



Refractory disease in antineutrophil cytoplasmic antibodies associated vasculitis

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Purpose of review

Induction treatment of antineutrophil cytoplasmic antibodies (ANCA) associated vasculitis (AAV) is not always successful and nonresponding patients are considered refractory.

Recent findings

Refractory disease should be subdefined to the treatment that was received. Cyclophosphamide refractory AAV occurs in up to 5% of patients. Many more patients develop contraindications to cyclophosphamide or relapse frequently. The latter two patient groups might also benefit from treatment used for cyclophosphamide refractory AAV.

Summary

The most promising drug for treating refractory AAV is rituximab.

Keywords

antineutrophil cytoplasmic antibodies, refractory, rituximab, vasculitis

INTRODUCTION

Refractory stems from the Latin *refractarius* and translates as stubborn or obstinate. Refractory disease relates to any form of resistance to therapy. The most important prerequisite for defining a disease as refractory is that the disease is active and that adequate therapy has been prescribed. Chronic sequelae of disease that do not reverse with therapy are defined as damage [1]. In some diseases, the differentiation between active disease and disease damage is difficult. Examples include nasal destruction in antineutrophil cytoplasmic antibodies (ANCA) associated vasculitis (AAV) [2] or persistent low-grade proteinuria in lupus nephritis [3].

Refractory disease should be further defined by the treatment that was given, for example, steroid refractory giant cell arteritis or cyclophosphamide refractory AAV. This is crucial as new treatment options may render a refractory disease treatable. Examples are the use of rituximab in cyclophosphamide refractory AAV [4[■]] and, perhaps, tocilizumab in steroid refractory giant cell arteritis [5[■]].

An important aspect in limiting therapeutic options is treatment toxicity or patient comorbidity. Examples are serious leukocytopenia due to earlier cytotoxic therapy or a preexisting bone marrow condition. This should be clearly distinguished from disease-related leukocytopenia in, for instance, lupus patients. We propose a clear definition of

refractory disease, subdefined to the treatment that was given. The disease can be refractory to conventional treatment or is refractory to any other form of therapy while conventional treatment is not possible. The latter patient group might also benefit from treatment used for disease refractory to conventional treatment.

Small-vessel vasculitis is a condition characterized by inflammation predominantly of vessels smaller than arteries, such as arterioles, venules, and capillaries. It is important to note that small-vessel vasculitis sometimes, but not always, also affects arteries, and thus the vascular distribution overlaps with that of the medium-sized-vessel and large-vessel vasculitides [6]. Its clinical manifestations are dependent on the localization of the involved vessels as well as on the nature of the inflammatory process. Vasculitis can be secondary

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to other conditions such as infections (e.g. hepatitis B or C), autoimmune rheumatic disease (e.g. rheumatoid arthritis and lupus), malignancies (e.g. hairy cell leukemia), drug reactions (e.g. hydralazine, propylthiouracil, and D-penicillamine) and substance abuse (e.g. cocaine) [7]. Primary small-vessel vasculitis can be divided in immune complex mediated (such as Henoch Schonlein Purpura, cryoglobulinemic vasculitis) and 'pauci immune' in which immune deposits are scarce or absent. The latter disease group consists of the ANCA-associated vasculitides (AAV), that is granulomatous inflammation with polyangiitis (GPA; formerly Wegener's disease), microscopic polyangiitis (MPA) and Churg–Strauss syndrome. In the next section, we will discuss the definition and treatment of refractory AAV [8,9].

REFRACTORY ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES ASSOCIATED VASCULITIS

Standard treatment of AAV with cyclophosphamide and oral corticosteroids is generally effective in inducing remission [10]. However, cyclophosphamide causes serious acute and long-term side-effects, such as hemorrhagic cystitis, leukopenia with opportunistic infections, gonadal failure, bladder cancer, bone marrow depression, myelodysplasia and myeloproliferative disease [11]. Patients can be refractory to standard therapy or can develop contraindications to this therapy.

