Clinical Kidney Journal, 2023, vol. 16, no. 4, 693–700

https://doi.org/10.1093/ckj/sfac257
Advance Access Publication Date: 7 December 2022
Original Article

ORIGINAL ARTICLE

Combining neutrophil and macrophage biomarkers to detect active disease in ANCA vasculitis: a combinatory model of calprotectin and urine CD163

Paula Anton-Pampols [1], Laura Martínez Valenzuela[1], Loreto Fernández Lorente[2], Maria Quero Ramos[1], Francisco Gómez Preciado[1], Irene Martín Capón[3], Francisco Morandeira[4], Joaquín Manrique Escola[2], Xavier Fulladosa[1], Josep Maria Cruzado[1,5], Joan Torras[1,5,*] and Juliana Draibe[1,*]

[1]Department of Nephrology, Bellvitge University Hospital, Bellvitge Biomedical Research Institute (IDIBELL), Hospitalet de Llobregat, Barcelona, Spain, [2]Department of Nephrology, Hospital Universitario de Navarra, Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain, [3]Department of Nephrology, Ramón y Cajal University Hospital, Madrid, Spain, [4]Department of Immunology, Bellvitge University Hospital, Hospitalet de Llobregat, Barcelona, Spain and [5]Department of Clinical Sciences, University of Barcelona, Barcelona, Spain

*These authors share seniorship.
Correspondence to: Laura Martinez Valenzuela; E-mail: lmartinezv@bellvitgehospital.cat

ABSTRACT

Background. CD163 and calprotectin have been proposed as biomarkers of active renal vasculitis. This study aimed to determine whether the combination of serum/urine calprotectin (s/uCalprotectin) and urinary soluble CD163 (suCD163) increases their individual performance as activity biomarkers.

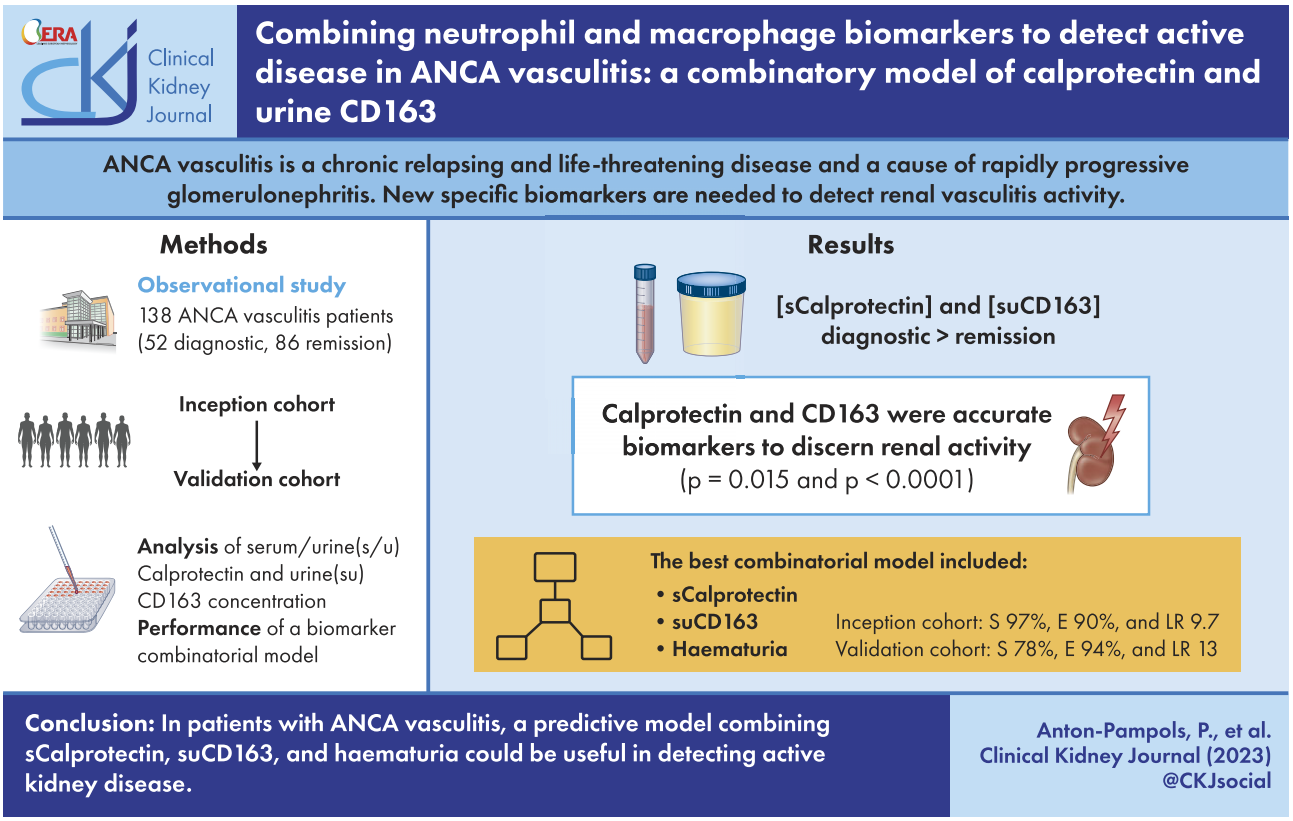
Methods. We included 138 patients diagnosed with ANCA vasculitis ($n = 52$ diagnostic phase, $n = 86$ remission). The study population was divided into the inception ($n = 101$) and the validation cohorts ($n = 37$). We determined the s/uCalprotectin and suCD163 concentration using enzyme-linked immunoassay at the diagnostic or at the remission phase. Receiver operating characteristic (ROC) curves were conducted to assess the biomarkers' classificatory values. We elaborated a combinatorial biomarker model in the inception cohort. The ideal cutoffs were used in the validation cohort to confirm the model's accuracy in the distinction between active disease and remission. We added the classical ANCA vasculitis activity biomarkers to the model to increase the classificatory performance.

