ANCA in systemic sclerosis, when vasculitis overlaps with vasculopathy: a devastating combination of pathologies

Review

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Abstract

In patients with systemic sclerosis (SSc), the coexistence of ANCA-associated vasculitis (SSc-AAV) has been reported to be associated with a severe disease course, including significant pulmonary and renal involvement. The presence of ANCA is not uncommon in patients with SSc and therefore clinicians must maintain a high index of clinical suspicion about SSc-AAV. p-ANCA and anti-MPO antibodies are the most common antibodies observed. Patients typically present with clinical features of microscopic polyangiitis or renal-limited vasculitis There are multiple areas of potential interaction in the pathogenesis of SSc and AAV which can exacerbate/compound vascular disease. In addition, similar patterns of major internal organ involvement (e.g., lung and kidneys) are seen in both conditions. We highlight a diagnostic approach to SSc-AAV and the paucity of data to inform management. As such, SSc-AAV is typically treated as per isolated AAV which can potentially be hazardous in patients with SSc (e.g., the association between high-dose steroid and scleroderma renal crisis). We propose that this rare clinical entity warrants rigorous investigation including definition of a therapeutic strategy to ameliorate the potentially devastating combination of pathologies in SSc-AAV.

Key words: Systemic sclerosis; scleroderma; ANCA; vasculitis; vasculopathy

Key messages:

- Presence of ANCA is not uncommon in patients with SSc.
- Vasculitis can overlap and compound vasculopathy in SSc.
- Coexistence of ANCA-associated vasculitis in SSc is associated with a severe disease course.

Introduction

Systemic sclerosis (SSc) is a complex autoimmune connective tissue disease (CTD) characterised by vascular abnormalities (often referred to as 'vasculopathy'), immune (both innate and adaptive) system activation, and widespread tissue fibrosis of the skin and major internal organs (1,2). SSc-vasculopathy is responsible for much of the non-lethal morbidity associated with the disease (e.g., Raynaud's phenomenon and digital ulcers) and mortality (e.g., pulmonary hypertension) (1–3). Antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) are a group of rare diseases that are characterised by small vessel inflammation and the development of antibodies towards myeloperoxidase (MPO) and proteinase 3 (4,5). The three major subtypes of AAV are granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA). AAV are complex multi-organ diseases which commonly affect the upper and lower respiratory tract and kidneys and without treatment are associated with high mortality.

In patients with SSc, the coexistence of AAV (SSc-AAV) has been reported to be associated with a severe disease phenotype and disease course, including from pulmonary and renal involvement, which in some cases demands very fast and prompt decisions. There are multiple areas of potential interaction in the pathogenesis of SSc and AAV that can exacerbate/compound underlying SSc-vasculopathy. In SSc and AAV small vessel disease is characteristic; however, involvement of the medium vessels can occur in both. Against this background, the aim of this viewpoint is to describe the pathogenesis, clinical features and treatment of SSc-AAV, highlighting the challenges posed by this rare disease entity, and the unmet needs therein.

Search strategy

Due to the rarity of SSc-AAV, the following search strategy was adopted in accordance with recommendations for narrative reviews (6). Data (224 citations) were identified within the National Institutes of Health's National Library of Medicine (PubMed) using the following search criteria (01/01/1970-19/12/2020):

(Systemic sclerosis OR scleroderma) AND (ANCA) AND ((pathogenesis) OR (management) OR (treatment))

Original research articles (including abbreviated reports, case reports, and reviews) identified were scrutinised for data relating to the major subheadings of this review. A grey search of manuscripts cited within these articles was also undertaken.

Pathogenesis of vascular disease in SSc and AAV

There are significant similarities and differences in the pathogenesis of SSc and AAV with multiple areas of potential interaction which can exacerbate vascular disease in SSc, resulting in a severe disease phenotype. For example, vascular injury is a central feature of both SSc and AAV. Whereas vascular inflammation is seldom seen in SSc and is a cardinal feature of AAV.

