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Original Article

Undetectable Mannose Binding Lectin and Corticosteroids Increase Serious Infection Risk in Rheumatoid Arthritis

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What is already known about this topic? Serious infections (SIs) are the leading cause of death in rheumatoid arthritis. Several clinical factors and most importantly prolonged corticosteroid use confer risk for SIs. So far, no serological predictor for SIs has been identified.

What does this article add to our knowledge? Mannose binding lectin (MBL) deficiency has been found to confer increased risk for SIs, comparable to the use of maintenance prednisolone.

How does this study impact current management guidelines? Knowledge of MBL status will emphasize SI risk and inform treatment decision making in rheumatoid arthritis. These findings are likely to inform clinical practice.

BACKGROUND: Infection is the leading cause of death in rheumatoid arthritis (RA). Corticosteroid (CS) use is a known and important risk factor for serious infections (SIs). Mannose binding lectin (MBL) is a genetically determined component of the innate immune system implicated in neonatal infections.

OBJECTIVE: Our aim was to determine whether MBL deficiency is a risk factor for SIs in RA and to compare it with CS use and also synthetic and biologic disease-modifying antirheumatic drug (DMARD) therapy.

METHODS: Data on 228 patients with RA were collected for up to 7 years (median = 5.9 years). Serum MBL concentrations were determined in all patients receiving synthetic (n = 96) or biologic (n = 132) DMARD therapy.

RESULTS: High rates of SIs were observed in RA irrespective of treatment (17%). Similar rates of SIs were observed in synthetic and biologic DMARD users. The rates of single and multiple SIs were similar, irrespective of the use of a biologic agent. Undetectable MBL (<56 ng/mL) concentrations and maintenance prednisolone at 10 mg per day or higher were associated with an increased risk for an SI, with incident risk ratio of 4.67 ($P = .001$) and 4.70 ($P < .001$), respectively.

CONCLUSIONS: Undetectable MBL and prednisolone confer a high risk for an SI. The use of biologic DMARDs did not confer substantial SI risk in this observational study. MBL deficiency is hitherto an unrecognized risk factor for an SI in RA. **(J Allergy Clin Immunol Pract 2017;5:1609-16)**

Key words: *Rheumatoid arthritis; Serious infection; Mannose binding lectin; Immune system; Risk factor*

Serious infections (SIs) are still the leading global cause of death in patients with rheumatoid arthritis (RA). Known risk factors for SIs in RA include increasing age, use of corticosteroids

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Abbreviations used
bDMARD- Biologic disease-modifying antirheumatic drug
COPD- Chronic obstructive pulmonary disease
CS- Corticosteroids
DMARD- Disease-modifying antirheumatic drug
ICC- Intraclass coefficient
IRR- Incident risk ratio
MBL- Mannose binding lectin
OR- Odds ratio
PY- Patient year
RA- Rheumatoid arthritis
sDMARD- Synthetic disease-modifying antirheumatic drug
SI- Serious infection
SNP- Single nucleotide polymorphism

(CS), and neutropenia, particularly in Felty's syndrome.[1,2] The inability to predict which patients will develop an SI represents an important unmet clinical need.

Mannose binding lectin (MBL) is a serum protein produced in the liver, which acts as a pattern recognition receptor.[3,4] MBL recognizes carbohydrate moieties on the surface of diverse microbes, including bacteria, viruses, fungi, and parasites. MBL binding results in the killing of microorganisms by the activation of the complement system with subsequent complement-mediated microbial lysis and phagocytosis of the microbe due to the opsonizing effect of C3b production.[5] The MBL glycoprotein in human serum is the product of the polymorphic MBL2 gene located on chromosome 10. Approximately 5% to 9% of the Caucasian population have very low concentrations of MBL (<56 ng/mL).[6] MBL deficiency has been associated with an increased susceptibility to infection in neonates, in young children with recurrent serious infections, and in adults with iatrogenic neutropenia.[7-9]

To evaluate risk factors for SIs in RA, an audit of an RA cohort was undertaken over a period of 7 years. In addition to established clinical and known laboratory parameters likely to increase risk, we also evaluated the potential for MBL serum concentration to confer the risk for an SI.

METHODS

The study was designed as an observational data audit. The project was evaluated by Fremantle Hospital and Sir Charles Gairdner Hospital Ethics and Human Rights Committees and given ethical approval (FH HREC 12/10 and SCGH HREC 2013-091). A waiver of consent was granted. No identifying details have been included in the article.

