

Macrophage Migration Inhibitory Factor in Systemic Lupus Erythematosus

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ABSTRACT. Objective. To examine associations between serum macrophage migration inhibitory factor (MIF) and disease-related variables and corticosteroid use in patients with systemic lupus erythematosus (SLE).

Methods. Serum MIF concentration was measured by ELISA in 90 female patients with SLE and 279 healthy controls. Univariate and multivariate regression analyses were used to examine the associations between serum MIF concentration and disease-related indices of SLE and corticosteroid use.

Results. Serum MIF concentrations were positively associated with SLE disease damage (SLICC/ACR index), and indices of disease damage were greater in SLE patients with serum MIF concentrations above the normal median value. Serum MIF concentration was also observed to be significantly greater in patients with SLICC/ACR damage index (DI) scores ≥ 3 . Serum MIF was also positively associated with current corticosteroid dose. Significantly higher SLICC/ACR DI scores were observed in patients with values of serum MIF above the normal median, and this remained significant after adjusting for corticosteroid dose. Serum MIF concentration was also predictive of SLICC/ACR index after 3 years of followup, but this association was partly confounded by corticosteroid dose. Serum MIF was also negatively associated with serum creatinine concentration, independent of disease damage and corticosteroid dose.

Conclusion. MIF is overexpressed in patients with SLE. While this can be partly explained by corticosteroid use, there is evidence of an association between MIF and lupus-related disease damage. (J Rheumatol 2004;31:268–73)

Key Indexing Terms:

SYSTEMIC LUPUS ERYTHEMATOSUS

CORTICOSTEROIDS

MACROPHAGE MIGRATION INHIBITORY FACTOR

The cytokine macrophage migration inhibitory factor (MIF) was first discovered in the 1960s, but it has not been until the last decade that its wide range of actions on the immune-inflammatory system has been fully appreciated. MIF is constitutively expressed by B cells, and antagonism of MIF inhibits B cell proliferation¹. It is also produced by T cells stimulated by recall antigens, mitogens, and anti-CD3 antibodies, and antagonism of MIF prevents T cell activation by these factors². MIF has a wide variety of proinflammatory actions in inflammatory lesions, including the upregulation of phospholipase A₂ and cyclooxygenase³. Studies in animal

models show a pivotal role for MIF in the pathogenesis of rheumatoid arthritis (RA) and glomerulonephritis^{4,5}, and detection of MIF in human RA and glomerulonephritis^{6,7} has led to increasing acceptance of MIF as a key cytokine in chronic inflammatory diseases. Of particular interest in systemic lupus erythematosus (SLE) is the observation that MIF is the only proinflammatory cytokine that is induced, rather than suppressed, by corticosteroids^{6,8}. In animal models and *in vitro*, moreover, MIF exerts an antagonistic effect on corticosteroid suppression of immune-mediated inflammation^{8,9}. The hypothesis that MIF operates as a physiological counter-regulator of corticosteroids suggests that therapeutic antagonism of MIF may have specific steroid-sparing benefits.

SLE is a chronic multisystem autoimmune disease with an unknown etiology, characterized by abnormalities of immune-inflammatory system function including altered B and T cell function, and by inflammation of organs including joints and kidneys¹⁰. Corticosteroids are a mainstay of the treatment of SLE, despite their widely known adverse effects including reduced bone mineral density¹¹. Despite the accumulation of evidence for a key role for MIF in autoimmune-inflammatory diseases, MIF has not been extensively investigated in SLE. The detection of MIF in

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Submitted October 4, 2002; revision accepted July 24, 2003.

humans with SLE would suggest that further research into the role of MIF in SLE is justified. To assess the potential involvement of MIF in SLE and its relationship with corticosteroids, we studied the relationship between serum MIF concentration, corticosteroid use, and disease-related variables in patients with SLE.

