EXPERT REVIEWS

Wegener's granulomatosis: an update on diagnosis and therapy

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Wegener's granulomatosis (WG) is a unique clinicopathological disease characterized by necrotizing granulomatous vasculitis of the respiratory tract, pauci-immune necrotizing glomerulonephritis and small-vessel vasculitis. Owing to its wide range of clinical manifestations, WG has a broad spectrum of severity that includes the potential for alveolar hemorrhage or rapidly progressive glomerulonephritis, which are immediately life threatening. WG is associated with the presence of circulating antineutrophil cytoplasm antibodies (c-ANCAs). The most widely accepted pathogenetic model suggests that c-ANCA-activated cytokine-primed neutrophils induce microvascular damage and a rapid escalation of inflammation with recruitment of mononuclear cells. The diagnosis of WG is made on the basis of typical clinical and radiologic findings, by biopsy of involved organ, the presence of c-ANCA and exclusion of all other small-vessel vasculitis. Currently, a regimen consisting of daily cyclophosphamide and corticosteroids is considered standard therapy. A number of trials have evaluated the efficacy of less-toxic immunosuppressants and antibacterials for treating patients with WG, resulting in the identification of effective alternative regimens to induce or maintain remission in certain subpopulations of patients. Recent investigation has focused on other immunomodulatory agents (e.g., TNF-α inhibitors and anti-CD20 antibodies), intravenous immunoglobulins and antithymocyte globulins for treating patients with resistant WG.

KEYWORDS: diagnosis • etiopathogenesis • therapy • update • Wegener's granulomatosis

Introduction & epidemiology

Wegener's granulomatosis

Wegener's granulomatosis (WG) belongs to the heterogeneous group of systemic vasculitides. It has been over half a century since Wegener first described a group of patients who presented with upper airways disease and died of renal failure [1,2]. In 1954, Godman and Churg delineated three criteria for WG: necrotizing granulomatous lesions of the upper airways, vasculitis and glomerulonephritis [3]. The American College of Rheumatology (ACR) published criteria for the classification of vasculitis in 1990 [4]. They were based on well-characterized patient cohorts, submitted for inclusion as cases of WG by rheumatologists from centers in Canada, Mexico and the USA [5]. The objective of the ACR was to provide diagnostic categories for individual patients, and not to institute a nomenclature system. An International Consensus Conference that convened in Chapel Hill addressed this issue [6]. According to the 1994 Chapel Hill Consensus Conference (CHCC), WG is defined as an antineutrophil cytoplasm antibody (ANCA)-associated small-vessel vasculitis with granulomatous inflammation involving the respiratory tract, necrotizing vasculitis affecting small-to-medium-sized vessels, for example capillaries, venules, arterioles and arteries, and, commonly, necrotizing glomerulonephritis [7].

Wegener's granulomatosis is a relatively rare disease, with an incidence that varies from five to 15 cases per million people. Higher incidence has been reported in northern Europe compared with southern Europe [8]. The mean age of patients at initial diagnosis is 50–60 years but some cases have been described in very elderly subjects and in children [9]. Published series report a low prevalence in males [5,10]. Gonzales-Gay *et al.* have showed that the CHCC definitions may be more restrictive than the ACR criteria for WG and that the use of these definitions may underestimate the incidence of WG [11].

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Etiopathogenesis

The etiology of WG is unknown but it is thought that it results from an interplay between genetic susceptibility and environmental triggers, including certain infections, occupational risk factors and drugs [12]. In multifactorial diseases such as WG, interactions of many predisposing alleles are expected, comprising immune-related genes, such as common autoimmunity and autoimmune disease-specific genes, in addition to environmental factors. Similar to other autoimmune disorders, WG is genetically associated with the class II region of the MHC, such as *HLA-DPB1* [13]. A current hypothesis regarding genetic factors for autoimmune diseases on chromosome 6 include the involvement of several non-MHC genes in the pathophysiologic development of such disorders [14]. There are also a high number of other immunologically relevant genes, comprising cytokines, chemokines, lymphocyte receptors and signaling molecules, as well as their inhibitors or activators [15]. In WG, these genes include polymorphisms, such as in *PRTN3* or *IL10* [15]. Recently, the role of the intracellular tyrosine phosphatase gene *PTPN22* 620W allele polymorphism has been reported to be involved in the pathogenesis of WG [15,16]. A lack or decrease of *PTPN22* activity may result in enhanced proliferation and activation of subsets of T cells and in the formation of ectopic germinal cells, in which antigen-driven positive selection of B-cell receptors and specific antibody production occurs [15].

A number of studies have focused on other potentially important genetic markers and have showed a link between α-1-antitrypsin deficiency and WG. However, the frequency of Z phenotypes among patients with WG does not exceed 7–27% [12].

Infections have frequently been associated with WG. Several microorganisms, such as *Human parvovirus B19*, *Mycobacterium tuberculosis* and *Mycobacterium avium* species *intracellulare*, have been suggested as possible causes of WG but with fewer consistencies [12]. Particular interest has focused on *Staphylococcus aureus* [17–19]. In fact, although none of the evidence is conclusive for it as an inciting agent, a strong association has been reported between nasal carriage of *S. aureus* and an increased risk of WG relapse [20]. As far as environmental factors are concerned, crystalline silica has repeatedly been mentioned as an etiologic agent in small-vessel vasculitis [21]. Lane *et al*. have also linked WG with high exposure to organic solvents and farming [22]. Several other occupational risk factors have been invoked as possible causes of WG, including cadmium, industrial pollutants such as mercury, volatile hydrocarbons, pesticides and fumes, but further studies are needed to confirm these data [12,19]. Some therapeutic agents, such as propylthiouracil [23] and monocycline [24], have been associated with WG, but the precise risk of developing disease in association with any drug as yet remains unknown [12,25].

