



HAL
open science

Serum calprotectin and renal function decline in ANCA-associated vasculitides: a post hoc analysis of MAINRITSAN trial

Xavier Romand, Marie H el ene Paclet, Minh Vu Chuong, Philippe Gaudin, Christian Pagnoux, Lo ic Guillevin, Benjamin Terrier, Athan Baillet

► To cite this version:

Xavier Romand, Marie H el ene Paclet, Minh Vu Chuong, Philippe Gaudin, Christian Pagnoux, et al.. Serum calprotectin and renal function decline in ANCA-associated vasculitides: a post hoc analysis of MAINRITSAN trial. *RMD Open*, 2023, 9 (4), pp.e003477. 10.1136/rmdopen-2023-003477. hal-04724529

HAL Id: hal-04724529

<https://cnrs.hal.science/hal-04724529v1>




Submitted on 6 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destin ee au d ep ot et  a la diffusion de documents scientifiques de niveau recherche, publi es ou non,  emanant des  tablissements d'enseignement et de recherche fran ais ou  trangers, des laboratoires publics ou priv es.

ORIGINAL RESEARCH

Serum calprotectin and renal function decline in ANCA-associated vasculitides: a post hoc analysis of MAINRITSAN trial

Xavier Romand ¹, Marie H el ene Paclet,¹ Minh Vu Chuong,¹ Philippe Gaudin,¹ Christian Pagnoux,² Lo c Guillevin,^{3,4} Benjamin Terrier ^{3,4}, Athan Baillet ¹

To cite: Romand X, Paclet MH, Chuong MV, *et al*. Serum calprotectin and renal function decline in ANCA-associated vasculitides: a post hoc analysis of MAINRITSAN trial. *RMD Open* 2023;**9**:e003477. doi:10.1136/rmdopen-2023-003477

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/rmdopen-2023-003477>).

BT and AB contributed equally.

BT and AB are joint senior authors.

Received 7 July 2023
Accepted 2 October 2023



  Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Dr Xavier Romand;
xromand@chu-grenoble.fr

ABSTRACT

Objective Serum calprotectin appears to be an interesting biomarker associated with renal vascular disease activity in antineutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV). The aim of this study was to assess whether serum calprotectin levels can predict decline in renal function in AAV patients receiving maintenance therapy.

Methods Serum calprotectin levels were assessed at inclusion and month 6 in AAV patients, in complete remission after induction therapy, randomly assigned to rituximab or azathioprine. Renal function decline was defined as a 25% decrease in estimated glomerular filtration rate (eGFR) and a change in the eGFR category, or a decrease of 15 mL/min/1.73 m². Relapse was defined as a Birmingham Vasculitis Activity Score >0 attributable to active vasculitis.

Results Seventy-six AAV were included. Serum calprotectin increased from baseline to month 6 in patients with renal function decline (7940 (–226.0, 28 691) ng/ml vs –4800 (–18 777, 3708) ng/ml; p<0.001). An increase of calprotectin level was associated with a higher risk of subsequent renal function decline even after adjustment (OR 6.50 (95% CI 1.7 to 24.9) p=0.006). A significantly higher risk of relapse was observed in proteinase 3- AAV patients with an increase of serum calprotectin levels (OR 5.6 (95% CI 1.0 to 31.2), p=0.03).

Conclusion An increase in serum calprotectin by month 6 compared with inclusion during remission-maintenance therapy in AAV was associated with a higher risk of renal function decline in the following 12 months.

Trial registration number NCT00748644.

INTRODUCTION

Antineutrophil cytoplasm antibody (ANCA)-associated vasculitides (AAV) are severe diseases with life-threatening complications, including renal function impairment leading to end-stage renal disease (ESRD). Predicting relapses and complications in patients with AAV remains a challenge. Proteinase 3 (PR3)-ANCA at diagnosis and persistent positive

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Antineutrophil cytoplasm antibody (ANCA) monitoring is widely used as an indicator of disease activity and to predict ANCA-associated vasculitides (AAV) relapse, but the ability of this biomarker to predict relapse remains limited.
- ⇒ Renal relapse remains difficult to predict in AAV patients, leading to the need of a tight monitoring of the renal function.
- ⇒ Serum calprotectin levels monitoring during induction therapy of AAV predicts the risk of relapse in patients with AAV.

WHAT THIS STUDY ADDS

- ⇒ An increase in serum calprotectin during the first 6 months following the initiation of maintenance therapy in AAV patients was associated with a higher risk of subsequent renal function deterioration.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ These results suggest that serum calprotectin is a biomarker of interest to monitor AAV activity and identify patients at risk of relapse during maintenance treatment.
- ⇒ These findings reinforce the contribution of calprotectin in the pathophysiology of AAV-associated organ damage.

ANCA at the time of switching from induction to maintenance therapy are associated with a higher risk of relapse.^{1,2} However, the usefulness of monitoring ANCA titres as a marker of relapse in clinical practice remains controversial.^{3,4} Renal relapse, creatine clearance at baseline and proportion of normal glomeruli on kidney biopsy are associated with a higher risk of ESRD in AAV.^{5–7} However, renal relapse remains difficult to predict in AAV patients, leading to the need of a tight monitoring of the renal function.⁵ The discovery of new biomarkers predictive of relapse could help

physicians optimise maintenance therapy, especially its duration, thereby avoiding unnecessary treatment and side effects and life-threatening complications through an adapted and personalised therapeutic strategy.

