Review Article

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# Biomarkers in ANCA-Associated Vasculitis: Potential Pitfalls and Future Prospects

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## Abstract

**Over the past 3 decades, significant advancements in the understanding of the pathophysiology of ANCA-associated vasculitis has led to the development of a multitude of potential candidate biomarkers. Accompanied by the advent of increasingly effective therapeutic strategies, the need for a dependable biomarker to help determine the extent of disease activity and risk of relapse is ever present. Implementation of such a biomarker would enable tailored therapy, optimizing disease control while helping to mitigate unnecessary exposure to therapy and potential treatment-related damage. Although far from perfect, ANCA serology and B-cell population are the two main staple biomarker tools widely used in practice to help supplement clinical assessment. Over recent years, the application and progress of more novel biomarker tools have arisen in both organ-limited and multisystem disease, including genomics, urinary proteins, degradation products of the alternative complement system, cytokines, metabolomics, and biospectroscopy. Validation studies and clinical translation of these tools are required, with serial assessment of disease activity and determination of therapy according to biomarker status correlated with patient outcomes.**

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## Introduction

Pauci-immune small-vessel vasculitis characterizes a group of relapsing diseases with potential multiorgan involvement, typically with circulating ANCA. The last decade has seen the advent of less toxic therapies and the move to a more tailored approach in the ANCA-associated vasculitides (AAV). These advances have improved patient outcomes, especially in the elderly and those with significant comorbidity, although treatment toxicity remains a significant risk (1–3). Since their first description nearly 40 years ago, ANCA have been used to aid initial diagnosis, with limited utility for disease monitoring. Given the continued therapeutic advances over the same period, it is surprising that comparatively little progress has been made in the use of laboratory biomarkers. This is particularly relevant given the role of histopathology to monitor disease activity is restricted by the risks of biopsy, and the accessibility and potential low diagnostic yield contingent on any extrarenal biopsy site (4–8). The use of imaging to monitor disease activity is equally limited. Computerized tomography is commonly used in patients with respiratory tract disease, but the radiologic features described are not specific to vasculitis (4,9,10). The time to interval change and repeated exposure to ionizing radiation further limits the use of such serial imaging for disease monitoring. More modern imaging techniques, such as positron emission tomography, are only validated for large-vessel vasculitis. These limitations are even more relevant in relapsing disease, where clinicians want to avoid both under- or over-treatment, and in distinguishing active AAV from infection. Therefore, a practical and specific biomarker that accurately correlates with systemic-disease activity remains a significant unmet need in the field. Its absence presents a significant challenge to clinicians when gauging the presence of relapsing or persistent disease. This unmet need also contrasts with the move toward less toxic and more individualized options for immunosuppressive therapy. In this primer for treating clinicians, we review currently used biomarkers in AAV and discuss their limitations. We also discuss novel biomarkers, highlight potential avenues for further research and aim to define the ideal biomarker for AAV (Figure 1). In the review that follows, PubMed and Cochrane databases were each searched using the search criteria: "ANCA" OR "anti-neutrophil cytoplasmic antibody" OR "vasculitis" OR "PR3" OR "MPO" OR "ANCA-associated" OR "renal vasculitis" AND "biomarker" OR "marker" OR "activity" OR "relapse," with further literature searches according to the presented subsections identified. The included articles reported potential biomarker application in AAV after assessment by two authors *via* a consensus process. Case reports, editorials, letters to the editor, review articles, conference abstracts, and studies not published in English were excluded. Table 1 provides a summary of the current and prospective noninvasive biomarkers in AAV discussed.