Wegener's granulomatosis is associated with the presence of circulating ANCAs (c-ANCAs), whose target autoantigens are primarily contained within azurophilic granules in neutrophils, and lysosomes in monocytes [10,26]. The major antigen specificities of ANCAs in patients with WG are for proteinase 3 (PR3-ANCA), but antibodies toward myeloperoxidase (MPO-ANCA) are seen in 20% of WG patients [27,28]. Much *in vitro* and experimental animal model evidence points to the potential mechanisms by which ANCAs could induce neutrophil-mediated vascular injury [27]. The most widely accepted pathogenetic model suggests that ANCAs activate cytokine-primed neutrophils, which express the ANCA antigen PR3 on their surface [9]. Reactive oxygen species, proinflammatory mediators and proteolytic enzymes are then released by c-ANCA-activated cytokine-primed neutrophils [29]. In this way, neutrophils induce microvascular damage and a rapid escalation of inflammation with recruitment of mononuclear cells. Although all studies on c-ANCAs target cells have concentrated on neutrophils, there is evidence that monocytes also express PR3 and that c-ANCA-induced monocyte activation can lead to release of mediators [30]. However, the clinical and pathologic hallmark of WG is the coexistence of vasculitis and granuloma. The causative agent(s) leading to granuloma formation is/are still unknown, but the presence of T cells in the granulomatous inflammation indicates T-cell hyperactivity [31].

Clinical manifestations

Ear, nose & throat manifestations

There is a high prevalence of head and neck manifestations of WG (72.3–99%) [32–36].

Nasal obstruction, pain, ulceration, edema, discharge, an altered sense of smell, epistaxis and deformity are manifestations of naso–sinusal involvement of WG. The most common site of active nasal disease is the area of Kiesselbacch locus, where necrosis of the septal cartilage is visible. The typical G saddle nose deformity often ensues but does not necessarily indicate the presence of an active disease. The remaining mucosa and turbinates are also frequently involved [34,37,38]. Otological symptoms can represent the onset of WG in 20–25% of cases, whereas aural lesions may develop during the course of the disease in 15–45% of cases [33–39]. In the former situation, conductive or mixed uni- or bilateral hearing loss associated with other objective signs of otitis media with effusion can be the presenting features [39,40]. Granulomatous obstruction of the eustachian tube often constitutes the pathological basis of the disease. Sensor neural hearing loss due to vascular damage, deposits of immune complexes at the cochlear site or granulomatous involvement of the cochlear nerve are less frequent [41].

Facial palsy occurs in association with middle-ear disease, but is extremely rare as a presenting sign [42,43]. Facial palsy usually improves with cytotoxic therapy, but it is often permanent when middle-ear surgery has been incorrectly performed or treatment has been delayed. In a very few instances, other cranial nerves, such as VI, IX, X and XI, are affected [44–46].

Peculiar gingival hyperplasia, known as 'strawberry gum', is pathognomic, but occasionally presents in the early stages of WG [47]. Deep mucosal ulcers of the tongue, cheek, gingiva and palate are also rare, but distinct, signs.

Occasionally, patients develop severe subglottic stenosis [48]. According to US NIH experience, subglottic stenosis was present in 16% of patients, half of whom needed a tracheostomy [49]. Laryngeal symptoms of subglottic stenosis in WG patients are stridor, pain, dyspnea, wheezing and altered phonation [32,50,51].

Pulmonary manifestations

Lung involvement is a characteristic feature of WG. In fact, it is described in 90% of WG patients. All parts of the respiratory tract can be affected, resulting in a variety of symptoms, including cough, dyspnea, thoracic pain and hemoptysis [52]. Approximately 7–45% of patients will present with diffuse alveolar hemorrhage as a consequence of pulmonary capillaritis [52]. However, asymptomatic pulmonary involvement is a common manifestation, occurring in over 30% of patients [52]. Typical findings on plain radiographs include isolated or multiple (in general, fewer than ten) nodules, ranging from a few millimetres to 10 cm [6]. These nodules tend to cavitate. Small pleural effusion may also be seen. Sometimes, bronchoscopy shows ulcerative tracheobronchitis and tracheobronchial stenosis [6].

Renal manifestations

Renal disease develops in 77% of patients with WG and is a potential source of life-threatening complications [53]. In fact, rapidly progressive renal failure is a common outcome (~20–40% of patients with renal involvement) [54] that may require dialysis in the short term, and the prognosis correlates closely with the time to treatment initiation [10]. Therefore, it is important to precociously recognize renal disease. Microhematuria or red cell casts in urine sediment, proteinuria and elevated serum creatinine are the most common manifestations [9,10]. Kidney biopsies usually reveal a focal segmental necrotizing glomerulonephritis, with or without crescents, and are rarely accompanied by necrotizing granulomas [28]. It has been described rarely as a solitary renal mass, which pathological examination reveals to be WG [28].

Articular involvement

Arthralgia or arthritis develops in 30–60% of WG patients. Small or large joints can be affected. Arthritis that antedates the WG can lead to a mistaken diagnosis of nondestructive rheumatoid disease, especially if the test for rheumatoid factor is positive. Therefore, a search of cardinal signs of WG should be performed in cases of unexplained arthritis [10].

Ocular disease

Ocular abnormalities in the form of conjunctivitis, orbital pseudotumors, episcleritis, proptosis, peripheral ulcerative keratitis, dacryocystitis and optic nerve ischemia have been noted in 15% of patients with WG and half of them develop ocular lesions during the course of the disease [6,29].

Intestinal involvement

Intestinal involvement is an uncommon manifestation in WG. Clinically, it mimics inflammatory and infectious bowel diseases. Pathological findings include multiple small ulcerations and intestinal perforation. Histological examination usually shows marked mixed inflammatory infiltrate associated with necrotizing and granulomatous vasculitis of small-to-medium-sized vessels. The small bowel is the most common site [55].

Other manifestations

The heart, CNS, genital tract and skin may also be involved in WG. General signs of the disease may include weight loss, fever and fatigue [10].

