Development and standardization of solid-phase assays for the detection of anti-neutrophil cytoplasmic antibodies (ANCA) for clinical application: report of a large clinical evaluation study

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In previous phases of this project, proteinase 3 (PR3) and myeloperoxidase (MPO), the main antigenic target molecules of anti-neutrophil cytoplasmic antibodies, were isolated and applied in standardized ELISAs.

In this study, standardized ELISAs with three PR3 preparations (from Copenhagen (CO), Raisdorf (RS) and Leiden (LF)) and one MPO preparation (from Copenhagen), were evaluated in a large retro-and prospective clinical study.

New patients (n=174) with primary systemic vasculitis (Wegener's granulomatosis, microscopic polyangiitis and idiopathic rapidly progressive glomerulonephritis, classical PAN and Churg-Strauss Syndrome) were included. Retrospectively, another 190 patients were evaluated. Furthermore control sera were obtained from patients with other forms of vasculitis, glomerulonephritis or granulomatous diseases (disease controls, n = 184) and healthy donors (healthy controls, n = 728). All patients were categorized by a system based on clinical and histological data. Patients were followed up for at least 1 year after diagnosis in order to evaluate a possible correlation between ANCA levels and disease activity.

The sensitivity of the anti-PR3 assays for histologically proven WG was between 59% and 69% in new patients, with a sensitivity of 22% for the anti-MPO assay. Similar figures were found for patients with clinically suspected WG. This was comparable with the results of the IIF test. In MPA and IRPGN a larger percentage of patients had anti-MPO antibodies than in WG. Only a few patients with PAN and CSS were investigated, and most of these were negative in the ELISAs.

The specificity of the assays for disease controls was 89–91% for the anti-PR3 assays and 95% for the anti-MPO assay. In the healthy controls the specificity was 98–99%. The specificity of the IIF test was 97% for a cANCA pattern and 81% for a pANCA pattern in disease controls. The combination of cANCA with anti-PR3 and pANCA with anti-MPO both had a specificity of 99%.

Further details will be presented during the meeting, in addition to the results of a follow-up study with correlation of disease activity and ANCA level. From this study we can conclude that ELISAs using purified PR3 or MPO are not more sensitive than the IIF test. However, the anti-MPO assay is more specific for systemic vascultis as compared to disease controls with related diseases. Furthermore, the combination of the IIF test with antigen-specific ELISAs is very specific for the diagnosis Wegener's granulomatosis, microscopic polyanglitis and idiopathic rapidly progressive glomerulonephritis.

European therapeutic trials in ANCA-associated systemic vasculitis: disease scoring, consensus regimens and proposed clinical trials

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Introduction

The systemic vasculitides are a significant health issue in the European Union (EU) with an annual incidence of approximately 40 per million and estimated direct health costs to the EU of 500 million ECU per year [1]. The relative rarity and heterogeneous nature of systemic vasculitis have impeded controlled clinical trials and dictate a mulidisciplinary and multicentre approach to their clinical investigation, which has been assisted by recent advances in classification and diagnosis [2,3]. In response to these problems, the ECSYSVASTRIAL group has been assembled, under European Community funding (BIOMED 1 concerted action programme). This group has grown from a previous European initiative concerned with the standardisation of ANCA assays which has successfully collected prospective clinical data and performed histological and serological analysis at a multicentre, international level [3]. The aims of the ECSYSVASTRIAL study group are the design and standardisation of disease scoring, data collection tools and databases, the design of clinical trials and the creation of national and European networks to facilitate clinical trials and thus harmonise treatment and improve outcome in ANCA-associated systemic vasculitis (Wegener's granulomatosis, microscopic polyangiitis and renal-limited vasculitis).

Strategy

Current treatment regimens, according to a round table discussion, showed marked differences between European centres in the doses of steroids used, in the choice and duration of cytotoxic therapy, in relapse rates and in second line or salvage therapies (Table 1 and Fig. 1). This indicated the need for a first wave of trials aimed at harmonising treatment with current agents before newer therapeutic strategies could

Table 1. Current approaches to the treatment of ANCA-associated systemic vasculitis with "generalised" disease according to a round table discussion between 15 European centres. The relapse rates are estimates. (/, - alternative drug. /-, - alternative is no drug. TAP, - tapering dose. A - azathioprine, ATG - anti-thymocyte globulin, C - oral cyclophosphamide, C_{IV} - pulse IV cyclophosphamide, CB - chlorambucil, CYA - cyclosporin, IA - immunoadsorption, IVIg - intravenous immunoglobulin, MAb - monoclonal antibody therapy, MP - pulse IV methyl prednisolone, MTX - methotrexate, P - prednisolone, PE - plasma exchange, ST - sulfamethoxazole/trimethoprim.)

