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Urinary soluble CD163 and monocyte chemoattractant protein-1 in the identification of subtle renal flare in anti-neutrophil cytoplasmic antibody-associated vasculitis

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ABSTRACT

Background. Prior work has shown that urinary soluble CD163 (usCD163) displays excellent biomarker characteristics for detection of active renal vasculitis using samples that included new diagnoses with highly active renal disease. This study focused on the use of usCD163 in the detection of the more clinically relevant state of mild renal flare and compared results of usCD163 testing directly to testing of urinary monocyte chemoattractant protein-1 (uMCP-1).

Methods. Patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV, $n = 88$) were identified within a serially sampled, longitudinal and multicentre cohort. Creatinine-normalized usCD163 and uMCP-1 levels were measured by enzyme-linked immunosorbent assay and, both alone and in combination, were compared between times of active renal AAV and during remission and/or active non-renal AAV.

Results. Samples from 320 study visits included times of active renal vasculitis ($n = 39$), remission ($n = 233$) and active extrarenal vasculitis ($n = 48$). Median creatinine levels were 0.9 mg/dL [interquartile range (IQR) 0.8–1.2] in remission and 1.4 mg/dL (IQR 1.0–1.8) during renal flare. usCD163 levels were higher in patients with active renal vasculitis compared with patients in remission and those with active extrarenal vasculitis, with median values of 162 ng/mmol (IQR 79–337), 44 (17–104) and 38 (7–76), respectively ($P < 0.001$). uMCP-1 levels were also higher in patients with active renal vasculitis compared with patients in remission and those with active extrarenal vasculitis, with median values of 10.6 pg/mmol (IQR 4.6–23.5), 4.1 (2.5–8.4) and 4.1 (1.9–6.8), respectively ($P < 0.001$). The proposed diagnostic cut-points for usCD163 and uMCP-1 were 72.9 ng/mmol and 10.0 pg/mmol, respectively. usCD163 and uMCP-1 levels were marginally correlated ($r^2 = 0.11$, $P < 0.001$). Combining novel and existing biomarkers using recursive tree partitioning indicated that elevated usCD163 plus either elevated uMCP-1 or new/worse proteinuria improved the positive likelihood ratio (PLR) of active renal vasculitis to 19.2.

Conclusion. A combination of usCD163 and uMCP-1 measurements appears to be useful in identifying the diagnosis of subtle renal vasculitis flare.

Keywords: ANCA, biomarkers, CD163, MCP-1, vasculitis

INTRODUCTION

Anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV) is frequently characterized by the presence of glomerulonephritis. This glomerular inflammation may progress to cause necrotizing lesions associated with crescents, macrophage-rich inflammatory infiltrates associated with glomerular destruction and rapid loss of kidney function [1–3].

AAV is a chronic relapsing and remitting autoimmune disease that is often treated with intense immunosuppression, leaving patients open to adverse events such as infection that may cause more harm than the underlying disease itself [4].

We have previously demonstrated that active glomerulonephritis in the context of small-vessel vasculitis can be identified by measuring the level of the protein soluble CD163 (sCD163) in the urine [5, 6]. This protein is expressed on the surface of macrophages and is cleaved from the cell surface by the action of a disintegrin and metalloprotease domain 17 (ADAM17)/tumour necrosis factor (TNF)-α-converting enzyme (TACE) [7]. Relatively high levels of sCD163 can be found in the serum, particularly in macrophage-rich diseases such as macrophage activation syndrome and various forms of liver disease [8]. When the protein is cleaved from glomerular macrophages it appears in the urine, where it shows potential as a biomarker to identify patients with active glomerular inflammation.

Our prior work was performed in patients with both AAV and anti-glomerular basement membrane disease [5], and other work shows high urine levels of sCD163 in patients with active lupus nephritis [9]. Most patients studied thus far were sampled at the time of diagnosis, when disease was severe and there was little doubt about the presence of active glomerulonephritis based on existing clinical parameters. The unmet need for clinical biomarkers in AAV is in identifying patients with a prior confirmed diagnosis who may be suffering a flare of vasculitis in the kidney. Unless there is overt rapidly progressive glomerulonephritis with marked loss of kidney function, the diagnosis is often difficult, with a broad differential diagnosis and frequent requirement for kidney biopsy. For example, dipstick positive haematuria and proteinuria, while useful in patients at the onset of disease, remain elevated for a median of 448 and 346 days, respectively, after diagnosis of AAV [10], thereby reducing their utility as discriminators of active glomerular inflammation.

Tam et al. [11] previously showed potential utility for urinary monocyte chemoattractant protein-1 (uMCP-1) in active renal vasculitis, with some indication that the levels fall in remission [11]. This biomarker is currently being used as a secondary outcome measure in clinical trials (https://clinicaltrials.gov/ct2/show/NCT02222155), although it has not entered routine clinical practice. Therefore, to address this clinical unmet need, we sought to test the utility of sCD163 and MCP-1, or a combination of the two, to diagnose subtle flares of renal vasculitis. The Vasculitis Clinical Research Consortium (VCRC) has access to a rich longitudinal clinical and biological sample set, including patients suffering from a wide range of levels of renal disease in AAV. We used this unique resource to determine sCD163 and MCP-1 biomarker characteristics in this setting and to explore ways in which they could be integrated with existing clinical biomarkers to maximize utility for the identification of patients with mild flares of renal vasculitis.