As treatment is aimed at controlling disease, most clinical trials in vasculitis take achievement of remission as one of their main outcome measures. Disease remission is mostly defined as reaching absence or a low level of disease activity while using no or a predefined amount of medication (e.g. low-dose steroids) at a certain point in time. Nowadays, remission is achieved in over 90% of patients. In patients who have refractory disease this is, logically, much lower. In AAV patients that are refractory to conventional treatment with cyclophosphamide and glucocorticoids, remission is achieved in only 35–83% of patients by using various second-line therapies [12].

DEFINITION OF REFRACTORY DISEASE IN ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES ASSOCIATED VASCULITIS

The European vasculitis study group (EUVAS) [13] defines five disease categories in AAV: localized, early systemic, generalized, severe renal, and refractory disease. The EUVAS disease categorization has

been adopted by the European League Against Rheumatism (EULAR) recommendations in the management of AAV [14].

Refractory AAV was defined by consensus within EUVAS [12,13,15] as either

- (1) unchanged or increased disease activity after 4 weeks of treatment with daily oral cyclophosphamide (2–3 mg/kg) and glucocorticoids or pulse-intermittent high-dose cyclophosphamide (15 mg/kg or 0.6–0.7 g/m² body surface area) with glucocorticoids; or
- (2) lack of response, defined as less than 50% reduction in the disease activity score and lack of improvement of at least one major item after 4–6 weeks of treatment; or
- (3) chronic persistent disease, defined as presence of at least one major or three minor items on the disease activity score list (according to the Birmingham Vasculitis Activity Score; BVAS) despite 8 weeks of treatment.

In addition, patients who are intolerant to treatment with daily oral or pulse-intermittent cyclophosphamide and glucocorticoids or who have contraindications against the use of cyclophosphamide can be regarded as having refractory disease, if the disease is not controlled with the best available alternative standard therapy for a defined duration of treatment [12].

OCCURRENCE OF REFRACTORY DISEASE IN ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES ASSOCIATED VASCULITIS

Many studies investigating different treatment modalities within patients with AAV have focused on achieving disease remission. This is mostly analyzed at 6 months after starting treatment. As stated above, refractory disease is encountered earlier than these 6 months and is defined differently. When re-analyzing the major published randomized treatment studies for the occurrence of refractory vasculitic disease, in general 4–5% of refractory cases could be identified (Table 1) [16–20]. This is likely an underestimation of refractory disease as a number of patients were lost to follow-up and not all deaths could be attributed to either infection or active vasculitis. Moreover, from most studies inadequate data were present to evaluate the response to treatment at 4–8 weeks separately.

The CYCLOPS study (cyclophosphamide oral versus pulse) [16] randomized 149 patients with newly diagnosed AAV with renal involvement for either oral or intravenous pulse cyclophosphamide. Of the 76 patients treated with pulse therapy, three

Table 1. Numbers and percentages of refractory cases in the different trials on patients with AAV

Study	Refractory/total	%	
CYCLOPS [16]	7/150	4.7%	3.8%
MEPEX [17]	6/137	4.4%	
NORAM [18]	2/46	4.3%	
RITUXVAS [19]	0/11 (1/33)	0% (3%)	
RAVE [20]	2/98	2.0%	

Part of the NORAM and RAVE patients received induction therapy without a cyclophosphamide component; these were excluded from this analysis. The RITUXVAS patients received either two or three pulses of intravenous cyclophosphamide together with 4 weekly rituximab infusions ($n=33$) or received intravenous pulses of cyclophosphamide for 3–6 months ($n=11$).

had uncontrolled disease and one did not tolerate treatment; in the oral group, three out of 73 patients had uncontrolled disease. The MEPEX study [17] (methylprednisolone or plasma exchange) investigated the benefit of adding plasma exchange versus methylprednisolone to standard therapy in patients with severe renal vasculitis. A total of 137 patients were randomized to receive methylprednisolone ($n=67$) or plasma exchange ($n=70$). Within 3 months, 11 patients in each group died mostly because of infectious complications, but also because of pulmonary hemorrhage in six patients. The latter might very well reflect refractory vasculitis. Another EUVAS study [18], Non-Renal Wegener's granulomatosis treated Alternatively with Methotrexate, compared the use of cyclophosphamide versus methotrexate (MTX) for induction of remission in patients with early systemic AAV. A total of 49 patients were randomized to receive MTX and 46 patients received oral cyclophosphamide. From the MTX group, three patients had to stop the medication because of side-effects versus two from the cyclophosphamide group. Treatment failure was observed in one patient in the MTX group. Rituximab versus cyclophosphamide in ANCA-associated vasculitis (RITUXVAS) was the latest EUVAS study comparing a rituximab-based regimen with a standard cyclophosphamide/azathioprine regimen in the treatment of active, 'generalized' AAV [19]. A total of 33 patients were randomized to receive rituximab, of which five died before the fourth month of treatment. None of the deaths was attributed to active vasculitis. One patient was classified as having treatment failure and received an additional dose of cyclophosphamide. None of the 11 cyclophosphamide randomized patients had treatment failure; one died before the fourth month of treatment.

