Disease-activity in ANCA-associated vasculitis

Sanders, Johannes Stephanus Franciscus

6

Patient outcome on maintenance therapy with azathioprine as compared to cyclophosphamide in ANCA-associated vasculitis: long-term single center experience

J.S.F. Sanders[1], P.M. Stassen[2], J.W. Cohen Tervaert[2], C.G.M. Kallenberg[3], C.A. Stegeman[1]

Department of Internal Medicine, Divisions of [1]Nephrology and [3]Rheumatology and Clinical Immunology, University Medical Center Groningen, The Netherlands. [2]Department of Internal Medicine, University Hospital Maastricht, The Netherlands

In preparation

ABSTRACT

Objective: We retrospectively evaluated long-term disease-free survival in patients with ANCA-associated vasculitis (AAV) on cyclophosphamide maintenance treatment as compared to azathioprine maintenance treatment.

Methods: Patients diagnosed with AAV between January 1990 and December 2007 were included (n=247). All patients received induction with cyclophosphamide. From 1990 to 1996 patients were treated with cyclophosphamide maintenance after induction of remission (n=113). From 1996 on patients were switched to azathioprine (n=34). Disease-free survival was analysed by logrank test.

Results: 176 patients were diagnosed Wegener's Granulomatosis, 52 Microscopic Polyangiitis, and 19 idiopathic Necrotizing Crescentic Glomerulonephritis. ANCA were directed against proteinase 3 (n=180) and myeloperoxidase (n=59), and their distribution did not differ significantly between the two groups. In the cyclophosphamide group mean follow up was 111 months and in the azathioprine group 68 months ($p<0.001$).

At 18 months and 60 months after diagnosis relapse-free survival was 85% and 54%, respectively, in the cyclophosphamide group, and 89% and 44%, respectively, in the azathioprine group (at five years, RR 1.22; 95% confidence interval 0.83-1.81; $p=0.32$). Leukopenia, incidence of infections, VDI-scores, and mortality did not differ significantly between the two cohorts.

Conclusion: This single center retrospective study shows that azathioprine maintenance is not inferior to cyclophosphamide maintenance during long-term follow-up in patients with AAV.

INTRODUCTION

Antineutrophil cytoplasmic auto-antibodies against myeloperoxidase (MPO-ANCA) and proteinase 3 (PR3-ANCA) are associated with primary vasculitides affecting small to medium sized blood vessels. Multiple organs can be involved, but the kidney and respiratory tract are predominantly affected. The introduction of cyclophosphamide has resulted in marked improvement of survival in these life-threatening diseases [1]. However, both short and long-term cyclophosphamide maintenance therapy is toxic. In the short term cyclophosphamide is associated with frequent infectious complications. In the long-term especially bladder and bone marrow toxicity and carcinogenesis are feared complications. Recently, potential improvement in outcome has been realized by a staged treatment approach with cyclophosphamide induction during a limited treatment period, followed by azathioprine or methotrexate maintenance therapy [2, 3]. The CYCAZAREM trial previously showed equivalence in relapse rate of cyclophosphamide maintenance versus maintenance with azathioprine after induction of remission. However, this study was limited by a follow-up of 18 months only, and failed to show differences in short-term therapy associated toxicity. Thus, whether these regimens differ during long-term follow-up has not been studied. Meanwhile, most relapses in ANCA-associated vasculitis occur after tapering and discontinuation of immunosuppressive therapy. During five year follow-up approximately 50% of patients experience a relapse [4-6]. We previously reported that azathioprine maintenance compared to cyclophospamide maintenance therapy might be associated with an increased relapse risk in ANCA-associated vasculitis [7]. Additionally, in ANCA-associated vasculitis long-term data of outcome and toxicity of prolonged cyclophosphamide therapy compared with azathioprine maintenance is absent [4, 8, 9].

In this single center study we compared the outcome of cyclophosphamide and azathioprine maintenance regimens in two historic cohorts during extended follow-up. We studied potential differences of the regimens regarding prevention of disease activity and compared damage inflicted by treatment modality.