Results. The concentrations of sCalprotectin and suCD163 were higher in the diagnostic compared with the remission phase ($P = .013$ and $P < .0001$). According to the ROC curves, sCalprotectin and suCD163 were accurate biomarkers to discern activity [area under the curve 0.73 (0.59–0.86), $P = .015$ and 0.88 (0.79–0.97), $P < .0001$]. The combinatory model with the best performance in terms of sensitivity, specificity and likelihood ratio included sCalprotectin, suCD163 and haematuria. Regarding the inception and the validation cohort, we obtained a sensitivity, specificity and likelihood ratio of 97%, 90% and 9.7, and 78%, 94% and 13, respectively.

Received: 29.7.2022; Editorial decision: 18.11.2022

Conclusions. In patients with ANCA vasculitis, a predictive model combining sCalprotectin, suCD163 and haematuria could be useful in detecting active kidney disease.

GRAPHICAL ABSTRACT



Keywords: ANCA, biomarkers, calprotectin, CD163, vasculitis

INTRODUCTION

Antineutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV) is an autoimmune disease that causes inflammation of small vessels. AAV presents specific antibodies against components of neutrophils and monocytes [myeloperoxidase (MPO) and proteinase 3 (PR3)] [1]. Glomerulonephritis is common and is characterized by the infiltration of monocytes/macrophages and glomerular T-cells. Berden *et al.* [2] described a histological classification to predict renal outcomes. They classified renal biopsies into four classes (focal, crescentic, mixed and sclerotic), according to the glomerular damage. The predominance of crescents or focal necrosis confers a better renal prognosis than mixed or sclerotic damage, suggesting that these lesions respond better to treatment [3]. Therefore, early detection of disease activity would allow prompt initiation of treatment limiting kidney damage.

The relationship between ANCA titres and the recurrence of kidney disease has been described. However, patients in clinical remission often have persistent ANCA positivity. So ANCA values do not always correspond to disease activity [4, 5]. Serial ANCA measurements are of limited utility to guide treatment during disease remission. Using other inflammatory markers like C-reactive protein (CRP) is also controversial as these markers are unspecific and may also be influenced by infections or other inflammatory conditions [4].

Therefore, specific biomarkers are needed to detect renal vasculitis activity, avoiding a kidney biopsy.

Calprotectin is a heterodimer formed by two intracellular calcium-binding proteins, S100A8(MRP 8)/S100A9(MRP 14), produced by activated circulating monocytes and neutrophils that can also be found infiltrating tissues. S100A8/S100A9 binds to activated endothelial cells through heparan sulfate, increases inflammatory cytokines such as tumour necrosis factor-α, interleukin (IL)-1β, IL-6, IL-23 and IL-8, and upregulates intercellular adhesion molecule 1 (ICAM-1) and leucocyte recruitment. This inflammatory set leads to endothelial damage resulting in necrosis [6].

The rise of serum calprotectin (sCalprotectin), measured by enzyme-linked immunoassay (ELISA), is higher in patients with active AAV than in remission patients. Pepper *et al.* [3] demonstrated that patients with focal or crescent glomerular injury, presented a higher expression of sCalprotectin by immunochemistry of renal biopsies. In contrast, those with sclerotic lesions presented less sCalprotectin, suggesting that sCalprotectin could be a biomarker of inflammation activity. In addition, the

increase in sCalprotectin during follow-up is an independent risk factor for disease recurrence in PR3-ANCA-positive patients treated with rituximab [7]. These findings indicate that sCalprotectin could help to identify those patients who need more intensive or prolonged treatment. Moreover, sCalprotectin was higher in patients with greater levels of proteinuria, persistent haematuria, positive ANCA and glomerular filtration rate (GFR) decline in a 2-year follow-up period [8]. These findings support that sCalprotectin may be useful to identify remission patients with subclinical inflammation and worse renal prognosis.

Also, we studied the usefulness of urinary Calprotectin (uCalprotectin) as a biomarker of disease activity in a small cohort. We found no differences between uCalprotectin concentration and clinical parameters. However, patients with sclerotic histology presented lower uCalprotectin excretion [8]. Furthermore, Heller *et al.* [9] noticed an elevated concentration of uCalprotectin in parenchymatous renal failure.

CD163 is a glycosylated membrane protein expressed on monocytes and macrophages. Soluble CD163 form is produced by enzymatic cleavage via ectodomain in response to proinflammatory stimuli [10]. As described by O'Reilly *et al.*, activated macrophages infiltrate the glomerulus and secrete soluble CD163 into the urine. They found a significant increase in this biomarker in patients with active vasculitis compared with patients in remission [11]. Besides being related to active ANCA-associated glomerulonephritis, high levels of soluble urinary CD163 (suCD163) are associated with higher histological activity [12].

This study aims to determine whether the combination of sCalprotectin and suCD163—together with the addition of the classical biomarkers—can improve their individual performance in the detection of renal flares of the disease.

