Increased matrix metalloproteinase-3 serum levels in rheumatic diseases: relationship with synovitis and steroid treatment

C Ribbens, M Martin y Porras, N Franchimont, M-J Kaiser, J-M Jaspar, P Damas, F A Houssiau, M G Malaise

EXTENDED REPORT

Objective: To determine matrix metalloproteinase-3 (MMP-3) serum levels in patients with rheumatic diseases and to study the relation between MMP-3 and C reactive protein (CRP) levels.

Methods: MMP-3 serum levels were determined by enzyme linked immunosorbent assay (ELISA) in (a) patients with active inflammatory rheumatic diseases: rheumatoid arthritis (RA), psoriatic arthritis, polymyalgia rheumatica, acute crystal arthritis, and anklyosing spondylitis; (b) patients with active inflammatory systemic diseases: cutaneo-articular or renal systemic lupus erythematosus (SLE), systemic sclerosis, and vasculitides; (c) patients with non-inflammatory rheumatic diseases: osteoarthritis and fibromyalgia; (d) critically ill patients without rheumatic diseases, representing an acute inflammatory control group; (e) healthy controls.

Results: MMP-3 serum levels were significantly increased in patients with active RA, psoriatic arthritis, and polymyalgia rheumatica, whether treated or not by corticosteroids, and in female patients with acute crystal arthritis. MMP-3 serum levels were normal in steroid-free patients with active cutaneo-articular or renal SLE, systemic sclerosis, and vasculitides but were significantly increased in steroid treated patients. MMP-3 levels were normal in fibromyalgia, osteoarthritis, anklyosing spondylitis, and acute inflammatory controls. MMP-3 was significantly correlated with CRP in RA (r=0.5, p=0.0004) but not in any of the other disease groups.

Conclusions: MMP-3 serum levels are increased in inflammatory rheumatic diseases characterised by joint synovitis, such as RA, polymyalgia rheumatica, psoriatic arthritis, and acute crystal arthritis—that is, whether the diseases are acute or chronic, erosive or not. They are normal in SLE, systemic sclerosis, and vasculitides as well as in non-rheumatic inflammatory controls, but are significantly increased by steroids. These data strongly suggest that serum MMP-3 reflects synovial inflammation.
Because the patients' serum levels of MMP-3 did not follow a normal distribution, they were significantly higher in men than in women (mean (SD) 20.1 (7.1) vs 9.2 (2.7) ng/ml, p<0.0001). MMP-3 levels for our patient groups were therefore compared with those of sex matched controls. Abnormal MMP-3 values were defined as levels higher than the mean + 2SD of healthy controls, yielding cut off points of 14 ng/ml in women and 34 ng/ml in men, respectively.

### RESULTS

Serum levels of MMP-3 assayed in healthy controls were normally distributed. However, they were significantly higher in men than in women (mean (SD) 20.1 (7.1) vs 9.2 (2.7) ng/ml, p<0.0001). MMP-3 levels for our patient groups were therefore compared with those of sex matched controls. Abnormal MMP-3 values were defined as levels higher than the mean + 2SD of healthy controls, yielding cut off points of 14 ng/ml in women and 34 ng/ml in men, respectively.

### Table 1  Characteristics of the patients with active rheumatic diseases

<table>
<thead>
<tr>
<th>Diagnostic criteria</th>
<th>Female patients</th>
<th>Male patients</th>
<th>Age Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoarthritis</td>
<td></td>
<td></td>
<td>60 (39–91)</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td></td>
<td></td>
<td>45 (19–65)</td>
</tr>
<tr>
<td>Acute crystal arthritis</td>
<td></td>
<td></td>
<td>59 (22–86)</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td></td>
<td></td>
<td>36 (26–54)</td>
</tr>
<tr>
<td>Polymyalgia rheumatica</td>
<td></td>
<td></td>
<td>70 (65–76)</td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td></td>
<td></td>
<td>46 (19–79)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td></td>
<td></td>
<td>53 (18–76)</td>
</tr>
<tr>
<td>Cutaneous arteritis lupus</td>
<td></td>
<td></td>
<td>34 (15–57)</td>
</tr>
<tr>
<td>Renal lupus</td>
<td></td>
<td></td>
<td>30 (15–54)</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td></td>
<td></td>
<td>58 (37–83)</td>
</tr>
<tr>
<td>Vasculitides</td>
<td></td>
<td></td>
<td>51 (22–84)</td>
</tr>
</tbody>
</table>

CS+/-: treated or not by corticosteroids.