Centre	Initial therapy	Additional therapy for severe disease	Maintenance therapy 1st year	Maintenance therapy 2nd year	Influence of ANCA on therapy	Relapse rate over 2 years (%)	Alternative therapies
1	P C	MP	P C	P C	+/-	10	CYA/MTX
2	P C	more C	$P_{tap\backslash ads\ C}$	MTX	+	25	IVIg
3	P C	MP	P C	P C/-	+/-	27	-
4	C_{IV}	PE/IVIg	P A/C_{IV}	PA	-	40	MTX/CYA
5	P C STR	MP C_{IV}	P_{tap} C/C_{IV}	-	+	?	PE
6	P C	PE	P A	-	+	25	MAb/IVIg
7	P C	PE/MP	P C	P	+	25	CYA/CB
8	P C ST/-	MP	$P_{tap\backslash ads\ C}$	C_{tap}	++	20	PE
9	PC	PE	P A	P A	+	20	CB
10	MP C_{IV}	MP	P C	C/-	+	25	-
11	P C	PE/MP	P C	P C/-	+	25	PE/ATG
12	P C	PE/IA/MP	P C	PA	+	25	-
13	P C ST/-	MP/IVIg	P_{tap} C_{tap}	-	+	20	A/MTX
14	MP C/C_{IV}	PE	P C_{tap}/A	-	+	10	PE
15	P C_{IV}	MP	P A/C_{IV}	P A	+	25	-

be tested. After adopting the Chapel Hill classification of primary systemic vasculitis, clinical subgroups were determined according to disease extent and severity with the purpose of agreeing a consensus "standard" regimen for each subgroup and a "best alternative" regimen which could be compared to the standard therapy by randomised trial (Tables 2 and 3) [2]. The workplan was divided into three areas: disease assessment, induction and remission therapy trials and salvage therapies. The resulting clinical trials form the core activity of the group and support the refining of disease scoring tools and establishment of a European database, with additional protocols examining the role of laboratory variables.

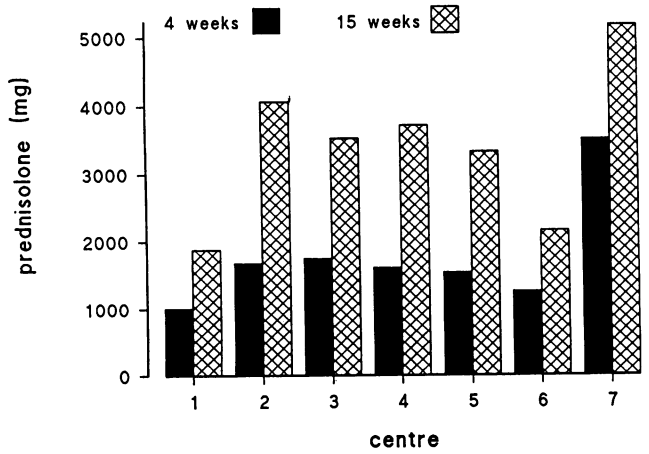

Fig. 1. Comparison of estimated 4- and 15-week cumulative steroid doses (prednisolone, mg) in the current regimens for ANCA-associated systemic vasculitis with "generalised" disease of seven European centres.

Disease assessment

A system of scoring tools was chosen to assess vasculitic activity, damage, patient function and adverse-effects. The activity score was derived from the Birmingham Vasculitis Activity Score (BVAS) which was extended to score both the presence of individual activity parameters and whether they were new or worse [4]. Although already validated at a single centre level, BVAS has required international validation which has indicated the need for training and experience with this tool in order to achieve comparable results [5]. The Vasculitis Damage Index (VDI) scores any damage resulting from vasculitis or other causes since the disease onset, with a similar list of variables to the BVAS score, and has also undergone validation. The BVAS and VDI will be scored at regular intervals for disease assessment in the clinical trials designed by the ECSYSVASTRIAL group. The nature of the data collection for BVAS and VDI allows weighting to be subsequently applied to the scores as well as the derivation of other activity indiced such as the Disease Extension Index [6]. Patient function will be scored using the Short Form 36 (SF-36) questionnaire from the Medical Outcomes Trust; an existing, validated, score available in several European languages [7]. Adverse drug reactions are recorded in a standardised form which assesses the severity and consequences of the reaction and is complimented by a glossary of definitions of the more frequent adverse effects.