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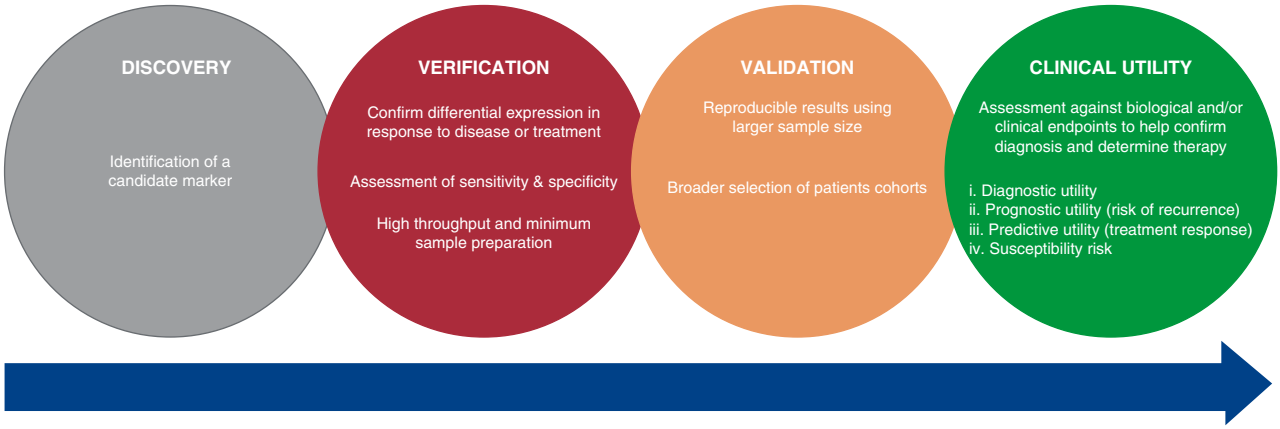


**Figure 1.** | **The required stages of biomarker development**.

## ANCA

The diagnostic value of ANCA in the context of clinical symptoms is well established, yet its role in the prediction of relapsing disease remains debatable. Numerous studies have attempted to delineate the role of serial ANCA monitoring with varying results and a lack of consensus on reported outcomes. The subsequent discordance between ANCA serology and disease activity limits any support for its use as a reliable biomarker.

Early retrospective studies supported the relationship between ANCA and disease activity; however, their sensitivity and positive predictive value for relapse remained relatively low at 23%–28% (11–15). An initial systematic review in 2006 attempted to provide more insight, but was unable to undertake a meta-analysis and offer any meaningful conclusion due to the considerable method heterogeneity and suboptimal design of most studies for the assessment of test accuracy (16). A subsequent meta-analysis by Tomasson *et al.* (17) was more stringent in its study selection, identifying a modest association, at best, for persisting ANCA positivity or rising titers with the risk of relapse. Several studies with more longitudinal data, including follow-up data from large trials, have since corroborated earlier findings that persistent ANCA positivity, ANCA reappearance, and the presence of anti–proteinase 3 (anti-PR3) antibodies are risk factors for relapsing disease (18–24). Similarly, seronegativity after remission-induction therapy is associated with a longer relapse-free survival period (18,19). The positive predictive value of detectable ANCA and disease relapse increases when combined with the clinical index of suspicion for active disease (25).

Ultimately, it is the presence of seronegative disease and positivity in the absence of disease that limits the use of ANCA as a functional biomarker. Despite the compelling *in vitro* and limited *in vivo* evidence base for the pathogenicity of ANCA, the reported rate of *de novo*, seronegative, pauci-immune GN varies from 12% to 30%, with up to 54% of patients with limited extrarenal disease and a significant proportion of those with relapsing disease exhibiting undetectable circulating ANCA (26–33). This prompts reconsideration of the current putative pathogenesis and assays. One possibility is the presence of a novel autoantibody. One candidate is anti–tissue plasminogen autoantibodies, which are thought to be integral to the fibrinolytic system and have been observed in up to 25% of patients with anti-PR3 and anti-myeloperoxidase (anti-MPO) positivity (34). These patients tended to display more severe glomerular inflammation, microthrombi, and increased thrombotic events; however, future study is required to further evaluate its role in disease. Another potential candidate is the anti–lysosomal-associated membrane protein-2 (anti-LAMP2) autoantibody. LAMP2 is a heavily glycosylated membrane protein that plays a key role in cellular homeostasis and is coexpressed on neutrophils with MPO and PR3 as a target of ANCA. Initial studies suggested a high degree of correlation with disease activity and a potential pathogenic role in ANCA-negative disease; however, these findings were not validated on subsequent study (35–37). A second consideration is that anti-PR3 and anti-MPO antibodies may be present, but either remain below the detection limit of current enzyme immunoassays or epitope masking may confound their detection (38,39). In 2013, Roth *et al.* (39) undertook a study in linear ANCA epitope mapping and disease correlation. In doing so, they identified a pathogenic anti-MPO autoantibody to a new, immunodominant, sole, linear-sequence epitope in seronegative disease—the detection of which is obscured by a fragment of ceruloplasmin in serum on conventional tests (39). It is also possible that disease is mediated by IgA ANCA (40). Current mainstream ELISAs for ANCA detect IgG. Kelley *et al.* (40) identified the presence of IgA ANCA in a significant proportion of patients who otherwise tested negative for IgG ANCA, and demonstrated the ability of IgA ANCA to mediate disease through neutrophil stimulation.

