Mannose-Binding Lectin and Susceptibility to Infection in Chinese Patients with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To test the hypothesis that low serum mannose-binding lectin (MBL) levels, as a result of the single-nucleotide polymorphisms in the promoter region (–221 X/Y) and exon 1 (codon 54 A/B) of the MBL2 gene, predispose to infection in Chinese patients with systemic lupus erythematosus (SLE).

Methods. Two hundred forty-five patients with SLE were prospectively followed for the development of major infective episodes that required hospitalization and antibiotic treatment during 1992-2005. MBL genotypes were determined by polymerase chain reaction and serum MBL levels were measured by ELISA.

Results. In total, 254 major infections developed in 130 patients. Serum MBL levels were shown to correlate inversely with the number of bacterial infections ($r = –0.13$, $p = 0.03$). The distribution of MBL genotypes was similar in patients with and without major infection ($p = 0.84$). Patients with major infection also had more major lupus exacerbations that required daily prednisolone dose ≥ 15 mg. Logistic regression showed that log MBL level (odds ratio 0.516, 95% confidence interval 0.305–0.873; $p = 0.01$) and major lupus exacerbation (OR 1.382, 95% CI 1.154–1.654; $p < 0.001$) were independent risk factors to major bacterial infection after adjustment for age and disease duration. Multiple regression analysis showed an increase in risk of bacterial infection by 34.2% for every decrease in serum MBL level by one log, and by 22.8% for each increase in number of major lupus exacerbations.

Conclusion. Low serum MBL level predisposes Chinese patients with SLE to more major infections, in particular bacterial ones. (J Rheumatol 2007;34:1270–6)

Key Indexing Terms:
COMPLEMENT DEFICIENCY HOSPITALIZATION IMMUNOCOMPROMISED HOST
IMMUNOSUPPRESSANT INFECTION

Mannose-binding lectin (MBL) is a serum C-type lectin that plays an important role in innate immunity[1,2]. Upon binding to targets, MBL can activate the complement cascade called lectin pathway via MBL-associated serine proteases[3]. The lectin exhibits antibacterial activity through killing mediated by the terminal lytic complement components or by promoting phagocytosis[4,5]. MBL is able to promote opsonization either by activation of complements and generation of C3b fragments via the lectin pathway[6] or by acting directly as an opsonin interacting with surface receptors on phagocytic cells[7,8]. MBL binds to glycoprotein terminated with mannose and N-acetylglucosamine that is present on a variety of microorganisms including bacteria, yeasts, parasitic protozoa, and viruses[6,9-13]. MBL deficiency was first identified in individuals with recurrent infections and having serum defect in opsonophagocytosis[14]. Indeed, MBL deficiency as a result of polymorphisms of the MBL (MBL2) gene has been known to be associated with frequent infections in children[15,16], adults[17,18], and those with immunodeficiency[19,20]. These patients were particularly prone to both gram-positive and gram-negative bacterial infection[21]. A mouse model deficient in MBL was also shown to be susceptible to infection by gram-positive bacteria[22].

Beyond infection, MBL deficiency has also been shown to predispose to autoimmune diseases like systemic lupus erythematosus (SLE)[23,24]. Some studies have also suggested a role of MBL as a disease modifier in lupus[25,26]. Mutation of any of the 3 codons 52, 54, and 57 of exon 1 of the MBL2 gene has been shown to be associated with low MBL levels in different ethnic populations[27-29]. Codon 54 mutation (A/B) has been shown by our group to be the most prevalent form of mutation found in our local Chinese population, with a gene

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frequency of 0.11[30]. It involves a single point mutation (GGC → GAC) of exon 1 of the MBL2 gene replacing glycine with aspartic acid residue. The other 2 known haplotypes carrying structural polymorphism, C (codon 57) and D (codon 52), have been found to occur at an extremely low frequency and are probably absent in the Chinese population[28,29]. Our group and others have demonstrated that low serum MBL level can also occur as a result of single-nucleotide polymorphism (SNP) upstream of the gene at position –221 (X/Y alleles), leading to poor transcription of the MBL2 gene[31,32].