MATERIALS AND METHODS

MIF enzyme linked immunosorbent assay (ELISA). Sera were separated from peripheral blood samples obtained between 9 A.M. and 12 P.M. and stored at -20°C prior to assay. A sandwich ELISA was utilized to determine the MIF concentration of the samples. Ninety-six well ELISA plates (Immunoplates; Nunc, Roskilde, Denmark) were coated with monoclonal mouse anti-human MIF antibody (R&D Systems, Minneapolis, MN, USA) and incubated for 24 h before being blocked with 1% bovine serum albumin in 5% sucrose-phosphate buffered saline. After washing, samples and standards of recombinant human MIF (R&D Systems) were added in duplicate and incubated overnight. MIF was detected with biotinylated goat anti-human MIF antibody (R&D Systems) and streptavidin conjugated to horseradish peroxidase (Silenus, Melbourne, Australia). Color was developed with 3,3',5,5'-tetramethylbenzidine (Sigma, Sydney, Australia) and read at 450 nm against a standard curve for human MIF. The detection limit of the assay was 31.25 pg/ml, the intraassay coefficient of variability was < 10%, and the interassay coefficient was < 6%.

Determination of a normal range for serum MIF concentration. The normal range for serum MIF concentration was determined using blood donors from the Red Cross Blood Bank in Melbourne, Australia. Recruitment was undertaken over a 2 month period, and was limited to women ($n = 279$), as all patients with SLE examined in this study were women.

SLE patients. All patients fulfilled the American College of Rheumatology criteria for the classification of SLE¹², and were originally recruited for studies of bone density and body composition^{11,13}. Disease-related variables included disease damage, measured by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR DI)¹⁴ and disease activity assessed using the clinical components of the Systemic Lupus Activity Measure (SLAM)¹⁵; laboratory endpoints of the SLAM were not obtained at the time of clinical data collection¹³. Variables relating to corticosteroid use included current corticosteroid dose, defined as the daily dose of prednisolone (mg/day) at the time of study. Additional variables examined included duration of SLE, age, body mass index (BMI), and serum creatinine concentration. The study was approved by the Human Research Ethics Committee of Monash Medical Centre, Melbourne, Australia.

Statistical methods. Serum MIF concentrations in both the controls and the patients with SLE were not normally distributed and therefore required natural logarithmic transformation. Transformed results were used for all analyses. A normal range for serum MIF was defined as the 2.5th to 97.5th percentile range in the control group. Two-sample unpaired t tests were used to compare continuous variables, and chi-square tests used for categorical variables. Univariate and multivariate regression analysis was used to examine the relationship between serum MIF concentrations, disease-related variables, and corticosteroid dose in patients with SLE.

A cross-sectional analysis was undertaken in patients with SLE ($n = 90$) to examine the associations between serum MIF concentrations, disease-related variables, and corticosteroid use. To ascertain the prognostic value of serum MIF concentration, a predictive analysis was also undertaken in a subgroup of these patients ($n = 27$) who had undergone repeat clinical assessment after a 3 year interval¹¹.

Statistical analyses were performed using Stata version 6.0. P values less than 0.05 were considered to be statistically significant.

RESULTS

Figure 1 shows the serum MIF concentration in the 279 control samples. It ranged from 0.8 to 19.6 ng/ml. The median value was 3.3 ng/ml and the normal range (2.5th to 97.5th percentile range) was 1.1 to 13.1 ng/ml.

Patient clinical characteristics are outlined in Table 1. The mean (\pm SEM) values of serum MIF for controls was 4.0 ± 0.2 ng/ml, compared to SLE patients' 5.9 ± 0.8 ng/ml (not significant, NS). A greater than expected proportion of patients with SLE (17.0%) had concentrations of serum MIF that exceeded the upper limit of the normal range (control 2.2%, $p < 0.001$). Table 2 outlines corticosteroid use and disease-related variables for patients with serum MIF concentrations below or above the median value (3.3 ng/ml) in the normal population. SLICC/ACR DI scores ($p = 0.020$), but not disease activity scores ($p = 0.365$), were significantly greater among SLE patients with serum MIF concentration above, compared to those below, the normal median value. Current corticosteroid doses were also significantly higher among SLE patients with serum MIF above the normal median value ($p = 0.010$). The higher SLICC/ACR DI scores for patients with serum MIF concen-

