**Effect of disease activity at three and six months on long-term outcomes in**

**ANCA associated vasculitis.**

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**Abstract:**

**Introduction:**

The treatment of ANCA associated vasculitis (AAV) aims to suppress disease activity and prevent subsequent disease flare. We explored the association of early disease control with long-term outcomes to validate early disease control as end-points for future clinical trials.

**Methods:**

Data from four inception trials (CYCAZAREM, NORAM, MEPEX & CYCLOPS) and subsequent long-term registry data were studied. Clinical parameters at baseline, three and six months were assessed to study the risk of death and end stage renal failure (ESRF). At six months, outcomes were defined as: sustained remission (remission by three, sustained to six months), late remission (remission after three and by six months), relapsing disease (remission by three months but relapse by six months), or refractory disease (no remission by six months).

**Results:**

Of 354 patients followed for a median of 5.7 years, 46(13%) developed ESRF, 66(18.6%) died and 89(25.1%) suffered either death or ESRF. At six months, age (HR=1.02(1-1.05), p=0.012\*), eGFR (HR=0.94(0.92-0.95), p=0.001\*), disease status at six months: late remission (HR=2.94(1.1-7.85), p=0.031\*), relapsing disease (HR=8.21(2.73-24.65), p=0.001\*), refractory disease (HR=4.89(1.96-12.18), p=0.001\*), predicted the composite end-point of death or ESRF. Similar results were observed when these analyses were performed separately for death and ESRF.

**Conclusions:**

This study suggests that disease status at three and six months may predict the risk of long-term mortality and ESRF in AAV, and that these time points may be valid end-points for induction trials in AAV. These results need to be validated in a larger dataset.

**Introduction:**

Anti-neutrophil cytoplasm antibody (ANCA) associated vasculitis (AAV) is an autoimmune condition associated with necrotising inflammation of predominantly small blood vessels leading to organ damage and death if left untreated. ANCA autoantibodies with pathogenic potential are directed against neutrophil proteinase 3 (PR3) and myeloperoxidase (MPO). Granulomatosis with polyangiitis (GPA, previously Wegener’s) and microscopic polyangiitis (MPA), the two main subsets of AAV share clinical and pathological characteristics and are often studied together.

The European Vasculitis Society (EUVAS) conducted four clinical trials in AAV between 1995 and 2002 that have informed the current management of AAV patients1–4. A registry has been established to collate longer-term outcome data from patients participating in these studies. Mortality of AAV has declined to less than 20% at two years over the last 20 years. However mortality remains higher than an age and sex matched general population5. Severe renal involvement and higher disease activity at diagnosis are risk factors for death. Relapsing disease contributes to the accrual of damage, cumulative drug toxicity and end stage renal failure (ESRF) risk.

Clinical trials in AAV typically use remission or relapse as primary end-points. This study aimed to define the predictive value of disease control at three and six months in order to validate the use of early disease control as an end-point. This has the potential to improve the quality of clinical trials and shorten their duration.

**Patients and methods:**

The EUVAS trials (Table 1) aimed to establish evidence in the treatment of varying AAV disease severity subgroups as defined by a European League Against Rheumatism (EULAR) statement67. Patients with a new diagnosis of AAV according to the 1994 Chapel Hill Consensus criteria were included at diagnosis. Patients were excluded if they had a co-existent multisystem autoimmune disease, active infection, pregnancy, life threatening pulmonary haemorrhage or were <18 years or >80 years. Long-term data were obtained from a sub study, which collected data by use of questionnaires sent to the participating doctors.

Baseline demographic and clinical data were obtained from the trial databases. Three and six month disease activity and damage were quantified using the Birmingham Vasculitis Activity Score, version 2, (BVAS) and Vasculitis Damage Index (VDI), two validated complementary scoring tools documenting disease activity and damage respectively8,9.