Participants were patients with RA of at least 6 months' duration who met the 1987 American College of Rheumatology diagnostic criteria[10] and who presented to private, public, or rural rheumatology clinics after January 1, 2007. Data were collected up to the April 30, 2014 (Table I). Data collection began when the patients either commenced a disease-modifying antirheumatic drug (DMARD) other than hydroxychloroquine or prednisolone, such as azathioprine, cyclosporine, leflunomide, methotrexate, or sulfasalazine, or when they commenced a biologic agent. Prednisolone is commonly used in Australia and is available in the form of 1 mg, 5 mg, and 25 mg. Its potency is similar to that of prednisone (1:1).[11] Patients who changed category, for example, a synthetic to biologic agent, were analyzed thereafter as biologic recipients. When participants switched to another biologic, they continued to be studied, and for the purpose of further analyses such as those shown in Table II, the biologic used for the longest period of time was assigned to those participants.

Biologic DMARDs (bDMARDs) include adalimumab, etanercept, infliximab, abatacept, rituximab, and tocilizumab. The internationally accepted definition for an SI was used, that is, infections that required hospital admission or treatment with intravenous antibiotics, with or without hospital admission.[12]

From January 1, 2007, to April 30, 2014, details concerning SIs were obtained by questioning at the time of the clinical review. Accordingly, every 3 to 6 months for the duration of the study, SIs were solicited by the attending physician. In May-June 2014, we conducted a retrospective audit of hospital admission records for all participants. A state-wide government health database was interrogated by 2 members of the investigative team. When an SI was confirmed, its nature and the outcome of treatment was recorded. In the month before commencement of synthetic or biologic DMARD therapy, the following investigations were performed: hepatitis B and C serology, QuantiFERON Gold testing for latent tuberculosis, serum MBL, serum immunoglobulins, and total neutrophil and lymphocyte counts. All participants had an MBL assay for the first time in conjunction with the other preliminary presynthetic DMARD or prebiologic tests as cited above. The choice of treatment was not influenced by the serum MBL concentration.

Measurement of serum MBL

The MBL Oligomer ELISA kit (Bioporto, Hellerup, Denmark) was used to determine the concentration of oligomerized MBL in human serum as per the manufacturer's instructions. In brief, microwells coated with a monoclonal antibody against the MBL carbohydrate-binding domain were incubated with diluted patient serum. Bound MBL was detected with a biotinylated MBL antibody and subsequent development using a horseradish peroxidase conjugated streptavidin tetramethylbenzidine substrate. In this assay, the intensity of the resulting colored product is directly proportional to the concentration of MBL in the serum. It should be noted that the assay determines MBL concentrations and does not assess MBL function. The lower limit of detection for this assay was 56 ng/mL.[13] The threshold of 56 ng/mL is based on a historical and current cutoff reported by the Western Australian Pathology Laboratory. When the assay was first introduced, the lower limit of detection in our laboratory was determined to be <56 ng/mL, based on the standards included in the measurements. As some of the samples used in this study were measured at this initial time, we maintained this threshold for all samples. We acknowledge that the assay can measure lower values, and the currently most used cutoff in the literature is 50 ng/mL.

The reference intervals for MBL concentrations are based on published data from comparable populations.[14,15]

Statistical analysis

Descriptive statistics are presented as means and standard deviations for continuous variables and as percentages for categorical variables. Statistical analysis was carried out using simple parametric tests as well as negative binomial regression models (to investigate factors related to the incidence of SIs) and logistic regression models (to investigate factors related to multiple SIs). Rate ratios and 95% confidence intervals are reported. All the statistical analyses were carried out using Stata v13.7.[16]

TABLE I. Participant characteristics

Caucasian 99.6%	Synthetic DMARD (n = 96)			Biologic DMARD (n = 132)			*P* value
Male (n = 74)	42 (44%)			32 (25%)			.002
Female (n = 154)	54 (56%)			100 (76%)			
Male MBL level	<56 5	56-399 4	399-1299 33	<56 4	56-399 5	399-1299 23	
Female MBL level	<56 11	56-399 6	399-1299 37	<56 13	56-399 19	399-1299 68	
Median age	68 (range 24-90)			64 (range 21-93)			.031
Rural	13 (14%)			28 (21%)			.136
Urban	83 (86%)			104 (79%)			
Median disease duration at baseline (y)	10 (range 0.5-52)			11 (range 1-46)			
Median number of DMARDs	1 (range 0-5)			2 (range 0-8)			.004
Corticosteroid at baseline	9 (9%)			23 (17%)			
Rheumatoid factor positive	49 (67%* of 73 samples)			77 (74%* of 104 samples)			.317
CCP† antibody positive	17 (41%* of 41 samples)			28 (67%* of 42 samples)			
Neutropenia ($\leq 2.0 \times 10^9$/L)	3 (3%)			4 (3%)			
Diabetes type 1 or 2	10 (13%)			7 (7%)			.084
Smokers (past and present)	34 (49%)			35 (31%)			.019
COPD	9 (10%)			11 (8%)			.638
Interstitial lung disease	5 (6%)			9 (7%)			.709

