Urinary monocyte chemoattractant protein-1 (MCP-1) is a marker of active renal vasculitis

Frederick W. K. Tam, Jan-Stephan Sanders, Abraham George, Tarig Hammad, Caroline Miller, Tammy Dougan, H. Terence Cook, Cees G. M. Kallenberg, Gill Gaskin, Jeremy B. Levy and Charles D. Pusey

1Renal Section, Division of Medicine and 2Department of Histopathology, Faculty of Medicine, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK and 3Department of Clinical Immunology, University Hospital Groningen, The Netherlands

Abstract

Background. Macrophage infiltration and cytokine production are important in the pathogenesis of crescentic glomerulonephritis in anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis. The aim of this study was to investigate whether urinary levels of chemokines, monocyte chemoattractant protein-1 (MCP-1) and fractalkine, were useful tools for non-invasive assessment of renal vasculitis.

Methods. In a prospective study, concentrations of chemokines were measured in urine and serum samples using specific enzyme-linked immunosorbent assays, and related to the patients’ clinical status. Renal expression of MCP-1 was studied by immunohistochemical staining of renal biopsies.

Results. Urinary levels of MCP-1 were significantly higher in patients with active \((P<0.01)\) or persistent \((P<0.05)\) renal vasculitis, in comparison with healthy volunteers, control patients, patients with inactive vasculitis and patients with extra-renal disease only. There were no differences in serum concentrations of MCP-1 between these groups. Reduction in urinary MCP-1 levels following treatment preceded the improvement of renal function by a median of 2 weeks. In one patient, rising urinary levels of MCP-1, despite immunosuppressive therapy, was associated with progression to severe renal failure. There were no differences in urinary fractalkine levels between the different groups of patients and controls. Immunohistology of renal biopsies from patients with crescentic glomerulonephritis showed increased staining for MCP-1 in glomerular and interstitial cells. Urinary MCP-1 levels correlated with glomerular, but not tubulointerstitial, macrophage infiltration \((P<0.05)\).

Conclusions. This study shows that measurement of urinary MCP-1, but not fractalkine, is a useful non-invasive technique for the assessment of renal involvement and monitoring the response to therapy in ANCA-associated vasculitis.

Keywords: ANCA; chemokine; crescent; glomerulonephritis; MCP-1; vasculitis

Introduction

Anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis typically causes focal segmental necrotizing glomerulonephritis with crescent formation [1]. Current treatment consists of immunosuppressive therapy which is often associated with significant side effects [1,2]. Some patients develop progressive renal injury resulting in end-stage renal failure despite therapy. Those who respond to treatment remain at risk of further relapses. Investigations that could be used to monitor patients’ responses to treatment and allow early detection of relapses would be very useful in optimization of therapy. However, assessment of renal function is an indicator of end-organ damage rather than of ongoing inflammatory activity. Markers of inflammation, such as C-reactive protein and erythrocyte sedimentation rate, are non-specific. Although ANCA titres correlate broadly with disease activity, they are not specific for renal involvement [3].

Infiltration of monocytes/macrophages into the glomeruli and interstitium is critical in the pathogenesis of renal vasculitis [4]. The intensity of cellular infiltration has been shown to correlate with severity of clinical
disease [5]. C-C chemokines, particularly monocyte chemoattractant protein-1 (MCP-1; CCL2), and the CX3C chemokine (fractalkine, CX3CL1), are potent chemoattractants for monocytes/macrophages in vitro and in vivo [6,7]. Increased expression of MCP-1 has been reported in renal biopsies of patients with vasculitis [8,9]. Similarly, increased expression of fractalkine was detected in glomeruli of patients with vasculitis by in situ hybridization and immunostaining, and was associated with monocyte/macrophage infiltration [10].

In experimental glomerulonephritis, there has been increased glomerular synthesis of both MCP-1 [9,11–13] and fractalkine [14]. Systemic administration of an anti-MCP-1 antibody has been shown to reduce the severity of acute glomerulonephritis and subsequent scarring [11,13]. Expression of fractalkine mRNA has been detected in the glomerular endothelium in experimental crescentic glomerulonephritis in WKY rats [14]. Administration of antibodies to the fractalkine receptor (CX3CR1) reduced macrophage infiltration and the severity of glomerular injury [14].

