EXTENDED REPORT

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

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Ann Rheum Dis 2002;**61**:161–166

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Accepted 23 July 2001

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 ankylosing 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, ankylosing 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.

Matrix metalloproteinase (MMP)-3 or stromelysin-1 is an enzyme which plays a part in the destruction of cartilage and bone in rheumatoid arthritis (RA).[1 2] Indeed, MMP-3 can degrade many components of the extracellular matrix [3] and also activate other pro-MMPs, including pro-MMP-1 and pro-MMP-9.[4 5] Patients with RA have increased serum levels of MMP-3,[6–10] which is thought to originate from the synovium as serum levels are 250 times lower than synovial levels.[6 11] In addition, serum levels of MMP-3 are correlated with the number of joints affected[6–8 10] and are decreased after an intra-articular injection of steroids[7] or of yttrium.[12] Furthermore, MMP-3 serum levels are correlated with parameters of inflammation such as the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), or interleukin (IL)6 levels.[6–10 13–15] Serum MMP-3 has therefore been proposed as a synovial derived marker of inflammation.[7 10 14] However, increased serum MMP-3 levels are not specific for RA because they are also found in patients with lupus,[16–18] connective tissue diseases,[13] or glomerulonephritis.[19] Corticosteroids increase MMP-3 serum levels in RA,[13 20] but this treatment was not taken into account in previous work studying MMP-3 levels in systemic diseases.[13 16] Therefore, the disease specificity of raised MMP-3 levels in systemic diseases remains unclear.

To test the hypothesis that MMP-3 is a synovial derived inflammatory parameter, we measured MMP-3 serum levels in patients presenting with various rheumatic diseases, inflammatory or not, associated or not with synovitis, and treated or not by steroids. MMP-3 serum levels were also measured in inflammatory non-rheumatic patients with increased CRP levels.

PATIENTS AND METHODS

Patients

Serum samples were collected from 376 patients with active rheumatic diseases whether inflammatory or not. Table 1 gives the characteristics, sex ratio, and the number of patients taking corticosteroids. Active RA was defined by the presence of at least three of the following four criteria: ⩾6 tender joints, ⩾3 swollen joints, ESR ⩾28 mm/1st h, and morning stiffness ⩾45 minutes' duration. For the other patients, an active disease was defined according to the respective criteria (table 1) by the presence of typical clinically relevant manifestations and was confirmed by synovial fluid analysis for acute crystal arthritis, by biological parameters for polymyalgia rheumatica, psoriatic arthritis, cutaneo-articular lupus, systemic sclerosis, and vasculitides, and by urine analysis for renal lupus (a proteinuria higher than 500 mg/24 h defining an active nephritis). Serum was also collected in patients with inactive RA—that is, without clinically detectable synovitis (n=21, mean age 60 years (30–87)), inactive cutaneo-articular lupus (n=11, 37 years (18–74)), and inactive renal lupus

Abbreviations: CRP, C reactive protein; IL, interleukin; MMP-3, matrix metalloproteinase-3; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TNFα, tumour necrosis factor α

Table 1 Characteristics of the patients with active rheumatic diseases

	Age Mean (range)	Female patients		Male patients		Diagnostic criteria (ref)
		CS–	CS+	CS–	CS+	
Osteoarthritis	60 (39–91)	28		9		21
Fibromyalgia	45 (19–65)	31				22
Acute crystal arthritis	59 (22–86)	16		12		23
Ankylosing spondylitis	36 (26–54)			9		24
Polymyalgia rheumatica	70 (65–76)	5	4	4	4	25
Psoriatic arthritis	46 (19–79)	5	4	9		26
Rheumatoid arthritis	53 (18–76)	31	64	10	17	27
Cutaneo-articular lupus	34 (15–57)	7	22			28
Renal lupus	30 (15–54)	5	16	2		28
Systemic sclerosis	58 (37–83)	16	8	3		29
Vasculitides	51 (22–84)	10	9	7	9	30–33

CS+/– , treated or not by corticosteroids.

(n=11, 34 years (19–48)); all were women owing to the paucity of men with these diseases. Serum samples from 30 critically ill patients (10 F/20 M, mean age 56 years (17–83)) admitted to hospital in the intensive care unit for multiple organ failure of infectious, traumatic, or postoperative, but non-rheumatic, origin were obtained. These patients had increased CRP levels and served as an acute inflammatory group. Serum samples from 96 healthy controls (46 F/50 M, mean age 44 years (25–64)) were used for the determination of "normal" MMP-3 levels.