CCP, Cyclic citrullinated peptides; *COPD*, chronic obstructive pulmonary disease; *DMARD*, disease-modifying antirheumatic drug; *MBL*, mannose binding lectin.
*Indicates the number positive as a percent of the number of available samples. Some participants did not have rheumatoid factor or CCP sera available for assays.
†CCP are frequently used to detect autoantibodies in patient serum or plasma (then referred to as anticitrullinated peptide antibodies).[10]

RESULTS

In total, 228 participants who satisfied the 1987 American College of Rheumatology criteria for the diagnosis of RA were audited, the demographic data for whom are shown in Table I. Ninety-six participants (42 males and 54 females), median age 68 years, received synthetic DMARDs (sDMARDs) alone; and 132 participants (32 males and 100 females), median age 64 years, received bDMARDs with or without sDMARDs. Of the cohort, 18% lived in rural areas of Western Australia, and 82% lived in urban areas. Socioeconomic, education, employment status, income, or area-based measures of disadvantage were not collected.

Seven participants, 4 of whom did not develop SIs, had neutropenia (1.5 to 2.0 neutrophils/nL; normal 2.0 to 7.5 $\times$ 10^9/L) in the full blood count performed closest to the time of the MBL measurement. None had Felty's syndrome. Two had hypogammaglobulinemia with IgG concentrations of 5.9 and 5.5 g/L (normal 6.10 to 13.00 g/L) and IgM concentrations of 0.3 and 0.9 g/L (normal 0.83 to 3.32 g/L), respectively. Twenty-two participants died (9.6%) and 18 participants were lost to follow-up (7.9%).

The mean duration of observation was 5.92 years (SD = 1.63, n = 228). The 132 participants in the bDMARD group received the bDMARD, either alone (n = 42) or in combination with one or more sDMARDs (n = 90). The types of DMARDs and the relative proportion of each are shown in Table II. Single SIs were observed in 48 (21%) participants (18/96 in the sDMARD group and 30/132 in the bDMARD group). Multiple SIs (2 or more, median = 2, range 2-14) were observed in 30 (13%) participants (11/96 and 19/132, respectively).

Thus, patients receiving bDMARDs, compared with sDMARDs, had a similar incidence of SIs (incident risk ratio [IRR] = 0.90; 95% CI 0.64-1.27), after controlling for age and gender.

In 18 study participants, the MBL was measured at more than 1 time point. These patients were identified retrospectively to have had repeat testing in the same laboratory; hence they were not selected based on clinical characteristics. We did not deliberately choose to repeat the assay in any participants in the study and in particular not in those who were found to have SIs. The repetition was inadvertent and coincidental. The intraclass coefficient (ICC) was calculated to assess the reliability of the MBL assay. The ICC value using a 2-way random effect model was found to be 0.93, 95% CI 0.67 to 0.98, indicating a high degree of measurement reliability.

Additionally, we audited data in our laboratory to assess the changes in MBL measurements in an individual over time. Over the past 10 years, 5761 MBL measurements were performed in our routine diagnostic laboratory. In 331 subjects, at least 1 repeat measurement of the MBL concentration was performed. A strong correlation between repeated measures was observed over time. Spearman's rank-order correlation coefficient = 0.9258 (CI 0.9081-0.9402; $P < .0001$). In those with more than 1 repeat testing, the correlation between the first 5 tests ranged from 0.860 to 0.968 ($P < .01$). The range of time between measurements was 0 to 9.1 years. The greatest fluctuation in concentration was detected in those with higher concentrations of serum MBL (Figure 1). The least fluctuation was observed in persons with low concentrations, which strengthens our confidence in the reliability of single measurements that yield low concentrations compatible with genetic MBL deficiency.

Furthermore, whether testing for genetic variation within the MBL gene in conjunction with serum MBL concentrations provides additional information remains unclear. There are 5 known single nucleotide polymorphisms (SNPs) that affect the level of serum MBL concentrations. Codon 52 (minor allele D),

TABLE II. Types of serious infections according to treatment and MBL concentration (undetectable or detectable)

	Synthetic DMARD		Biologic DMARD		
Type of Infection	**No. (n = 63)**	**No. (%) with MBL <56**	**No. (n = 92)**	**No. (%) with MBL <56**	***P* value**
Respiratory	33	19 (58)	23	7 (30)	.045
Urological	6	3 (50)	13	1 (8)	.426
Gastrointestinal	4	0 (0)	7	3 (43)	.216
Skin	7	0 (0)	21	4 (19)	.545
Septicemia	6	1 (17)	8	1 (13)	1.0
Joint, bone, and muscle combined	4	3 (75)	11	8 (73)	1.0
Septic arthritis	2	1 (50)	6	5 (83)	
Other	1	0 (0)	3	0 (0)	