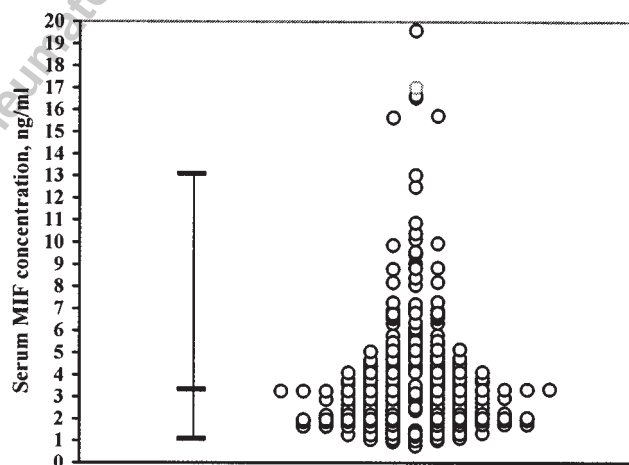


Figure 1. Serum MIF concentration in controls. Serum MIF was measured by ELISA in 279 female blood donors. The median and normal range are shown as bars, together with a scatter plot of all values.

Table 1. Clinical characteristics of patients with SLE.

	Median	Range
Age, yrs	45.0	18–81
Body mass index, kg/m ²	25.2	16.4–35.4
Serum creatinine	0.07	0.04–0.14
Disease duration, yrs	6.7	0.1–32.8
SLICC/ACR DI score	0	0–7
SLICC/ACR DI score > 0, %	43.6	
SLAM score	3.0	0–9
Corticosteroid dose at time of the study, mg/day	2	0–60
Corticosteroid dose at time of the study > 7.5 mg/day, %	22.3	

Table 2. Disease and corticosteroid variables by serum MIF concentration for patients with SLE. Data are median (interquartile range).

	< 3.3 ng/ml, n = 49	≥ 3.3 ng/ml, n = 41	p
SLICC/ACR DI score	0.0 (0.0–1.0)	1.0 (0.0–2.0)	0.020
Disease activity score	3.0 (1.0–4.0)	3.0 (1.5–5.0)	0.365
Corticosteroid dose at time of study, mg/day	0.0 (0.0–4.5)	5.0 (0.0–9.4)	0.010

trations above the normal mean value remained significantly greater even after adjusting for current corticosteroid doses ($p = 0.034$). Adjusting for corticosteroid use did not alter the findings for disease activity score.

Figure 2 shows the median and interquartile range of serum MIF concentration according to corticosteroid dose at the time of study. On univariate analysis, a significant trend in serum MIF concentration was seen across categories of current corticosteroid dose ($p = 0.003$). Figures 3 and 4 show the median and interquartile range of serum MIF concentrations for patients categorized according to SLICC/ACR DI and disease activity scores. There was a trend for serum MIF concentration to increase across SLICC/ACR DI ($p = 0.070$), but not disease activity scores ($p = 0.360$). Serum MIF concentration was also observed to be significantly greater in patients with SLICC/ACR DI scores ≥ 3 , compared to those with scores < 3 ($p = 0.045$). No similar threshold value of disease activity score above which serum MIF concentration was significantly increased was observed.

No association was seen between serum MIF concentration and duration of SLE ($p = 0.186$), age ($p = 0.777$), or

BMI ($p = 0.662$). Serum MIF concentration decreased significantly, however, with increasing serum creatinine concentration ($p = 0.038$). No association was detected between serum MIF and the presence of hematuria or proteinuria on urinalysis.