Patients with SLE are intrinsically immunocompromised and the corticosteroids and immunosuppressants used as treatment for major organ involvement further predisposed them to infection. Studies have shown that MBL2 gene polymorphism led to higher risk of infection in Caucasian[23] and Japanese[25] patients with SLE. However, the predisposition to infection by MBL phenotype and various genotypes in relation to immunosuppressive use had not been explored. We sought to test the hypothesis that low serum MBL level, as a result of mutations involving the promoter region (–221 X/Y) and exon 1 (codon 54 A/B) of the MBL2 gene, predisposes to major infection in Chinese patients with SLE and to evaluate their interaction with immunosuppressive use in their contribution to infection.

MATERIALS AND METHODS

Consecutive patients who satisfied the 1982 revised American College of Rheumatology (ACR) classification criteria for SLE[33] were recruited at the university affiliated lupus clinic of Queen Mary Hospital, Hong Kong. The study was approved by the Clinical Research Ethics Committee of the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster. Demographic data including sex, age at onset of SLE, duration of disease, cumulative clinical manifestations, immune profiles including antinuclear antibodies (ANA), anti-double stranded (ds) DNA antibodies, and anti-extractable nuclear antigen (ENA) antibodies were recorded. The main outcome of our study was the development of major infective episodes that were defined as infections that warranted hospitalization and antibiotic treatment. The causative microorganisms, if known from septic investigations, and the dose of corticosteroids and immunosuppressants at the time of infection were recorded. The number of major lupus exacerbations during the followup period was also recorded for each patient. As immunosuppressive use was a confounder of lupus exacerbation, major lupus exacerbations were defined as the requirement of an immunosuppressive dose of prednisolone for treatment, taken to be ≥ 15 mg daily in our study.

Information on all major infective episodes was prospectively collected during the period 1992-2005 for an inception cohort recruited since 1992 and other patients with SLE whose disease was diagnosed before 1992. The number of major infective episodes/patient-year for patients in the inception cohort was derived using the followup period as the time between disease onset and the time of the study. For patients whose disease was diagnosed before 1992, the average number of major infective episodes/patient-year was derived using the followup period from 1992 until the time of the study.

Serum MBL assay. Blood samples were obtained from patients at clinic visit when the disease activity was quiescent and in the absence of concurrent infection. Serum levels of MBL were determined by ELISA in which a mouse monoclonal anti-human MBL antibody (HYB-131-01; Antibody Shop, Copenhagen, Denmark), either unlabeled or labeled with biotin, was used as the primary or secondary antibody, respectively, as described[16]. Horseradish peroxidase-conjugated streptavidin and substrate solution containing tetramethylbenzidine (R&D Systems, Minneapolis, MN, USA) were used for detection of bound secondary antibody, in accord with the manufacturer's instructions.

MBL genotyping and haplotyping. The SNP in the promoter (–221 X/Y) and exon 1 (codon 54 A/B) of the MBL2 gene were genotyped by the use of 2 separate cycling reactions of the TaqMan allele-discrimination system (Applied Biosystems, Foster City, CA, USA), as described[34]. The frequencies of 2-locus haplotypes composed of the promoter and a structural allele were also estimated by performing the haplotype procedure of ASA/Genetic Software (version 8.2; SAS Institute, Cary, NC, USA), as described[35]; the expectation-maximization algorithm was used to generate maximum-likelihood estimates of haplotype frequencies, given that genetic-marker genotypes are under the assumption of Hardy-Weinberg equilibrium (HWE).