Lastly, circulating ANCA has been detected in individuals without any known history of disease. Two case-control studies confirmed positive ANCA serology from biobank samples of asymptomatic individuals up to 19 years before disease onset (41,42). In this context, it is possible that these individuals might have been lacking the "second hit" required for disease at the time of sample collection. Similarly, anti-PR3 and anti-MPO positivity have been detected in healthy individuals and with other diagnoses, including inflammatory bowel disease, liver disease, rheumatic disease, and infection, such as tuberculosis (39,43,44). The

**Table 1. Summary of current and prospective noninvasive biomarkers in ANCA-associated vasculitis**

| Biomarker | Source | Organ System | Comment | Reference |
|---|---|---|---|---|
| ANCA | Serum | Multisystem disease | Diagnostic value in the context of clinical symptoms is well established. | |
| | | | Persistent ANCA positivity, ANCA reappearance and the presence of anti–proteinase 3 antibodies are risk factors for relapsing disease. | 18–24 |
| | | | Discordance with serology and disease activity, with seronegative disease and positivity in the absence of disease restricting its use as a reliable biomarker. | 26–33,39,41–44 |
| Anti-LAMP2 Ab | Serum | Multisystem disease | Initial studies suggested a potential role in pathogenesis and association with disease activity, although these findings were not corroborated in subsequent study. | 35–37 |
| Anti–tissue plasminogen Ab | Serum | Multisystem disease | Associated with ANCA seropositivity and a higher degree of acute inflammatory renal lesions. Validation studies are required along with determination of its prognostic and predictive utility. | 34 |
| CD19+ B-cell population | Serum | Multisystem disease | Conflicting data on the prognostic utility of B-cell reconstitution from follow-up data of several large trials. | 22,24,46,47 |
| | | | Relapsing disease can occur despite peripheral B-cell depletion with B-cells present in tissue sites of active disease. | 48 |
| | | | B-cell depletion should not provide reassurance of a reduced relapse risk and repopulation may indicate susceptibility when taken into account with other clinical parameters. | |
| Cytokines | Serum | Multisystem disease | CXCL-13, TIMP-1, and MMP-3 each distinguish active disease from remission with a high degree of accuracy. Further validation study is required to assess their use. | 55 |
| | | | Conflicting data exist on the association of BAFF with disease activity. | 51–54 |
| | | | Conflicting data exist on the association of Bregs, such as CD5+ B-cells, with disease activity and its prognostic utility. | 49,50 |
| T-cells | Serum | Multisystem disease | T-cell activity is associated with disease activity, with elevated levels of IL-2 and CD30; further validation study and assessment of its clinical utility are required. | 54 |
| ESR and acute-phase proteins | Serum | Multisystem disease | ESR and acute-phase proteins—including CRP, calprotectin, hepcidin and procalcitonin—remain nonspecific for active AAV with limited clinical use. | 55–64 |
| N/L and P/L ratio | Plasma | Multisystem disease | Both the N/L and P/L ratio are potential predictors of disease severity, but both require larger prospective study. | 57,65,66 |

