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Organisation of lymphocytic infiltrates in ANCA-associated glomerulonephritis

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Aims: Renal involvement in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis contributes to significant morbidity and mortality in patients. In chronic inflammation, B cells are recruited to the inflamed tissue and organised lymphoid structures have been described in several autoimmune diseases. The aim of this study was to correlate the lymphoid organisation in renal biopsies with renal outcome in ANCA-associated glomerulonephritis (GN).

Methods and results: We investigated 112 renal biopsies from patients with newly diagnosed ANCA-associated necrotising GN. We identified four different levels of the intrarenal organisation of lymphocytes: T cells without B cells, scattered B and T cells, clustered lymphocytic infiltrates and nodular compartmentally arranged B and T cell aggregates. Almost half the patients showed clusters of B and T lymphocytes in their biopsies. In 15 of these biopsies, a higher degree of organisation with lymphocytic compartments was detected. Inflammatory cell organisation was associated with renal failure, but not with tubular atrophy and interstitial fibrosis. Patients with organised lymphocytic infiltrates in their biopsy had worse renal function during follow-up and were more likely to develop end stage renal disease.

Conclusions: In the present study, we show that the renal lymphocytic organisation is associated with renal outcome in ANCA-associated GN. The organisation of the lymphocytic infiltrate may be a morphological correlate of a perpetual and exaggerated inflammation in renal ANCA disease. Classifying the lymphocytic infiltrate could help to predict renal outcome, and might therefore be used for individualised adjustments in the intensity and duration of immunosuppressive therapy.

Keywords: ANCA-associated vasculitis, crescentic glomerulonephritis, lymphocytic organisation, renal outcome

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis is the major cause of crescentic necrotising glomerulonephritis (GN).[1] Substantial advances in treatment during the last decades have improved patient outcome.[2–5] However, a significant percentage of patients reach end stage renal disease (ESRD) or suffer from relapsing disease.[2,6]

In inflamed tissue, it has been observed that inflammatory cells can organise themselves anatomically and functionally, as in secondary lymphoid

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organs.[7,8] These structures are often called tertiary lymphoid organs.[9] In several chronic inflammatory conditions, organised lymphoid structures have been described as a manifestation of lymphoid neogenesis maintaining immune responses against persistent antigens.[10]

In autoimmunity, lymphocytic organisation is most prominent in thyroid disorders for which the antigenic targets of the humoral immune response have been characterised.[11] Ectopic lymphoid tissue has been implicated as promotor of destruction and loss of organ function in several autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis.[12,13] It has been described in the kidney in different conditions, e.g. renal allograft rejection, interstitial nephritis, immunoglobulin (IgA) nephropathy, lupus nephritis and ANCA-associated GN.[14–21]

In renal transplantation, lymphocytic organisation was thought initially to correlate with steroid-resistant rejection and graft loss.[22] Validation studies could not confirm these findings.[23,24] In ANCA-associated glomerulonephritis, several studies have searched for histological predictors of renal outcome, concentrating on localisation and quantity of inflammatory cells.[25–31] Complicating these analyses is the heterogeneity in the organisational structure of intrarenal lymphocytes. We have previously revealed the presence of intrarenal lymphoid structures of four different organisational stages in renal ANCA disease.[17] It is, however, still unknown whether the intrarenal lymphoid neogenesis contributes to the exacerbation of chronic inflammation in ANCA-associated vasculitis. The aim of this study was to investigate the local lymphocytic organisation in the kidney for a possible correlation with clinical outcome in renal ANCA disease.

Materials and methods

STUDY DESIGN

A total of 112 patients were recruited in this prospective, observational study between March 2009 and January 2016 from 10 centres in Germany. Inclusion criteria were an ANCA detected in the sera, a necrotising and crescentic GN in the kidney biopsy and, to achieve tissue adequacy, a biopsy with at least nine glomeruli. The trial was performed in accordance with the Declaration of Helsinki. After informed consent was obtained, patient data and renal biopsies were collected according to the guidelines of the respective local ethics committees (PV3162). Long-term follow-up data were acquired from a questionnaire. The participating physicians performed disease assessments regularly every 3 months during the trial.

IMMUNOHISTOCHEMISTRY

Formalin-fixed paraffin-embedded (FFPE) tissue sections (2 μm) were stained for CD3, CD20 and CD21. The following antibodies were used: CD3 (CI597R06SG; DCS Innovative Diagnostik Systeme, Hamburg, Germany), 1:150, incubated overnight at 4°C; CD20 (M0755; Dako, Glostrup, Denmark), 1:800, incubated for 30 min at 37°C; and CD21 (M0784; Dako), 1:200, incubated overnight at 4°C. FFPE tissue sections were deparaffinised and rehydrated through graded ethanol solutions. Antigen retrieval was performed by placing sections in citrate buffer (pH 6.1) and heating in the autoclave for 15 min. Detection was achieved using ZytochemPlus/POLAP100 (Zytomed Systems, Berlin, Germany) for CD20, Vectastain ABC-AP kit (Vector Laboratories, Burlingame, CA, USA) for CD21 and StreptABC-complex/horseradish peroxidase (HRP) (K0377; Dako) for CD3. Nuclei were stained with Mayers' Haemalaun solution.