MATERIALS AND METHODS

Study population

This is an observational study performed in two centres, the Bellvitge University Hospital (HUB) and the Hospital Universitario de Navarra (HUN). AAV diagnosis was established according to the Chapel Hill consensus criteria [13]: gathering of typical symptoms, positivity of serum ANCA and renal histology [14]. The third version of Birmingham Vasculitis Activity (BVAS) was used to determine disease activity [15]. Active renal vasculitis was defined according to clinical practice as a new increase in haematuria, and/or proteinuria and/or increase of ANCA and/or increase of creatinine serum along with/without new clinical symptoms. Patients in remission were defined as BVAS = 0, including stable urinary sediment and creatinine levels. All the patients were under a similar therapeutic scheme. Remission was achieved by treatment with plasmapheresis or intravenous methylprednisolone bolus followed by cyclophosphamide or rituximab. Maintenance treatment was oral prednisone (2.5–5 mg daily), mycophenolate mofetil/mycophenolic acid (500 or 360 mg bis in die), azathioprine (50 mg daily) or rituximab (1 g every 6 months) as supported by the EULAR/ERA-EDTA guidelines [16].

A total of 138 patients with ANCA-associated glomerulonephritis (diagnostic phase $n = 52$ and remission phase $n = 86$) were recruited. Patients in diagnostic phase were treatment-naïve.

Active neoplasm or infection, other autoimmune diseases and end-stage kidney disease are contraindications for participation in the study.

Samples and data patients from HUB included in this study were provided by the Biobank HUB-ICO-IDIBELL (PT20/00 171) and those from HUN were provided by the Biobank Navarrabiomed (PT13/0010/0051). Biologic products were processed according to established procedures with the approval of the Ethics and Scientific Committees.

This study was conducted in accordance with the amended Helsinki Declaration and was approved by HUB and HUN ethical review boards.

Biomarker assays

We determined s/uCalprotectin and suCD163 in samples at the time of the inclusion in the study. We used a commercial ELISA kit (Legend Max Human MRP8/14, Biolegend Inc.; for Calprotectin, and the Quantikine® Human CD163 immunoassay, Bio-Techne R&D Systems, for CD163) following the manufacturer's instructions.

Medical data collection

We obtained clinical and analytical data from electronic medical records. We recorded sex, age, ANCA specificity (MPO/PR3) and titres, the presence of haematuria and proteinuria, CRP values and time since diagnosis. We compiled analytical data at the diagnosis and at the time of recruitment. We evaluated the outcome renal function as serum creatinine and GFR using the Chronic Kidney Disease Epidemiology Collaboration formula. Histology at diagnosis was analysed according to Berden's classification [2]. The immunosuppressive agents administered, and the patient and renal survival were also registered.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables as total number (n) and percentage (%). For comparison between two groups, when variables were quantitative and normally distributed, Student's t-test was used, and Mann–Whitney U was performed when the variables were not normally distributed. Chi-square test was used for qualitative variables. Spearman's correlation was applied to correlate not normally distributed variables. To assess the biomarker's ability to predict active renal vasculitis, receiver operating characteristic (ROC) curves were estimated. The Youden's J statistic yielded the optimum cut-offs to predict active renal vasculitis and estimate sensitivity, specificity, positive/negative predictive value, and positive/negative likelihood ratios (PLR/NLR) for the individual biomarkers. In the inception cohort biomarker combination models presented as a decision tree were elaborated using the recursive partition method. The ideal cut-offs obtained in this model were validated in a validation cohort to assess the reliability of the model.

P-values were two-tailed and statistical significance level was established at $P < .05$.

IBM SPSS Statistics Version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 8.00 (GraphPad Software, La Jolla, CA, USA) were used for data analysis.

RESULTS

Inception cohort

Demographic, serological and clinical characteristics

One hundred and one AAV patients were recruited as inception cohort from HUB (diagnostic $n = 31$, remission phase $n = 70$). Table 1 details the main clinical characteristics.

Table 1: Baseline characteristics of the inception cohort.

$n = 101$	Diagnostic phase, $n = 31$	Remission phase, $n = 70$	P-value
Sex (% male)	55	36	.072
Mean age ± SD (years)	68 ± 13.60	63 ± 13.81	.043
Median time from diagnosis (range) (months)	0	81.90 ± 71.54	
ANCA type (%MPO)	77	76	.76
Mean ANCA titres ± SD (karbU/L)	588.1 ± 1177	57.41 ± 115.1	<.0001
Mean serum creatinine ± SD (μmol/L)	312.7 ± 272.9	150.8 ± 74.38	<.0001
Haematuria (%)	97	21	<.0001
Mean proteinuria ± SD (g/day)	1.46 ± 1.39	0.42 ± 0.47	<.0001
Mean CRP ± SD (mg/L)	40.84 ± 36.02	7.76 ± 28.72	<.0001
Berden histopathologic classification (%)			
Focal	19	19	
Crescentic	26	29	
Mixed	39	31	
Sclerotic	10	6	
Induction therapy:			
Cyclophosphamide (%)	39	67	.011
Rituximab (%)	39	4	<.0001
Plasma exchange (%)	29	30	.92
Treatment in remission patients			
Without treatment (%)		17	
ST (%)		73	
MMF (%)		60	
AZA (%)		4	
RTX (%)		6	

ST, steroids; MMF, mycophenolate mofetil; AZA, azathioprine; RTX, rituximab.

