# The evolving pathogenesis of systemic vasculitis

## Caroline OS Savage

**ABSTRACT** – The primary small vessel systemic vasculitides are disorders that target small blood vessels, inducing vessel wall inflammation and associated with development of antineutrophil cytoplasmic antibodies. Multiple organs are attacked including the lungs and kidneys. Increasing knowledge of pathogenesis suggests that the antibodies activate neutrophils inappropriately, leading to endothelial and vascular damage. Cytokines such as tumour necrosis factor (TNF) can facilitate the damage by priming neutrophils and activating endothelial cells. Understanding pathogenesis can help to rationalise existing therapies and indicate new approaches to therapy such as the use of agents that inhibit the effects of TNF.

KEY WORDS: antineutrophil cytoplasmic antibodies (ANCA), autoantibodies, endothelium, microscopic polyangiitis, neutrophils, vasculitis, Wegener's granulomatosis

Vasculitis is a disease process that targets all parts of the arterial tree from aorta to capillaries and also often venules, with leukocyte infiltration and necrosis. Different forms of vasculitis attack different vessels and are classified accordingly (Table 1)[1]. The main focus here will be on a triad of vasculitides that affect small vessels in the kidney and lungs, as well as other organs. This triad comprises Wegener's granulomatosis (WG), microscopic polyangiitis and Churg-Strauss syndrome. WG and microscopic polyangiitis have an annual incidence exceeding 20 per million population in the UK; they are encountered much more frequently than Churg-Strauss syndrome which will not be discussed further.

## Clinical features

In 1939 Friedrich Wegener described a granulomatous vasculitis affecting small to medium sized vessels. The vasculitis can affect any organ, but the lungs and kidneys are particularly vulnerable resulting in pulmonary haemorrhage and renal failure.

Microscopic polyangiitis was at first called microscopic polyarteritis. Until the 1980s it was often confused and grouped with the separate disorder, polyarteritis nodosa. Microscopic polyangiitis has many similarities to WG but has no granulomas in the respiratory tract. It, too, targets kidneys and lungs. The clinical features of both WG and microscopic polyangiitis are given in Table 2.

## Pathology

Small vessels anywhere in the body may be affected by focal necrotising lesions. In the kidney, the earliest lesions pick off single capillary loops, causing necrosis of the tuft; these lesions contain a few neutrophils but no other inflammatory cells. In untreated disease, the capillaries rupture and cells spill into Bowman's space surrounding the glomerulus. Inflammatory cells, particularly monocytes, are recruited in large numbers and proliferate to form crescents around the glomerulus.

In 1976, Donald and colleagues drew attention to the electron microscopic appearance of early vasculitic lesions affecting pulmonary capillaries in WG[2]. They described endothelial cell damage and white blood cells undergoing lysis, with free nuclei and cytoplasmic organelles in the vascular lumen. They suggested that the effects might be due to a cytophilic antibody.

## Evolving understanding of pathogenesis

Patients with small vessel vasculitides have an extremely poor outlook if untreated, with fewer than 20% and 10% alive at one year and two years, respectively. The introduction of the alkylating agent, cyclophosphamide, together with cortisone in the

**Table 1. Classification of primary systemic vasculitis** (see Ref 36 for further details about nomenclature).

| Vasculitis type | Classification |
|---|---|
| **Large vessel** | Giant cell (temporal) arteritis |
| | Takayasu's arthritis |
| **Medium-sized vessel** | Polyarteritis nodosa (classic polyarteritis nodosa) |
| | Kawasaki disease |
| **Small vessel** | Wegener's granulomatosis |
| | Churg-Strauss syndrome |
| | Microscopic polyangiitis (microscopic polyarteritis) |
| | Henoch-Schönlein purpura |
| | Essential cryoglobulinaemic vasculitis |
| | Cutaneous leucocytoclastic angiitis |

**Table 2. Clinical features of Wegener's granulomatosis (WG) and microscopic polyangiitis.**

| General | Major | Other | Features of WG only |
|---|---|---|---|
| Weight loss | Glomerulonephritis: | Skin: | Proptosis |
| Malaise | renal failure | purpura | Ocular palsies |
| Fever | Lung: | splinter haemorrhages | Tracheal stenosis |
| Arthralgia | haemorrhage | Ocular: | Nasal: |
| Myalgia | granulomas | episcleritis | bleeding |
| Mouth ulcers | | uveitis | collapse of bridge |
| | | Deafness | |
| | | Peripheral neuropathy | |

1970s dramatically improved the prognosis and over 80% survival at one year can now be expected. Cyclophosphamide and steroids suppress immune responses, leading to a suspicion that vasculitis is immunologically mediated. Early studies on serum sickness, a type of vasculitis, suggested that deposition of immune complexes in vessel walls triggers vasculitic lesions, so similar pathogenic processes were initially believed to underlie WG and microscopic polyangiitis. The discovery of autoantibodies against neutrophil components challenged this view, particularly when it became apparent that such antibodies have major effects on neutrophil function.