HISTOPATHOLOGICAL EVALUATIONS AND GRADING

Renal biopsies were classified according to the organisational stage of inflammatory cell infiltrates. Using CD3–CD20 co-staining, four distinct categories could be distinguished. Biopsies with $CD3^+$ T cells without intrarenal $CD20^+$ B cells were graded as zero. A scattered pattern of $CD20^+$ cells was graded as one. Cell clusters consisting of $CD3^+$ T cells and $CD20^+$ B cells without a micro-anatomical compartmentalisation were graded as two. Nodular aggregates of lymphocytes with a clearly distinct and central B cell zone surrounded by T cells were graded as three. CD21 staining was performed to detect a central network of follicular dendritic cells within the lymphocytic aggregates identifying the highest level of lymphoid organisation in the sense of tertiary lymphoid tissue.

To determine possible histopathological correlations, slides were evaluated further by a nephropathologist who was blinded to the patient's data and research results. Periodic acid-Schiff (PAS) and Giemsa staining were applied. Glomerulosclerosis and crescents were counted, and the severity of interstitial fibrosis and tubular atrophy (IFTA) as the percentage of the affected cortical area was estimated.

STATISTICAL ANALYSES

Data were analysed using Graph Pad Prism version 5.01 software. Values are expressed as mean ± 95% confidence interval (CI) or median with interquartile range (IQR) for continuous normal and non-normal distributed variables, respectively. Differences between two groups were compared using a two-tailed *t*-test. In case of multiple comparisons, one-way analysis of variance (ANOVA) with Bonferroni's multiple comparisons test was performed. Correlation analyses of the lymphocytic organisation with other histopathological variables were performed using Spearman's rank correlation. Renal survival was assessed by the Kaplan–Meier method. A two-sided *P*-value less than 0.05 was considered statistically significant.

Results

PATIENT COHORT DESCRIPTION

One hundred and twelve patients with a new diagnosis of ANCA-associated GN were included in the present study. Table 1 summarises their baseline clinical characteristics. Median follow-up time was 32 months. All patients received immunosuppressive induction therapy with steroids and cyclophosphamide (n = 103, median dosage = 4.3 g, IQR = 3–5.4 g) and/or rituximab (n = 14, median dosage = 2 g, IQR = 2–2.1 g). Forty patients (35.7%) underwent additional plasmapheresis. Table 2 depicts patients' outcome. Thirty patients (26.8%) developed a renal relapse during follow-up. A total of 25 patients (22.3%) reached ESRD. Thirteen patients (11.6%) were lost during follow-up.

Table 1. Clinical baseline characteristics of the patients

Patients, *n*	112
Age, median years (IQR)	66 (54–72)
Male sex, *n* (%)	88 (78.6)
ANCA-type	
Proteinase 3, *n* (%)	58 (51.8)
Myeloperoxidase, *n* (%)	54 (48.2)
Renal function at time of diagnosis	
Creatinine clearance,* median ml/min (IQR)	28 (21–44)
Dialysis dependence, *n* (%)	26 (23.2)

ANCA, antineutrophil cytoplasmic antibody; IQR, interquartile range.
*Patients not on dialysis.

Table 2. Outcome of the patients

Follow-up, median months (IQR)	32 (15–55)
ESRD, *n* (%)	25 (22.3)
Renal relapses, *n* (%)	30 (26.8)
Deaths, *n* (%)	19 (17.0)

IQR, interquartile range; ESRD, end stage renal disease.

Nineteen patients (17%) died. Nine of these patients were dialysis-dependent at the end.