MATERIALS AND METHODS

Patients and clinical data collection

Patients were enrolled in the VCRC longitudinal study of patients with granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) or eosinophilic granulomatosis with polyangiitis (EGPA) from eight referral centres in the USA and Canada. All patients were enrolled using protocols approved by the institutional review boards or ethics committees of all participating sites and written informed consent documents were obtained in keeping with the Declaration of Helsinki. All enrolled patients met the American College of Rheumatology (ACR) criteria for GPA modified to include ANCA or the Chapel Hill Consensus Conference definition of MPA or the ACR Classification Criteria for EGPA (adapted so that biopsy proof of small-vessel vasculitis was not required). Clinical data, including measures of disease activity and immunosuppression, were collected on a quarterly or annual basis. We identified patients with a visit associated with a flare of AAV (either renal or non-renal) and targeted for analysis up to two encounters during remission both before and after the flare visit. We also included patients initially presenting with renal vasculitis but with only remission encounters thereafter.

Measures of vasculitis disease activity

Information on specific manifestations of vasculitis was recorded using the Birmingham Vasculitis Activity for Wegener's Granulomatosis Score (BVAS/WG) [12], urinalysis for blood and protein, red blood cell (RBC) casts, C-reactive protein (CRP) and serum creatinine [13]. Active renal disease was determined by the physician-investigator and was informed by the presence of new or worse haematuria, new or worse proteinuria, urinary RBC casts and/or an increase in serum creatinine >30% (interpreted by the clinician as being due to active vasculitis).

Collection and storage of urine samples

Urine was collected by the patients in sterile cups and aliquoted without further manipulation, frozen at −80°C at each participating clinical site, shipped on dry ice to the VCRC specimen repository and stored at −80°C until used for this study.

Clinical laboratory tests

Serum creatinine and urinalyses (dipstick and microscopy) were performed in Clinical Laboratory Improvement Amendments–approved laboratories at the clinical sites per standard practice for collection and processing of outpatient specimens. Results of dipstick and microscopic urinalyses were recorded in the VCRC database as positive or negative (without further quantification) for blood, protein, RBCs and RBC casts. RBCs and RBC casts could also be noted as positive based on examination by the investigator or a nephrologist colleague at the time of the patient visit, but such an examination was not required. It was not recorded whether assessment for RBCs or casts was made by the clinical laboratory or the investigator. The presence or absence of dysmorphic RBCs was not recorded. Glomerular filtration rate (GFR; mL/min/1.73 m^2 body surface area) was calculated from serum creatinine using the Modification of Diet in Renal Disease formula [14].

Urinary biomarker assays

sCD163 is produced following inflammatory cleavage from the surface of glomerular macrophages, whereas MCP-1 is

derived from intrinsic renal cells in response to inflammatory stimuli and results in recruitment of monocytes. As these are discrete elements in the process of renal macrophage accumulation, we were interested in determining whether the pattern of urinary excretion was different and whether urinary soluble CD163 (usCD163) could identify cases mischaracterized by uMCP-1 and vice versa. We addressed the following experimental questions:

1. Does usCD163 aid in the detection of renal vasculitis flare?
2. Does measurement of uMCP-1 aid in the diagnosis of renal flare, either alone or in combination with usCD163?

usCD163 was measured by commercially available enzyme-linked immunosorbent assay (ELISA) (Duoset DY1607; R&D Systems, Minneapolis, MN, USA) at a 1:4 dilution [5]. MCP-1 was measured by commercially available ELISA (Duoset DY279; R&D SYstems) at a 1:5 dilution [11]. Urine creatinine and protein were measured by a Cobas Creatinine Plus (0566127) and Total Protein (11877801) modules (Roche, Rotkreuz, Switzerland), respectively. Urine biomarker values were normalized to urine creatinine. As any new biomarker will not be likely be used in isolation from existing best practice, we tested both usCD163 and uMCP-1 in combination with new haematuria, creatinine increase, CRP and new proteinuria. To identify the optimal combination of tests, we used unbiased recursive partitioning to generate a combination of markers that maximized specificity, that is, to 'rule out' the presence of active renal vasculitis.

Statistical methodology

Clinical, laboratory data and ELISA results were analysed using GraphPad Prism version 6 (GraphPad Software, La Jolla, CA, USA). Biomarker values were non-normally distributed and are thus reported as median and interquartile range (IQR). Kruskal–Wallis and Mann–Whitney U tests were used to determine the significance of associations. Correlations were measured using the Spearman correlation coefficient. Decision tree and optimal cut-off ranges were performed using R Studio version 0.99.902 (RStudio, Boston, MA, USA). Rpart and party packages were used [15, 16]. To generate receiver operating characteristics (ROC) curves and determine the best cut-points we used the OptimalCutpoints package. The Youden calculation [17] was selected to maximize the sum of sensitivity and specificity; other methods include MaxSp (to maximize specificity), MaxSe (to maximize sensitivity), MaxProdSpSe (to maximize the product of specificity and sensitivity) and SpEqualSe (using criterion of equality of sensitivity and specificity), but the Youden calculation yielded the most clinically relevant cut-off values. Decision trees were generated using the rpart package and included the clinical variables new/worse proteinuria, new/worse haematuria, serum creatinine, CRP, age and sex, in addition to the biomarkers of interest, usCD163 and uMCP-1. These clinical variables were included in the decision tree irrespective of whether or not they were recorded as an active BVAS/WG item. To confirm that the association of usCD163 and uMCP-1 with active renal vasculitis remained robust after taking into account the intra-individual repeated measures employed in this study, the transformed trajectories of CD163 were modelled using mixed effects models. For this purpose, the group with active renal disease was compared with all other groups combined. Within-patient errors were modelled as longitudinal using an autoregressive correlation structure (Order 1). The autocorrelation parameter was estimated. This parameter can be thought of as a measure of memory, with a value of zero corresponding to the more usual assumption of independent errors. Model fitting was carried out using the nlme package in R. To quantify the performance of the regression tree outputs in three different prevalence scenarios we used the formula $P1 = (LR \times P0)/(1 - P0 + LR \times P0)$, where P0 is pre-test probability and P1 is post-test probability. The three scenarios were (i) high probability of stable remission (P0 = 5%), (ii) clear extra-renal flare with some urinary abnormalities (P0 = 40%) and (iii) high probability renal flare (P0 = 70%).