Biologic agent	No.(percentage of total)	Female/Male	% Female	Rural/Urban	Percentage of Rural	Age (y) Median (range)	DAS28 Median (range)	% RF positive/% CCP positive
Etanercept	91 (69%)	65/26	71	18/73	20	65 (26-95)	2.64 (0.14-7.22)	74/70 (30*)
Adalimumab	28 (21%)	25/3	89	8/20	29	73 (43-94)	3.68 (1.02-4.93)	61/40 (5*)
Rituximab	5 (4%)	4/1	80	0/5	0	64 (49-83)	4.4 (1.84-5.88)	75/67 (3*)
Abatacept	4 (3%)	3/1	75	2/2	50	61 (35-65)	3.32 (1.25-4.5)	67/50 (2*)
Tocilizumab	3 (2%)	3/0	100	0/3	0	75 (54-78)	NA	100/100 (2*)
Infliximab	1 (1%)	1/0	100	0/1	0	79	NA	100/NA
Total	132 (100%)	100/32	76	28/104	21	65 (27-95)	2.85 (0.14-7.22)	74/67 (42*)

The biologic DMARD used for the longest period of time for any given patient was recorded in those patients who changed agents. Biologic DMARDs were not used in combination.

CCP, Cyclic citrullinated peptides; *DMARD*, disease-modifying antirheumatic drug; *MBL*, mannose binding lectin; *NA*, not available due to small numbers or no testing; *RF*, rheumatoid factor.

*Refers to the number of patients who had sera available for CCP assays.

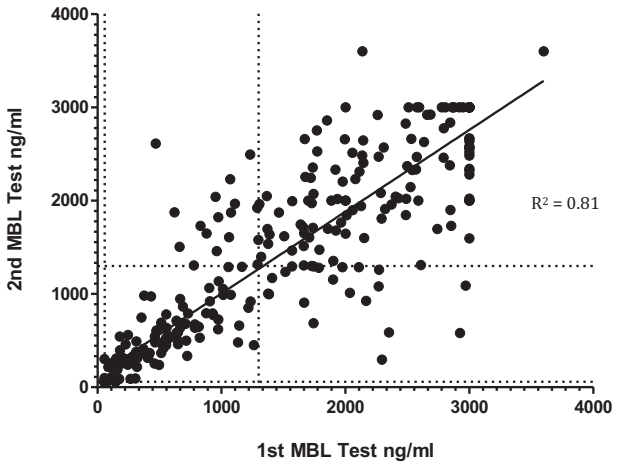

FIGURE 1. Correlation between the 1st and 2nd mannose binding lectin (MBL) test (n = 331). R^2 = 0.81 (complete data set). The dotted lines indicate the grouping (namely <56 ng/mL and <1300 ng/mL) used in our study.

54 (minor allele B), and 57 (minor allele C) SNPs are all located on exon 1. The major allele is named allele A (no SNP). The presence of any minor allele (sometimes collectively named the O allele) results in a significant reduction of serum MBL concentrations, with homozygosity or a combination of minor alleles resulting in very low or almost absent serum MBL concentrations. There are also additional SNPs in the promoter region of the gene that can influence MBL concentration, resulting in some individuals with AA or AO genotypes that also have reduced concentrations.[17] In the laboratory, 731 specimens were screened for the presence of homozygous or heterozygous minor allele while concurrently measuring serum MBL concentrations. In this laboratory cohort 132 of 731 had an MBL concentration of <56 ng/mL; 128 of 132 (97%) carried at least 1 minor allele; mutations in minor allele B (codon 54) were the most common; with the homozygous presence of minor allele B in 28 of 132 (21%) and the heterozygous presence of minor allele B in 58 of 132 (44%), 34 of these 58 had an additional variation in codon 52 or 57.

MBL genotyping

Genotyping was performed by PCR-restriction fragment length polymorphism based on the method described by Madsen et al.[18]

In the RA cohort, we performed MBL genotype testing in 19 of the 34 participants with MBL concentrations <56 ng/mL. All 19 carried at least 1 minor allele (5 homozygous variation and 7 heterozygous variation at minor allele B; 1 homozygous variation and 3 heterozygous variation at minor allele D; 2 with heterozygous variation at minor allele B and D; 1 with heterozygous variation at minor allele C).