Antineutrophil cytoplasmic antibodies (ANCA) were described in association with glomerulonephritis by Davies et al[3] and Hall et al[4] in the early 1980s. However, Van der Woude firmly associated them with WG in 1985[5], and their occurrence in microscopic polyangiitis (and Churg-Strauss syndrome) was recognised later. Indirect immunofluorescence staining of alcohol-fixed normal neutrophils revealed two major patterns of staining:

- a cytoplasmic stain, or cANCA pattern: the antigen specificity is almost always against the enzyme proteinase 3 (PR3-ANCA) which is contained in neutrophil azurophil granules
- a perinuclear stain, or pANCA pattern: the antigen specificity of pANCA in the context of vasculitis is usually myeloperoxidase (MPO-ANCA) which also locates to azurophil granules.

When neutrophils are primed with inflammatory cytokines, such as tumour necrosis factor (TNF), granule contents become expressed at the neutrophil surface. PR3 and MPO can then be bound by specific antibodies.

The ANCA are useful to aid diagnosis, and titres are helpful when monitoring responses to therapy. The diagnosis of ANCA-associated vasculitis is made on the basis of the clinical findings, by biopsy of a relevant involved organ (typically kidney, nasal mucosa or occasionally lung) and the presence of ANCA. These antibodies have a specificity approaching 100% and a sensitivity of 70% or better for diagnosis of vasculitis in patients with compatible symptoms and signs[6]. Following therapy to induce remission of acute disease, relapse remains a threat with about 50% of patients relapsing during the first five years after diagnosis. Patients who have persistent ANCA detectable in serum samples or those who display intermittent positivity are more likely to relapse. There has been controversy about the value of monitoring ANCA titres, but new antigen-specific assays for measurement of ANCA titres (such as the capture ELISA) are highly reliable[7]. In many patients, a rise in ANCA titre precedes disease relapse. The close association between ANCA and disease activity has provoked an interest in the relationship between ANCA and disease pathogenesis.

## Functional effects of antineutrophil cytoplasmic antibodies

The electron microscopic findings caused Donald to suggest in 1973 that the effects might be due to a circulating cytophilic antibody. They continue to raise provocative questions about what is occurring in the microvasculature[2], in particular the cause of endothelial cell damage and leukocyte lysis.

### Effects of antineutrophil cytoplasmic antibodies on neutrophil activation

In patients with acute vasculitis, neutrophils are primed (a preparatory state for activation) and have increased levels of PR3 and other activation markers on their cell surface[8]. A similar effect can be obtained in vitro by treating neutrophils with inflammatory cytokines such as TNF. TNF priming followed by ANCA activation leads to:

- a respiratory burst with release of reactive oxygen species
- degranulation with release of proteolytic enzymes
- increased production of nitric oxide
- increased production of chemokines and cytokines such as interleukin (IL)-8 and IL-1[9–11].

Neutrophils can also sequester exogenous MPO to their surface and become activated following binding by MPO-ANCA[12]. Collectively, these responses concentrate injury to bystander cells and amplify inflammation. PR3 can induce endothelial cell apoptosis via a mechanism involving PR3 internalisation which is independent of its proteolytic function[13]. Subthreshold concentrations of PR3-ANCA can themselves prime neutrophils for activation by chemoattractants such as N-formyl-methionyl-leucyl-phenylalanine (fMLP)[14], enhancing leukotriene generation and chemotaxis, but suppressing the respiratory burst and degranulation. These responses could encourage neutrophil accumulation at sites of infection but suppress concurrent

antimicrobial responses. Monocytes also express PR3 and MPO, and ANCA-induced monocyte activation can lead to mediator release including IL-8[15].

### Effects of antineutrophil cytoplasmic antibodies on neutrophil–endothelial cell interactions

If neutrophils are to be instrumental in provoking significant endothelial cell damage, they must be brought into close proximity to endothelial cells. Endothelial cells in fresh vasculitic lesions are activated and express enhanced levels of adhesion molecules such as E-selectin and intercellular adhesion molecule (ICAM)-1 which are important for neutrophil recruitment. The endothelial activation is a response to the enhanced local levels of inflammatory cytokines such as TNF[16]. Antiendothelial cell antibodies, which can develop in these patients, are also able to activate endothelial cells[17], as does PR3 which appears to bind an endothelial cell receptor[18].

