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Renal MCP-1: an emerging universal biomarker and therapeutic target for kidney diseases?

(Invited editorial for NDT)

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Biological function

Monocyte chemoattractant protein-1 (MCP-1) (also known as CCL2) is a member of large family of chemotactic cytokines (chemokines) known to be important soluble mediators of innate immunity and tissue inflammation. MCP-1 was one of the earliest cytokines found to have a selective role in controlling monocytes/macrophage migration (although it is now known to act also on T cells and basophils). This is in contrast to other inflammatory mediators, such as complements and platelet activity factor (PAF), which have a broader chemoattractant activity.

Origin and regulation of renal MCP-1

MCP-1 is synthesised by a range of cell types in the kidney including intrinsic kidney cells and infiltrating leucocytes (Figure 1A). Glomerular mesangial cells release MCP-1 *in vitro* following stimulation with a variety of stimuli include inflammatory cytokines (IL-1 and TNFα), immune complexes, metabolic factors (glucose, glycation end products), danger associated molecular patterns (DAMP), such as extracellular ATP [1]. Podocytes release MCP-1 following stimulation with glycation end products, TNFα and TGF-β in vitro [2-4]. Stimulation of podocytes (which express CCR2) by MCP-1 has also been shown to downregulate nephrin, an alteration that could contribute to development of proteinuria in diabetic nephropathy and FSGS [5]. Renal tubular cells also produce MCP-1 in response to IL-1 and TNF [6], thrombin [7], and albumin [8] . The wide variety of cells and stimuli that are known to release and respond to MCP-1 highlight its importance but also raise questions as to whether it could ever be useful as a specific disease biomarker or selective therapeutic target (recently reviewed) [9].

Ligand receptor relationships

Although MCP-1 and CCR2 is the best-known ligand-receptor interaction, it is not an exclusive one. MCP-1 has been shown to interact with multiple receptors ie CCR1, CCR2, CCR3 and CCR5. Conversely, CCR2 can bind to other chemokines apart from MCP-1 such as CCL7, CCL8, CCL11, CCL12, CCL13, CCL24 and CCL26 (Figure 1B). Therefore, selective CCR2 blockade should not be considered the same as MCP-1 neutralisation (with an anti-MCP-1 antibody). Nevertheless, the severity of kidney injury (macrophage infiltration, type I collagen expression and tubulointerstitial fibrosis) was reduced in *Ccr2* knockout mice or following treatment with a CCR2 antagonist [10] suggesting that CCR2 could be the major receptor mediating the effect of increased MCP-1 in kidney disease.

In this month's issue of NDT, three papers highlight the role of MCP-1 in a range of kidney diseases both as a clinical biomarker of disease activity or in disease pathogenesis. The first two studies report that the combination of urinary MCP-1 (uMCP-1) with other soluble mediators can improve its performance as a biomarker of disease activity. In the first study, Moran and colleagues found that the combination of urinary soluble CD163 (sCD163) and uMCP-1 improved the diagnostic accuracy of renal flares in patients with anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis [11]. In the second study, Wu and colleagues report a significant association between urinary epidermal growth factor (uEGF) and uMCP-1 with disease progression in diabetic nephropathy [12]. Finally, Wilkening and colleagues demonstrate increased expression of the MCP-1 receptor CCR2 in kidney biopsies of patients with focal segmental glomerulosclerosis (FSGS) and found a positive effect of CCR2 deletion in a murine model of FSGS [13].

Expression of MCP-1 in kidney diseases (Table 1)

Immune mediated diseases

Increased glomerular synthesis of CC chemokines, including MCP-1, has been shown to correlate with monocyte/macrophage infiltration in experimental models of antibody and immune complex mediated glomerulonephritis [14, 15] . In clinical diseases, increased MCP-1 expression in human renal biopsies and excretion in urine has been detected in a number of proliferative glomerulonephritis associated with monocyte/macrophage infiltration, such as IgA nephropathy, lupus nephritis, renal vasculitis and Goodpasture's disease [16, 17].