On multivariate analysis, corticosteroid dose remained significantly associated with serum MIF concentration after the possible confounding effects of SLICC/ACR DI score and serum creatinine were controlled for in the analysis ($p = 0.019$). The association between serum MIF concentration and SLICC/ACR DI score remained significant when controlled for the possible confounding effects of serum creatinine concentration ($p = 0.045$), but not when controlled for current corticosteroid dose ($p = 0.269$). The association of serum MIF concentration and serum creatinine concentration was independent of both corticosteroid and disease-related variables.

On univariate analysis, an association between initial serum MIF concentration in 1994 and the value of SLICC/ACR DI measured after a 3 year interval was observed ($p = 0.003$). In addition, there was an association between initial serum MIF concentration in 1994 and the change in SLICC/ACR DI over the 3 year period ($p = 0.021$). No association was found with disease activity score. The potential confounding effect of the corticosteroid dose at the time of initial study was controlled for in a multivariate analysis. Although there was a trend for initial serum MIF concentration and subsequent SLICC/ACR DI to remain associated after adjusting for corticosteroid dose ($p = 0.098$), the association between initial serum MIF concentration and the change in SLICC/ACR DI between examinations was no longer significant ($p = 0.268$). Therefore, after correction for corticosteroid dose, serum

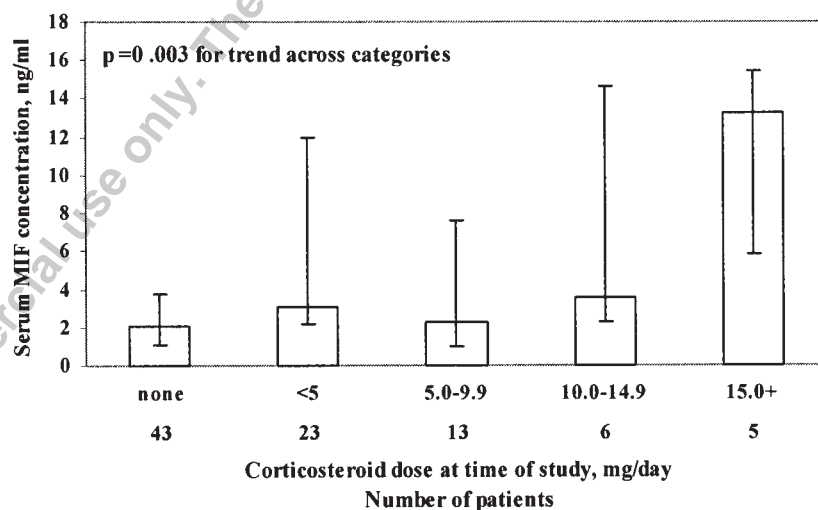


Figure 2. Association of serum MIF concentration and corticosteroid dose. Serum MIF was measured by ELISA in 90 women with SLE and categorized according to the dose of corticosteroid (prednisolone) taken at the time of study. A significant association between corticosteroid dose and serum MIF concentration was observed ($p = 0.003$).

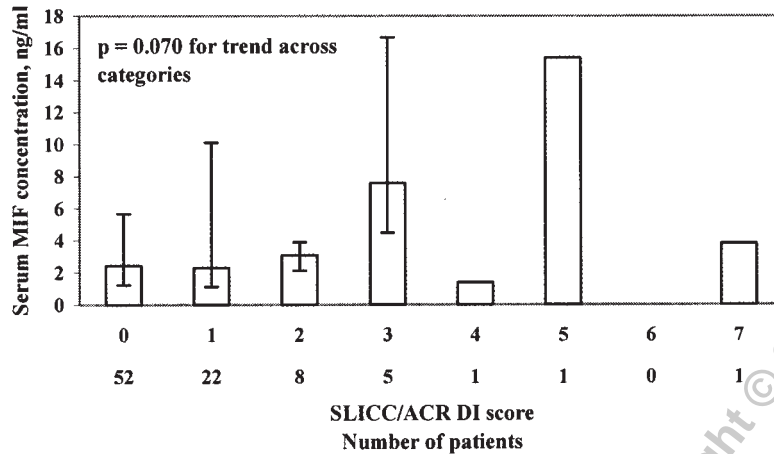


Figure 3. Association of serum MIF concentration and SLICC/ACR DI. Serum MIF was categorized according to the value of the SLICC/ACR DI disease damage score. A trend was observed towards an association between serum MIF and SLICC/ACR DI ($p = 0.070$).