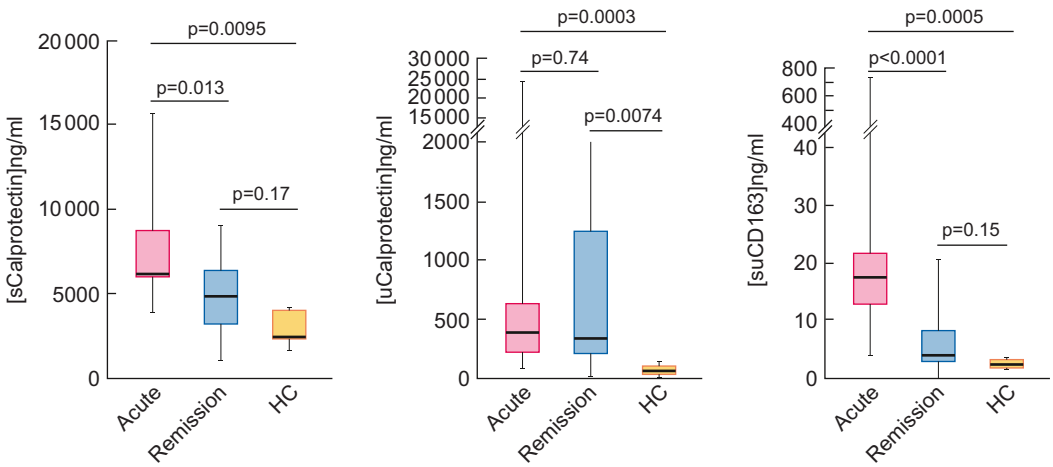


Figure 1: Mean of sCalprotectin, uCalprotectin, and suCD163 of the acute AAV patients (patients in diagnostic stage), remission patients, and healthy controls (HC). Values correspond to inception cohort.

Acute patients showed higher values of ANCA, creatinine, haematuria, proteinuria and CRP. Anti-MPO was the most frequent specificity of ANCA antibodies. Kidney biopsies, categorized by Berden's classification, presented mostly crescentic and mixed lesions.

Induction therapy mostly included cyclophosphamide and rituximab. Regarding maintenance treatment of patients in remission, most patients were treated with steroids and mycophenolate mofetil.

sCalprotectin and suCD163 levels are elevated in active renal disease

We determined the concentration of s/uCalprotectin and the suCD163 using ELISA. In the inception cohort, the concentration of sCalprotectin and suCD163 was higher in patients with active AAV than in patients in remission (sCalprotectin 7436 ± 3423 ng/mL in diagnostic and 4784 ± 2037 ng/mL in remission phase, $P = .013$; and suCD163 44.05 ± 138.7 ng/mL in diagnostic and 6.26 ± 5.79 ng/mL in remission stage, $P < .0001$). The concentration of uCalprotectin was not different between patients in acute or remission phases (diagnostic 1376 ± 4776 ng/mL and remission phase 1283 ± 2166 ng/mL, $P = .74$) (Fig. 1).

We plotted the ROC curves of the biomarkers and we observed that sCalprotectin and suCD163 were accurate biomarkers to discern AAV activity while uCalprotectin was not (Fig. 2).

Youden's J statistic conceded the optimum cut-offs to predict active renal vasculitis and the values of sensitivity, specificity and PLR as shown in Table 2.

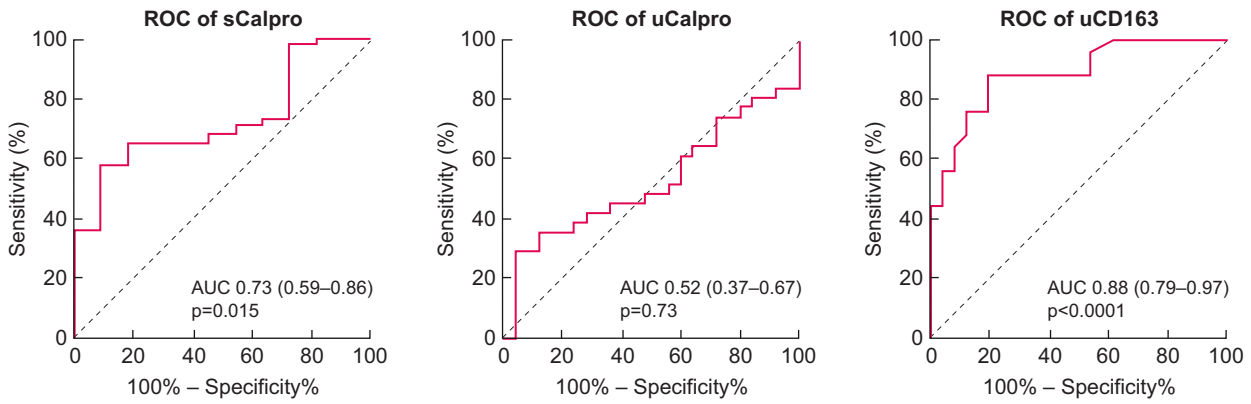


Figure 2: ROC curve for the levels of sCalprotectin (sCalpro), uCalprotectin (uCalpro), and uCD163 as a biomarker to determine disease activity, in the inception cohort.

Table 2: Statistics and optimum cut-off of biomarkers in inception cohort.