RESULTS

Both usCD163 and uMCP-1 are elevated in the presence of active renal flare

Urine and clinical data were obtained at 320 clinical encounters from 88 patients, with active renal disease present in 39 (12.2%) of the encounters (Tables 1 and 2). These encounters were characterized as follows: (i) patients with renal flare ($n = 39$) and remission encounters selected from either before or after the renal flare visit ($n = 116$ in 39 patients); (ii) patients with non-renal flare ($n = 48$) and remission encounters ($n = 106$ in 48 patients) selected from before or after this flare encounter and (iii) patients with remission encounters only, having presented with renal vasculitis at diagnosis ($n = 10$).

The study population included many patients with GPA (82.9% of total study population) with minimal kidney impairment. Renal flares were often subtle, as indicated by median creatinine levels in remission and during renal flare of 0.9 mg/dL (IQR 0.8–1.2) and 1.4 (1.0–1.8), respectively. usCD163 levels were higher in patients with active renal vasculitis compared with patients in remission and those with active extra-renal vasculitis, with median values of 162 ng/mmol (IQR 79–337), 44 (17–104) and 38 (7–76), respectively ($P < 0.001$) (Figure 1A). The area under the ROC curve for distinguishing patients with active renal flare from those without active renal vasculitis was 0.794 (Figure 1B). uMCP-1 levels were also higher in patients with active renal vasculitis compared with patients in remission and those with active extra-renal vasculitis, with median values of 10.6 (IQR 4.6–23.5), 4.1 pg/mmol (2.5–8.4) and 4.1 (1.9–6.8) ($P < 0.001$), respectively (Figure 1C). The area under the respective ROC curve was 0.687 (Figure 1D). The proposed diagnostic cut-points in this setting for usCD163 and uMCP-1 were 72.9 ng/mmol and 10.0 pg/mmol, respectively (Table 3). The correlation between usCD163 and uMCP-1 was weakly positive ($r^2 = 0.11$, $P < 0.001$; Figure 2A). There was also a weak correlation of each parameter with the urine protein excretion rate ($r^2 = 0.02$, $P < 0.001$ for usCD163 and $r^2 = 0.14$, $P < 0.001$ for uMCP-1; Figure 2B and C).

Table 1. Demographic and clinical characteristics of study subjects

Characteristics	Active renal ($n = 39$)	Active non-renal ($n = 48$)	Remission ($n = 10$)
Demographics			
Female, *n* (%)	14 (35.9)	34 (70.8)	9 (90.0)
Age (years), median (IQR)	56.0 (45.9–64.2)	39.3 (27.8–62.9)	51.6 (43.3–60.7)
Ethnicity (white), %	92.3	91.7	100
Disease, *n* (%)			
GPA	34 (87.2)	33 (68.8)	6 (60.0)
MPA	2 (5.1)	1 (2.1)	4 (40.0)
EGPA	3 (7.7)	14 (29.2)	0
Disease duration (years), median (range)	4.8 (2.5–8.7)	6.1 (2.3–11.1)	6.2 (0.2–21.7)
ANCA specificity, *n* (%)			
Anti-PR3	27 (69.2)	19 (39.6)	5 (50.0)
Anti-MPO	7 (17.9)	11 (22.9)	4 (40.0)
ELISA negative	3 (7.7)	15 (31.2)	0
ELISA unknown	2 (5.1)	3 (6.3)	1 (10.0)

The active renal and active non-renal patients contributed sample and clinical data at the time of flare, as well as one to three samples at visits both before and after the flare event.
PR3, proteinase 3; MPO, myeloperoxidase.

Table 2. Clinical characteristics according to disease activity

Characteristics ($n = 320$ encounters in 88 patients)	Active renal ($n = 39$)	Active non-renal ($n = 48$)	Remission ($n = 233$)	P-value
C-reactive protein (mg/dL), median (IQR)	1.4 (1–1.8)	0.8 (0.7–0.9)	0.9 (0.8–1.2)	0.0028
Physician global assessment (0–10), mean (SD)	5.7 (1.9)	4.4 (1.3)	0.1 (0.7)	<0.0001
Current IS?, *n* (%)	28 (71.8)	36 (85.1)	169 (72.8)	ns
Renal parameters				
eGFR baseline[a] (mL/min), median (IQR)	54 (37.2–90.5)	101 (83.1–121)	79 (54.4–98.2)	<0.0001
Serum creatinine (mg/dL), median (IQR)	1.4 (1.0–1.8)	0.8 (0.7–0.9)	0.9 (0.8–1.2)	<0.0001
Creatinine level change (%), median (IQR)	6.5 (−1.2–13.3)	−2.8 (−13.3–7.5)	0 (−7.2–8.3)	ns
Dipstick haematuria, *n* (%)	26 (66)	12 (25)	65 (27)	<0.0001
Dipstick proteinuria, *n* (%)	29 (74.5)	6 (12.5)	79 (33.9)	<0.0001
RBC casts present, *n* (%)	33.3 (13)	2.1 (1)	7 (2.6)	<0.0001
New proteinuria, *n* (%)	14 (35.9)	2 (4.2)	23 (8.5)	<0.0001
New haematuria, *n* (%)	12 (30.8)	12 (25)	24 (8.9)	0.0011
Acute kidney injury (%)	10.3	4.2	3.4	0.0813

IS, immunosuppression; ns, not significant.
Acute kidney injury determined by Acute Kidney Injury Network criteria.
[a]Refers to the eGFR at the earliest time point.

