>MASP 2 Allele</b>	<b>Cases n (%)</b>	<b>Controls n (%)</b>	<b>p</b>
<b>A</b>	78 (100)	103 (95.37)	0.14
<b>G</b>	0	5 (4.63)	na
<b>Genotype</b>			
<b>A/A</b>	39 (100)	54 (91.53)	0.16
<b>A/G</b>	0	5 (8.47)	na
<b>G/G</b>	0	0	na

## 14. Figures

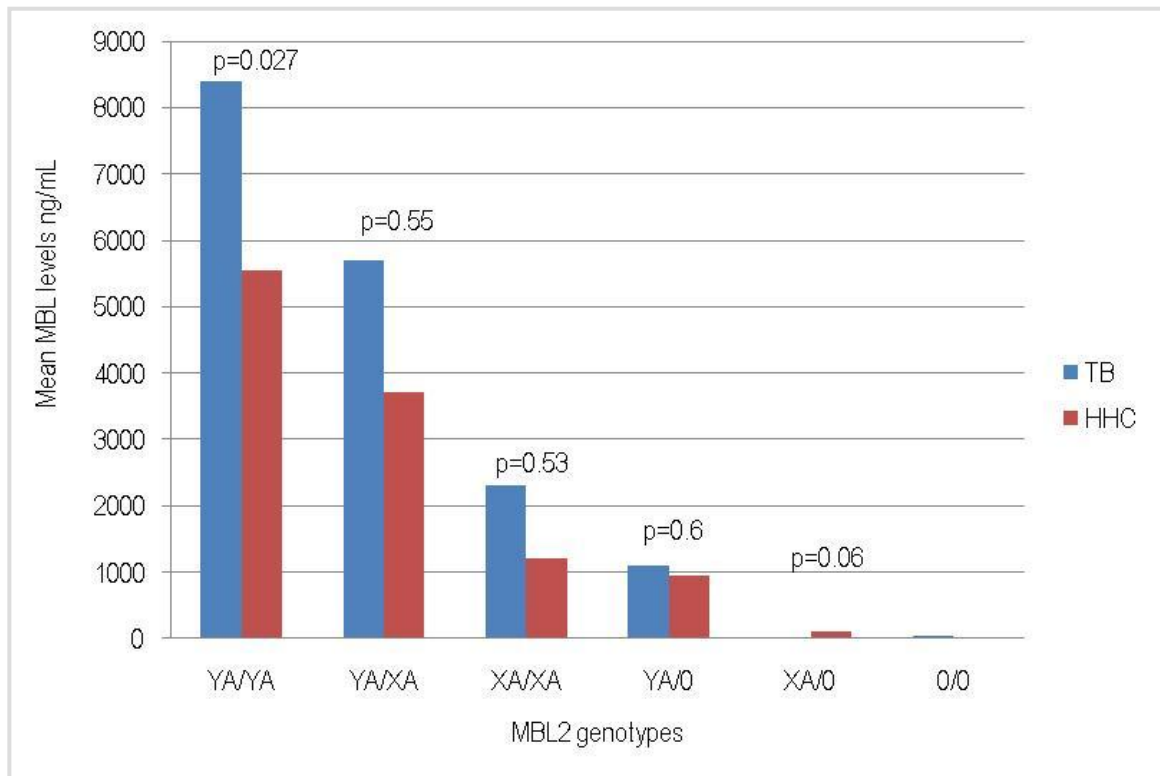


Figure 1: Influence of MBL2 genotype variants on serum MBL levels (ng/mL) in TB and HHC