To mimic events occurring in vessels more closely, neutrophil behaviour was examined under flow conditions[19]. Initially, platelet monolayers were used as a surrogate vessel wall within a glass microslide. Neutrophils were perfused over the platelet monolayer which can support P-selectin mediated rolling and integrin-dependent firm adhesion. Video recordings were made of the neutrophil behaviour and analysed off-line to determine the percentage of neutrophils that were rolling or stationary. Normal immunoglobulin (Ig) G or ANCA-IgG was superfused over the rolling neutrophils. Rolling is the baseline normal behaviour.

When neutrophils are perfused over the platelet monolayer they bind efficiently; approximately 90–95% of the adherent neutrophils are rolling at any one time and the rest are stationary. ANCA-IgG has major effects on the rolling neutrophils. They stop rolling, become stationary and change their shape, becoming irregular with pseudopodia instead of round. These effects are maximal at six minutes, at which time 80–90% of neutrophils are stationary. Superfusion of normal IgG resulted in only 10–15% conversion to stationary adhesion. Both MPO-ANCA and PR3-ANCA caused the same extent of conversion from rolling to stationary adhesion. Pretreating platelets with ANCA had no effect, so the effects do not depend on ANCA–platelet interactions, confirming that ANCA-IgG recognises antigen on the surface of the neutrophils and does not act via presentation of ANCA-IgG Fc portions from the monolayer. Furthermore, the effects of ANCA are apparent on single cells – neutrophil clumping or cross-linking is not required. Two important ligands are required for the conversion from rolling to stationary adhesion: the neutrophil antibody receptor FcγRIIa and the integrin receptor CD11b. Antibodies to these ligands could completely block conversion.

Platelets are deposited early at vasculitic sites, so neutrophil–platelet interactions are not without pathophysiological significance. However, neutrophil-endothelial cells may be key during development of vasculitis. Thus, monolayers of TNF-activated endothelial cells were substituted for the platelets in further studies using the flow model[20]. Endothelial cells were activated with TNF (2 ng/ml) to upregulate E-selectin and ICAM-1 adhesion molecule expression. As with the platelet monolayer, activated endothelial cells can support neutrophil rolling. Following exposure to ANCA, neutrophils changed shape and became firmly adherent. Over 15 minutes the majority of neutrophils maintained contact with the endothelial cells and a high proportion migrated underneath the monolayer. Thus, ANCA-IgG stabilises neutrophil adhesion to activated endothelium and promotes neutrophil migration through endothelium so that the number of migrating cells can increase 10-fold. In this way, ANCA may potentiate early vasculitic lesions by promoting recruitment and adhesion between neutrophils and endothelial cells.

### Effects of antineutrophil cytoplasmic antibodies on neutrophil survival

Neutrophils can die by apoptosis (planned or programmed cell death) or necrosis. An apoptotic cell can be eaten and cleared by a phagocyte so that cell contents are not released and inflammation not provoked. A necrotic cell releases inflammatory contents into the environment.

ANCA accelerates apoptosis of TNF-primed neutrophils[21]. Twelve hours after priming with TNF and treating with PR3-ANCA or MPO-ANCA, more neutrophils display nuclear features of early apoptosis than do cells treated with TNF only or TNF and normal Ig. By 18 hours there is an excess of cells displaying late nuclear apoptotic features after exposure to ANCA-IgG. If preparations are run out on gels, ladders of fragmented DNA are seen in neutrophils treated with TNF and ANCA-IgG for 18 hours but not in neutrophils treated with TNF and normal IgG. Since ANCA initially activate TNF-primed neutrophils to undergo a respiratory burst, and since reactive oxygen species can induce apoptosis in other cell types, it was logical to test whether reactive oxygen species induce the accelerated apoptosis. Neutrophils from patients with chronic granulomatous disease cannot generate reactive oxygen species since they lack the enzyme, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Acceleration of apoptosis was not seen with these neutrophils in response to ANCA-IgG, confirming that the respiratory burst drives the accelerated apoptosis.

One feature of the accelerated apoptosis was perplexing. When a neutrophil becomes apoptotic, normally there is enhanced expression of phosphatidylserine molecules on its surface which allows it to be recognised and eaten by a phagocytic cell. ANCA-treated neutrophils did not show enhanced expression of phosphatidylserine on their surfaces[21]. This suggested that the cell membrane changes were dissociated from the morphological and nuclear changes of apoptosis and, perhaps more importantly, that the apoptotic neutrophils might not be efficiently cleared by phagocytic cells such as macrophages. *In vitro*, human monocyte-derived macrophages were, as predicted, less efficient at removing ANCA-treated apoptotic neutrophils than untreated apoptotic neutrophils. The consequences of this failure of removal may be profound since late apoptotic

neutrophils disintegrate with release of cell contents into the microenvironment. These observations may also explain the fragmentation of white blood cells seen in electron microscopy studies of early vasculitic lesions.