Calprotectin (protein S100A8/A9) is an alarmin released by neutrophils and monocytes during inflammation. Calprotectin amplifies inflammation by activating the Toll-like receptor 4 (TLR4)⁸ and advanced glycation end-products receptor (RAGE), leading to endothelial and mesangial cell damage in the kidney.^{9,10} Calprotectin correlates with disease activity and severe outcomes in multiple inflammatory disorders, such as chronic inflammatory rheumatic diseases^{11,12} and more recently SARS-CoV-2 infection.^{13,14}

In active AAV patients, calprotectin is elevated in the serum and highly expressed on neutrophils and monocytes cell surface, and in active glomerular lesion.^{15–18} TLR4 and RAGE are also upregulated in glomeruli, tubulointerstitial tissues and infiltrating cells in the kidney of AAV patients.^{16,19} Page *et al* published data suggesting that the calprotectin-activated TLR4/RAGE axis is an important pathway in AAV as it leads to the release of proinflammatory cytokines from peripheral blood mononuclear cells into the kidney, causing kidney damage.¹⁶

An increased level of serum calprotectin in patients with active AAV receiving induction of remission therapy by cyclophosphamide or rituximab was observed in patients with a higher risk of relapse.²⁰ There is currently scarce knowledge about the usefulness of the monitoring of serum calprotectin during remission maintenance therapy.^{16,21} Patients in remission have lower circulating calprotectin levels than active patients.^{16,21} It appears that patients in remission who relapsed within a short period of time after calprotectin measurement tended to have a higher level of calprotectin than patients who remained in remission.¹⁶ The limited number of patients included in these studies has not allowed to draw conclusions on the relevance of calprotectin monitoring during remission in patients with AAV.^{16,21}

We propose in this study to assess the predictive value of serum calprotectin in a large cohort of AAV patients in remission. Since calprotectin plays a role in the development of glomerulonephritis and reflects the severity of the inflammatory process, we hypothesised that serum calprotectin changes in AAV patients in remission could be associated with renal function decline and disease relapse in the following 12 months.

METHODS

Study design and patients

Selected stored serum samples from patients enrolled in the Maintenance of Remission using Rituximab in Systemic ANCA-associated Vasculitis (MAINRITSAN) trial, whose design details have been reported previously, were retrieved and analysed.²² Briefly, calprotectin assays were performed in sera of patients with granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA)

or renal-limited ANCA-AAV, in complete remission (Birmingham Vasculitis Activity Score (BVAS) of 0) after induction therapy with cyclophosphamide and included in the MAINRITSAN trial. After inclusion, patients were randomised to either receive rituximab (RTX) or azathioprine (AZA) to maintain remission, during 18 and 22 months, respectively.

Serum calprotectin assessment

Sera from AAV patients were prospectively collected at inclusion (month 0, ie, on achievement of remission) and month 6 after initiation of maintenance therapy and stored in aliquots at -80°C at the Cochin Hospital (Paris, France). Calprotectin serum concentrations were assessed with an in vitro diagnostic (IVD) ELISA assay kit according to the manufacturer's method (IDK Calprotectin ELISA kit K6935, Immunodiagnostik). Serum samples were diluted 1:100 with dilution buffer samples. If the concentration of calprotectin was above the measurement range (3.9–250 ng/mL), the serum was further diluted and assayed again. The serum calprotectin assay was centralised and performed by the same technician.

Study parameters

Significant renal function decline was based on the National Institute for Health and Care Excellence (NICE) 2015 guideline definition as (1) a decrease in estimated glomerular filtration rate (eGFR) $\geq 25\%$ and a change in eGFR category or (2) a decrease in eGFR $\geq 15\text{ mL/min/1.73 m}^2$ occurring during the follow-up.²³ Serum creatinine levels were assessed every 3 months and eGFR was calculated using the modification of diet in renal disease equation. Disease relapse was defined as the reappearance or worsening of manifestations attributable to active vasculitis (BVAS > 0) and (1) involvement of at least one major organ, a life-threatening manifestation or both (major relapse) or (2) reappearance or worsening of manifestations attributable to active vasculitis, not corresponding to a major relapse but requiring mild treatment intensification (minor relapse). A calprotectin ratio (calprotectin M6/M0 ratio) was calculated by dividing the calprotectin level at month 6 (M6) by the calprotectin level at inclusion (M0). The calprotectin difference was calculated by subtracting the calprotectin level at month 6 (M6) by the calprotectin level at inclusion (M0). An increase in calprotectin level was defined as a calprotectin M6/M0 ratio > 1 . Disease relapse and significant renal function decline were retained if occurring between M6 and M18 of follow-up. The following data were collected at inclusion and used in this study: age, gender, disease status (newly diagnosed vs relapse), AAV clinical phenotype (GPA vs MPA), ANCA subtype at diagnosis (PR3-ANCA vs myeloperoxidase (MPO)-ANCA), ANCA evolution after induction of remission, maintenance treatment (RTX vs AZA), neutrophil count, C reactive protein (CRP) level, dose of prednisone, eGFR and Vasculitis Damage Index (VDI).

Objectives

The primary objective was to evaluate if serum calprotectin level variation by month 6 during maintenance therapy was associated with renal function decline within the following 12 months.