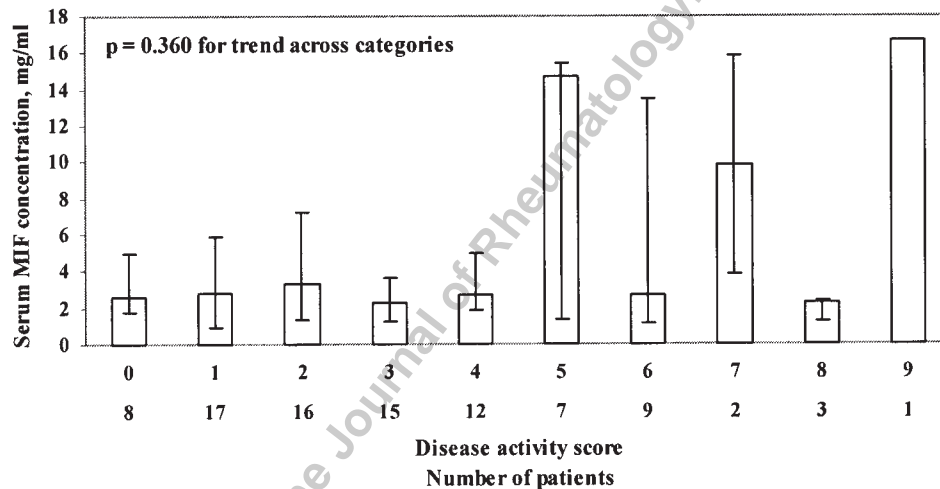


Figure 4. Association of serum MIF concentration and disease activity scores. Serum MIF was categorized according to the value of the disease activity score, based on the clinical elements of the SLAM¹⁵. No trend was observed towards an association between serum MIF and disease activity score ($p = 0.360$).

MIF was not predictive of 3 year SLE damage accumulation in the small number of patients available for this aspect of the study.

DISCUSSION

Although the etiology of SLE remains unknown, it is clear that patients with SLE have a wide variety of immunoregulatory abnormalities leading to autoimmune mediated organ injury. Immunoregulatory abnormalities observed in SLE include hyperresponsive B cells and abnormal antibody production, as well as abnormal T cell responses¹⁰. MIF has been identified as a mediator of activation of B and T cells^{1,2}, as well as of synovial cells, endothelium, and glomerular cells^{3,16,17}. In addition, MIF is expressed in inflammatory lesions in organs targeted by SLE including

joints, kidney, bowel, skin, and brain. It is increasingly accepted that MIF contributes to the pathogenesis of autoimmune inflammatory diseases including RA, immune glomerulonephritis, inflammatory bowel disease, psoriasis, and multiple sclerosis^{5,18-21}. A study that examined the expression of MIF in human glomerulonephritis found that renal MIF expression was significantly upregulated in forms of proliferative glomerulonephritis that included lupus nephritis⁷.

The range of effects of MIF in the immune system, and its expression in target organs of SLE, led us to the hypothesis that MIF is involved in the pathogenesis of inflammatory organ injury in SLE. Our results show that patients with SLE were more likely to have elevated serum MIF concentrations than controls. Disease related damage, as measured

using the SLICC/ACR DI, was greater in patients with a serum MIF concentration above the median value of the normal population. This association was independent of current corticosteroid dose. Similarly, patients with high SLICC/ACR DI scores were more likely to have abnormally elevated values of serum MIF. These data suggest that serum MIF is associated with disease severity in SLE. Possible explanations of this could include *mif* gene polymorphisms, such as those recently described in patients with inflammatory arthritis²².