Statistical analysis. The values quoted in the study were expressed as mean ± standard deviation (range) unless otherwise specified. Statistical analysis was performed by SPSS 11.0 (SPSS, Chicago, IL, USA). Comparisons of demographic data and genotypes of MBL were performed by the use of chi-square test. Pearson correlation was used to determine any relationship of serum MBL level and the number of major infections. Serum levels of MBL between patients with and without major infection were compared by Mann-Whitney U-test. Other scalable variables such as age at onset, duration of SLE, and serum MBL were analyzed by unpaired t test. Analysis of variance (ANOVA) was used to compare the number of major bacterial infections between groups of patients with serum MBL levels defined by arbitrary cut-offs. Logistic regression analysis was used to investigate the association between the number of major infections and MBL genotypes or phenotype, after adjustment for independent variables found to be statistically significant from the chi-square test. As serum MBL levels were not normally distributed, serum MBL levels were transformed to a log base 10 before the variable was put into the regression model. A significance level of $p < 0.05$ was used for all analyses.

RESULTS

Demographic data of patients. Two hundred forty-five consecutive patients with SLE were studied, of whom 155 (63.3%) belonged to the inception cohort whose disease was diagnosed after 1992. There were 234 female and 11 male patients. Age of patients was 37.8 ± 10.1 (range 17–77) years with disease duration of 8.8 ± 6.7 (1–43) years. Patients in the inception cohort were found not to differ from the other patients whose disease was diagnosed before 1992 in terms of their demographic characteristics and disease profile, except for younger age ($p < 0.001$) and shorter duration of followup ($p < 0.001$). Table 1 summarizes the demographic data of the patients with SLE in our study.

MBL genotype and phenotype. The serum MBL level of these patients was 1077.8 ± 863.2 μg/l (median 959.0). The MBL genotypes YA/YA, YA/XA, XA/XA, YA/YB, XA/YB, and YB/YB were found in 37.6% (n = 92), 29.8% (n = 73), 3.3% (n = 8), 23.3% (n = 57), 4.5% (n = 11), and 1.6% (n = 4) of patients, respectively. The high, intermediate, and low MBL-producing haplotypes YA, XA, and YB were found in 64.1% (n = 314), 20.4% (n = 100), and 15.5% (n = 76), respectively. Figure 1 shows the serum MBL levels of SLE patients with different MBL haplotypes. Patients with YB haplotype were found to have significantly lower serum MBL levels (229.9 ± 186.4 μg/l) than patients who were non-YB carriers (1430.7 ± 785.2 μg/l) ($p < 0.001$).

Major infective episodes in patients with SLE. Two hundred

Table 1. Demographic data of patients with SLE.

Characteristics	N
Patients with SLE	245
Diagnosed before 1992	90 (36.7%)
Diagnosed after 1992	155 (63.3%)
Age at study, yrs	37.8 ± 10.1 (17–77)
Age at onset of SLE, yrs	29.0 ± 9.8 (10–66)
Duration of disease, yrs	8.8 ± 6.7 (1–43)
Female:male ratio	234:11
Cumulative clinical features	N (%)
Polyarthralgia	203 (82.9)
Lymphopenia	173 (70.6)
Malar rash	164 (66.9)
Thrombocytopenia	93 (38.0)
Renal involvement	111 (45.3)
Cutaneous vasculitis	89 (36.3)
Neutropenia	87 (35.5)
Serositis	56 (22.9)
Oral ulceration	55 (22.4)
Discoid rash	50 (20.4)
Nervous system involvement	40 (16.3)
Autoimmune hemolytic anemia	33 (13.5)
Serological features	
ANA positivity	241 (98.4)
Anti-dsDNA	165 (67.3)
Anti-Ro	148 (60.4)
Anti-RNP	64 (26.1)
Anti-La	23 (9.4)
Anti-Sm	26 (10.6)
Medications ever used	
Prednisolone	217 (88.6)
Azathioprine	141 (57.6)
Cyclophosphamide	64 (26.1)

SLE: systemic lupus erythematosus; ANA: antinuclear antibodies; RNP: ribonuclear protein.