Secondary objectives were (1) to determine if calprotectin level could predict relapse within the 12 months follow-up and (2) to assess whether calprotectin levels correlated with inflammatory parameters.

Statistical analysis

Statistical analysis was performed using R software V.3.5.1 and GraphPad Prism V.9.0.0 (GraphPad Software, San Diego, USA). Mann-Whitney tests and t-tests were used, respectively, to compare two groups with non-normally or normally distributed data. The D'Agostino-Pearson test was used to determine the normal distribution of data. Continuous variables were expressed as mean±SD for normally distributed or median and IQR for non-normally distributed variables. Correlations between calprotectin level and neutrophil count, CRP level, VDI, eGFR and age were assessed with non-parametric Spearman's correlation tests (r_s). Calprotectin variation and risk of renal function decline and relapse were investigated using (1) Kaplan-Meier survival analysis and the Gehan-Beslow-Wilcoxon test, (2) OR calculation and χ^2 test. The last observation carried forward technique was applied to impute missing data for eGFR occurring during the follow-up. Age, sex, disease status (relapser), maintenance treatment (rituximab), ANCA status at diagnosis, VDI at inclusion, neutrophil count at inclusion, eGFR at inclusion, CRP at inclusion, ANCA status at diagnosis, ANCA evolution after induction of remission, prednisone dose at inclusion, and an increase of calprotectin were evaluated as potential factors predictive of renal function deterioration and relapse using univariate logistic regression analysis. Variables with $p < 0.2$ in univariate analysis were selected for multivariate analysis. Moreover, ANCA status and maintenance treatment (rituximab), factors known as relevant in the literature,^{1 24 25} were also added in the multivariate model. Multivariate logistic regression was performed to build the final combined model. A $p < 0.05$ was considered significant.

RESULTS

Baseline characteristics

Out of the 115 patients included between October 2008 and June 2010 in the MAINRITSAN trial, 76 patients included had serum samples available for calprotectin determination at both inclusion and month 6 and were included in this study (online supplemental figure S1). Mean age of patients was 55.7±13.1 years, 50% were female and 69.8% had GPA. Mean eGFR was 64.5±31.8 mL/min/1.73 m² and 22 patients (28.9%) had moderately to severely decrease of eGFR (eGFR<45 mL/min/1.73 m²) at month 0. Other baseline characteristics of these 76 AAV patients are described in [table 1](#). There

was no statistically significant difference in baseline characteristics between included and non-included patients (data not shown).

Serum calprotectin level in AAV patients in remission

Median serum calprotectin level was similar at month 0 and month 6 (median (IQR), 11 250 (5498–26 475) vs 10 450 (3700–31 525) ng/mL; $p=0.53$). Patients with positive ANCA at inclusion had similar levels of calprotectin compared with ANCA-negative patients (9411 (4285–24 725) vs 13 000 (5928–28 250) ng/mL; $p=0.49$). Serum calprotectin at month 0 was associated with higher neutrophil counts ($r_s=0.25$, $p=0.03$), higher CRP level ($r_s=0.27$, $p=0.03$) but not with gender, age, disease status (newly diagnosed vs relapse), AAV clinical phenotype (GPA vs MPA), ANCA serotype (PR3 vs MPO), maintenance treatment (RTX vs AZA) and VDI.

Serum calprotectin variation at month 6 and renal function decline

Nineteen of the 76 studied patients (25.0%) (12 in the AZA group and seven in the RTX arm, including 15 PR3-ANCA patients) developed significant renal function decline between month 6 and month 18. Of these, five patients (26.3%) also had a relapse during this period. The calprotectin level at month 0 was significantly lower in patients developing significant renal function decline (5283 (3406, 12 100) vs 14 500 (7067, 33 300) ng/mL, $p=0.002$). In contrast, the serum calprotectin level at month 6 was not different. Calprotectin increased from month 0 to 6 in patients with renal function decline (7940 (–226.0, 28 691) ng/mL, calprotectin ratio M6/M0: 2.86 (0.94, 4.27)) and decreased in those without deterioration of renal function between month 6 and month 18 (–4800 (–18 777, 3708) ng/mL, calprotectin ratio M6/M0: 0.57 (0.22, 1.78); $p < 0.001$) ([figure 1A](#)). An increase in serum calprotectin during the first 6 months of maintenance therapy, observed in 33 patients (43.4%), predicted a significant deterioration of renal function within the following 12 months in univariate analysis (OR 5.60 (95% CI 1.8 to 17.9; $p=0.002$) ([figure 1B](#), [table 2](#)). eGFR at inclusion, gender, ANCA serotype, rituximab and calprotectin level increase were included in the multiple logistic regression analysis. Multivariate logistic regression showed that male (OR 0.14 (95% CI 0.03 to 0.68); $p=0.01$) and serum calprotectin level (OR 6.50 (95% CI 1.69, 24.9) $p=0.006$) were independently associated with subsequent renal function decline ([table 2](#)). The area under the receiver operating characteristic (ROC) curve for predicting renal function worsening using the ratio calprotectin M6/M0 was 0.77 (95% CI 0.66 to 0.88, $p < 0.001$). After determining the optimal cut-off for calprotectin ratio M6/M0 at 2.07 using the Youden index, sensitivity was 68.4% (95% CI 46.0% to 84.6%), specificity 79.0% (95% CI 66.7% to 87.5%), positive predictive value 52.0% (95% CI 31.8% to 71.7%), negative predictive value 88.2% (95% CI 75.4% to 95.1%), positive likelihood ratio of 3.3 (95% CI 1.8 to 5.9) and