The other large rituximab-based study [20] in AAV, the Rituximab in ANCA-Associated Vasculitis

(RAVE) study, randomized 99 patients to receive rituximab and 98 patients to receive standard treatment with oral cyclophosphamide. Patients were classified as having early treatment failure if at 1 month their disease activity score had not decreased by at least 1 point on BVAS-Wegener's or a new manifestation of disease had emerged. Seven of the rituximab-treated patients and two of the patients treated with cyclophosphamide experienced early treatment failure and thus refractory vasculitis (see also Table 1).

In conclusion, the refractory disease rate was low in these well studied but selected patient groups.

TREATMENT OF REFRACTORY ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES ASSOCIATED VASCULITIS

For many decades, standard induction treatment of AAV has been cyclophosphamide with prednisolone followed by maintenance therapy with azathioprine. However, with the results of two randomized AAV trials, RITUXVAS and RAVE, treatment with rituximab and prednisolone has been demonstrated to have equal efficacy, with perhaps better performance in patients who experience relapse of their disease. Also, the results of these trials at 24 and 18 months, respectively, are comparable to standard treatment [20–22,23[□],24[□]]. Moreover, single-center experience with rituximab in AAV is reported to be excellent [23[□]]. Rituximab has been successfully used in cyclophosphamide refractory AAV [23[□],25[□]]. These data challenge the concept of cyclophosphamide-based regimens as the sole standard therapy of AAV [25[□]].

When evaluating published studies on treatment of cyclophosphamide refractory AAV, 19 relevant clinical trials could be identified. All were open-label studies. The agents described as being successful in patients with refractory AAV included rituximab, antithymocyte globulin (ATG), alemtuzumab, autologous nonmyeloablative hematopoietic stem cell transplantation (HSCT), high-dose azathioprine, anti-TNF α , mycophenolate mofetil (MMF), and 15-deoxyspergualin.

Rituximab

Rituximab is a genetically engineered chimeric murine–human monoclonal IgG-1 kappa antibody directed against the CD20 antigen expressed on the surface of B lymphocytes. Upon intravenous administration, it rapidly depletes circulating B cells. In AAV, ANCA produced by activated B cells and/or B-cell-derived plasma cells play an important role. Also, B cells themselves are important in various

immune regulating functions relevant for auto-immune diseases [26].

In 2001, the first report of successful treatment in a patient with relapsing Wegener’s granulomatosis and contraindications to cyclophosphamide was described [27]. In the years thereafter, many case reports and case series have established a role for rituximab in cyclophosphamide refractory AAV. Nine such studies are summarized in Table 2 [4^{••},28–34,35^{••},36].

Antithymocyte globulin

ATG is a polyclonal preparation of antibodies directed against surface antigens of activated lymphocytes and results in profound lymphocyte depletion of rapid onset. Mechanisms of action of ATG include classic complement-mediated lysis of lymphocytes, clearance of lymphocytes because of reticuloendothelial uptake, masking of T-cell antigens, or expansion of negative regulatory cells [15,37]. Schmitt *et al.* treated seven cyclophosphamide unresponsive GPA patients with ATG; six obtained partial response and one patient died 3 days after first ATG dose because of pulmonary hemorrhage.

Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody directed against CD52 (alemtuzumab, CAMPATH-1H) and depletes circulating lymphocytes and macrophages. Recovery of peripheral T lymphocytes after treatment takes months to even years. In

addition to T lymphocytes, alemtuzumab is known to deplete other lymphocytes and monocytes, both of which may be important to the pathogenesis of AAV. Walsh *et al.* [38] treated 71 refractory or relapsing AAV patients with one or more courses of alemtuzumab. A total of 79% of these patients had used large doses of cyclophosphamide prior to the infusion of alemtuzumab (range 100–200 g). A total of 60 patients (85%) obtained remission after treatment but 43 relapsed (median 9.2 months); 24 had a remission longer than 1 year, of which 10 had a remission of at least 3 years. A total of 31 patients died (median survival time of 106 months). Up to 38% of patients with AAV treated with alemtuzumab experienced (severe) infections.

The major disadvantages of this monoclonal antibody are infectious complications [38].

Autologous nonmyeloablative hematopoietic stem cell transplantation

HSCT might ameliorate vasculitis by hoarse immunosuppressive conditioning resulting in deletion of almost all T and B cells, including autoreactive cells with substitution by ‘newly primed’ nonautoreactive cells after HSCT [39]. Statkute *et al.* [40] successfully treated one patient with GPA with HSCT with a cyclophosphamide and ATG conditioning regime; this patient experienced a 24-month sustained remission. Kotter *et al.* [41] treated one GPA patient with HSCT with a comparable condition regime and achieved complete remission for an observed 6 years.

Table 2. Rituximab as treatment option for refractory vasculitis as described in the literature

Treatment	Patients	Complete response (%)	Partial response (%)	Refractory disease (%)	Reference
Rituximab	19	10 (53%)	3 (16%)	6 (31%)	[28]
	65	49 (75%)	15 (23%)	1 (2%)	[29]
	7	7	0	NR	[30]
	8	8 (100%)	0 (0%)	0 (0%)	[31]
	11	9 (82%)	1 (9%)	1 (9%)	[32]
	3	2 (66%)	1 (34%)	0 (0%)	[33]
	10	10 (100%)	0 (0%)	0 (0%)	[34]
	11	11 (100%)	0 (0%)	0 (0%)	[35 ^{••}]
	16 ^a	12 (75%)	3 (18.8%)	0 (%)	[36]
	9	9 (100%)	0 (0%)	0 (0%)	[30]
	59 ^b	4 (6.8%)	31 (52.5%)	22 (37.3%)	[4 ^{••}]
	218	60.1%	24.8%	13.8%	

ANCA, antineutrophil cytoplasmic antibodies.

^aFour patients were cyclophosphamide intolerant. One patient died because of fatal sepsis; no data are available on whether this patient was refractory or not.

^bIn this table, only the first round of rituximab per patient was described; two patients died after receiving rituximab. Granulomatous manifestations such as orbital granuloma were more frequently refractory to rituximab than vasculitis or other granulomatous manifestations. Twenty-seven of the fifty-nine (45.7%) patients in this study had orbital masses; this could explain the lower remission rate compared with the other studies in this table.

High-dose azathioprine

Studies with high-dose intravenous azathioprine have been conducted in selected patients with inflammatory bowel diseases and in patients with refractory rheumatoid arthritis. In the latter patients, azathioprine was given as a loading dose intravenously at 22.5–36 mg/kg, after which oral azathioprine was continued. Treatment toxicity was low. However, azathioprine at the doses studied did not appear to enhance control of disease enough to warrant additional studies [42]. Thiopurine methyltransferase activity should be normal; otherwise, patients are at increased risk for severe neutropenia. Benenson *et al.* [43] treated four cyclophosphamide refractory GPA patients with 1200 mg azathioprine intravenously at monthly intervals which resulted in complete response in two patients and refractory disease in the remainder.

Antitumor necrosis factor alpha

Anti-TNFalpha treatment is now widely used to treat rheumatoid arthritis and inflammatory bowel disease. Evidence suggests that TNFalpha plays a central role in the pathogenesis of AAV. *In vitro*, TNFalpha priming of activated neutrophils markedly enhances the ability of ANCA to stimulate degranulation of neutrophils. There is increased expression of TNFalpha at the sites of vasculitic injury, and circulating levels of both TNFalpha and TNFalpha receptors are increased during disease activity and normalize with disease remission [44–47]. Furthermore, treatment with anti-TNFalpha therapy diminishes the inflammation in an animal model of human renal vasculitis [48] and decreases the formation of granulomas [49].