**Table 1. (Continued)**

| Biomarker | Source | Organ System | Comment | Reference |
|---|---|---|---|---|
| NGAL | Serum | Multisystem disease | Higher levels of NGAL are associated with relapsing disease, but this remains nonspecific and should be cautiously interpreted. | 67 |
| Endothelial cells | Plasma | Multisystem disease | Circulating necrotic endothelial cells offer a direct index of vascular damage, with a high degree of correlation in active AAV, although intensive resource requirements limit its clinical application and possibility for validation study. | 68 |
| Angiopoietin 2 | Plasma | Multisystem disease | Limited ability to distinguish active from quiescent disease or predict relapse. | 69 |
| Complement | Serum | Multisystem disease | Higher plasma concentrations of alternative complement-pathway degradation products in active disease. Prospective study is required with assessment in relapsing disease. | 71–76 |
| | Urine | Renal-limited disease | Higher urinary degradation products associated with active renal vasculitis, with urinary Bb inversely correlated with the percentage of normal glomeruli. These results require validation study. | 70 |
| mRNA | Plasma | Multisystem disease | Autoantigen gene expression is a risk factor for disease through histone depletion, hypomethylation and impaired transcriptional repression. Lower levels of DNMT1 mRNA and subsequent DNA hypomethylation is associated with active disease and a higher risk of relapse. | 88,89 |
| | | | CD8+ T-cell transcriptional profile is predictive of relapsing disease. Further prospective validation studies of gene-expression profiles are required. | 90 |
| MCP-1 | Urine | Renal-limited disease | Prospective validation studies have demonstrated a positive association of urinary MCP-1 levels with active renal vasculitis, with a corresponding fall after remission-induction therapy. Evaluation of its clinical utility now required. | 79–82 |
| Soluble CD163 | Urine | Renal-limited disease | Higher urinary levels of soluble CD163 cleaved from macrophages and monocytes conferred a high sensitivity and specificity for active renal vasculitis compared with remission. This correlates with the degree of inflammatory lesions on histopathology in both new and relapsing ANCA-associated GN. Potential elevation can occur in infection with study of its clinical utility required. | 83,84 |

**Table 1. (Continued)**

| Biomarker | Source | Organ System | Comment | Reference |
|---|---|---|---|---|
| Metabolomics | Serum | Multisystem disease | Active vasculitis is associated with a distinctive metabolomic profile of raised N-acetyl glycoproteins, LDLs/VLDLs, choline, and glycerophosphocholine; whereas glucose and amino acids were reduced compared with control groups. | 91 |
| | Urine | Renal-limited disease | Raised urinary myo-inositol and hypocitraturia is present in active disease, and a ratio of the two closely associated with active renal vasculitis. Validation study and evaluation in relapsing disease required. | 92 |
| Biospectroscopy | Urine | Renal-limited disease | Biospectroscopy offers a novel and low-cost surrogate technique of determining a sample's metabolomic profile. One study observed the 1545 $cm^{-1}$ spectral band increasing in intensity in line with glomerular inflammation and treatment response, whereas 1033 $cm^{-1}$ was inversely related with the degree of fibrosis. | 101 |

Anti-LAMP2, anti–lysosomal-associated membrane antibody; Ab, antibody; CXCL-13, chemoattractant chemokine (C-X-C motif) ligand 13; TIMP-1, tissue inhibitor of metallopeptidase inhibitor 1; MMP-3, matrix metalloproteinase 3; BAFF, B-cell activating factor; Breg, regulatory B cells; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; N/L, neutrophil/lymphocyte; P/L, platelet/lymphocyte; NGAL, neutrophil gelatinase-associated lipocalin; DNMT1, DNA methyltransferase 1; MCP-1, monocyte chemoattractant protein-1.

presence of anti-MPO ANCA in healthy individuals may represent differing epitope specificity (39).