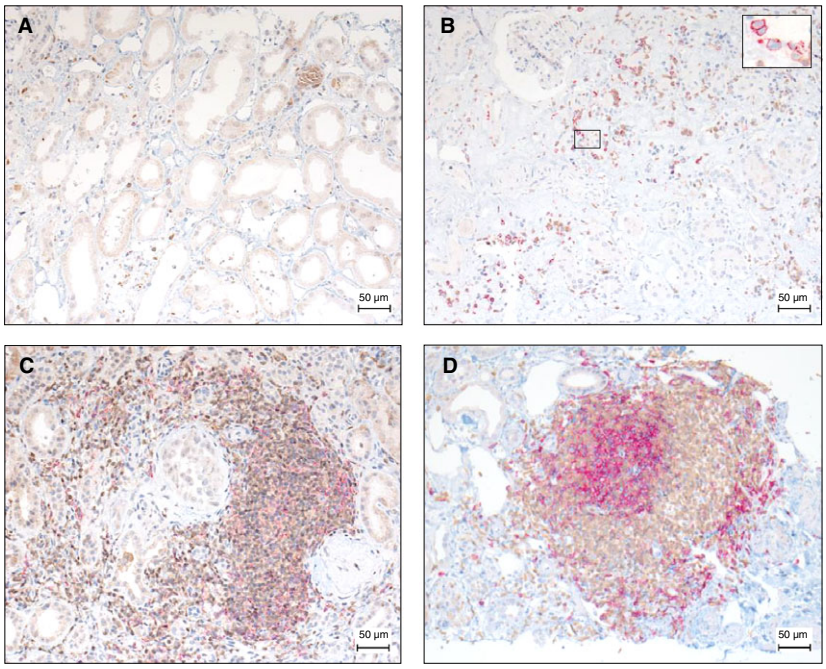
INTRARENAL LYMPHOCYTIC ORGANISATION

Immunohistochemistry presented different degrees of intrarenal lymphocytic organisation: T cells without intrarenal B cells (grade 0), scattered B and T cells (grade 1), clustered B and T cells (grade 2) and nodular aggregates of B and T lymphocytes with a distinct and central B cell zone surrounded by T cells (grade 3). Figure 1 illustrates the micro-anatomical organisation of inflammatory infiltrates, giving an example of all four types of infiltrates as well as an overview of the findings in the study cohort. Many biopsies contained lymphocytic infiltrates of various degrees of organisation. Patients were classified according to the highest organisational grade of lymphocytic infiltrate in the biopsy. Ten tissue specimens did not contain any B cells. Forty-nine biopsies showed scattered B and T cells. Clusters of B and T lymphocytes without compartmentalisation were found in 38 specimens. Nodular lymphocytic aggregates with distinct B and T cell zones were present in 15 biopsies.

Supporting information, Figure S1 shows a representative biopsy with a grade 3 infiltrate with compartmentalised lymphocytes with a central network of follicular dendritic cells (fDCs). This classical feature of a germinal centre of lymph follicles in secondary lymphoid tissue was found in only three of 15 biopsies with distinct B and T cell regions. Given the scarcity of these cases, they were grouped together with biopsies containing nodular lymphocytic aggregates lacking fDCs.

HISTOPATHOLOGICAL CORRELATIONS – INTRARENAL LYMPHOCYTIC ORGANISATION IS NOT ASSOCIATED WITH TUBULAR ATROPHY, INTERSTITIAL FIBROSIS, GLOMERULOSCLEROSIS OR CRESCENTS

Correlation analyses of the micro-anatomical organisation of inflammatory cells with other histological



E

Level	B Cell Infiltrate	*n*
0	None	10
1	Scattered	49
2	Clustered	38
3	Nodular	15

Figure 1. Lymphocytic infiltration in anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis. Representative examples of lymphocytic infiltrates ($CD3^+$ T lymphocytes in brown, $CD20^+$ B lymphocytes in red). **A**, T cells without significant interstitial B lymphocytes. **B**, Scattered B and T lymphocytes. **C**, Clustered B and T lymphocytes. **D**, Organised lymphocytic infiltration with compartmental B and T lymphocytes with a central B cell zone surrounded by T cells (nodular infiltrate). **E**, List of biopsies graded according to their highest level of intrarenal lymphocytic organisation ranging from none to nodular appearance with compartmentally arranged cells.

changes in necrotising ANCA-associated GN were performed (Figure 2). The degree of lymphocytic organisation was not associated with the severity of tubular atrophy and interstitial fibrosis or the percentage of glomerulosclerosis (Figure 2A,B). We also looked for a correlation with glomerular inflammation, e.g. fibrinoid necrosis, cellular and fibrocellular crescents. The level of organisation of intrarenal lymphocytes was not associated with the percentage of glomerular crescents and did not differ in histological classes for ANCA-associated GN (Figure 2C,D).

CLINICAL CORRELATION TO RENAL FUNCTION – LYMPHOCYTIC ORGANISATION IS ASSOCIATED WITH RENAL FUNCTION

Figure 3A–C depicts renal function at the time of biopsy and after 2 and 4 years' follow-up. Patients with T cells without significant intrarenal B cells had the best renal function. Patients with scattered intrarenal B lymphocytes had a worse glomerular filtration rate. Patients with nodular infiltrates with compartmentalised B and T cells in the biopsy had the lowest renal function (at time of biopsy $P < 0.01$, during follow-up $P < 0.05$, respectively). At the time of biopsy, fewer patients with scattered lymphocytes than patients with organised intrarenal lymphocytes in the biopsy needed renal replacement therapy ($P < 0.05$) (Figure 3D). During follow-up, more patients with higher degrees of intrarenal lymphocytic organisation developed end stage renal disease ($P < 0.05$) (Figure 3E). Numerically, more renal relapses were found in patients with an organised lymphocytic infiltrate during follow-up; this was not statistically significant (Figure 3F).

