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;■:■-■)**

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]