Full Review

Twenty-five years of European Union collaboration in ANCA-associated vasculitis research

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ABSTRACT

This special edition reviews the progress in understanding of systemic vasculitis associated with autoantibodies to neutrophil cytoplasmic antigens (ANCA). European research groups have contributed to this research including the original observations of the association between ANCA and vasculitis. Areas of interest include classification and epidemiology, genetics and pathogenesis, disease assessment, histology and long-term outcomes. Clinical trials conducted by the European Vasculitis Study group have helped to define the current standard of care for the treatment of patients with vasculitis and provided a platform for the investigation of newer therapies. The prognosis of patients with ANCA-associated vasculitis has improved over this period as a result of facilitated diagnosis and development of consensus, evidence-based, treatment recommendations. The ANCA story represents an example of the power of a biomarker in influencing a disease area, inspiring research and providing physicians with better tools to treat patients with these disorders.

Keywords: ANCA vasculitis, clinical trial, genetics, rituximab, vasculitis

INTRODUCTION

Vasculitis remains a challenging disease to diagnose and manage, and surviving vasculitis patients are usually damaged by their disease. Yet there have been recent advances with noticeable improvements in the survival for patients with severe renal and pulmonary diseases. European physicians have played central roles in vasculitis research over this period, and the reviews in this special edition highlight the areas of activity. This special edition of Nephrology Dialysis and Transplantation focusses on the progress made in vasculitis research by European researchers over the past 25 years and comprises a series of invited reviews by those involved in this research complimented by 11 papers submitted in response to a call related to this edition.

The discovery of the biomarker, autoantibodies to neutrophil cytoplasmic antigens (ANCA) by a Dutch/Danish collaboration 30 years ago (reviewed by Rasmussen et al.), enabled improved classification and diagnosis, and understanding of its contribution to pathogenesis has provided a rationale for plasma exchange and B-cell depletion therapy (reviewed by Schonermark et al. and Szpirt et al.). The association of ANCA with vasculitis also inspired international consensus on classification (reviewed by Mahr et al.) that permitted patient subgrouping for clinical studies (Table 1). Disease assessment criteria and the evolution of clinical trial methodology provided treatment response measures and outcome tools for these complex multisystem disorders (reviewed by Luqmani et al.) [2].

Building on the European collaborative group that defined the clinical utility of ANCA, the European Vasculitis Study group (EUVAS), developed to conduct interventional clinical trials aimed at harmonizing and optimizing treatments and outcomes for vasculitis patients. Particular areas of interest were the reduction in cyclophosphamide exposure, plasma exchange, remission maintenance strategies and the development of newer therapies. A sequence of 12 clinical trials involving over 1300 patients has been conducted that have defined the standard of care and permitted consensus treatment recommendations (Table 2). The large-scale assembly of clinical data also supported parallel studies on nephropathology (reviewed by Bajema et al.), clinical epidemiology (reviewed by Westman et al.) and serology.

A genome-wide association survey was performed by the European Vasculitis Genetics Group on almost 2500 Northern European samples from patients with granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) in 2011...
This has firmly established the genetic contribution to these disorders and provided momentum for more detailed genetic studies in GPA and MPA and study of less frequent vasculitides (reviewed by Vaglio et al.). Intriguingly, the genomic studies found a stronger distinction on subgrouping by ANCA serotype [proteinase 3 (PR3) or myeloperoxidase (MPO)] than clinical phenotype (GPA or MPA) that will influence future classification systems.

Children were included in clinical trials for the first time in the MYCYC (MYCophenolate mofetil versus CYClophosphamide) study (Table 2), and related European paediatric studies have included classification and disease assessment (reviewed by Eleftheriou et al.). The European Renal Association registry has reported on the consequences of end-stage renal failure in vasculitis patients with respect to survival and transplantation (reviewed by Tesar et al.).