Table 1 Baseline characteristics of ANCA-associated vasculitis patients from the MAINRITSAN trial eligible for this study

Patient characteristics	All patients n=76	eGFR decline* n=19	No eGFR decline* n=57	P value
Age (years)	55.7 (13.1)	55.0 (11.0)	56.0 (13.8)	0.8
Female (n (%))	38 (50.0)	14 (73.7)	24 (42.1)	0.02
Granulomatosis with polyangiitis (n (%))	53 (69.8)	15 (78.9)	38 (66.7)	0.3
Microscopic polyangiitis (n (%))	19 (25.0)	3 (15.8)	16 (28.1)	0.3
Renal-limited ANCA-associated vasculitis (n (%))	4 (5.2)	1 (5.3)	3 (5.3)	1.0
Disease status: newly diagnosed (n (%))	57 (75.0)	15 (78.9)	42 (73.7)	0.6
ANCA-positivity at inclusion (n (%))	50 (65.8)	12 (63.2)	32 (56.1)	0.6
C reactive protein (mg/L)†	2.5 [1.0 ; 5.0]	2.7 [1.0 ; 5.2]	2.3 [1.0 ; 5.0]	0.7
C reactive protein elevated (n (%))‡	16 (21.1)	5 (27.8)	11 (19.7)	0.5
Serum calprotectin at inclusion (ng/mL)	11250 [5498; 26475]	5283 [3406; 12 100]	14500 [7067; 33 300]	0.002
Serum calprotectin at month 6 (ng/mL)	10450 [3700; 31 525]	18000 [4574; 39 600]	7148 [3058; 27 500]	0.2
eGFR (mL/min/1.73 m ²)	64.5 (31.8)	75.9 (40.3)	60.7 (27.7)	0.07
Prednisone (mg/day)	17.0 [12.5; 20.0]	15.0 [13.0; 17.5]	20.0 [12.5; 20.0]	0.5
Rituximab remission treatment (n (%))	37 (48.7)	7 (36.9)	30 (52.6)	0.2
Neutrophil count (10 ⁹ /L)	5.3 [3.7; 7.9]	5.5 [2.8; 9.3]	5.3 [4.0; 7.4]	0.8
Vascular Disease Index	2 [1; 3]	2.0 [1; 3]	2.0 [1; 3]	1.0

†C reactive protein was missing for two patients.

*estimated Glomerular Filtration Rate (eGFR) decline was defined as a decrease in eGFR \geq 25% and a change in eGFR category or a decrease in eGFR \geq 15 ml/min/1.73m² occurring during the follow-up ‡Neutrophil count was missing for three patients. Normally distributed continuous data were described as mean (SD) and non-normally distributed variables as median (Q1; Q3). t-test and Mann-Whitney U test for continuous data and χ^2 test for dichotomous data were used.

ANCA, antineutrophil cytoplasm antibody; eGFR, estimated glomerular filtration rate; MAINRITSAN, Maintenance of Remission using Rituximab in Systemic ANCA-associated Vasculitis.

negative likelihood ratio of 0.4 (95% CI 0.2 to 0.8) to predict subsequent renal function decline (figure 1C). Similar results were observed in PR3-ANCA patients

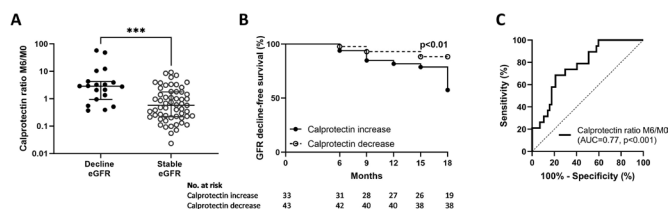


Figure 1 Serum calprotectin change predicting renal function decline in ANCA-associated vasculitis patients. (A) Calprotectin ratio M6/M0 in AAV patients with renal function decline (decline estimated glomerular filtration rate (eGFR)) or not (stable) between month 6 and month 18. Data are shown as median with IQR. Mann-Whitney U test, ***p<0.001. (B) Kaplan-Meier survival curves patients with an increase in serum calprotectin at month 6 (solid line) or not (dotted line) remaining without eGFR decline between month 6 and month 18. The time is the number of months without the occurrence of renal function decline. Renal function decline was retained if it occurred between month 6 and month 18 of follow-up. Gehan-Beslow-Wilcoxon test. (C) Receiver operating characteristic curves of the predictive value of eGFR decline of calprotectin ratio M6/M0. ANCA, antineutrophil cytoplasm antibody; AUC, area under the curve.

(online supplemental figure S2 and online supplemental table 1).

Serum calprotectin variation at month 6 was independent of age, gender, disease status (newly diagnosed vs relapse), AAV clinical phenotype (GPA vs MPA), ANCA serotype (PR3 vs MPO), increased CRP, and maintenance treatment used (RTX vs AZA). Calprotectin level was not correlated with eGFR and was not significantly different in patients with or without haematuria. We observed no difference in patient's characteristics in patients with calprotectin increase without renal function decline (online supplemental table S2 and S3).

Serum calprotectin variation by month 6 and risk of disease relapse

Ten patients (13.1%) (seven in the AZA group and three in the RTX arm, including eight PR3-ANCA patients) experienced a relapse between month 6 and month 18. The serum calprotectin levels at month 0 and 6 were not significantly different between relapsing patients and those without relapse.

