Biomarkers in ANCA-associated vasculitis: potential pitfalls and future prospects

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Abstract

Over the past three 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. Their implementation would enable tailored therapy, optimising disease control whilst 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 are required, with serial assessment of disease activity and determination of therapy according to biomarker status correlated with patient outcomes.
Introduction

Pauci-immune small vessel vasculitis characterises a group of relapsing diseases with potential multi-organ involvement, typically with circulating anti-neutrophil cytoplasmic autoantibodies (ANCA). The last decade has seen the advent of less toxic therapy 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 co-morbidity, 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 that the role of histopathology to monitor disease activity is restricted by the risks of biopsy, as well as the accessibility and potential low diagnostic yield contingent on extrarenal biopsy site\(^{4–8}\). The use of imaging to monitor disease activity is equally limited. Computerised tomography (CT) is commonly used in patients with respiratory tract disease, but the radiological features described are not specific to vasculitis\(^{4,9,10}\). The time to interval change and repeated exposure to ionising 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, as well as distinguishing active AAV from infection. A practical and specific biomarker that accurately correlates with systemic disease activity therefore 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 towards less toxic and more individualised 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. In the review that follows Pubmed and Cochrane databases were each searched using the following 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. Included articles reported potential biomarker application in AAV following 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.

**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 Tomassen et al was more stringent in its study selection, identifying a modest association at best for persisting ANCA positivity or rising titres with the risk of relapse\(^{(17)}\). 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 (PR3) antibodies are risk factors for relapsing disease\(^{(18–24)}\). Similarly, seronegativity following 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 glomerulonephritis varies from 12-30%, with up to 54% of patients with limited extra-renal disease and a significant proportion of 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-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 anti-lysoosomal-associated membrane protein-2 (LAMP2) autoantibody. LAMP2 is a heavily glycosylated membrane protein that plays a key role in cellular homeostasis and is co-expressed 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 that epitope masking may confound their detection(38,39). In 2013 Roth et al 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 immunoglobulin A (IgA) ANCA(40). Current mainstream ELISAs for ANCA detect immunoglobulin G (IgG). Kelley et al identified the presence of immunoglobulin A (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\(^{40}\).

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 nineteen years prior to 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 other diagnoses including inflammatory bowel disease, liver disease, rheumatic disease and infection such as tuberculosis\(^{39,43,44}\). The presence of anti-MPO ANCA in healthy individuals may represent differing epitope specificity\(^{39}\).

**B-cell population & Cytokines**

Data from observational studies suggests that incomplete B-cell depletion and B-cell repopulation post rituximab 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. Amongst the RAVE cohort, B-cell population did not predict relapse with disease occurring 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 that demonstrated relapsing disease with B-cells present in tissue sites of active disease despite peripheral depletion\(^{48}\). 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 (Bregs), such as CD5+ B-cells. The CD5 protein attenuates activating signals from the B-cell receptor, downregulating B-cell activity. Measurement of Bregs showed initial promise with a lower CD5+ B-cell count correlating with active disease, while 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\(^{(51,52)}\). Although the few studies evaluating its predictive value found no association with disease activity and a potential inverse correlation with ANCA titre, 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 six months post remission. This identified three candidate markers; the B-lymphocyte chemoattractant chemokine (C-X-C motif) ligand 13 (CXCL13), matrix metalloproteinase 3 (MMP-3) and tissue inhibitor of metallopeptidase inhibitor 1 (TIMP-1). These distinguished active disease from remission with an AUC >0.8 and likelihood ratio of 4.3-4.6, warranting future assessment\(^{(55)}\).

**Inflammatory markers**

Traditional inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are non-specific 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 CRP with a limited role for such inflammatory markers in assessing disease activity\(^{(58)}\). Other inflammatory markers such as calprotectin, hepcidin and procalcitonin have 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 2015 observed significantly higher counts in active AAV, although there was an irregularity in their findings with relatively lower levels in more severe disease\(^{(57)}\). Park et al evaluated the platelet:lymphocyte ratio, identifying a value >272 as an independent predictor of severe disease, however confounding factors could not be accounted for\(^{(65)}\). Similarly, Ahn et al 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\(^{(66)}\). Application of the N:L 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 relapsing AAV\(^{(67)}\). NGAL has also been extensively investigated as an early predictor of acute kidney injury and as with other inflammatory markers discussed, if employed its use should be interpreted alongside an array of other clinical parameters to help inform an assessment 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 glomerulonephritis compared to 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 following 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 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[70]. The same group subsequently demonstrated that these degradation products were also significantly higher in plasma amongst patients with active multisystem disease[71]. Several retrospective studies have since analysed circulating levels of C3 and their relation to patients 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 concluded that plasma levels of complement factor H (CFH), a negative regulator of the alternative pathway, were lower in patients with active disease and inversely correlated with renal function, renal inflammation and BVAS[77]. 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 whether or not a subgroup of patients have 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 & chemokines

Elevated levels of urinary monocyte chemoattractant protein-1 (uMCP-1) have been found amongst
patients with active or persistent renal vasculitis, correlating with upregulated macrophage
infiltration in severely inflamed glomeruli and a corresponding fall in uMCP-1 following successful
treatment\(^{(79–82)}\). CD163 is expressed on monocytes and macrophages, functioning as scavenger
receptors for the haemoglobin-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 \textit{et al} detected higher levels from urine in SVV compared to other glomerular
pathologies\(^{(83)}\). 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 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 glomerulonephritis
compared to 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\(^{(85,86)}\).