Modelling of longitudinal usCD163 and uMCP-1 values against disease activity

The serial measurements of usCD163 and uMCP-1 are depicted in Figure 3. To validate the association between usCD163 and uMCP-1 levels and active renal vasculitis, while taking into account the intra-individual repeated measures in the study dataset, we generated and fitted a mixed effects model that incorporated both clinical covariates and accounted for the serial nature of the measurements. This demonstrated that the only parameters significantly associated with the presence of active renal flare were usCD163 and uMCP-1 (Table 4). This confirmed that the presence of active renal flare was a significant determining factor in observing elevated usCD163 and uMCP-1 levels (Table 4).

Combining usCD163 and uMCP-1 with existing biomarkers improves diagnostic fidelity

To determine whether combining the two biomarkers enhances diagnostic fidelity we generated a recursive partitioning tree that sought to maximize the distinction between 'active renal vasculitis' and 'no active renal vasculitis' by sequentially adding parameters that improved prediction. In addition to usCD163 and uMCP-1, we included new-/worse-onset proteinuria, new/worse haematuria, CRP and an increase in creatinine level in the model. This was an attempt to place the novel biomarkers within the context of existing clinical approaches for identifying renal flare. In all models tested, usCD163 was identified as the first node in the tree (Table 3). A subsequent testing strategy in usCD163-positive individuals that incorporated uMCP-1 and then new-onset proteinuria maximized diagnostic fidelity and increased specificity to 97.9% and the positive likelihood ratio (PLR) to 19.2. As these biomarker statistics are intimately dependent on pretest probability (Figure 4B) and to provide an estimate of real-world diagnostic accuracy, we estimated the post-test probability of confirming or excluding renal flare in three scenarios: (i) some degree of proteinuria or haematuria but nothing else to suggest active disease (pretest probability of active renal vasculitis 5%), (ii) clear extrarenal flare with some degree of proteinuria or haematuria (40%) and (iii) high clinical probability of renal flare (70%). In the latter two scenarios, the post-test probability of a positive result following

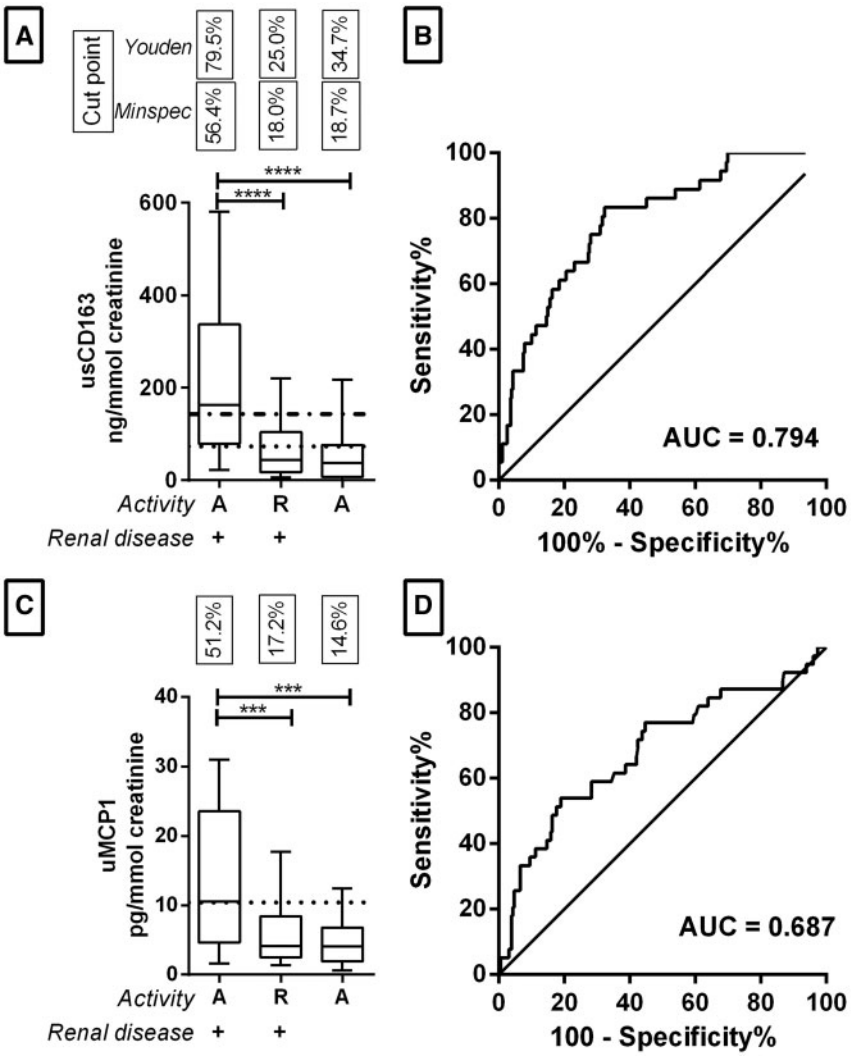

FIGURE 1: usCD163 and uMCP-1 levels across the cohort. **(A)** usCD163 levels in patients with active [A] renal vasculitis compared with those in remission [R] and those with active non-renal vasculitis. Upper line denotes the cut-off of 143 ng/mmol [optimizing sensitivity derived from the Rule Out decision tree (Figure 4)] and the lower line denotes the Youden index cut-off of 72.9 ng/mmol. The boxes in panels (A) and (C) indicate the fraction of positive samples in each group. **(B)** ROC curve of usCD163 comparing active renal vasculitis with remission and active non-renal vasculitis. **(C)** uMCP-1 levels in patients with active [A] renal vasculitis compared with those in remission [R] and those with active non-renal vasculitis. Upper line denotes the cut-off of 20 ng/mmol [optimizing sensitivity derived from the Rule Out decision tree (Figure 4)] and the lower line denotes the Youden index cut-off of 10 ng/mmol. **(D)** ROC curve of uMCP-1 comparing active renal vasculitis with remission and active non-renal vasculitis. ****$P < 0.0001$, ***$P < 0.001$.