Disease severity

The European Vasculitis Study (EUVAS) group defined several subgroups of patients, covering the spectrum of ANCA-associated vasculitis (AAV) severity [56]. Patients who develop a disease restricted to only one or two target areas (in particular upper and lower airways) without constitutional symptoms or systemic vasculitis, are defined patients with a 'localized form' of WG [56]. The term 'limited form' or 'initial phase' of WG is used to refer to patients with localized WG and constitutional symptoms or patients with multifocal WG without threatened organ function. Indeed, patients with involvement of threatened organs are traditionally defined as subjects with 'generalized' disease [56]. The systemic form of WG is more frequent than the localized form [57] but, since determination of c-ANCAs was introduced as a diagnostic tool, in combination with histology, an increasing number of cases have been identified at an early stage [37]. Such early identification is of great importance in order to implement stage-adapted therapy. In terms of disease severity, we define a 'severe form' when there is rapidly progressive renal failure, with or without diffuse alveolar hemorrhage [56].

Disease activity & damage

Any chronic disease can be described in terms of two aspects: activity and damage [54]. Disease activity should be evaluated at diagnosis and at regular intervals during follow-up. The Birmingham Vasculitis Activity Score (BVAS) is a validated instrument used in clinical trials and everyday practice [10]. Signs of disease activity must be differentiated from sequalae related to the disease (e.g., renal or neurological impairment) or treatment (e.g., vertebral fractures). The vasculitis damage index is useful for quantifying the impact of sequalae [10]. It gives a cumulative record of damage that must have been present for at least 3 months [58]. Organ involvement is defined according to disease extent index, which records vasculitis activity in ten organ

systems; the maximum number of points is 20 and one point is added for constitutional symptoms with malaise [58]. Complete remission was defined as the absence of active disease, complete resolution of pulmonary infiltrates or evidence of stable scarring, absence of constitutional symptoms and stabilization or improvement in renal fuction without active urinary sediment. Relapse is defined as the re-emergence of clinically detectable vasculitis or the worsening of previous manifestations [58].

Diagnosis

The diagnosis of WG usually depends on the presence of a combination of findings with both clinical and laboratory features; because of the lack of a single pathognomonic test, several criteria have been developed for diagnosis or classification. The ACR in 1990 proposed classification criteria for rheumatic diseases (mainly to standardize clinical definition for use in research studies), while, in 1994, the CHCC defined characteristic vascular or glomerular abnormalities of systemic vasculitis [59,60]. Nevertheless, physicians often misused these classification criteria as diagnostic ones. Clinical suspicion for WG should be considered in patients with unexplained refractory head and neck symptoms and systemic complaints. Diagnosis of this rare disorder is supported by histological features of vascular inflammation, granulomatosis and/or pauci-immune glomerulonephritis. Although a biopsy is often helpful for diagnosing vasculitis, some tissue specimens yield nonspecific findings because of sampling of focal and segmentally distributed vascular lesions. In order to facilitate WG diagnosis, in 2000, Sørensen *et al.* proposed diagnostic criteria based upon the CHCC, concerning histological inflammatory findings in the respiratory system (or a surrogate parameter), and in small-to-medium-sized vessel glomerular nephritis or positive PR3-ANCA; in spite of the exclusion criterion of the peripheral blood eosinophilia for WG, Lane *et al.* suggested that a modification of the acceptable level of eosinophilia (for a value of $<1.5 \times 10^9$/l is still a compatible diagnosis of WG [61,62]. Nevertheless, no validated diagnostic criteria are currently available. Thus, a definitive diagnosis can be made on some combination of clinical, laboratory and biopsy (or angiographic) data by histological examination of suspicious lesions (in general, lung nodule, nasal–sinus, kidney and muscle) in conjunction with positive serological analysis [29], as in approximately 90% of cases. Among these 90%, c-ANCAs are frequently recognized with an immunofluorescence labeling pattern and the ELISA test always shows a PR3 target in them [63]. For a correct diagnosis, interpretation of serological data is necessary to carefully assess the clinico–pathological relationship.

Also, if not included in ACR classification criteria and CHCC definitions, ANCA is useful in diagnosis and patient monitoring [9]. Indeed, the presence of ANCAs is important in the diagnosis and follow-up of patients with vasculitides, on clinical manifestations and on treatment strategies, which have benefited recently from the introduction of biotherapies. ANCAs can be subdivided by indirect immunofluorescence tests (IFTs) and by ELISAs. IFTs can be distinguished by two major fluorescence patterns on ethanol-fixed human neutrophils: one of these patterns, classic c-ANCA, is highly specific of WG, while the other, perinuclear ANCA (p-ANCA), is commonly seen in microscopic polyarteritis and rarely in WG. ELISAs are used to further specify the target antigens of ANCA, namely PR3, which detects ANCAs specific for PR3 [64–66]; ELISAs are mandatory when vasculitis is present, as they are positive in approximately 5% of patients with negative IFT findings [67–69]. Recently, Lee *et al.* reported that serum and plasma samples can be used interchangeably for measuring ANCA [68].

Sensitivity & specificity of ANCA detection

The clinical utility of c-ANCA as a diagnostic marker for WG was recently confirmed in a large prospective European study using sera from vasculitis patients (sensitivity 60% and specificity 95%). The sensitivity rose to 83% if only biopsy-proven WG was considered [70]. These data show that the value of c-ANCA testing is limited by a rather low sensitivity. The greatest utility of c-ANCA testing may be in patients with suspected, but not yet proven, WG; negativity of the c-ANCA test does not necessarily exclude the diagnosis [71]. These antibodies, in fact, are present in more than 80% of the systemic form of the disease but only in 50% of the localized forms. Even patients with generalized WG may be ANCA negative or may show anti-MPO-ANCA positivity. There is some evidence that these ANCA-negative or anti-MPO/p-ANCA-positive patients represent clinically distinct subtypes and that the outcomes of patients with anti-PR3/c-ANCA may differ from that of anti-MPO/p-ANCA-positive patients [72]. Moreover, active WG is strongly associated with the presence of these antibodies. ANCAs are often present before a relapse and become undetectable during remission in approximately 50% of patients [20]. International recommendations mandate ANCA titer determination at regular intervals during follow-up, although interpretation of the results is not standardized [68]. A review of 15 studies showed that no relapses occurred after 155 (42%) of 365 ANCA increases by indirect immunofluorescence, and 75 (25%) of 295 increases by ELISA [66]. Thus, approximately a third of patients would receive unnecessary treatment if ANCA testing was used alone to diagnose relapses [73]. ANCA titers correlate poorly with disease activity, and titer increases do not reliably predict relapses. A recent multicenter study demonstrated that the ANCA levels were only weakly associated with disease activity across patients; although the longitudinal association within patients was stronger, changes in ANCA levels explained less than 10% of the variation in disease activity. The authors described that decreases in PR3-ANCA levels were not statistically significantly associated with shorter time to remission, and increases in PR3-ANCA levels were not associated with relapse, suggesting that ANCA levels cannot be used to guide immunosuppressive therapy [74].