CLINICAL CORRELATION TO RENAL SURVIVAL – PATIENTS WITH AN ORGANISED INTRARENAL LYMPHOCYTIC INFILTRATE ARE MORE LIKELY TO DEVELOP ESRD

The primary outcome measure was the development of ESRD. Patients divided according to their lymphocytic infiltrate differed in renal survival (Figure 4). Kaplan–

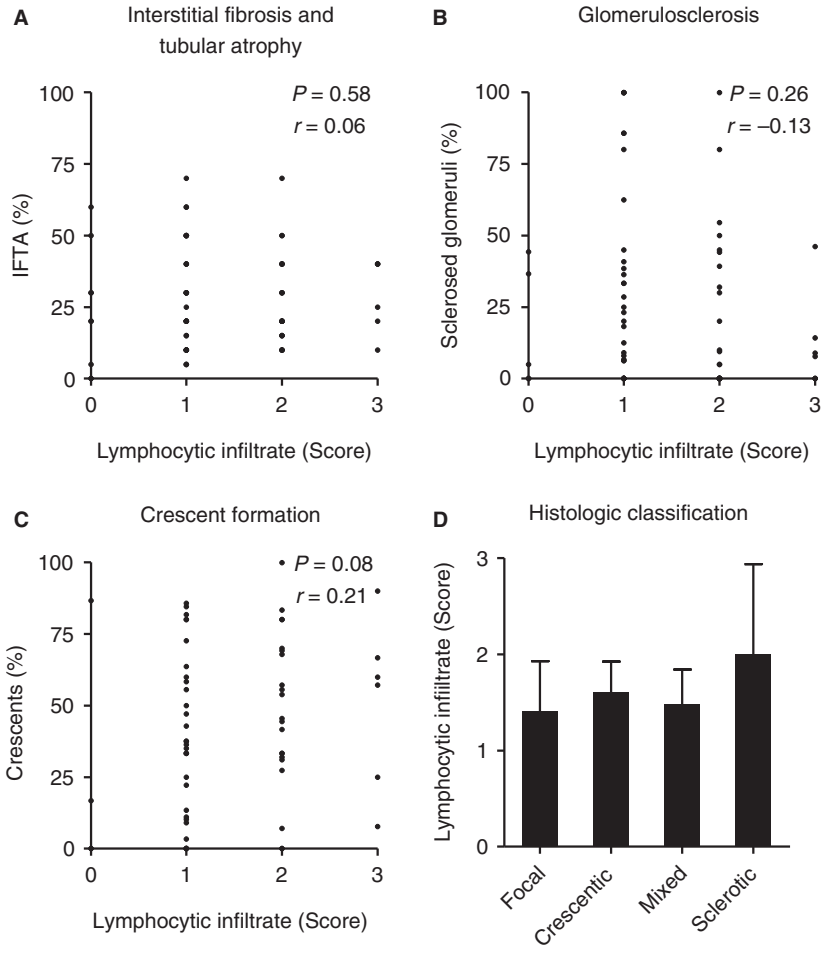


Figure 2. Correlation analyses of the intrarenal lymphocytic organisation with interstitial fibrosis and tubular atrophy, glomerulosclerosis and crescent formation. Spearman's correlation analyses of levels of lymphocytic organisation with (**A**) interstitial fibrosis and tubular atrophy, (**B**) glomerulosclerosis and (**C**) crescent formation (including fibrinoid necrosis, cellular and fibrocellular crescents). **D**, Analysis of variance (ANOVA) of the lymphocytic infiltration in biopsies divided according to the Berden classification. Depicted are means and 95% confidence interval (CI).

Meier curves demonstrate the percentage of patients reaching ESRD during follow-up. Here, patients with only T cells or scattered B lymphocytic infiltrates in their biopsy needed less renal replacement therapy during follow-up than patients with clustered infiltrates, and patients with nodular infiltrates developed ESRD more often than patients with less organised infiltrates ($P < 0.0001$) (Figure 4A). The differing renal survival was even more noticeable when merging patient groups. Patients were grouped according to their infiltrate in only T cells and scattered B lymphocytes as well as in clustered and nodular B and T cell aggregates in the biopsy. More than 90% of patients with only T cells or scattered B lymphocytes in the biopsy preserved their renal function during follow-up. Patients with a higher degree of lymphocytic organisation in their initial biopsy had a higher risk of persistent renal failure. Almost half this patient cohort developed ESRD ($P < 0.0001$) (Figure 4B).

The lymphocytic organisation did not differ in the subanalyses investigating the influences of antibody subtypes, gender and age of patients (Supporting information, Figure S2). Patients with myeloperoxidase (MPO)-positive ANCA-associated GN showed a similar organisation of intrarenal lymphocytes when compared with patients with proteinase 3 (PR3)-positivity.