### Table 2  MMP-3 and CRP serum levels in non steroid-treated patients with active diseases

<table>
<thead>
<tr>
<th>MMP-3 levels (ng/ml)</th>
<th>Abnormal MMP-3 levels</th>
<th>Abnormal CRP levels (mg/l)</th>
<th>Abnormal CRP levels</th>
<th>Concomitant abnormal MMP-3 and CRP levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women (median [range])</td>
<td>No (%)</td>
<td>Women (median [range])</td>
<td>No (%)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>46 (9.1 [7.4–11.2])</td>
<td>28/96 (2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>28 (10.8 [7.1–16.2])</td>
<td>9/56 (14.2–26.4)</td>
<td>3/37 (22)</td>
<td>3/37 (5)</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>31 (9.8 [8.8–12.5])</td>
<td>12/14 (9.9–26.3)</td>
<td>11/28 (29)</td>
<td>11/28 (18)</td>
</tr>
<tr>
<td>Acute crystal arthritis</td>
<td>16 (13.3)</td>
<td>19/14 (10.2–36.8)*</td>
<td>12/14 (9.9–26.3)</td>
<td>12/14 (9.9–26.3)</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>5 (28 [25.9–44.5]*</td>
<td>9/15 (12.2–21.5)</td>
<td>1/9 (11)</td>
<td>8/10 (11)</td>
</tr>
<tr>
<td>Polymyalgia rheumatica</td>
<td>4 (60)</td>
<td>60/1 (42.9–69.5)*</td>
<td>4/60 (1)</td>
<td>6/60 (1)</td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td>5 (29.5 [19.9–48.1])</td>
<td>9/35 (22.8–69.5)*</td>
<td>10/14 (71)</td>
<td>10/14 (71)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>31 (30 [18.5–47.9]*</td>
<td>10/35 (26.101)</td>
<td>33/41 (80)</td>
<td>33/41 (80)</td>
</tr>
<tr>
<td>Cutaneous arteritis lupus</td>
<td>7 (8 [6–10.6])</td>
<td>8/60 (1)</td>
<td>0/7 (0)</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>Renal lupus</td>
<td>5 (11 [8.7–14.7])</td>
<td>2/8 (4–12)</td>
<td>1/7 (14)</td>
<td>4/31 (3)</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>16 (10.2 [7–14.7])</td>
<td>3/17 (16.6–22.5)</td>
<td>6/19 (31)</td>
<td>6/19 (31)</td>
</tr>
<tr>
<td>Vasculitides</td>
<td>10 (12 [15–52])</td>
<td>7/16 (12.7–23.3)</td>
<td>23/35 (5–74)</td>
<td>23/35 (5–74)</td>
</tr>
<tr>
<td>Acute inflammatory controls</td>
<td>10 (9.8 [5–12.5])</td>
<td>20/13 (10.4–18.7)</td>
<td>3/30 (10)</td>
<td>3/30 (10)</td>
</tr>
</tbody>
</table>

*p<0.05 compared with sex matched healthy controls using the Mann-Whitney U test (¶p<0.05 after correction for multiple testing). Abnormal MMP-3 levels are defined as levels higher than the mean + 2SD of normal controls—that is, 14 ng/ml in women and 34 ng/ml in men. Abnormal CRP levels are defined as levels higher than 5 mg/ml. ND, not determined.
MMP-3 levels were first determined in patients with an active rheumatic disease not treated with corticosteroids. Table 2 shows that MMP-3 levels were significantly increased in male and female patients with RA, polymyalgia rheumatica, or psoriatic arthritis, 70–100% of patients displaying highly abnormal MMP-3 values. MMP-3 levels were similar to the controls in patients presenting with fibromyalgia, osteoarthritis, anklyosing spondylitis, cutaneo-articular and renal lupus, systemic sclerosis, and vasculitides. A modest but significant increase in MMP-3 levels was found in female patients with acute crystal arthritis, 6/16 (37%) patients displaying abnormal MMP-3 values, while levels were normal in male patients with acute crystal arthritis (table 2). Critically ill patients in the acute inflammatory group had normal MMP-3 levels.

MMP-3 levels were further analysed according to treatment by corticosteroids. Figure 1 shows that MMP-3 levels were significantly increased in steroid treated women with RA, vasculitides, systemic sclerosis, cutaneo-articular and renal lupus compared with corresponding patients not treated with steroids. For each disease, corticosteroid treated and untreated subgroups had comparable age, disease duration, disease activity, and inflammatory parameters (data not shown). In particular, CRP levels were not significantly different between patients with RA receiving steroids (median 24 mg/l) and those not receiving steroids (median 28 ng/ml, p=0.5). MMP-3 levels were also increased in steroid treated men with vasculitides (median 63 ng/ml v 16 ng/ml, p=0.009) as well as in men with RA, though the difference was not significant in the latter group (median 60 ng/ml v 36 ng/ml, p=0.079) (not illustrated). In patients with polymyalgia rheumatica and with psoriatic arthritis, the presence of steroids did not further increase MMP-3 levels (data not shown).