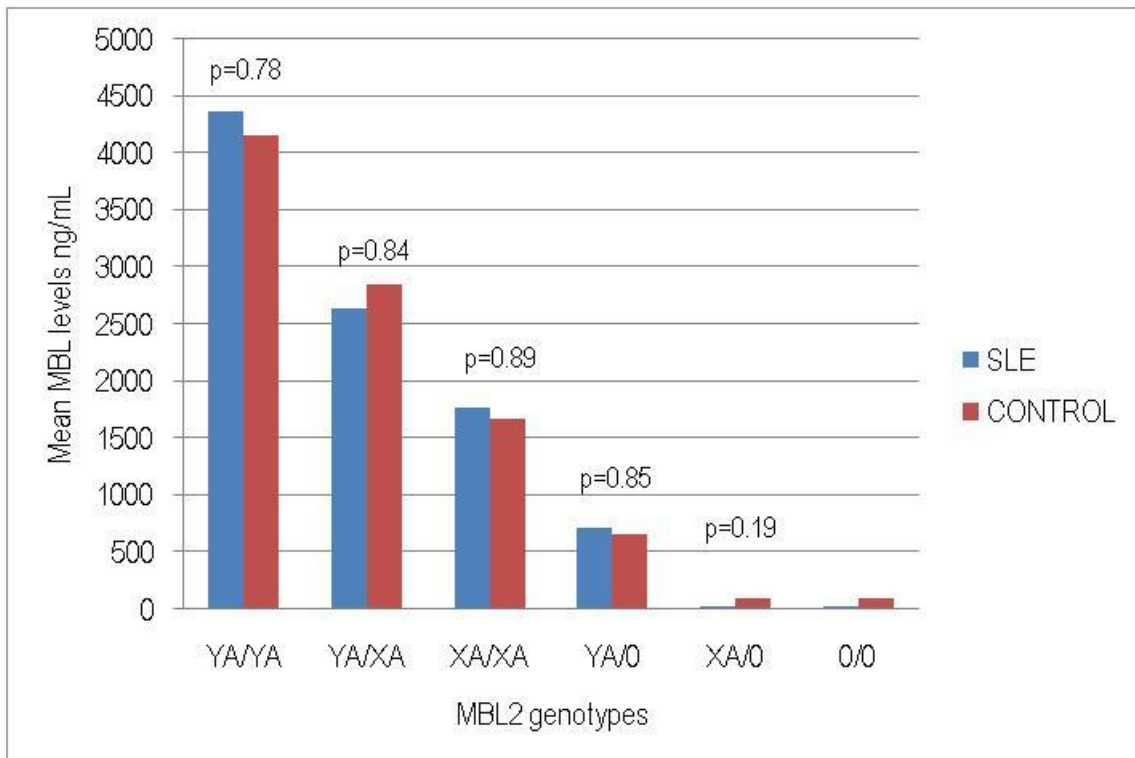


Figure 2. Influence of MBL2 genotype variants on serum MBL levels (ng/mL) in SLE and healthy controls

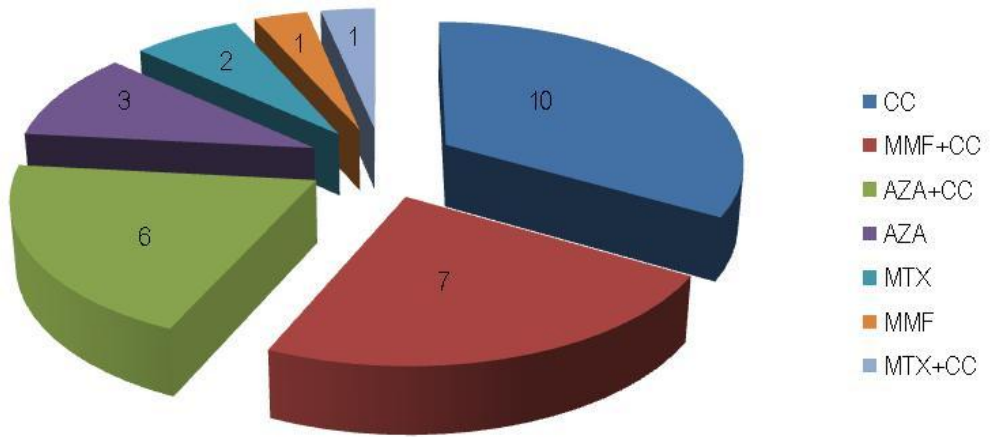


Figure 3: Immunosuppressive treatment in SLE patients

## **15. Publicacations**

### 11.1. First study

Mannose-Binding lectin exon 1 and promoter polymorphisms in tuberculosis disease in a Mediterranean area. *International Journal of Immunogenetics*. In press.

### 11.2. Second study

Mannose binding lectin polymorphisms in systemic lupus erythematosus in Spain.

### 11.3. Third study

Mannan-binding lectin serine peptidase 2 (MASP-2) in tuberculosis disease.

### 11.4. Fourth study

MASP2 polymorphisms in a multi-ethnic lupus cohort. *Scandinavian Journal of Rheumatology*. Submitted, under review.

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