PATIENTS AND METHODS

Patients

All two hundred forty-seven consecutive patients diagnosed with ANCA-associated vasculitis and treated with induction therapy with cyclophosphamide and corticosteroids at our center between January 1990 and December 2007 were included in this retrospective study. Data of the patients are given in table 1. Patients were followed until death (n=73), loss to follow-up (n=17) or December 31st 2008 (n=157), whichever came first. Patients were classified as Wegener's Granulomatosis (WG), Microscopic Polyangiitis (MPA) and Necrotizing Crescentic Glomerulonephritis (NCGN) according to Chapel-Hill criteria [10]. In all patients a positive ANCA in indirect immunofluorescence (IIF) was confirmed by antigen specific enzyme-linked immunosorbent assay (ELISA). Induction treatment consisted of oral cyclophosphamide (2 mg/kg) and prednisolone (1 mg/kg; maximal dose of 60 mg/day). Doses of cyclophosphamide were adjusted to maintain the white blood cell count above 4 x 10^9/L. After 4 to 6 weeks, the daily prednisolone dose was tapered by 10 mg every 2 weeks until the dose reached 30 mg, and thereafter by 5 mg every 2 to 4 weeks. In patients diagnosed before 2000 reductions of daily prednisolone from 10 mg to discontinuation were often made over an extended time interval.

During the period 1990-1996 once remission was achieved the daily dose of oral cyclophosphamide was tapered by 25 mg every 3 months. Patients treated solely with cyclophosphamide for maintenance were included in the cyclophosphamide cohort (CYC, n=113). From 1997 on, patients were switched to azathioprine (1.5-2 mg/kg body weight daily) after three months of stable remission following induction treatment with cyclophosphamide (AZA, n=134). From one year after diagnosis azathioprine was tapered by 25 mg every 3 months. Patients who died within six months after diagnosis (n=22) were included in the cyclophosphamide group when diagnosed before 1997 (n=6), and in the azathioprine group when diagnosed after 1997 (n=16). All patients received *Pneumocystis jirovecii* prophylaxis with co-trimoxazole (three times per week 800/160 mg) during therapy with cyclophosphamide and/or azathioprine.

At diagnosis, during follow-up and at relapse, disease activity was scored using the Birmingham Vasculitis Activity Score (BVAS) [11]. At 6, 12 months after diagnosis and subsequently at 2, 3 and 5 years after diagnosis damage was assessed by the Vasculitis Damage Index (VDI) [12]. VDI-scores were subdivided according to critical damage (16 items) and treatment related damage (9 items). Additionally, VDI was scored the year before and after the occurrence of a relapse.

Table 1. Patient Characteristics at baseline

Characteristic	Cyc	Aza	p (cyc vs aza)
Patients, n	113	134	
Men, n (%)	67 (59%)	76 (57%)	0.68
Mean age at diagnosis (SD), years	59 (13)	55 (18)	0.07
Mean follow-up (SD), months	111 (58)	68 (49)	< 0.001
ANCA-status, n (%)			
PR3 ANCA	84 (74%)	96 (72%)	0.64
MPO ANCA	24 (21%)	35 (26%)	0.37
HNE ANCA	2 (2%)	2 (2%)	0.86
None	6 (5%)	3 (2%)	0.20
Diagnosis, n (%)			
WG	84 (74%)	92 (69%)	0.33
MPA	21 (19%)	31 (23%)	0.38
NCGN	8 (7%)	11 (8%)	0.74
Organ involvement, n (%)			
Ear, nose, throat	90 (80%)	82 (61%)	0.002
Pulmonary	54 (48%)	72 (54%)	0.35
Renal	85 (75%)	108 (81%)	0.31
BVAS at diagnosis, mean (SD)	20 (8)	22 (8)	0.24
CRP, mean (SD), mg/l	86 (79)	112 (98)	0.04
Creatinine, mean (SD), µmol/l	296 (301)	239 (263)	0.13
Hemodialysis, n (%)	17 (15%)	15 (11%)	0.37

Cyc, cyclophosphamide maintenance; Aza, azathioprine maintenance

Definitions

Remission was defined as the absence of clinical signs and symptoms of active vasculitis (Birmingham Vasculitis Activity Score (BVAS)=0) in combination with a normal C-reactive protein level (<10 mg/l). A relapse was defined as clinical signs of vasculitic activity in combination with biopsy proven vasculitic disease activity, or the occurrence of nodular pulmonary lesions after exclusion of infectious or malignant diseases. Renal vasculitic disease was defined as biopsy proven necrotizing glomerulonephritis or a combination of microscopic glomerular erythrocyturia, erythrocyte cell casts, proteinuria, and a decrease in creatinine clearance [13]. The time point of relapse was the first day of renewed or intensified therapy for disease activity.