## B-Cell Population and Cytokines

Data from observational studies suggest that incomplete B-cell depletion and B-cell repopulation after rituximab treatment is associated with a significantly higher relapse rate (18,45). Supporting this, follow-up data from RITUXVAS observed that B-cell repopulation accompanied all cases of relapsing disease (46). However, the trial was not powered to draw any significant conclusion from this subgroup and follow-up data from several other larger trials did not corroborate this finding. Among the RAVE cohort, B-cell population did not predict relapse with disease occuring despite undetectable CD19+ B-cells in the vast majority of relapsing cases and the presence of B-cell detectability in quiescent disease (22). Similarly, data from both the MAINRITSAN and MAINRITSAN 2 trials found that CD19+ B-cell reconstitution was not predictive of relapse (24,47). Confirmation of the tenuous association of B-cell population with disease activity comes from a case report by Ferraro *et al.* (48) that demonstrated relapsing disease with B-cells present in tissue sites of active disease, despite peripheral depletion. As such, B-cell depletion should not provide reassurance of a reduced relapse risk and repopulation may indicate susceptibility when taken into account with other clinical parameters.

B-cell subset populations that have drawn interest include regulatory B-cells (B*regs*), such as CD5+ cells. The CD5 protein attenuates activating signals from the B-cell receptor, downregulating B-cell activity. Measurement of B*regs* showed initial promise with a lower CD5+ B-cell count correlating with active disease, whereas maintaining a normal count conferred a longer relapse-free survival period (49). Data from the RAVE study observed similar findings, but subsequent analysis found that serial CD5+ B-cell count was not predictive of disease relapse, severity, or treatment failure (50).

Key cytokines may offer another predictive tool. Elevated levels of B-cell activating factor (BAFF) have been found in active disease, with a corresponding fall post-treatment (51,52). However, the few studies evaluating its predictive value found no association with disease activity and a potential inverse correlation with ANCA titer, bringing into question its role in autoantibody production (53,54). Indicators of T-cell activation have also been observed, with elevated levels of soluble IL-2 receptors and CD30 in active disease, but further study is required to elicit their potential biomarker role (54). A panel of chemokines and circulating proteins have been prospectively evaluated from patients enrolled in the RAVE trial at presentation and 6 months postremission. This identified three candidate markers: the B-lymphocyte chemoattractant chemokine (C-X-C motif) ligand 13, matrix metalloproteinase 3 (MMP-3) and tissue inhibitor of metallopeptidase inhibitor 1 (TIMP-1). These distinguished active disease from remission with an area under the curve of >0.8 and likelihood ratio of 4.3–4.6, warranting future assessment (55).

## Inflammatory Markers

Traditional inflammatory markers, such as erythrocyte sedimentation rate and C-reactive protein, are nonspecific for AAV with limited clinical use (55–57). A large, cross-sectional study of the Birmingham Vasculitis Activity Score 3 (BVAS) provided confirmation of this, demonstrating a poor correlation between BVAS and C-reactive protein, with a limited role for such inflammatory markers in assessing disease activity (58). Other inflammatory markers—such as calprotectin, hepcidin, and procalcitonin—ave also been evaluated, but face the same limitation (57,59–64).

The function of activated platelets in disease propagation has drawn attention to their level as a potential gauge of disease activity. Willeke *et al.* (57) observed significantly higher counts in active AAV, although there was an irregularity in their findings with relatively lower levels in more severe disease. Park *et al.* (65) evaluated the platelet/lymphocyte ratio, identifying a value >272 as an independent predictor of severe disease; however, confounding factors could not be accounted for. Similarly, Ahn *et al.* (66) observed that patients exhibiting a neutrophil/lymphocyte ratio >5.9 at diagnosis tended to present with more severe disease and have a higher frequency of future relapse. Application of the neutrophil/lymphocyte ratio is needed in larger prospective studies to determine its reliability.

Neutrophil gelatinase-associated lipocalin (NGAL) provides a marker of neutrophil degranulation, with significantly higher levels found at the time of diagnosis and in relapsing AAV (67). NGAL has also been extensively investigated as an early predictor of AKI and as with the other inflammatory markers discussed, if used it should be interpreted alongside an array of other clinical parameters to help inform an assessment of disease activity.

More direct indices of vascular damage have been investigated. Analysis of circulating necrotic endothelial cells yielded promising results, with higher levels in ANCA-associated GN compared with remission and control groups, although its intensive resource requirements may have restricted clinical application (68). Investigation of angiopoietin 2 was of limited clinical utility, failing to discriminate disease activity after clinically successful treatment or to predict relapses (69).