Thus, in these data sets, undetectable concentrations of serum MBL of <56 ng/mL were highly indicative of the presence of minor alleles and the allele determination in those with low serum levels offered very little additional clinical information. On the other hand, participants with very high concentrations of MBL above 1300 ng/mL can still have heterozygous variation within codons 52, 54, and 57, but in this concentration range, no homozygosity for minor alleles was detected in this cohort. This indicates that genotyping alone without performing serum MBL concentrations may be misleading, unless variation in the

promoter regions, which influence expression levels, is also being considered.

Thirty-three participants in this study had undetectable MBL (14.5%), which is higher than the background rate in comparable Caucasian populations for whom such data have been reported and also higher than in the RA cohort studied by Saevarsdottir et al,[13] who reported undetectable MBL in just under 10% of 330 healthy volunteers and blood donors and in just under 8% of an early RA cohort in Iceland. In this study, 20 of the 33 patients with undetectable MBL (61%) developed 1 or more SIs. Patients with RA with undetectable MBL had an odds ratio (OR) for an SI of 4.67. As can be seen in Figure 2, participants with undetectable MBL had significantly higher rates of SIs in the whole group and also in the sDMARD and the bDMARD subsets.

The incidence of SIs by MBL concentration and prednisolone dose was examined (Figure 3). It can be seen that the SI rate was a function of both MBL concentration and corticosteroid dosage. Participants with an undetectable MBL who were taking prednisolone at a dose greater than 5 mg per day were found to have the highest susceptibility to SIs with a rate of 68 per 100 patient years (PYs) in comparison to 6 per 100 PYs for those taking less than 5 mg per day of prednisolone in whom MBL was detectable and 20 per 100 PYs in those taking greater than 5 mg per day of prednisolone in whom MBL was detectable. The possibility of synergy between undetectable MBL and higher dose prednisolone was considered. Tests for an interaction between MBL and prednisolone did not support synergy ($P = .203$). During the almost 6-year period of observation, approximately 12 versus 9 SIs per 100 PYs were recorded for synthetic and biologic DMARD recipients, respectively. Thus, no substantive difference in SI rates was observed.

The types and frequencies of SIs according to treatment category are shown in Tables II and III. Amongst this cohort, pneumonia and other respiratory tract infections were the most common SI occurring in 39.2% of all participants with 1 or more SIs and dominating in both the synthetic and biologic treatment groups. A significantly higher frequency of respiratory tract infections was observed in recipients of sDMARDs ($P < .045$). Skin infections of all types accounted for 19.6% of SIs and trended toward a higher frequency in biologic recipients, but the difference was not statistically significant. Urological infections occurred in 13.3% and joint, bone, and muscle infections combined occurred in 10.5%. There was no significant difference in the rate of septic arthritis between the 2 groups; rates observed were 7.2% for the bDMARD group and 3.3% for the sDMARD group ($P = .48$). The type and frequency of SIs in bDMARD recipients are set out in more detail in Table III. Musculoskeletal infections were overrepresented in the etanercept recipients.

There were 22 deaths over the observation period: 11 (8.3%) in the biologic DMARD group (n = 132) and 11 (11.4%) in the synthetic DMARD group (n = 96). Of these 22 deaths, in 7 patients an SI was the primary cause of death (6 respiratory tract infections of which 4 were in the biologic DMARD group and 2 in the synthetic DMARD group; and 1 urinary tract infection with septicemia in the synthetic DMARD group). Amongst these 7 infection-related deaths, 3 had undetectable MBL and 3 had MBL in the range 56 to 1300 ng/mL.

Age was another significant predictor of SIs. For every 5-year increase in age, there was a 19% increase in the risk of an SI (IRR = 1.19, 95% CI 1.06-1.33) using negative binomial regression. For every 10-year increase in age, there was a 41% increase in the risk of an SI (IRR = 1.41, 95% CI 1.12-1.77). Gender, smoking, diabetes, chronic obstructive pulmonary disease (COPD), low neutrophil or lymphocyte counts, and low immunoglobulin concentrations did not confer increased risk for SIs in our cohort.

SIs occurred in 23 of the 30 participants who were receiving CS (77%). The median dose of prednisolone in these 23 participants was 7 mg per day (range 3-20 mg/day). IRRs for SIs according to corticosteroid dosage were determined by a negative binomial model. Compared with no CS, the IRR for an SI with a dose of 1 to 4.5 mg of prednisolone per day was 2.57, for 5 to 9.5 mg per day it was 3.38, and for doses of 10 mg per day or more it was 4.70. Thus, severe MBL deficiency confers a risk for an SI similar to that of regular use of prednisolone $\geq$10 mg per day.

Multiple SIs (2 or more) were observed in 30 patients with RA (13.2%), 9 of whom had undetectable MBL concentrations and 16 of whom had MBL concentrations $\leq$600 ng/mL (OR = 4.01, CI 1.40-11.45, $P = .009$).