Pathogenesis of SSc-associated vasculopathy

The pathogenesis of the vascular disease in SSc appears to involve many cell types and signalling pathways. Endothelial cell (EC) injury leads to decreased capillary density and to fibroproliferative intimal lesions in larger blood vessels. This vascular remodelling is systemic as depicted by the widespread intimal proliferation in the pulmonary, coronary, and renal arteries (7). The vascular endothelium is the primary target in the disease, and EC interactions with other cells and pathways, including the innate and adaptive immune system, platelets and coagulation factors, smooth muscle cells, pericytes, and fibroblasts mediate SSc pathogenesis ((8) The exact mechanism for the widespread SSc vascular disease is still unknown. Disorganised microvasculature and vascular dysfunction occur early before the onset of fibrosis. The downstream effects of vascular dysfunction adversely affect organ function and determine clinical outcomes.

Endothelial injury

The event that initiates vascular injury in SSc is currently unknown. Microbial triggers such as *CMV*, *Parvovirus B19* and *Helicobacter pylori* are suggested ((9) EC apoptosis and cytotoxicity are well documented in the early stages of the disease. Some studies suggested that cytotoxic T cells or natural killer (NK) may mediate the injury, while other studies implied a role for the Fas/FasL pathway in cytotoxicity ((10) Circulating autoantibodies to EC are reported to induce cell apoptosis ((11)

Endothelial dysfunction

Increased vascular permeability is seen early leading to the “puffy” hand’s appearance (12) Vascular tone imbalance favouring excess vasoconstrictors and diminished vasodilators results

in heightened vasospastic propensity. A proinflammatory role is demonstrated by increased expression of endothelial cellular adhesion molecules, intercellular adhesion molecule-1, vascular adhesion molecule-1, E-selectin and P-selectin. Platelet activation, aggregation, and granular release are reported. Microvascular thrombosis and enhanced fibrin deposition are frequently encountered in SSc. Shorter fibrinogen half-life and enhanced fibrinolytic system activity are associated with high levels of fibrin breakdown products (D-dimers) (13)

Defective angiogenesis

Despite reduced capillary density due to injury, there is paradoxically an insufficient angiogenic response in SSc. Hypoxia and tissue ischemia typically lead to robust angio/vasculogenesis and vascular repair, but new capillaries are rarely seen in SSc, where instead there are substantial avascular areas suggesting defective angiogenesis and vascular-repair pathways (14).

Pathogenesis of AAV

AAVs target small-medium vessels. In GPA and MPA, neutrophils primed by TNF-α, IL-1β or ligation of toll-like receptors, and further activated by ANCA, localise to cytokine-activated vascular endothelium. These close interactions predispose to endothelial injury and monolayer disruption. As the disease evolves a necrotising vasculitis develops with fibrinoid necrosis of the vessel wall. In GPA, pathogenesis is further associated with granulomatous inflammation. EGPA pathogenesis is distinct, dominated by eosinophil-associated vascular injury resulting in necrotising granulomatous inflammation.

ANCA

ANCA development reflects loss of immune tolerance to two predominant antigens proteinase-3 and myeloperoxidase. Classical GPA associates with a PR3-ANCA in ~70%, MPO-ANCA ~25%, while in MPA MPO-ANCA is seen in 60%, PR3-ANCA 25%, with 5-10% of patients ANCA negative. In contrast, 60% of EGPA is ANCA negative, 35-40% MPO-ANCA positive and <5% PR3-ANCA positive (4) Although low-titre non-pathogenic ANCA can be identified in healthy individuals, pathogenic ANCA are of high affinity, driven in part by epitope spreading (15) Loss of tolerance predates detectable disease and important underlying mechanisms include defective Tregs (16) and Bregs ((17) with the former exhibiting a Th17, IL-17 synthesising phenotype (18)Autoreactive B cells, stimulated by cytokines including B

cell activating factor (BAFF, also known as BLys and TNF ligand superfamily member 13B), help maintain disease ((19)