Laboratory analysis

Serum levels of MMP-3 were measured by ELISA using a one step sandwich method, as previously described.[10 34] This assay measures pro-MMP-3, the predominant form in serum,[9 16] active MMP-3, and active MMP-3 complexed with tissue inhibitors of MMP but not with α_2 macroglobulin. The range of the assay was 1.25–20 ng/ml. Serum levels of CRP were measured by nephelometry using specific antisera.

Statistical analysis

Because the patients' serum levels of MMP-3 did not follow a Gaussian distribution, results are expressed as median values with the 25–75 centiles ("interquartile" range). Between-group differences were analysed with the non-parametric Mann-Whitney U test. p Values were further corrected for multiple testing. For comparisons of 10 (male) or 11 (female) disease groups with the sex matched healthy control group, a p value of 0.0050 (0.05/10) or 0.0045 (0.05/11), respectively, was necessary to reach the 95% significance level. The Wilcoxon rank sum test was used to compare paired populations. p Values of less than 0.05 were considered significant. Correlations between MMP-3 and CRP levels were sought using linear regression, after logarithmic transformation. p Values of less than 0.05 were considered significant.

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) *v* 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 2 MMP-3 and CRP serum levels in non steroid-treated patients with active diseases

	MMP-3 levels (ng/ml)				Abnormal MMP-3 levels	CRP levels (mg/l)	Abnormal CRP levels	Concomitant abnormal MMP-3 and CRP levels
	Women		Men		Women and men	Women and men	Women and men	Women and men
	No	Median (interquartile range)	No	Median (interquartile range)	No (%)	Median (interquartile range)	No (%)	No (%)
Healthy controls	46	9.1 (7.4–11.2)	50	19.2 (14–26.4)	2/96 (2)	ND		ND
Osteoarthritis	28	10.8 (7.1–16.2)	9	15.6 (10.8–25.9)	8/37 (22)	3 (1–8)	13/37 (35)	2/37 (5)
Fibromyalgia	31	9.8 (6.8–12.5)			4/31 (13)	3 (1–6)	8/31 (26)	1/31 (3)
Acute crystal arthritis	16	13.3 (10.2–36.8)*¶	12	14.9 (10.9–26.3)	8/28 (29)	11 (3–38)	17/28 (61)	5/28 (18)
Ankylosing spondylitis			9	15 (12.2–21.5)	1/9 (11)	8 (4–15)	5/9 (55)	0/9 (0)
Polymyalgia rheumatica	5	28 (25.9–44.5)*¶	4	60.1 (42.9–69.5)*¶	9/9 (100)	76 (29–114)	9/9 (100)	9/9 (100)
Psoriatic arthritis	5	29.5 (19.9–48.1)*¶	9	39.5 (22.8–69.5)*	10/14 (71)	14 (6–34)	9/14 (64)	6/14 (43)
Rheumatoid arthritis	31	30 (18.5–47.9)*¶	10	36.5 (26–101)*¶	33/41 (80)	22 (9–41)	35/41 (85)	29/41 (71)
Cutaneo-articular lupus	7	8 (6.1–10.6)			0/7 (0)	0 (0–2)	0/7 (0)	0/7 (0)
Renal lupus	5	11 (8.7–14.7)	2	8 (4–12)	1/7 (14)	4 (3–31)	2/7 (28)	1/7 (14)
Systemic sclerosis	16	10.2 (7.1–14.7)	3	17 (11.6–22.5)	6/19 (31)	4 (1–14)	7/19 (37)	3/19 (16)
Vasculitides	10	12 (12–15.5)	7	16.3 (12.7–23.3)	6/17 (35)	23 (5–74)	13/17 (76)	4/17 (23)
Acute inflammatory controls	10	9.8 (5.8–12.5)	20	13.2 (10.4–18.7)	3/30 (10)	266 (241–292)	30/30 (100)	3/30 (10)

*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/l.
ND, not determined.