We next evaluated the influence of disease activity on MMP-3 levels. Figure 2 shows that women with inactive RA not treated with steroids had significantly lower MMP-3 levels than women with active RA not treated with steroids (median 5 ng/ml (n=7) v 30 ng/ml (n=31)), while their levels were comparable with those found in healthy sex matched controls (p=0.13). As for active RA, the use of steroids in inactive RA significantly increased MMP-3 levels (median 23 ng/ml, n=14, p=0.001 v inactive RA women without steroids, p<0.0001 v normal healthy women). Patients with inactive lupus had normal MMP-3 levels (median 8 ng/ml in 10 patients with inactive cutaneo-articular lupus, median 11 ng/ml in five patients with inactive renal lupus, p>0.1 v normal healthy women) as did patients with active lupus. Patients with a history of lupus nephritis, treated with steroids but having a quiescent disease, had increased MMP-3 levels (median 38 ng/ml, n=6, p<0.01 v patients with inactive renal lupus not treated with steroids, p<0.0001 v normal healthy women).

In addition, nine patients developing a lupus nephritis were studied longitudinally. MMP-3 levels were determined at baseline—that is, at the time of the renal biopsy leading to the diagnosis of glomerulonephritis and three months later after pulse IV cyclophosphamide (500 mg/m²) and steroid treatment. Corticosteroid treatment was started in three patients and the level was increased in six patients previously treated with low dose prednisolone (mean 4.4 mg/day). After three months’ treatment the nephritis had improved as assessed by a decrease of the proteinuria, but MMP-3 levels had risen significantly from 30 to 83 ng/ml (median levels, p=0.0077 using Wilcoxon’s rank sum test) (fig 3A). Concomitantly, the prednisolone dose was also significantly increased to 17.8 mg/day (mean dose, p=0.0117 using Wilcoxon’s rank sum test) (fig 3A). When day 0 and month 3 time points were studied together (n=18), we found a significant positive linear correlation between MMP-3 levels and prednisolone dose (r=0.55, p=0.02) (fig 3B).

Correlations were sought between MMP-3 and CRP levels in each disease group. A significant positive correlation

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**Figure 1** MMP-3 serum levels in female patients with various systemic diseases treated or not with corticosteroids. The horizontal line represents the median level of each group. The dotted line represents the upper normal limit of female healthy controls—that is, 14 ng/ml. Patients treated with steroids and those not treated were compared by the Mann-Whitney U test.

**Figure 2** MMP-3 serum levels in female patients with active or inactive RA not treated with corticosteroids. The horizontal line represents the median level. The dotted line represents the upper normal limit of female healthy controls—that is, 14 ng/ml. The two groups were compared by the Mann-Whitney U test.
Much emphasis has been placed on the use of anti-inflammatory drugs in RA. We have recently shown that MMP-3 serum levels are increased in patients with active RA as well as in patients with active peripheral psoriatic arthritis and reactive arthritis. Moreover, we have also shown that patients with active RA had raised serum levels of MMP-3 compared with patients with active peripheral psoriatic arthritis and reactive arthritis. Furthermore, we found that 80% of patients with active RA had raised serum levels of MMP-3 compared with patients with active peripheral psoriatic arthritis and reactive arthritis. These results were confirmed by the use of a rank sum test. (A) MMP-3 serum levels in nine patients with an active lupus nephritis (grey symbols, dotted line) or increased in six patients treated at baseline with low-dose prednisolone (black symbols, continuous line, mean dose 4.4 mg/day). The prednisolone dose was significantly higher at three months (mean dose 17.8 mg/day). (B) Positive linear correlation between MMP-3 levels and prednisolone dose in the nine lupus nephritis patients at baseline (white symbols) and at month 3 (black symbols).

**DISCUSSION**

Our results show that serum levels of MMP-3 are increased in diseases presenting with a synovitis independently of steroid treatment. Indeed, we found that 80% of patients with active RA as well as 70% of patients with active peripheral psoriatic arthritis had raised MMP-3 values. These two diseases have in common chronic synovitis, even though psoriatic arthritis lesions are less erosive. Moreover, increased MMP-3 levels were also found in polymyalgia rheumatica, an inflammatory disease characterised by the presence of shoulder and pelvic girdles synovitis but by the absence of erosions. Furthermore, female patients with an inflammatory acute synovitis seen in crystal arthritis also had significantly higher MMP-3 levels. On the contrary, patients with ankylosing spondylitis, osteoarthritis, and fibromyalgia, rheumatic diseases with no synovitis, had normal MMP-3 values. Lastly, patients with inactive RA—that is, without clinically detectable synovitis, had normal MMP-3 serum levels.

