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The Clinical Application of Urine Soluble CD163 in ANCA-Associated Vasculitis

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Abstract: Background Up to 70% of patients with ANCA-associated vasculitis (AAV) develop glomerulonephritis, with 26% progressing to ESKD. Diagnostic-grade and noninvasive tools to detect active renal inflammation are needed. Urinary soluble CD163 (sCD163) is a promising biomarker of active renal vasculitis, but a diagnostic-grade assay, assessment of its utility in prospective diagnosis of renal vasculitis flares, and evaluation of its utility in proteinuric states are needed.

Methods We assessed a diagnostic-grade urinary sCD163 assay in (1) a real-world cohort of 405 patients with AAV and 121 healthy and 488 non-AAV disease controls; (2) a prospective multicenter study of 84 patients with potential renal vasculitis flare; (3) a longitudinal multicenter cohort of 65 patients with podocytopathy; and (4) a cohort of 29 patients with AAV (with or without proteinuria) and 10 controls.

Results We established a diagnostic reference range, with a cutoff of 250 ng/mmol for active renal vasculitis (area under the curve [AUC], 0.978). Using this cutoff, urinary sCD163 was elevated in renal vasculitis flare (AUC, 0.95) but remained low in flare mimics, such as nonvasculitic acute kidney injury. Urinary sCD163â€™s specificity declined in AAV patients with nephrotic-range proteinuria and in primary podocytopathy, with 62% of nephrotic patients displaying a â€œpositiveâ€ urinary sCD163. In AAV patients with significant proteinuria, urinary sCD163 normalization to total urine protein rather than creatinine provided the greatest clinical utility for diagnosing active renal vasculitis.

Conclusions Urinary sCD163 is elevated in renal vasculitis flare and remains low in flare mimics. Nonspecific protein leakage in nephrotic syndrome elevates urinary sCD163 in the absence of glomerular macrophage infiltration, resulting in false-positive results; this can be corrected with urine protein normalization.

Significance Statement

In ANCA-associated vasculitis (AAV), noninvasive biomarkers of active renal inflammation, such as urinary soluble CD163, are needed for early detection of active disease before irreversible end-organ damage occurs. Clinical translation requires a diagnostic-grade assay, prospective assessment of its diagnostic utility in AAV flare, and assessment of its utility in proteinuric states. The authors report use of an accredited diagnostic-grade assay for urinary sCD163, derivation of cutoff values, and application of the assay to a prospective cohort of patients with potential renal vasculitis flare. They found that urinary sCD163 displays high precision in separating RV flare from flare mimics. They also observed increased false-positive results in the setting of high-grade proteinuria, which they demonstrated can be effectively corrected by normalization to the urine protein value, thereby restoring diagnostic accuracy.

The Clinical Application of Urine Soluble CD163 in ANCA-Associated Vasculitis

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Abstract

Background Up to 70% of patients with ANCA-associated vasculitis (AAV) develop glomerulonephritis, with 26% progressing to ESKD. Diagnostic-grade and noninvasive tools to detect active renal inflammation are needed. Urinary soluble CD163 (sCD163) is a promising biomarker of active renal vasculitis, but a diagnostic-grade assay, assessment of its utility in prospective diagnosis of renal vasculitis flares, and evaluation of its utility in proteinuric states are needed.

Methods We assessed a diagnostic-grade urinary sCD163 assay in (1) a real-world cohort of 405 patients with AAV and 121 healthy and 488 non-AAV disease controls; (2) a prospective multicenter study of 84 patients with potential renal vasculitis flare; (3) a longitudinal multicenter cohort of 65 patients with podocytopathy; and (4) a cohort of 29 patients with AAV (with or without proteinuria) and 10 controls.

Results We established a diagnostic reference range, with a cutoff of 250 ng/mmol for active renal vasculitis (area under the curve [AUC], 0.978). Using this cutoff, urinary sCD163 was elevated in renal vasculitis flare (AUC, 0.95) but remained low in flare mimics, such as nonvasculitic acute kidney injury. Urinary sCD163's specificity declined in AAV patients with nephrotic-range proteinuria and in primary podocytopathy, with 62% of nephrotic patients displaying a "positive" urinary sCD163. In AAV patients with significant proteinuria, urinary sCD163 normalization to total urine protein rather than creatinine provided the greatest clinical utility for diagnosing active renal vasculitis.

Conclusions Urinary sCD163 is elevated in renal vasculitis flare and remains low in flare mimics. Nonspecific protein leakage in nephrotic syndrome elevates urinary sCD163 in the absence of glomerular macrophage infiltration, resulting in false-positive results; this can be corrected with urine protein normalization.

Background

ANCA-associated vasculitis (AAV) is a chronic disease with periods of remission and relapse. Kidney involvement is characterised by focal necrotising glomerulonephritis[1]. There is a need for non-invasive methods of detection of active renal inflammation to prevent irreversible end organ damage including end stage kidney disease. 40% of patients with AAV experience a relapse within the first five years after initial diagnosis[2] and 26% develop ESKD[3]. It is estimated that 43% patients with renal AAV who progress to ESKD do so without clinically detected renal vasculitis activity[4].

The gold standard for diagnosis of kidney involvement in AAV is biopsy. However, a kidney biopsy is an invasive procedure and sequential biopsies are not feasible in routine clinical practice[5]. Non-invasive clinical tools for detection of active renal inflammation include serum creatinine, hematuria, proteinuria and red blood cell casts. However, these biomarkers lack sensitivity for detection of early renal structural and functional loss, and do not reliably differentiate active vasculitis from flare mimics[6,7].

Urine is an ideal biospecimen as it is readily available and biomarkers in urine may reflect local kidney injury. Retrospective studies raise the possibility that urine biomarkers may obviate the need for kidney biopsy[8,9]. CD163 functions as a monocyte/macrophage-specific scavenger receptor for haemoglobin–haptoglobin complexes, which is cleaved from the surface of activated macrophages[13]. Soluble CD163 is present at relatively high concentration in serum, playing a role in innate defense by reversibly binding bacteria and free hemoglobin[10,11]. sCD163 appears in the urine when activated glomerular macrophages are present. sCD163 possesses many ideal biomarker properties with low levels in health, stability at room temperature for up to 7 days, and ease of measurement by enzyme linked immunosorbent assay (ELISA)[12]. Prior work in retrospective cohorts has shown elevated concentrations of usCD163 in active renal vasculitis at diagnosis, in subtle renal vasculitis flare and in lupus nephritis[12-16].

In order to further develop usCD163 as a clinical biomarker, a diagnostic grade assay is required. A clinical ELISA platform was developed by Euroimmun GMBH. In this study, we validate its use in real-world samples to facilitate clinical accreditation. Few biomarkers of kidney disease have made this transition to clinical practice with the recent exception of serum anti-phospholipase A2 antibody in membranous glomerulonephritis (MN)[17].

A clinical concern remains that usCD163 quantification may be a sophisticated measure of proteinuria, reflecting glomerular leak of the protein through the damaged glomerular filtration barrier. The optimal setting to assess the contribution of serum protein leakage is nephrotic syndrome. Primary podocytopathies, such as minimal change disease and focal segmental glomerulosclerosis, characteristically lack inflammatory infiltrates (including macrophages) on light microscopy so local sCD163 production should be minimal[18]. Leakage of serum sCD163 across the glomerular filtration barrier could occur in this setting due to extensive foot process effacement[151519]. Protein-normalised usCD163 has been reported in lupus nephritis[15,16]. Thus, to fully define the clinical role for usCD163 use as a biomarker of renal vasculitis, in whom glomerular scarring without active inflammation may cause non-specific glomerular protein leak,

we conducted a prospective study to assess its utility using a clinical grade analytic platform in renal vasculitis flare and assessed mechanisms for accounting for elevated levels of proteinuria.

Methods

Patient identification and recruitment

Cohort 1: Real world assessment of a diagnostic grade assay. To support the use of a clinically validated diagnostic grade ELISA we prospectively used a novel clinical grade sCD163 assay in a cohort of unselected patients attending the Vasculitis Ireland Network (VINE) service (https://www.tcd.ie/medicine/thkc/vasculitis/), including patients with chronic kidney disease with varying levels of proteinuria[19]. Clinical status was adjudicated following chart review blinded to usCD163 concentration. To establish a reference range, healthy volunteers were recruited at Galway University Hospitals/National University of Ireland Galway as previously described[20,21]. Between March 2016 and December 2018 a cross-sectional study was carried out. Participants were identified through poster advertisement/word of mouth. Each participant completed a comprehensive health questionnaire and underwent a battery of investigations to ensure that participants did not have any medical conditions/recent illnesses that could impact on study validity.

Cohort 2: Prospective diagnosis of renal vasculitis flare. To prospectively evaluate the utility of usCD163 in the diagnosis of renal vasculitis (RV) flare, we enrolled patients previously recruited to the Irish Rare Kidney Disease biobank (www.tcd.ie/medicine/thkc/research/rare.php) who presented to one of seven VINE centres with a clinical suspicion of RV flare. All enrolled patients met Chapel Hill Consensus Conference classification criteria for small vessel vasculitis[22]. All patients provided informed consent and the study was approved by the local research ethics committee in each hospital.

Cohort 3: Effect of nephrotic syndrome on usCD163 excretion. To investigate the impact of proteinuria on usCD163 concentration in the absence of glomerular macrophage infiltration, we leveraged a cohort of patients with primary podocytopathy (minimal change disease (MCD) and focal segmental glomerular sclerosis (FSGS)) and membranous nephropathy (MN). These were obtained from the NEPTUNE programme, a multi-centre international longitudinal study of primary nephrotic syndrome[23]. Recruits provided paired urine samples from active (urine protein >3.5g/day) and remission (urine protein <0.5g/day) nephrotic syndrome.

Cohort 4: Assessment of methods to correct usCD163 value in the setting of heavy proteinuria. Patients with ANCA vasculitis and healthy controls were identified from the Irish Rare Kidney Disease biobank. Inclusion criteria: (a) Active renal vasculitis (haematuria and proteinuria on urinalysis), (b) Remission vasculitis with persistent proteinuria (proteinuria >2+ on urinalysis), (c) Remission vasculitis without proteinuria (proteinuria 0 on urinalysis) and (d) Healthy controls. All recruits provided synchronous paired urine and serum samples.