Only low-level evidence is available to support treatment intensification in patients with isolated ANCA titer increases [75]. There is widespread agreement that an isolated

ANCA titer increase should be viewed as indicating a risk of relapse and, therefore, a need for close monitoring rather than treatment intensification, in the absence of other signs [67,68,73]. Thus, the utility of ANCA levels to guide the management of patients with WG remains controversial.

Therapy

Treatment of WG is stage adapted, for there is a clear distinction among induction of remission and maintenance of remission strategies, which may vary according to the extension of disease. The therapeutic goals in WG have expanded dramatically over the past 30 years. Prolongation of patient survival was the primary objective prior to the 1970s as 82% of patients with active WG died within 1 year [76]. Long-term survival became possible with the introduction of prednisone and cyclophosphamide (CYC) [33], although morbidity and mortality continued to occur as a result of treatment-induced toxicity and disease relapse. This prompted the search for safer treatment options that reduce disease recurrence. The opportunity to explore new therapies in WG has, however, brought challenges in clinical trial design. As always, therapy for each patient affected by WG must be individualized, taking into account clinical presentation, comorbid conditions, concomitant medications, clinician experience and comfort. Outcome measures play an important role in the evaluation of treatment efficacy in WG. Remission and relapse are the most frequently used outcome terms, which are based on disease activity. There remains no unequivocally reliable means by which to confirm active disease, and assessment is based on clinical parameters from physical examination, from the laboratory and from radiographic studies. The difficulty in determining disease activity is further compounded in multicenter trials, as definitions may not be universal between investigators. A significant area of progress in the conduct of clinical trials for WG has been the recognized necessity of having predefined outcome measures and their definition in the published methods. As a means of standardizing the assessment of disease activity, the development and validation of instruments has been actively sought.

Conducting therapeutic studies in WG is additionally complicated by the rarity of the disease. The estimated prevalence of WG in the USA is 13–30 cases per million individuals per 5-year period [32,77]; whereas, annual incidence rates of WG per million are estimated to be 12 in Norway [78], 10.3 in England and 4.1 in Spain [79].

Currently, a regimen consisting of daily CYC and corticosteroids, which induces complete remission in the majority of patients, is considered standard therapy. Since approximately 50% of patients experience a relapse following discontinuation of therapy, alternative regimens designed to maintain remission after using CYC and corticosteroids are usually necessary. This 'induction maintenance' approach to treatment has emerged as a central premise in planning therapy for patients with WG [80].

A number of trials have evaluated the efficacy of less-toxic immunosuppressants (e.g., methotrexate [MTX], azathioprine [AZA] and mycophenolate mofetil [MM]) and antibacterials (i.e., cotrimoxazole [CTR; trimethoprim/sulfamethoxazole]) for treating patients with WG, resulting in the identification of effective alternative regimens to induce or maintain remission in certain subpopulations of patients. Given the efficacy of MTX (for early systemic WG) and CTR (in WG limited solely to the upper airways) to induce remissions, and the relatively decreased associated morbidity compared with CYC, these alternative regimens are preferred in certain patients. Recent investigation has focused on other immunomodulatory agents (TNF-α inhibitors [infliximab {IN} and etanercept {ET}] and anti-CD20 antibodies [rituximab {RTX}]) for treating patients with WG [81].

Therapy for the induction of remission in the active phase of WG

A summary of appropriate pharmacological protocols to induce remissions in the active phase of WG is presented in Table 1 [32,82–93]. The therapy for the induction of remission in the active phase of WG is usually followed by less-aggressive treatment for the maintenance of remission.

Corticosteroids & CYC

Corticosteroids were employed initially and ameliorated many of the inflammatory manifestations of WG. Nevertheless, steroids alone are insufficient to control WG in the active phase; this monotherapy prolonged the median survival from 5 to 12.5 months only (mean value of 7.5 months) [94] and the patients died after a median of 5 months following diagnosis [76].

The immunosuppressive properties of cytotoxic agents became appreciated during the 1950s and 1960s. Emerging in concert with these findings was evidence that immunologic mechanisms played an important role in the pathophysiology of WG.

Cyclophosphamide is an alkylating agent that results in crosslinking of DNA, decreased DNA synthesis and apoptosis. CYC also impacts on a number of components of the immune response, including decreasing the number of T and B lymphocytes, reducing lymphocyte proliferation, decreasing antibody production, suppressing delayed hypersensitivity to new antigens and interfering with the function of both resting and stimulated B lymphocytes.

In the 1970s, Fauci and Wolff first introduced a therapeutic regimen of oral daily CYC (2 mg/kg) combined with prednisone (initial dose of 1 mg/kg) [33], inducing remission rates of 75–100%, as observed in large cohort studies, and reducing mortality by up to 15% [32,84,95,96]. Among responders, prednisone was gradually tapered and discontinued by 6–9 months; CYC was continued for at least 1 year after remission. Therefore, oral daily CYC is still the standard treatment for severe active generalized WG. The use of CYC is, however, limited by often serious morbidity from the side effects of treatment,

Table 1. Protocol of the treatment for the induction of remission of Wegener's granulomatosis.