There have been reports indicating that MBL deficiency is associated with chronic lung infections such as in cystic fibrosis.[19] When we examined the relationship between MBL deficiency and all nonpulmonary SIs in this cohort, a strong association between undetectable MBL concentrations and SIs was still observed for all of these infections taken together, but most commonly for bone, joint, and muscle infections combined (11 of the 15 participants with bone, joint, and muscle infection had an undetectable MBL concentration).

DISCUSSION

Serious infections are a major cause of morbidity and mortality in RA. This observational data audit shows that undetectable MBL (OR = 4.67) and CS use (prednisolone dose of 10 mg per day or higher, OR = 4.70) confer high risk for an SI. Increasing age was also found to be another significant, but lesser predictor of an SI. MBL deficiency is a hitherto unrecognized risk factor for an SI in RA.

Crowson et al in a series of studies addressing SIs in RA identified several major risk factors, notably increased age and CS use. They also showed that extra-articular manifestations of RA and comorbidities such as chronic lung disease, coronary artery disease, peripheral vascular disease, organic brain disease, diabetes mellitus, chronic renal disease, and alcoholism were associated with increased SI risk.[1,2] Furthermore, a previous SI was shown to predict subsequent SIs, a risk that we were unable to evaluate in this cohort.

The findings reported here indicate that an undetectable MBL concentration is an important risk factor for both single and multiple or recurrent SIs in RA with IRRs comparable with those of moderate dose maintenance CS therapy. Amongst those patients with RA with an undetectable MBL concentration, the rate of SIs was 25 per 100 PYs (95% CI 18.1-31.9), which was substantially higher than that for patients with RA with a detectable MBL (8 per 100 PYs, $P < .001$). Furthermore, these findings indicate that MBL deficiency confers a higher risk for an SI than age, tobacco consumption, COPD, or type 1 diabetes mellitus and type 2 diabetes mellitus. It is worth noting that no patient aged less than 50 years with MBL concentrations greater than 56 ng/mL and taking <5 mg of prednisolone per day