To complicate matters, ANCA can also bind to neutrophils that have become naturally apoptotic. Neutrophils undergoing normal ageing can express phosphatidylserine receptors on their surface and also express enhanced amounts of PR3 and MPO. ANCA can bind to the PR3 and MPO. An apoptotic neutrophil coated by ANCA is *more likely* to be eaten by a macrophage, but eating antibody-coated apoptotic neutrophils induces the macrophage to release cytokines such as IL-1, IL-8 and TNF that enhance inflammation[8]. Therefore, there may be several pathways to inflammation and tissue damage in vasculitis. In studies some years ago, addition of ANCA to endothelial cells co-cultured with primed neutrophils indeed precipitated endothelial cell injury[22].

## Signal transduction and neutrophil activation by antineutrophil cytoplasmic antibodies

Vasculitic lesions may be initiated by the inappropriate activation of neutrophils by ANCA in the microvasculature (Fig 1). Cytokines such as TNF may play an important facilitating role by priming neutrophils and activating endothelial cells. How does ANCA induce disordered neutrophil activation?

A neutrophil constitutively expresses receptors for the Fc portions of antibody molecules. When a bacterium is coated by several antibodies, the neutrophil can bind multiple antibody Fc tails through its Fc receptors, and the Fc receptors become cross-linked together on the cell surface. The neutrophil will then be appropriately activated to engulf and destroy the bacterium. Fc receptors are important also for activation of neutrophils by ANCA-IgG as the effects of ANCA can be prevented by blocking the receptors. In addition, if the Fc tails are removed from ANCA-IgG, ANCA can no longer activate neutrophils. PR3 or MPO binding is also important because antibodies that recognise other antigens are ineffective. Cross-linking Fc recep-

tors with PR3 or MPO on the neutrophil surface by ANCA-IgG seems to activate neutrophils inappropriately. In the flow model, individual neutrophil behaviour is affected by ANCA-IgG.
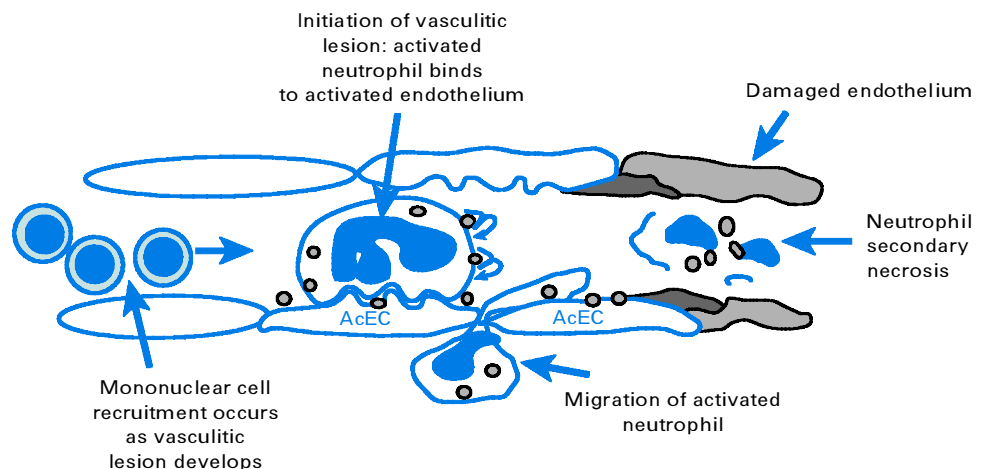
When the F(ab')2 portions (or the head) of ANCA-IgG binds to PR3 or MPO, Gi proteins are recruited, while a tyrosine kinase, Syk, is activated when ANCA Fc tails bind to Fc receptors (Ben-Smith and Hewins; unpublished observations). Further downstream, ANCA activate a kinase, phosphatidylinositol (PI)-3kinase and its downstream mediator phosphokinase B[23]. Both the Gi protein-dependent and the Syk-dependent pathways are necessary for PI-3kinase activation. Neutrophil activation by ANCA-IgG differs from cross-linking Fc receptors on the neutrophil both in its independence from phosphokinase D and because a different isoform of PI-3kinase is recruited[23].