In the clinical situation, serial assessment of renal inflammation would be informative in guiding therapy. However, serial renal biopsies are not appropriate in clinical practice. A non-invasive approach is therefore needed for early diagnosis and monitoring of renal disease activity in vasculitis. Raised urinary levels of MCP-1 have been reported in a variety of renal diseases, including lupus nephritis [15,16], IgA nephropathy [17], crescentic glomerulonephritis [18] and diabetic nephropathy [19], and rejection of renal allograft [20]. However, the role of measuring urinary MCP-1 in the clinical management of ANCA-associated vasculitis needs to be investigated. MCP-1 is synthesized as a secreted protein by intrinsic renal cells and infiltrating leukocytes. Fractalkine is synthesized as a transmembrane protein, with its chemotactic module attached to a mucin stalk [7], but the chemotactic module of fractalkine may be cleaved into a soluble form. These differences between MCP-1 and fractalkine may affect their application in clinical measurement. Urinary fractalkine in glomerular diseases has not been reported. In this study, we investigated the measurement of urinary MCP-1 and fractalkine, in relation to disease activity, at both initial presentation and follow-up of patients with renal vasculitis.

**Subjects and methods**

The protocol of this study was approved by Hammersmith, Queen Charlotte’s and Chelsea Hospital Research Ethics Committee.

**Patient selection**

All subjects gave consent for this study. We studied 28 control subjects (including 20 healthy volunteers and eight patients from an endocrinology clinic with no evidence of diabetes mellitus, renal disease or hypertension) and 52 patients with ANCA-associated vasculitis who attended in-patient or out-patient services at Hammersmith Hospital between June 1999 and July 2003. Vasculitis disease activity was assessed by the Birmingham Vasculitis Activity Score (BVAS) [21]. The vasculitis patients were divided into four categories according to assessment of their disease activity: inactive disease (BVAS = 0) (n = 27); renal active disease (positive BVAS with renal involvement, n = 14); extra-renal disease only (positive BVAS with no active renal involvement, n = 6); and persistent renal disease (n = 5). Among the 14 patients with active renal vasculitis, 13 had renal biopsies. The patients with persistent renal disease had haematuria and variable proteinuria despite stable immunosuppressive therapy. Persistent vasculitis was assessed by BVAS2, which represents ongoing disease activity [22]. The clinical features of these subjects are summarized in Table 1. Clinical assessment of the vasculitis patients was performed independently of, and prior to, measurement of urinary chemokines. All patients received immunosuppressive therapy, corticosteroid, cyclophosphamide and plasmapheresis, according to the standard protocol of Hammersmith Hospital Renal Unit [1].

**Collection of specimens**

Freshly voided urine samples and 10 ml of serum were collected from each subject. Aliquots of urine were sent for quantification of protein and creatinine concentrations, microscopy and bacterial culture. Samples from patients with urinary sepsis were excluded from analysis. Urine and serum samples were centrifuged at 2500 r.p.m. for 10 min at 4°C, then aliquoted and stored at −20°C until tested by enzyme-linked immunosorbent assay (ELISA).

**ELISA**

This was performed using paired antibodies (R and D Systems, Oxon, UK) for human MCP-1 and fractalkine according to the manufacturer’s instructions. The sensitivity of the assays was 8 pg/ml for MCP-1 and 60 pg/ml for fractalkine. Chemokines in spot urine samples were expressed as ng/mmol creatinine.

**Immunohistology**

Paraffin sections of routine renal biopsies surplus to clinical requirements were used. Specific goat anti-human MCP-1 antibody (R and D Systems, Oxon, UK) was used at 1:40 dilution. The secondary antibody was biotinylated horse anti-goat at 1:200 and this was detected using a streptavidin–peroxidase technique. The complex was visualized using 0.5 mg/ml of diaminobenzidine (DAB; Sigma, Poole, UK), with 0.01% hydrogen peroxidase as the substrate. Biopsies were scored by a blinded observer on a semi-quantitative scale as follows: glomerular staining (scores 1–3); tubular intensity of staining (scores 1–3); and proportion of tubules stained (scores 1–5). Total tubular staining = tubular intensity x proportion of tubules stained.

Macrophages were detected with an antibody to CD68 (PGM1) at 1:100 dilution using a standard streptavidin–peroxidase technique. The number of macrophages present in each glomerular section was counted and averaged over the
entire number of glomeruli in the biopsy. Interstitial macrophage infiltration was scored 1–3.

Statistics

Results were analysed by non-parametric tests using GraphPad Prism software (version 3.02 GraphPad Software Inc, San Diego, CA). Results between groups are examined by Kruskal–Wallis test with Dunn’s multiple comparison test. Longitudinal follow-up of patients was analysed with the paired Wilcoxon rank sum test. Relationships between urinary MCP-1 levels and clinical parameters were analysed using Spearman correlation.