In inflammatory diseases, the long-term effects of renal injury are determined by a balance between inflammation-induced injury and cellular repair or regeneration. Epidermal growth factor (EGF) is produced by the thick ascending limb of Henle's loop and distal renal tubule and has tubular-trophic effects. In a study of patients with IgA nephropathy, Torres and colleagues found that the uEGF/uMCP-1 ratio provided a better prognostic assessment of renal outcome at 4 and 8 years after biopsy than the uEGF/creatinine or uMCP-1/creatinine ratio [18].

The role of uMCP-1 as a disease marker has also been investigated in ANCA associated renal vasculitis. In an early study, uMCP-1 was found to be elevated in patients with active renal vasculitis but not in patients with systemic vasculitis sparing the kidney. Most patients had a decrease in uMCP-1 following successful broad immunosuppressive treatment except interestingly in one patient who had further rise in uMCP-1 and progressed to renal failure despite immunosuppressive treatment [17]. Although the number of patients reported in this initial study was small, these findings have since been replicated by several other groups [19-21]. Recently, uMCP-1 has also been used successfully as a pre-specified secondary endpoint in a randomised clinical trial of a complement C5a receptor inhibitor in patients with ANCA associated renal vasculitis [22].

In the latest study by Moran and colleagues, the combination of creatinine-normalised urinary soluble CD163 (usCD163) and uMCP-1 was more sensitive than either alone in detecting subtle renal flares in ANCA associated renal vasculitis (n=88) in the longitudinal VCRC (Vasculitis Clinical Research Consortium) multicentre North American cohort [11]. Mechanistically, this could reflect distinct

independent aspects of macrophage activation (CD163) and recruitment (MCP-1) since there was a poor correlation between them. The algorithm performed better when new-onset proteinuria was included as an independent variable. A major limitation of the study is the reliance on clinical assessment alone without histological confirmation leading to potential misclassification. There was also a discrepancy with uMCP-1 results reported on a subset of patients with non-renal disease by a different group [20]. Finally, although the post-test probabillity of detecting a positive renal flare using this algorithm was >90% in patients with either moderate or high pre-test probability of having a renal flare, the equivalent post-test probability of a negative test excluding a flare was 22% (moderate) or 58% (high) respectively indicating that the proposed algorithm will need much refinement and validation prior to clinical use.

Of interest, MCP-1 may also play a role in the pathogenesis of chronic allograft failure. In a recent study, uMCP-1 was prognostic of renal allograft failure [23].

Diabetic nephropathy

Although diabetic nephropathy is now the most common cause of kidney failure, the contribution of monocytes/macrophages in its pathogenesis has not been studied until recently. In renal biopsies of patients with diabetic nephropathy, increased expression of MCP-1 and macrophage infiltration in the glomeruli and tubulointerstitium has been reported. Similarly, increased numbers of glomerular macrophages have been detected in rodent models of diabetic nephropathy [24]. Proteinuria and glomerular macrophages were significantly reduced in *Mcp1* knockout mice following the experimental induction of diabetes. Increased levels of uMCP-1 as well other cytokines and profibrotic growth factors (TGF-β, CTGF) have been detected in patients with diabetic nephropathy. In a longitudinal study over 6 years, the uMCP-1/creatinine ratio (uMCP-1/Cr) was found to be predictive of the rate of eGFR decline [25]. These results have been validated in several other studies even when adjusted for baseline covariates, such as diabetic control, blood pressure, age and sex [26].