We believe that recruitment of these pathways is responsible for the dysregulated and disturbed behaviour of neutrophils activated by ANCA that leads to a respiratory burst, with activation of NADPH oxidase and degranulation in the wrong place at the wrong time, and hence to endothelial cell damage.

Once a vasculitic lesion begins, other inflammatory cells are quickly recruited including mononuclear cells such as monocytes and T cells (Fig 1). These cells require different chemo-attractant and adhesion molecules to neutrophils. Fractalkine and monocyte chemotactic protein (MCP)-1 are important in recruitment[24]. Vascular cell adhesion molecule (VCAM) and CS-1-peptide-containing isoform of fibronectin (CS-1Fn) are important for adhesion[25]. T cell mediated immunity is thought to contribute to the pathogenesis of ANCA-associated vasculitis[26]. An advanced vasculitic lesion has many similarities to delayed hypersensitivity reactions which promote severe tissue injury[27].

Thus, there is strong circumstantial evidence, but no direct evidence, that small vessel vasculitides are mediated through autoimmune responses[28]. However, a recently developed animal model strongly supports the pathogenic potential of ANCA autoimmunity[29]. The agents that provoke the autoimmune response are unknown, although certain drugs, infections and environmental toxins have been implicated[26].



**Fig 1. Initiation and development of the vasculitis lesion**
(AcEC = activated endothelial cells).

Initiation of vasculitic lesion: activated neutrophil binds to activated endothelium

Damaged endothelium

Neutrophil secondary necrosis

AcEC

AcEC

Mononuclear cell recruitment occurs as vasculitic lesion develops

Migration of activated neutrophil

# Therapy for vasculitis

Is it possible to translate the better understanding of pathogenic mechanisms underlying vasculitis into more effective therapies? It would seem beneficial to prevent development of early lesions by:

- removing or halting the production of ANCA
- targeting pro-inflammatory cytokines such as TNF, or
- inhibiting neutrophil–endothelial cell interactions.

More advanced disease might benefit from anti-monocyte or T cell therapy.

Clinically, a number of problems must also be addressed. Although it is not yet possible to address all of them, they are the goals against which future therapies must be tested:

1. It would be helpful if treatments could be tailored according to whether a patient has severe, mild or organ-limited disease.
2. Curative treatments are needed because about 50% of patients relapse during the five years after the initial acute presentation, causing further tissue damage.
3. Current therapies are toxic and poorly tolerated.

## Cyclophosphamide and corticosteroids

Treatment with cyclophosphamide and corticosteroids has turned an almost universally fatal disease into one with a better than 80% five-year survival. Recent improvements have in part been due to more considered use of cyclophosphamide. Cyclophosphamide alkylates DNA guanidine nucleotides, induces lymphopenia, particularly of B lymphocytes, and sup-

presses Ig responses, but it has a narrow therapeutic index. Toxicity includes bladder and lymphoproliferative malignancy, and infertility.

A commonly used approach for initial therapy in patients with non-organ threatening renal involvement (creatinine <5.6 mg/100 ml, 500 µmol/l) is daily oral prednisolone and cyclophosphamide (Table 3). A recently reported multicentre randomised controlled trial, Cyclophosphamide versus Azathioprine during Remission of Systemic Vasculitis (CYCAZAREM), organised through the European Vasculitis Study group (EUVAS), supported this approach[30]. The trial compared three or 12 months' therapy with cyclophosphamide followed by conversion to azathioprine for remission therapy. Patients converted to azathioprine at three months entered remission as readily as those receiving more protracted cyclophosphamide therapy, with over 94% of patients achieving remission within three months.

A recent meta-analysis suggested that pulsed cyclophosphamide is less toxic than continuous oral cyclophosphamide, with fewer adverse effects, and is at least as potent an inducer of remission but possibly at the expense of a higher relapse rate[31]. However, existing studies are flawed. The CYCLOPS trial, also organised by EUVAS, is testing whether intermittent pulse therapy is as effective at inducing remission and less toxic.

## Severe disease

Therapy for the patient with more severe disease is outlined in Table 3. In this situation, the goal is to control inflammation quickly to reduce tissue injury. Plasma exchange removes ANCA-IgG and other inflammatory mediators from the circula-

