Nephro-Urology Monthly
Volume 17 · Issue 3 · e161826

OPEN PEER REVIEW

Clinical Profile, Treatment Outcomes, and Complement Pathway Involvement in Idiopathic ANCA-negative Renal-Limited Pauci-immune Crescentic Glomerulonephritis: A Single-Centre Prospective Study

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SUBMITTED	REVISED	ACCEPTED	DOI
19 Apr 2025	2 Jul 2025	7 Jul 2025	10.5812/numonthly-161826

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1 Original submission

6 comments · 18 May 2025 – 20 May 2025

Referee 1 Reviewer

MAJOR REVISION 18 May 2025

Dear AE,
Thank you very much for giving me this opportunity to review this manuscript.
This prospective single-center study investigates idiopathic ANCA-negative renal-limited pauci-immune crescentic glomerulonephritis (GN), focusing on clinical profiles, treatment outcomes, and the potential role of the alternative complement pathway. The study addresses a rare but clinically significant subset of GN, contributing to the limited literature on ANCA-negative cases.

@ Key strengths include:
* Clear inclusion/exclusion criteria and rigorous ANCA negativity confirmation.
* Detailed histological and biochemical characterization.
* Exploration of complement pathway involvement, a novel aspect in ANCA-negative GN.

@ Limitations:
* Small sample size (n=21)
* Lack of a control group (e.g., ANCA-positive GN)
* Incomplete mechanistic insights into complement activation

@ Major Concerns
1. Sample Size and Generalizability: The small cohort (n=21) limits statistical power and external validity. Larger multicenter studies are needed to validate findings.
2. Complement Pathway Analysis: While reduced C3 levels suggest alternative pathway involvement, the study lacks direct evidence (e.g., factor B or properdin staining). Include immunohistochemistry or functional assays to strengthen claims.
3. Treatment Heterogeneity: Combined rituximab/plasma exchange therapy obscures individual treatment effects. Stratify outcomes by monotherapy vs. combination therapy.

@ Minor Concerns
4. Statistical Analysis: Chi-square tests for small subgroups (e.g., fibrous crescents, n=2) are unreliable. Use Fisher's exact test where appropriate.
5. Confounding Variables: Adjust for baseline creatinine/proteinuria in remission analyses to account for disease severity.

6. Why was cyclophosphamide not used, given its role in ANCA-associated GN?
7. How were C3 levels measured (threshold for "low" C3)? Provide assay details.
8. Were complement inhibitors (e.g., anti-C5a) considered for refractory cases?

9. Abstract: Clarify "partial remission" criteria (e.g., proteinuria reduction threshold).
10. Methods: Specify ANCA/anti-GBM testing protocols (ELISA/IIF?).
11. Results: Report p-values for Table 3/4 associations (currently missing).
12. Figures: Label arrows/structures in histology images (Fig 1–3) for clarity.
13. Discussion: Contrast findings with ANCA-positive GN (e.g., remission rates).

Final Decision: Major Revision

Kind Regards | Publisher Supervisor

Referee 2 Reviewer

MAJOR REVISION 18 May 2025

This study investigates ANCA-negative renal-limited pauci-immune crescentic glomerulonephritis, focusing on clinical outcomes and complement pathway involvement. However, major revisions are needed to improve the paper:

-The abstract lists complement pathway analysis as an objective, but the Methods section lacks details on how complement activation was assessed (e.g., assays for C3, C5b-9, or factor B). This omission undermines the validity of claims about complement involvement.
-With only 21 patients, statistical power is limited, especially for subgroup analyses (e.g., 38% with complement pathway involvement = 8 patients). A power calculation is absent.
-The study excludes patients who later tested positive for immune markers but does not specify how many were excluded for this reason, risking selection bias.
-The "Statistical method" section ends abruptly without describing the tests used (e.g., regression for prognostic factors). No p-values or confidence intervals are reported for outcomes like remission rates.
-The claim that "the alternative complement pathway is implicated in pathogenesis" is not fully supported by the data. No mechanistic evidence (e.g., C5a or factor B levels) is provided, only indirect associations.
-The "Objectives" section (page 7) is incomplete, listing only "3. Methods" as a header. A numbered list of specific aims is needed.
-"Alternative complement pathway" is included in the keywords but not sufficiently addressed in the results or discussion.
-The Background section excessively reviews general crescentic GN pathophysiology (pages 4–6). Condense it to focus on knowledge gaps specific to ANCA-negative cases.
-Uploaded files (Fig 1.jpg, Fig 2.jpg) are not cited in the text. Integrate these with captions explaining their relevance.
-The Conflict of Interests section repeats the same statement three times. Consolidate to a single, concise declaration.
-The statement "No new data were created" contradicts the prospective study design. Clarify whether data will be shared upon request.