The idea of measuring the uEGF/uMCP-1 ratio was first described in a study of IgA nephropathy [18]. Two later studies (including one in this current issue) have now demonstrated the prognostic value of uEGF/uMCP-1 in diabetic nephropathy [12, 27]. The first study from the Joslin Diabetic Centre reported a negative correlation between uEGF/Cr and uEGF/uMCP-1 and the occurrence of diabetic kidney disease [27]. In longitudinal follow up, the uEGF/uMCP-1 ratio correlated more closely to the rate of loss of eGFR than uEGF/Cr or uMCP-1/Cr. When a composite end point (end stage kidney disease or 30% reduction in baseline eGFR) was studied, uEGF/Cr and uEGF/uMCP-1 were negative predictors whereas uMCP-1/Cr was an independent positive predictor of disease progression [27]. In this issue, Wu and colleagues confirm that the ratio of uEGF/uMCP-1 in the urine is a better prognostic biomarker than the uEGF/Cr or uMCP-1/Cr ratio [12]. However, there are several major limitations to the current study. First, the conclusions were based on a cross-sectional analysis of two very different Chinese population-based cohorts with early (INDEED, n=1811) and advanced (C-STRIDE, n=208) diabetic kidney disease (DKD). Second, over 90% of patients had Type 2 diabetes mellitus and therefore the conclusions may not apply to Type 1 patients. Third, although statistically significant, the use of uEGF/Cr or uEGF/uMCP-1 only marginally added to the discriminatory value of urine albumin creatinine ratio (uACR) is distinguishing DKD from those without DKD in the INDEED cohort. Similarly, uEGF/Cr, uMCP-1/Cr or uEGF/uMCP-1 showed limited clinical value (overlapping confidence intervals) in predicting composite kidney outcomes (end-stage renal failure and 30% decline in eGFR) in the C-STRINE cohort. Although promising, these results will clearly need to be validated in a larger multi-ethnic cohort with longer term follow-up.

The clinical relevance of the MCP-1/CCR2 pathway has been tested in a trial of CCX140B, a small molecule inhibitor of CCR2, in type 2 diabetic patients with well-controlled blood pressure (average age 62.5, women 15%, ethnic origin 97% Caucasian, average BP 137/78 blood pressure, average eGFR 62 ml/min/1.73 m^2) and receiving maximal tolerable dose of angiotensin converting enzyme inhibitor or angiotensin receptor inhibitor. Low dose, but not high dose, treatment with CCX140B resulted in a significant reduction in urinary albuminuria in comparison to placebo [28]. However, the clinical

development of CCX140B requires further evaluation because of the lack of efficacy of high dose treatment. In a Phase 2A clinical trial in type 2 diabetic patients, a different CCR2 antagonist, Emapticap pegol (NOX-E36), was studied. Although the drug was safe and well tolerated, the reduction in albuminuria (29% treated v 16% placebo) observed did not reach significance in comparison to the placebo treatment [29].

Focal segmental glomerulosclerosis (FSGS)

Increased uMCP-1 had been previously reported in patients with FSGS [30] and increased renal tubular expression of MCP-1 was detected in FSGS renal biopsies [6]. Wilkening and colleagues now show that there is increased gene expression for MCP-1 and CCR2 in microdissected FSGS human glomeruli. This is an interesting finding since FSGS is not a typical proliferative glomerulonephritis [13]. However, these results were based on microarray analysis without validation by other techniques. The authors did not determine the intrinsic glomerular cell types expressing MCP-1 and CCR2 in diseased glomeruli. However, they did show that soluble urokinase-type plasminogen activator receptor (suPAR) and recombinant TNFα stimulated podocytes to produce MCP-1 in vitro. Importantly, their study demonstrated that genetic deletion of CCR2 in the adriamycin-induced murine FSGS model reduced macrophage numbers in the glomeruli and tubulointerstitium, and simultaneously reduced the severity of glomerular and tubulointerstitial fibrosis [13].