Statistical analysis

Groups were compared using the *t* test or the chi-square test. For comparison of non-continuous data the Mann Whitney U test was used. For paired data the Wilcoxon signed rank test was used. Relapse free survival was assessed by actuarial survival curves calculated using Kaplan-Meier estimates for survival distribution. Differences between groups in survival were analysed with log-rank test. Patients lost to follow-up or who died before December 31st 2008 without relapse were censored. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Patient and treatment characteristics

In table 1 clinical characteristics of patients in the cyclophosphamide (n=113) and azathioprine cohort (n=134) at the time of diagnosis are presented. Patients in the cyclophosphamide cohort tended to be older than patients in the azathioprine cohort (59 versus 55 years, p=0.07). No differences in diagnosis or ANCA status were present. In both the cyclophosphamide and azathioprine group one patient was simultaneously PR3-, MPO-, and HNE-ANCA positive. In the cyclophosphamide group one additional patient was simultaneously MPO- and HNE-ANCA positive. In the cyclophosphamide cohort significantly more patients presented with ENT involvement (p=0.002) while there was no difference in ANCA specificity or classification as WG, MPA or NCGN. Mean follow-up was more than 9 years in the cyclophosphamide group and more than 5.5 years in the azathioprine group.

As expected patients in the cyclophosphamide cohort were treated significantly longer with cyclophosphamide than patients in the azathioprine cohort (22.6 versus 5.4 months, p<0.001). However, in addition to cyclophosphamide, patients in this cohort received prednisolone during an extended period compared to patients switched to azathioprine (22.2 versus 13.3 months, p<0.001). A major proportion of patients in the cyclophosphamide group received low dose steroids during long-term follow-up. As shown in table 2 mean dosages of prednisolone at 3 and 12 months did not differ significantly. However, at 6 months after diagnosis mean prednisolone dosage in the cyclophosphamide group was significantly higher than in the azathioprine group (p<0.001). In contrast, in the azathioprine group at diagnosis more patients were treated by high-dose methylprednisolone (p=0.014), and plasma exchange (p=0.005) than in the cyclophosphamide group.

Table 2. Treatment characteristics

Treatment	Cyc	Aza	p (cyc vs aza)
Plasmaferesis, n (%)	11 (10%)	31 (23%)	0.005
Methylprednisolone, n (%)	20 (18%)	42 (31%)	0.014
Co-trimoxazole therapeutic, n (%)	13 (12%)	12 (9%)	0.51
Duration of cyclophosphamide (SD), months	21.4 (19.8)	5.2 (3.1)	< 0.001
Duration of cyclophosphamide, median (25%-75%), months	17.4 (11.4-24.4)	4.6 (3.4-6.0)	
Duration of prednisolone (SD), months	21.2 (19.9)	11.9 (9.1)	< 0.001
Duration of prednisolone, median (25%-75%), months	12.1 (9.7-25.3)	9.1 (5.9-13.9)	
Prednisolone dosage at, mean (SD), mg			
3 months	26.8 (13.5)	25.6 (12.5)	0.54
6 months	11.9 (6.3)	7.9 (5.6)	< 0.001
12 months	5.0 (6.9)	3.6 (9.6)	0.27
Duration of azathioprine (SD), months		20.1 (14.8)	
Duration of azathioprine, median (25-75%), months		17.5 (10.6-21.2)	

Cyc, cyclophosphamide maintenance; Aza, azathioprine maintenance
Co-trimoxazole therapeutic stands for a dosage of 960 mg twice daily