## Complement

Alternative complement-pathway activation is fundamental for the development of disease and urinary degradation products provide a potential biomarker of renal vasculitis. Gou *et al.* (70) found that urinary levels of Bb, C3a, C5a, and soluble C5b-9 were significantly higher in active disease, in addition to Bb—in effect—providing a surrogate marker of renal histopathology with inverse correlation with the percentage of normal glomeruli. The same group subsequently demonstrated that these degradation products were also significantly higher in plasma among patients with active multisystem disease (71). Several retrospective studies have since analyzed circulating levels of C3 and their relation to patient outcomes. A low level is present in up to 35% of patients at the time of initial diagnosis, with a higher likelihood of more severe disease and poorer renal function at presentation (72,73). Prognostically, this has been associated with a poorer renal and patient survival (74–76). Circulating markers of alternative complement-pathway activation holds promise, but studies assessing their use in relapsing disease are lacking and their role as a functional biomarker of disease activity requires further study.

In 2015, Chen *et al.* (77) concluded that plasma levels of complement factor H (CFH), a negative regulator of the alternative pathway, were lower in patients with active disease and were inversely correlated with renal function, renal inflammation, and BVAS. An *in vitro* study by the same group supported the hypothesis that higher CFH inhibited ANCA-induced neutrophil activation with reduced functional activity in patients with active disease (78). This raises the question of whether a subgroup of patients has a predisposition to disease due to an absolute or functional deficiency of CFH. Measurement of circulating CFH may help identify those patients who may be more susceptible to disease and subsequent future potential relapse.

## Urinary Proteins and Chemokines

Elevated levels of urinary monocyte chemoattractant protein-1 (MCP-1) have been found among patients with active or persistent renal vasculitis, correlating with upregulated macrophage infiltration in severely inflamed glomeruli and a corresponding fall in urinary MCP-1 after successful treatment (79–82). CD163 is expressed on monocytes and macrophages, functioning as a scavenger receptor for the hemoglobin-haptoglobin complex. It also provides a surrogate marker of cell activity, with cleavage to soluble CD163 (sCD163) in a proinflammatory state. In a rodent model of disease, O'Reilly *et al.* (83) detected higher levels from urine in small-vessel vasculitis compared with other glomerular pathologies. Subsequent human study with an external validation cohort confirmed noticeably higher urinary levels in active disease (likelihood ratio, 20.8) (83). Evaluation of urinary sCD163 with serial renal biopsy specimen data has since demonstrated a high degree of correlation with fibrinoid necrosis and cellular crescents in those with both *de novo* and relapsing ANCA-associated GN compared with remission and healthy controls (84). This also lends support to the position that serial comparative analysis of non-invasive biomarkers and histopathology in renal vasculitis is potentially feasible and, arguably, needed as the ideal reference standard when determining their clinical utility in predicting outcomes and disease recurrence (85,86).

Moran *et al.* (87) combined urinary MCP-1 in patients positive for urinary sCD163, with a 98% specificity and positive likelihood ratio of 19.2 for relapsing disease in the presence of new-onset proteinuria, subject to pretest probability. Both urinary proteins offer a promising non-invasive candidate biomarker that could be translated into clinical practice, although their use is limited to renal vasculitis with potential elevation in the context of infection.

## mRNA

Variation in autoantigen gene expression has been confirmed as a risk factor for disease through histone depletion, hypomethylation, and impaired transcriptional repression due to reduced RUNX3 at the MPO and PRTN3 gene loci. Jones *et al.* (88) confirmed this link by investigating the DNA methyltransferase 1 (DNMT1) gene expression required for DNA methylation and downregulation of autoantigen expression. In doing so, they found the degree of DNMT1 mRNA positively correlated with DNA methylation and negatively correlated with PRTN3 and MPO gene expression (88). As such, a reduction in DNMT1 mRNA and DNA hypomethylation was associated with active disease and predicted a higher risk of relapse (hazard ratio, 4.55; 95% CI, 2.09 to 9.91), whereas patients exhibiting increased DNA methylation at the PRTN3 promoter in remission had a greater likelihood of a longer relapse-free survival period (88). Contrary to this, Kurz *et al.* (89) concluded that elevated leukocyte PR3 mRNA was not predictive of relapsing disease, although this may reflect the transrepressive effect of concurrent glucocorticoid therapy.