CLINICAL CORRELATION ACCORDING TO IMMUNOSUPPRESSIVE THERAPY – PATIENTS TREATED WITH RITUXIMAB HAVE SIMILAR RENAL OUTCOME COMPARED WITH PATIENTS RECEIVING CYCLOPHOSPHAMIDE INDUCTION

To detect differences in treatment-related outcomes, patients receiving rituximab for induction therapy ($n = 14$) were paired with patients treated with cyclophosphamide according to clinical and histological parameters (Supporting information, Table S1). Patients were matched for age, gender and disease, as well as for grade of lymphocytic organisation, interstitial fibrosis and tubular atrophy, crescents and initial

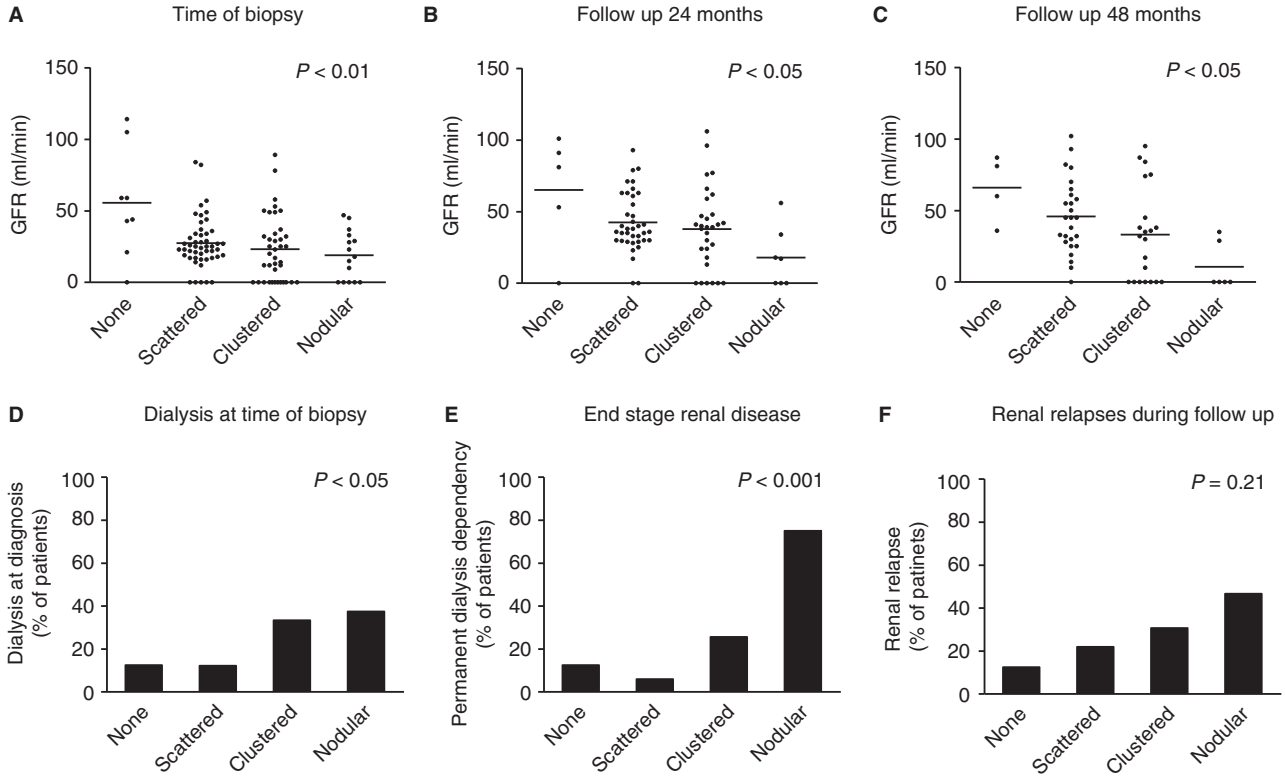


Figure 3. Organisation of B and T lymphocytic infiltrates is associated with renal function in anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis. Patients are divided according to their lymphocytic infiltration in their renal biopsy in T cells without B cells, scattered, clustered and compartmentally organised (nodular) T and B cell infiltrates. **A**, Glomerular filtration rate (GFR) at time of biopsy according to the lymphocytic organisation. **B**, **C**, Renal function after 24 and 48 months. **D**, Patients with dialysis dependency at time of diagnosis. **E**, Patients developing end stage renal disease during follow-up. **F**, Patients developing a renal relapse during follow-up. Depicted are analyses of variance (ANOVA) for multiple comparisons.

glomerular filtration rate. Median GFR at baseline was 32 ml/min in the rituximab-treated patients and 27 ml/min in patients receiving cyclophosphamide induction therapy. Of these 28 paired patients, eight developed ESRD during follow-up. Four of these patients had been treated with rituximab and four patients had been treated with cyclophosphamide. Renal function during follow-up (GFR at 24 months) was compared. In the rituximab-treated patients, five had better and eight had worse renal function than did the matched patients with cyclophosphamide induction therapy. Median GFR at follow-up was 44 ml/min in the rituximab-treated patients and 35 ml/min in the cyclophosphamide-treated patients (Supporting information, Table S1).

Discussion

Renal involvement in ANCA-associated vasculitis causes necrotising crescentic glomerulonephritis often accompanied by considerable tubulointerstitial inflammation.[25,26] Despite extensive treatment, a significant proportion of patients have resistant or recurrent disease activity and develop organ damage and ESRD.[32] Markers for refractory disease activity are needed to identify patients at risk of perpetual inflammation to improve patient care and outcome.