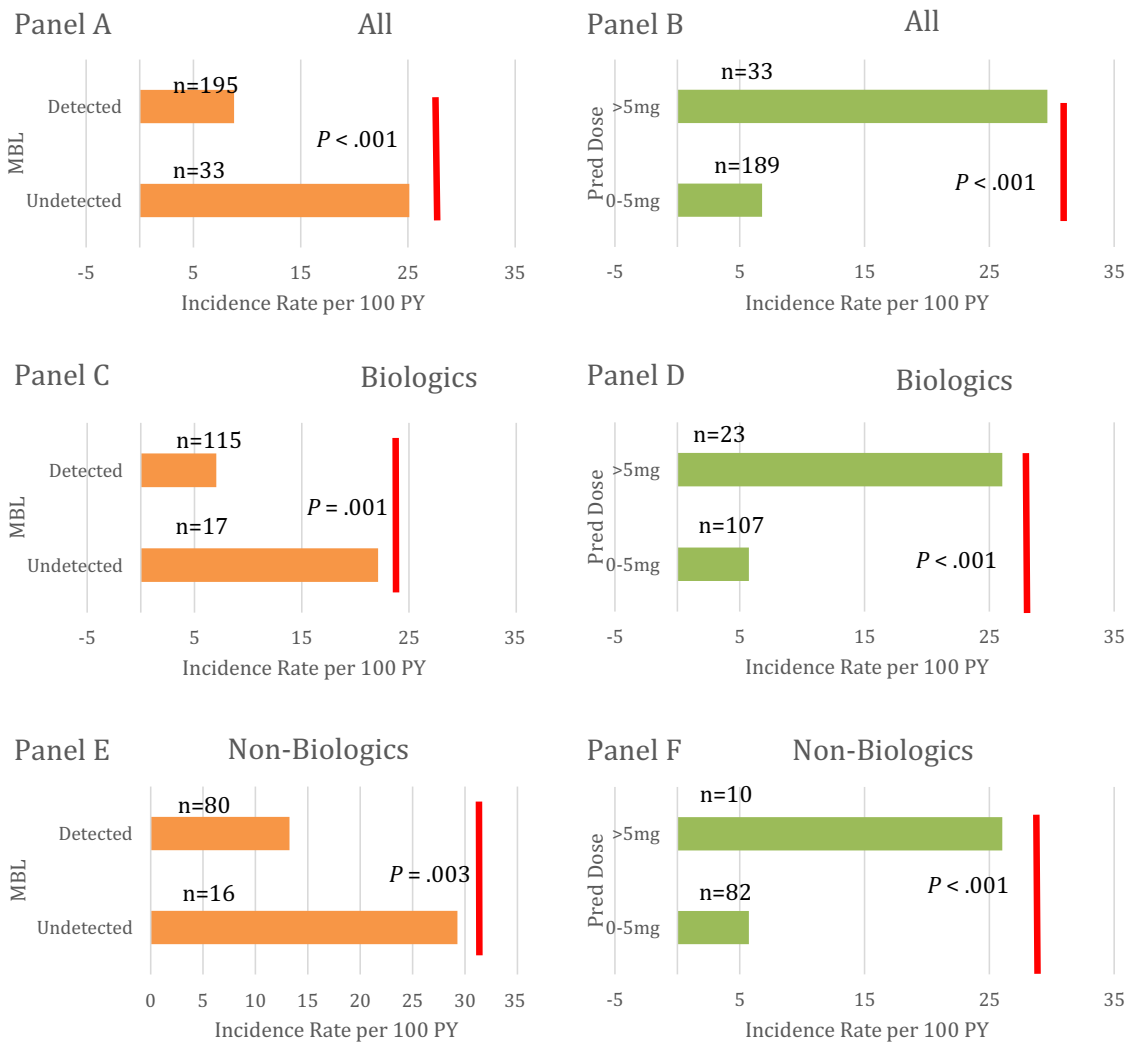

FIGURE 2. Number of serious infections per hundred patient years according to MBL concentrations or prednisolone dose; participants treated with biologic DMARDs (n = 132) or synthetic DMARDs (n = 96). "Pred" denotes prednisolone, "Biologics" denotes biologic DMARDs, and "Non-Biologics" denotes synthetic DMARDs. *DMARD*, Disease-modifying antirheumatic drug; *MBL*, mannose binding lectin; *PY*, patient year.

(n = 16) developed an SI over the median 5.9 years of observation, whereas all 6 patients aged over 65 years with undetectable MBL concentrations and taking ≥5 mg of prednisolone each day developed 1 or more SIs (6.5-fold increase in the risk of an SI [IRR = 6.49, 95% CI 4.07-9.97, $P < .001$]).

To date, there is limited published evidence addressing changes in MBL concentration over time and in particular, during the time of acute infection or disease flares. Longitudinal data by Auriti et al[20] showed that neonates with nosocomial sepsis had a significantly higher median MBL concentration after the onset of infection compared with the admission concentration. The MBL concentration decreased commonly after resolution of infection, although a large variation in pattern was seen overall. Takahashi et al[21] investigated changes in MBL concentration over time in a subset of 14 patients with newly diagnosed systemic lupus erythematosus to assess the impact of treatment on MBL. In this study, MBL concentrations rose in 6 of 14 patients and declined in 7 patients, whereas 1 patient showed no change. There was no significant association between an increase or a decrease of serum MBL concentration, MBL genotype, or clinical characteristics. We addressed the question of variability of MBL concentrations over time, by analyzing retrospective data from the local immunodiagnostic laboratory, in addition to randomly performed repeat measurements of MBL in this RA cohort. Both datasets showed that repeat measurements were highly correlated, especially in persons with lower MBL concentrations or undetectable MBL. Additionally, the genotypic analysis presented here indicated that undetectable MBL was highly indicative of the presence of minor alleles. Allele determination in those persons provided little additional information.

On the basis of the limited evidence available so far, the authors recommend that the concentration of MBL should be determined when the patient is clinically well with no evidence of an acute infection or disease flare, for example, normal range C-reactive protein, as occurred mostly in this study.

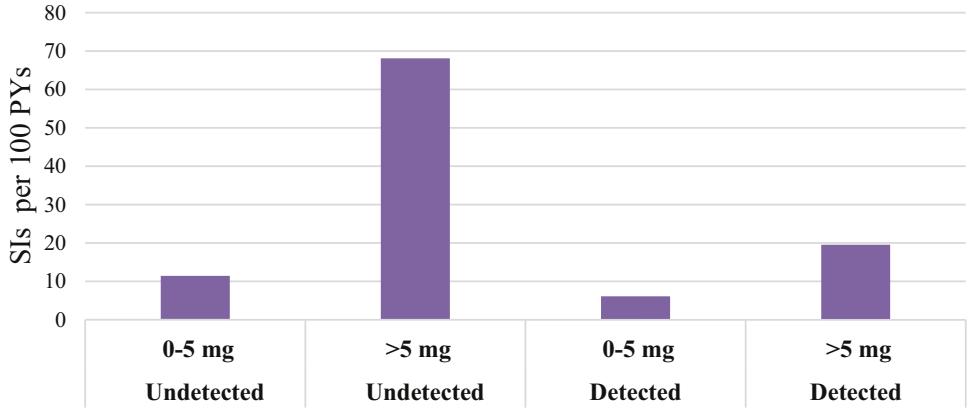

FIGURE 3. The incidence of serious infections (SIs) by mannose binding lectin (MBL) concentration (undetectable or detectable) and dose of prednisolone (0-5 mg and >5 mg). A much higher rate of SIs was observed in participants with undetectable MBL who were receiving a prednisolone dose >5 mg (68 per 100 patient years of treatment or observation). Statistical tests for an interaction between MBL and prednisolone did not support synergy ($P = .203$).