The definitions of remission and relapse are primary end-points for the proposed trials and have been agreed with semi-objective criteria; they are assisted by, but not reliant on, the BVAS score. It is anticipated that, with further experience, objective criteria for remission and relapse may be derived from these indices.

A software package has been written (Vasculitis Integrated Total Assessment Log - VITAL) which combines the four scoring systems

Table 2. Clinical subgroupings of primary systemic vasculitis agreed by the ECSYSVASTRIAL group. (WG - Wegener's granulomatosis; MPA - microscopic polyartyeritis; RLV - renal-limited vasculitis; Cr - serum creatinine in umol/l; OCS - oral corticosteroids; CYC - cyclophosphamide.)

Subgroup	Disease	Organ involvement	Constitutional symptoms	ANCA status	Trial
Predominantly small-vessel primary systemic vasculitis					
Localised	WG	upper and/or lower respiratory tract	no	+/-	MAYO
Early systemic	WG, MPA	any, except renal or imminent vital organ failure	yes	+/-	NORAM
Generalised (or renal-limited)	WG,MPA,RLV	renal with Cr < 500 and/or imminent vital organ failure	yes	+	CYCAZAREM
Severe renal	WG,MPA,RLV	renal with Cr > 500	yes	+	MEPEX
Salvage	WG,MPA	progressive disease after OCS + CYC	yes	+/-	WARCRY
Predominantly medium-sized vessel systemic vasculitis					
Polyarteritis nodosa and Churg-Strauss angitis		good and poor prognostic groups	yes	+/-	CHUSPAN

with other trial information and facilitates data collection removing the need for paper records. VITAL will allow local, national and European vasculitis databases to be created and the transmission of trial data by disk or network.

Emergence of 'standard' treatment regimens

In the process of designing the clinical trials "standard" regimens were agreed reflecting a consensus view of the group on the best current practice. Steroids and cyclophosphamide were accepted as the standard induction and remission treatment for 'early systemic' disease and as essential induction treatment if renal vasculitis or imminent failure of another vital organ was present, i.e. 'generalised' or 'severe renal disease'. Major areas of discussion in these three subgroups were the steroid dosage, the use of intravenous pulse versus daily oral cyclophosphamide, the use of alternatives to cyclophosphamide, the choice of additional treatment in severe renal disease and the total duration of treatment.

Despite the wide variation in current steroid dosage, common regimens using intermediate doses were agreed (Fig. 2). Several studies have addressed the use of intravenous cyclophosphamide with varying conclusions; it appears equally effective for induction treatment in renal vasculitis and some patients with Wegener's granulomatosis with a lower cumulative dosage and reduced adverse-effect rates; however, it may be less effective than daily oral cyclophosphamide for patients with more extensive disease and for the maintenance of remission [8-10]. Oral cyclophosphamide was accepted as the current standard in the knowledge that further comparative trials were nearing completion, but the group intends to test intravenous pulse cyclophosphamide as a second wave study when the subgroups most likely to benefit might be identified more clearly.

Table 3. Protocol overview for the randomised clinical trials designed by the ECSYSVASTRIAL group, and the MAYO and CHUSPAN trials. (OCS - oral corticosteroids; CYC - oral cyclophosphamide; IVCYC - intravenous pulse cyclophosphamide; MTX - methotrexate; AZA - azathioprine; IVMeP - intravenous bolus methyl prednisolone; PE - plasma exchange.)

Trial name	Clinical subgrouping	Standard limb	Alternative limb	End-points
ECSYSVASTRIAL treatment protocols				
NORAM	early systemic	OCS + CYCx 12 months	OCS + MTXx 12 months	Remission induction Relapse rate Adverse effects
CYCAZAREM	generalised or renal-limited	OCS + CYC x 12 months	OCS + AZA for remission phase	Relapse rate Adverse effects
MEPEX	severe renal	IVMeP + OCS + CYC	PE + OCS + CYC	Renal survival Adverse effects
WARCRY	salvage	not applicable	CAMPATH-1H + 9H vs. ATG	Remission induction Adverse effects
ECSYSVASTRIAL participation in existing studies				
MAYO	localised WG	OCS x 12 months	sulfamethoxazole/trimethoprim x 12 months	Treatment success/ failure
CHUSPAN	PAN + CSA	Good prognosis: OCS, IVCYC x 6 for relapse Poor prognosis: OCS + IVCYC x 6	OCS, AZA for relapse OCS + IVCYC x 12	Relapse/failure rate Adverse effects