BVAS comprises 65 items in nine domains related to different organ systems and items are scored as actively involved or not. This produces a weighted, summary score that reflects disease activity. VDI is designed to capture ‘all cause’ damage that occurs from the time of diagnosis. It incorporates 62 items in 10 domains and the score is the summation of the checked items. Remission in this study was defined as a BVAS of zero, reflecting absence of disease activity. BVAS of greater than 0 was considered to represent active disease.

**Statistical analyses:**

Missing data were estimated by probability imputation techniques 10. At six months patients were divided into four groups depending on BVAS at three and six months. The groups were: 1) Sustained remission: those that achieved remission at three months (BVAS=0) and sustained it to six months without relapse 2) Late remission: those that achieved remission after three and before six months 3) Relapsing disease: those that achieved remission by three months (BVAS=0) but relapsed by 6 months (BVAS>0) 4) Refractory disease: those that had not achieved remission by six months.

Continuous variables were expressed as medians with 25th and 75th quartiles within brackets. Categorical variables were presented as percentages and frequencies. Chi-square test was used for categorical variables. The following predetermined parameters were included in a Cox regression analyses: age at diagnosis in years, sex, disease subtype (GPA or MPA), eGFR (MDRD) in ml/min, disease activity (categorised into the above mentioned 4 groups), damage index (categorised into two groups VDI <= 2 or >2), ANCA specificity (PR3, MPO, negative), trial, year of enrolment; a minority of patients with double positive and missing ANCA data were included in the MPO sub-group as this disease phenotype was more consistent with MPA. Multiple regression analyses were performed separately for mortality, ESRF and for the composite end-point of mortality & ESRF. For ESRF analyses, the late remission group was not included in the disease activity sub-group as there were no events in this group. Entry eGFR was not included in the regression models due to multi-collinearity with eGFR at six months (Pearson correlation coefficient 0.799, p<0.001). All covariates in the multiple regression analyses were entered simultaneously. The proportional hazards assumption for Cox regression models was tested by the weighted scaled Schoenfeld residuals test and visual inspection of log-log plots. Hazard ratio of age (HR) was expressed in relation to change in age by one year, for eGFR, it was in relation to change in eGFR by 1ml/min. Data were analysed using SPSS version 23 and the R software (R foundation for Statistical Computing, Vienna, Austria) version 3.1.2. A two-tailed p value of ≤0.05 was considered significant.

**Results:**

**Patient characteristics:**

535 patients with a new diagnosis of AAV from the four trials were studied. Patient clinical characteristics at diagnosis and during follow-up are shown in Table 2. Of the 535 patients, disease status was available at both 3 months and 6 months in 354 patients. Complete data on all variables to perform regression analyses was available in 329 patients for mortality, 325 for ESRF and 329 patients for the composite of ESRF or death. It may sometimes be difficult to differentiate between ongoing disease activity and damage especially in relation to renal items. Whilst 65% of the patients at entry had at least one renal item scored, only 2.9% of the patients at 3 months and 3.5% at 6 months had at least one scored renal item. This suggests that the risk of misclassifying damage as disease activity was small.

At three and six months, 84.8% and 89.8% patients achieved remission respectively. At six months, of the 354 patients, 283 (79.9%) were in sustained remission, 35 (10%) had late remission, 18 (5.2%) had relapsing disease and 18 (5.2%) refractory disease.

**Patient survival:**

Over a median follow-up of 5.7 years, 66 out of 354 (18.6%) patients died. Forty eight (16.9%) of the 283 patients in the sustained remission group, 7 of 35 (20%) in the late remission group, 5 of 18 (27.8%) in the relapsed disease group, and 6 of 18 (33.3%) in the refractory disease group died. Of the 354 patients, complete data on all variables to perform regression analysis was available on 329 patients with 62 events.