\textbf{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 \textit{et al} confirmed this link by investigating DNA methyltransferase
1 (DNMT1) gene expression required for DNA methylation and downregulation of autoantigen
expression. In doing so they found that 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 (HR 4.55, 95% CI 2.09 – 9.91), while 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 concluded that elevated leukocyte PR3 mRNA was not predictive of relapsing disease, although this may reflect the transrepressive effect of concurrent glucocorticoid therapy (89).

In 2010, McKinney et al quantified the gene expression profiles from purified leukocytes amongst patients with active AAV to prospectively predict future relapse risk (90). This identified that transcriptional profiling of CD8+ T-cells with overexpression of mRNA encoding proteins for interleukin-7 receptor pathway, T-cell receptor signalling 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 physiological processes or pathological states. Studies applying metabolomics in AAV are limited. In 2016 Al-Ani et al analysed the urinary metabolomic profile in a rodent model of disease using nuclear magnetic resonance spectroscopy and chemometric analysis (91). This identified a distinctive metabolomic profile in active disease, which resolved following successful treatment with subsequent recurrence in relapsing disease. A large patient cohort study by the same group yielded similar results (91). Gupta et al has since evaluated metabolomics in serum, identifying a profile that was specific to active AAV with good
separation from control groups including Takayasu’s arteritis and systemic lupus erythematosus\(^{(92)}\).

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 pathophysiological state. Advancements in instrumentation and standardised 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 utilising ATR-FTIR to identify potential urinary biomarkers in an animal model of crescentic glomerulonephritis and patients with ANCA-associated glomerulonephritis\(^{(101)}\). This identified the 1545 cm\(^{-1}\) spectral marker as a key wavenumber variable, increasing in intensity in line the degree of glomerular injury and subsiding following 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 that can be readily translated to clinical practice requires further study.

**Conclusion**

The remarkable progress in treatment strategies over the past three 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 individualisation of treatment. Alongside the use of more innovative analytical 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 favourable initial results, further validation studies with longitudinal data are required to elicit their potential role and translation into clinical practice.

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F Martin: Supervision; Writing - review and editing
A Woywodt: Writing - review and editing
A Dhaygude: Supervision; Writing - review and editing.


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Table 1: Summary of current and prospective non-invasive biomarkers in ANCA-associated vasculitis

