Curr Rheumatol Rep (2013) 15:363
DOI 10.1007/s11926-013-0363-x

VASCULITIS (LR ESPINOZA, SECTION EDITOR)

Biomarkers in ANCA-Associated Vasculitis

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Published online: 17 August 2013

Abstract Despite recent advances in the treatment of anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV), relapse remains common and patients often experience a variable clinical course after initial treatment. New biomarkers are needed to aid the management of these complex diseases. Discoveries regarding the pathogenesis of AAV, from the importance both of activated B and T cells and the alternative complement pathway to genomic data, may lay the groundwork for identification of novel biomarkers.

Keywords ANCA · AAV · ANCA-associated vasculitis · Biomarkers · Granulomatosis with polyangiitis · Wegener's granulomatosis · Microscopic polyangiitis · Complement

Introduction

Granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA) constitute antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides (AAV), a group of systemic diseases characterized by pauci-immune necrotizing inflammation of small to medium vessels. AAV affects multiple organ systems, with glomerulonephritis and pulmonary involvement being frequent manifestations. Present in most patients, ANCAs directed against proteinase-3 (PR3) or myeloperoxidase (MPO) are often associated with GPA and MPA respectively [1]. Relapses of the disease are common, occurring in half of patients with GPA after induction of remission [2, 3]. Predicting relapse before development of overt clinical symptoms remains a challenge. Changes in ANCA titers or a rise in traditional acute phase indicators, for example erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), lack the sensitivity and specificity for clinically relevant prediction of relapse [4–6]. The importance of serial ANCA measurements in disease monitoring remains controversial. Given the highly variable disease course which can result in organ or life-threatening disease and development of permanent damage, biomarkers that would enable distinction of active disease from damage or infection, herald relapse, or predict treatment response and prognosis are areas of active investigation. Robust databanks of longitudinal clinical information and bio-repositories compiled from therapeutic trials in AAV are a rich resource from which to identify and verify potential biomarkers which could fill this unmet need in management of AAV [7, 8•]. Potential biomarkers include lymphocyte subsets implicated in AAV pathogenesis, markers of vascular activation or damage, and organ-specific markers.

ANCA

ANCA directed against PR3 or MPO are of undisputed importance in the diagnosis of AAV; however, data supporting use of ANCA as a biomarker of disease activity are less clear. There is substantial evidence of involvement of ANCA in the pathogenesis of AAV via interactions with primed neutrophils both in vitro and in animal models [9, 10]. Interaction with ANCA leads to neutrophil activation and initiation of the oxidative burst culminating in endothelial damage. Irrespective of the critical function of ANCA in disease development and propagation, observational cohorts have failed to show a definitive correlation between ANCA levels and disease activity. In a systematic review addressing the use of serial ANCA measurements to monitor disease activity, Birck et al. analyzed 22

This article is part of the Topical Collection on *Vasculitis*

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studies and found wide variation in reported clinical utility of ANCA titers for monitoring of disease activity [11]. Reported sensitivity of an increasing ANCA titer for diagnosis of relapse ranged from 24 % to 100 %. Variations in definition of remission and relapse, different follow up time and time between serial ANCA measurements, different techniques for ANCA measurement, and how the rise in titer is defined can account for discrepancies between studies. In the right clinical setting and with supporting pathology when available, ANCA remain a cornerstone for AAV diagnosis but are insufficiently sensitive or specific for monitoring disease activity and predicting relapse. A meta-analysis by Tomasson et al. focused on the utility of serial ANCA measurements for predicting relapse for patients with either rising ANCA titer or persistently positive ANCA [12•]. Trying to account for heterogeneity between studies, the authors found that both rising ANCA or persistently positive ANCA were associated with flare (positive likelihood ratio 2.84 and 1.97, respectively); however, they concluded that ANCA titer alone was insufficient to guide treatment decisions. Furthermore, persistently negative ANCA in a patient who was previously ANCA-positive does not guarantee disease quiescence. In a study of 100 AAV patients followed prospectively with serial ANCA, 13 of the 37 patients who relapsed were ANCA-negative at time of relapse [13].