Biomarker	Cutoff	Sensitivity (%)	Specificity (%)	PLR	AUC	P-value
sCalpro	<5135 ng/mL	58	90	6.40	0.73	..015
suCD163	<12 ng/mL	88	80	4.57	0.88	<..0001
uCalpro	>1101 ng/mL	29	96	7.25	0.52	..73
ANCA titres	<49.05 karbU/L	74	70	2.55	0.78	<..0001
Haematuria		96	78	4.45		<..0001
CRP	<11 mg/L	91	70	3.14	0.87	<..0001

sCalpro, sCalprotectin; uCalpro, uCalprotectin.

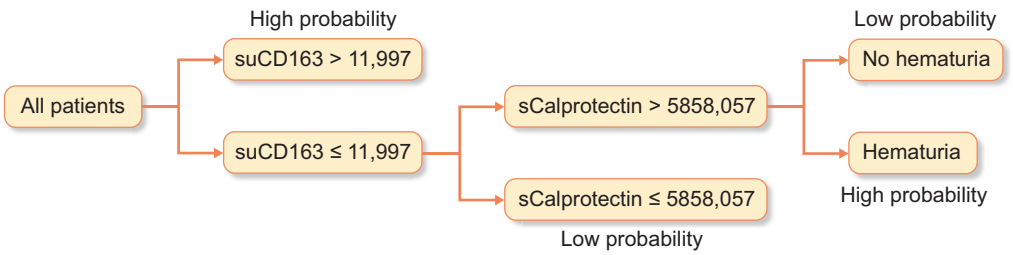


Figure 3: Recursive partitioning was applied to the dataset. The best tree obtained (model-2) combined sCalprotectin, suCD163, and hematuria.

Combining sCalprotectin and suCD163 improves diagnostic reliability

A predictive combinatory biomarker model was developed in the inception cohort to classify patients with AAV into those with active disease and remission. By combining sCalprotectin and suCD163 we obtained a combinatorial model with a sensitivity of 68%, a specificity of 96%, and a PLR of 17 (Supplementary data, Fig. S1). Statistics from the individual biomarkers are listed in Table 2.

Subsequently, to obtain a model with a higher sensitivity we added the classical ANCA vasculitis activity biomarkers. Multiple decision trees were generated by recursive partitioning (data not shown). The best model obtained, named Model 2, combined sCalprotectin, suCD163 and haematuria (Fig. 3). This presented a sensitivity of 97%, a specificity of 90% and a PLR of 9,7.

Validation cohort

Patients and clinical data collection

We recruited 37 patients followed at the HUN to establish the validation cohort (diagnostic $n = 21$, remission stage $n = 16$). Supplementary data, Table S1 shows the baseline characteristics.

Patients in the validation cohort in the diagnostic phase had higher levels of ANCA titres, haematuria, proteinuria, CRP and creatinine than patients in remission. Anti-MPO specificity of ANCA antibodies predominated in the population and crescentic and mixed lesions prevailed.

Most patients had been treated with cyclophosphamide as induction therapy and had mycophenolate mofetil as maintenance treatment.

Table 3: Performance of the combinatory model (sCalprotectin, suCD163 and haematuria) in the inception and validation cohorts.

Cohort analysed	Sensitivity (%)	Specificity (%)	PPV	NPV	PLR	NLR
Inception cohort	97	90	81	0.98	9.7	0.03
Validation cohort	78	94	93	0.79	13	0.23

Statistics were determined by first analysing data from the inception cohort and applying them to the validation cohort.
PPV, positive predictive value; NPV, negative predictive value.

External validation of Model 2

We appraised the concentration of s/uCalprotectin and suCD163 using ELISA in the population from HUN. In this validation cohort the concentration of sCalprotectin and suCD163 was more elevated in patients in diagnostic than in patients in remission stage (sCalprotectin 8571 ± 4583 ng/mL in diagnostic and 4270 ± 2020 ng/mL in remission stage, $P = .0006$; and suCD163 18.20 ± 18.96 ng/mL in diagnostic and 7.72 ± 1.25 ng/mL in remission phase, $P = .0004$). In contrast to the inception cohort, patients in the acute phase of the validation cohort showed higher levels of uCalprotectin compared with remission phase (diagnostic 794.7 ± 488.4 ng/mL and remission phase 518.2 ± 484.1 ng/mL, $P = .048$) (Supplementary data, Fig. S2).

The ROC curves determined that sCalprotectin and suCD163 were precise biomarkers to detect AAV activity [AUC sCalprotectin 0.82 (0.68–0.96), $P = .0009$; and AUC suCD163 0.84 (0.70–0.97) (Supplementary data, Fig. S3)].

Finally, we implemented the Model 2 obtained in the inception cohort, which combined sCalprotectin, suCD163 and haematuria, in the validation cohort to perform an external validation of our data. We maintained excellent sensitivity and specificity and increased the PLR to 13 (Table 3, Fig. 3).

Clinical significance of the classification established by Model 2

We further divided the all patients in clinical remission into two groups: those designated as active and those designated as remission AAV according to our Model 2. The patients in clinical remission that were designated as acute by this model presented higher proteinuria values, ANCA titres, serum creatinine and CRP compared with those classified as remission AAV (Fig. 4).

We followed-up for 1 year a small cohort after diagnosis. We performed a Student's *t*-test of paired data showing a downward trend in biomarkers, obtaining statistical significance in sCalprotectin ($P = .0005$).

Moreover, we studied biomarker levels according to Berden's histological classification and no significance differences were found.