### THE DEVELOPMENT OF A NETWORK

The discovery of autoantibodies in vasculitis inspired collaborative research and the initiation of a sequence of International ANCA Workshops, commencing in Copenhagen in 1988 [12]. At the second International Workshop in Nordwijkerhout, the Netherlands in 1989, it was agreed to launch a European study to standardize ANCA testing. Funding was secured from the European Community Bureau Central de Reference and the project launched the following year on the occasion of Friederich Wegener’s last public lecture in Saarbrucken, Germany. After several cycles of sera sample and assay exchanges and meetings in Brussels, it was determined that the indirect immunofluorescence (IIF) assay was difficult to standardize, but solid-phase ELISAs for PR3 and MPO-ANCA could be standardized with acceptable levels of variability [13]. The diagnostic value of ANCA testing for the diagnosis of GPA and MPA was confirmed, and consensus recommendations for how ANCA testing should be performed were published [14, 15]. A key supportive element of clinical research at this time was the agreement by international consensus of a common system of nomenclature, the Chapel Hill Conference, in 1993 that was supported by many European Union (EU) delegates [16].

With the success of the ANCA project, growth in membership of the network and an awareness of variability in treatment approaches between centres, a proposal to harmonize

### Table 1. Classification of vasculitis according to extent and severity at presentation [1].

<table>
<thead>
<tr>
<th>Disease subgroup</th>
<th>Definition</th>
<th>ANCA status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localized</td>
<td>Vasculitis confined to one organ system, no systemic disturbance.</td>
<td>Often negative</td>
</tr>
<tr>
<td>Early systemic</td>
<td>Vasculitis in at least one organ system with systemic features without threatened vital organ function.</td>
<td>Usually positive</td>
</tr>
<tr>
<td>Generalized</td>
<td>Vasculitis in at least one organ system with systemic features and threatened vital organ function.</td>
<td>Usually positive</td>
</tr>
<tr>
<td>Severe</td>
<td>Vasculitis in at least one organ system with systemic features and vital organ function failure (e.g. serum creatinine &gt; 500 μmol/L).</td>
<td>Usually positive</td>
</tr>
<tr>
<td>Refractory</td>
<td>Failure of standard induction regimen.</td>
<td>Positive or negative</td>
</tr>
</tbody>
</table>

### Table 2. Prospective interventional clinical trials conducted by the EUVAS.

<table>
<thead>
<tr>
<th>Trial acronym</th>
<th>Trial question</th>
<th>Disease subgroup</th>
<th>Sample size</th>
<th>End point</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECYSVASTRIAL</td>
<td>Malthotrexate or cyclophosphamide for induction</td>
<td>New-onset ‘early systemic’ GPA/MPA</td>
<td>100</td>
<td>Remission at 6 months</td>
<td>[3]</td>
</tr>
<tr>
<td>NORAM</td>
<td>3 months or 12 months cyclophosphamide for induction</td>
<td>New-onset ‘generalized’ GPA/MPA</td>
<td>144</td>
<td>Relapse by 18 months</td>
<td>[4]</td>
</tr>
<tr>
<td>CYCAZAREM</td>
<td>Addition of plasma exchange or high-dose methyl prednisolone for induction</td>
<td>New-onset ‘severe renal’ GPA/MPA</td>
<td>137</td>
<td>Renal survival</td>
<td>[5]</td>
</tr>
<tr>
<td>MEPEX</td>
<td>Anti-thymocyte globulin for induction</td>
<td>‘Refractory’ GPA/MPA</td>
<td>15</td>
<td>Remission</td>
<td>[6]</td>
</tr>
<tr>
<td>SOLUTION</td>
<td>Pulsed or daily oral cyclophosphamide for induction</td>
<td>New-onset ‘generalized’ GPA/MPA</td>
<td>160</td>
<td>Remission at 6 months</td>
<td>[7]</td>
</tr>
<tr>
<td>AVERT project</td>
<td>MMF or azathioprine for relapse prevention after cyclophosphamide induction</td>
<td>New-onset ‘generalized’ GPA/MPA</td>
<td>171</td>
<td>Relapse by 48 months</td>
<td>[8]</td>
</tr>
<tr>
<td>CYCLOPS</td>
<td>Long-term immunosuppression for relapse prevention</td>
<td>Remission ‘generalized and severe renal’ GPA/MPA</td>
<td>120</td>
<td>Relapse by 48 months</td>
<td>Completed</td>
</tr>
</tbody>
</table>