Here, we investigated the lymphocytic organisation in renal biopsies as a prognostic marker for renal failure in patients with ANCA-associated GN. In our patient cohort, the degree of the local organisation of lymphocytes in the kidney biopsy was indeed more pronounced in patients developing ESRD during follow-up. The Kaplan–Meier survival curves demonstrate the usefulness of defining separate classes for the intrarenal lymphocytic organisation. According to the organisational degree of inflammatory infiltrates, patients differ in the risk for persistent renal failure. Interestingly, the lymphocytic organisation did not correlate with interstitial fibrosis and tubular atrophy. Therefore, the association with renal failure was not

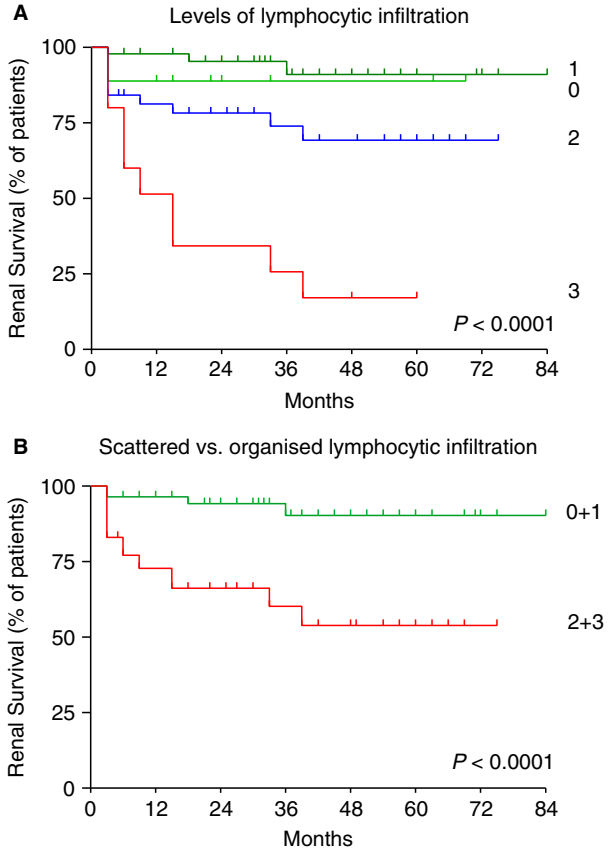


Figure 4. Renal survival according to the local lymphocytic response in anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis. Kaplan–Meier survival curves demonstrating the percentage of patients with end stage renal disease (ESRD) according to their intrarenal lymphocytic infiltration. **A**, Patients divided according to the lymphocytic organisation in the renal biopsy in T cells without a B cell infiltrate (0), scattered T and B cells (1), clustered (2) and compartmentally organised (nodular) infiltrates (3). **B**, Patients grouped according to their lymphocytic infiltration in T cells without B cell infiltrates and scattered T and B cell infiltrates (0 + 1) as well as clustered and compartmentally organised (nodular) T and B cell infiltrates (2 + 3).

due to the established prognostic marker of tubulointerstitial scarring.[25,26]

In humans, B cells are recruited to most chronically inflamed tissues, and the formation of tertiary lymphoid organs has been described in chronic diseases involving organs such as lung, gut, thyroid and synovia.[10] Data indicate that lymphoid neogenesis is a dynamic process during which sparse lymphocytic infiltrates evolve into aggregates that eventually organise in secondary B cell follicles, with follicular dendritic cells surrounded by a T cell zone and containing high endothelial venules.[33] In this way, B cell proliferation might lead to expansion and perpetuation of inflammatory cell aggregates, resulting in persistence and chronicity of inflammation. At present, the functional competence of these ectopic lymphoid structures remains unknown. The presence in renal ANCA disease suggests that they are an inductive site for self-reactive T cells and antibody production that contribute to pathology.

In the kidney, compartmentalisation processes of inflammatory cells have been described in different disorders, and we have proposed a classification of the intrarenal lymphocytic organisation in renal ANCA disease in the past.[17,19] A clinical correlation has not been determined so far.[21,23,24] In several mouse models of inflammation, ectopic B cell follicles have been associated with more severe disease.[34,35] In humans, lymphocytic organisation might contribute to disease progression. Local production of tissue-specific, disease-relevant autoantibodies has been shown in autoimmune thyroid disorders, myasthenia gravis, Sjögren's syndrome and rheumatoid arthritis.[11,13,36,37] A correlation was described between the formation of ectopic lymphoid tissue and antibody serum levels.[11] Anti-neutrophil cytoplasmic antibodies are thought to be pathogenic, and have been shown to cause small vessel vasculitis.[38] In this study, we focused upon tertiary lymphoid organs and did not investigate plasma cells and their antibody production. Plasma cells are, however, found frequently in diseased organs and have been described in the inflamed kidney.[36,39–41] This indicates the possibility that the lymphocytic organisation contributes to tissue destruction and loss of organ function.[42] It is not unlikely for the renal lymphocytic neogenesis to be a local source of plasma cells and their antibodies. The renal plasma cell niche has not been well studied in ANCA-associated vasculitis.