Cox proportional hazards regression model using the above-mentioned pre-determined parameters at six months was performed to identify mortality predictors. Age (HR=1.09(1.05-1.13), p<0.001\*); eGFR at 6 months (HR=0.96(0.94-0.98), p=<0.001\*); disease status at six months: compared to sustained remission, late remission (HR=3.31(1.28-8.57), p=0.013\*), relapsing disease (HR=6.59(2.18-19.87), p=0.001\*) and refractory disease (HR=6.15(2.26-16.73), p<0.001\*) predicted mortality. The trial in which patients were enrolled was also predictive of mortality (Table 1) but it was interesting to note that patients in trials enrolling severe disease patients did better. This may be due to the fact that the models are adjusted for eGFR. This analysis shows that patients in the sustained remission group have improved survival (large effect size) compared to patients in the other groups. Disease sub-group, sex and entry BVAS were other significant predictors and the results are summarised in Table 3 and Cox regression curves are shown in Figure 1(a).

**End stage renal failure (ESRF):**

Of the 354 patients, 9 patients developed ESRF before 6 months and were not included in ESRF analyses. Of the 345 patients for whom disease status was available, 37 (10.4%) went on to develop late ESRF. 32 of the 276 patients (10.6%) in the sustained remission group, no patients in the late remission group, 2 of the 18 patients (11.1%) in the relapsed disease group, and 3 of the 18 (16.6%) in the refractory disease group developed late ESRF.

As there were no events in the late remission group (35 patients) , this group was not included in the regression analysis. Data on all variables for regression analysis was available in 290 patients with 35 events. In the Cox regression analysis, eGFR at six months (HR=0.9(0.87-0.93), p<0.001\*), and disease status at six months: relapsing disease (HR=34.22(4.72-247.8), p<0.001\*) and refractory disease (HR=9.64(2.25-41.29), p=0.002\*) predicted ESRF. Other significant variables include male sex and year of enrolment (see Table 3). Those in the sustained remission group had a lower probability of ESRF compared to the relapsing disease and refractory disease group. The results are summarised in Table 3 and Cox regression curves are shown in Figure 1(b).

In a sensitivity analysis we performed competing risk regression analysis as proposed by Fine and Gray11 for the estimation of the semi-parametric proportional hazards for ESRF with death as a competing event. In this analysis, eGFR at 6m (sub-HR=0.9(0.88-0.92), p<0.001\*), and disease status at six months: relapsing disease (sHR=HR=15.75(2.53-97.92), p=0.003\*) and refractory disease (sHR=HR=5.98(1.32-26.97), p=0.02\*) were co-variates that independently predicted ESRF (Table 3).

**Composite end-point:**

We further analysed the impact of these predictors on a composite end-point of death or ESRF whichever occurred earlier.

Of the 345 patients that had not developed ESRF by six months, 80 (23.2%) went on to develop the composite end-point. 62/276 patients (22.4%) in the sustained remission group, 6/33 (15.6%) patients in the late remission group, 5/18 patients (27.7%) in the relapsed disease group, and 7/18 (38.8%) in the refractory disease group developed the composite end-point. Data on all variables to perform regression analysis was available on 321 patients with 75 events.

Results from the Cox proportional hazards model showed that age (HR=1.02(1-1.05), p=0.012\*), eGFR (HR=0.94(0.92-0.95), p=0.001\*), disease status at six months: late remission (HR=2.94(1.1-7.85), p=0.031\*), relapsing disease (HR=8.21(2.73-24.65), p=0.001\*), refractory disease (HR=4.89(1.96-12.18), p=0.001\*), predicted the composite end-point (Table 3 and Figure 1(c)). Other predictors include disease subtype, male sex, trial enrolled in (Table 3).