In 2010, McKinney *et al.* (90) quantified the gene-expression profiles from purified leukocytes among patients with active AAV to prospectively predict future relapse risk. This identified that transcriptional profiling of $CD8^+$ T-cells with overexpression of mRNA encoding proteins for the IL-7 receptor pathway, T-cell receptor signaling, and expanded $CD8^+$ T-cell memory population conferred a poorer prognosis (90). This finding has the potential for translation to clinical practice and requires validation in a prospective study with longitudinal data.

## Metabolomics

Metabolomics enables the quantitative analysis of the substrates and products of metabolism to directly reflect the biochemical activity within a sample. Variation in the metabolomic profile will be reflective of changes in the underlying biochemical composition caused by physiologic processes or pathologic states. Studies applying metabolomics in AAV are limited. In 2016, Al-Ani *et al.* (91) analyzed the urinary metabolomic profile in a rodent model of disease using nuclear magnetic resonance spectroscopy and chemometric analysis. This identified a distinctive metabolomic profile in active disease, which resolved after successful treatment, with subsequent recurrence in relapsing disease. A large patient-cohort study by the same group yielded similar results (91). Gupta *et al.* (92) has since evaluated metabolomics in serum, identifying a profile that was specific to active AAV with good separation from control groups, including Takyasu arteritis and SLE. The role of metabolomics as a robust and relatively non-invasive biomarker of disease activity in AAV merits further study, but its associated costs may limit its potential application.

## Biospectroscopy

Biospectroscopy provides a novel and low-cost surrogate technique of determining the metabolomic profile of a sample through one of two primary techniques: attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy and Raman spectroscopy (93,94). Irrespective of the modality used, biochemical changes caused by disease will result in a unique spectral fingerprint that is representative of the underlying pathophysiologic state. Advancements in instrumentation and standardized chemometric analysis have enabled the successful application of biospectroscopy across numerous areas of medicine, including rheumatic disease, lymphocyte subsets, cytokine monitoring, and nephrology (95–100). Its application in vasculitis is emerging, with one previous study using ATR-FTIR to identify potential urinary biomarkers in an animal model of crescentic GN and in patients with ANCA-associated GN (101). This

identified the 1545 $cm^{-1}$ spectral marker as a key wavenumber variable, increasing in intensity in line with the degree of glomerular injury and subsiding after treatment. In parallel, the intensity of 1033 $cm^{-1}$ was inversely related with the degree of fibrosis. These findings suggest that ATR-FTIR could be used as a fast, innovative method of monitoring disease progression and treatment response in renal vasculitis. The promising use of biospectroscopy to provide a robust biomarker of disease activity in AAV, which can be readily translated to clinical practice requires further study.

## Conclusions

The remarkable progress in treatment strategies over the past 3 decades has been accompanied by a rising disease prevalence. Yet a reliable biomarker to detect relapsing or persistent disease is lacking, risking increasing morbidity and mortality from suboptimal disease control or unnecessary patient exposure to potentially harmful therapy. As such, there is a need for the development of a functional biomarker to enable risk stratification and individualization of treatment. Alongside the use of more innovative analytic tools, an improved understanding of the underlying immunopathogenesis and treatment targets has led to the identification of several promising novel candidate markers for renal-limited and multisystem disease. With favorable initial results, further validation studies with longitudinal data are required to elicit their potential role and translation into clinical practice.

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### Author Contributions

A.P. Dhaygude, F.L. Martin, A.D. Morris, A.W. Rowbottom, and A. Woywodt reviewed and edited the manuscript; A.P. Dhaygude, F.L. Martin, and A.W. Rowbottom provided supervision; and A.D. Morris conceptualized the study, was responsible for data curation, and wrote the original draft.

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