In renal ANCA disease, the persistent source of antigen and continued need for leucocyte extravasation could result in the structural organisation found in our cohort.[43] Therefore, the intrarenal lymphocytic organisation in ANCA-associated GN perhaps represents an adaption of the body to the increased demand for a localised immune response in the kidney. In conformity with this hypothesis, in our matched patient subcohort renal outcome did not differ, despite different immunosuppressive induction regimens.

Here, we show that organised lymphoid tissue in the kidney is associated with renal impairment and, therefore, it might contribute to the inflammatory process in ANCA-associated vasculitis. The appearance of lymphocytic clusters could indicate the need for an intensified induction treatment and a prolonged maintenance therapy in renal ANCA disease.

These results need confirmation in an independent prospective patient cohort. The classification of the lymphocytic infiltrate could then be used as an additional prognostic tool to develop an individualised risk prediction for ESRD, and future clinical trials might use this score for grading and outcome analyses.

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Conflicts of interest

The authors declare no financial or commercial conflicts of interest.

References

1. Jennette JC, Falk RJ. Small-vessel vasculitis. *N. Engl. J. Med.* 1997; **337**; 3.
2. Luqmani R. Maintenance of clinical remission in ANCA-associated vasculitis. *Nat. Rev. Rheumatol.* 2013; **9**; 127–132.
3. Wallace ZS, Lu N, Unizony S, Stone JH, Choi HK. Improved survival in granulomatosis with polyangiitis: a general population-based study. *Semin. Arthritis Rheum.* 2016; **45**; 483–489.
4. Jones RB, Tervaert JW, Hauser T *et al.* Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N. Engl. J. Med.* 2010; **363**; 211–220.
5. Stone JH, Merkel PA, Spiera R *et al.* Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N. Engl. J. Med.* 2010; **363**; 221–232.
6. Specks U, Merkel PA, Seo P *et al.* Efficacy of remission–induction regimens for ANCA-associated vasculitis. *N. Engl. J. Med.* 2013; **369**; 417–427.
7. Prineas JW. Multiple sclerosis: presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. *Science* 1979; **203**; 1123–1125.
8. Young CL, Adamson TC III, Vaughan JH, Fox RI. Immunohistologic characterization of synovial membrane lymphocytes in rheumatoid arthritis. *Arthritis Rheum.* 1984; **27**; 32–39.
9. Ruddle NH. Lymphoid neo-organogenesis: lymphotoxin's role in inflammation and development. *Immunol. Res.* 1999; **19**; 119–125.
10. Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. *Nat. Rev. Immunol.* 2006; **6**; 205–217.
11. Armengol MP, Juan M, Lucas-Martin A *et al.* Thyroid autoimmune disease: demonstration of thyroid antigen-specific B cells and recombination-activating gene expression in chemokine-containing active intrathyroidal germinal centers. *Am. J. Pathol.* 2001; **159**; 861–873.
12. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol.* 2004; **14**; 164–174.
13. Takemura S, Braun A, Crowson C *et al.* Lymphoid neogenesis in rheumatoid synovitis. *J. Immunol.* 2001; **167**; 1072–1080.
14. Kerjaschki D, Regele HM, Moosberger I *et al.* Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. *J. Am. Soc. Nephrol.* 2004; **15**; 603–612.
15. Martins HL, Silva C, Martini D, Noronha IL. Detection of B lymphocytes (CD20 +) in renal allograft biopsy specimens. *Transplant Proc.* 2007; **39**; 432–434.
16. Steinmetz OM, Panzer U, Kneissler U *et al.* BCA-1/CXCL13 expression is associated with CXCR5-positive B-cell cluster formation in acute renal transplant rejection. *Kidney Int.* 2005; **67**; 1616–1621.
17. Steinmetz OM, Velden J, Kneissler U *et al.* Analysis and classification of B-cell infiltrates in lupus and ANCA-associated nephritis. *Kidney Int.* 2008; **74**; 448–457.
18. Heller F, Lindenmeyer MT, Cohen CD *et al.* The contribution of B cells to renal interstitial inflammation. *Am. J. Pathol.* 2007; **170**; 457–468.
19. Segerer S, Schlondorff D. B cells and tertiary lymphoid organs in renal inflammation. *Kidney Int.* 2008; **73**; 533–537.
20. Chang A, Henderson SG, Brandt D *et al. In situ* B cell-mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. *J. Immunol.* 2011; **186**; 1849–1860.
21. Shen Y, Sun CY, Wu FX *et al.* Association of intrarenal B-cell infiltrates with clinical outcome in lupus nephritis: a study of 192 cases. *Clin. Dev. Immunol.* 2012; **2012**; 967584.
22. Sarwal M, Chua MS, Kambham N *et al.* Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. *N. Engl. J. Med.* 2003; **349**; 125–138.
23. Mengel M, Gwinner W, Schwarz A *et al.* Infiltrates in protocol biopsies from renal allografts. *Am. J. Transplant.* 2007; **7**; 356–365.
24. Kayler LK, Lakkis FG, Morgan C *et al.* Acute cellular rejection with CD20-positive lymphoid clusters in kidney transplant patients following lymphocyte depletion. *Am. J. Transplant.* 2007; **7**; 949–954.
25. Bajema IM, Hagen EC, Hermans J *et al.* Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis. *Kidney Int.* 1999; **56**; 1751–1758.
26. Hauer HA, Bajema IM, Van Houwelingen HC *et al.* Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. *Kidney Int.* 2002; **62**; 1732–1742.
27. Weidner S, Carl M, Riess R, Rupprecht HD. Histologic analysis of renal leukocyte infiltration in antineutrophil cytoplasmic antibody-associated vasculitis: importance of monocyte and neutrophil infiltration in tissue damage. *Arthritis Rheum.* 2004; **50**; 3651–3657.
28. Aasarod K, Bostad L, Hammerstrom J, Jorstad S, Iversen BM. Wegener's granulomatosis: inflammatory cells and markers of repair and fibrosis in renal biopsies–a clinicopathological study. *Scand. J. Urol. Nephrol.* 2001; **35**; 401–410.
29. de Lind van Wijngaarden RA, Hauer HA, Wolterbeek R *et al.* Clinical and histologic determinants of renal outcome in ANCA-associated vasculitis: a prospective analysis of 100