Other chronic kidney diseases

Increased renal tubular expression of MCP-1 has been detected in the renal biopsies of patient with a variety of glomerulonephritis (IgA nephropathy, membranous nephropathy, membranoproliferative glomerulonephritis and FSGS) in close proximity to infiltrating monocytes/macrophages [31]. Furthermore, inflammatory cytokines such as IL-1 and TNF are capable of stimulating cultured renal

tubular epithelial cells to release MCP-1 [6]. In another study of patients with a range of kidney diseases (including thin glomerular basement disease, IgA nephropathy, ischaemic/hypertensive nephropathy, FSGS, membranous nephropathy, diabetic nephropathy, minimal change nephropathy, primary amyloidosis, light-chain nephropathy), uMCP-1 was shown to correlate with the number of interstitial macrophages [30] (Table 1). This suggests that diseases of diverse aetiology may share a final common pathway for disease progression characterised by monocyte/macrophage infiltration and chronic tubulointerstitial injury.

Of interest, the uMCP-1/Cr ratio was found to be elevated in patients with congenital obstructive nephropathy and surprisingly improved following surgical treatment [32]. Unexpectedly, very high levels of uMCP-1 were also detected in patients with Fanconi syndrome due to Dent's disease, Lowe's syndrome and autosomal-dominant idiopathic Fanconi syndrome [33]. It is unclear whether these high levels relate to increased local tubular synthesis or decreased tubular reabsorption of filtered MCP-1.

Polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) is the third or fourth most common cause of kidney failure world-wide [34]. The major research focus in ADPKD research has been on understanding how increased tubular cell proliferation and fluid secretion lead to cyst growth. Nonetheless, there is now increasing evidence that other factors (including metabolism, autophagy, inflammation and innate immunity, oxidative stress, epigenetic modification) could contribute to the severity of disease progression in ADPKD (recently reviewed [35]) .

Although ADPKD is not an obvious disease associated with monocyte/macrophage infiltration, early data from experimental models had indicated a modifying role for MCP1 in disease pathogenesis. Increased renal expression of MCP-1 was detected and localised to the cyst-lining epithelium in a non-orthologous rat PKD model (Han:SPRD), [36] and in murine *Pkd1* models [37]. Significantly tubule-

specific deletion of *Mcp1* or administration of a CCR2 inhibitor to *Pkd1* mutant mice reduced the number of pericystic macrophages, cyst formation, tubular injury and improved kidney function [38]. MCP-1 has also been detected in renal cyst fluid, cyst lining of end-stage ADPKD kidneys and in urine (but not serum) samples of ADPKD patients indicating *de novo* tubular synthesis [39]. Importantly, uMCP-1 levels were positively correlated with the rate of loss of eGFR in another study [40]. In the pivotal TEMPO3/4 trial in patients with ADPKD, there was a 11-24% reduction in uMCP-1 over 36 months in the tolvaptan-treated group [41] raising the possibility that MCP-1 could be a biomarker of therapeutic response in ADPKD trials.

Challenging issues in blocking MCP-1 and CCR2

In the CCX140B trial in type 2 diabetic patients with nephropathy, blocking CCR2 resulted in a dose-dependent increase in plasma MCP-1 in comparison to the placebo group [28]. Only the lower dose of the CCR2 blocker reduced albuminuria significantly. The increase in plasma MCP-1 observed at the higher dose could have led to an increase in systemic inflammation counter-balancing the beneficial effects on renal inflammation observed at the lower dose.

Conversely, the systemic delivery of MCP-1 inhibitor or a neutralisation antibody could increase the concentration gradient between circulating plasma and tissue levels paradoxically leading to increased leukocyte recruitment. In addition, circulating antibody-MCP-1 immune complexes may still retain biological activity. These potential issues have been highlighted by results of a phase 2 clinical trial of a human monoclonal antibody (mAb) to MCP-1 (ABN912) in rheumatoid arthritis [42]. After two infusions of ABN912, no clinical benefits were observed in the treated patients. However there were very high serum levels of ABN912-MCP-1 immune complexes and synovial biopsies showed that the group treated with the highest dose of anti-MCP-1 mAb showed a paradoxical rise in the number of CD68+ macrophages in the synovium lining [42].