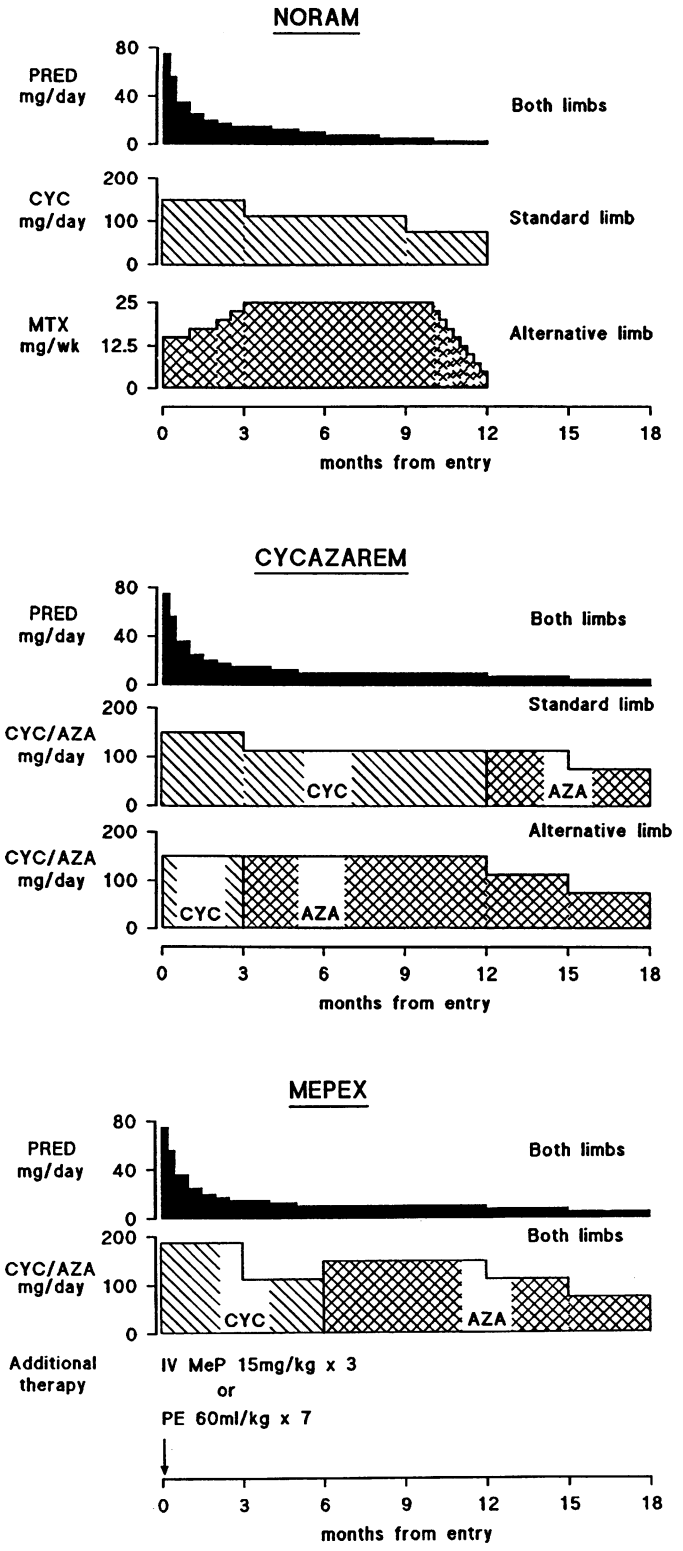

Fig. 2. Proposed standard and alternative regimens for "early systemic" disease (NORAM), 'generalised' disease (CYCAZAREM) and severe renal vasculitis (MEPEX). (PRED - oral prednisolone; CYC - oral cyclophosphamide; MTX - oral, weekly, methotrexate; AZA - oral azathioprine; IV MeP - pulse intravenous methyl prednisolone; PE - plasma exchange).

Induction and remission trials

A series of three core protocols were developed for new patients with ANCA-associated, systemic vasculitis and 'early systemic', 'generalised' or 'severe renal' disease (Table 3). Furthermore, patients could be entered in two existing studies for 'localised' Wegener's granulomatosis, and Churg Strauss angiitis and ployarteritis nodosa (Table 3). They share overlapping inclusion and exclusion criteria and treatment limbs and form a continnuum covering the entire spectrum of new presentations of these disorders.