**Table 3. Therapy.**

|  | Dose |
|---|---|
| **Induction therapy** | |
| Prednisolone | 1 mg/kg/day (maximum 80 mg) |
|  | Tapering to 10 ng/day by 5–6 months |
| Cyclophosphamide | 2 mg/kg/day for 3 months, then discontinue if remission achieved |
|  | If disease activity, reduce dose to 1.5 mg/kg/day and continue for up to 3 months |
|  | Maximum 200 mg/day |
|  | Reduce dose by 25% if >60 years, by 50%   if >75 years |
|  | Stop if white cell count <4 × 10$^9$/l |
| Prophylaxis | Consider gastric, fungal, pneumocystis, fertility, and osteoporosis protection, |
|  | also bladder protection with MESNA |
| **Induction therapy for severe disease (creatinine >500 µmol/l; lung haemorrhage)** | |
| Cyclophosphamide | 2.5 mg/kg/day |
| Methylprednisolone OR | 15 mg/kg as three daily pulses |
| Plasma exchange | Seven 4 litre exchanges (for plasma albumin and/or fresh frozen plasma) over 14 days |
| **Maintenance therapy (3–24+ months)** | |
| Azathioprine | 2 mg/kg/day |
| Prednisolone | 5–10 mg/day |

MESNA = sodium-2-mercaptoethanesulfonate.

tion, and may confer additional benefit when patients are dialysis dependent[32]. Neutrophils from patients with vasculitis produce more oxygen free radicals and have a greater inflammatory potential than healthy cells[8]. Methylprednisolone may improve inflammation by increasing the production of the antioxidant, superoxide dismutase, normalising superoxide anion production and reducing damage by neutrophils. The Methylprednisolone or Plasma Exchange trial (MEPEX) (which has completed recruitment), organised by EUVAS, is considering whether adjuvant therapy with plasma exchange or methylprednisolone has added benefit for severe disease.

Encouraging results have also been obtained from pilot studies in which patients were given anti-TNF therapy, infliximab or enteracept[33]. Therapies to block neutrophil interactions with endothelium or to inhibit T cell functions are other plausible approaches.

### Maintenance therapy

The risks of maintenance therapy have to be balanced against the risks of disease relapse. Azathioprine has been extensively used for maintenance (Table 3)[34,35]. Colonisation of the upper respiratory tract by *Staphylococcus aureus* may increase the risk of disease relapse. Sulphamethoxazole/trimethoprim added to conventional therapy may reduce the risk of respiratory relapse[36].

## The future

The challenge for the future will be to find therapies that can re-establish tolerance to PR3 and MPO following the development of autoimmune disease and to identify the factors that trigger breakdown of tolerance. Until then, the immediate goals must remain targeting therapy to break the vicious cycle of injurious events that cause vascular injury in a manner tailored to the severity of disease in the individual patient, and to reduce toxicity and further tissue damage.

## References

1 Jennette JC, Falk RJ, Andrassy K, Bacon PA *et al*. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Review. *Arthritis Rheum* 1994;**37**:187–92.

2 Donald KJ, Edwards RL, McEvoy JDS. An ultrastructural study of the pathogenesis of tissue injury in limited Wegener's granulomatosis. *Pathology* 1976;**8**:161–9.

3 Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotising glomerulonephritis with antineutrophil antibody: a possible arbovirus aetiology? *BMJ* 1982;**285**:606.

4 Hall JB, Wadham B McN, Wood CJ, Ashton V, Adam WR. Vasculitis and glomerulonephritis: a subgroup with an antineutrophil cytoplasmic antibody. *Aust NZ J Med* 1984;**14**:277–8.

5 van der Woude FJ, Rasmussen N, Lobatto S, Wiik A *et al*. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1985;**i**:425–9.

6 Hagen EC, Daha MR, Hermans J, Andrassy K *et al*. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int* 1998;**53**:743–53.

7 Westman K, Selga D, Bygren P, Segelmark M *et al*. Clinical evaluation of a capture ELISA for detection of proteinase-3 antineutrophil cytoplasmic antibody. *Kidney Int* 1998;**53**:1230–6.

8 Harper L, Cockwell P, Adu D, Savage COS. Neutrophil priming and apoptosis in anti-neutrophil cytoplasmic autoantibody-associated vasculitis. *Kidney Int* 2001;**59**:1729–38.

9 Brooks CJ, King WJ, Radford DJ, Adu D *et al*. IL-1$\beta$ production by human polymorphonuclear leucocytes stimulated by anti-neutrophil cytoplasmic autoantibodies: relevance to systemic vasculitis. *Clin Exp Immunol* 1996;**106**:273–9.

10 Cockwell P, Brooks CJ, Adu D, Savage COS. Interleukin-8: a pathogenetic role in antineutrophil cytoplasmic autoantibody-associated glomerulonephritis. *Kidney Int* 1999;**55**:852–63.

11 Tse WY, Williams J, Pall A, Wilkes M *et al*. Antineutrophil cytoplasm antibody-induced neutrophil nitric oxide production is nitric oxide synthase independent. *Kidney Int* 2001;**59**:593–600.