Future perspectives

uMCP-1 has unexpectedly emerged as a sensitive biomarker of disease activity in a range of kidney diseases, not just in those characterised by acute inflammation but also in others of a more chronic nature. The latter observation thus supports a disease-modifying role for chronic inflammation due to monocyte/macrophages in a common pathway of renal disease progression regardless of the initial aetiology. Definitive proof of this hypothesis is awaited since to date, clinical trials attempting to block MCP-1 action (through CCR2 blockade) in diabetic nephropathy have been equivocal, possibly due to the redundancy of ligand-receptor interaction and/or unanticipated effects on systemic MCP-1 levels. Nonetheless, based on our emerging knowledge of disease pathogenesis, it seems likely that a combinatorial therapeutic approach targeting several key cytokines apart from MCP-1 in chronic kidney disease will be required. Other chemokines with powerful chemoattractant effects on monocytes/macrophages (such as RANTES, MIP-1α and MIP-1β) as well as pro-fibrotic cytokines (TGF-β, CTGF) have been shown to be upregulated in preclinical and clinical studies. The simultaneous analysis of multiple urinary chemokines, including MCP-1 using multiplex assays is now achievable and may add to the utility of uMCP-1 as a prognostic, activity or therapeutic biomarker.

These three papers add to the emerging body of evidence that uMCP-1, probably in combination with other soluble mediators (such as CD163, EGF and others), could be a clinically useful biomarker for disease onset or progression in acute and chronic kidney disease from multiple aetiologies. Future studies in larger multi-ethnic longitudinal patient cohorts should clarify whether it is a sufficiently sensitive biomarker especially for chronic kidney disease. Whether inhibiting MCP1 synthesis or action is beneficial in any form of kidney disease in man remains to be shown.

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Competing interest

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Legend to Figure 1

The renal MCP-1 and CCR2 axis

A. Cytokines, inflammatory cells & the pathogenesis of kidney disease. MCP-1 can be synthesised by a range of cell types including macrophages, podocytes, mesangial cells, endothelial cells and tubular cells.