In addition to environmental factors ((20) evidence exists for a genetic predisposition to AAV. Although both closely associated with MHC Class II genes, GPA and MPA are genetically distinct. Moreover, GWAS reveal that genetic associations relate to ANCA specificity rather than the clinical syndrome *per se* ((21). Non-MHC genes identified include PRTN3 (encoding PR3), PTPN22 (tyrosine-protein phosphatase non-receptor type 22 and SERPINA1 (α_1 anti-trypsin) ((21,22) A recent study has identified 11 loci associated with EGPA. Intriguingly, clear clinical and genetic differences between ANCA negative and MPO-ANCA positive EGPA were revealed, suggestive of different pathogenic mechanisms and hence the need for alternative treatment approaches ((21)

Vascular injury

Vascular injury in AAV is multi-factorial with ANCA at its centre. ANCA may directly bind MPO or PR3 expressed on the surface of primed neutrophils and/or engage Fc receptors. In addition to the release of proteolytic enzymes and reactive oxygen species, activated neutrophils extrude extracellular traps (NETs) ((23) NETs may directly injure the endothelium, expose ANCA antigens to the immune system and activate the complement cascade. The subsequent release of C5a leads to further neutrophil priming and a vicious cycle is established (24). Activated intermediate monocytes may also contribute to pathogenesis. Through secretion of chemokine ligand-2 and IL-8 they amplify injury via recruitment of neutrophils (25), while activated macrophages play a critical role in fibrotic damage.

Inflammation

EGPA, is a Th2-cytokine-driven disease in which eosinophils predominate. Raised IL-5 levels mobilise eosinophils, while eotaxin released by vascular endothelium recruits activated dysfunctional eosinophils into the vascular wall and surrounding tissues. Release of eosinophilic granules predisposes to tissue injury. While the efficacy of anti-IL-5 monoclonal antibody mepolizumab confirms the role of these pathways (26), as discussed above disease subsets within EGPA require further mechanistic investigation and likely, distinct, targeted treatment approaches.

<u>Epidemiology and antigenic target of ANCA in SSc</u>

Epidemiology of ANCA in SSc

ANCA positivity has been reported in 0 to 12% of patients with SSc (27–29), with one study reporting positivity as high as 35% (30). In a controlled study of a 5-year inception cohort of US-based tertiary-care university teaching hospitals, ANCA (perinuclear or cytoplasmic) was not detected by IIF in any of the patients with SSc (n=45). However, of note, 7 patients (15.6%) displayed an atypical IIF ANCA pattern (and with negative anti-MPO and PR3 by ELISA) (28). A number of authors have reported that the prevalence of p-ANCA in SSc is around 5% (29,31,32). Whereas, in a recent publication from the Australian Scleroderma Cohort Study, which obtained clinical data (n=1303) from 5 centres, 116 (8.9%) were found to be ANCA positive (33). p-ANCA and anti-MPO antibodies are most commonly observed in SSc. However, p-ANCA with anti-PR3 antibodies has been rarely reported. Anti-MPO positive (compared to negative) patients were more likely to be anti-Scl-70 positive and less likely to be ANA positive (33). Whereas, anti-PR3 positive patients were more likely to have an overlap with rheumatoid arthritis and a trend (but not reaching statistical significance) towards the presence of interstitial lung disease (33). C-ANCA (PR3) positivity is extremely vanishingly rare in SSc.

Detection and elucidation of the antigenic target of ANCA in SSc

The presence of anti-MPO antibodies has been reported to range between 0 to 22% in SSc (28,29,33). High concordance of 60% to 100% between p-ANCA positivity and the presence of anti-MPO antibodies has been observed (29,32,34). However, anti-PR3 antibodies have also been reported in SSc (35). In the previously described study from the Australian Scleroderma Study cohort, in ANCA-positive patients, anti-PR3 was more common than anti-MPO (13.8% vs. 11.2%) (33). Ruffatti et al (29), identified 5 patients with p-ANCA positive sera (out of 115 patients with SSc, 4.3%) and detected anti-MPO (n=1), anti-PR3 (n=2) and both anti-MPO/PR3 antibodies. Therefore, there is a clear need for a rigorous testing system which combines the results of IIF and ELISA. It has been postulated that alternative antigenic targets may be implicated in SSc-AAV including bactericidal/permeability-increasing protein and cathepsin G. In a study which included 24 patients with ANCA detected by IIF, antibodies were found toward bactericidal/permeability-increasing protein (n=14), cathepsin G (n=13) and MPO (n=8) (30).