Sample Processing

Real world VINE (cohort 1) and Rare Kidney Disease biobank (cohorts 2 and 4) urine and serum samples were centrifuged at 2000g for 10 minutes at 4°C, with storage of the supernatant at –80°C until assay. Healthy controls (cohort 1) serum samples were allowed to clot for 1 hour, then centrifuged at 800g for 15 min, aliquoted and stored at −80 °C. Urine was centrifuged at 2,000g for 10 min at 4°C, aliquoted and stored at -80°C. NEPTUNE (cohort 3) samples were collected according to pre-determined protocolized procedures. Urine was spun at 1000g for 12 minutes and serum samples were allowed to clot for 30 minutes, then centrifuged at 2000g for 12 minutes. Both sample types were then frozen at -80^{o}C until shipping on dry ice.

sCD163 measurement, establishment of reference range and normalisation techniques

Two sCD163 assays were used in this study: the assay used in prior work[14] and a newly developed diagnostic grade assay. In all cohorts usCD163 was initially measured by the capture ELISA used in our prior experimental work [12,13,15,24] (R&D Systems, human sCD163 DuoSet, DY1607 ELISA). This assay was developed for use in serum and has manufacturing steps in its protocol. To facilitate clinical translation of usCD163 we partnered with Euroimmun GMBH to develop a pre-coated diagnostic grade sandwich sCD163 ELISA specifically optimised for measurement of usCD163. Batch numbers E190708BA, E181018CD and E180424BD were used. These assays were performed centrally in a diagnostic laboratory (Clinical Immunology, St. James's Hospital). As per prior studies of usCD163 we normalized the urine sCD163 level to the creatinine level as determined by a modified Jaffe technique. Urine creatinine and protein were measured by Roche Cobas Creatinine plus (05 6612 7) and Total Protein (11877801) modules, respectively. To assess inter-assay performance, we measured usCD163 measured using both ELISA methods in cohorts 1 and 2. The upper limit of normal in the Euroimmun assay was defined as the 97.5th centile of healthy control values.

Various techniques were used to incorporate data regarding non-specific urine protein leak:

- Normalisation of usCD163 concentration to total urine protein (pg/mg).
- Fractional excretion of sCD163:
  $urine\ creatinine\left(\frac{\mu mol}{L}\right) \text{X}\ serum\ CD163\ (\frac{ng}{mL}) / urine\ sCD163\left(\frac{ng}{mL}\right) \text{X}\ serum\ creatinine\left(\frac{\mu mol}{L}\right)$
- Albumin:CD163 ratio of serum to urine:
  $urine\ sCD163\left(\frac{ng}{L}\right) \text{X}\ serum\ albumin\left(\frac{g}{L}\right) / urine\ albumin\left(\frac{g}{L}\right) \text{X}\ serum\ sCD163\ (\frac{ng}{L})$

Clinical assessment

Cohort 1: Real world assessment of a diagnostic grade assay. Vasculitis disease activity was recorded using the Birmingham Vasculitis Activity Score (BVAS). Active renal vasculitis was defined as BVAS>0 with one or more renal items, including the presence of haematuria by urine dipstick; urine samples were not routinely assessed for the presence of casts or dysmorphic red cells. The presence or absence of active vasculitis was adjudicated without knowledge of the sCD163 concentration.

Cohort 2: **Prospective diagnosis of renal vasculitis flare.** At the time of potential RV flare detailed demographic and phenotypic information was collected. A challenge we faced in our study design was in accurately determining a diagnosis of RV flare. There are no consensus criteria for this diagnosis and, in clinical practice, a combination of serum creatinine, urine sediment analysis for hematuria, proteinuria and RBC casts are used. This pragmatic approach has also been taken in clinical trials with BVAS/WG major renal criteria (which do not require biopsy) used as inclusion criteria[25,26]. In this prospective observational study kidney biopsy was not mandated. Biopsy interpretation is limited by the focal nature of histologic findings, with false negative results in 1-3% of biopsies and reported sensitivity of 91%[27,28]. Final diagnostic category (RV flare or no RV flare) was adjudicated by committee six weeks after the encounter, which was blinded to usCD163 results. This committee reviewed renal major and minor BVAS criteria, trends in serum creatinine, urinary protein, red blood cell casts (if available), subsequent clinical management (response to immunosuppression), and renal biopsy (if available). The absence of tissue confirmation of crescentic glomerulonephritis is both a limitation of this study and a strength as it reflects clinical and research practice definitions of active RV. Physicians were asked, at the time of clinical review, to classify the likelihood of vasculitis flare as "*possible*" or "*highly probable*." Clinical information was collected in real time as well as retrospectively from the clinical encounter prior to the potential flare visit and from subsequent clinical encounters. Vasculitis disease activity was recorded using the BVAS.

Cohort 3: **Effect of nephrotic syndrome on usCD163 excretion.** Patients were considered actively nephrotic if their urine protein was >3.5g/day and in remission if their urine protein was <0.5g/day.

Cohort 4: **Assessment of methods to correct usCD163 value in the setting of high-grade proteinuria.** Vasculitis activity was recorded as per Cohort 1.

Statistical Methodology

Clinical, laboratory data and ELISA results were analysed using GraphPad Prism version 9 and R. Biomarker values were non-normally distributed and are thus reported as median and interquartile ranges. Kruskal Wallis, Mann Whitney U tests, chi-squared, unpaired t-tests were used to determine the significance of associations for non-normally and normally distributed data. Correlations were measured using Spearman correlation coefficient. OptimalCutpoints R package was used to determine diagnostic cut off ranges and generate receiver-operator characteristic (ROC) curves[29,30]. Two methods were used to determine the most clinically relevant diagnostic cut-offs: the Youden calculation was selected to maximize the sum of sensitivity and specificity and yield the most clinically relevant cut-off values[31,32]. Net reclassification index was calculated as per Pencina et al[33]. We developed an interactive web app using Shiny (Version 1.6.0) in R (https://thkc.shinyapps.io/usCD163/)[34].

Results

Baseline Characteristics

We first sought to develop and validate an accredited sCD163 kit that is validated for testing in urine and could be deployed in clinical practice. The characteristics of cohort 1 comprised

healthy controls (n=121) and sequential clinic patients with systemic vasculitis (n=274 in remission, n=131 with active RV) and disease controls without ANCA vasculitis (n=488), as described in supplemental table 1. Cohort 2 comprised patients enrolled prospectively in our study evaluating RV flare: 121 encounters were screened for inclusion and 37 were excluded, leaving 84 cases available for analysis (**Supplemental Figure 1**). Baseline demographics and clinical characteristics are summarised in **Table 1**, and clinical characteristics at the time of potential RV flare are outlined in supplemental **Table 2**. Those who were subsequently adjudicated as having a RV flare had higher absolute and delta serum creatinine, and higher levels of proteinuria and hematuria on dipstick. Physician impression of high flare probability was more frequent in those with RV flare and these recruits were more also likely to undergo renal biopsy. Cohort 3 (NEPTUNE, primary nephrotic syndrome) included 65 patients (MCD n=20, FSGS n=23, and MN n=22 for comparison, supplemental table 4). Cohort 4 (ANCA vasculitis & proteinuria) included 39 patients: ten with active renal vasculitis, ten in remission with residual proteinuria, nine in remission with no proteinuria, and ten healthy controls (**Supplemental Table 5**).

Optimisation of clinical grade sCD163 ELISA

In order to use usCD163 as a clinical biomarker, an accredited, validated ELISA platform is required. In collaboration with Euroimmun GMBH, who have generated such a platform, we validated its use in real-world samples to facilitate clinical accreditation (cohort 1). The upper limit of normal, defined as the 97.5th centile in healthy controls, was 1.32ng/mL or 254.9ng/mmol (normalised to urine creatinine, **Figure 1A**). In sequential patients with systemic vasculitis, the 97.5th centile in patients in remission was 3.2ng/mL or 520.3ng/mmol. The 2.5th centile in patients with active renal vasculitis was 0.75ng/mL or 194.9ng/mmol. In 22 (18%) of healthy control samples, the value obtained was below the lower limit of detection. Median (IQR) non-normalised usCD163 values were 0.29ng/mL (0.2 to 0.4), 0.64ng/mL (0.4 to 1.1) and 5.0ng/mL (2.6 to 9.4) in healthy control, remission vasculitis and active RV, respectively. The corresponding respective values normalised to urine creatinine were 33.7ng/mmol (19.2 to 69.4), 97.8ng/mmol (51.0 to 147.6) and 938.9ng/mmol (491.8 to 1469). The values for the disease control samples are summarised in **Supplemental Figure 2**. Based on these values we propose an upper limit of normal cut-off for use in clinical practice of 250ng/mmol (non-normalised 1.3 ng/mL), which we applied in the prospective study. When distinguishing active renal vasculitis from remission vasculitis in this real-world cohort, the AUC of the receiver-operator characteristic (ROC) curve of non-normalised usCD163 was 0.945 (**Figure 1B**) and the AUC of normalised usCD163 was 0.978 (**Figure 1C**). usCD163 values measured in a subgroup of these cases (n=97) using both the Euroimmun and R&D Duoset assay methods were highly correlated (r=0.87, **Figure 1D**).

usCD163 is elevated in renal vasculitis flare

We then applied this diagnostic grade assay to a prospective cohort of patients with a clinical suspicion of renal flare of ANCA vasculitis (cohort 2). Of the 84 patients assessed, 31 (36.9%) were adjudicated (blind to usCD163 values) as suffering from a RV flare (**Table 2**). The usCD163 values in the 19 patients undergoing kidney biopsy are summarised in **Supplemental Figure 3**. In those adjudicated as having a RV flare the median usCD163 concentration was

805.8ng/mmol creatinine (IQR 439 to 1705) and in those without RV flare the median usCD163 concentration was 100.0ng/mmol creatinine (IQR 52-174, p<0.0001, Fig 2A). The area under the curve for detection of active renal vasculitis in this prospective study was 0.95, p<0.0001, **Figure 2B**. The optimal diagnostic cut-off was 253ng/mmol, virtually identical to our previously defined optimal cut-off (250ng/mmol). Biomarker characteristics in this clinical setting are summarised in **Table 3**.

There was no difference in usCD163 concentrations between diagnostic categories in those adjudicated as non-RV flare, as summarised in **Table 2** and **Figure 2C**. The treating physician thought that RV flare was "*possible*" in 45 (53.6%, median usCD163 95.8ng/mmol, IQR 53.4 to 173.9) and "*highly probable*" in 39 (46.4%, median usCD163 524.9ng/mmol, IQR 271.7 to 1134, **Figure 2D**). Of the "*possible*" renal flares, 42 (93.3%) were adjudicated as not having a RV flare; the negative predictive value for usCD163 in this setting was 95.2%. Of the "*highly probable*" renal flares, 11 (28.2%) were adjudicated as not having a renal flare; the positive predictive value of usCD163 in this setting was 84.4%. usCD163 concentration at diagnosis did not predict subsequent development of ESKD (supplemental **Figure 3B-D**).