GPA differs from MPA by the presence of granulomatous inflammation, with predominantly upper airway involvement, concurrent with necrotizing vasculitis. Patients with GPA, which is associated with PR3 ANCA in more than 80 % of cases, are at higher risk of relapse than patients with MPA [14]. In a cohort of over 500 patients with AAV and biopsy-proved glomerulonephritis, PR3 specificity conferred a twofold risk of relapse compared with MPO [15]. ANCA specificity for PR3 was independently associated with relapse, irrespective of pathology or clinical classification using the Chapel Hill Consensus Conference definitions of AAV. Because patients with PR3 are more likely to relapse, attributes specific to PR3 have been investigated as potential predictors of disease activity and relapse. Using serologic and disease activity data from the Wegener's Granulomatosis Etanercept Trial (WGET), Finkielman et al. compared the utility of pro-PR3, an inactive pro-enzyme, with PR3, the active serine protease, as a marker of disease activity [4]. There was no significant difference between pro-PR3 and mature PR3 for measurement of disease activity in this prospective cohort; furthermore, in a longitudinal analysis of individual patients, fluctuation in ANCA levels accounted for less than 10 % of observed changes in disease activity.

Glycosylation and sialylation of the Fc portion of antibodies can alter clearance and ability to become deposited in tissue, thereby potentially altering pathogenicity. Espy et al. investigated the effects of modification of PR3 via sialylation on pathogenicity and correlation with disease activity among 42 GPA patients [16]. Statistically significant differences in sialylation of PR3 were detected in patients with active GPA compared with those in remission. In-vitro studies showed a correlation between sialylation of PR3 and neutrophil oxidative burst, suggesting involvement in pathogenesis. These techniques are not commercially available and clinical applicability longitudinally and in a larger patient population are unknown.

Ongoing recognition of the clinical distinctions between PR3 and MPO could result in greater insight into the mechanisms of disease relapse; this may, in turn, lead to novel biomarkers. A recent genome-wide association study of patients with AAV established that GPA and MPA have distinct genetic backgrounds with different associations in both major histocompatibility complex (MHC) and non-MHC genes, depending on ANCA specificity [17••]. This finding further supports a difference between GPA and MPA and may provide the basis for future genetic markers to aid diagnosis, therapy, and disease monitoring. The clinical and pathogenic significance of auto-antibodies other than traditional ANCAs are being investigated. One such autoantibody is human lysosome-associated membrane protein-2 (LAMP-2). LAMP-2 is co-expressed with MPO and PR3 in neutrophils; however, unlike MPO and PR3, LAMP-2 is also expressed in glomerular endothelial cells, a key site of injury in AAV. LAMP-2 antibodies are pathogenic in vitro and in animal models and share 100 % homology with a bacterial adhesion protein, FimH [18]. LAMP-2 autoantibodies were initially reported to be present in >85 % of patients with untreated AAV [19]. The same investigators measured LAMP-2 autoantibodies in a cohort of 84 AAV patients with renal involvement and, by using several techniques to measure LAMP-2, confirmed prevalence of 93 % at disease presentation [20]. Once remission was achieved, only 6 % of patients remained LAMP-2 positive. LAMP-2 antibodies were not detected in any of the healthy controls studied and were positive in only 1 of 30 patients with other renal disease. These promising results were not replicated in a study by another group who found LAMP-2 positivity in only 21 % of AAV patients compared with 16 % of controls [21]. Use of different LAMP-2 assays and patient populations (active vs. quiescent) are likely to explain the discrepant results of these studies [22]. Given these conflicting results and lack of commercial assays, the value of LAMP-2 measurement in AAV remains unknown.

B and T lymphocytes

B lymphocytes are implicated in the pathogenesis of AAV by giving rising to autoantibody-producing plasma cells, contributing to local cytokine production, acting as antigen-presenting cells, and through the T cell co-stimulatory pathway. Importance of B lymphocytes in the pathogenesis of

AAV is strengthened by the clinical efficacy of B-cell-targeted therapy. Rituximab (RTX), the monoclonal anti-CD20 antibody, was demonstrated to be non-inferior to cyclophosphamide for induction of remission in severe AAV and possibly superior to cyclophosphamide for treatment of relapsing disease [23]. B lymphocyte subsets and B cell-associated factors have been investigated as potential biomarkers in AAV.