Study	Protocol	Stage of disease	Dose	Recommendations*	Ref.
Fauci *et al.* Hoffmann *et al.* Guillevin *et al.* Reinhold-Keller *et al.* Jayne *et al.*	CYC (daily, orally)	Generalized	2 mg/kg/day, orally	A	[81] [32] [82] [83] [84]
de Groot *et al.* Adu *et al.* Guillevin *et al.* de Groot *et al.*	CYC and PR	Generalized	15–20 mg/kg intravenous CYC + 1 mg/kg oral PR every third week	A	[85] [86] [82] [87]
Gaskin *et al.*	CYC, PR and plasmapheresis	Severe	2 mg/kg daily, orally, 1 mg/kg oral PR and plasmapheresis 40–60 ml/kg (4–7 ×)	A	[88]
de Groot *et a l.* Sneller *et al.* de Groot *et al.*	MET and PR	Early systemic, nonlife-threatening disease	0.3 mg/kg intravenously and 1 mg/kg/week oral PR	A	[89] [90] [91]
Reinhold-Keller *et al.*	TR/SULF	Localized	2 × 960 mg/day, orally	A	[92]

*Grade A recomendations: high-quality study evidence (type I).
CYC: Cyclophosphamide; MET: Methotrexate; PR: Prednisone; TR/SULF: Trimethoprim/sulfamethoxazole.

including infections, leukopenia related to bone marrow failure, infertility, hemorragic and recurrent cistitis and a high increased risk of bladder cancer [97].

Cyclophosphamide is metabolized in the liver to various chlormethine metabolites and acrolein [98]. The chlormethine metabolites are responsible for the therapeutic effects; the toxic effects on the urinary tract are thought to be mediated by the acrolein metabolite [99]. Although the entire urinary collecting system is at risk for acrolein-mediated toxicity, the bladder is most susceptible because of its prolonged exposure to the drug [97].

The Cyclophosphamide and Azathiopirine in Maintenance of Remission trial conducted by the EUVAS group suggested that CYC exposure should be limited to the active phase of WG and that it should be replaced by a less-toxic pharmacological therapy as soon as remission is accomplished [84].

The use of intermittent high-dose intravenous 'pulse' CYC is another strategy to reduce the cumulative exposure to the drug. In several trials, rates of remission with the intermittent intravenous pulses of CYC were similar to those using daily oral CYC, but relapses appear to be more likely following pulse administration [82,87,100]. According to the recently presented preliminary analysis of a large randomized trial (Daily Oral Pulse Cyclophosphamide as Therapy for ANCA-Associated Systemic Vasculitis [CYCLOPS]), although the disease-free periods did not differ significantly between patients receiving oral compared with pulse CYC therapy, a regimen containing steroids and daily oral CYC should still be the treatment of choice in generalized, severe WG [91]. A complete data report from CYCLOPS is still awaited.

Methotrexate

Methotrexate is a folic acid that inhibits dihydrofolate reductase and, at pharmacologic concentrations, can increase adenosine accumulation and release from cultured fibroblasts and endothelial cells [101]. Adenosine inhibits neutrophil adhesion to endothelial cells, inhibits generation of toxic oxygen metabolites, inhibits TNF production, and may increase the secretion of IL-10.

According to the recent prospective, unblinded, randomized controlled trial (Non-Renal Wegener's Granulomatosis Treated Alternatively with Methotrexate [NORAM]), MTX can be used in association with steroids for inducing and maintaining remission in select patients with non-life-threatening WG. This drug may be used instead of CYC in patients with early disease or in those without lower respiratory tract involvement. The NORAM trial highlights the need for maintenance therapy beyond 12 months after induction of remission. Compared with CYC, MTX presents with a low incidence, and a minor severity, of adverse events [91,102–104]. The same trial also demonstrated that there is a high incidence of relapse at 6 months after complete discontinuation of treatment, both for MTX (69.5%) and CYC (46.5%). The unfavorable relapse rate observed highlights the need for the maintenance therapy in WG beyond 12 months.

Plasma exchange

Plasma exchange is supposedly effective in WG patients with severe disease. Its mechanism of action is not well understood but presumably it works by removing ANCAs [56]. The role of plasma exchange is still far from clear [105] and there is disagreement among the authors [81].

A retrospective review conducted on patients with diffuse alveolar hemorrage suggests that plasma exchange represents a good adjuctive therapy in patients treated with traditional immunosuppressants [106]. Another randomized, controlled study on WG patients with advanced renal disease (two-third dialysis-dependent) showed that plasma exchange plus immunosuppressants, compared with immunosuppressants alone, may lead to higher rates of renal recovery and dialysis

independence [56,106]. Although these preliminary results are promising, the same authors did not find a difference between the two groups in prognosis (mortality was 25% in both groups) [106]. In addition, Aasarød *et al.*, in a controlled trial on 29 subjects with severe renal involvement (of whom 17 patients were dialysis dependent at presentation), showed that, although a considerable fraction of patients regain independent renal function, few will have normal serum creatinine concentration at follow-up, despite the addition of PE as adjunctive therapy [106]. However, plasma exchange would appear to be useful in association with intensive immunosuppressive therapy for the treatment of WG patients with acute renal failure and/or severe pulmonary [105]. Its mechanism of action is not well known, but it is presumed that it works by removing ANCAs [56].

Therapy for maintenance of remission

A summary of the therapeutic protocols for the maintenace of remission is described in Table 2.

Azathioprine

Azathioprine is a purine analog and a prodrug of 6-mercaptopurine. Metabolites of AZA have been shown to inhibit *de novo* synthesis of purine ribonucleotides, inhibit purine ribonucleotide interconversion and incorporate themselves into cellular DNA and RNA. Mechanisms through which AZA may impact on immune function include suppression of lymphocyte proliferation, suppression of natural killer cell activity, inhibition of monocyte and antibody production and inhibition of cell-mediated and humoral immune responses.