Effect of proteinuria on usCD163 false positive rate

Urine sCD163 concentration was strongly correlated with urine protein:creatinine ratio (PCR) in this prospective cohort (**Figure 3A**). We hypothesised that urine sCD163 concentration reflects glomerular protein leak from the serum. If this hypothesis is true, the fraction of false positive results would rise as total urine protein rises. To test this, we first quantified the fraction of false positives with increasing urine protein excretion in the prospective RV flare cohort (cohort 2). We found that, in the setting of clinical suspicion for RV flare, the fraction of false positives does not rise with increased proteinuria (**Figure 3B**). Increasing proteinuria in this restricted setting was almost invariably due to active RV. We then analysed the fraction of false positives in cohort 1 (a large mixed unselected real-world cohort of patients with vasculitis). In this setting, which more accurately reflects widespread use of the test in clinical practice, the fraction of false positive results did increase with increasing proteinuria (**Figure 3C**), suggesting that non-specific protein excretion in nephrotic syndrome elevates usCD163 in the absence of active glomerular macrophage infiltration. To assess the effect of nephrotic range proteinuria on usCD163 concentration, in the absence of glomerular macrophage infiltration or CD163 mRNA transcription (**Supplemental Figure 4A**), we next tested paired urine samples from patients with biopsy proven primary podocytopathy using samples from both nephrotic and remission periods. Median usCD163 concentration (**Figure 3D**) in nephrotic syndrome was 654.9ng/mmol creatinine (IQR 305-1505) and in remission was 9.1ng/mmol (IQR 1-47). Similar values were obtained in membranous nephropathy (**Supplemental Figure 4B**). In active nephrotic syndrome, 56 (86.1%) of cases were positive for usCD163, which most likely reflected glomerular leakage of the protein rather than its intra-glomerular production. Supporting this hypothesis, urine protein concentration was correlated with usCD163 in active nephrotic syndrome (but not in remission (**Figure 3E**)), and in patients with chronic kidney disease, where an inflection point at a urine albumin level of 50mg/L was noted (supplemental figure 4C).

Clinical application of usCD163 measurement in the setting of proteinuria

To systematically assess methods of proteinuria correction in ANCA vasculitis, we first estimated the fractional excretion of sCD163 (FE-CD163) in a group of patients who were in remission with either minimal or heavy proteinuria (cohort 4, **Figure 4A**). The FE-CD163 value in patients in remission without proteinuria (2.0, IQR 0.7 to 63) was comparable to the value in healthy controls (2.6, IQR 0.3 to 12.5) and >2-log lower than patients in remission with heavy proteinuria (345.7, IQR 33 to 658, p<0.005), confirming glomerular leak of sCD163 in these patients. The log2 FE-CD163 was correlated with log2 uPCR (r=0.58, p=0.0001, **Figure 4A**). We then compared the utility of normalisation of usCD163 to total urine protein or urine albumin, and the albumin:sCD163 ratio of serum to urine (which provides as estimate of local glomerular production of sCD163), to the standard approach of normalising to urine creatinine (**Figure 4B**). To distinguish patients in remission, but with persistent heavy proteinuria, from patients with active RV, the three approaches were similar, with simple normalisation to total urine protein (**Figure 4D**) slightly outperforming the more complex albumin:sCD163 ratio method (**Figure 4E**) and albumin normalisation (**Figure 4F**). This suggests that, in patients with heavy proteinuria, normalisation of the usCD163 value to total urine protein is the method of greatest clinical utility for non-invasive identification of active renal vasculitis. The optimal usCD163:protein cut-off ratio in these patients was 2.5ng/mg. Applying this cut-off value to patients with nephrotic syndrome from cohort 3 led to all patients being re-classified as usCD163 negative (supplemental **Figure 4**C) and, when applied to real-world AAV patients with >0.5g/L total protein from Cohort 1, sensitivity was 90% and specificity 85.7% (**Figure 4G**).

Discussion

One of the greatest challenges in caring for patients with AAV is the early detection of active disease prior to accrual of irreversible end organ damage. usCD163 is a promising biomarker of crescentic glomerulonephritis with elevated concentrations in active renal vasculitis and lupus nephritis[12-14,16]. This is the first study of usCD163 using a diagnostic grade assay which allows evidence-based translation into clinical practice, the establishment of a real-world reference range as well as information on the potential caveats of interpretation in the setting of high-grade proteinuria. We prospectively used usCD163 to distinguish active renal vasculitis flare from flare mimics in a clinical context, where it performed with sufficient accuracy to potentially reduce the need for kidney biopsy in this setting. We noted in our real word data analysis that false positive events occurred when the patient had heavy proteinuria due to leakage of the sCD163 protein across the injured glomerular basement membrane. Systematic comparison of several normalisation strategies identified simple normalisation to total urine protein as the technique of greatest utility when nephrotic range proteinuria is present.

The diagnostic grade sCD163 assay was validated in 487 AAV patient samples and 121 healthy controls and compared to the previously used research grade assay. These two assay results were highly correlated. The reported usCD163 concentrations with the diagnostic grade assay in active renal vasculitis were similar to our prior work studying the time of diagnosis and subtle flare[12,14]. Derived cut-off values were similar to prior published work with the current proposed cut-off of >250ng/mmol comparing to >300ng/mmol[12] and >143ng/mmol[14] (when used in

combination with uMCP-1). The validation of this clinical grade ELISA allows clinical translation of usCD163 from a research test to a clinical diagnostic tool.

We demonstrated in a multicentre prospective study that usCD163 levels are elevated in RV flare, remain low in clinically relevant flare mimics, and are superior to currently used non-invasive clinical tools. usCD163 had favourable biomarker characteristics for the detection of RV flare, the proposed cut-off value of >250ng/mmol being associated with sensitivity, specificity and AUC of 96.8%, 86.8%, 0.95, respectively. Importantly, these biomarker metrics differ from previous published work in this field by taking as the comparator (against which to test the ability to identify renal vasculitis flare) an unselected cohort of patients with vasculitis who *displayed a variety of flare mimics*, as opposed to a cohort of healthy controls or patients in long term remission. This directly reflects the proposed use of the test in clinical practice alongside traditional biomarkers. We identified two clinical scenarios that further refine use of the test. Firstly, about half of the study cohort were recruited because the physician felt there was a high chance that the patient was suffering from a RV flare. In this context, usCD163 displayed high positive predictive value (84.4%), suggesting that the test was useful for confirming the physician's assessment. Secondly, in the other half of the cohort, the physician felt there was a "possible" renal flare (with a lower pre-test probability), in which context the negative predictive value was 95.2%, suggesting that the test may be useful to "rule out" in this clinical setting. In both scenarios, measurement of usCD163 has the potential to reduce the need for kidney biopsy.

We sought to explore the effect of high-grade proteinuria on potential leakage of serum sCD163 across the glomerular filtration barrier leading to detection of non-locally produced sCD163 in urine. In healthy glomeruli, usCD163 does not cross the glomerular filtration barrier due to its high molecular weight[35]. To explore this potential caveat, we studied the fraction of false positives in prospectively recruited patients with potential renal vasculitis flares (cohort 2). The fraction of false positives did not rise when stratified by proteinuria in this cohort. However, increasing proteinuria in this restricted setting was almost invariably due to active RV. We then analysed the fraction of false positives in an unselected longitudinal cohort of patients with vasculitis (cohort 1). In this setting, which reflects the use of the test in routine clinical practice, the fraction of false positive results did increase with increasing proteinuria (PCR >300mg/mmol, Figure 3C), suggesting that non-specific protein excretion increases usCD163 concentration even in the absence of glomerular macrophage infiltration. To explore this hypothesis further the clinical scenario of nephrotic syndrome was selected due to glomerular filtration barrier disruption from foot process effacement with an absence of glomerular macrophage infiltration. usCD163 concentrations were elevated in active nephrotic syndrome with median values of 655ng/mmol, which is comparable to median levels of 939ng/mmol in our active vasculitis population and exceeds the diagnostic threshold for active vasculitis of >250ng/mmol. In paired samples from the same patients in remission we found that usCD163 levels returned to the normal range.

We then explored different methods of correcting for a potential leak of sCD163 across the glomerular basement membrane. The fractional excretion (FE)-CD163 in patients in remission

without proteinuria and healthy controls were comparable but were >2-log higher than in patients in remission with heavy proteinuria, confirming glomerular leak of sCD163 in this setting. Correction for urinary protein and albumin (instead of creatinine normalisation) led to attenuation of the increased signal seen in active nephrotic syndrome. We also assessed the effect of fractional excretion relative to paired urine and serum albumin, mirroring techniques used to assess renal fractional excretion of sodium, as well as quantification of cerebrospinal fluid glucose and protein[36,37]. Normalising usCD163 to total urine protein, albumin, and sCD163 ratio of serum to urine albumin values yielded broadly similar results. However, simple normalisation to total urine protein (AUC 0.91) was marginally better than both albumin normalisation (AUC 0.89) and the more complex albumin:sCD163 ratio equation (0.88). Therefore, we propose that, in high grade proteinuria, normalisation of the usCD163 value to total urine protein is the method of greatest clinical utility for non-invasive identification of active renal vasculitis, with a potential cut-off of 2.5ng/mg. We propose applying this technique only in those with total urine protein value >0.5g/L as normalisation against lower protein values causes artificial inflation of the result. Use of these values will need to be validated in larger cohorts. To aid in the translation of our findings to clinical practice, we have developed an online calculator (https://thkc.shinyapps.io/usCD163/) that provides clinical context to urine creatinine and protein normalised usCD163 concentrations in AAV.

In conclusion, this study has further defined use of usCD163 as a diagnostic tool for detection of renal vasculitis flare using a diagnostic grade assay, thereby allowing translation of usCD163 from a research assay at the bench to a clinical grade test to be used at the bedside. In the setting of high-grade proteinuria, normalisation of sCD163 to urine protein rather than creatinine improves diagnostic accuracy. Future research directions include assessment of the utility of serial usCD163 measurement to aid in early identification of sub-clinical flare, assessment of usCD163 as an early marker of response to, or resistance to, immunotherapy, and in the identification of treatment futility in dialysis-dependent RV. Beyond AAV, the role of usCD163 in lupus has been described but its utility in other glomerular diseases with crescentic glomerulonephritis requires further investigation. Broader use of usCD163 as a screening test to identify AKI of glomerular origin (perhaps using a point-of-care test) and prompt nephrology referral has the potential to lead to earlier identification of treatment-responsive glomerular diseases.