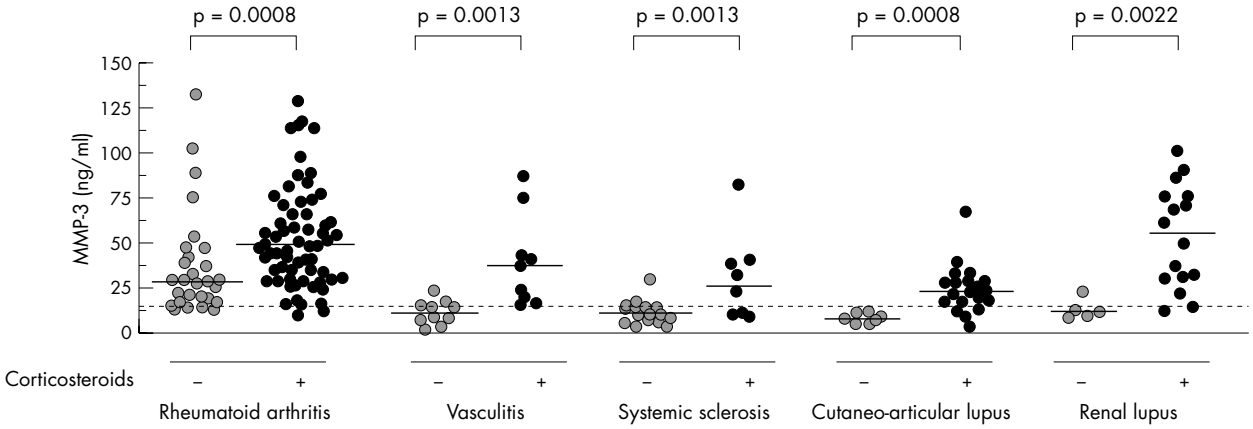

Figure 1 MMP-3 serum levels in female patients with various systemic diseases treated or not with corticosteroids. The horizontal line represents the median level of each group. The dotted line represents the upper normal limit of female healthy controls—that is, 14 ng/ml. Patients treated with steroids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

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, ankylosing 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 (table 2).

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/m^2) 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

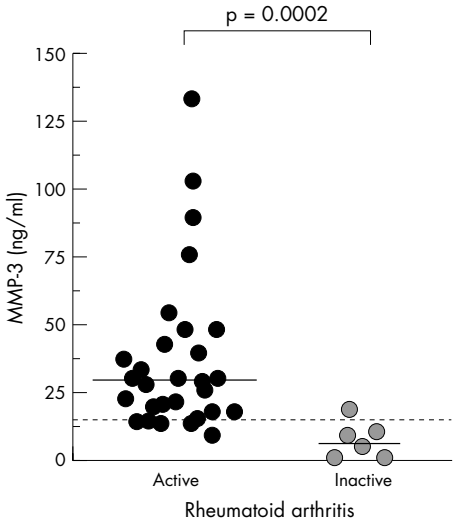

Figure 2 MMP-3 serum levels in female patients with active or inactive RA not treated with steroids. The horizontal line represents the median level. The dotted line represents the upper normal limit of female healthy controls—that is, 14 ng/ml. The two groups were compared by the Mann-Whitney U test.

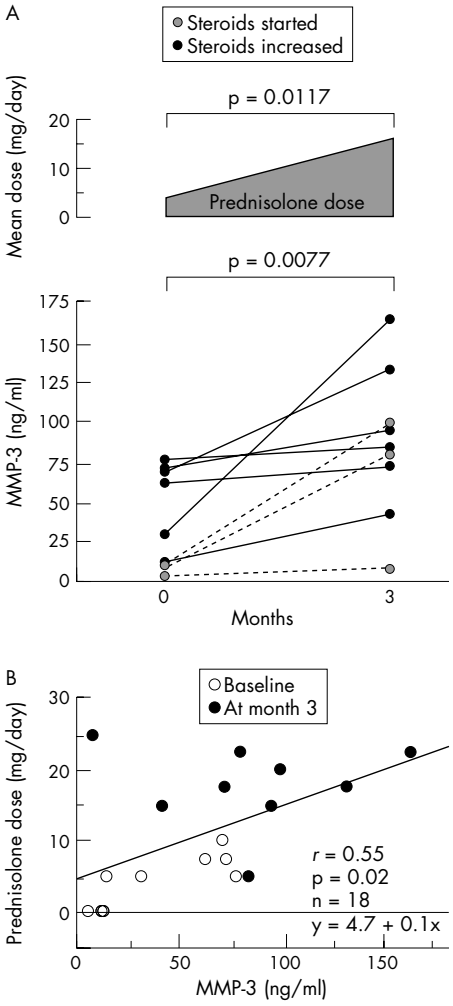

Figure 3 (A) MMP-3 serum levels in nine patients with an active lupus nephritis at the time of the renal biopsy (month 0) and after three months' treatment. Steroids were started in three patients (grey symbols, dotted line) or increased in six patients treated at baseline with low dose prednisolone (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). Paired samples of MMP-3 and of prednisolone were compared using the Wilcoxon rank sum test. (B) Positive linear correlation between MMP-3 levels and mean prednisolone dose in the nine lupus patients at baseline (white symbols) and at month 3 (black symbols).