Extrarenal involvement in diagnostic phase (inception and validation cohorts)

Of the 52 patients in the diagnostic phase of the entire population, 25% had lung involvement. uCalprotectin and suCD163 were slightly increased in patients with lung involvement compared with those without lung involvement (*P*-value non-significant). As was expected in extrarenal involvement, sCalprotectin was higher in patients with lung involvement than in patients without lung involvement (12829 ng/mL vs 6371 ng/mL), but no statistical significance was found ($P = .1$). No differences

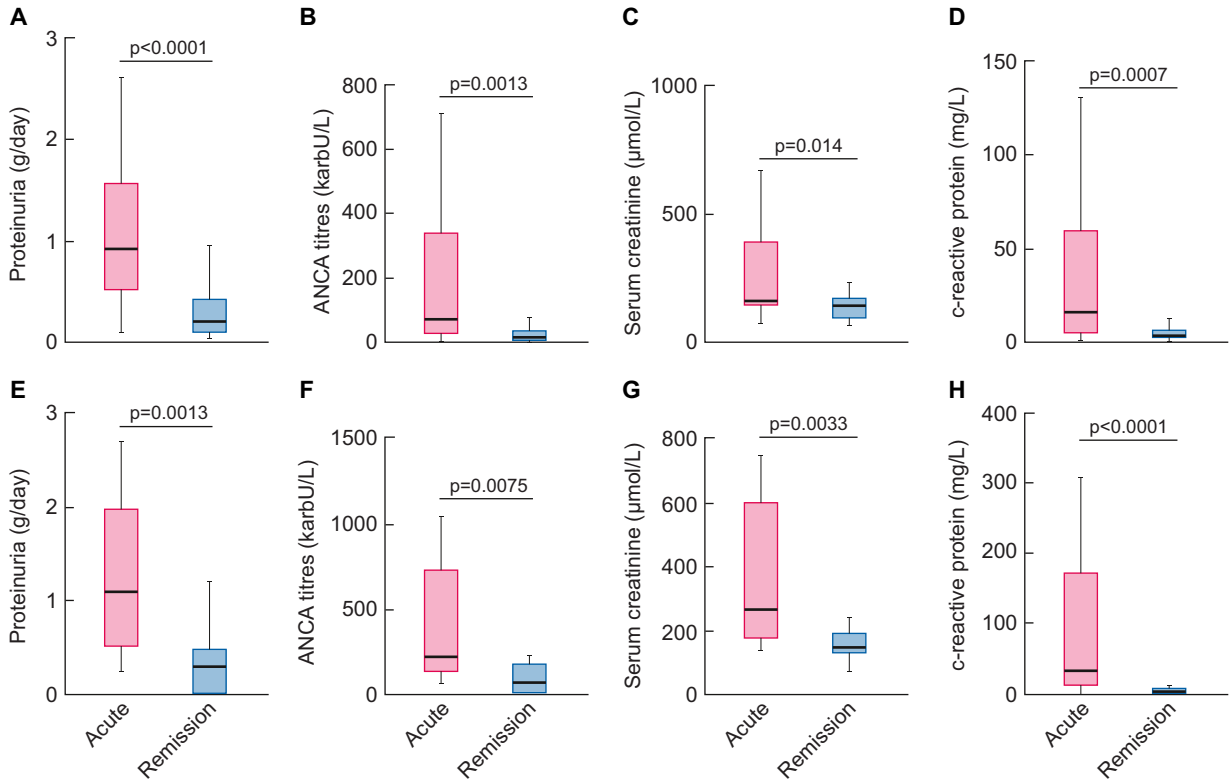


Figure 4: Comparison of clinical parameters among the two groups of AAV patients designed by the decision tree as positive (acute in the legend of the figure corresponding to diagnostic phase) and negative (remission). Designated by the model as acute is a positive in the decision tree and designated by the model as remission is a negative in the decision tree. A, B, C, and D are clinical parameters of inception cohort and E, F, G, and H of validation cohort.

between biomarker levels and other extra-renal involvement were found.

Moreover, we studied whether there was a correlation between the increase in BVAS value and the increase in biomarkers. We found a positive correlation between markers and the score, obtaining statistical significance in uCalprotectin ($P = .039$) and suCD163 ($P = .027$).

DISCUSSION

There is an urgent need to find specific reliable biomarkers to precisely identify renal AAV activity. Our study's results support the role of suCD163 and sCalprotectin as prognostic biomarkers in AAV with renal involvement.

Previous studies suggested sCalprotectin's role as a biomarker of active AAV and described its association with acute glomerular lesions in renal biopsies [3]. Also, sCalprotectin levels were associated with GFR decline in remission patients [8]. In our study we were able to confirm that sCalprotectin rather than uCalprotectin is an accurate biomarker for detecting disease activity. In contrast to the inception cohort, patients of the validation cohort in acute stage presented elevated levels of uCalprotectin compared with remission phase. We hypothesize that this difference is due to the higher acute histological damage (crescentic lesions inception cohort 26% vs validation cohort 48%).

Many studies have revealed the role of suCD163 as a biomarker of active kidney disease in AAV. O'Reilly *et al.* [11], defined an optimal cut-off of suCD163 (0.3 ng/mmol) to detect active renal vasculitis in patients with a known diagnosis. In our study, we found that suCD163 had a slightly inferior LR (4.57) to detect active disease. One explanation could be that in their study most acute patients were recruited at the time of diagnosis presenting a severe disease, compared with our cohort composed of patients with different degrees of disease severity. This may have diluted the differences between patients in the acute phase and those in remission.

