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Urinary cell mRNA profiling distinguishes disease activity in antineutrophil cytoplasmic antibody-associated glomerulonephritis

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Keywords

ANCA; Vasculitis; Disease activity; Urinary markers

Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies with a predilection to affect small vessels, giving rise to a group of diseases broadly classified as ANCA-associated vasculitis (AAV). Glomerulonephritis (GN) is a hallmark of AAV and progression to end-stage renal disease is common if not treated promptly. While kidney biopsy is the gold standard to diagnose active GN, repeat biopsies are not practical.

Previous studies have implicated the role of B cells, neutrophils, and macrophages in AAV pathogenesis [1–5]. A role for T cells is increasingly being recognized [S1–S15]. While non-invasive urinary cell messenger ribonucleic acid (mRNA) profiling for T cell markers has been employed in discerning lupus flares and allograft rejection [S16–S22], it has yet to be studied in ANCA-associated glomerulonephritis (ANCA GN). In this study we explored the utility of urinary cell levels of a panel of mRNAs encoding proteins implicated in the innate and adaptive immune systems to discern active and remission phases in ANCA GN.

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Declarations

Ethical statement All procedures performed in in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

With approval from the Johns Hopkins Institutional Review Board, we conducted a prospective cohort study of 28 patients with biopsy-proven ANCA GN. Urine samples were collected during the active and remission phase. Disease activity was scored using the Birmingham Vasculitis Activity Score (BVAS). Total RNA was isolated from the urinary cells and mRNA expression of 22 genes was examined by reverse transcription and polymerase chain reaction (PCR). Mann–Whitney *U* test and AUC were used to evaluate the ability of mRNA to discriminate between groups. Methods are detailed in Supplementary Methods.

Eleven patients with active disease and 17 in remission were included. There were no significant differences between the groups with respect to age, ANCA subtype, and mean glomerular filtration rate (GFR). The majority of patients in both groups received glucocorticoids and rituximab for remission induction (Supplementary Table 1).

Urinary expression of mRNAs for Perforin ($p = 0.001$), Granzyme B ($p = 0.02$), CD20 ($p = 0.012$), Vimentin ($p = 0.0068$), and RANTES ($p = 0.05$) was significantly higher in the active disease group compared to the remission group (Fig. 1). The urinary expression of mRNA for FOXP3 was numerically higher in the active samples with a tendency to significance ($p = 0.06$). There were no significant differences between the groups for mRNAs encoding TGFβ1, CD25, Tbet, CD3GB, MCP1, MIG, C3, CXCR3, E-cad, NKCC2, VEGF, CD46, and IP10. ROC curve analysis highlights the urinary markers that were able to discriminate vasculitic activity (Supplementary Fig. 1).

Distinguishing active from remission phase remains a challenge in ANCA GN. While hematuria, proteinuria, and rise in serum creatinine are non-invasive, they can be unreliable indicators of ANCA GN activity. There is an ultimate reliance on renal biopsy, but this is associated with multiple potential risks as well as sampling bias. Previous studies have demonstrated the potential use of urine soluble CD163 (usCD163), a secretory product of activated macrophages [4, S26], as well as serum soluble CD25 (ssCD25) and urine soluble CD25 (usCD25), molecules generated from activated T cells (S12), in detecting active renal vasculitis. On the other hand, other studies have demonstrated the limitations of these markers, highlighting their increase in sepsis and lupus nephritis [S27–S29].

In our study, perforin and granzyme were found to be potent markers of active disease. These markers have been shown to be involved with cytotoxic CD8 + T cell and CD4 + T cell activities [S30, S31]. The presence of CD4 + effector memory T (TEM) cells in the urine has been demonstrated to reflect renal involvement in AAV [S8]. Moreover, vimentin is an intermediate filament component of the cellular cytoskeleton that is expressed in mesenchymal cells [S32]. There is data highlighting the involvement of vimentin in regulatory T cell (Treg) activity [S33] which is of interest, as functional Treg deficiency may play a role in the pathogenesis of AAV [S5, S6]. FOXP3 is also a critical regulator for Treg development and has been shown to be upregulated in patients with active lupus nephritis [S18]. Our finding of significantly increased urinary mRNA expression of vimentin and numerically higher FOXP3 in patients with active disease adds further to this axis of cell-mediated immune regulation in AAV.

RANTES is a chemokine that is predominantly secreted by CD8 + T cells and mediates the trafficking of various immune cells and potentially leukocyte activation to directly induce inflammation [S34, S35]. RANTES was able to detect active vasculitis in our study, supporting the role of this chemokine in T cell-generated leukocyte activation and infiltration. Additionally, the finding of high urinary CD20 mRNA in active vasculitis further supports the established role of B-cell dysregulation and autoantibody production in AAV.