Subsequent trials

| RITUXVAS      | Rituximab or cyclophosphamide for induction | New-onset ‘generalized and severe renal’ GPA/MPA | 44 | Sustained remission by 12 months | [9] |
| MYCYC         | MMF or cyclophosphamide for induction | New-onset ‘early systemic and generalized’ GPA/MPA | 140 | Remission at 6 months | Completed |
| PEXIVAS       | Plasma exchange and reduced-dose glucocorticoids for induction | New-onset or relapsing renal (GFR < 50 mL/min) or lung haemorrhage GPA/MPA | 500 (target) | Time to death or ESRD | Recruiting |
| RITAZAREM     | Rituximab or azathioprine for relapse prevention after rituximab induction | Relapsing GPA/MPA | 190 (target) | Relapse by 24 months | Recruiting |

Note: Disease subgroups as defined in Table 1.
and standardize treatment approaches across 14 EU centres was funded under the BIOMED 1 programme in 1994, the ECSYSVASTRIAL project. The application proposed investigation of the newer agents, intravenous immunoglobulin and trimethoprimsulfamethoxazole as additions to current therapies to improve the speed of remission induction and to reduce relapse risk. The IVISTAT trial used a factorial design to test both these interventions but was abandoned when industry support was withdrawn. The evolved workplan then subcategorized patients by disease extent and severity, into five subcategories and designed a randomized controlled trial for each category (Tables 1 and 2). A common theme was to agree on a consensus, control, treatment regimen and then to select the best alternative treatment regimen for which there were experience and preliminary evidence. The trial activities were supported by development of disease assessment methodology, the VITAL project, a histology review process and serum bank [1, 17]. Forty centres contributed patients to this ‘first wave’ of EU clinical trials that recruited 400 GPA and MPA patients between 1995 and 2003.

With success in the recruitment to the ‘first wave’ of trials, an application to launch a ‘second wave’ of three trials, the AVERT project, was funded through the EU BIOMED 2 programme in 1999. This aimed to further investigate lower cyclophosphamide exposure protocols, to examine remission maintenance strategies through longer-term trials and to look again at infection control in GPA (Table 2). A trial of nasal mupirocin to reduce relapse in GPA was initiated but then closed for logistic reasons, but three other trials were completed.

The clinical trial datasets were merged, and a cross sectional survey of longer-term outcomes was conducted in 550 patients in 2005. This aimed to define the long-term patient and renal survival of ANCA associated vasculitis (AAV) and to explore cardiovascular and malignancy risks and ‘all cause’ damage [18, 19]. It also supported the development of a renal histopathology subclassification system that associated with renal prognosis and newer approaches to patient classification [20]. The clinical trial experience since 1995 was used to inform the European League Against Rheumatism (EULAR) recommendations on how to perform clinical investigations in vasculitis and how to manage small- and large-vessel vasculitides [2, 21, 22]. Deficiencies in the classification systems in use were reviewed in a ‘Points to consider’ EULAR report that led to the 2012 Chapel Hill consensus nomenclature update (15) [23, 24].

Through the ‘2000s’, two investigator-initiated clinical trials were conducted with funding from the pharmaceutical industry to examine rituximab and mycophenolate mofetil (MMF) as induction therapy. The trials network extended from the EU to include centres in Australia and New Zealand. The rituximab trial (RITUXVAS) compared a rituximab-based induction regimen with cyclophosphamide in severe renal GPA/MPA and ran in parallel to a larger North American study, RAVE [9, 25]. The latter study led to the licensing of rituximab as the first drug licensed for AAV in 2011, and these two trials underpin the first major change in induction treatments since the introduction of cyclophosphamide in 1970.