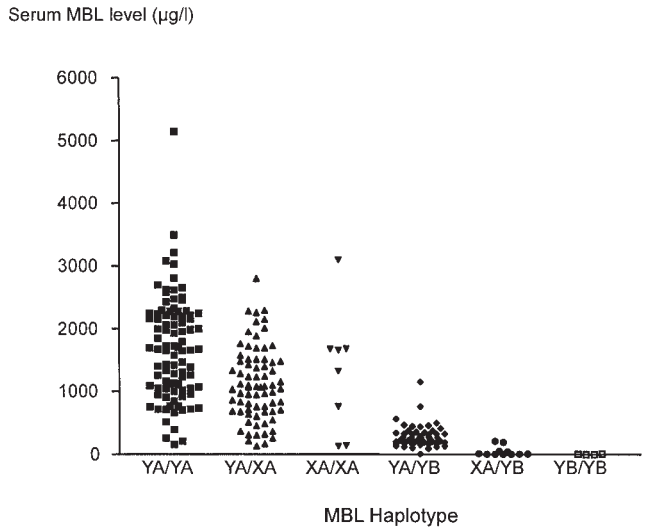

Figure 1. Serum mannose-binding lectin (MBL) levels of SLE patients with different MBL haplotypes.

fifty-four episodes of infection that warranted hospitalization and antibiotic treatment were identified in 130 (53.1%) patients. Sixty patients had recurrent infection. The average number of infective episodes of the 130 patients was 0.35 ± 0.37 (0.11–2.00)/patient-year. Most patients presented with urinary tract infection (n = 81) followed by cutaneous infection (n = 70), pneumonia (n = 55), gastroenteritis (n = 36), and infection of the central nervous system (n = 7), and less common, cholangitis (n = 1), otitis media (n = 1), parotitis (n = 1), osteomyelitis (n = 1), and disseminated cytomegalovirus infection (n = 1). Most hospital admissions were caused by bacterial infections (179/254, 70.5%), of which 64 (35.8%) episodes were by gram-positive and 115 (64.2%) by gram-negative bacteria. Viral and *Mycobacterium tuberculosis* infections that led to admission were found in 20.9% (53/254) and in 7.9% (20/254) of patients, respectively. *M. tuberculosis* was the causative agent for pneumonia, meningitis, skin ulcers, tenosynovitis, and lymphadenitis in patients with SLE in this cohort. Fungal infection was not common (2/254, 0.8%).

There were 2 infection-related deaths (2/245, 0.8%), one from bacterial meningitis and one from severe pneumonia. The patients were the only 2 in the cohort with endstage renal failure. One patient was on hemodialysis and the other on continuous ambulatory peritoneal dialysis as renal replacement therapy. Both patients had no codon 54 gene mutation and had serum MBL levels above median (959 µg/l): 1699 µg/l and 1681 µg/l, respectively.

Major infection and MBL phenotype. Serum MBL levels were shown to correlate in an inverse relationship with the number of major infective episodes. This relationship was most apparent for bacterial infection (r = –0.133, p = 0.03; Figure 2). Serum MBL levels of these patients were subcategorized as "sufficient," "low," and "very low" levels using arbitrary cutoffs at ≥ 1000 µg/l, 400–1000 µg/l, and < 400 µg/l. The number of major bacterial infections for patients in these 3 groups was significantly different using ANOVA (p = 0.015), and those for the "very low" and "sufficient" groups were also found to be significantly different after post-hoc Tukey test (1.05 ± 1.56, 0.67 ± 1.12, and 0.55 ± 0.93, respectively; Figure 3). Serum MBL was not found to correlate with tuberculous infection (p = 0.049).