between MMP-3 and CRP was found only in RA. The best correlation was seen in patients with both active and inactive RA and not treated with steroids (r=0.5, p=0.0004, n=46) (fig 4). A correlation was also found when only considering patients with active RA (r=0.37, p=0.02, n=39). A significant correlation was also found in the steroid treated group whether patients with active RA were studied separately (r=0.26, p=0.02, n=79) or together with patients with inactive RA (r=0.26, p=0.007, n=104). On the contrary, MMP-3 and CRP levels were not associated in the group of critically ill patients with acute inflammatory disease who displayed highly increased CRP levels (table 2) but normal MMP-3 values (table 2). Although MMP-3 levels were increased in patients treated with corticosteroids, we found no correlation between serum levels of MMP-3 and prednisolone dose, except for the lupus nephritis group studied longitudinally.

DISCUSSION

Our results show that serum levels of MMP-3 are increased in diseases presenting with a synovitis independently of steroid

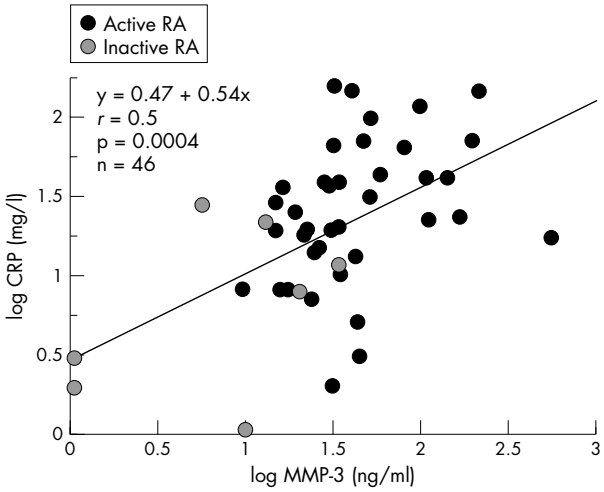

Figure 4 Positive linear correlation between MMP-3 and CRP serum levels in non-steroid treated female and male patients with active (n=39) or inactive (n=7) rheumatoid arthritis.

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.[35] Moreover, increased MMP-3 levels were also found in polymyalgia rheumatica, an inflammatory disease characterised by the presence of shoulder and pelvic girdles synovitis[36] 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).[6–12] 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, of erosive potential or not, confirming previous studies.[7 13 16] MMP-3 serum levels were twice as high in RA as in acute crystal arthritis, although synovial fluid levels are similar in these diseases.[23] 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.[6–8 10] We have recently shown that synovial fluid MMP-3 levels in inflammatory arthritides such as RA, reactive arthritis and acute crystal arthritis were very significantly correlated with synovial IL6 levels as well as with serum CRP levels.[23] Furthermore, serum levels of MMP-3 and CRP are closely correlated with each other.[6–10 13 14] 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[37] 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.[7 14 23] 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.[13 16–19] 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[38] as well as in mesangial cells.[18 39] Patients with mesangial proliferative glomerulonephritis, such as IgA nephritis and lupus nephritis, have increased MMP-3 serum levels.[18–19] MMP-3 has also been identified in skin lesions of lupus patients.[18] 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 TNFα.[40] However, we found that patients with active renal lupus, active cutaneo-articular 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. Sharif *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.[20] 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 characterised 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 serum determination of MMP-3 levels is an easy method for quantifying synovial inflammation and should complete the biological assessment of synovial inflammatory diseases.

ACKNOWLEDGMENTS

The authors thank A Desoroux, S Gaspard, and A Villers for technical assistance and A Albert for expert statistical advice. C Ribbens and N Franchimont are post-doctoral researchers of the Belgian National Fund for Scientific Research (FNRS).

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