Coser collaboration with North American centres had developed through regular international ANCA vasculitis workshops, and the first formal joint proposal between the EUVAS and the Vasculitis Clinical Research Consortium (VCRC) was the PEXIVAS trial in 2007 [10]. The trial was funded by joint applications to the Food and Drug Administration (FDA) in the USA and Medical Research Council in the UK. Subsequent national networks were supported in Canada by a grant from the Canadian Institute of Health Research (CIHR) and Australia by the National Health Medical Research Council (NHMRC). This demonstrated the growth of the collaborative network across three continents, the progressive alignment in the approach to clinical problems and serves as a model for the conduct of rare disease research. A second rituximab trial, RITAZAREM, co-funded by a UK charity and industry has since been launched across the global network, and Japanese centres have become involved in both studies.

In 2011, the European Vasculitis Society was founded to place coordination of European vasculitis research on a more structured foundation [26]. A scientific council comprising nine areas of research activity was established (Figure 1), and common facilities for data storage, histology review and a serum bank were developed.

**REDUCING CYCLOPHOSPHAMIDE EXPOSURE**

Although the addition of immunosuppressives, such as nitrogen mustard in the 1950s and methotrexate and azathioprine in the 1960s, to glucocorticoids had a therapeutic effect in systemic vasculitis, it was the addition of cyclophosphamide, reported by Novack and Pearson, and Fauci, both in 1971, in the treatment of Wegener’s granulomatosis (now GPA) that had the biggest impact [27, 28]. The combination of daily oral cyclophosphamide and glucocorticoids led to remission of disease activity in the majority of patients and became the ‘standard of care’. Longer-term follow-up of these patients

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**FIGURE 1:** Areas of research activity coordinated by the European Vasculitis Society in 2015.
reported high rates of haemorrhagic cystitis and bladder and lymphoproliferative malignancies consequent on the high cumulative cyclophosphamide exposures [29].

Experience of shorter cyclophosphamide courses in renal vasculitis and an appreciation, following the discovery of ANCA, that the renal lesions of GPA and MPA were similar, inspired study of the two subgroups together in the CYCAZAREM trial (CYClophosphamide or AZThioprine for REMission) [4]. This trial recruited 144 new patients with GPA/MPA and compared a 3–6-month cyclophosphamide course, stopping when remission was achieved to a ‘standard’ 12-month course, both groups then receiving azathioprine. No differences in remission or relapse rates were observed, although a long-term extension study found a trend towards more relapses in the short-course cyclophosphamide group [30]. The validation of a sequential cyclophosphamide induction followed by azathioprine maintenance harmonized the ‘standard of care’ and has served as the basis for subsequent trials [31].

Intravenous (IV) administration of cyclophosphamide permits improved bladder protection through prehydration and use of MESNA. Following three small randomized trials, the EUVAS group found a novel pulsed IV regimen (CYCLOPS, CYCLOphosphamide Oral versus PulSed) to have an identical remission rate to daily oral cyclophosphamide, with a 50% reduction in cyclophosphamide exposure and fewer adverse events [7, 32]. As with the CYCAZAREM trial, the higher-dosed cyclophosphamide regimen was associated with a lower relapse risk after 5 years [33]. Whether the lower-dosed cyclophosphamide regimens will reduce malignancy risk is uncertain, although a trend towards an increased risk of bladder cancer was seen in a pooled analysis of these cohorts at 5 years with a relative risk of 2.4 and an average cyclophosphamide exposure of 15 g, implying a dose-dependent effect on risk is likely [34].

B-cell depletion with rituximab is an alternative to cyclophosphamide for remission induction for GPA and MPA but was evaluated in combination with two IV cyclophosphamide doses in the RITUXVAS trial of severe renal disease [9]. This regimen had no benefit over a standard IV cyclophosphamide regimen in terms of efficacy or adverse event rate but demonstrated cyclophosphamide sparing. Whether low-dose cyclophosphamide has a role in induction therapies alongside rituximab is uncertain [35].