Results

Urinary and serum MCP-1

Patients with active renal vasculitis had significantly higher urinary MCP-1 (median 139.8, range 42.3–3483 ng/mmol creatinine) than healthy volunteers (median 8.8, range 2.2–24.3 ng/mmol creatinine), control patients (median 13.6, range 3.9–18.5 ng/mmol creatinine), patients with inactive vasculitis (median 8.5, range 2.0–31.5 ng/mmol creatinine) or patients with active extra-renal vasculitis only (median 14.8, 3.4–22.5 ng/mmol creatinine) ($P < 0.01$) (Figure 1).

In patients with persistent renal vasculitis (median 42.4, range 31.2–55.2 ng/mmol creatinine), the urinary MCP-1 levels overlapped with the lower range of active renal vasculitis patients, and was significantly higher than in the healthy volunteers, control patients, patients with inactive vasculitis or patients with active extra-renal vasculitis only ($P < 0.05$). There were no significant differences in urinary MCP-1 levels between normal volunteers, control patients, patients with inactive vasculitis and patients with active extra-renal vasculitis only ($P < 0.05$). There were no significant differences in urinary MCP-1 levels between normal volunteers, control patients, patients with inactive vasculitis and patients with extra-renal vasculitis. Therefore, measurement of urinary MCP-1 levels was proved to be effective in identifying renal involvement in vasculitis patients, as there was no overlap in the range of urinary MCP-1 levels in patients with active renal vasculitis in comparison with that in patients with inactive vasculitis or extrarenal vasculitis only.

There were similar levels of MCP-1 in the serum of different groups of patients (Figure 1B). These results support the hypothesis that the higher level of urinary MCP-1 in active renal vasculitis was due to increased renal production rather than to changes in the circulating concentration of MCP-1.

The relationship between urinary MCP-1 and the clinical and laboratory features of vasculitis patients was analysed by Spearman correlation test. Raised urinary MCP-1 levels correlated positively with ANCA titre measured by ELISA ($r = 0.33$, $P < 0.05$), total BVAS ($r = 0.76$, $P < 0.0001$) and with the renal component of the BVAS ($r = 0.83$, $P < 0.0001$). Raised urinary MCP-1 also significantly correlated with proteinuria ($r = 0.65$, $P < 0.05$) (Figure 2) and the number of urinary leukocytes ($r = 0.38$, $P < 0.005$), but not...
serum creatinine levels ($r = 0.52, P = 0.058$) in patients with active renal vasculitis. Raised levels of urinary MCP-1 were not a consequence of renal impairment or proteinuria. Some patients in the inactive vasculitis group (nine of 27) had stable renal impairment and/or proteinuria from previous renal damage. They had low levels of urinary MCP-1 in the same range as the control groups. Therefore, in this study, raised urinary MCP-1 levels related to active glomerulonephritis but not to chronic renal damage. However, in other conditions, the aetiology of raised urinary MCP-1 may be of different mechanisms. For example, previously we reported that the urinary MCP-1 level was raised in patients with Fanconi syndrome [23].

**Response to treatment**

For patients with active renal vasculitis, disease activity, renal function, and serum and urinary MCP-1 levels were monitored while patients received immunosuppressive therapy according to the established protocols at Hammersmith Hospital. In 11 patients, regular follow-up samples were obtained (Figure 3). One month after the initial presentation, urinary MCP-1 levels were reduced in 10 patients (reduction of median by 59%, paired Wilcoxon test, $P < 0.01$) after 1 month. In one patient with renal vasculitis (solid line, black filled circles), urinary MCP-1 levels continued to rise despite immuosuppressive therapy. This patient had further deterioration of renal function and became dialysis dependent.

---

**Fig. 1.** Urinary and circulating MCP-1 levels in patients with ANCA-associated vasculitis. (A) Urinary levels of MCP-1 were significantly higher in patients with active (Kruskal–Wallis test with Dunn’s multiple comparison test, $P < 0.01$) or persistent ($P < 0.05$) renal vasculitis. (B) There were no significant differences in serum MCP-1 concentrations between groups of subjects.

**Fig. 2.** Relationship between urinary MCP-1 levels and proteinuria in ANCA-associated vasculitis. There is a significant correlation between proteinuria and urinary MCP-1 levels in patients with active renal vasculitis (Spearman correlation $r = 0.65, P < 0.05$).

