

Short communication

Matrix metalloproteinase-9: a novel biomarker for monitoring disease activity in patients with chronic urticaria patients?

Background: Matrix metalloproteinase (MMP)-9, an enzyme that contributes to inflammatory responses and subsequent tissue remodelling, has recently been suggested to be a good biomarker for monitoring disease activity in patients with chronic urticaria (CU). Here, we assessed whether total MMP-9 and/or active MMP-9 plasma levels are increased and correlated to disease activity in patients with CU.

Methods: Total MMP-9 and active MMP-9 plasma levels were determined by ELISA in 70 CU patients and control subjects (patients with psoriasis and healthy controls). CU activity was measured using weekly and daily composite symptom scores (urticaria activity score) calculated from the number of wheals and the intensity of pruritus.

Results: Significantly increased levels of total and active MMP-9 were detected in patients with CU as compared to healthy controls. Interestingly, patients with psoriasis also had clearly elevated plasma levels of total and active MMP-9, indicating that MMP-9 plasma levels do not specifically reflect CU activity. Most notably, total and active MMP-9 levels were not correlated with disease activity in CU or psoriasis patients.

Conclusion: Plasma MMP-9 is not a good CU biomarker and should not be used for assessing the efficacy of treatment in CU patients or their spontaneous changes in disease activity.

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Matrix metalloproteinase 9 (MMP-9) is a zinc- and calcium-dependent enzyme that cleaves extracellular proteins. It is thought to contribute to inflammatory responses and subsequent tissue remodelling and repair. MMP-9 is produced by several different cell-types involved in the induction of inflammation including fibroblasts, macrophages, T cells, and mast cells (MCs) (1). Interestingly, it was reported that MCs can enhance their MMP-9 secretion upon activation (2). Several studies suggest a crucial role of MMP-9 in allergic diseases such as asthma (3) or atopic dermatitis (4). Chronic urticaria (CU), one of the most common inflammatory skin diseases, is characterized by itchy wheals and sometimes angioedema, which result from the activation and subsequent release of histamine by skin MCs (5). Recently, plasma levels of MMP-9 were reported to be increased in patients with CU, and MMP-9 levels in these patients were claimed to correlate

positively with disease activity (6, 7). Based on these findings, MMP-9 has been called 'the first CU biomarker' and MMP-9 plasma levels have been suggested to be used as the primary outcome parameter for assessing the efficacy of novel therapies in the context of controlled clinical trials.

As both, the inactive pro-form (92 kDa) and the active form (82 kDa) of MMP-9 can easily be detected in the plasma of patients (8), we assessed the levels of total and active MMP-9 in a sizeable number of CU patients. Healthy individuals and patients with plaque-type psoriasis, a chronic inflammatory, non allergic skin disease served as controls. The aim of our study was to test MMP-9 for its qualities as a 'CU biomarker', specifically its value for assessing disease activity in CU patients.

Patients and methods

Patients

We collected plasma in lithium-heparin tubes from CU patients ($n = 70$), healthy volunteers ($n = 39$), and patients with plaque-type psoriasis ($n = 38$). CU was diagnosed and CU activity was assessed according to the recent EAACI/GA²LEN/EDF guidelines (9). Severity of psoriasis was defined using the PASI score (10) as

Abbreviations: Ab, antibody; CRP, C-reactive protein; CU, chronic urticaria; ELISA, enzyme-linked immunosorbent assay; kDa, kiloDalton; MC, mast cell; MMP, matrix metalloproteinase; PASI, psoriasis area and severity index; SCORAD, SCORing Atopic Dermatitis; SD, standard deviation; TIS, Three-Item Severity score; UAS, urticaria activity score.

Table 1. Mean age and sex of all subjects tested, including the mean urticaria activity scores or mean PASI scores if applicable

	Patients [number]	Age (mean) [years]	Sex	UAS day (mean)	UAS week (mean)	PASI (mean)
Chronic urticaria	70	45.2	79% female 21% male	2.3	15.9	–
Psoriasis	38	51.6	45% female 55% male	–	–	19.3
Healthy subjects	39	41.8	74% female 26% male	–	–	–

previously described. Briefly, patients with PASI scores of 0–9.9 were considered to have mild psoriatic symptoms, whereas patients with PASI scores of 10–16.9 and >17 were considered to be moderately and severely affected respectively.

The age and gender of all subjects tested, including the mean urticaria activity scores (UAS) and PASI scores are presented in Table 1. CU patients were assessed for the duration of disease and all patients discontinued systemic immunosuppressive therapy, phototherapy or antihistamines at least 2 weeks before enrolment.