Author Contributions

SM and ML devised and conducted studies. SM, JD, EG, KML conducted usCD163 assays. JD, EG and KML optimised the Euroimmun assay. MK and members of the NEPTUNE consortium enrolled patients with nephrotic syndrome. JS, ML, NC, SM were members of the adjudication committee. TG, MG recruited healthy and disease controls. SM, JS, MRC, NC, JH, POH, CJ, MG, ML recruited study candidates across all sites. CJ developed the online calculator.

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Disclosures

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Tables.

BASELINE CHARACTERISTICS (N=84)	**RENAL FLARE (N=31)**	**NO RENAL FLARE (N=53)**	**P-VALUE**
GENDER %, (N)	Female 48.4% (15)	Female 35.8% (19)	0.2586
AGE MEAN (SD)	63.1 yrs. (SD 16.2)	60.3yrs (SD 13.5)	0.3926
DIAGNOSIS			0.9594
MPA, %, (N)	54.8% (17)	54.7% (29)	-
GPA, %, (N)	38.7% (12)	35.8% (19)	-
EGPA, %, (N)	3.2% (1)	3.8% (2)	-
AAV/AGBM, %, (N)	3.2% (1)	5.7% (3)	-
DISEASE DURATION MEDIAN (IQR)	4.0 years (IQR 1.7-7.8)	4.1 years (IQR 1.7-6.5)	0.8124
PRIOR RENAL INVOLVEMENT %, (N)	87.1% (27)	83.0% (44)	0.6180
BASELINE EGFR MEAN (SD)	48.2mls/min (SD 27.5mls/min)	54.1mls/min (SD 19.7mls/min)	0.4384
BASELINE HAEMATURIA MEDIAN (IQR)	2+ (IQR 0-3+)	1+ (0-2+)	0.0516
BASELINE PROTEINURIA MEDIAN (IQR)	1+ (IQR 0-3+)	0+ (IQR 0-2+)	0.0870
BASELINE PROTEIN: CREATININE RATIO MEDIAN (IQR)	96mg/mmol (IQR 36-329mg/mmol)	34mg/mmol (IQR 17-55mg/mmol)	0.0088
CURRENT IMMUNOSUPPRESSION			0.3269
NONE	54.8% (17)	41.5% (22)	-
AZATHIOPRINE	16.1% (5)	33.9% (18)	-
MMF	6.4% (2)	13.2% (7)	-
MTX	3.2% (1)	3.7% (2)	-
RITUXIMAB	3.2% (1)	3.7% (2)	-
OTHER	9.6% (3)	9.4% (5)	-
NO DATA AVAILABLE	12.9% (4)	0% (0)	
CURRENT CORTICOSTEROIDS (N=83)	45.2% (14)	43.4% (23)	0.8174

Table 1: Cohort 2. Baseline characteristic derived from clinical parameters from last review prior to study visit (flare or flare mimic). MPA= microscopic polyangiitis, GPA = granulomatosis with polyangiitis, EGPA= eosinophilic granulomatosis with polyangiitis, AAV/aGBM denotes overlap syndrome of dual positive ANCA and anti-GBM antibodies, SD= standard deviation, IQR = interquartile range.

FINAL ADJUDICATED DIAGNOSIS	USCD163 (NG/MMOL, MEDIAN, IQR)	N (%)
RENAL FLARE	805.8 (439.1 -1705)	31 (36.9)
SYSTEMIC FLARE	88.2 (71.6 – 197.8)	13 (15.5)
ACUTE KIDNEY INJURY*	105.4 (48.4 – 191.0)	7 (8.3)
SEPSIS	141.0 (75.5 – 232.7)	11 (13.1)
CKD PROGRESSION	103.8 (54.7 – 524.9)	3 (3.6)
ISOLATED HEMATURIA	53.0 (24.4 – 95.8)	11 (13.1)
OTHER	122.9 (42.1 – 201.3)	8 (9.5)

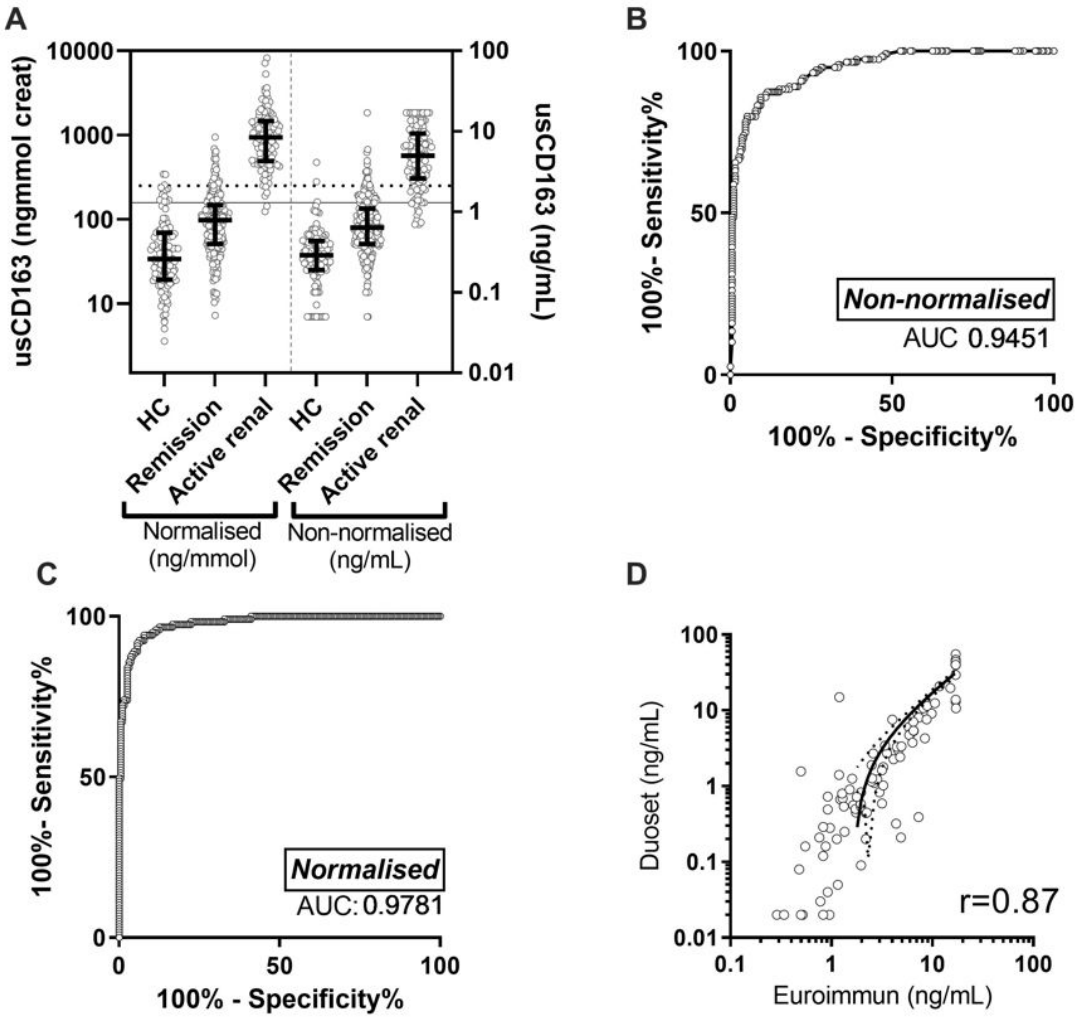
*Table 2. Final adjudicated diagnosis and usCD163 values in renal vasculitis flare and renal vasculitis flare mimics. CKD=Chronic kidney disease. *Not due to renal vasculitis*

	SENSITIVITY	SPECIFICITY	PPV	NPV	FP/FN	AUC
USCD163 >250 NG/MMOL (95% C.I.)	96.8% (83.3-99.9%)	86.8% (74.7-94.5%)	81.1% (68.2-89.6%)	97.9% (82.1-95.8%)	7/1	0.950 (0.90-0.99)
PHYSICIAN IMPRESSION of HIGH PROBABILITY (95% C.I.)	90.3% (74.2-97.9%)	79.2% (65.9-89.2%)	71.8% (56.3-92.3%)	93.3% (81.2-96.7%)	11/3	0.848 (0.771,0.924)

Table 3: Biomarker characteristics compared to adjudication committee diagnosis of renal vasculitis flare or non-renal vasculitis flare (Cohort 2, n=84). BVAS = Birmingham Vasculitis Activity Score. PPV = positive predictive value. NPV= negative predictive value. FP= false positive. FN= false negative. AUC = area under the curve, C.I.= confidence interval.