A further analysis performed on a cohort of these patients followed up after a 3 year interval revealed that serum MIF concentration was predictive of future SLICC/ACR DI, although this result was partly confounded by corticosteroid use. Prognostic factors other than current disease burden have been difficult to define in the clinical assessment of patients with SLE, and to our knowledge MIF is the first cytokine described with such an association.

We were unable to find a relationship between concurrent serum MIF and disease activity score. However, patients did not have laboratory documentation of disease activity, preventing a full analysis of any associations between disease activity and serum MIF. In addition, as our study included a mostly ambulant community-based population with very low disease activity scores, the potential to find an association between serum MIF and disease activity may have been limited. Larger prospective studies, including patients studied during periods of active disease and incorporating a more complete assessment of disease activity, are required to further examine any potential associations between MIF and disease activity.

One of the intriguing aspects of MIF biology is its relationship with corticosteroids. Unlike most proinflammatory molecules, the synthesis and release of MIF is increased by corticosteroids *in vitro* and *in vivo*^{2,6,8,18}. Moreover, MIF has the ability to override or antagonize the antiinflammatory effects of corticosteroids^{2,8,9}. Taken together, these observations suggest that MIF acts as a physiological counter-regulator of endogenous corticosteroids²³. In the case of a patient receiving therapeutic corticosteroids, the persistence and induction of MIF would serve to antagonize the therapeutic effects of corticosteroids on inflammation. Our analysis of the relationship between serum MIF and corticosteroid use in SLE patients showed that corticosteroid use was positively associated with serum MIF, particularly at higher doses, and that this association remained significant even when the possible confounding effects of variables such as disease damage and serum creatinine were controlled for in the analysis. This is the first time a relationship between corticosteroid use and MIF has been described *in vivo* in humans. No patient in this study was taking very high doses of corticosteroids that could suppress serum MIF.

An unexpected finding was the inverse relationship between serum creatinine and serum MIF, which has not

been previously examined. One possible explanation for the positive association between serum MIF concentration and renal function is that renal cells, or factors influencing renal function, may contribute to serum MIF. In a study in 1996, Imamura, *et al* showed that MIF was synthesized *de novo* in renal tubular epithelial cells²⁴. We were unable to detect a difference in serum MIF in patients with active renal lupus, but increases in urinary MIF were recently reported in renal transplant patients during rejection episodes, and in active glomerulonephritis^{25,26}. While animal studies suggest that the principal source of serum MIF in healthy animals is the pituitary, the only such information available for humans suggests a non-pituitary source of serum MIF²⁷. Our results suggest that the kidney may be a significant source of serum MIF in humans. It would have been preferable to measure creatinine clearance in order to confirm this observation, but this was not performed. At the very least, our results suggest that serum creatinine is a critical covariate to control for in studies reporting on serum MIF concentrations in humans. In our study, all samples were obtained between 9:00 A.M. and noon in case diurnal variation contributed to variation in MIF concentrations. In a study published after ours was completed, Malmegrim, *et al* reported the absence of a significant cortisol-dependent diurnal variation in serum MIF concentrations²⁸. Similar findings were reported by Isidori, *et al*²⁷.

Our data suggest that the serum concentration of MIF is more likely to be elevated above the normal range in SLE, as in other inflammatory diseases. The data also indicate that any future studies of the expression of MIF *in vivo* in humans should control for the confounding effects of corticosteroids and of serum creatinine. That serum MIF was also associated with corticosteroid use represents the first report of an association between corticosteroid use and serum MIF *in vivo* in humans. Prospective studies of the association between MIF and disease activity using validated indices of disease activity, and including patients with active disease, are required.

ACKNOWLEDGMENT

We are grateful to the Victorian Red Cross Blood Bank, Victoria, Australia, for supplying us with samples to use as control sera in this study. We also acknowledge the assistance of Drs. Geoff Littlejohn, Stephen Hall, Rachele Buchbinder, Christopher Mack, David Baraclough, Andrea Bendrups, Michael Burke, Sam Engel, John Findeisen, Ben Ihle, Peter Kerr, Leslie Koadlow, Peter Meyer, and Evange Romas.

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