12 Hess C, Sadallah S, Schifferli JA. Induction of neutrophil responsiveness to myeloperoxidase antibodies by their exposure to supernatant of degranulated autologous neutrophils. *Blood* 2000;**96**:2822–7.

13 Yang JJ, Preston GA, Pendergraft W, Segelmark M *et al*. Internalization of proteinase 3 is concomitant with endothelial cell apoptosis and internalization of myeloperoxidase with generation of intracellular oxidants. *Am J Pathol* 2001;**158**:581–92.

14 Hattar K, Sibelius U, Bickenbach A, Csernok E *et al*. Subthreshold concentrations of anti-proteinase 3 antibodies (c-ANCA) specifically prime human neutrophils for fMLP-induced leukotriene synthesis and chemotaxis. *J Leukoc Biol* 2001;**69**:89–97.

15 Ralston DR, Marsh CB, Lowe MP, Wewers MD. Antineutrophil cytoplasmic antibodies induce monocyte IL-8 release. Role of surface proteinase-3, $\alpha$1-antitrypsin, and Fc$\gamma$ receptors. *J Clin Invest* 1997;**100**:1416–24.

16 Noronha IL, Kruger C, Andrassy K, Ritz E, Waldherr R. *In situ* production of TNF-$\alpha$, IL-1$\beta$ and IL-2R in ANCA-positive glomerulonephritis. *Kidney Int* 1993;**43**:682–92.

17 Carvalho D, Savage CO, Isenberg, D, Pearson JD. IgG anti-endothelial cell autoantibodies from patients with systemic lupus erythematosus or systemic vasculitis stimulate the release of two endothelial cell-derived mediators, which enhance adhesion molecule expression and leukocyte adhesion in an autocrine fashion. *Arthritis Rheum* 1999;**42**:631–40.

18 Taekema-Roelvink ME, Kooten CV, Kooij SV, Heemskerk E, Daha MT. Proteinase 3 enhances endothelial monocyte chemoattractant protein-1 production and induces increased adhesion of neutrophils to endothelial cells by upregulating intercellular cell adhesion molecule-1. *J Am Soc Nephrol* 2001;**12**:932–40.

19 Radford DJ, Savage CO, Nash GB. Treatment of rolling neutrophils with antineutrophil cytoplasmic autoantibodies causes conversion to firm integrin-mediated adhesion. *Arthritis Rheum* 2000;**43**:1337–45.

20 Radford DJ, Luu NT, Hewins P, Nash GB, Savage CO. Antineutrophil cytoplasmic antibodies stabilize adhesion and promote migration of flowing neutrophils on endothelial cells. *Arthritis Rheum* 2001;**44**:2851–61.

21 Harper L, Ren Y, Savill J, Adu D, Savage CO. Antineutrophil cytoplasmic antibodies induce reactive oxygen-dependent dysregulation of primed neutrophil apoptosis and clearance by macrophages. *Am J Pathol* 2000;**157**:211–20.

22 Savage CO, Pottinger BE, Gaskin G, Pusey CD, Pearson JD. Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am J Pathol* 1992;**141**:335–42.

23 Ben-Smith A, Dove SK, Martin A, Wakelam MJ, Savage CO. Antineutrophil cytoplasm autoantibodies from patients with systemic vasculitis activate neutrophils through distinct signalling cascades: comparison with conventional Fc$\gamma$ receptor ligation. *Blood* 2001;**98**:1448–55.

24 Cockwell P, Howie AJ, Adu D, Savage CO. *In situ* analysis of C-C

chemokine mRNA in human glomerulonephritis. *Kidney Int* 1998;**54**:827–36.

25 Chakravorty SJ, Howie AJ, Cockwell P, Adu D, Savage CO. T lymphocyte adhesion mechanisms within inflamed human kidney: studies with a Stamper-Woodruff assay. *Am J Pathol* 1999;**154**:503–14.

26 Savage CO, Harper L, Holland M. New findings in pathogenesis of anti-neutrophil cytoplasm antibody-associated vasculitis. Review. *Curr Opin Rheumatol* 2002;**14**:15–22.

27 Cunningham MA, Huang XR, Dowling JP, Tipping PG, Holdsworth SR. Prominence of cell-mediated immunity effectors in 'pauci-immune' glomerulonephritis. *J Am Soc Nephrol* 1999;**10**:499–506.

28 Hewins P, Tervaert JW, Savage CO, Kallenberg CG. Is Wegener's granulomatosis an autoimmune disease? Review. *Curr Opin Rheumatol* 2000;**12**:3–10.