Higher levels of activated (CD19+/CD38+) B cells have been detected in the serum of patients with active GPA compared with inactive GPA and healthy controls [24]. Activated B cells did not correlate with ANCA level. More recent studies have used flow cytometry to characterize circulating lymphocytes in patients with AAV, with a focus on regulatory B cells (Bregs) [25, 26]. Wilde et al. report decreased levels of IL-10-producing Bregs in AAV patients compared with healthy controls. The percentage of circulating Bregs could not be used to discriminate active disease from remission, although the number of patients with active disease was small. Conversely, using CD25+ as a marker of Bregs, Eriksson et al. demonstrated that CD25+ B cells occurred significantly more frequently in patients in remission than in patients with active AAV and healthy controls. CD25+ was inversely related to CRP and disease activity; however, it is unknown whether this CD25+ population reflects a regulatory mechanism or is merely the effect of recent treatment with cytotoxic medications.

Neutrophils activated by ANCA have been shown to upregulate production of BAFF (B lymphocyte activating factor), a key B cell survival factor [27]. BAFF levels are increased in the serum of patients with AAV compared with healthy controls. However, BAFF levels are affected by corticosteroid treatment and do not correlate with disease activity, rendering it an inadequate biomarker in AAV [28, 29].

A recently published observational study reporting the Mayo Clinic's decade-long experience with repeated RTX treatment for refractory GPA suggests utility in the combination of B lymphocyte reconstitution and ANCA level in predicting relapse [30]. In this historical cohort of chronically relapsing patients, all observed relapses occurred after B cell reconstitution and were temporally accompanied by an increase in PR3 levels (except for one patient who was ANCA negative). While time to B cell reconstitution varied between patients, the authors concluded that "preemptive retreatment decisions can be individualized based on serial B lymphocyte and PR3-ANCA monitoring." Although the overall generalizability of this conclusion can be questioned, because of the refractory nature of included patients, in addition to racial and ethnic homogeneity, this suggests that in a subset of PR3-positive patients with relapsing disease treated with RTX, the combination of B cell reconstitution and PR3 level can serve as a biomarker for relapsing disease and provide information on which a therapeutic strategy can be based.

T cells are also involved in the pathogenesis of AAV. T cells are the predominant cells in inflammatory tissue infiltrates in AAV. The number of circulating CD4+ effector T cells is also increased in sera from AAV patients. Unlike activated B cells, which are increased in the sera of active but not quiescent AAV, abnormally activated T cells were detected during periods of disease activity and remission [31]. McKinney et al. studied the transcriptome of purified cells (T cells, B cells, neutrophils, and monocytes) from 59 patients with active AAV in an attempt to identify biomarkers [32]. CD8+ T cell expression profiles divided patients into two distinct subgroups; although the two groups did not differ in disease activity scores, inflammatory markers, or immunosuppressive therapy, patients in one group were at significantly increased risk of disease flare or relapse. The CD8+ T cells associated with risk of relapse displayed markers associated with T cell survival and a memory T cell population. If validated in prospective cohorts, CD8+ T cell profile may be able to identify a group of AAV patients more likely to relapse, which could lead to a more customized approach to maintenance therapy.

T regulatory cells (Tregs) have also been studied in AAV. Tregs are present in lower numbers and have less functional capacity in AAV [33]. Moreover, Treg proportion was inversely correlated with relapse and positively associated with time to remission. A more comprehensive understanding of the mechanisms of action of Tregs in facilitating disease remission and examination of Treg numbers and functionality in larger populations of AAV patients may lead to identification of biomarkers with prognostic and therapeutic importance.

Complement

Complement activation, particularly through the alternative pathway, has been implicated in the pathogenesis of AAV in animal models [34]. In a mouse model of glomerulonephritis (GN) initiated by inoculation with MPO antibodies, complement depletion resulted in a completely normal phenotype. Inhibiting the alternative pathway but not the classical or lectin pathways could block development of GN. The common pathway component C5a was subsequently identified as a key complement component in AAV-associated GN in murine models. C5a is thought to prime neutrophils for an ANCA-induced respiratory burst, which has been demonstrated in-vitro in a dose-dependent manner by use of recombinant C5a [35]. Recently, complement activation and C5a levels were studied in human AAV patients for the first time. In the initial study of C5a in human AAV, Yuan et al. demonstrated higher levels of C5a in patients with active AAV compared with AAV in remission, lupus nephritis, and healthy controls [36]. Similarly, expression of C5a receptor (CD88) was lower in renal tissue of patients with active AAV. The authors suggest that beyond a proposed pathogenic effect of C5a–CD88 interaction in AAV, these molecules may serve as