**Fig. 3.** Longitudinal follow-up of urinary MCP-1 levels in patients with active renal vasculitis. Regular follow-up urine samples were available in 11 patients who presented with active glomerulonephritis. In 10 patients with renal vasculitis (dotted line), reduction of urinary MCP-1 levels was associated with improved renal function during therapy (reduction of median by 59%, paired Wilcoxon test, $P < 0.01$) after 1 month. In one patient with renal vasculitis (solid line, black filled circles), urinary MCP-1 levels continued to rise despite immuosuppressive therapy. This patient had further deterioration of renal function and became dialysis dependant.
creatinine or resolution of microscopic haematuria) in six of 10 patients. Four patients had reduction of urinary MCP-1 and improvement of renal function at the same time (two patients had reduction of serum creatinine and resolution of microscopic haematuria, another two patients had resolution of microscopic haematuria only). Comparing laboratory results at presentation and those at first laboratory features of improvement, reduction of urinary MCP-1 levels is the most significant laboratory marker; urinary MCP-1 levels were reduced in all the patients (45% reduction of median, \( P < 0.005 \)). Six of the 10 patients had reduction of ANCA titre (22% reduction of the median, \( P < 0.05 \)). There was no significant reduction of urinary protein/creatinine ratio or serum creatinine at the same time. Therefore, urinary MCP-1 level is a more sensitive marker of patients’ responses to therapy than proteinuria, serum creatinine or ANCA titre (Table 2). One patient had rising urinary MCP-1 accompanied by further deterioration of renal function despite treatment, and subsequently became dialysis dependent (Figure 3).

Relapse of renal vasculitis

During regular follow-up of the patients with inactive vasculitis, one of them had a relapse of renal vasculitis (BVAS = 6; anti-proteinase 3 antibodies = 100% by ELISA), which was associated with a rise in urinary MCP-1 (Figure 4). The other inactive patients continued to have low urinary MCP-1 levels during follow-up.

Immunohistology of MCP-1 in crescentic glomerulonephritis. In order to analyse the relationship between urinary MCP-1 and renal production of MCP-1, we examined renal biopsies from 12 patients with crescentic glomerulonephritis (11 patients with renal vasculitis and one patient with Goodpasture’s disease). MCP-1 protein was detected in renal tissue from patients with active renal vasculitis and Goodpasture’s disease, but not in control tissue (Figure 5). MCP-1 protein was detected in most cell types, including parietal epithelial cells, infiltrating mononuclear cells in the capillary loops and tubules, and in glomerular crescents. MCP-1 was also detected in vessel walls and in the interstitium.

Table 2. Summary of laboratory results of 10 patients who responded to immunosuppressive therapy

<table>
<thead>
<tr>
<th>Initial presentation</th>
<th>At first laboratory response</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary MCP-1/creatinine (ng/mmol creatinine)</td>
<td>117 (42–863)</td>
<td>64 (24–332)</td>
</tr>
<tr>
<td>Urine protein/creatinine ratio (mg/mmol)</td>
<td>182 (3–987)</td>
<td>110 (37–529)</td>
</tr>
<tr>
<td>Serum creatinine (( \mu \text{mol/l} ))</td>
<td>169 (75–649)</td>
<td>151 (74–529)</td>
</tr>
<tr>
<td>ANCA (% assessed by ELISA)</td>
<td>95 (23–100)</td>
<td>78 (0–100)</td>
</tr>
</tbody>
</table>

Data are shown as median and range. Statistical analysis was by paired Wilcoxon rank sum test (two-tailed) between results at initial presentation and those at first laboratory features of improvement (25% reduction of urinary MCP-1 levels, urinary protein/creatinine ratios, serum creatinine concentrations or ANCA titre).

Fig. 4. Urinary MCP-1 during relapse of renal vasculitis. One of the patients under follow-up for vasculitis developed a relapse of renal disease (BVAS = 6) (●). Four months preceding relapse, the patient had a low level of urinary MCP-1. At the time of relapse, there was 6-fold increase in urinary MCP-1 levels. The urinary MCP-1 levels of patients with inactive vasculitis remained low during follow-up as shown in the example (▲).