Although several studies support the use of MTX and steroids (prednisone) in maintaning remission in patients with WG, even AZA is considered to be an effective drug for the maintenance of remission [107]. Moreover, in contrast to CYC, AZA lacks bladder toxicity and has low oncogenic potential.

Recent data have shown that AZA induces the apopotosis of T cells at a molecular level through the modulation of RAC-1 activation upon CD28 stimulation [108]. This study supports the possibility of the use of AZA, not only for maintenance therapy but also for the active phase of WG, in which activated T cells play a central role [109,110]; remission was achieved with high-dose intermittent intravenous AZA (1200 mg/month).

Cotrimoxazole

Cotrimoxazole (trimethoprim/sulfamethoxazole) has been found in several reports to be beneficial in treating WG limited to the upper and/or lower airways. Interpretation of these results is confounded by their retrospective nature, the use of concurrent immunosuppressive agents, the difficulty in defining active upper airways disease and the lack of controlling for infection. Regarding remission, maintenance studies have shown that CTR is less effective than MTX following initial treatment with CYC plus steroids [111].

The mechanism of CTR is not know but could reflect the antimicrobial or immunomodulatory effect; it may abrogate the pathological action of *S. aureus*, thus limiting neutrophil activation and further tissue damage. The most important therapeutic role of CTR in WG is to prevent pneumonia caused by *Pneumocystis carinii*, a well-known complication of immunosuppressive treatment. Its incidence in patients with WG is 1–20% [112,113]. Prophylaxis against *P. carinii* pneumonia with CTR is highly efficacious, well tolerated and should be given to all patients treated with aggressive immunosuppressive therapy [114].

Another study supports the possible role of CTR in reducing relapse rates, especially in those patients with WG limited to the upper respiratory tract [115]

Advanced therapy with emerging drugs

Leflunomide

Leflunomide (LE) is an immunomodulatory drug that inhibits dihydro-orotate dehydrogenase, an enzyme involved in *de novo* pyrimidine synthesis. LE also inhibits the responses of activated T and B cells. Recent trials have shown that treatment with LE is effective for the maintenance of remission after CYC induction therapy, showing a significantly lower rate of severe relapses compared with MTX [116,117]; however, this is associated with an increased frequency of adverse events. Further studies testing other dosing regimens of lower doses of LEF are needed to confirm these promising results in larger patients cohorts. Although encouraging results have been found, the experience with this agent remains limited [118].

Table 2. Protocol of the treatment for the maintenance of remission of Wegener's granulomatosis.

Study	Protocol	Dose	Recommendations*	Ref.
de Groot *et al.*	Methotrexate	0.3 mg/kg/week, intravenously/orally	A	[101]
Jayne *et al.*	Azathioprine	2 mg/kg/day, orally	A	[84]
Reinhold-Keller *et al.*	Trimethoprim/ sulfamethoxazole	2 × 960 mg/day, orally	A	[92]
Metzler *et al.*	Leflunomide	30–40 mg/day	A	[116, 117]
Schmitt *et al.*	15-deoxyspergualin	0.5 mg/kg/day	C	[147]
Nowack *et al.*	Mycophenolate mofetil	Mycophenolate mofetil	B	[119]

*Grade A recomendations: high-quality study evidence (type I); grade B recommendations: inconsistent or limited evidence (type II); grade C recommendations: lacking direct evidence (type III).

Mycophenolate mofetil

Mycophenolate mofetil is an ester prodrug of mycophenolic acid, a noncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase. Inhibition of inosine monophosphate dehydrogenase blocks the *de novo* synthesis of guanosine nucleotides, necessary substrates for DNA and RNA synthesis. Unlike other cell types, lymphocytes rely solely on the *de novo* pathway for the generation of guanosine. MM has been used in small, nonrandomized trials as both a remission-inducing and remission-maintaining agent for WG [119,120]. This drug may play a role in remission induction in patients with WG refractory to conventional therapeutic protocols but larger studies will be needed to confirm these findings before this therapy can be recommended. A retrospective study observed varying efficacy of MM in antibody-associated systemic vaculitis, with over 50% of patients with relapsing disease achieving remission and marked falls in concomitant steroid doses. However, longer follow-up indicates a subsequent relapse rate of over 50% that may be associated with low MMF dosing [121]. A recent trial demonstrated that oral administration of MM 500 mg twice daily could significantly improve BVAS in patients in whom remission was not maintained with CC and/or AZA [122]. A large study of MM as maintenance therapy is ongoing in the EUVAS groups [123].

Immunomodulatory agents

TNF-α antagonists

Monoclonal antibody and recombinant DNA technology has led to an expanding range of therapies capable of directly targeting components of the immune response. The evaluation of these agents in WG may provide insights into disease pathogenesis and new treatment options and. Antibodies against TNF-α play a critical therapeutic role in a variety of inflammatory conditions.

Tumor necrosis factor-modulatory agents have raised interest in the treatment of WG because of the potential role of Th1 cytokines in this disease. Activated peripheral blood $CD4^+$ T lymphocytes from patients with active WG have been found to produce higher levels of IFN-γ [124]. Increased production of TNF by activated CD4 T lymphocytes and increased production of IL-12 by purified monocytes was also noted. A Th1 pattern of cytokine expression has also been exhibited by T-cell clones isolated from WG nasal biopsy specimens displaying granulomatous inflammation [125]. Based on these observations, a hypothetic pathogenic mechanism would be that patients with WG have an immunoregulatory defect that, following exposure to an infection and/or autoantigen, leads to an unbalanced production of Th1 cytokines with initiation and perpetuation of the granulomatous vascular lesion that is characteristic of WG. Experimental investigation into ANCA-associated vasculitis has highlighted a potential pathogenic role for both TNF-α and B-cell-derived ANCA. In animal models, inhibition of TNF-α markedly decreases the formation of granulomas [126,127]. $CD4^+$ T cells from patients with WG produced elevated levels of TNF-α [124]. Serum levels of the TNF-α receptor correlate with disease activity [128]. *In vitro*, TNF-α priming of activated neutrophils markedly enhances the ability of ANCA to stimulate the degranulation of neutrophils [129]. Several recent studies have evaluated the efficacy of TNF blockade in ANCA-associated diseases [130,131].