the recursive tree algorithm was 93% and 98%, respectively (Table 5), potentially obviating the need for kidney biopsy in these settings.

DISCUSSION

It remains difficult to distinguish active vasculitis in the kidney from other causes of renal injury, such as infection, acute tubular necrosis, allergic interstitial nephritis and paraprotein-mediated kidney disease. We examined the ability of testing for usCD163 and uMCP-1 to identify subtle active renal vasculitis in a large multicentre cohort. We found that both biomarkers were elevated in the presence of active renal vasculitis, with usCD163 displaying a slightly larger area under the ROC than uMCP-1. The low degree of correlation between usCD163 and uMCP-1 highlights the fact that each reflects a different component of the glomerular macrophage recruitment and activation pathway. In this setting of subtle clinical evidence of active renal vasculitis, the moderate clinical utility of each biomarker in isolation was enhanced by using usCD163 to exclude active vasculitis and then grouping the '$usCD163^{+}$/$uMCP\text{-}1^{+}$' and 'usCD163/new proteinuria' as the two 'yes' nodes, giving a PLR of 19. This decision tree approach reflects more accurately the use of novel biomarkers in clinical practice.

We have previously identified usCD163 as a promising biomarker in glomerulonephritis [5] and it is similarly emerging as a potentially useful test in lupus nephritis [18]. In our prior study, we observed excellent biomarker characteristics, with a PLR for usCD163 alone of 20.7, an NLR of 0.17 and an area under the ROC curve of 0.93. However, most patients with active

Table 3. Biomarker characteristics

Biomarker	Cut-point method	Cut-point	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV	NPV (95% CI)	PLR (95% CI)	NLR (95% CI)	AUC
usCD163	Youden	72.9 ng/mmol	79.5 (63.5–90.7)	67.3 (61.4–72.7)	25.2	95.9 (92.7–97.8)	2.4 (1.9–3.1)	0.3 (0.2–0.6)	0.79
uMCP-1	Youden	10.0 pg/mmol	53.9 (37.2–69.9)	82.2 (77.2–86.5)	29.6	92.8 (90.1–94.8)	3.0 (2.1–4.4)	0.6 (0.4–0.8)	0.68
Decision Tree	Recursive partitioning	CD163 >143 ng/mmol[a] MCP-1 >20 pg/mmol[a] New/worse proteinuria	41.0	97.9	72.7	92.3	19.2	0.6	NA

In each case, the ability of the biomarker to correctly classify patients with active renal vasculitis was tested within a heterogeneous cohort including patients in remission and those with active extrarenal disease.
[a]The decision tree cut-points were determined by recursive partitioning.

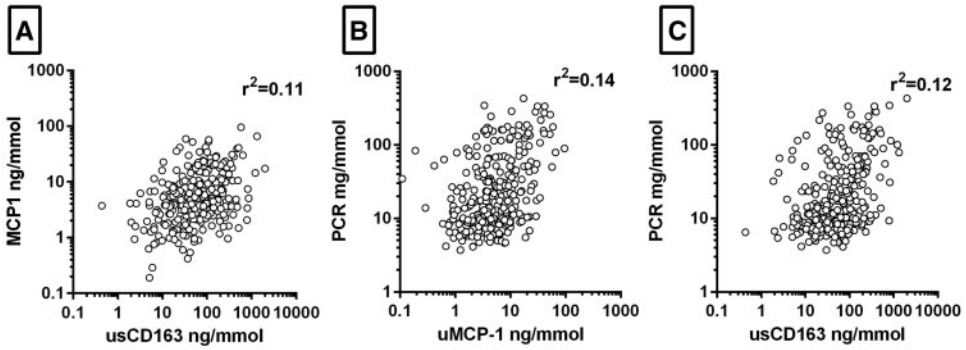

FIGURE 2: (**A**) Correlation between usCD163 and uMCP-1 (r^2=0.11). (**B**) Correlation between urine protein:creatinine and uMCP-1 ($r^2 = 0.14$). (**C**) Correlation between urine protein:creatinine and usCD163 ($r^2 = 0.12$).