While our study is limited by its small sample size and lack of paired urine samples in all patients, it is the first study to evaluate the utility of urinary cell mRNA profiling to discriminate between active and remission phases of ANCA GN. Further studies are needed to confirm the association of urinary cell mRNA excretion profile to histologic indices of disease activity. Additionally, our study uniquely identifies potential markers connected to the largely unexplored role of T cells in disease activation. Future studies investigating the mechanisms of T cell subsets, along with their interactions with established perpetrators of disease, may yield exciting results for translation into potential therapeutics for ANCA GN.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Conflict of interest

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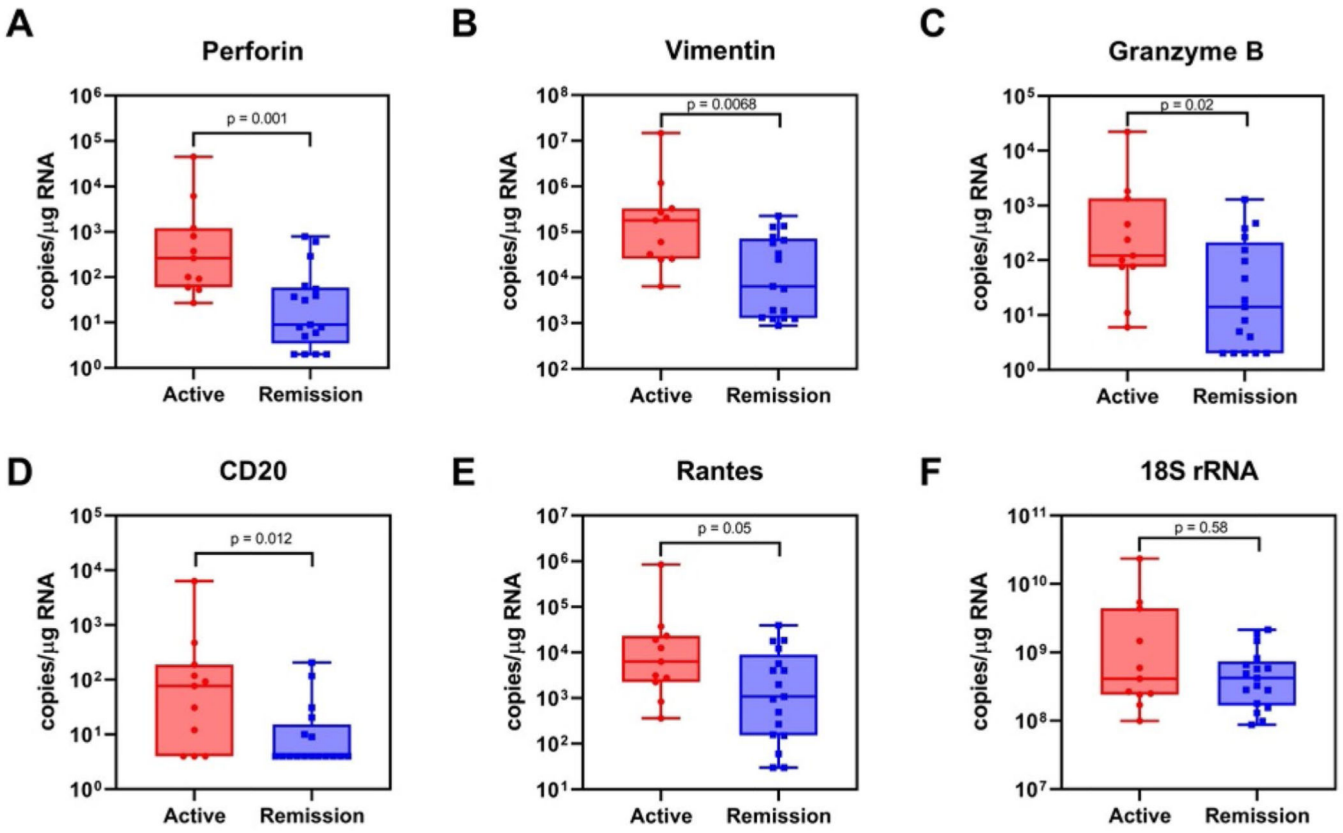

Fig. 1.

Levels of urinary cell mRNA transcripts in active and remission phases of disease. Box and Whisker plots show the minimum, 25th percentile, median, 75th percentile, and maximum for **A** Perforin, **B** Vimentin, **C** Granzyme B, **D** CD20, **E** Rantes, and **F** 18 s rRNA in urine samples collected during the Active ($n = 11$) and Remission ($n = 17$) phases. The levels of Perforin ($p = 0.001$), Vimentin ($p = 0.0068$), Granzyme B ($p = 0.02$), CD 20 ($p = 0.012$), and Rantes ($p = 0.05$) mRNAs, but not 18S rRNA ($p = 0.58$) were significantly higher in urine matched to the Active phase compared to urine matched to the Remission phase. *P* values were calculated using Mann–Whitney Test