Assessment of urticaria activity using the Urticaria Activity Score

Urticaria activity was assessed by using the UAS as recommended by the current EAACI/GA²LEN/EDF guidelines (9). Briefly, CU patients documented the severity of their symptoms in a 'urticaria diary' the week before and on the day of blood withdrawal. On each day, patients recorded the number of their wheals and the intensity of pruritus. Daily UAS scores (maximum = 6) were calculated from these recordings by adding the score values for wheal numbers (no wheals = 0, 1–10 wheals = 1, 11–50 wheals = 2, >50 wheals = 3) and pruritus intensity (no pruritus = 0, mild pruritus = 1, moderate pruritus = 2, severe pruritus = 3).

A daily UAS day of 0–2 was considered to represent mild disease activity, 3–4 moderate and 5–6 severe urticarial symptoms. Consequently, weekly UAS scores of 0–14, 15–29 and 30–42 were considered to represent mild, moderate, and severe disease activity, respectively.

ELISAs for MMP-9 detection in human plasma

Total human MMP-9 was determined using the Quantikine[®] Human MMP-9 Immunoassay (R&D Systems, Abingdon, Oxfordshire, UK). Active human MMP-9 was determined using the Fluorokine[®] E Human Active MMP-9 Enzyme Activity Assay (R&D Systems). The assays were performed according to the manufacturer's instructions. Data are presented as mean ± standard deviation (SD).

C-reactive protein (CRP)

C-reactive protein serum levels were measured by the central laboratory of the Charité-Universitätsmedizin Berlin, Germany. CRP serum levels below 0.5 mg/dl were rated as normal.

Autologous serum skin test (ASST)

The autologous serum skin test was performed as described previously (11). In brief, venous blood was drawn into sterile 10 ml vacuum tubes without a clot accelerator (Vacutainer; Becton

Dickinson, Franklin Lakes, NY, USA), and allowed to clot at room temperature for 30 min. The serum was separated by centrifugation at 550 g for 15 min and collected under sterile conditions. 0.05 ml histamine (1 mg/ml, ALK-Scherax, Hamburg, Deutschland) as positive control, 0.05 ml of fresh undiluted sterile autologous serum, 0.05 ml of 1 : 1 diluted autologous serum and 0.05 ml of physiological saline solution were injected, each intradermally, into the patients' forearm. The reactions were evaluated after 10 and 30 min. A wheal diameter of at least 1.5 mm greater than that of the control wheal induced with the saline solution was recorded as positive.

Statistics

Statistical analysis was performed by using the two sided Student's *t*-test and Pearson's correlation. *P*-values of <0.05 were considered statistically significant.

Results

Total and active MMP-9 plasma levels are elevated in CU and psoriasis patients

Using ELISA technology, we detected significantly (*P* = 0.0001) increased plasma levels of total MMP-9 (*n* = 70; 241 ng/ml SD ± 299) in patients with CU as compared to healthy controls (HC) (*n* = 39; 74 ng/ml

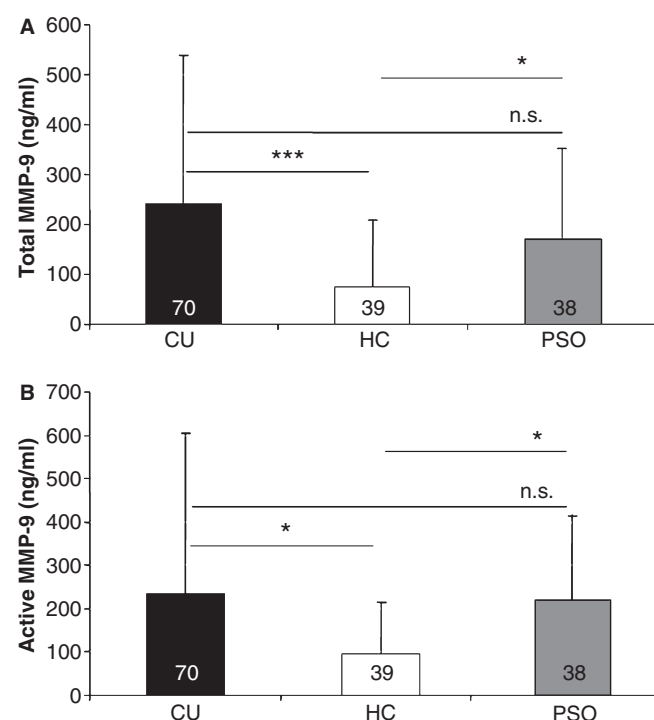


Figure 1. Matrix metalloproteinase (MMP)-9 plasma levels are increased in chronic urticaria and psoriasis patients. Plasma levels of total (A) and active (B) MMP-9 were measured by ELISA in healthy controls and in patients with chronic urticaria (CU) or psoriasis (PSO). Numbers of tested subjects are indicated inside the bars. ****P* < 0.001, **P* < 0.05, n.s. = not significant.