Major infection and MBL genotype. Table 2 shows the clinical characteristics of patients who developed major infection and major bacterial infection, and those who did not develop any major infection. Patients who had major infection were younger at onset of SLE (p = 0.01) and had longer disease duration (p = 0.01) compared to those without major infection. Serum MBL levels (p = 0.25) and the distribution of MBL genotypes (p = 0.84) were not different between the 2 groups. The low MBL-producing YB haplotype was found in 16.1% of patients without major infection and 15.0% of patients with major infection (p = 0.78). There was a slightly higher percentage of YB carrier (37.7%) among patients who

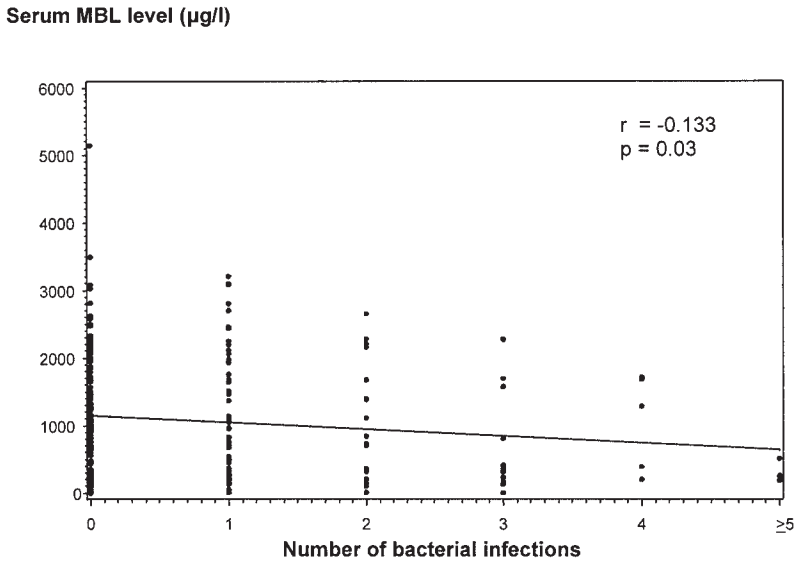

Figure 2. Correlation between serum MBL levels and the number of major bacterial infections in patients with SLE.

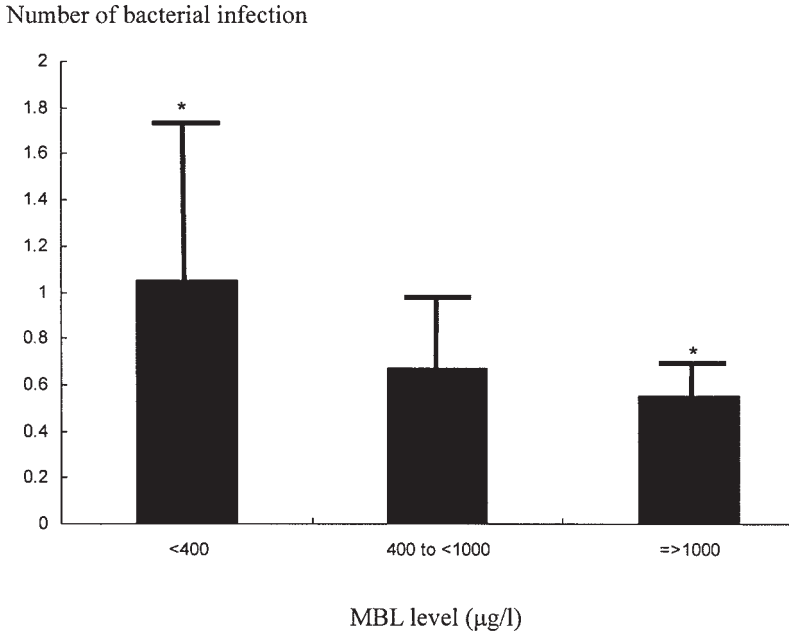

Figure 3. Association of number of major bacterial infections with serum MBL levels defined as sufficient (≥ 1000 µg/l), low (400–1000 µg/l), and very low (< 400 µg) using arbitrary cutoffs. ANOVA p = 0.015. *Significantly different after post-hoc test.

had bacterial infection compared to those without major infection, although the difference was not statistically significant (p = 0.51). Patients with major infection (2.03 ± 1.73) were found to have higher number of major lupus exacerbations that required daily prednisolone > 15 mg than those patients without major infection (1.04 ± 1.22) (p < 0.001).