Serum calprotectin level increased from month 0 to 6 in relapsing patients (5578 (–3402, 46 338) ng/mL), whereas a decline was observed in patients without subsequent disease relapse (–3100 (–14 975, 7594) ng/mL, p=0.04). Calprotectin ratio M6/M0 was not increased in relapsing patients (2.2 (0.45, 6.3) vs 0.71 (0.23, 2.6),

Table 2 Univariate and multivariate logistic regression analysis of renal function decline in AAV patient

Factors	Univariable analysis OR (95% CI)	P value	Multivariable models* OR (95% CI)	P value	Multivariable models† OR (95% CI)	P value
Male	0.26 (0.08 to 0.82)	0.02	0.14 (0.02 to 0.56)	0.007	0.14 (0.03 to 0.68)	0.01
Age	0.99 (0.96 to 1.03)	0.78				
Rituximab	0.53 (0.18 to 1.53)	0.24			0.40 (0.10 to 1.59)	0.19
Relapser	0.75 (0.21 to 2.61)	0.64				
MPO positivity at diagnostic	0.35 (0.09 to 1.34)	0.12	0.38 (0.76 to 1.94)	0.25	0.31 (0.06 to 1.67)	0.17
VDI at inclusion	1.03 (0.76 to 1.40)	0.84				
Neutrophil count at inclusion	1.00 (0.99 to 1.00)	0.64				
CRP at inclusion	1.00 (0.89 to 1.13)	0.96				
eGFR at inclusion	1.02 (0.99 to 1.04)	0.08	1.02 (1.00 to 1.05)	0.07	1.02 (1.00 to 1.05)	0.07
ANCA positivity at inclusion	0.85 (0.28 to 2.53)	0.77				
Negativation ANCA at remission	0 to64 (0 to21 to 1.94)	0.43				
Prednisone at inclusion	0.98 (0.90 to 1.05)	0.53				
Calprotectin increase	5.6 (1.76 to 17.87)	0.004	6.16 (1.64 to 23.06)	0.007	6.50 (1.69 to 24.9)	0.006

Logistic regression models were applied for univariate and multivariate analysis.

Bold values indicate statistical significance

*P values are from a multivariate logistic regression model with factors including sex, eGFR at inclusion, MPO positivity at diagnostic and calprotectin increase.

†P values are from a multivariate logistic regression model with factors including sex, eGFR at inclusion, calprotectin increase, MPO positivity at diagnostic, rituximab.

AAV, associated renal vasculitis; ANCA, antineutrophil cytoplasm antibodies; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; MPO, myeloperoxidase; VDI, Vascular Disease Index.

$p=0.08$) (figure 2A). An increase in serum calprotectin, occurred in 33 patients (33/76, 43.4%) but was not associated in the whole study population (OR 3.6 (95% CI

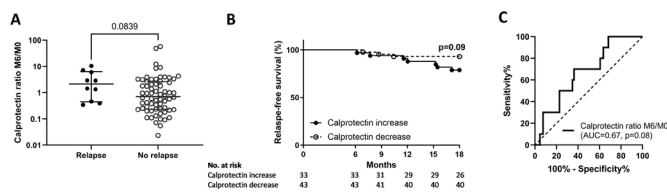


Figure 2 Serum calprotectin change in ANCA-associated vasculitides (AAV) patients and risk of relapse. (A) Calprotectin ratio month 6/month 0 in AAV patient relapsing or not between month 6 and month 18. Data are shown as median with IQR. Mann-Whitney U test. (B) Kaplan-Meier survival curves of AAV patients with an increase in serum calprotectin at month 6 (solid line) or not (dotted line) remaining relapse-free between month 6 and month 18. The time is the number of months of remission during 18 months of follow-up. Disease relapse was retained if it occurred between month 6 and month 18 of follow-up. (A) Gehan-Beslow-Wilcoxon test was performed. (C) Receiver operating characteristic curves of the predictive value of relapse of serum calprotectin ratio month 6/month 0. ANCA, antineutrophil cytoplasm antibody.

0.9 to 15.1), $p=0.07$) with disease relapse (figure 2B, table 3). The association was statistically significant in AAV patients with PR3-ANCA positive at diagnosis (OR 5.6 (95% CI 1.0 to 31.2, $p=0.03$)) in contrast to patient with MPO-ANCA (OR 1.1 (95% CI 0.06 to 20.1, $p=0.9$). After adjustment calprotectin increase was not significantly associated with relapse (OR 3.20 (95% CI 0.60, 16.52, $p=0.12$) (table 3). The area under the ROC curve of calprotectin ratio M6/M0 was 0.67 ($p=0.08$) to predict disease relapse (figure 2C).

DISCUSSION

This study shows that an increase in serum calprotectin during the first 6 months following the initiation of maintenance therapy in AAV patients identifies patients at risk of subsequent renal function deterioration. Calprotectin variation was also associated with risk of disease relapse but only in PR3-ANCA patients at diagnosis.