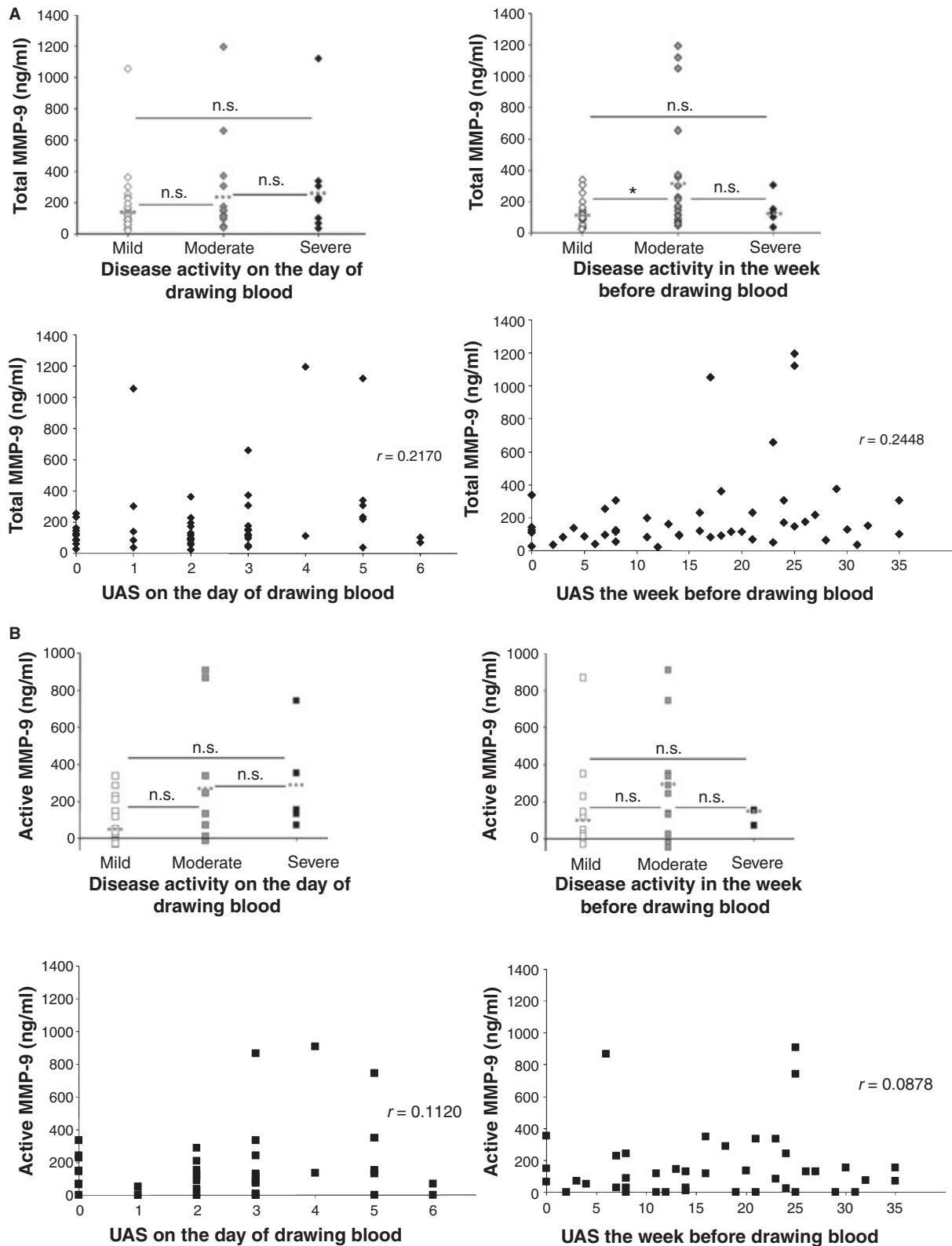


Figure 2. Total matrix metalloproteinase (MMP)-9 plasma levels (A) or active MMP-9 plasma levels (B) and urticaria activity are not correlated chronic urticaria patients were assessed for their daily or weekly urticaria activity score and are represented as diamonds or squares. * $P < 0.05$, n.s. = not significant.