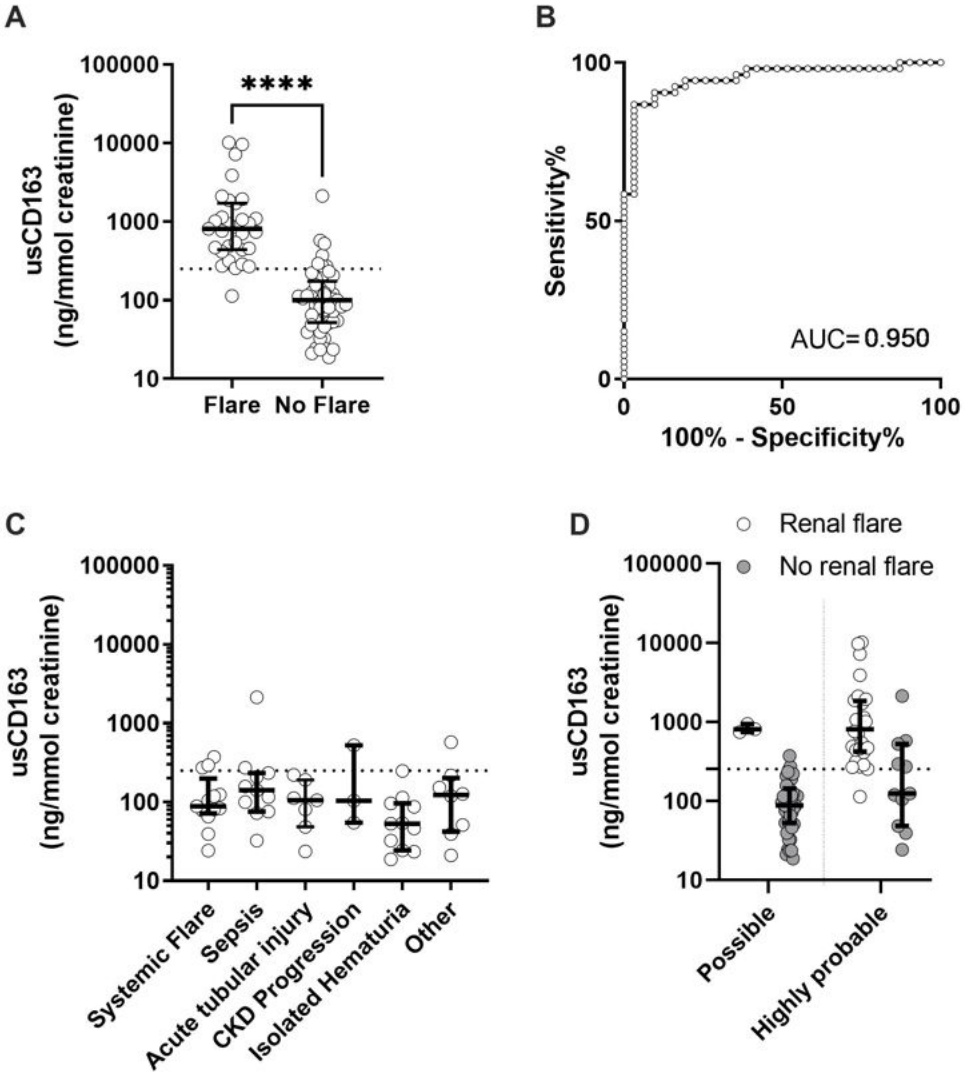
Figure legends

Figure 1. Real world clinical testing for usCD163 using a clinically accredited ELISA test (Cohort 1).



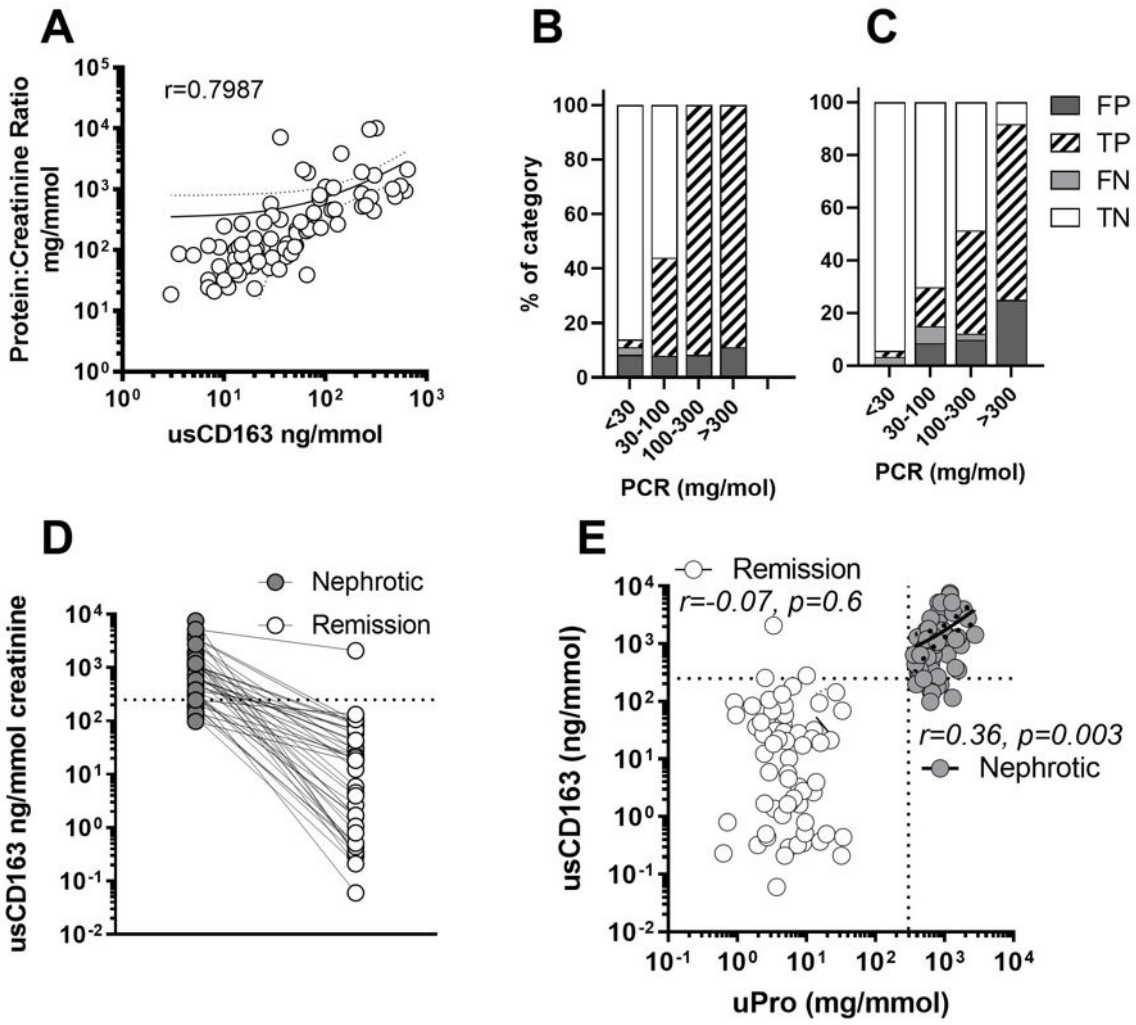
A: Comparison of all usCD163 values in healthy control (HC) and patients with vasculitis in remission and with active renal vasculitis. Both normalised (to urine creatinine, left axis) and non-normalised (right axis) values are presented. The dotted line reflects the optimal cut point for normalised values (250ng/mmol) and the solid line reflects the optimal cut point for non-normalised values (1.3ng/mL). Receiver-operator characteristic curves area under the curve (AUC) values for non-normalised **(B)** and normalised **(C)** values, comparing active renal vasculitis and remission vasculitis, are included. **D:** Correlation of clinically accredited (Euroimmun) pre-coated ELISA kit with the R&D duoset kit (as used in prior work).

Figure 2. Prospective evaluation of usCD163 testing in the clinical setting of possible renal vasculitis flare (Cohort 2).



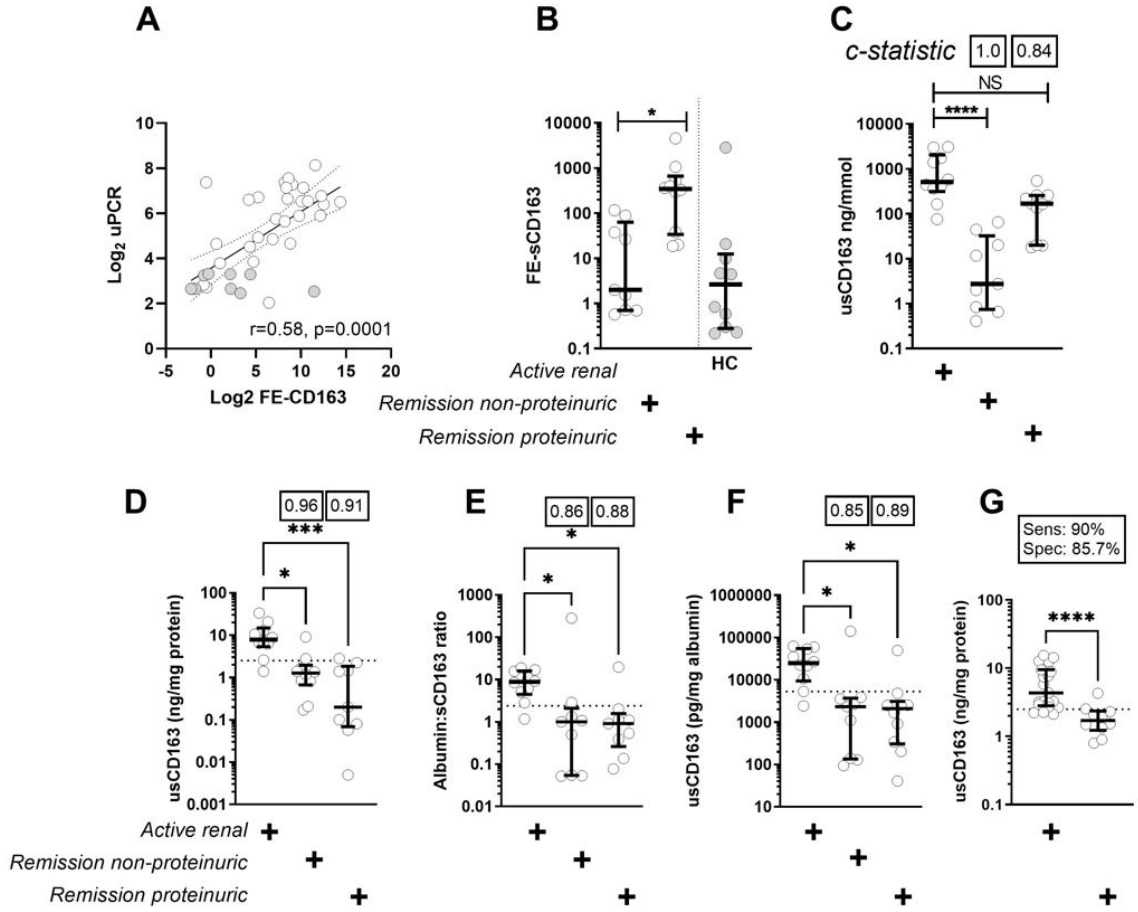
A: Comparison of normalised usCD163 values in those subsequently adjudicated as having suffered a renal vasculitis flare versus those that did not have a renal vasculitis flare; Mann-Whitney test, ****p<0.0001. **B:** Receiver-operator characteristic curve for normalised usCD163 comparing renal vasculitis flare with no renal vasculitis flare in this prospective cohort; AUC= Area under curve. **C:** Normalised usCD163 values in patients subsequently evaluated as not having suffered a renal vasculitis flare; CKD= Chronic Kidney Disease. **D:** Normalised usCD163 values according to the pre-test physician assessment of “possible” or “highly probable” renal vasculitis flare. In each case the dotted line represents the upper limit of normal (250ng/mmol).

Figure 3. Assessment of the impact of proteinuria on usCD163 estimation.