<u>SSc-AAV: a true overlap of rheumatological diseases or co-existence by chance alone?</u>

Although the presence of ANCA in SSc is not uncommon and likely underrecognised, the development of systemic vasculitis (i.e., AAV) is relatively rare. For example, in a study from the Royal Free clinical database which included 2200 patients, only 1.6% had evidence of vasculitis (35). SSc-AAV has been described in patients with both limited and diffuse cutaneous SSc and some authors have proposed a predilection for the two major subsets of the disease (34,36,37). Akin to SSc and many other rheumatological disorders, there is a predominance of females affected by SSc-AAV (34,36). The presence of anti-Scl-70 antibodies has been reported to be associated with the development of AAV in SSc (34,36,37). For example, two cases series of the extant literature identified that the presence of anti-Scl-70 antibodies was as high as 58.8% and 77.7% in SSc-AAV (34,36). Other antibodies have been reported in SSc-AAV including (but not limited to) anti-U1RNP and U3RNP, and also in the context of overlap disease (35). Indeed, it has been reported that patients with SSc-CTD overlap have a higher prevalence of SSc-AAV (35,38). A reversible membranous glomerulonephritis has been reported with penicillamine and has been implicated in some patients with SSc-AAV. However, a definitive causative association has yet to be determined and SSc-AAV has been reported many years after treatment has been discontinued. However, this now represents a largely historical concern as the drug is no longer used in the treatment of SSc (34,36,39).

Clinical sequelae of SSc-AAV

SSc-AAV can result in a devastating clinical phenotype. Patients invariably present with clinical features of MPA (MPO-ANCA) or renal-limited vasculitis, rather than GPA (PR3-ANCA) vasculitis including upper respiratory tract involvement with granulomatous inflammation (33,34,36,40) There is only one case report of the development of GPA in a patient with SSc. Table 1 compares major organ involvement in SSc and SSc-AAV.

Kidney involvement

The kidney is a key target organ for SSc-AAV and involved in approximately 75 to 80% of patients (34,36,37). The typical picture is of pauci-immune glomerulonephritis/rapidly progressive glomerulonephritis with glomerular crescent formation (34,36,37). This is easily distinguishable on biopsy from the typical characteristic features of scleroderma renal disease which include (but are not limited to) thrombotic microangiopathy with myxoid intimal changes, onion-skin lesions and fibrointimal sclerosis (35]). Background sclerodermatous renal involvement has been described in SSc-AAV and it has been postulated that established SSc-vasculopathy could exacerbate the interaction between ANCA and the endothelium resulting

in neutrophil activation (41). Features of arteritis of the arcuate artery have been reported in SSc-AAV. The arcuate arteries are typically involved (along with the intralobular arteries) in the classical scleroderma renal crisis (SRC). An example of SSc-ANCA renal disease is presented in Figure 1.

Lung involvement

Pulmonary (alveolar) haemorrhage is seen in one-third of SSc-AAV patients (34,36), while interstitial lung disease (ILD) has been reported in up to 80% of patients with SSc-AAV, although this remains a controversial issue (35). The authors of the previously described Australian cohort study identified that ANCA was independently associated with ILD (OR 2.63, 95% CI 1.72–4.0) after accounting for anti-Scl-70 antibodies (33). They also observed an association (OR 3.11, 95% CI 1.49-6.48) between ANCA and PE, including in those who were anti-PR3 positive (compared to negative), which was independent of the presence of anti-phospholipid antibodies (33).