SD $\pm$ 130). Interestingly, patients with psoriasis also exhibited clearly ($P = 0.007$) elevated plasma levels of total MMP-9 ($n = 38$; 172 ng/ml SD $\pm$ 176) (Fig. 1A). Active MMP-9 levels were also significantly ($P = 0.01$) increased in CU (234 ng/ml SD $\pm$ 372) and psoriasis patients (220 ng/ml SD $\pm$ 194) compared to HC (96 ng/ml SD $\pm$ 117) (Fig. 1B), indicating that total and active MMP-9 levels are elevated in at least two very different chronic skin disorders.

Severity of disease and MMP-9 levels are not correlated in patients with chronic urticaria

Next, we performed detailed statistical analyses to evaluate whether total or active MMP-9 plasma levels are correlated with disease severity, as suggested for CU by two earlier reports (6, 7).

Out of 53 CU patients that had documented their disease activity on the day blood was drawn for subsequent MMP-9 measurements using the UAS, 31 (= 59%) patients had mild symptoms, 14 (= 26%) had moderate and 8 (= 15%) had severe symptoms. Surprisingly, we did not detect significant differences of total or active MMP-9 plasma levels between CU patients with mild or severe symptoms (Fig. 2), and there was no correlation of disease activity and MMP-9 levels (total or active). We also did not find a correlation of MMP-9 plasma levels with patients' urticaria activity as assessed by calculating the cumulative UAS for the 7 days prior to taking the blood for MMP-9 measurements.

In addition, MMP-9 plasma levels in the psoriatic patient control group also did not show a correlation with disease activity (PASI score).

MMP-9 levels are elevated in CU patients that exhibit elevated CRP serum levels

Thereafter, we assessed the association of gender, age, duration of disease, ASST responses and CRP serum levels in CU patients with their MMP-9 plasma levels. Except for the latter, we did not detect a significant correlation. Interestingly, higher MMP-9 plasma levels (386.5 ng/ml total MMP-9 $\pm$ 431.5) were found in CU patients with elevated CRP serum levels compared to CU patients with normal or low CRP serum levels (172.9 ng/ml total MMP-9 $\pm$ 194.6) ($P < 0.05$).

Discussion

Matrix metalloproteinase-9 is known to be produced by MCs (1) and it is thought to play a relevant role in the

pathogenesis of allergic diseases (3, 4, 12) and urticaria. MMP-9 was suggested to be a specific biomarker for disease activity by the authors of a previous report on a study in 29 CU patients (6). In line with this report, we detected elevated, i.e. doubled, total and active MMP-9 plasma levels in both, patients with CU and psoriasis, a chronic non allergic skin disease. In contrast to the previous study we could not detect, in a bigger cohort of CU patients, a correlation of MMP-9 plasma levels with disease severity, as determined by using the UAS. Our findings are in accordance with other recent studies. For example, in patients with atopic dermatitis, plasma MMP-9 levels were also found to be significantly elevated, but no correlation could be detected between MMP-9 levels and their symptom scores (SCORAD and TIS) (4).

Although our patients were not perfectly matched, the influence of age and gender on MMP-9 levels is thought to be minimal (13). Differences between our results and the previous study (6) are also not explainable by the different clinical scores used (Breneman's score (14) and UAS), as the symptoms are evaluated in a similar way. In our study a big cohort of non selected CU patients were tested with two different ELISA systems in a blinded fashion, suggesting that the independence of MMP-9 level and CU severity in these patients is statistically valid. A strong preselection of urticaria patients in the other study (6) might explain the differences obtained.

Although MCs are well known to react to various stimuli with the secretion of MMP-9 (2, 15, 16), other cell types including T cells, fibroblasts, keratinocytes, macrophages, endothelial cells also express this proteinase. Thus, elevated MMP-9 levels in CU and psoriasis patients' plasma are probably the result of a general inflammatory status, i.e. the combination of cell destruction, migration of inflammatory cells and tissue remodelling in these patients, and not due to a specific release of active MMP-9 by MCs or other pro-inflammatory cells. Significantly increased total MMP-9 levels in CU patients with elevated CRP serum levels support the idea of an unspecific inflammatory stimulus as the cause of systemic MMP-9 release. Our study demonstrates that MMP-9 plasma levels do not specifically reflect CU activity and are not suitable to evaluate disease severity in CU patients.

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