biomarkers of disease severity. The same group also measured a circulating complement activation profile in the plasma of patients with active AAV compared with AAV patients in remission [37]. Plasma levels of fragment Bb, a measure of alternative pathway activation, were significantly higher in active AAV than in patients in remission or in healthy controls. Fragment Bb levels correlated with the number of crescents on renal histology, vasculitis activity score, and serum inflammatory markers. Again, the authors conclude circulating levels of Bb may prove to be a useful biomarker of disease activity in AAV. Although these small studies offer insight into pathogenic mechanisms of AAV and suggest potential therapeutic targets, use of complement components as biomarkers in AAV must be evaluated among larger cohorts and in non-renal disease.

Table 1 Summary of biomarkers/potential biomarkers in AAV

Biomarker	Comment
Inflammatory markers (ESR, CRP)	Low sensitivity and lack of specificity for active AAV
ANCA	Change in titer or persistent positivity alone insufficient to guide treatment according to recent meta-analysis
Sialylation of PR3	Not commercially available: statistically significant difference in sialylation between active disease and remission in single study
LAMP-2 antibody	Standardized detection techniques needed: titers fall rapidly after treatment and suitability for predicting relapse is unknown
B lymphocytes:	
Activated B cells	Higher levels of activated B cells in active disease vs remission
CD25+ B regs	Higher levels in remission compared with active disease, though may reflect treatment effect
B cell reconstitution and rising ANCA following RTX	Serial measurements can aid retreatment decisions on individual basis
T lymphocytes:	
CD8+ expression profiles	Able to identify group of patients more likely to relapse in small study; must be validated prospectively
Complement:	Technically difficult to measure and results yet to be replicated for larger cohorts
C5a	Higher levels in active disease compared with remission, may correlate with disease severity
Fragment Bb	Higher levels in active disease compared with remission, may correlate with renal disease severity
Urine MCP-1	Elevated levels associated with relapse, serum levels inversely related to disease activity

Kidney-Specific Markers

GN is a common manifestation of AAV which can result in end-stage renal disease or chronic renal impairment. Renal involvement can occur at first presentation; in GPA, however, 60 % of renal disease arises with subsequent relapse after initial diagnosis. Although kidney biopsy remains the method of choice for diagnosis of GN, gauging disease activity with repeated kidney biopsies is not clinically feasible. Elevation of serum creatinine cannot distinguish active vasculitis from damage or other causes of renal injury; furthermore, creatinine elevation may not be detected until significant or even permanent damage has already occurred. Measurement of cells and protein in urine also lacks sensitivity and specificity for determination of GN from active vasculitis. There is, therefore, a need for biomarkers for early detection of GN which can be measured without invasive biopsies and before accrual of permanent damage.

Lieberthal et al. investigated potential urinary biomarkers in AAV by studying four urinary proteins known to be associated with active renal disease in other forms of GN [38]. Of the four candidate proteins studied, monocyte-chemoattractant protein-1 (MCP-1) was most suitable for discrimination between active disease and remission. Urinary MCP-1, a chemokine which promotes monocyte recruitment to areas of inflammation, has previously been shown to be increased in AAV patients in remission compared with controls [39]. Elevated urine levels of MCP-1 were associated with poor prognosis and relapse. In a longitudinal study, plasma MCP-1 levels were inversely correlated with disease activity in GPA patients; levels of plasma MCP-1 were lower in patients with active disease than in patients for whom remission was achieved [40].

Conclusions

Clinically useful biomarkers for prediction of relapse and response to therapy are needed for management of AAV. New insights into the pathogenesis of these complex diseases, including genomic data, may ultimately lead to the identification of novel biomarkers (Table 1). Clinical judgment and vigilance remain paramount in the treatment of AAV.

Compliance with Ethics Guidelines

Conflict of Interest Robert F. Spiera has served as a consultant and on advisory boards for Roche/Genentech and has received grant support from Roche/Genentech and Human Genome Sciences.

Lindsay Lally declares that she has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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