Fig. 5. Immunostaining of MCP-1 in renal biopsies from patients with active renal vasculitis. (A) MCP-1 protein was detected in most cell types, including parietal epithelial cells (Pa), and infiltrating mononuclear cells in the capillary loops (Ma) and tubules (T). (B) MCP-1 staining was seen in the glomerular crescent (arrows). (C) MCP-1 was detected in renal glomeruli (G), tubules (T) and interstitium (In). Positive MCP-1 staining was also found in the endothelial cells (E) of the vessels together with staining of mononuclear cells in the media (M) and adventitia (A). (D) MCP-1 staining was seen in swollen and distended tubules (T) and in mononuclear cells in the interstitium (In).
or tubulointerstitial macrophage infiltration (data not shown).

**Urinary fractalkine levels.** Fractalkine was detected in the urine of both control groups and different groups of vasculitis patients. The median and range of urinary fractalkine levels in different groups of subjects were: 43 (6–145) ng/mmol creatinine in healthy volunteers; 21 (2–303) ng/mmol creatinine in control patients; 20 (0–413) ng/mmol creatinine in patients with inactive vasculitis; 14 (3–84) ng/mmol creatinine in patients with active renal vasculitis; and 47 (3–520) ng/mmol creatinine in patients with active extra-renal vasculitis only. There was a wide range of overlap between groups, and no significant differences were detected. We did not measure serum fractalkine concentrations or perform immunostaining for fractalkine.

**Discussion**

In this study, we have detected increased urinary levels of MCP-1 in patients with active or persistent renal vasculitis, in comparison with healthy volunteers, control patients, patients with inactive vasculitis and patients with vasculitis confined to extra-renal sites. There were no significant differences in circulating concentrations of MCP-1 among the different groups, supporting the hypothesis that increased urinary MCP-1 in active renal vasculitis is due to increased synthesis in the kidney. Using renal biopsies from patients with crescentic glomerulonephritis, we confirmed by immunohistology that there was increased expression of MCP-1 in both the glomeruli and the
One problem in the management of renal vasculitis is the early detection of relapse. A rising serum creatinine in vasculitis patients may be due to active disease or progressive renal scarring. However, serial renal biopsy is associated with significant risk, making a non-invasive test desirable. In this study, one patient had a relapse of renal vasculitis in association with a rise in urinary MCP-1 levels. This observation needs to be confirmed by our ongoing serial studies. It is speculative and remains to be investigated whether a rise in the urinary MCP-1 level may be detected before clinical relapse. The raised urinary MCP-1 levels in patients with persistent renal vasculitis suggest that monitoring urinary MCP-1 levels is also of value in the assessment of patients with ongoing disease activity in the kidney despite the lack of significant disease elsewhere.

We examined whether urinary MCP-1 levels may be used in monitoring patients’ responses to treatment. We found that urinary MCP-1 levels generally decreased before improvement of renal function and resolution of microscopic haematuria. In this study, we found that reduction of urinary MCP-1 levels is a more useful early laboratory marker of response to therapy than reduction of proteinuria, serum creatinine, ANCA titre or resolution of microscopic haematuria. Wada and colleagues showed reduction in urinary MCP-1 levels following corticosteroid therapy in patients with crescentic glomerulonephritis [18]. In this study, a patient with renal vasculitis, in whom urinary MCP-1 remained elevated despite immunotherapy, showed progression of disease to end-stage renal failure. Further study is needed to investigate whether monitoring urinary MCP-1 levels may be useful in detecting patients who do not respond to therapy.

Glomerular fractalkine expression has been shown to increase in renal biopsies from patients with renal vasculitis [10]. In this study, we did not find any significant differences in urinary fractalkine levels between the different groups of subjects. This may be due to the observation that: (i) a large proportion of fractalkine exists as transmembrane protein [7]; and (ii) expression of fractalkine is restricted to endothelial surfaces [10,14].

In conclusion, we found that urinary MCP-1, but not fractalkine, is a useful non-invasive approach to assessment of the activity of renal vasculitis and monitoring response to therapy. Further study is needed to investigate whether rising urinary MCP-1 levels is useful in identifying patients who do not respond to therapy or preceding a renal relapse.

Acknowledgements. F.W.K.T. was a National Kidney Research Fund Senior Fellow. This work was supported by Henry Smith’s Charity. Part of this work was presented at the 33rd American Society of Nephrology Annual Meeting at Toronto, Canada, 2000.

Conflict of interest statement. None declared.
References

11. Lloyd CM, Minto AW, Dorf ME et al. RANTES and monocyte chemoattractant protein-1 (MCP-1) play an important role in the inflammatory phase of crescentic nephritis, but only MCP-1 is involved in crescent formation and interstitial fibrosis. J Exp Med 1997; 185: 1371–1380

Received for publication: 19.3.04
Accepted in revised form: 30.7.04