29 Xiao H, Heeringa P, Liu Z, Hu P *et al*. Adoptive transfer of myeloperoxidase reactive lymphocytes into recombinant activating gene-2 deficient (RAG-2 -/-) mice induces severe crescentic glomerulonephritis. *J Am Soc Nephrol* 2001;**12**:644A.

30 Jayne DRW, Rasmussen N, Andrassy K, Cohen Tervaert JVV *et al*. Randomised trial of cyclophosphamide versus azathioprine as remission maintenance therapy for ANCA-associated vasculitis (CYCAZAREM). *N Engl J Med* (submitted).

31 de Groot K, Adu D, Savage CO. The value of pulse cyclophosphamide in ANCA-associated vasculitis: meta-analysis and critical review. *Nephrol Dial Transplant* 2001;**16**:2018–27.

32 Pusey CD, Rees AJ, Evans DJ, Peters DK, Lockwood CM. Plasma exchange in focal necrotizing glomerulonephritis without anti-GBM antibodies. *Kidney Int* 1991;**40**:757–63.

33 Kamesh L, Harper L, Savage CO. ANCA-positive vasculitis. *J Am Soc Nephrol* 2002;**13**:1953–60.

34 Reinhold-Keller E, Fink CO, Herlyn K, Gross WL, de Groot K. High rate of renal relapse in 71 patients with Wegener's granulomatosis under maintenance of remission with low-dose methotrexate. *Arthritis Rheum* 2002;**47**:326–32.

35 Nowack R, Gobel U, Klooker P, Hergesell O *et al*. Mycophenolate mofetil for maintenance therapy of Wegener's granulomatosis and microscopic polyangiitis: a pilot study in 11 patients with renal involvement. *J Am Soc Nephrol* 1999;**10**:1965–71.

36 Stegeman C, Cohen Tervaert J, de Jong P, Kallenberg CG. Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. *N Engl J Med* 1996;**335**:16–20.

# Shakespeare the physician

Shakespeare's plays contain over 450 medical references, including mention of most of the drugs and diseases of the early seventeenth century, and numerous references to medical men and their social standing. He comments on many medical doctrines, including those of the humours, the values of therapeutic herbs and plants, the influence of the planets on disease, blood-letting and quackery.

Shakespeare was a brilliant observer of the clinical signs and symptoms of disease. In the tragedy *Julius Caesar* there is a clear description of Caesar's epilepsy:

> CASCA: He fell down in the
> market-place, and foam'd at the mouth
> and was speechless.
>
> BRUTUS: He hath the falling sickness[1].

Of palpitations in *The winter's tale*:

> I have tremor cordis on me. My heart
> dances,
> But not for joy[2].

Of angina in *Henry VI part 2*:

> Suddenly a grievous sickness took him
> That made him gasp, and stare, and
> catch the air[3].

In a passage full of vitriol and anger, from *Troilus and Cressida*, Shakespeare uses a curse to display an amazing knowledge of diseases of his day:

> Now the rotten diseases of the south
> [syphilis], the guts griping ruptures
> [herniae], catarrhs, loads o'gravel in the
> back [kidney stones], lethargies, cold
> palsies, raw eyes, dirt-rotten livers,
> wheezing lungs, bladders full of
> imposthume [absesses], sciaticas,
> lime-kilns i' the palms [calcinosis], incur-
> able bone ache [syphilitic periostitis],
> and the rivelled fee-simple of the tetter
> [permanent sores], take and take again
> such preposterous discoveries[4]!

Whilst broadly upholding the position of medical men in Elizabethan society, Shakespeare was not entirely without critical comment. Many of these criticisms are echoed in charges laid against modern medicine:

> He hath abandoned his physicians,
> madam; under whose practices he hath
> persecuted time with hope, and finds no
> other advantage in the process but only
> the losing of hope by time[5].

And:

> Trust not the physician;
> His antidotes are poison, and he slays
> More than you [bandits] rob.
> For he takes wealth and life together[6].

And finally, from *King Lear*, as a warning to those who charge excessively:

> Kill the physician
> and thy fee bestow upon the foul disease[7].

## References

1. Shakespeare W. *Julius Caesar* I. ii. 240.
2. Shakespeare W. *The winter's tale* I. ii. 110
3. Shakespeare W. *King Henry VI part 2* III. ii. 368
4. Shakespeare W. *Troilus and Cressida* V. i. 16
5. Shakespeare W. *All's well that ends well* I. i. 12
6. Shakespeare W. *Timon of Athens* IV. iii. 427
7. Shakespeare W. *King Lear* I. i. 162

**Adam Stone**
Special Registrar in Gastroenterology,
St Georges Hospital, London