Referee 3 Reviewer

MINOR REVISION 19 May 2025

title: Clinical profile, treatment outcomes, and complement pathway involvement in idiopathic ANCA-negative renal-limited pauci-immune crescentic glomerulonephritis: A single-centre prospective study

Suggestion to rewrite the final sentence:
Given these gaps in the literature, the present prospective single-center study was designed to characterize the clinical profile, treatment response, and prognostic implications of idiopathic ANCA-negative pauci-immune crescentic glomerulonephritis, and to evaluate the possible involvement of the alternative complement pathway.

2. Objectives: Nothing is written.

The sample size is only 21, but ANOVA was used. With this small sample size, it would have been better to consider nonparametric tests such as Kruskal–Wallis, or at least to include the limitation of the sample size in the discussion section.

The treatment method is not fully described. The doses, duration of treatment, and criteria for choosing between plasmapheresis and rituximab are not clear.

The duration and method of follow-up are not clear (e.g., every few months? With what tools?).

The treatments (plasma exchange and rituximab) are reported along with the number of recipients (13 and 14, respectively)
but further information on:
Dose of drugs
Duration of treatment
Basis for choosing the treatment
is not available and should be provided in the "Discussion" or "Methods" section.

Referee 4 Reviewer

MAJOR REVISION 19 May 2025

Dear Associate Editor
I thank the authors and express my appreciation to the researchers.
1-The title is overly long and detailed. A concise and focused title would better reflect the study's core.

2-Some sentences lack references (for example, the first sentence of the background). All sentences quoted from other articles must have references at the end of the sentence.
3-Some key references are outdated, and recent literature on the topic has not been adequately cited.

4-The manuscript contains numerous grammatical, syntactical, and stylistic errors.
5-In all tables, abbreviations must be explained in a legend.

Associate Editor

MAJOR REVISION 19 May 2025

Dear EIC,
Thanks to the authors and appreciation to the researchers. The manuscript is a valuable piece of research. But some revisions are needed. The authors should revise it based on the comments. Also, minor changes should be made to the abstract:
1- The study design should be mentioned in the first line of the method section of ABSTRACT.
2- A summary of the statistical analysis results should be mentioned in the result section of ABSTRACT.

Editor-in-Chief

MAJOR REVISION 20 May 2025

It requires a major revision.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reply to all reviewer's comments:

Reviewer 1:

1) Comment: The abstract states complement pathway analysis as an objective, but the Methods section lacks details on how complement activation was assessed (e.g., assays for C3, C5b-9, or factor B). This omission undermines the validity of claims about complement involvement.

   Answer: We have added the methods of assessment. C3 was measured using immunoturbidimetry method. The assay was based on the reaction between C3 and its antibody. The insoluble complex in case of a positive reaction shall produce a turbidity which was read with a spectrophotometry. The normal reference range for serum C3 was 90-180mg/dl. We did estimate C5b-9 levels in some patients, say nearly 11 patients of our study. We also did the same in nearly 19 out of 37 other patients who were initially included in the study but later turned out to be ANCA positive and hence were excluded. As our institution runs mainly on government-aided funds and we could not afford the test for all, we had to exclude it from our study in order to maintain a uniformity. (marked in red in text)

2) With only 21 patients, statistical power is limited, especially for subgroup analyses (e.g., 38% with complement pathway involvement = 8 patients). A power calculation is absent.

   Answer: A proper re-analysis has been done and statements added.

3) The study excludes patients who later tested positive for immune markers but does not specify how many were excluded for this reason, risking selection bias.

   Answer: Our study purely concentrated on the demographic, clinical profile and the prognostic implications of ANCA-Negative pauci-immune glomerulonephritis alone and hence we didn't include the ANCA-positive patients. However, approximately 37 patients were excluded from our study who turned out to be ANCA-positive over the follow-up period.

4) The "Statistical method" section ends abruptly without describing tests used (e.g., regression for prognostic factors). No p-values or confidence intervals are reported for outcomes like remission rates.

Answer: All the tests are written in detail and mentioned in the text. P values reported for remission rates.

5) The claim that "the alternative complement pathway is implicated in pathogenesis" is not fully supported by the data. No mechanistic evidence (e.g., C5a or factor B levels) is provided, only indirect associations.