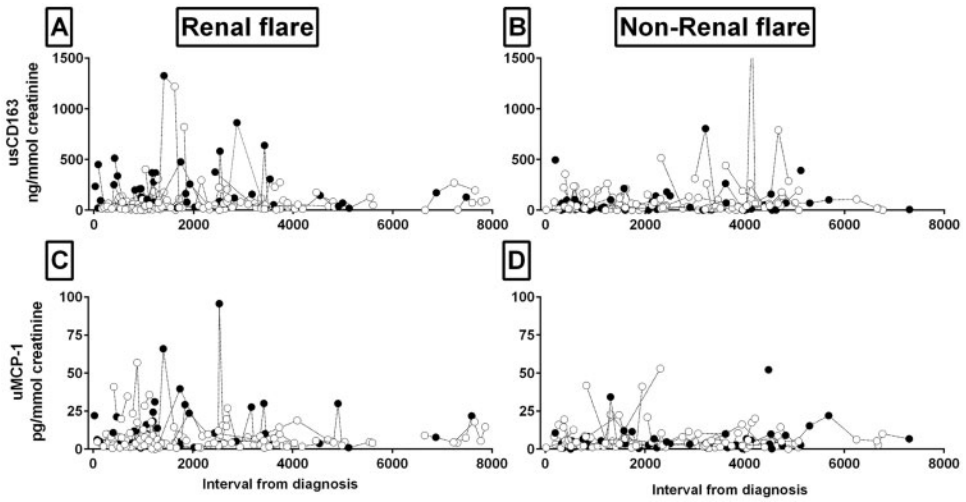

FIGURE 3: Levels of usCD163 in serial patient samples plotted over time from date of diagnosis (depicted as time 0). In each case, samples taken at the time of active vasculitis are marked in black. **(A)** usCD163; black circles depicting active renal vasculitis. **(B)** usCD163; black circles depicting active non-renal vasculitis. **(C)** uMCP-1; black circles depicting active renal vasculitis. **(D)** uMCP-1; black circles depicting active non-renal vasculitis.

disease in the prior study were recruited at the time of diagnosis, had severe disease (often mandating dialysis) and included patients with anti-glomerular basement antibody disease. Although this demonstrated the proof of concept, there is little clinical unmet need for diagnosis of active glomerulonephritis in this patient group, with conventional kidney biomarkers usually being adequate. Also, in the presence of such severe glomerular disease, most biomarkers of kidney inflammation are likely to perform well. A subset of the current sample set has been tested previously for uMCP-1 (among other biomarkers) by Lieberthal *et al.* [13]. Although a similar degree of elevation in uMCP-1 was observed in active renal disease, this study differed from the current one in identifying positive uMCP-1 results in active non-renal disease. A slightly different assay was used [pre-coated Quantikine DCP00, versus Duoset DY279 (R&D Systems)]in this study, which may have contributed to the

divergent results. Of note, Tam *et al.* [11] reported low uMCP-1 levels in patients with active non-renal disease using the same Duoset assay used in this study, although there were only six patients in that group.

To assess the true clinical utility of these novel biomarkers, we chose to test them in a more clinically challenging and relevant environment, seemingly mild (or early) renal flare. In this setting, PLR/NLR values of 2.4/0.3 and 3.0/0.6 for usCD163 and uMCP-1, respectively, suggest borderline clinical utility and reflect the fact that these patients may require kidney biopsy to make a definitive diagnosis. However, when unbiased recursive tree partitioning was applied to the novel biomarkers alongside conventional markers, usCD163 and uMCP-1 emerged as the first and second nodes, respectively, with new-onset proteinuria adding additional fidelity. The PLR and NLR of the novel algorithm were 19.2 and 0.6, respectively, indicating high potential clinical value. In the setting of intermediate or high pretest clinical probability, this algorithm provided an estimated post-test probability in excess of 90%. It is not uncommon currently for such patients to undergo kidney biopsy; our results suggest that in many cases it may not be necessary to perform a biopsy and to embark on appropriate treatment based on the algorithm results.

Although both usCD163 and uMCP-1 are linked to the recruitment of activated macrophages to the injured glomerulus, these markers reflect different stages of this process. sCD163 is actively shed from the surface of glomerular macrophages in the presence of pro-inflammatory peptides, particularly ADAM17/TACE [6]. The level of usCD163 is presumed to reflect the burden of activated macrophages in situ. In contrast, MCP-1 (CCL2) is a chemokine that specifically attracts blood monocytes and tissue macrophages to its source, via interaction with its cell surface receptor, CCR2. Renal cells produce MCP-1 in response to various pro-inflammatory stimuli. Indirectly, MCP-1 has the potential to drive renal fibrosis by macrophage recruitment and via direct induction of a fibrotic response in glomerular mesangial cells. Therefore it is conceivable that MCP-1 appears in the urine earlier in the process than sCD163, providing a rationale for testing for both peptides when clinical uncertainty remains after the use of traditional biomarkers. This premise was supported by our finding of a low degree of correlation between the two biomarkers in urine. The moderate correlation between the urine protein:creatinine ratio and the novel biomarkers suggests that in cases of heavy proteinuria

Table 4. Mixed effects modelling of novel biomarkers in longitudinal samples

	usCD163		uMCP-1	
	Coefficient value	P-value	Coefficient value	P-value
Renal flare	4.362	<0.0001	1.850	<0.0001
No renal flare	−0.881	0.002	−0.332	0.016
Gender (male)	0.105	0.747	−0.010	0.949
Age	−0.001	0.916	0.005	0.295
ANCA positive	−0.100	0.798	0.074	0.692
New proteinuria	0.085	0.777	0.141	0.328
New haematuria	0.131	0.630	−0.136	0.319

The coefficient value for renal flare gives the typical level of transformed biomarker for a female, ANCA-negative renal patient in a renal flare episode. This also represents the baseline level for the other explanatory variables, that is, it represents the typical level for females, ANCA-negative, no new proteinuria and no new haematuria. The remaining coefficient values represent differences from this baseline for a patient with the given characteristic. For example, a male patient in remission will typically have a transformed sCD163 level of 3.586 (4.362 − 0.881 + 0.105). The P-values confirm that renal flare episodes are the only significant characteristic in explaining the transformed biomarker values through mixed effects modelling.