patients with severe renal involvement. *J. Am. Soc. Nephrol.* 2006; **17**; 2264–2274.
30. Berden AE, Jones RB, Erasmus DD *et al.* Tubular lesions predict renal outcome in antineutrophil cytoplasmic antibody-associated glomerulonephritis after rituximab therapy. *J. Am. Soc. Nephrol.* 2012; **23**; 313–321.
31. Geetha D, Sethi S, De Vriese AS *et al.* Interstitial immunostaining and renal outcomes in antineutrophil cytoplasmic antibody-associated glomerulonephritis. *Am. J. Nephrol.* 2017; **46**; 231–238.
32. Rhee RL, Hogan SL, Poulton CJ *et al.* Trends in long-term outcomes among patients with antineutrophil cytoplasmic antibody-associated vasculitis with renal disease. *Arthritis Rheumatol.* 2016; **68**; 1711–1720.
33. Neyt K, Perros F, GeurtsvanKessel CH, Hammad H, Lambrecht BN. Tertiary lymphoid organs in infection and autoimmunity. *Trends Immunol.* 2012; **33**; 297–305.
34. Kendall PL, Yu G, Woodward EJ, Thomas JW. Tertiary lymphoid structures in the pancreas promote selection of B lymphocytes in autoimmune diabetes. *J. Immunol.* 2007; **178**; 5643–5651.
35. Magliozzi R, Howell O, Vora A *et al.* Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* 2007; **130**; 1089–1104.
36. Salomonsson S, Jonsson MV, Skarstein K *et al.* Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjogren's syndrome. *Arthritis Rheum.* 2003; **48**; 3187–3201.
37. Roxanis I, Micklem K, McConville J, Newsom-Davis J, Willcox N. Thymic myoid cells and germinal center formation in myasthenia gravis; possible roles in pathogenesis. *J. Neuroimmunol.* 2002; **125**; 185–197.
38. Jennette JC, Falk RJ. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. *Nat. Rev. Rheumatol.* 2014; **10**; 463–473.
39. Sekine H, Watanabe H, Gilkeson GS. Enrichment of anti-glomerular antigen antibody-producing cells in the kidneys of MRL/MpJ-Fas(lpr) mice. *J. Immunol.* 2004; **172**; 3913–3921.
40. Teng YK, Levarht EW, Toes RE, Huizinga TW, van Laar JM. Residual inflammation after rituximab treatment is associated with sustained synovial plasma cell infiltration and enhanced B cell repopulation. *Ann. Rheum. Dis.* 2009; **68**; 1011–1016.
41. Chu VT, Enghard P, Schurer S *et al.* Systemic activation of the immune system induces aberrant BAFF and APRIL expression in B cells in patients with systemic lupus erythematosus. *Arthritis Rheum.* 2009; **60**; 2083–2093.
42. Kang S, Fedoriw Y, Brenneman EK, Truong YK, Kikly K, Vilen BJ. BAFF induces tertiary lymphoid structures and positions t cells within the glomeruli during lupus nephritis. *J. Immunol.* 2017; **198**; 2602–2611.
43. Steinmetz OM, Stahl RA, Panzer U. Formation of lymphoid-like tissue in the kidney – is there a role for chemokines? *Nephrol. Dial. Transplant.*. 2007; **22**; 350–352.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Ectopic lymphoid tissue in ANCA-associated glomerulonephritis.

Figure S2. Lymphocytic organisation in subgroup analyses

Table S1. Comparison of treatment outcomes in matched patients