A: Correlation of usCD163 with urine total protein concentration. **B**. Effect of increasing amount of proteinuria on false positive (FP) rate in cohort 2, a restricted clinical setting of clinical suspicion of renal vasculitis flare, and in cohort 1, a real world cohort reflecting the general use of the test in clinical practice (**C**). TP=True positive, TN=True negative, FN=False negative. **D**. urine sCD163 values in paired active and remission nephrotic syndrome in patients with biopsy proven podocytopathy. **E**. Correlation of urine sCD163 values in patients with active nephrotic syndrome and in remission.

Figure 4. Assessment of methods for correction of usCD163 in the setting of heavy proteinuria (Cohort 4).



A. Correlation of fractional excretion of usCD163 (FE-CD163) with urine protein:creatinine ratio (PCR). Light grey data points reflect healthy controls. B. FE-CD163 in patients with vasculitis in remission with, and without, heavy proteinuria. HC=Healthy control, shown for comparison. C. Urine sCD163, normalised to creatinine, in patients with active and remission renal vasculitis (with and without proteinuria). The c-statistic refers to the area under the respective ROC curve in attempting to differentiate active renal vasculitis from both proteinuric and non-proteinuric patients in remission. D-F. Various mechanisms for normalising urine sCD163 in the setting of proteinuria: D. Normalised to total urine protein, E. Normalised using the albumin:sCD163 ratio of serum to urine and F. Normalised to urine albumin. G. The total protein normalised cut-off of 2.5ng/mg was applied to those cohort 1 AAV cases with a urine protein value >0.5g/L (Sens = sensitivity, Spec = Specificity) *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, Kruskal-Wallis test, with Dunn's multiple comparisons test.

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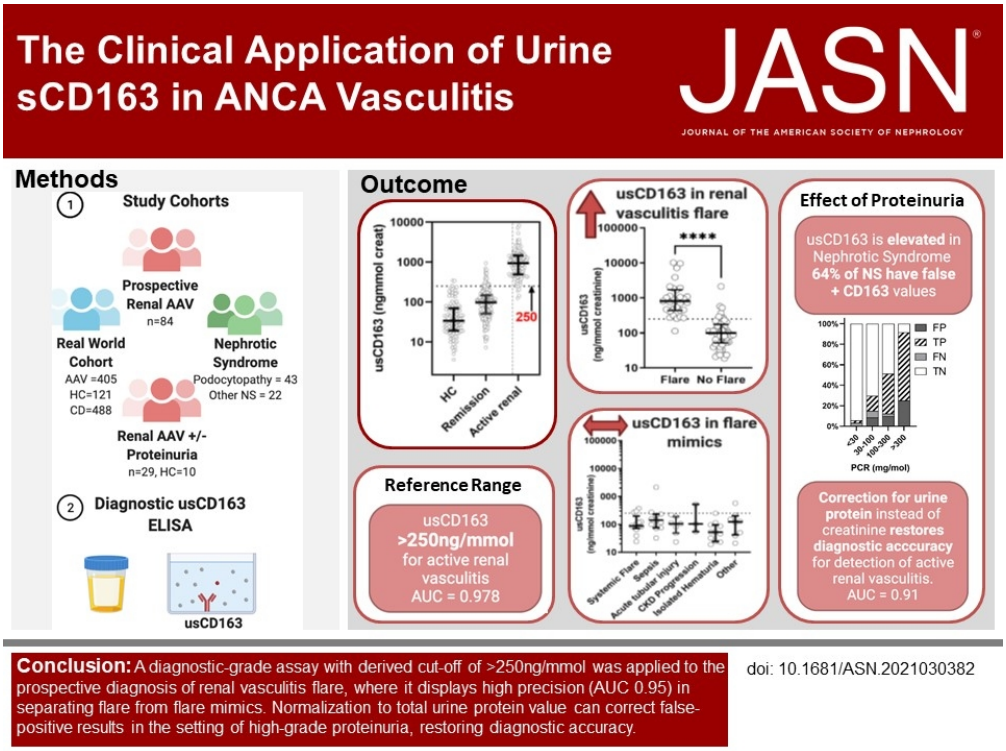

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	Healthy Control (n=121)	Active Renal vasculitis (n=131)	Remission vasculitis (n=274)	Disease Control (n=488)
Gender, (N)	Female 58.7% (71)	Female 52.7% (69)	Female 38.3% (105)	Female 31.0% (155)
Age, mean (SD)	34.1 (13.6)	65.2 (14.0)	62.3 (13.2)	58.9 (17.5)
Diagnosis				
MPA, %*, (N)	N/A	61.8% (81)	53.6% (147)	N/A
GPA, %*, (N)	N/A	31.2% (41)	37.6% (103)	N/A
EGPA, %*, (N)	N/A	0.7% (1)	2.9% (8)	N/A
AAV/AGBM, %*, (N)	N/A	6.9% (9)	5.8% (16)	N/A
Prior renal involvement %, (N)	N/A		92.3%* (253)	
CKD1 (N)	N/A			138
CKD2 (N)	N/A			103
CKD3 (N)	N/A			126
CKD4 (N)	N/A			50
CKD5 (N)	N/A			5
TIN (N)	N/A			8
Lupus nephritis (N)	N/A	13	10	23
IgA vasculitis (N)	N/A			15
Other glomerular disease~ (N)	N/A			20

Supplemental Table 1: Characteristics of cohort 1, used to develop reference ranges for the Irish National Accreditation Board. *MPA=Microscopic polyangiitis, GPA, Granulomatosis with polyangiitis, EGPA=Eosinophilic granulomatosis with polyangiitis, AGBM=Anti-GBM. CKD=Chronic kidney disease. TIN=Tubulointerstitial nephritis. *As a fraction of patients with vasculitis. ~C3 glomerulopathy, membranoproliferative glomerulonephritis, Behcet's disease nephropathy, post-infectious glomerulonephritis, transplant glomerulopathy, eclampsia, endocarditis associated glomerulonephritis*.

	Renal Flare (n=31)	No Renal Flare (n=53)	p value
SERUM			
Creatinine at study visit median, (IQR)	240 umol/L (115-445)	121 umol/L (97-155)	0.0012
Creatinine % Change from Baseline (IQR)	72.9% (15-118%)	1.1% (-7 to 11%)	<0.0001
eGFR at study visit, median (IQR)	22.0 mls/min (10.5 – 52.3)	53.5 mls/min (5.9 to 9.2)	<0.0001
CRP at study visit, median (IQR)	30mg/dL (8-82)	5 mg/dL (4-26)	0.0013
ANCA at study Visit, median (IQR)	64 IU/ml (13-124)	19 IU/ml (5-54)	0.0088
ANCA % Change from Baseline, median (IQR)	0% (27 to 89)	16% (-1 to 51)	0.7533
URINE			
RBC Casts Detected on Microscopy %, (n)	Yes 30% (3) No 70% (7)	Yes 22% (7) No 78% (18)	0.6728
Urinalysis Protein (Scale 0 to 3)	3+ (IQR 2-3)	1+ (IQR 0-2)	<0.0001
UA Hematuria (Scale 0 to 3)	2+ (IQR 2-3)	1+ (IQR 0-2)	0.0002
New/Worse Proteinuria	Yes 60% (12)	Yes 36.2% (17)	0.0716
PCR at study visit (IQR)	124 mg/mmol (64 -281)	29 mg/mmol (13-52)	<0.0001
PCR % Change from Baseline (IQR)	21.3% (-7.5-362%)	20.5% (-41.7- 57.1%)	0.3405
BVAS CRITERIA			
New/Worse Haematuria %, (n)	Yes 80.6% (25)	Yes 43.4% (23)	0.0009
Creatinine >30% %, (n)	Yes 58.1% (18)	Yes 9.4% (5)	<0.0001
BVAS **Total** **Renal**	 12 (IQR 12-15) 12 (IQR 10-12)	 6 (IQR 4-10) 6 (IQR 4-10)	 <0.0001 <0.0001
Physician Impression of flare probability at time of clinic visit, (n)	High Probability 90.3% (28) Possible 9.7% (3)	High Probability 20.7% (11) Possible 79.3% (42)	<0.0001
TISSUE			
Renal biopsy performed % (n)	54.9% (17)	3.8% (2)	<0.0001
Active vasculitis on biopsy % (n)	88.2% (15)	0 (0%)	0.0351

Supplemental table 2. Clinical characteristics of cohort 2 at time of study visit (flare or flare mimic).

eGFR=estimated glomerular filtration rate, CRP=c-reactive protein, ANCA=anti-neutrophil cytoplasmic antibody, PCR=protein: creatinine ratio, UA= dipstick urinalysis, RBC=red blood cell, BVAS=Birmingham Vasculitis Activity Score as scored at the time of clinic visit, SD= standard deviation, IQR = interquartile range.

	CLASSIFICATION	USCD163 VALUE (ng/mmol)	CLINICAL COURSE	RENAL BIOPSY	BVAS	SERUM CREATININE (umol/L, % CHANGE)	ANCA (FOLD INCREASE)	URINE PROTEIN	PROTEIN CORRECTED*
CASE1	False Negative	112.9	New Hematuria & Proteinuria	N/A	10	89 (+8.5%)	aMPO 89 (3.2-fold)	14mg/mmol UA 1+	N/A
CASE 2	False Positive	2126	Severe AKI, stroke, lung mass, sudden death.	N/A	12	425 (+150%)	aMPO 8.3 (NA)	647mg/mmol UA 3+	0.33ng/mg
CASE 3	False Positive	575.5	Papillary Necrosis, 50% fall in GFR	No CGN	12	157 (+40.2%)	aPR3 54 (no change)	102mg/mmol UA 3+	0.5ng/mg
CASE 4	False Positive	524.9	Rising creatinine	N/A	10	225 (+27.1%)	aMPO 2.5 (0.25-fold)	231ng/mmol UA 2+	0.22ng/mg
CASE 5	False Positive	371.9	Systemic Flare, rise in creatinine	N/A	6	116 (+9.3%)	aMPO 5.6 (0.04)	114mg/mmol LV UA 4+	1.2ng/mg
CASE 6	False Positive	270.9	Urinary tract infection	N/A	10	102 (+3.03%)	0 (no change)	No PCR UA 1+	N/A
CASE 7	False Positive	271.7	Systemic Flare post transplant	N/A	6	127 (-18.1%)	aMPO 0.8 (no change)	No PCR UA 0+	N/A
CASE 8	False Positive	292.6	Systemic Flare	N/A	19	155 (+7.6%)	aPR3 6.1 (0.15-fold)	57mg/mmol UA 2+	0.51ng/mg