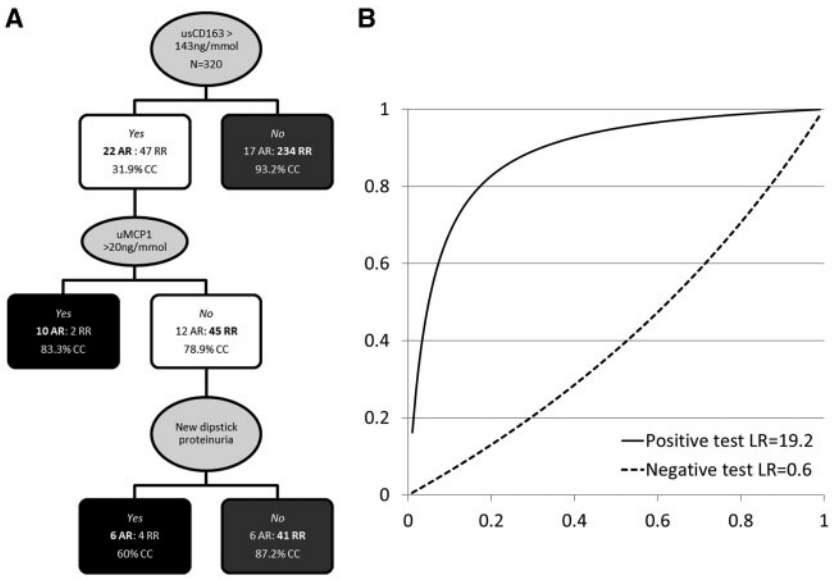

FIGURE 4: Recursive partitioning was applied to the dataset to identify variables that maximized correct classification of patients. **(A)** In the resulting decision tree variables are depicted in ovals. Black cells indicate tree termination with classification of the patient as having active renal disease ($n = 22$), whereas grey cells indicate tree termination with the patient classified as not having active renal disease ($n = 298$). CC, correctly classified; AR, active renal vasculitis; RR, remission renal vasculitis/active non-renal vasculitis. **(B)** Likelihood ratio plot depicting the change in post-test probability (y-axis) following application of the algorithm in a range of pretest probability scenarios (x-axis).

Table 5. Post-decision tree probability in a series of hypothetical scenarios

Pretest probability (odds)	Post-test probability of having renal flare if test positive	Post-test probability of not having renal flare if test negative
0.05 (0.052)	0.51	0.03
0.40 (0.67)	0.93	0.22
0.70 (2.33)	0.98	0.58

Using the calculated PLRs and NLRs, post-test probability was compiled for situations where the pretest probability varied between 5, 40 and 70%.

there may be leakage of the sCD163 or MCP-1 from serum into the urine, but that this is a minor consideration overall, with most of the measured protein coming from the inflamed nephron.

The principal limitation of our study is the lack of a gold standard kidney biopsy to diagnose active renal vasculitis. This is likely to have led to misclassification of some cases and may account for some of the high values observed in remission and active extrarenal disease. For example, in our prior published work examining usCD163, using a Youden cut-point, the test was positive in 3.7%, <1% and 1.8% of patients in remission, those with active extrarenal vasculitis and healthy controls, respectively. However, we observed a positive test in 25% and 34.7% of patients in remission and with active extrarenal disease using a similar cut-point method in the current cohort. Even using a cut-point that maximized specificity, much higher false-positive rates were observed in this study (18.0–18.7%). In some of these cases the patient was classified by the treating physician as having active extrarenal vasculitis, and satisfied BVAS/WG criteria for the same, but had new-onset haematuria by dipstick and/or slight elevation in creatinine level on the visits after treatment. One may infer that these cases may also have had subtle renal vasculitis. This limitation probably falsely reduced the biomarkers' performance. In addition, the use of ROC curve analysis has variable utility in datasets with repeated measures, but it does represent the best option for describing biomarker performance. An additional limitation is that we have not included a validation cohort for the classification algorithm, and it should be noted that the high likelihood ratio is derived from a relatively small number of cases ($n = 22$).

The presence of active BVAS/WG items informed the definition of patient groups with active renal and extrarenal vasculitis. These were recorded by the investigator physician only if they were considered, in their clinical judgement, to be due to active vasculitis. This clinical judgement was disregarded for the purpose of the decision tree, so changes in serum creatinine, proteinuria and haematuria were included at face value. When treated in this manner, usCD163 and uMCP-1 were selected in an unbiased fashion as the first and second nodes in the model and proteinuria was the only clinical marker that added utility, whereas haematuria, creatinine level and CRP did not. This analysis does not include the physician assessment of whether observed changes were due to active vasculitis; however, in clinical practice, such an assessment would be added on top of such a decision tree, as it would be added on top of the individual parameters.

In summary, an algorithm that combines usCD163 with uMCP-1 and new-onset proteinuria aids the diagnosis of subtle renal flare in AAV. The biomarkers of the future will be incorporated into machine learning algorithms that incorporate existing clinical parameters and additional variables, such as environmental changes. We have taken the first steps in the vasculitis field to realize this novel approach. The ability of this approach to reduce the need for kidney biopsy will need to be tested in a prospective clinical study.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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CONFLICT OF INTEREST STATEMENT

None declared. The results presented in this article have not been published previously in whole or part, except in abstract format.