Higher number of major bacterial infections in low serum MBL and lupus exacerbation. To explore the predisposition of low serum MBL level to higher number of major bacterial infection, logistic regression analysis was performed using number of bacterial infections as dependent variable and age of patients, duration of SLE, serum MBL level, YB carrier status, and number of major lupus exacerbations as independent variables. Serum MBL level and major lupus exacerbations,

Table 2. Characteristics of patients without/with major infection.

	Patients with SLE (n = 245)			
	Without Major Infection, n = 115	With Major Infection, n = 130	With Major Bacterial Infection, n = 61	p†
Female:male	110:5	124:6	59:2	0.58
Age, yrs	37.9 ± 9.5	37.5 ± 10.6	38.4 ± 10.8	0.41
Age at onset, yrs	30.5 ± 9.7	27.8 ± 9.9	29.4 ± 9.5	0.01
Duration of disease, yrs	8.3 ± 6.0	9.3 ± 5.9	8.6 ± 5.1	0.01
Any codon 54 mutation of the MBL gene, n (%)				0.84
YAYA	43 (37.4)	49 (37.7)	24 (39.3)	
YAXA	35 (30.4)	38 (29.2)	14 (23.0)	
XAXA	3 (2.6)	5 (3.8)	1 (1.6)	
YAYB	25 (21.7)	32 (24.6)	17 (27.9)	
XAYB	6 (5.2)	5 (3.8)	4 (6.6)	
YBYB	3 (2.6)	1 (0.8)	1 (1.6)	
YB carrier	34 (29.6)	38 (29.2)	23 (37.7)	
MBL haplotype				
YA	146 (63.5)	168 (64.6)	79 (64.8)	0.66
XA	47 (20.4)	53 (20.4)	20 (32.8)	0.90
YB	37 (16.1)	39 (15.0)	23 (37.7)	1.00
Median serum MBL level, μg/l	1030.0	822.5	673.0	0.25
Log MBL level, μg/l	2.78 ± 0.56	2.89 ± 0.53	2.68 ± 0.66	0.07
No. of major lupus exacerbations requiring prednisolone ≥ 15 mg daily (mean ± SD)	1.04 ± 1.22 (median 1.0)	2.03 ± 1.73 (median 2.0)	2.23 ± 1.82 (median 2.0)	< 0.001
No. of major lupus exacerbation/patient-yr requiring prednisolone ≥ 15 mg daily (mean ± SD)	0.33 ± 0.55 (median 0.14)	0.32 ± 0.32 (median 0.22)	0.39 ± 0.38 (median 0.33)	0.86

† Comparison between patients with and without major infection.

but not YB carrier status, were found to be independent risk factors for major bacterial infection after adjustment for age and duration of disease. Serum MBL level was shown to decrease risk of bacterial infection, with odds ratio (OR) 0.516, 95% confidence interval (95% CI) 0.305–0.873 (p = 0.01) compared to an increase in risk of bacterial infection for major lupus exacerbation that required daily prednisolone ≥ 15 mg (OR 1.382, 95% CI 1.154–1.654, p < 0.001). The interactive term of serum MBL level and major lupus exacerbation was not statistically significant (p = 0.61), suggesting absence of synergistic effect of these 2 factors. Multiple regression analysis using number of major bacterial infections demonstrated an increase in risk of bacterial infection by 34.2% for every decrease in serum MBL level by one log, and an increase in risk of bacterial infection by 22.8% for each additional major lupus exacerbation that required daily prednisolone ≥ 15 mg.

Time to first major infection. Patients in the inception cohort (n = 155) were prospectively followed for development of the first major infective episode from onset of SLE. Patients with YB haplotype (n = 49, 31.6%) (5.4 ± 2.6 yrs) were found not to differ in time to the development of the first major infection from those who were non-YB carriers (n = 106) by Cox regression analysis (5.6 ± 2.4 yrs) (p = 0.92).