Two EUVAS trials evaluated whether cyclophosphamide could be replaced by methotrexate or MMF. The NORAM trial (Non-Renal Alternative treatment with Methotrexate) found that remission rates for early systemic, or non-severe, GPA/MPA were similar at 6 months between an oral methotrexate and an oral cyclophosphamide regimen, but that late relapse was more common after methotrexate [3]. Long-term follow-up found higher glucocorticoid and cyclophosphamide exposure in the methotrexate induction group indicating poorly controlled disease [36]. MMF also proved not inferior to an IV cyclophosphamide regimen at 6 months in the MYCYC trial with a higher relapse rate in the mycophenolate group. However, it was notable that the excess relapses associated with mycophenolate induction were confined to the PR3-ANCA-positive subgroup, and no differences in remission or relapse were seen in the MPO-ANCA subgroup.

INTRODUCTION OF NOVEL THERAPIES INTO VASCULITIS THERAPY

The demand for newer therapies in 2015 is similar to that of 20 years ago, to reduce the need for toxic agents and to secure a more rapid and prolonged disease remission. High-dose pooled intravenous immunoglobulin (IVIg) and the antibiotic combination sulfamethoxazole/trimethoprim were ‘add-on’ therapies considered in the ECSYSVASTRIAL IVISTAT project. A rationale for IVIg developed from the detection of anti-idiotypic antibodies to ANCA, its success in the paediatric vasculitis, Kawasaki’s disease and other potential modes of action relevant to vasculitis [37]. Infection of the upper respiratory tract with Staphylococcus aureus has been linked with relapse in GPA. In the event, IVISTAT did not proceed, and separate randomized studies supported an efficacy for IVIg in refractory vasculitis, and sulfamethoxazole/trimethoprim was shown to reduce relapse rates in GPA [38, 39].

Lymphocyte depletion with alemtuzumab (anti-CD52) and anti-thymocyte globulin (ATG) was employed in compassionate use settings with reports of some useful sustained remissions [40]. The EXSYSVASTRIAL WARCY study aimed to compare these approaches in patients with refractory ANCA vasculitis but did not proceed, although a smaller, open-label study of ATG, SOLUTION, demonstrated the efficacy and toxicity of ATG in 15 patients [6]. This reported a failure to induce prolonged remission, the goal of the study, and poor tolerance of ATG. After a review of the compassionate use experience of alemtuzumab, a small, dose-ranging trial has been launched, ALEVIA (NCT01405807) [41].

The oral immunosuppressive MMF was shown to be more effective than azathioprine in the prevention of renal transplant rejection and more recently, in the prevention of flare in lupus nephritis. After several smaller studies, the EUVAS group found MMF not to be superior for relapse prevention of AAV after cyclophosphamide induction in the IMPROVE trial [8]. In fact, relapses were more frequent in the MMF treatment group, an observation that was a surprise and has raised concerns as to whether MMF dosing or another aspect of the trial design was responsible. As a result, MMF has not been recommended as a routine remission maintenance agent but can be used when azathioprine or methotrexate have failed. A related EUVAS trial examined whether MMF could replace cyclophosphamide as an induction agent in ANCA vasculitis in the MYCYC trial (NCT00414128). MYCYC aimed to demonstrate non-inferiority of MMF, and this was shown at 6 months. Beyond this point, all patients received azathioprine, and more relapses were seen in those who had received MMF, in support of observation from earlier trials that induction regimens with lower cyclophosphamide exposure had higher late relapse rates. The higher relapse rate in the MYCYC trial was confined to the PR3-ANCA subgroup, which raises the question as to whether MMF induction could still be considered an option for the MPO-ANCA subgroup, as shown in smaller studies [42].