Supplemental table 3. Clinical characteristics of false positive and false negative usCD163 values in Cohort 2 (prospective assessment of renal vasculitis flare). **Only applied if urine protein > 50 mg/mmol; proposed cut-off=2.5ng/mg. GFR=glomerular filtration rate*

N=65	**PRIMARY PODOCYTOPATHY**	***FSGS* *(N=23)***	***MCD* *(N=20)***	**MN (N=22)**
AGE AT DIAGNOSIS, Y (IQR)	15 (4-29)	13 (3-30)	16 (4-27)	58 (39-65)
EGFR, MLS/MIN (SD)	89.9 (30.7)	77.3 (30.3)	96.5 (29.6)	68.3 (19.7)
PROTEINURIA (DURING NEPHROSIS) G/L (IQR)	7.3 (5.6-11.3)	5.6 (3.8-6.1)	10.1 (7.0-11.7)	5.6 (4.8-7.4)
PROTEINURIA (DURING REMISSION) G/L (IQR)	0.04 (0.02-0.07)	0.05 (0.03-0.11)	0.07 (0.04-0.3)	0.06 (0.03-0.1)

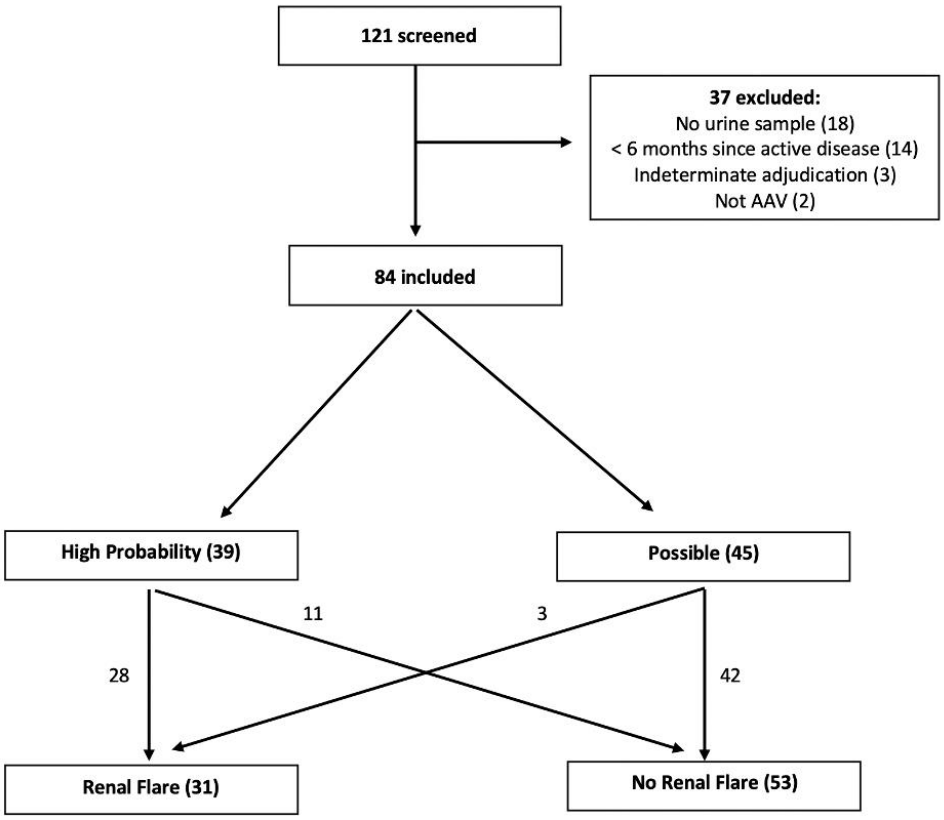
Supplemental Table 4: Characteristics of cohort 3 used to investigate the effect of proteinuria on urine sCD163 excretion. *Podocytopathy includes focal segmental glomerulosclerosis (n=23) and minimal change disease (n=20). MN = membranous nephropathy, eGFR= estimated glomerular filtration rate, IQR = interquartile range, SD=standard deviation.*

	ACTIVE RENAL (N=10)	REMISSION PROTEINURIC (N=10)	REMISSION NON-PROTEINURIC (N=9)	HEALTHY CONTROL (N=10)	P-VALUE
AGE, MEAN, SD	64.8 (12.1)	52.1 (14.4)	55.7 (17.4)	68.2 (14)	0.0609
MALE SEX, (N)	90% (9)	100% (10)	44.4% (4)	50% (5)	0.0105
UA PROTEIN (MEDIAN, IQR)	2.5+ (1.8 to 3+)	3+ (2 to 3+)	0+ (0 to 0+)	N/A	<0.0001
UA BLOOD (MEDIAN, IQR)	3+ (2.8 to 3+)	1+ (0 to 2.3+)	0+ (0 to 1+)	N/A	0.0003
URINE PCR, *MG/MMOL* **(**MEDIAN, IQR)	58 (47-167)	168 (98-212)	19 (9-27)	9.7 (5.7-9.9)	0.0002
SERUM CREATININE, *µMOL/L* (MEDIAN, IQR)	220 (128-291)	198 (128-312)	100 (80-141)	76 (62-79)	<0.0001

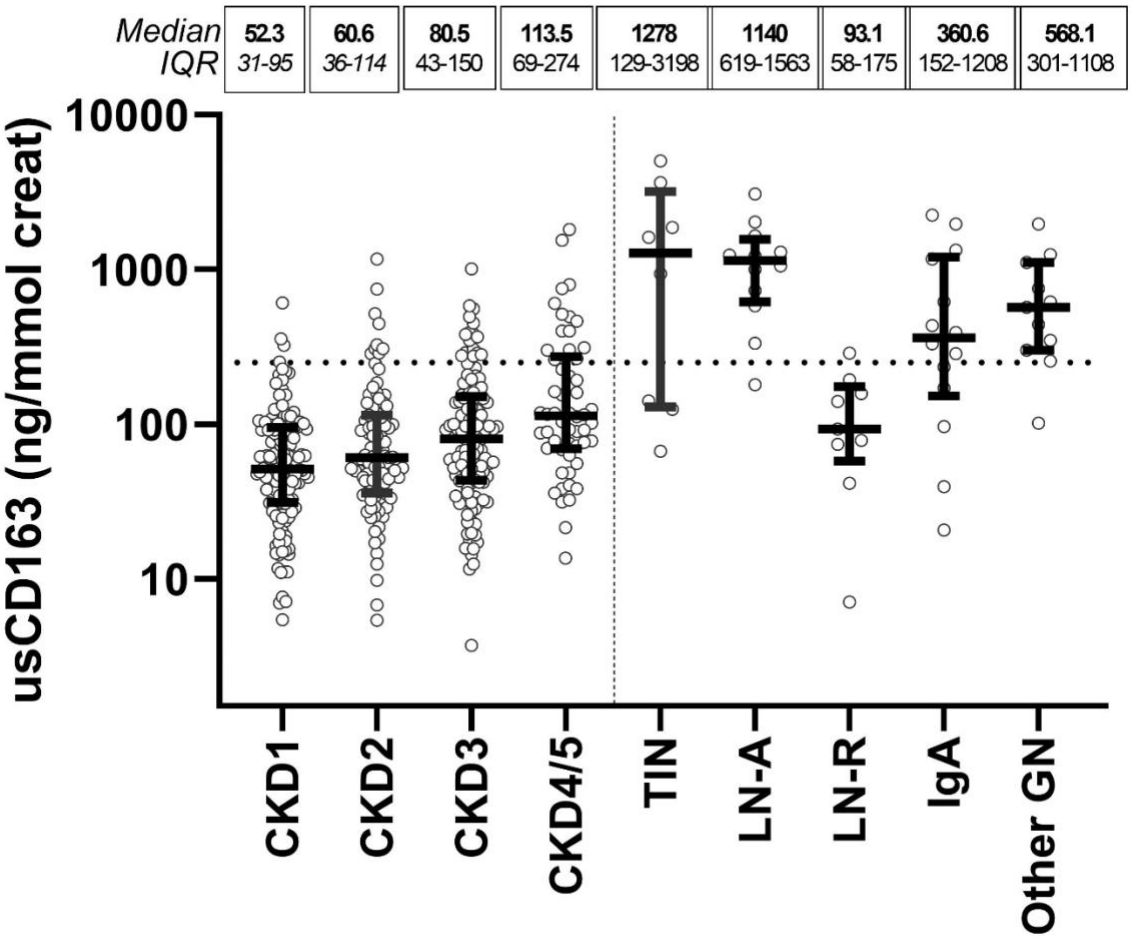
Supplemental Table 5: Characteristics of cohort 4, patients with ANCA vasculitis and disease controls used to investigate the impact of proteinuria on false positive diagnostic rate.

	ACTIVE RENAL	REMISSION PROTEINURIC	REMISSION NON-PROTEINURIC	HEALTHY CONTROL
SERUM SCD163, NG/ML	83.5 (78.6-99.3)	101.5 (99.3-115.4)	102.7 (70.6-109.3)	101.4 (3.6-111)
URINE SCD163, PG/ML	4395 (1805-9898)	1700 (243-2386)	4 (4-134)	4 (4-45)
FRACTIONAL EXCRETION OF SCD163	1500 (414-5162)	346 (33-658)	2.0 (0.7-63)	2.6 (0.3-12.5)
URINE CREATININE NORMALISED, NG/MMOL	510 (313-2048)	167 (20-257)	2.8 (0.7-32)	0.9 (0.5-5.7)
URINE PROTEIN NORMALISED, PG/MG	7860 (5336-14717)	1265 (666-1959)	200 (69-1844)	90 (72-691)
URINE:SERUM SCD163:ALBUMIN RATIO	8.8 (4.5-16.0)	0.9 (0.3-1.6)	1.0 (0.1-2.1)	2.0 (0.1-23.6)
URINE ALBUMIN NORMALISED, PG/MG	24759 (9406-54897)	2091 (307-3111)	2316 (135-3702)	3611 (400-7854)