TABLE III. Types of serious infections and biologic DMARDs

	Resp	Urological	Gastro	Skin	Sept	Joint	Bone	Other
Abatacept			2					
Adalimumab	5	7	2	4	5			1
Etanercept	14	4	3	16	2	11	6	2
Infliximab	1							
Rituximab		1						
Tocilizumab	3	1		1	1			
Total	23	13	7	21	8	11	6	3

Resp denotes respiratory; Gastro denotes gastrointestinal; Sept denotes septicemia.
DMARD, Disease-modifying antirheumatic drug.

Interestingly, musculoskeletal SIs amongst bDMARD users were restricted to etanercept recipients. A similar observation was made in the German Biologics Registry, referred to as RABBIT, in which 5 of 512 etanercept-treated patients with RA developed septic arthritis, whereas only 1 of 346 infliximab recipients and 1 of 601 RA controls not taking tumor necrosis factor inhibitors developed septic arthritis.[22] In RABBIT, the difference was not statistically significant. Further studies are required to determine whether etanercept confers increased and selective risk for musculoskeletal sepsis.

It is well recognized that the use of CS is associated with an increased risk for serious infection, both in RA and other diseases. Doses of prednisolone 10 mg or more were associated with 88 SIs per 100 PYs in this study, which is well above the rate of 7 per 100 PYs encountered amongst patients with RA taking prednisolone at a dose of less than 5 mg per day ($P = .012$).

One of the strengths of this observational data audit is the longitudinal observation, which has allowed SIs to be captured well beyond the usual time frame applicable to clinical trials and in particular randomized controlled trials. Furthermore, the ascertainment of SIs did not rely heavily on self-reporting, as is the case for some registries or on physician reporting, where compliance can be an issue, but rather is the product of regular questioning by a physician, cross-referencing against hospital discharge summaries and a comprehensive audit. Importantly, no patient was excluded from cohort entry because of previous SIs. Thus, a high degree of SI ascertainment, reflecting real-world experience, is likely to have been achieved. Weaknesses include the absence of a large non-RA or untreated RA control group and the heterogeneous nature of the RA study population with a bias toward more severe biologic DMARD-treated RA. Many biologic agents are underrepresented because of the data audit commencement date in 2007. Furthermore, over time, changing from one agent to another became increasingly common, thus complicating between biologic comparisons. Small numbers limited the assessment of combinations of risk factors, such as the combination of undetectable MBL concentrations and prednisolone dose of 10 mg or more in the same participant (n = 2). It is also noteworthy that the number of participants in special interest subsets, such as Felty's syndrome, RA with hypogammaglobulinemia, and RA with neutropenia, were too few to permit deep analysis. Because of the predominant Caucasian ethnicity of the participants, the findings may not be generalizable to other racial groups.

The observation of a similar rate of SIs in recipients of synthetic and biologic DMARDs was unexpected, but not entirely out of keeping with the longer term observational nature of this study

and the rigorous review of SIs that was undertaken by means of hospital record audit. Large meta-analyses, such as that of Singh et al,[23] showed no statistically significant increase in SIs for low dose biological drugs with or without synthetic DMARD therapy, but did note increases for standard dose, high dose, and especially combination biological drugs. It should be noted, however, that most of the studies on which they relied were short-term controlled trials (less than 1 year) with a placebo comparator and from which an indeterminate number of patients including the elderly may have been excluded. A higher rate of SIs in biologic recipients in the first year of therapy may contribute to the differences between meta-analyses based mainly on controlled trials and the findings in this longer term observational study.

Whether MBL replacement can protect against SIs or be useful for the treatment of an established SI in a critically ill patient remains unknown, but warrants further consideration in the light of these findings and the studies already underway to ascertain whether MBL replacement may improve outcomes in MBL-deficient neonates with septicemia.[19,24]

In the meantime, because serious infections in RA are common and potentially life threatening, clinicians should carefully consider SI risk factors before making therapeutic decisions in patients about to begin or change synthetic or biologic DMARDs. Throughout therapy, a high level of vigilance is required for both synthetic and biologic DMARD recipients, but especially in those who are elderly, receiving corticosteroids and now also we suggest, those with undetectable MBL concentrations.

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