Pepper *et al* demonstrated that an increase in serum calprotectin levels at 2 or 6 months, compared with baseline, in active PR3-ANCA patients treated with rituximab for induction of remission is associated with a higher risk of relapse during 18 months of follow-up, whereas

Table 3 Univariate and multivariate logistic regression analysis of total relapse in AAV patient

Factors	Univariable analysis		Multivariable models*		Multivariable models†	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Male	0.63 (0.16 to 2.43)	0.50				
Age	1.00 (0.95 to 1.06)	0.92				
Rituximab	0.40 (0.10 to 1.70)	0.22			0.12 (0.18 to 0.85)	0.03
Relapser	0.30 (0.04 to 2.51)	0.26				
PR3 positivity at diagnostic	2.29 (0.45 to 11.65)	0.32			4.38 (0.60 to 31.70)	0.14
VDI at inclusion	0.90 (0.69 to 1.38)	0.64				
Neutrophil count at inclusion	1.00 (0.99 to 1.00)	0.08	1.00 (0.99 to 1.00)	0.11	1.00 (1.00 to 1.00)	0.03
CRP at inclusion	1.01 (0.87 to 1.18)	0.88				
eGFR at inclusion	1.02 (1.00 to 1.04)	0.11	1.01 (0.99 to 1.04)	0.19	1.01 (0.99 to 1.04)	0.23
ANCA positivity at inclusion	0.82 (0.21 to 3.21)	0.78				
Negativation ANCA at remission	0.94 (0.24 to 3.65)	0.93				
Prednisone at inclusion	0.97 (0.89 to 1.08)	0.61				
Calprotectin increase	3.59 (0.85 to 15.15)	0.08	2.60 (0.56 to 12.15)	0.22	3.20 (0.60 to 16.52)	0.14

Logistic regression models were applied for univariate and multivariate analysis.
 Bold values indicate statistical significance.
 *P values are from a multivariate logistic regression model with factors including neutrophil count at inclusion, eGFR at inclusion and calprotectin increase.
 †P values are from a multivariate logistic regression model with factors including neutrophil count at inclusion, eGFR at inclusion, rituximab, PR3 positivity at diagnostic and calprotectin increase.
 AAV, associated renal vasculitis; ANCA, antineutrophil cytoplasm antibodies; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; MPO, myeloperoxidase; VDI, Vascular Disease Index.

baseline calprotectin level did not predict subsequent relapse.²⁰ In contrast, a previous small study including 27 patients in persistent remission failed to demonstrate that serum calprotectin levels identified patients that relapsed during 24 months of follow-up.²¹ Here, on a larger population, we show that calprotectin variation may predict the risk of subsequent disease relapse in PR3-ANCA patients. Monitoring the evolution of calprotectin seems particularly relevant in this population known to be at high risk of relapse,^{1,26} thus helping the physician to identify the most at-risk patients who will then be able to have a tightened follow-up.

Martinez Valenzuela *et al* showed that during remission, serum calprotectin at inclusion was significantly higher in AAV patients with subsequent eGFR decrease and failed to predict relapse, which contrasts with our findings.²¹ In this study, the definition of eGFR worsening was less stringent, since a decrease >2 mL/min/year (compared with a physiological eGFR loss of 1 mL/min/year) was sufficient to determine renal function decline. A small variation in eGFR could be explained by analytical and within-subject biological variability. A decrease of eGFR by 13.1% could be considered as

a clinically relevant renal function deterioration that cannot be explained by normal biological variability or analytical variation.²⁷ Therefore, we decided to use a stringent definition of renal function decline following the NICE 2015 guideline. Importantly, serum calprotectin was not correlated with eGFR value as previously described,²¹ allowing the use of this biomarker in AAV patients with renal impairment.

In our study, the serum calprotectin level was independent of ANCA status at remission suggesting that calprotectin could be useful in addition to ANCA to predict the subsequent risk of disease relapse.³ As in the active phase of the disease,²⁰ calprotectin levels correlated with inflammatory parameters such as neutrophil count and CRP level in AAV patients in remission. CRP is a widely used biomarker to assess disease activity in AAV, but its predictive value for disease relapse is limited since its elevation remains very acute. In contrast, the CRP level during remission does not predict relapse in GPA patients²⁸ which tempered its interest during AAV monitoring. Calprotectin seems to reflect the subclinical inflammation occurring during the remission phase and leading to AAV relapse and renal damage.

C5a receptor inhibitor, avacopan, by blocking neutrophil activation and chemoattraction, induced remission and improve kidney function in AAV patients.²⁹ The complement component C5a mediates the release of calprotectin from neutrophils and induced glomerulonephritis in an animal model.^{30 31} Calprotectin could be an interesting candidate biomarker of neutrophil activation for monitoring the efficiency of avacopan treatment.

We observed that female gender was associated with an increased risk of occurrence of renal function decline. These results contrast with previous studies showing that male gender was associated with more severe vasculitis and more renal involvement.^{32 33} Other studies did not show an association.^{1 34} The impact of gender on ANCA vasculitis severity remains controversial. Further studies are needed to clarify this relationship.

The major strength of our study is to assess for the first-time serum calprotectin in a large cohort of AAV patients in remission in the RTX era. Also, in contrast with previous studies, we used a stringent definition of renal function decline. The major limitation of this study is the low number of disease relapses (n=10) and renal function decline (n=19) to perform a multivariate logistic regression analysis. Therefore, the logistic regression model used in this study may overfit the data with the risk that these results may not be generalisable to the entire population. At baseline, the eGFR decline group had a statistical tendency to have a higher eGFR than the group without eGFR decline. Although no statistically significant difference was found, we cannot exclude that this difference in eGFR at baseline is not a confounding factor that could bias the observed results due to an increased probability of being able to observe a decline in renal function in the eGFR decline group. Nevertheless, the association between changes in calprotectin and a decline in renal function persisted even after adjustment for eGFR at inclusion. We were unable to confirm our results using a replication cohort, as AAV is a rare disease, it is difficult to obtain large cohorts of patients.