Peripheral vascular and cutaneous

Around 10% of patients with SSc-AAV have been reported to develop necrotizing vasculitis (13%) resulting in limb ischemia and a vasculitic skin rash (10%) (34). Ischaemic digital ulcers have been reported in SSc-AAV and may contribute to the development of critical digital ischaemia (38,41).

CNS involvement

Quéméneur et al (36), reported neurological involvement in 15.7% of patients included in their case series and reviewed the extant literature which included mononeuritis multiplex and/or peripheral neuropathy and myopathies. Isolated cerebral vasculitis and subarachnoid haemorrhage have also been rarely reported in SSc-AAV (35).

Diagnostic approach and treatment of SSc-AAV

The key to diagnosis is to maintain a high index of clinical suspicion as to the potential development of AAV in patients with SSc. In patients with acute renal failure and features not in keeping (or less frequently observed) with SRC and/or pulmonary haemorrhage, AAV should be strongly suspected. ANCA (both IIF and ELISA) and renal biopsy examination should be performed. In SSc-AAV, there may be other non-specific features of systemic

inflammation (e.g., elevation in inflammatory markers). Potential ‘red flags’ when to suspect SSc-AAV are depicted in Table 2.

Table 3 depicts a number of clinical features that can help to distinguish between SRC and SSc-AAV renal disease. Understanding the epidemiology including timing of onset and clinical features can help to distinguish SSc-associated renal disease, in particular SRC, from SSc-AAV glomerulonephritis and renal vasculitis. SRC is most likely to occur during the first 5 years. Whereas, in patients with established disease (>5 years) presenting with acute or rapidly progressive renal failure, alternative aetiologies to SRC should be strongly considered including AAV (34,36).

It is essential to distinguish between SRC and SSc-AAV renal involvement because the management differs significantly. Thus, immunosuppression is used in the management of SSc-AAV, while the late diagnosis and introduction of specific treatment for SRC results in a poor clinical outcome (42). Examination of the urine is mandatory. Proteinuria can be seen in both conditions; however, this is typically <1g/day in SRC (43). In SSc-AAV renal disease haematuria including red cell casts is seen and approximately 10% of patients are nephritic. Malignant hypertension is typical of SRC; however, approximately 10% of patients may present with normotensive SRC (36,42). Arad et al (34), reported that only around one-third of patients with ANCA-associated glomerulonephritis in SSc were hypertensive and none displayed features of end-organ damage from malignant hypertension. Microangiopathic haemolytic anaemia is observed in approximately 50% of patients with SRC (44). Renal biopsy is not usually required for ‘classic’ SRC; however, this is mandatory for patients with atypical features (e.g., normal blood pressure and absence of microangiopathic haemolytic anaemia).

Lung disease is very common, being present in up to ~50% of patients with SSc with approximately one-third developing progressive ILD (45,46), and is reported in the majority (80%) of those with SSc-AAV. Subacute alveolar haemorrhage with worsening dyspnoea and alveolar infiltrates could be mistaken for ILD (34). Chest imaging by computerised tomography could help to differentiate SSc-ILD from pulmonary haemorrhage. Patients with SRC may develop left ventricular failure and pulmonary oedema can resemble pulmonary haemorrhage (34). Acute or worsening anaemia may be an indicator of pulmonary haemorrhage in AAV; however, microangiopathic haemolytic anaemic can occur in SRC.

Determining when AAV-associated digital vasculitis complicates SSc-vasculopathy is challenging and often not possible based upon clinical grounds. Similar to SSc-vasculopathy complicated by overlap with other rheumatological conditions including CTDs (e.g., RA and SLE), there is no single diagnostic test. The presence of other active 'inflammatory' features suggestive of AAV can help raise the index of suspicion of digital vasculitis and should be systematically investigated (e.g., urine examination for the presence of protein and/or blood).

Patients with SSc-AAV may become critically ill requiring intensive care admission for intensive monitoring and organ support (48). Clinicians should be aware that thrombotic complications including digital ulceration have been reported after arterial line cannulation in patients with SSc (49,50).