Many studies provide arguments in favour of a synovial origin of serum MMP-3 in patients with RA (see introduction). Our data, obtained in various rheumatic diseases, clearly show that raised MMP-3 serum levels are associated with the presence of synovitis, reflecting the inflammatory reaction occurring in the joints, whether acute or chronic, or erosive potential or not, confirming previous studies. MMP-3 serum levels were twice as high in RA as in acute crystal arthritis, although synovial fluid levels are similar in these diseases. This might be explained by the polyarticular involvement of RA in contrast with the monoarticular presentation of most acute crystal arthropathies in our work. Indeed, serum levels of MMP-3 have been positively correlated with the number of joints affected. We have recently shown that synovial fluid MMP-3 levels in inflammatory arthropathies such as RA, reactive arthritis and acute crystal arthritis were very significantly correlated with synovial IL6 levels as well as with serum CRP levels. Furthermore, serum levels of MMP-3 and CRP are closely correlated with each other. However, these two parameters do not strictly convey the same information. CRP is produced by the liver in response to circulating IL6, tumour necrosis factor α (TNFα), or IL1 and is a marker of systemic inflammation, including that originating in the joint. In contrast, MMP-3 is produced in the joint in response to local IL6, TNFα, and IL1 and is a marker of synovial inflammation. Although in this work MMP-3 and CRP were both increased in patients with active RA, psoriatic arthritis, and polymyalgia, they were correlated with each other only in the RA group. Furthermore, the percentage of patients displaying concomitant abnormal levels of MMP-3 and CRP was lower than the percentage of patients with an increase of each parameter alone. In addition, critically ill
patients with multiple organ failure but devoid of any joint inflammation had highly increased serum levels of CRP but normal levels of MMP-3, a lack of correlation between these two parameters which has already been shown.\(^7\)

Although our data show that an increase in serum MMP-3 levels is restricted to diseases with synovitis, they are in contradiction with reports showing raised serum levels in lupus, connective tissue diseases, or glomerulonephritis.\(^8\) In chronic inflammatory diseases without synovitis, tissues other than the synovium may be sites of production of MMP-3 and contribute to increased MMP-3 serum levels. Indeed, the production of MMP-3 has been identified in glomerular and tubular epithelial cells\(^9\) as well as in mesangial cells.\(^10\) Patients with mesangial proliferative glomerulonephritis, such as IgA nephritis and lupus nephritis, have increased MMP-3 serum levels.\(^11,10\) MMP-3 has also been identified in skin lesions of lupus patients.\(^10\) Patients with vascular disorders may also display increased levels of MMP-3 because endothelial venous and arterial cells can also produce MMP-3 when activated by the proinflammatory cytokine TNF-\(\alpha\).\(^8\) However, we found that patients with active renal lupus, active cutaneous-artericlar lupus, active vasculitides, and systemic sclerosis not receiving steroids had normal serum MMP-3 levels. On the contrary, patients with these diseases treated with steroids had increased MMP-3 levels, although their disease activity and inflammatory parameters were comparable with those of patients not treated with steroids. Furthermore, we found in the longitudinal study that starting or increasing steroids in patients with a newly diagnosed lupus nephritis was accompanied by an increase in MMP-3 levels while disease activity was reduced. Therefore, although this study was not designed to study the influence of steroids on MMP-3 levels and although these data have been obtained in small groups of patients, they strongly suggest that MMP-3 levels are increased in these diseases by steroids, a criterion not taken into account in previous studies. Such an increase of MMP-3 serum levels by corticosteroids is also found in patients with active or inactive RA. Interestingly, we noted that in patients with polymyalgia rheumatica, psoriatic arthritis, and male patients with RA where MMP-3 values were particularly high, levels did not differ between steroid treated patients and those not treated.

The mechanisms by which steroids increase MMP-3 serum levels remain unknown. Sharifi \textit{et al.} observed a doubling of serum MMP-3 levels with 7.5 mg of prednisolone given daily while clinical and biological parameters of RA disease activity were reduced with the treatment.\(^10\) The authors suggest that steroids influence the clearance of pro-MMP-3 from the circulation. Our data do not formally prove that steroids directly increase MMP-3 levels, and additional studies are required to identify the mechanisms. However, our results favour a mechanism which is independent of the anti-inflammatory properties of steroids because the effect of steroids is found in diseases without clinical or biological evidence of joint inflammation. In patients with lupus nephritis, one cannot exclude the possibility that the increase of MMP-3 levels after steroid treatment might be linked to an improvement of the nephrotic syndrome and therefore to an increase in serum levels of proteins which may bind MMP-3.

In conclusion, our results show that MMP-3 serum levels are increased in inflammatory rheumatic diseases characterized by joint synovitis. They are normal in non-inflammatory rheumatic diseases and in inflammatory diseases without synovitis, but are significantly increased in patients treated with steroids. Our data therefore strongly suggest that the determination of MMP-3 levels is an easy method for quantifying synovial inflammation and should complete the biological assessment of synovial inflammatory diseases.

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