The rational of combining sCalprotectin and suCD163 is based on the fact that activation of both neutrophils and macrophages plays a key role in the pathogenesis of the disease. suCD163 is a surrogate marker of the activation of M2 macrophages in kidney, as this molecule's shedding leads to its presence in urine [11]. Macrophages are the most representative cells of crescents and take part in renal fibrosis [17]. Neutrophils cause vascular endothelial damage through degranulation by ANCA binding against MPO/PR3 autoantigens. Glomerular infiltration by macrophages and neutrophils producing calprotectin was associated with high calprotectin levels in AAV [3]. Therefore, suCD163 and sCalprotectin concentration are markers of two different effector cells involved in the pathophysiology of the disease and their use in combination may further improve diagnostic accuracy.

Other studies have evaluated combinations of different biomarkers to improve the predictive value of active vasculitis disease. In the study by Moran *et al.* [18] the combination of suCD163 and uMCP-1 appeared to be helpful in identifying the diagnosis of the subtle flare vasculitis. By means of a recursive tree partitioning applied to the new biomarkers, a better fidelity was obtained by adding proteinuria, improving the LR (19.2). The main limitation was that these findings were not verified on an independent validation cohort.

In our research, the decision tree that combined sCalprotectin and suCD163 presented a high PLR of 17. However, despite its statistical power, it was a model with high specificity (96%) but lower sensitivity (68%). Therefore, we combined both biomarkers with haematuria, obtaining the Model 2 with high sensitivity and a good specificity, and PLR (97%, 90% and 9.7, respectively), which were confirmed in the validation cohort.

Dekkema *et al.* [19] also assessed T-lymphocyte biomarkers, such as usCD25 and ssCD25, combined with suCD163. They found that, although suCD163 was a precise biomarker for detecting active renal vasculitis, some patients with active disease were suCD163 negative. The combination of suCD163, usCD25 and ssCD25 increased the sensitivity compared with suCD163 alone (84.7% versus 72.2%). In our model, suCD163 alone had a sensitivity of 88% in the inception cohort, which increases to 97% when sCalprotectin, suCD163 and haematuria were combined.

Other combinations of proteins have been proposed as biomarkers of active AAV. Kronbichler *et al.* [20] used Salford Predictive Modelar Software to reproduce a biomarker panel involving CRP and urinary MCP-1. With cut-offs of 21.6 mg/dL for the former and 0.53 ng/mL for the latter biomarker, a sensitivity and specificity of 76% was obtained for the combination of both. Other studies have used conditional logistic regression and the Cox model to explore the combination of markers. In this field, Monach *et al.* [21] observed that the combination of CRP, IL-18BP, neutrophil gelatinase-associated lipocalin and sIL-2Rα improved the strength of association with active vasculitis disease.

Of note, in contrast to the O'Reilly study, we did not normalize biomarker values by urinary creatinine concentration, [uCr]. As has been described in the literature [22, 23], normalization by [uCr] can result in an underestimation or an overestimation of biomarker excretion depending on the clinical context. Under non-steady state [uCr] values, such as acute kidney injury, urinary creatinine excretion can vary over time and overestimate the value of the biomarker. Accordingly, we decided to perform our decision tree predictive model with non-normalized biomarkers.

Our study presents the following limitations. First, sCalprotectin and suCD163 are not specific markers of vasculitis. Elevated levels of sCalprotectin and suCD163 have been observed in other diseases, such as lupus nephritis [24, 25]. Therefore, the best utility of these markers and the combination of them with existing biomarkers would be to detect a flare of the disease during follow-up in patients already diagnosed. Secondly, we do not have a biopsy to verify that patients clinically in remission are in histological remission. Thirdly, the implementation of our combinatorial biomarker model to patients clinically in remission, especially in patients with haematuria, would demonstrate its applicability in daily clinical practice. Unfortunately, due to lack of long-term data, we could not apply the model in patients clinically in remission to assess long-term evolution. Finally, the validation cohort is smaller than the inception cohort. The biomarker model could be validated in a larger validation cohort in future projects.

The strength of our study is that although AAV is an uncommon disease and obtaining large cohorts is challenging, we were able to recruit a significant number of patients. Generating new studies with a larger number of patients and with longer follow-up and implementing the new biomarkers in clinical practice is within our prospects.

In conclusion, suCD163 and sCalprotectin are accurate relapse biomarkers in patients with AAV. The information added by the presence of haematuria to these novel biomarkers increases its ability to detect patients in acute or in remission phase. The duration of immunosuppressive maintenance therapy has not yet been defined. Therefore, implementing this

model in clinical practice would help clinicians to guide and individualize treatment in ANCA-associated glomerulonephritis, and to avoid invasive diagnostic procedures such as kidney biopsies.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

ACKNOWLEDGEMENTS

We want to particularly acknowledge patients and Biobank HUB-ICO-IDIBELL (PT20/00171) integrated in the ISCIII Biobanks and Biomodels Platform, Biobank Navarrabiomed (PT13/0010/0051) integrated in the Spanish National Biobank Network and the ISCIII RETICS RedinRen RD16/0009/0003 and CERCA Programme/Generalitat de Catalunya for institutional support.

FUNDING

This study has been funded by Instituto de Salud Carlos III through the grant CM21/00170 and JR21/00059 [co-funded by European Social Fund (ESF investing in your future)]. This work was supported by the Ministerio de Ciencia, Innovación y Universidades (Madrid, Spain) (Grants FIS-ISCIII PI20/00812, co-funded by FEDER funds/European Regional Development Fund—a way to build Europe).