As added evidences to substantiate the alternative complement pathway involvement, we also estimated the terminal complement components c5b-9 as well as serum complement factor B. Both were measured using Sandwich ELISA method.
The reference range for c5b-9 was <250ng/ml and for factor B it was 120-330 µg/ml. Nearly 11 patients who got enrolled in our study had their terminal complement components measured while nearly 19 out of 37 patients who were later excluded from the study for turning out to be ANCA positive also got them measured. Due to financial constraints, these tests could not be done for all the 21 enrolled patients and hence these tests had to be excluded from the study to maintain a uniformity. Serum C3 and immunofluorescent staining of C3 stand as convincing evidences of the alternative complement pathway involvement in our study.

6) The "Objectives" section (page 7) is incomplete, listing only "3. Methods" as a header. A numbered list of specific aims is needed.

The objectives have been properly added and numbered. (marked in red inside the text).

7) Alternative complement pathway" is included in keywords but not sufficiently addressed in results or discussion.

Sufficient data have been added in discussion and results.

8) The Background section excessively reviews general crescentic GN pathophysiology (pages 4–6). Condense to focus on knowledge gaps specific to ANCA-negative cases.

   The redundant and the excessive part has been removed and adequate thrust has been added to ANCA-negative variety.

9) Uploaded files (Fig 1.jpg, Fig 2.jpg) are not cited in the text. Integrate these with captions explaining their relevance.

   Uploaded figures are properly cited in the text in the result section at the appropriate places. Marked in red in the text.

10) The Conflict of Interests section repeats the same statement three times. Consolidate to a single, concise declaration.

    Consolidated into one single statement.

11) The statement "No new data were created" contradicts the prospective study design. Clarify whether data will be shared upon request.

    The data will be shared upon request.

Reviewer 2:

1) The title is overly long and detailed. A concise and focused title would better reflect the study's core.

   The title has been made short and precise.

2) Some sentences lack references (for example first sentence of background). All sentences quoted from other articles must have references at the end of themselves.

References have been added and numbered.

3) Some key references are outdated, and recent literature on the topic has not been adequately cited.

The old references have been removed and the latest have been added. (marked in red inside the text of the references).

4) The manuscript contains numerous grammatical, syntactical, and stylistic errors.

We have corrected the grammatical, syntactical and stylistic errors to the best of our ability.

5) In all Tables, the abbreviated words must be explained, in a Legend.
The abbreviated words have been explained clearly in a legend.

Reviewer 3

1) Sample Size and Generalizability: The small cohort (n=21) limits statistical power and external validity. Larger multicenter studies are needed to validate findings.

We agree to the fact that the sample size is small. It was the same reason that we could not attach a statistical significance to several parameters and the outcome, though we could observe a demonstrable clinical change. We have quoted this in the limitations of the study clearly.

2) Complement Pathway Analysis: While reduced C3 levels suggest alternative pathway involvement, the study lacks direct evidence (e.g., factor B or properdin staining). Include immunohistochemistry or functional assays to strengthen claims. As added evidences to substantiate the alternative complement pathway involvement, we also estimated the terminal complement components c5b-9 as well as serum complement factor B. Both were measured using Sandwich ELISA method.

Nearly 11 patients who got enrolled in our study had their terminal complement components measured while nearly 19 out of 37 patients who were later excluded from the study for turning out to be ANCA positive also got them measured. Due to financial constraints, these tests could not be done for all the 21 enrolled patients and hence these tests had to be excluded from the study to maintain a uniformity. Serum C3 and immunofluorescent staining of C3 stand as convincing evidences of the alternative complement pathway involvement in our study.

3) Treatment Heterogeneity: Combined rituximab/plasma exchange therapy obscures individual treatment effects. Stratify outcomes by monotherapy vs. combination therapy.

Outcomes stratified as combination vs monotherapy. Table 4 describes the outcome characteristics individually.

4) Statistical Analysis: Chi-square tests for small subgroups (e.g., fibrous crescents, n=2) are unreliable. Use Fisher's exact test where appropriate.

Chi-square test has been removed and data using Fisher's exact T test have been added.

5) Confounding Variables: Adjust for baseline creatinine/proteinuria in remission analyses to account for disease severity.

Baseline proteinuria and serum creatinine were compared to see the remission status. Data were expressed as median with interquartile range. Kruskal Wallis test with Dunn post hoc test was used to compare the mean rank between the three groups. Both the factors were not found to be predictive of the remission status. Table 6 in the result part describes in detail.

6) Why was cyclophosphamide not used, given its role in ANCA-associated GN?

We adopted a standard protocol of intensified immunosuppressive therapy

consisting of Injection methylprednisolone pulse therapy three pulses for all, along with either Inj Rituximab OR Injection Cyclophosphamide with or without plasma exchange. Hence, we chose Inj Rituximab in lieu of Inj cyclophosphamide and then supplemented it with plasma exchange.