MAYO — Participation in an existing trial of Wegener's granulomatosis limited to the upper and/or lower respiratory tract comparing steroids alone to sulfamethoxazole/trimethoprim for twelve months (co-ordinator, Dr Ulrich Specks, Mayo Clinic, USA) [11].

NORAM — Comparison of oral methotrexate and oral steroids to standard therapy with oral cyclophosphamide and oral steroids for the 'early systemic' subgroup, (i.e. patients with Wegener's granulomatosis or microscopic polyangiitis with constitutional symptoms but without renal involvement or other imminent vital organ failure) in both the induction and remission phases (Fig. 2) [12, 13].

CYCAZAREM — Standard therapy of oral cyclophosphamide and oral steroids for induction therapy for all patients with mild to moderate renal vasculitis (serum creatinine < 500 μmol/l) and those with other imminent vital organ failure in the context of Wegener's granulomatosis or microscopic polyangiitis, then comparison of oral azathioprine to continued oral cyclophosphamide for the maintenance of remission (Figure 2) [14].

MEPEX — Comparison of pulse intravenous methyl prednisolone to plasma exchange as additional therapy to oral cyclophosphamide and oral steroids for patients presenting with renal vasculitis and severe renal impairment (serum creatinine > 500 μmol/l) or requiring dialysis (Figure 2) [15,16].

CHUSPAN — Participation in an existing study of Churg Strauss Angiitis and classical polyarteritis nodosa comparing immunosuppressive regimens for good and poor prognostic groups (co-ordinator, Dr Loic Guillevin, Bobigny, France) [17].

The three core trials (NORAM, CYCAZAREM and MEPEX) will also contribute to the additional protocols:

RELANCA — Evaluation of the association between sequential ANCA levels and relapse [18]. This will require the regular collection of sera samples which will contribute to a European vasculitis sera bank.

SAVAS — Evaluation of the association between chronic nasal carriage of staphylococcus aureus and relapse [19].

Agreement could not be reached on a standard therapy beyond 18 months, due to the variety of possible outcomes by this time and the current remission regimens, as centres using less toxic drugs tend to treat for longer (Table 1). The maximum length of the protocols for NORAM, CYCAZAREM and MEPEX is therefore 18 months, but it is anticipated that long-term remission trials will have been designed before any patient reaches this point.

Salvage therapies

A minority of patients have disease that is difficult to control with standard agents, and of the multiple alternatives currently used for this indication (Table 1) recent interest has focused on anti-lymphocyte antibody therapies, as a result of the elucidation of the role of T- and B-cell autoimmunity in the pathogenesis of systemic vasculitis [21]. Two pilot studies, of monoclonal anti-T-cell therapy (MAb) and anti-thymocyte globulin (ATG), have demonstrated that disease remission can be induced in the majority of such patients without major adverse effects [22,23]. Both approaches induce marked lymphopaenia, and thus the risk of opportunistic infection; the MAbs, CAMPATH-1H and CAMPATH-9H, are "humanised" antibodies, and are capable of repeated administration because they do not induce an antiglobulin response. ATG, a heterologous antiserum, is an established preparation used for the treatment of renal allograft rejection but its repeated use is limited by serum sickness reactions.

WARCRY — In order to compare the efficacy and safety of these two approaches and to disseminate experience of their use in vasculitis, a randomised study has been designed for patients with ANCA associated Wegener's granulomatosis and microscopic polyarteritis with progressive disease and disabling organ damage resisitant to or intolerant of therapy with steroids and cyclophosphamide comparing CAMPATH-1H + CAMPATH-9H to ATG.

The future

The consensus recommendations for standard therapy will be updated in line with the results of the first wave trials. Under the existing three year programme a second wave of trials will be designed using refined clinical subgroupings, disease scoring tools and surrogate end-points derived from the first wave studies. Anticipated areas of interest will be studies of intravenous pulse cyclophosphamide, long-term remission therapies including sulfamthoxazole/trimethoprim, regimens for the elderly and introduction of newer agents such as intravenous immunoglobulin and mycophenolic mofetil [23]. Patient trial activity will contribute to a European vasculitis database which will provide a resource for physicians managing patients with vasculitis, for clinical research and for epidemiological and health economic studies. These factors have the common underlying ambition of achieving better care for patients with systemic vasculitis in Europe.

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