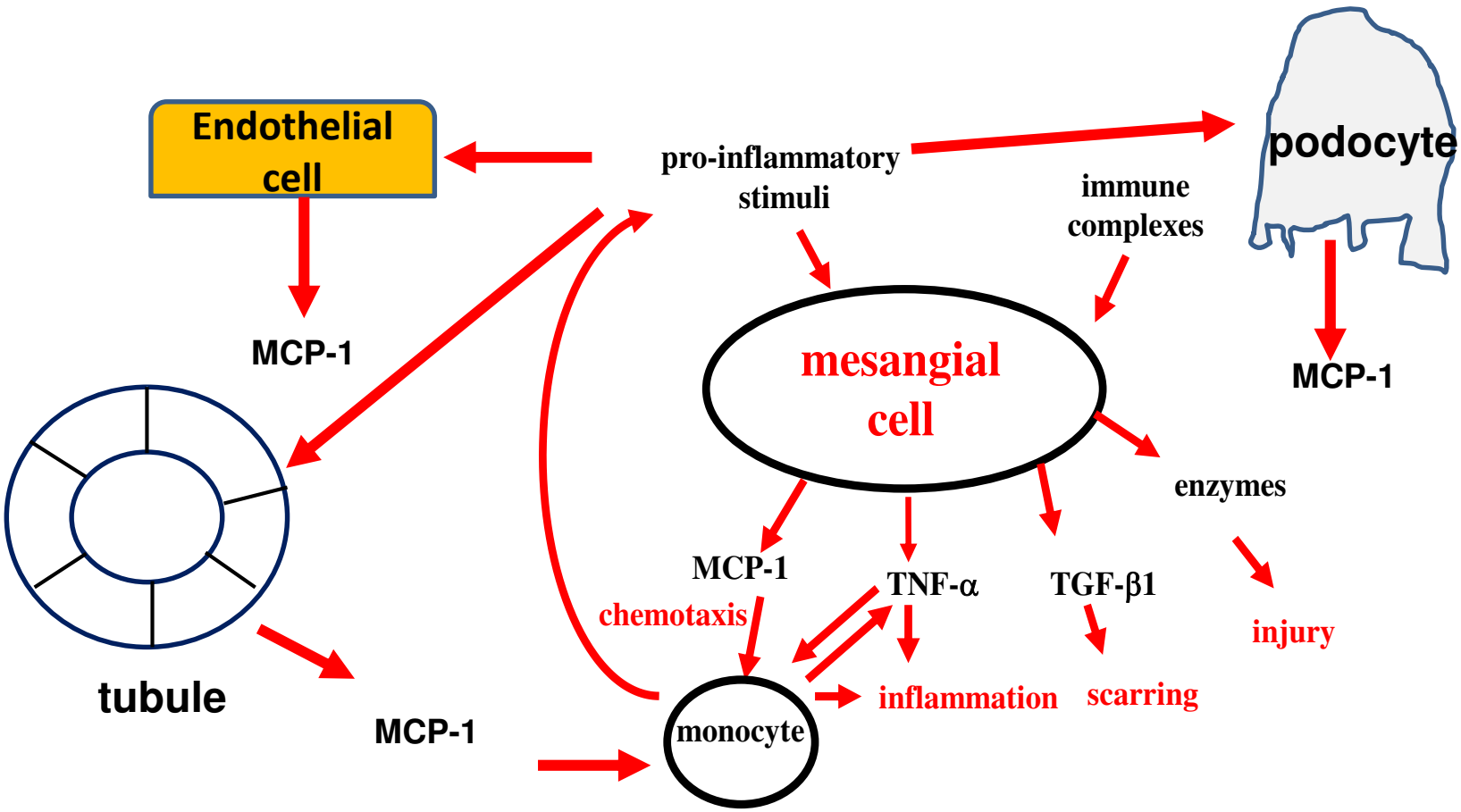
B. The interaction between MCP-1 and CCR2 is not exclusive as MCP-1 can also bind to CCR1, CCR3 and CCR5. Conversely, CCR2 has been shown to bind multiple chemokines such as CCL7, CCL8, CCL11, CCL12, CL13, CCL24 and CCL26.

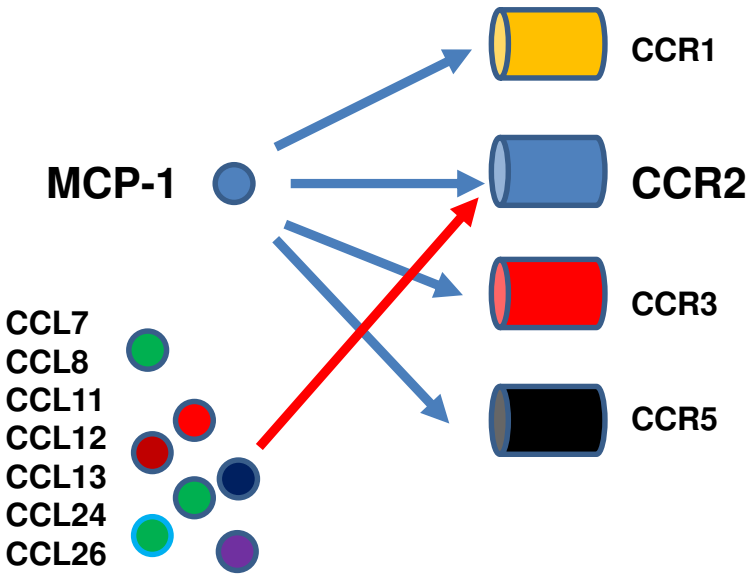
Table 1: Urinary and renal MCP-1 as a biomarker in studies of patients with kidney disease