(See related articles by Tam and Ong. Renal monocyte chemoattractant protein-1: an emerging universal biomarker and therapeutic target for kidney diseases? *Nephrol Dial Transplant* 2020; 35: 198–203; Wu *et al.* Associations of urinary epidermal growth factor and monocyte chemotactic protein-1 with kidney involvement in patients with diabetic kidney disease. *Nephrol Dial Transplant* 2020; 35: 291–297; and Wilkening *et al.* C–C chemokine receptor type 2 mediates glomerular injury and interstitial fibrosis in focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 2020; 35: 227–239)

REFERENCES

1. Duffield JS, Tipping PG, Kipari T *et al.* Conditional ablation of macrophages halts progression of crescentic glomerulonephritis. *Am J Pathol* 2005; 167: 1207–1219
2. Zhao L, David MZ, Hyjek E *et al.* M2 macrophage infiltrates in the early stages of ANCA-associated pauci-immune necrotizing GN. *Clin J Am Soc Nephrol* 2015; 10: 54–62
3. Rastaldi MP, Ferrario F, Crippa A *et al.* Glomerular monocyte-macrophage features in ANCA-positive renal vasculitis and cryoglobulinemic nephritis. *J Am Soc Nephrol* 2000; 11: 2036–2043
4. Little MA, Nightingale P, Verburgh CA *et al.* Early mortality in systemic vasculitis: relative contribution of adverse events and active vasculitis. *Ann Rheum Dis* 2010; 69: 1036–1043

5. O'Reilly VP, Wong L, Kennedy C *et al.* Urinary soluble CD163 in active renal vasculitis. *J Am Soc Nephrol* 2016; 27: 2906–2916
6. Moller HJ, Tesar V, Little MA. Urine sCD163: a window onto glomerular inflammation. *Nephrol Dial Transplant* 2016; 31: 1970–1972
7. Etzerodt A, Maniecki MB, Møller K *et al.* Tumor necrosis factor alpha-converting enzyme (TACE/ADAM17) mediates ectodomain shedding of the scavenger receptor CD163. *J Leukoc Biol* 2010; 88: 1201–1205
8. Moller HJ. Soluble CD163. *Scand J Clin Lab Invest* 2012; 72: 1–13
9. Endo N, Tsuboi N, Furuhashi K *et al.* Association of glomerular macrophage phenotypes and urine soluble Cd163 with disease activity in human lupus nephritis. *Nephrol Dial Transplant* 2014; 29: 435–436
10. Oomatia A, Moran SM, Kennedy C *et al.* Prolonged duration of renal recovery following ANCA-associated glomerulonephritis. *Am J Nephrol* 2016; 43: 112–119
11. Tam FW. Urinary monocyte chemoattractant protein-1 (MCP-1) is a marker of active renal vasculitis. *Nephrol Dial Transplant* 2004; 19: 2761–2768
12. Mukhtyar C, Lee R, Brown D *et al.* Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis* 2009; 68: 1827–1832
13. Lieberthal JG, Cuthbertson D, Carette S *et al.* Urinary biomarkers in relapsing antineutrophil cytoplasmic antibody-associated vasculitis. *J Rheumatol* 2013; 40: 674–683
14. Levey AS. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999; 130: 461–470
15. López-Ratón M, Rodríguez-Álvarez MX, Suárez CC *et al.* OptimalCutpoints: an R package for selecting optimal cutpoints in diagnostic tests. *J Stat Soft* 2014; 61: 1–36
16. Therneau TB, Atkinson B. Ripley *rpart: Recursive Partitioning and Regression Trees. R package version 4.1-11.* 2017; https://cran.r-project.org/package=rpart
17. Youden WJ. Index for rating diagnostic tests. *Cancer* 1950; 3: 32–35
18. Endo N, Tsuboi N, Furuhashi K *et al.* Urinary soluble CD163 level reflects glomerular inflammation in human lupus nephritis. *Nephrol Dial Transplant* 2016; 31: 2023–2033

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Associations of urinary epidermal growth factor and monocyte chemotactic protein-1 with kidney involvement in patients with diabetic kidney disease

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ABSTRACT

Background. In diabetic kidney disease (DKD), it is important to find biomarkers for predicting initiation and progression of the disease. Besides glomerular damage, kidney tubular injury and inflammation are also involved in the development of DKD. The current study investigated the associations of urinary epidermal growth factor (uEGF), monocyte chemotactic protein-1 (MCP-1) and the uEGF:MCP-1 ratio with kidney involvement in patients at early and advanced stages of DKD.

Methods. The concentration of uEGF and uMCP-1 was measured in two Chinese population-based studies. The associations of uEGF, uMCP-1 and uEGF/MCP-1 with occurrence of DKD were studied in a cross-sectional study ($n = 1811$) of early stage DKD. Associations of baseline uEGF, uMCP-1 and uEGF/MCP-1 with kidney outcome were assessed in a longitudinal cohort ($n = 208$) of advanced-stage DKD.

Results. In both studies, positive correlations were found between uEGF/urine creatinine (Cr) and estimated glomerular filtration rate (eGFR) at sampling and between uMCP-1/Cr and urinary albumin:creatinine ratio (uACR). In the cross-sectional study, uEGF/Cr and uEGF/MCP-1 were negatively associated with the occurrence of DKD {odds ratio (OR) 0.65 [95% confidence interval (CI) 0.54–0.79], $P < 0.001$; 0.82 (0.71–0.94), $P = 0.005$, respectively}. In the longitudinal cohort, the uEGF:MCP-1 ratio correlated more closely with the percentage change of eGFR slope ($r = 0.33$, $P < 0.001$) as compared with uEGF/Cr or uMCP-1/Cr alone. The composite endpoint was defined as end-stage renal disease or 30% reduction of eGFR. These three markers were independently associated with composite endpoint after adjusting for potential confounders [hazard ratio 0.76 (0.59–1.00), $P = 0.047$ for uEGF/Cr; 1.18 (1.02–1.38),