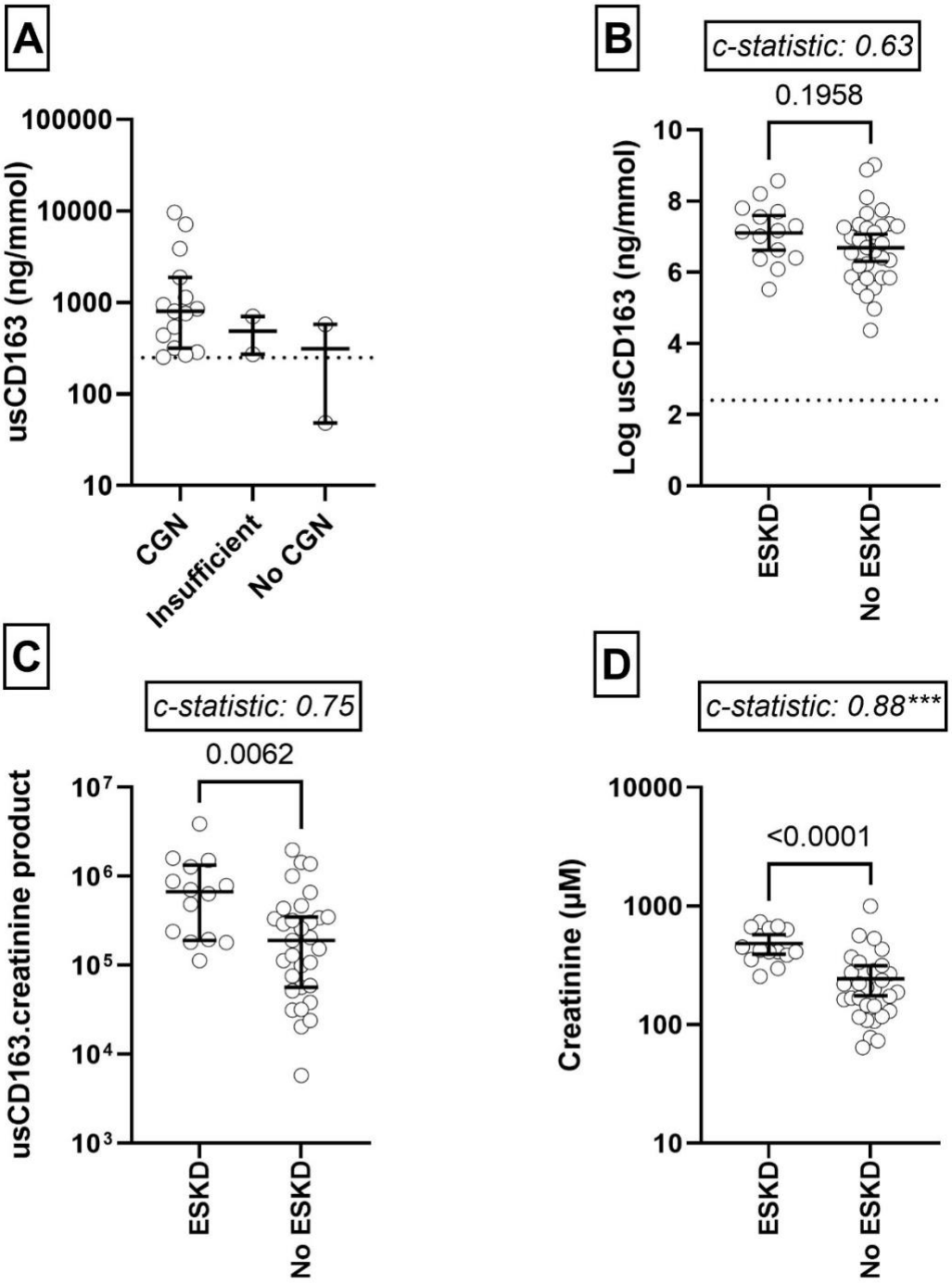
Supplemental Table 6: Cohort 4. Median and interquartile range levels of soluble CD163 values in active renal vasculitis, remission vasculitis with proteinuria, remission vasculitis without proteinuria. *Formulae for calculation of protein and albumin ratios included in methods.*



Supplemental Figure 1. Flow diagram of cohort 2 recruitment from screening to enrollment and subsequent diagnosis. High Probability and Possibly denote Physician impression at the time of study enrollment. Renal Flare and No Renal Flare denote final blinded retrospective adjudication by committee.

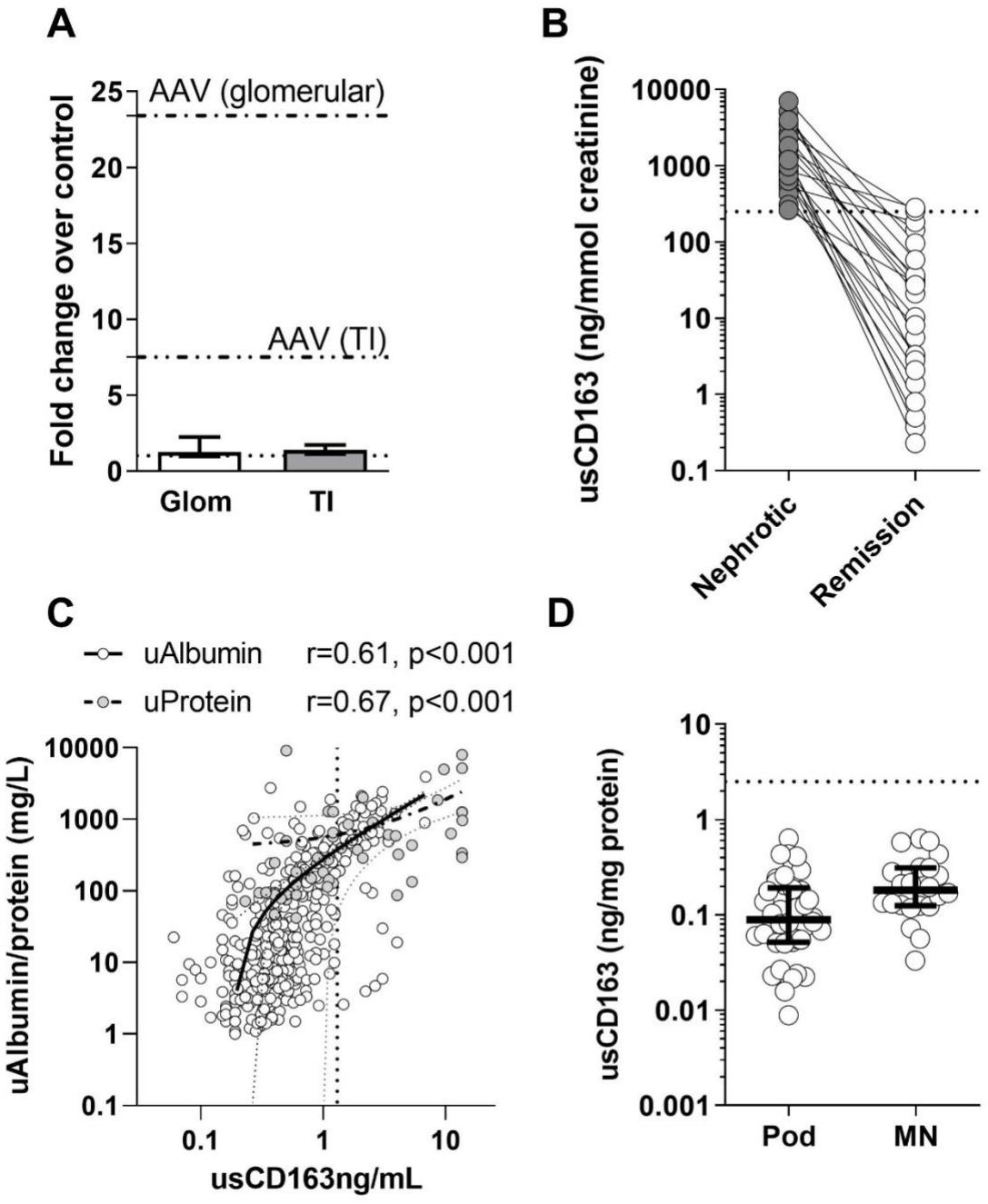


Supplemental Figure 2. Real world usCD163 values in a range of disease controls using the Euroimmun assay. CKD=Chronic Kidney Disease. TIN=Tubulointerstitial nephritis. LN-A=Active lupus nephritis. LN-R=Remission lupus nephritis. Other GN=Other forms of glomerular disease. Median = the median normalised usCD163 value (ng/mmol). IQR = interquartile range.



Supplemental Figure 3: usCD163 in those who underwent kidney biopsy (cohort 2) and relationship to subsequent development of ESKD. A. In a subgroup analysis of those who underwent kidney biopsy because of a suspicion of renal vasculitis flare (n=19), 15 (79%) had this diagnosis confirmed. Two had an insufficient sample and were adjudicated as having a renal vasculitis flare based on subsequent clinical course; all of these had a usCD163 value >250ng/mmol. Two participants had no evidence of crescentic GN (CGN) on biopsy and were thus adjudicated as having no renal vasculitis flare.

One of these two cases had a positive sCD163 test (575ng/mmol); they did not receive additional immunosuppression on the basis of the kidney biopsy and glomerular filtration rate dropped subsequently by 50%. Dotted line denotes cut off value for active renal vasculitis of >250ng/mmol. B. usCD163 (normalised to urine creatinine) at diagnosis of RV flare. Dotted line denotes diagnostic cut off for active renal vasculitis of >250ng/mmol. C. usCD163:creatinine product at diagnosis of RV flare. D. serum creatinine (umol/L) at diagnosis of RV flare. The c-statistic reflects the area under the respective ROC curve.



Supplemental Figure 4. Effect of proteinuria on usCD163 values. A. CD163 mRNA was measured in kidney tissue obtained from 7 of the 56 patients with primary podocytopathy using rtPCR. The dotted line indicates the null value of 1 and the respective glomerular and tubulointerstitial values from our previous work[1] is shown for comparison. B. usCD163 (normalised to urine creatinine) in paired samples from patients with Membranous nephropathy in active nephrosis and remission. Dotted line denotes diagnostic cut off for active renal vasculitis of 250ng/mmol creatinine. C. Correlation between usCD163 and urine

[1] J Am Soc Nephrol. 2016 Sep;27(9):2906-16. doi: 10.1681/ASN.2015050511

albumin (uALbumin) in patients with chronic kidney disease with a wide range of albuminuria levels, and total urine protein in real world non-AAV patients, both from Cohort 1. The dotted line depicts the proposed usCD163 cut-off value and the solid black line depicts the best fit regression line (95% confidence interval), with an “elbow” evident at UAlbumin level of 50mg/L. D. usCD163 (normalised to urine protein) in podocytopathy (FSGS and MCD) and MN (membranous nephropathy). Dotted line denotes diagnostic cut off for active renal vasculitis of 2.5ng/mg protein.