Unfortunately, randomized, controlled, clinical trials adding anti-TNF (etanercept, infliximab, or adalimumab) to the standard of care could not demonstrate increased response rates in patients with nonrefractory AAV [44,45,50]. On the contrary, in the etanercept-treated group, an increase in incidence of solid cancer could be observed [44].

Bartolucci *et al.* [45] described the use of infliximab against refractory AAV in seven patients; two of these patients were true cyclophosphamide refractory AAV patients. All patients reached complete or partial remission. Booth *et al.* [46] described the use of infliximab in 16 active AAV patients who had received at least 3 months of combination therapy with prednisolone and either cyclophosphamide, azathioprine, or MTX before enrollment and had not achieved remission. A total of 14 reached remission and two experienced treatment failure.

Mycophenolate mofetil

MMF is a pro-drug. Its active metabolite mycophenolic acid suppresses guanine synthesis in lymphocytes by inhibiting inosine monophosphate dehydrogenase, and blocks DNA synthesis and proliferation after activation. As most other cells, in contrast to lymphocytes, can synthesize guanine by using a different salvage pathway, the effect on lymphocytes is rather selective, with fewer side-effects. Different case studies demonstrate reasonable efficacy of MMF in inducing remission in patients with AAV (reviewed in [51]). Recent investigations have demonstrated that azathioprine is superior to MMF in maintaining remission in AAV patients [52]. MMF has not been described in cyclophosphamide refractory AAV; however, in cyclophosphamide intolerant patients, MMF was successful in achieving a complete response in 25 of 32 patients as reported by Stassen *et al.* [53]. Six patients reached partial remission and one patient was considered refractory.

Deoxyspergualin

15-Deoxyspergualin is an antiproliferative drug derived from *Bacillus laterosporus*. Although initially developed as an anticancer drug, strong immunosuppressive properties were discovered with effects on lymphocyte and macrophage function and neutrophil production. In clinical trials including patients with recurrent kidney transplant rejections, deoxyspergualin led to remission in 79% of cases [54]. Deoxyspergualin was also tested in five patients with various forms of proliferative glomerulonephritis. It demonstrated reduction of proteinuria, and reduced circulating and urinary CD16⁺ (FcgammaRIII) monocytes [55]. Birck *et al.* [56] described nine cyclophosphamide refractory AAV patients treated with 15-deoxyspergualin. One patient reached complete remission, whereas four others reached partial remission at 6 months. Two patients responded initially, but relapsed during treatment before 6 months. One patient stopped therapy because of severe thrombocytopenia and one patient stopped because of an infectious problem. Flossman *et al.* [57] described 32 mostly cyclophosphamide refractory AAV patients who were treated with 15-deoxyspergualin. A total of 95% of these reached complete or partial remission. Severe or life-threatening treatment-related adverse events occurred in over 50% of patients mostly because of leukopenias.

In conclusion, 15-deoxyspergualin can be a useful drug in reaching remission in patients refractory to cyclophosphamide. However, treatment-related

side-effects are very frequent, and alternative treatment modalities should be considered.

In the studies with the above-mentioned drugs for refractory AAV, complete response rates ranged from 20 to around 80%. When complete response was not achieved, many patients did achieve partial response and prednisolone dose could be lowered. Selection of patients with refractory disease might reflect a selection of patients who have a different pathogenesis more resistant to standard therapy. In the RAVE study [20], it was observed that patients who relapsed (after previous cyclophosphamide treatment) were more likely to be responsive to rituximab than to cyclophosphamide.

Differences between the therapies used for refractory disease are mostly reflected in the percentages of complete and partial remissions, but also in the number of serious side-effects. The studies presented in this literature overview on refractory AAV will likely include studies preferentially demonstrating a positive result from the investigational product, that is, a publication bias [58].

CONCLUSION

With the available data today, rituximab should be considered the most well tolerated and effective second-line therapy and should be advocated as a first alternative choice for cyclophosphamide in disease induction in refractory AAV. However, it would be prudent to confirm these findings in prospectively obtained data from multiple centers using a protocolized approach to refractory disease.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 342).

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