Diagnosis	Clinical correlation	N	Response to treatment and Prognosis	Reference
Crescentic GN due to ANCA and IgAN	uMCP-1 correlated with glomerular crescents and CD68+ cells in the interstitium	20	uMCP-1 fell in response to corticosteroid treatment	[43]
IgA nephropathy	uMCP-1 higher than controls	5		[16]
Lupus Nephritis	uMCP-1 higher than controls	3		[16]
IgA nephropathy	uEGF/uMCP-1 ratio	132	Lower uEGF/uMCP-1 correlated with more severe renal histopathology. Lower uEGF/uMCP-1 prognostic of worse renal survival over 4 and 8 years	[18]
ANCA associated renal vasculitis	uMCP-1 raised in patients with active renal vasculitis	52	uMCP-1 fell in response to immunosuppression except in a non-responder. Rising uMCP-1 in one patient who progressed to ESRD	[17]
ANCA associated vasculitis	Raised uMCP-1 in patients is prognostic of future relapse	99		[19]
ANCA associated vasculitis	Raised uMCP-1 in both renal flare and some cases of 50% of non-renal flares	50		[20]
ANCA associated vasculitis	Comparison of 161 biomarkers: uMCP-1 and CRP are the best biomarker to distinguish between active disease and remission	22		[21]
ANCA associated renal vasculitis	uMCP-1 as a secondary endpoint of the clinical trial	67	Fall in response to immunotherapy (including corticosteroid +/- C5a receptor inhibitor	[22]
ANCA associated renal vasculitis	Combined uMCP-1/Cr, uCD163/Cr and new onset proteinuria were effective in	88		[11]

	identifying patients with active renal vasculitis			
Diabetic nephropathy	uMCP-1 correlated with late stage diabetic nephropathy, with increased number of CD68+ macrophages on renal biopsies. MCP-1 positive cells were detected in the tubulointerstitium	45		[24]
Diabetic nephropathy	uMCP-1 raised in patients with established macroalbuminuria	43	Rate of loss of eGFR over 6 years	[25]
Diabetic nephropathy	Subsets of Patients from ACCORD study Baseline and year 2 uMCP-1	380	Prognostic of rate of loss of eGFR over 5 years	[26]
Diabetic nephropathy	uEGF/MCP-1 is prognostic of eGFR	1032		[27]
Diabetic nephropathy	uEGF/MCP-1 negatively associated with onset or progression of DKD	1811	Two cohorts with early (INDEED) or advanced DKD (C-STRINE). Negative correlation with incidence of DKD or renal survival using composite endpoint (end stage renal disease or 30% reduction eGFR	[12]
FSGS	Increased expression of mRNA for MCP-1 and CCR2	10		[13]
A range of kidney diseases	uMCP-1 correlated with the number of interstitial macrophage	215 (30 patients with FSGS)		[30]
Obstructive uropathy due to pelviureteric junction obstruction	Inverse relationship to kidney function (MAG3)	24	Reduction of uMCP-1 after pyeloplasty	[32]
Inherited renal Fanconi syndrome	Raised uMCP-1 in all the patients with inherited	10		[33]

	renal Fanconi syndrome			
ADPKD	Correlated with Serum creatinine concentration	55		[39]
ADPKD	Total kidney volume correlated with the amount of uMCP-1	102		[44]
ADPKD	Higher uMCP-1 in comparison to normal subjects	1307	Reduction of uMCP-1 in patients treated with Tolvaptan	[41]
ADPKD	uMCP-1 correlated with annual rate of loss of eGFR	104		[40]


Endothelial cell
pro-inflammatory stimuli
podocyte
immune complexes
MCP-1
MCP-1
mesangial cell
enzymes
MCP-1
TNF-α
TGF-β1
chemotaxis
injury
tubule
MCP-1
monocyte
inflammation
scarring


CCR1
MCP-1
CCR2
CCR3
CCR5
CCL7
CCL8
CCL11
CCL12
CCL13
CCL24
CCL26