DISCUSSION

Our study showed that serum MBL phenotype was associated with major infection in patients with SLE, whereas previous studies have related major infection to the frequency of MBL alleles rather than to the direct measure of MBL concentration[23,25]. Low serum MBL level was found to predispose our lupus patients to more major infection, in particular bacterial infection. This increase in risk was found to be independent of immunosuppressive use and was higher with further decrease in serum MBL level.

There have been no clinical data to suggest cutoff levels to define MBL deficiency or clinically relevant concentration of serum MBL. Different studies have used different cutoffs, for instance, < 500 μg/l[36] or < 1000 μg/l[37], for analysis. As MBL therapy has been suggested as immunotherapy to prevent infection in particular for immunocompromised patients[38-40], a clinically relevant MBL concentration would be useful to determine dosing of MBL therapy. A recent Phase I pharmacokinetic study on a single intravenous dose of recombinant

MBL (rhMBL) in healthy subjects with MBL deficiency showed a wide range of maximum observed plasma rhMBL concentrations: 0.5 mg/kg (9710 μg/l), 0.1 mg/kg (1987 μg/l), 0.05 mg/kg (1262 μg/l), and 0.01 mg/kg (200 μg/l)[41]. rhMBL was shown to be safe and had high tolerability[40,41] despite potential risk of complement activation[42]. Further studies are needed to evaluate its clinical efficacy and the optimal dose in relation to serum MBL level.

The association we found between MBL levels and infection has been shown to be stronger for bacterial infection than total major infection where intracellular microbes like *M. tuberculosis* were included. There were suggestions that MBL deficiency may be protective against obligate intracellular organisms, in contrast to the innate immunity of MBL against Gram-positive and Gram-negative bacteria, because MBL enhances opsonophagocytosis and thus access of these organisms to their intracellular niche[43,44]. There was no association found between serum MBL level and mycobacterial infection in our patients.

We demonstrated close association between serum MBL level and MBL genotypes and a strong relation between MBL level and the number of bacterial infections in our patients with SLE. However, we found no direct association between major infection and various MBL genotypes including the low MBL-producing YB haplotype. This discrepancy in MBL phenotype and genotype in predisposition to major infection can be due to the small number of patients with homozygous YB/YB genotype leading to significant type II error. Further, sera from subjects heterozygous and homozygous for MBL variant alleles have been found to contain low molecular weight dysfunctional MBL protein that may not be detected by an assay that depends on the repetitive structure of MBL[45]. Other factors that may affect serum MBL level include anti-MBL antibodies that have been described in some lupus patients[46,47], and different levels of inflammation associated with the disease as MBL is an acute-phase reactant. Comorbidities including steroid-induced diabetes mellitus and uremia from renal dysfunction related to lupus nephritis[48] may lead to further immunosuppression in these patients. The 2 infection-related deaths in this cohort involved patients who had endstage renal failure and serum MBL levels above median.

We showed lupus exacerbation with confounding high immunosuppressive use and MBL deficiency to be independent risk factors for major bacterial infections. Their effects were not shown to be synergistic. A number of previous studies suggested the clinical importance of MBL deficiency when the immune system was compromised after chemotherapy[36,49], whereas no relation was found between infection and low MBL level in immunosuppressed patients in other studies[50,51]. In the absence of MBL genotype confirmation, serum MBL level can be affected by inflammation associated with the underlying malignancy or by other factors such as the presence of anti-MBL antibodies in the serum.

Our study demonstrated that major infection was a significant morbidity in patients with SLE. Low serum MBL levels from MBL2 gene polymorphisms and major lupus exacerbation with confounding immunosuppressive use were both important predisposing factors to major bacterial infection in these patients. Comorbidities of these patients may add to further risk of infection.

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