**Discussion:**

Improvements in the diagnosis and classification of vasculitis following the discovery of ANCA, and the development of disease assessment tools have permitted large scale multicentre randomised clinical trials. However long term outcomes remain poor and the treatment itself contributes to adverse outcomes. Treatment aims to control disease activity and prevent disease relapse but there is no consensus as to the optimum end-point for use in induction clinical trials. We sought to determine the value of early disease remission in an association study of patients recruited to inception clinical trials of AAV for whom long-term outcome data were available. In addition to contributing to an understanding of the current clinical epidemiology of vasculitis the study aimed to validate disease remission at three and six months as clinical trials end-points. The principle observation is that disease remission at three months sustained to six months is the best predictor for a good outcome, as defined by death and ESRF.

Although there has been progressive improvement in outcomes of AAV over the last decades, the mortality risk remains elevated. A systematic review has shown that patients with MPA are at increased risk of death compared to GPA12, and also at increased risk of renal failure and cardiovascular events. In this study we showed that achieving remission at three months sustained to six months was more important than other baseline variables such as disease subtype or ANCA specificity. Advanced renal insufficiency and development of ESRF were shown to be risk factors for mortality in AAV patients12. However, even after correcting for renal function, disease status at six months remained an important predictor. It is evident from this analysis that those with any disease activity at or after 3 months will have poor prognosis, possibly due to a combination of accrual of disease related damage and drug toxicity.

In the Cox proportional hazards model, achieving remission reduced the risk of ESRF when compared to patients that do not achieve remission or develop early relapses. In this analysis patients that died before the development of ESRF were censored. In the sensitivity analysis this issue was addressed by competing risk regression modelling and the results were not dissimilar to the initial analysis, supporting the primary contention that disease status is an important predictor for ESRF.

Response to therapy varies widely within disease severity sub-groups and therefore looking at baseline characteristics does not have reliable prognostic value. On the other hand, using data from three and six months would give us an opportunity to assess the response to standard therapy, adding more weight to the prediction models. This study establishes the fact that three and six months data can be used as a surrogate for long-term mortality and ESRF. This emphasises the need for faster acting therapies: plasma exchange is currently being assessed in this context for severe AAV,13 while IVIg14and tumour necrosis factor blockade15 were evaluated for this purpose, and newer therapeutics, such as the complement inhibitor avacopan have shown, in a Phase II study a more rapid effect on disease activity than current standard of care 16.

The study is limited by the retrospective analyses of the data pooled from multiple clinical trials. There may be many confounders such as different immunosuppressive therapies, cumulative steroid dosage which are likely to influence sustained remission and consequently long term outcomes. EUVAS clinical trials were designed to study patients with varying disease severity and hence pooling them offers the chance to study the whole spectrum of patients with AAV. The steroid dosage has been reasonably consistent across CYCAZERAM, CYCLOPS & MEPEX, whilst in NORAM trial steroids were tapered by 12 months. However similar analyses were shown to be helpful in drawing important conclusions in patients with AAV17–19. Also, as highlighted, the disease severity subtypes and treatment options differed across the studies. It is worth noting that none of the patients were treated with rituximab, which may change remission and relapse rates. The mortality in AAV patients is lower with the current treatment regimens compared to historical cohorts, and this study needs to be validated on prospective clinical trial datasets, such as the PEXIVAS and MYCYC clinical trials20,21 and in prospective registry cohorts. A strength of this study lies in the fact that the threshold for disease activity was binary, BVAS above or equal to zero, which should help with comparisons of studies using different BVAS versions. Another strength of this study is that data comes from clinical trials where assessments were standardised and treatments were defined by the protocol.

**Conclusions:**

Objective parameters obtained early in the course of the disease course may predict long-term outcomes and early sustained remission may be an important goal of therapy. This study establishes early surrogate markers for long-term outcomes of value to future clinical trials shorter follow-up durations. However, these results should be viewed cautiously as the toxicity associated with therapy can influence the long term outcomes, the goal of achieving early remission by increased immunosuppression needs to be weighed carefully against the risk of drug toxicity.

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**Figure 1:** Cox proportional hazard curves based on disease status at 6 months depicting (a) the risk of mortality, (b) the risk of ESRF and (c) composite end-point of ESRF or death.