Currently, three TNF-α antagonists are marketed worldwide for use in patients: adalimumab, ET and IN, although none has an indication for use in treatment of WG.

Adalimumab

Adalimumab has never been studied in patients with ANCA-associated diseases.

Etanercept

Etanercept is a soluble TNF-α inhibitor consisting of two extracellular p75 TNF-α receptor domains linked to the Fc portion of human IgG1. In association with standard treatment (CYC, MTX, AZA and for steroids), ET was found to be effective in an open-label prospective trial involving patients with persistently active or new flares of WG [132]. In the only large-scale randomized trial (Wegener's Granulomatosis Etanercept Trial) to assess the efficacy of ET in the treatment of severe or nonlife-threatening WG, ET did not appear to have a role in the induction or the maintenance of remission in WG [133]. Unexpectedly, more solid malignancies were observed in the etanercept group than in the group treated with standard therapy alone.

This is the only study to suggest that ET increases the risk for solid malignant disease. As ET has not been found to cause solid cancer when used in patients with rheumatoid arthritis, these findings suggest an interaction between ET and immunosuppressive drugs, such as CYC [134,135].

Infliximab

Infliximab is a chimeric mouse/human monoclonal antibody directed against TNF-α that inhibits the action of TNF-α by binding to both soluble and transmembrane human TNF-α; thereby, preventing attachment of TNF-α to its receptors. IN was shown to effectively induce remission in Crohn's disease, whereas ET was found to be ineffective [136]. Only four clinical trials have been published regarding the use of IN in patients with WG. Although these studies appear to support a possible therapeutic role of IN, larger randomized, controlled trials will be necessary prior to recommending IN for standard use in WG therapy [129,130,137–139].

Rituximab

Rituximab is a chimeric mouse/human monoclonal antibody directed against the CD20 molecule on the surface of B lymphocytes. B cells are the primary source of circulating antibodies and are a primary source of ANCA in patients with WG. RTX, by binding CD20 on the surface of B cells, promotes a depletion of peripheral blood B cells and a decrease in ANCA titers [140,141]. RTX has been shown to be an effective rheumatoid arthritis treatment in three randomized, controlled

trials and it is now licensed for use in refractory rheumatoid disease. There is evidence for efficacy in a range of other autoimmune diseases, including idiopathic autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, Evans syndrome, vasculitis, bullous skin disorders, Type 1 diabetes mellitus, Sjogren's syndrome, Devic's syndrome and systemic lupus erythematosus. RTX is now being used in the management of renal transplant recipients. This drug is especially useful in transplants involving incompatible blood groups. It is also used as an induction therapy in highly sensitized patients who undergo renal transplantation. The evidence of the use of RTX in ANCA-associated vasculitides is limited to a small number of case reports and uncontrolled clinical studies. All, except one, of the published reports showed rituximab to be an effective and safe treatment in ANCA-associated vasculitides, with responses in excess of 90% of cases [142]. One case series reported a response in only three out of eight patients who had predominantly granulomatous disease manifestations [143]. These patients had previously failed to respond to standard treatment with CYC and steroids and a range of alternative treatments and, therefore, represent the treatment-resistant (refractory) end of the disease spectrum.

There are a number of considerations regarding the use of RTX in the treatment of ANCA-associated diseases in clinical practice. All published studies included patients who had active vasculitis and were intolerant of, or refractory to, standard treatment [144].

Relapses occurred after the reappearance of peripheral B cells and often in association with a rising ANCA titer. Most patients remained in remission with no treatment or when receiving small doses of corticosteroids, despite the reconstitution of peripheral B cells.

The use of RTX as a remission maintenance agent is less clear. In some case series, patients without clinical manifestations were retreated because of a rising ANCA titer. These patients remained in remission during the follow-up of 12 months [144]. Whether the initial treatment of patients with newly diagnosed RTX will lead to long-term remission is unknown. Two studies have reported that some clinical features, such as retro-orbital granulomatas, were less responsive than other disease manifestations [142,143]. By contrast, other granulomatous features, such as pulmonary nodules and endobronchial disease, responded well to treatment [141].

More work is needed to determine whether or not there is a differential response to RTX, dependent on the disease manifestation. This new drug cannot be recommended for routine use until vigorous clinical studies confirm its efficacy. Further studies are needed to assess the role of B cells in the pathogenesis of WG and the effect of RTX. A randomized, double-blind trial is currently underway [145].

15-desoxyspergualin

15-desoxyspergualin (15-DE) is a synthetic analog of spergualin, a product of the bacterium *Bacillus laterosporus* that has immunosuppressive properties. The mechanism of action of 15-DE is unclear, although effects on B-cell differentiation and T-lymphocyte maturation have been described [146,147]. 15-DE binds to chaperone proteins required for nuclear translocation of the transcription factor nuclear factor (NF)-κB, thereby preventing cell activation. Since T-cell proliferation and B-cell maturation are highly dependent upon nuclear translocation of NF-κB, 15-DE gained much interest as a new immunosuppressive agent for the treatment of transplant rejection and autoimmunity [148]. In fact, this agent is licensed in Japan for the treatment of recurrent kidney transplant rejection. Although 15-DE did not influence the expression of T-cell activation markers *in vivo*, its use in the therapy of refractory WG decreases ANCA titers and reduces ANCA-mediated neutrophil activation [149]. A large multicenter trial evaluating the efficacy of 15-DE in patients with refractory WG is currently in progress.

Intravenous immunoglobulin

Some preliminary trials on the use of immunoglobulin in the treatment of patients with refractory WG have evaluated the therapeutic efficacy of intravenous immunoglobulins, concerning constitutional symptoms, arthralgia and ear, nose and throat manifestations [56,106,150], but other studies are necessary. It is thought that intravenous immunoglobulin interferes with ANCAs binding to their antigens and inhibits ANCA-mediated neutrophil activation [56]. Immunoglobulins are administered intravenously at a dose of 2 g/kg/month [33].