Overall, an increase in serum calprotectin at month 6 after initiation of maintenance therapy was independently associated with a risk of subsequent renal function decline in AAV patients in the following 12 months. Serum calprotectin reflects the subclinical inflammation occurring during remission and could represent an interesting biomarker to predict subsequent renal function deterioration and relapses during maintenance therapy. These results need to be confirmed in a larger validation cohort.

Author affiliations

¹CNRS, UMR 5525, T-RAIG, TIMC, CHU Grenoble Alpes, Université Grenoble Alpes, Grenoble, France

²Vasculitis clinic, Mount Sinai Hospital, Toronto, Ontario, Canada

³Université de Paris, Paris, France

⁴Department of Internal Medicine, National Referral Center for Rare Systemic Autoimmune Diseases, Hôpital Cochin APHP-Centre Université Paris (CUP), Paris, France

Acknowledgements We would like to thank Anaïs Courtier for his preliminary assistance in the statistical analysis. This article is based on the thesis by Xavier Romand defended on 04 February 2022.

Contributors XR was responsible for data analysis. XR wrote the first draft of the manuscript. XR, MHP and MVC collected the data and performed the calprotectin assay. PG, CP, LG, BT and AB contributed to the conception and study design. CP, LG and BT provided and cared for the study patients. All authors contributed substantially to the interpretation of the data. All authors critically reviewed the work for important intellectual content. All authors approved the final version. XR is the guarantor.

Funding The MAINRITSAN trial was supported by a grant from the Hospital Clinical Research Program (Programme Hospitalier de Recherche Clinique), French Ministry of Health (2008-002846-51).

Competing interests Roche has provided rituximab for the MAINRITSAN trial. MAINRITSAN trial was supported by the French Ministry of Health. XR reported personal fees from Abbvie, MSD, UCB, Novartis, Celltrion Healthcare, Galapagos and CHUGAI pharma for presentations, speakers or educational events. MHP did not report any other conflicts of interest. MVC did not report any other conflicts of interest. PG did not report any other conflicts of interest. CP reported receiving grant from Pfizer, TEVA, Roche and personal fees from GSK, Roche, Amgen, Pfizer for lectures, presentations, speakers, manuscript writing or educational events and from Sanofi, ChemoCentryx, AstraZeneca, InflaRx and Otsuka for participation on a data safety monitoring board or advisory board. LG did not report any other conflicts of interest. BT reported receiving consulting fees from Roche, Chugai, GSK and AstraZeneca. AB did not report any other conflicts of interest.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and the MAINRITSAN study was approved by the Ethics Committee 'Comité de Protection des Personnes Ile de France III Paris' (CPP No Am5857-5-2988, No EUDRACT: 2012-001963-66). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The data underlying this article are available in the article and in its online supplemental material. The data underlying this article will be shared on reasonable request to the corresponding author.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Xavier Romand <http://orcid.org/0000-0003-2222-1456>

Benjamin Terrier <http://orcid.org/0000-0001-6612-7336>

Athan Baillet <http://orcid.org/0000-0001-6006-2519>

REFERENCES

- Walsh M, Flossmann O, Berden A, *et al*. Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 2012;64:542–8.
- Morgan MD, Szeto M, Walsh M, *et al*. Negative anti-neutrophil cytoplasm antibody at switch to maintenance therapy is associated with a reduced risk of relapse. *Arthritis Res Ther* 2017;19:129.
- 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.
- Charles P, Terrier B, Perrodeau É, *et al*. Comparison of individually tailored versus fixed-schedule Rituximab regimen to maintain ANCA-associated vasculitis remission: results of a Multicentre, randomised controlled, phase III trial (Mainritsan2). *Ann Rheum Dis* 2018;77:1143–9.