There are no specific data to inform the treatment of SSc-AAV including randomised controlled trials, which reflects the rare occurrence of underlying vasculopathy and concomitant vasculitis in SSc. Akin to AAV, the treatment of SSc-AAV consists of high-dose steroid and cyclophosphamide and subsequent maintenance therapy. Plasma exchange has been utilised and rituximab for refractory disease in SSc-AAV (34,36).

Conclusion

Although ANCA-vasculitis complicating SSc-vasculopathy is rare, the resulting clinical phenotype is severe and often extremely challenging to identify. In SSc, ANCA should be considered as a 'red flag', alerting the clinician to a likely poor prognosis and the need for rigorous monitoring. There are multiple areas of potential interaction in the pathogenesis and of SSc and AAV which can exacerbate vascular disease. Furthermore, there are similar patterns of major internal organ involvement (e.g., lung and kidneys) and co-existing pathology can result in a severe disease phenotype. Although the aetiopathogenesis of SSc-AAV is poorly understood, there might be a predilection for patients with overlap SSc-CTD. There are no specific data to inform the treatment of SSc-AAV and therefore management invariably follows that of isolated AAV. The treatment of AAV may be extremely hazardous in patients with SSc, for example, the strong association between high-dose steroid treatment and SRC. Many clinicians check ANCA status at baseline in patients with SSc and should be urgently ascertained if the evolving clinical picture suggests SSc-AAV. Further research is required to understand this complex clinical entity including aetiopathogenesis and to define the therapeutic strategy of SSc-AAV.

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	SSc	SSc-AAV
Renal	Scleroderma renal crisis including normotensive (~10%)	Pauci-immune glomerulonephritis/rapidly progressive glomerulonephritis
Pulmonary	Pulmonary hypertension Interstitial lung disease	Interstitial lung disease Pulmonary vasculitis - diffuse alveolar haemorrhage
Peripheral vascular	Raynaud's phenomenon Digital ulcers Critical digital ischaemia	Digital ulcers Critical digital ischaemia Necrotizing vasculitis resulting in limb ischemia
Cutaneous	Scleroderma Telangiectases Calcinosis	Vasculitic skin rash
Neurological	Carpal tunnel syndrome in early diffuse cutaneous SSc	Mononeuritis multiplex Peripheral neuropathy Rarely reported: Isolated cerebral vasculitis and subarachnoid haemorrhage

Table 1: Comparing major organ involvement in SSc and SSc-AAV.

Pulmonary haemorrhage
Significant abnormalities on urine analysis – marked proteinuria and/or haematuria
High ANCA or anti MPO/PR3 titres
Very high inflammatory markers - sedimentation rate/CRP
Vasculitic skin rash

Table 2. Potential ‘red flags’ when to suspect SSc-AAV. Clinical features which could help to determine whether ANCA is an innocent bystander or is actively participating in the disease process.

Scleroderma renal crisis	SSc-AAV renal disease
Typically occurs in patients with diffuse cutaneous SSc	Can occur in both subsets of the disease
Malignant hypertension (<10% of the patients are normotensive)	Absent or mild hypertension
Mild proteinuria (<1g/day)	Heavy proteinuria, haematuria including red cell casts, can be nephritic
Usually in patients with anti-RNA polymerase III antibodies	Usually in patients with anti-MPO p-ANCA antibodies
Acute onset of renal failure and severe hypertension	Subacute presentation with progressive renal failure
ACE-inhibitors are the first line treatment	Does not respond to ACE-inhibition
Steroids (≥15mg daily) are one of the major risk factors	Responsive to steroid treatment
Renal biopsy shows proliferative obliterative vasculopathy with crescentic 'onion skin' narrowing of arterioles with glomerular ischaemia	Renal biopsy shows focal segmental necrotizing lesions, crescent formation, inflammatory infiltrate, scarce deposits of immunoglobulins

Table 3: Distinguishing between scleroderma renal crisis and SSc-AAV renal involvement. Adapted from Chrabaszcz et al (47) and Woodworth et al (44).