7) How were C3 levels measured (threshold for "low" C3)? Provide assay details.

Serum C3 was measured using immunoturbidimetry method. The assay was based on the reaction between C3 and its antibody. The insoluble complex in case of a positive reaction shall produce a turbidity which was read with a spectrophotometry. The normal reference range for serum C3 was 90-180mg/dl. (Marked in red inside the text).

8) Were complement inhibitors (e.g., anti-C5a) considered for refractory cases?

We regret to inform that we couldn't give anti-C5a inhibitors to any of our patients due to financial reasons.

9) Abstract: Clarify "partial remission" criteria (e.g., proteinuria reduction threshold).

Complete remission is defined as normalization of serum creatinine, normal serum C3 levels and 24 -hour urine protein excretion of ≤ 300 mg per day. A repeat renal biopsy couldn't be done to demonstrate histopathological remission. ""No remission" is defined as dialysis dependence, persistent low serum C3, failure of serum creatinine to decrease by > 50% from the baseline towards the end of the treatment who were not on dialysis. Anyone who showed a tendency towards remission but didn't meet the above criteria were declared to have attained partial remission. (marked in red inside the methods section).

10) Specify ANCA/anti-GBM testing protocols (ELISA/IIF?).

c-ANCA and p-ANCA were measured by indirect ELISA method. A mixture of purified antigens namely proteinase 3 and myeloperoxidase was coated on to microwells. The specific antibodies if found in the patients' serum bound to the surface of the coated antigens. The added enzyme conjugate reacted with the immobile antigen-antibody complexes that finally imparted a yellow colour, the intensity of which was proportionate to the concentration of the complexes. It was read photometrically at 450nm in an ELISA reader. The normal range was 0.00-0.22 units/ml. Anti-GBM was measured using the indirect fluorescent antibody test. (all have been added inside the text in the "methods" section and marked in red).

11) Results: Report p-values for Table 3/4 associations (currently missing).

The statistical analyses have been changed and the new tables have been added accordingly.

12) Figures: Label arrows/structures in histology images (Fig 1–3) for clarity.

Arrows have been attached and the images have been marked appropriately.

Reviewer 4

1) Suggestion to rewrite the final sentence:

Sentence rewritten as "Given these gaps in the literature, the present prospective single-centre study was designed to characterize the clinical profile, treatment response, and prognostic implications of idiopathic ANCA-negative pauci-immune crescentic glomerulonephritis, and to evaluate the possible involvement of the alternative complement pathway". (Marked as red

inside the text at end of “The background” section.

2) Objectives Nothing is written.

Objectives have been clearly written. Marked in red inside the text.

3) The sample size is only 21, but ANOVA was used. With this small sample size, it would have been better to consider nonparametric tests such as Kruskal–Wallis, or at least to include the limitation of the sample size in the discussion section.

The statistical tests have been changed as accordingly mentioned above. All results presented as newer tables marked in red inside the “results” section.

4) The treatment method is not fully described. The doses, duration of treatment, and criteria for choosing between plasmapheresis and rituximab are not clear.

The treatment methods and the reason for the choices of treatments have been clearly explained inside “the materials and methods” section and marked in red.

5) The duration and method of follow-up are not clear (e.g. every few months? With what tools?)

The details of follow-up have been added and written at the end of the “Methods” section. Marked in red inside the text.

6) The treatments (Plasma exchange and Rituximab) are reported along with the number of recipients (13 and 14, respectively)
but further information on:
Dose of drugs
Duration of treatment

Basis for decision making for the choice of treatment
is not available and should be completed in the "Discussion" or "Methods" section.

All the details about the dose, duration, number of cycles of plasma exchange have been added inside the “methods” section and have been distinctly marked in red.

2 Revision 1

5 comments · 6 Jul 2025 – 7 Jul 2025

Referee 2 Reviewer — ACCEPT 6 Jul 2025

All concerns have been resolved.

Referee 3 Reviewer — ACCEPT 6 Jul 2025

Considering the amendments made to the article, it is approved by me as a referee.

Referee 4 Reviewer — ACCEPT 6 Jul 2025

Dear EIC.
Previous errors have been corrected, and there are no further recommendations.

Associate Editor — ACCEPT 6 Jul 2025

Dear EIC,
I read the manuscript, and the authors corrected the requested items. The article could be accepted at this stage.
Sincerely;

Editor-in-Chief — ACCEPT 7 Jul 2025

Accept