- 5 Wester Trejo MAC, Floßmann O, Westman KW, *et al.* Renal relapse in antineutrophil cytoplasmic autoantibody-associated vasculitis: unpredictable, but predictive of renal outcome. *rheumatology. Rheumatology (Oxford)* 2019;58:103–9.
- 6 Slot MC, Tervaert JWC, Franssen CFM, *et al.* Renal survival and Prognostic factors in patients with Pr3-ANCA associated vasculitis with renal involvement. *Kidney Int* 2003;63:670–7.
- 7 Brix SR, Noriega M, Tennstedt P, *et al.* Development and validation of a renal risk score in ANCA-associated glomerulonephritis. *Kidney Int* 2018;94:1177–88.
- 8 Vogl T, Tenbrock K, Ludwig S, *et al.* Mrp8 and Mrp14 are endogenous Activators of toll-like receptor 4, promoting lethal, Endotoxin-induced shock. *Nat Med* 2007;13:1042–9.
- 9 Viemann D, Barczyk K, Vogl T, *et al.* Mrp8/Mrp14 impairs endothelial integrity and induces a caspase-dependent and -Independent cell death program. *Blood* 2007;109:2453–60.
- 10 Pepper RJ, Wang H-H, Rajakaruna GK, *et al.* S100A8/A9 (Calprotectin) is critical for development of glomerulonephritis and promotes inflammatory Leukocyte-renal cell interactions. *Am J Pathol* 2015;185:1264–74.
- 11 Romand X, Bernardy C, Nguyen MVC, *et al.* Systemic Calprotectin and chronic inflammatory rheumatic diseases. *Joint Bone Spine* 2019;86:691–8.
- 12 Manfredi M, Van Hoovels L, Benucci M, *et al.* Circulating Calprotectin (cCLP) in autoimmune diseases. *Autoimmun Rev* 2023;22:103295.
- 13 Shi H, Zuo Y, Yalavarthi S, *et al.* Neutrophil Calprotectin identifies severe pulmonary disease in COVID-19. *J Leukoc Biol* 2021;109:67–72.
- 14 Silvin A, Chapuis N, Dunsmore G, *et al.* Elevated Calprotectin and abnormal myeloid cell Subsets discriminate severe from mild COVID-19. *Cell* 2020;182:1401–18.
- 15 Pepper RJ, Hamour S, Chavele K-M, *et al.* Leukocyte and serum S100A8/S100A9 expression reflects disease activity in ANCA-associated vasculitis and glomerulonephritis. *Kidney Int* 2013;83:1150–8.
- 16 Page TH, Chiappo D, Brunini F, *et al.* Danger-associated molecular pattern molecules and the receptor for advanced Glycation end products enhance ANCA-induced responses. *Rheumatology* 2022;61:834–45.
- 17 Michailidou D, Duvvuri B, Kuley R, *et al.* Neutrophil activation in patients with anti-neutrophil cytoplasmic autoantibody-associated vasculitis and large-vessel vasculitis. *Arthritis Res Ther* 2022;24:160.
- 18 Anton-Pampols P, Martinez Valenzuela L, Fernández Lorente L, *et al.* Combining neutrophil and macrophage biomarkers to detect active disease in ANCA vasculitis: a Combinatory model of Calprotectin and urine Cd163. *Clin Kidney J* 2023;16:693–700.
- 19 O'Sullivan KM, Ford SL, Longano A, *et al.* Intrarenal toll-like receptor 4 And toll-like receptor 2 expression correlates with injury in antineutrophil cytoplasmic antibody-associated vasculitis. *Am J Physiol Renal Physiol* 2018;315:F1283–94.
- 20 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.
- 21 Martinez 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:e0205982.
- 22 Guillevin L, Pagnoux C, Karras A, *et al.* Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. *N Engl J Med* 2014;371:1771–80.
- 23 National Institute for Health and Care Excellence. Chronic kidney disease in adults: assessment and management. 2015. Available: <https://www.nice.org.uk/guidance/cg182>
- 24 Smith RM, Jones RB, Specks U, *et al.* Rituximab versus azathioprine for remission of remission for patients with ANCA-associated vasculitis and relapsing disease: an international randomised controlled trial. *Ann Rheum Dis* 2023;82:937–44.
- 25 Charles P, Guillevin L. Long-term Rituximab use to maintain remission of antineutrophil cytoplasmic antibody-associated vasculitis. *Ann Intern Med* 2020;173:948.
- 26 Lionaki S, Blyth ER, Hogan SL, *et al.* Classification of antineutrophil cytoplasmic autoantibody Vasculitides: the role of antineutrophil cytoplasmic autoantibody specificity for myeloperoxidase or Proteinase 3 in disease recognition and prognosis. *Arthritis Rheum* 2012;64:3452–62.
- 27 Rowe C, Sitch AJ, Barratt J, *et al.* Biological variation of measured and estimated glomerular filtration rate in patients with chronic kidney disease. *Kidney Int* 2019;96:429–35.
- 28 Kälsch A-I, Csernok E, Münch D, *et al.* Use of highly sensitive C-reactive protein for followup of Wegener's granulomatosis. *J Rheumatol* 2010;37:2319–25.
- 29 Jayne DRW, Merkel PA, Bekker P. Avacopan for the treatment of ANCA-associated vasculitis. *N Engl J Med* 2021;384:10.1056/NEJMc2104672#sa3.
- 30 Hetland G, Talgö GJ, Fagerhol MK. Chemotaxins C5A and fMLP induce release of Calprotectin (Leucocyte L1 protein) from Polymorphonuclear cells in vitro. *Mol Pathol* 1998;51:143–8.
- 31 Xiao H, Dairaghi DJ, Powers JP, *et al.* C5A receptor (Cd88) blockade protects against MPO-ANCA GN. *J Am Soc Nephrol* 2014;25:225–31.
- 32 Bjørneklett R, Solbakken V, Bostad L, *et al.* Exploring sex-specific differences in the presentation and outcomes of ANCA-associated vasculitis: a nationwide Registry-based cohort study. *Int Urol Nephrol* 2018;50:1311–8.
- 33 Rathi M, Pinto B, Dhooria A, *et al.* Impact of renal involvement on survival in ANCA-associated vasculitis. *Int Urol Nephrol* 2016;48:1477–82.
- 34 Scott J, Canepa C, Buettner A, *et al.* A cohort study to investigate sex-specific differences in ANCA-associated glomerulonephritis outcomes. *Sci Rep* 2021;11:13080.
- 35 Romand X. Etude des Mécanismes Pathogéniques Impliquant L'Immunité Innée Dans LES Spondyloarthrites. 2022.