Antithymocite globulin

In 2004, an early, prospective and uncontrolled trial on the use of anti-thimocyte globulin in the treatment of WG patients demonstrated the usefulness of the drug in those who do not respond well to conventional treatment or have contraindications to CYC. It is thought that they work by blocking T cells. In fact, infusion with anti-thimocyte globulin causes T-cell depletion [56]. Its use in the treatment of WG is limited due to serious side effects, including pulmonary edema [56,106].

Abatacept

Abatacept is a soluble fusion protein consisting of the extracellular domain of human cytotoxic T-lymphocyte antigen 4 and a fragment of the Fc portion of human IgG_1 (hinge, CH2 and CH3 domains). It binds human B7 (CD80/86) more strongly than CD28 [151]. Abatacept is a safe and effective drug for the treatment of established rheumatoid arthritis. Other T-cell-mediated diseases are also to be investigated. There has been recent evidence for the use of abatacept in lupus [152] and it could have a role as a potential drug in other types of vasculitis.

Expert commentary & five-year view

In the past few years, many advances have been achieved in elucidating the pathogenesis of WG. Several genetic risk factors have been described. The structure of the granuloma and its possible function as ectopic lymphoid tissue have been

defined. Furthermore, the consecutive immunopathological reactions leading to induction of ANCAs directed against PR3 or MPO and the role of ANCA itself are becoming clearer. In the last 25 years, these antibodies were the subject of intensive studies and a growing body of evidence arose for a distinct role of ANCA in the pathogenesis of AAV. Yet, the evidence derived from clinical observations and *in vitro* studies remains circumstantial. Various animal models have provided substantial support for a pathogenic role of MPO-ANCA *in vivo*, but the debate as to whether ANCAs play a primary role in the pathogenesis of these diseases is still unresolved. Recent data have provided evidence that WG granulomatas might provide the necessary 'proinflammatory environment' for the break of tolerance and display features of lymphoid-like tissue neoformation, in which autoimmunity to 'Wegener's autoantigen' PR3 could be sustained. However, the initial events leading to granuloma formation are still widely unknown.

Concerning therapy, conventional immunosuppressive treatment of systemic vasculitides has improved their often fatal outcome but is burdened by cytotoxic side effects and frequent relapses. Recent advances in the therapy of systemic vasculitides with biological agents have helped to establish new options for patients resistant to conventional treatment. Moreover, early intervention aiming to interfere with specific targets important in the break of tolerance and/or persistence of the autoimmune response might improve the prognosis of autoimmune vasculitides. *In vitro* and *in vivo* studies suggest that the interaction of ANCA and cytokines (e.g., TNF-α and IL-1) results in premature neutrophil activation and degranulation, subsequent endothelial cell damage and further leukocyte recruitment. Blocking TNF-α and eliminating autoreactive B cells seem to be promising treatments to interfere with these fundamental disease processes. While the recombinant TNF-α receptor/IgG_1 fusion protein etanercept, in addition to standard therapy with subsequent tapering of standard medications, was found to be ineffective for maintenance of remission, open clinical studies suggest a beneficial effect of the anti-TNF-α antibody IN, in addition to standard therapy, for the induction of remission in patients with refractory AAV. Peripheral B-cell depletion with the anti-CD20 antibody rituximab also induces remission in AAV in uncontrolled trials. Remission following T-lymphocyte depletion can be achieved with alemtuzumab and antithymocyte globulin, but it is not yet clear what the clinical role will be for these agents in AAV. Acceptance for using these treatments could be accelerated over the next 5 years if several carefully designed and powered clinical trials confirm the current efficacy and safety data. Concomitant immunosuppressants and nonstandardized definitions are major limitations of the recent trials, and future studies of these agents and newer ones must follow agreed standards of study design and reporting to facilitate clearer interpretation of the circumstances (e.g., disease stage, severity or organ involvement) under which these agents perform optimally. Consequently, use of these agents is still limited to centers experienced in such agents and mostly in the context of clinical trials.

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Key issues

- Wegener's granulomatosis (WG) is an idiopathic, systemic vasculitis characterized by the formation of necrotizing granulomas of the respiratory tract in addition to focal or proliferative glomerulonephritis.
- Its etiology is unknown but it is thought that it results from an interplay between genetic susceptibility (association with the genes of the class II region of the MHC, such as *HLA-DPB1*) and environmental triggers, including certain infections, occupational risk factors and drugs.
- Wegener's granulomatosis is associated with the presence of circulating antineutrophil cytoplasmic antibodies (c-ANCAs); the most widely accepted pathogenetic model suggests that c-ANCA-activated cytokine-primed neutrophils induce microvascular damage and a rapid escalation of inflammation with recruitment of mononuclear cells.
- The diagnosis of WG is made on the basis of typical clinical and radiologic findings, the biopsy of involved organs (nasal mucosa, lung, kidney and muscle), the presence of ANCA and the exclusion of all other small-vessel vasculitis.
- Treatment of WG is stage adapted; there is a clear distinction between induction of remission and maintenance of remission strategies, which may vary according to the extension of disease.
- Currently, a regimen consisting of daily cyclophosphamide and corticosteroids, which induces complete remission in the majority of patients, is considered standard therapy.
- A number of trials have evaluated the efficacy of less-toxic immunosuppressants (e.g., methotrexate, azathioprine and mycophenolate mofetil) and antibacterials (i.e., cotrimoxazole [trimethoprim/sulfamethoxazole]) for treating patients with WG, resulting in the identification of effective alternative regimens to induce or maintain remission in certain subpopulations of patients.
- Recent investigations have focused on other immunomodulatory agents (TNF-α inhibitors [infliximab and etanercept] and anti-CD20 antibodies [rituximab]), intravenous immunoglobulins and antithymocyte globulins for treating patients with resistant WG.

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