The European experience with rituximab is discussed by Kallenberg and Hauser and comprised an induction study, RITUXVAS, that compared a rituximab/low-dose cyclophosphamide
combination with a standard-dose cyclophosphamide regimen and found similar efficacy [9]. RITUXVAS focussed on patients with severe renal vasculitis for whom there is a paucity of data concerning rituximab, and a debate continues as to the efficacy of rituximab in this presentation and the value of combining rituximab with cyclophosphamide [35]. With the licensing of rituximab for remission induction of AAV in the USA in 2011 and Europe in 2013, attention has turned to its role in relapse prevention and repeat-dose rituximab was shown to be associated with fewer relapses than azathioprine, following cyclophosphamide induction in the French MAINRICTSAN trial [43]. A combined EUVAS–VCRC study is asking a similar question after rituximab induction for relapsing disease in the RITAZAREM trial (NCT01697267) [44]. Other biologic agents that have been considered in AAV therapy are tumour necrosis alpha inhibitors, but these were abandoned after a negative result in relapse prevention in an etanercept study [31]. The co-stimulation inhibitor CTLA4-Ig or abatacept has been examined in non-severe GPA by a small American open-label study that has led to the launch of a larger randomized trial ABROGATE (NCT02108860) that will be conducted by investigators from both the EUVAS and VCRC networks [45]. EUVAS investigators contributed to the design and conduct of an open-label study of deoxyspergualin that demonstrated efficacy in relapsing and refractory GPA [46].

THE IMPACT OF EUROPEAN COLLABORATIVE VASCULITIS RESEARCH

The discovery of ANCA inspired an international consensus to classify vasculitis and research into pathogenesis; the definition of ANCA-associated vasculitis as a discrete group of vasculitis subgroups then permitted clinical and genetic studies [11]. Although diagnostic and classification criteria remain elusive, the combination of clinical phenotype, ANCA and histology from tissue biopsies has been sufficiently robust for clinical trials. European and North American investigators are conducting a large-scale prospective phenotyping study, DCVAS, aimed to de

national and international treatment recommendation statements that are widely cited and did not exist before these trials were performed [22, 49, 50]. The strongest evidence for an impact of this clinical research activity is the improvement in survival for patients with ANCA vasculitis diagnosed since 2000 as opposed to the decades before [51]. This is in contrast to lupus nephritis where advances have been harder to detect. Both earlier diagnosis and better handling of immunosuppression may have contributed to improvements in outcome, and better survival of patients with respiratory failure points to improvements in supportive care [52].

The long-term toxicity of cyclophosphamide was one reason for the search for different agents, but in the process, much has been learnt about managing cyclophosphamide, and head-to-head trials have found no difference in shorter-term toxicity between cyclophosphamide-based regimens and those containing methotrexate, MMF or rituximab [3, 25]. Yet high rates of severe adverse events are seen with current protocols, and attention is turning to reducing glucocorticoid exposure as the major modifiable factor contributing to toxicity. In a secondary hypothesis, the PEXIVAS trial is comparing a reduced oral glucocorticoid regimen with a standard-dose regimen [10].

The development of clinical trial methodology relied also on tools for disease assessment and reliable endpoints for clinical trials [2, 17]. These tools have also become key elements of clinical epidemiological studies, defining outcomes and becoming prognostic elements themselves. This methodology and trial experience has facilitated the pharmaceutical industry investment in vasculitis with studies in ANCA vasculitis of deoxyspergualin, rituximab, mepolizumab and complement blockade and in giant cell arteritis of tocilizumab. The licensing of rituximab in 2011 for ANCA vasculitis relied on investigator-initiated trials and represents both the first drug licensed for this indication and a pathway for other drug development.

A yardstick of international interest in vasculitis inspired by ANCA has been the international ANCA workshops. A sequence began in Copenhagen in 1988 and is continuing with the 18th ANCA workshop in London in 2015 with 350 abstracts and many hundreds of delegates [53]. The data from the clinical trials have been combined in a common database that has directly permitted a portfolio of parallel clinical studies and defined clearly the obstacles to optimal patient outcomes. Of most importance is diagnostic delay with irreversible damage at the time of diagnosis, next is the toxicity and limited efficacy of therapies, especially the difficulty in achieving sustained, ‘off-drug’, remission. Longer-term outcomes are dominated by depressed quality of life that has been quantified but is not fully understood and by increased cardiovascular and malignancy risks and reduced survival. These areas are the current challenges and research agenda for investigators in the future.

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Twenty-five years of vasculitis research
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