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Source</th>
<th>Organ System</th>
<th>Comment</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANCA</td>
<td>Serum</td>
<td>Multisystem disease</td>
<td>• Diagnostic value in the context of clinical symptoms is well established.</td>
<td>18-24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Persistent ANCA positivity, ANCA reappearance and the presence of anti-proteinase 3 antibodies are risk factors for relapsing disease.</td>
<td>26-33, 39 &amp; 41-44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Discordance with serology and disease activity with seronegative disease and positivity in the absence of disease restricts its use as a reliable biomarker.</td>
<td></td>
</tr>
<tr>
<td>Anti-LAMP2 Ab</td>
<td>Serum</td>
<td>Multisystem disease</td>
<td>• Initial studies suggested a potential role in pathogenesis and association with disease activity, although these findings were not corroborated in subsequent study.</td>
<td>35-37</td>
</tr>
<tr>
<td>Anti-tissue plasminogen Ab</td>
<td>Serum</td>
<td>Multisystem disease</td>
<td>• Associated with ANCA seropositivity and a higher degree of acute inflammatory renal lesions.</td>
<td>34</td>
</tr>
<tr>
<td>CD19+ B-cell population</td>
<td>Serum</td>
<td>Multisystem disease</td>
<td>• Conflicting data on the prognostic utility of B-cell reconstitution from follow up data of several large trials.</td>
<td>22, 24, 46 &amp; 47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Relapsing disease can occur despite peripheral B-cell depletion with B-cells present in tissue sites of active disease.</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 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.</td>
<td></td>
</tr>
<tr>
<td>Cytokines</td>
<td>Serum</td>
<td>Multisystem disease</td>
<td>• CXCL-13, TIMP-1 &amp; MMP-3 each distinguish active disease from remission with a high degree of accuracy. Further validation study is required to assess their use.</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Conflicting data exists on the association of BAFF with disease activity.</td>
<td>51-54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Conflicting data exists on the association of Bregs, such as CD5+ B-cells, with disease activity and its prognostic utility.</td>
<td>49 &amp; 50</td>
</tr>
<tr>
<td>T-cells</td>
<td>Serum</td>
<td>Multisystem disease</td>
<td>• T-cell activity is associated with disease activity with elevated levels of IL-2 and CD30 with further validation study and assessment of its clinical utility required.</td>
<td>54</td>
</tr>
<tr>
<td>ESR &amp; acute phase proteins</td>
<td>Serum</td>
<td>Multisystem disease</td>
<td>• ESR &amp; acute phase proteins including CRP, calprotectin, hepcidin, procalcitonin remain non-specific for active AAV with limited clinical use.</td>
<td>55-64</td>
</tr>
<tr>
<td>N:L &amp; P:L ratio</td>
<td>Plasma</td>
<td>Multisystem disease</td>
<td>• Both the N:L &amp; P:L ratio are potential predictors of disease severity, but both require larger prospective study.</td>
<td>57, 65 &amp; 66</td>
</tr>
<tr>
<td>NGAL</td>
<td>Serum</td>
<td>Multisystem disease</td>
<td>• Higher levels of NGAL are associated with relapsing disease, but remains non-specific and should be cautiously interpreted.</td>
<td>67</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>Plasma</td>
<td>Multisystem disease</td>
<td>• Circulating necrotic endothelial cells offer a direct indices of vascular damage with a high degree of correlation in active ANCA-associated vasculitis, although intensive resource requirements limit its clinical application and validation study.</td>
<td>68</td>
</tr>
<tr>
<td>Angiopoietin 2</td>
<td>Plasma</td>
<td>Multisystem disease</td>
<td>• Limited ability to distinguish active from quiescent disease or predict relapse.</td>
<td>69</td>
</tr>
<tr>
<td>Complement</td>
<td>Serum</td>
<td>Multisystem disease</td>
<td>• Higher plasma concentrations of alternative complement pathway degradation products in active disease. Prospective study is required with assessment in relapsing disease.</td>
<td>71-76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Higher urinary degradation products associated with active renal vasculitis, with urinary Bb inversely correlated with the percentage or normal glomeruli. These results require validation study.</td>
<td>70</td>
</tr>
<tr>
<td>mRNA</td>
<td>Plasma</td>
<td>Multisystem disease</td>
<td>• Autoantigen gene expression is a risk factor for disease through histone depletion, hypomethylation &amp; impaired transcriptional repression. Lower levels of DNMT1 mRNA &amp; subsequent DNA hypomethylation is associated with active disease and a higher risk of relapse.</td>
<td>88 &amp; 89</td>
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<tr>
<td></td>
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<td></td>
<td>• CD8+ T-cell transcriptional profile is predictive of relapsing disease.</td>
<td>90</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Further prospective validation studies of gene expression profiles are required.</td>
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</tr>
<tr>
<td>MCP-1</td>
<td>Urine</td>
<td>Renal limited disease</td>
<td>• Prospective validation studies have demonstrated a positive association of urinary MCP-1 levels with active renal vasculitis, with a corresponding fall following remission-induction therapy. Evaluation of its clinical utility now required.</td>
<td>79-82</td>
</tr>
</tbody>
</table>
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 to remission. This correlates with the degree of inflammatory lesions on histopathology in both new and relapsing ANCA-associated glomerulonephritis. Potential elevation can occur in infection with study of its clinical utility required.

Metabolomics
- Serum Multisystem disease
  - Active vasculitis is associated with a distinctive metabolomic profile of raised N-acetyl glycoproteins, low/very low density lipoproteins, choline and glycerophosphocholine, whereas glucose and amino acids were reduced compared to control groups.

- 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.
  - Further study of metabolomics is warranted, although associated costs may limit its potential application.

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. 1033 cm⁻¹ was inversely related with the degree of fibrosis.

ANCA, anti-neutrophil cytoplasmic autoantibody; anti-LAMP2 Ab, anti-lysosomal-associated membrane antibody; CXCL-13, chemoattractant chemokine (C-X-C motif) ligand 13; TIMP-1, tissue inhibitor of metalloproteinase 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 ratio, neutrophil:lymphocyte ratio; P:L ratio, platelet:lymphocyte ratio; NGAL, neutrophil gelatinase-associated lipocalin; DNMT1, DNA methyltransferase 1; MCP-1, monocyte chemoattractant protein-1.
Figure 1: Stages of biomarker development

**DISCOVERY**
- Identification of a candidate marker

**VERIFICATION**
- Confirm differential expression in response to disease or treatment
- Assessment of sensitivity & specificity
- High throughput and minimum sample preparation

**VALIDATION**
- Reproducible results using larger sample size
- Broader selection of patients cohorts

**CLINICAL UTILITY**
- Assessment against biological and/or clinical endpoints to help confirm diagnosis and determine therapy
  - i. Diagnostic utility
  - ii. Prognostic utility (risk of recurrence)
  - iii. Predictive utility (treatment response)
  - iv. Susceptibility risk