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and in the Supplementary data.

CONFLICT OF INTEREST STATEMENT

JMC is member of the CKJ editorial board.

REFERENCES

1. Jennette JC, Nachman PH. ANCA glomerulonephritis and vasculitis. *Clin J Am Soc Nephrol* 2017;**12**:1680–91.
2. Berden AE, Ferrario F, Hagen EC *et al*. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol* 2010;**21**:1628–36.
3. Pepper RJ, Hamour S, Chavele KM *et al*. Leukocyte and serum S100A8/S100A9 expression reflects disease activity in ANCA-associated vasculitis and glomerulonephritis. *Kidney Int* 2013;**83**:1150–8.
4. Tomasson G, Grayson PC, Mahr AD *et al*. Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis-a meta-analysis. *Rheumatology (Oxford)* 2012;**51**:100–9.
5. Birck R, Schmitt WH, Kaelsch IA *et al*. Serial ANCA determinations for monitoring disease activity in patients with ANCA-associated vasculitis: systematic review. *Am J Kidney Dis* 2006;**47**:15–23.
6. Ometto F, Friso L, Astorri D. *et al*. Calprotectin in rheumatic diseases. *Exp Biol Med* 2017;**242**:859–73.
7. Pepper RJ, Draibe JB, Caplin B. *et al*. Association of serum calprotectin (S100A8/A9) level with disease relapse in proteinase 3–antineutrophil cytoplasmic antibody–associated vasculitis. *Arthritis Rheumatol* 2017;**69**:185–93.
8. Martínez Valenzuela L, Draibe J, Quero Ramos M *et al*. Calprotectin as a smoldering activity detection tool and renal prognosis biomarker in ANCA associated vasculitis. *PLoS One* 2018;**13**:1–13.
9. Heller F, Frischmann S, Grünbaum M *et al*. Urinary calprotectin and the distinction between prerenal and intrinsic acute kidney injury. *Clin J Am Soc Nephrol* 2011;**6**:2347–55.
10. Møller HJ, Nielsen MJ, Maniecki MB *et al*. Soluble macrophage-derived CD163: a homogenous ectodomain protein with a dissociable haptoglobin-hemoglobin binding. *Immunobiology* 2010;**215**:406–12.
11. O'Reilly VP, Wong L, Kennedy C *et al*. Urinary soluble CD163 in active renal vasculitis. *J Am Soc Nephrol* 2016;**27**:2906–16.
12. Aendekerk JP, Timmermans SAMEG, Busch MH. *et al*. Urinary soluble CD163 and disease activity in biopsy-proven ANCA-associated glomerulonephritis. *Clin J Am Soc Nephrol* 2020;**15**:1740–8.
13. Jennette JC, Falk RJ, Bacon PA. *et al*. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013;**65**:1–11.
14. Berden A, Göçeroglu A, Jayne D. *et al*. Diagnosis and management of ANCA associated vasculitis. *BMJ* 2012;**344**:e26.
15. Mukhtyar C, Lee R, Brown D. *et al*. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis* 2009;**68**:1827–32.
16. Yates M, Watts RA, Bajema IM. *et al*. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. *Ann Rheum Dis* 2016;**75**:1583–94.
17. Vegting Y, Vogt L, Anders HJ *et al*. Monocytes and macrophages in ANCA-associated vasculitis. *Autoimmun Rev* 2021;**20**:102911.
18. Moran SM, Monach PA, Zgaga L. *et al*. Urinary soluble CD163 and monocyte chemoattractant protein-1 in the identification of subtle renal flare in anti-neutrophil cytoplasmic antibody-associated vasculitis. *Nephrol Dial Transplant* 2020;**35**:283–91.
19. Dekkema GJ, Abdulahad WH, Bijma T. *et al*. Urinary and serum soluble CD25 complements urinary soluble CD163 to detect active renal anti-neutrophil cytoplasmic autoantibody-associated vasculitis: a cohort study. *Nephrol Dial Transplant* 2019;**34**:234–42.
20. Kronbichler A, Kerschbaum J, Gründlinger G *et al*. Evaluation and validation of biomarkers in granulomatosis with polyangiitis and microscopic polyangiitis. *Nephrol Dial Transplant* 2016;**31**:930–6.
21. Monach PA, Warner RL, Lew R. *et al*. Serum biomarkers of disease activity in longitudinal assessment of patients with ANCA-associated vasculitis. *ACR Open Rheumatol* 2022;**4**:168–76.
22. Waikar SS, Sabbisetti VS, Bonventre JV. Normalization of urinary biomarkers to creatinine during changes in glomerular filtration rate. *Kidney Int* 2010;**78**:486–94.
23. Tang KW, Toh QC, Teo BW. Normalisation of urinary biomarkers to creatinine for clinical practice and research – When and why. *Singapore Med J* 2015;**56**:7–10.
24. Haga HJ, Brun JG, Berntzen HB *et al*. Calprotectin in patients with systemic lupus erythematosus: relation to clinical and laboratory parameters of disease activity. *Lupus* 1993;**2**:47–50.
25. Endo N, Tsuboi N, Furuhashi K. *et al*. Urinary soluble CD163 level reflects glomerular inflammation in human lupus nephritis. *Nephrol Dial Transplant* 2016;**31**:2023–33.