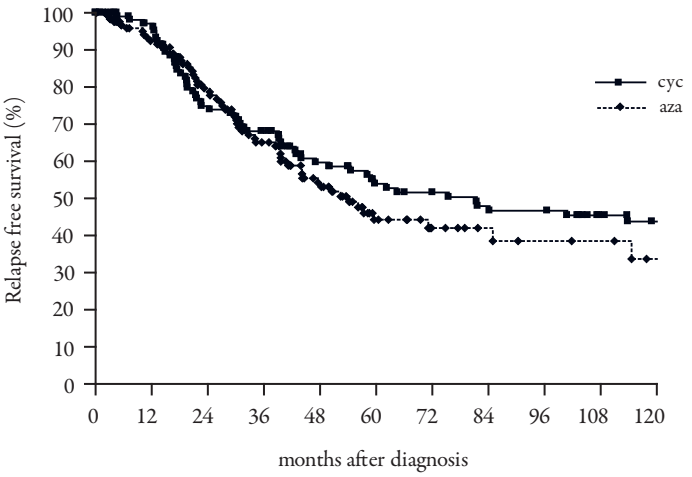
Table 3 shows clinical characteristics of the first relapse patients experienced. Again more patients in the cyclophosphamide group had ENT involvement (p=0.023). Also creatinine levels at the time of relapse were significantly higher in the cyclophosphamide group compared to the azathioprine group (p = 0.002).

Table 3. Patient Characteristics at first relapse

	Cyc	Aza	p (cyc vs aza)
1st relapse	51 (45%)	59 (44%)	
Organ involvement, n (%)			
Ear, nose, throat	36 (71%)	29 (49%)	0.023
Pulmonary	9 (18%)	10 (17%)	0.92
Renal	29 (57%)	25 (42%)	0.13
BVAS, mean (SD)	12 (6)	11 (5)	
CRP, mean (SD), mg/l	57 (57)	51 (56)	0.84
Creatinine, mean (SD), µmol/l	228 (251)	151 (122)	0.002

Relapse rate

Relapse rate of patients on azathioprine maintenance was not significantly higher as compared to patients on cyclophosphamide maintenance (RR 1.22; 95% confidence interval 0.83-1.81; p=0.32) (figure 1). Relapse free survival in the cyclophosphamide group was 85% at 18 months, and 54% at 60 months. Relapse free survival in the azathioprine group was 89% at 18 months, and 44% at 60 months.



Time	0	24	48	60	72	96	120
Cyc (N)	113	78	56	47	44	39	27
Aza (N)	134	85	47	26	17	11	7

Figure 1. Relapse free survival of cyclophosphamide and azathioprine cohort

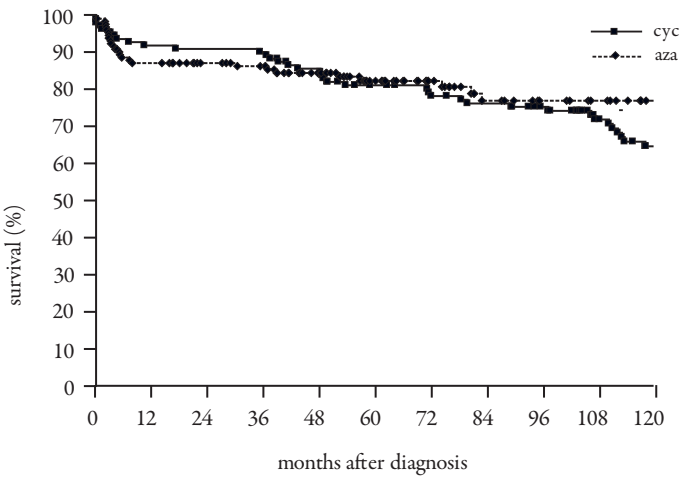
PR3-ANCA positive patients experienced significantly more relapses than MPO-ANCA positive patients (RR 2.90; 95% CI 1.93-4.36; p<0.0001). Also, patients classified as WG who were PR3-ANCA positive (n=161) experienced significantly more relapses at five years than MPO-ANCA positive or ANCA negative patients diagnosed WG (n=15) (RR 2.80; 95% CI 1.44-5.40; p=0.002). Finally, patients diagnosed MPA who were PR3-ANCA positive (n=14) were at increased risk to experience a relapse in comparison to MPO-ANCA positive MPA patients (n=33) (RR 4.53; 95% CI 1.57-27.94; p=0.01).

As for the whole group, the relapse rate was not significantly different in PR3-ANCA positive patients (RR 1.16; 95% CI 0.78-1.73; p=0.46), or MPO-ANCA positive patients

(RR 1.83; 95% CI 0.47-7.11; p=0.39) between patients on cyclophosphamide as compared to azathioprine maintenance therapy.

Mortality

In total 73 (29.5%) patients died during follow-up, 47 patients in the cyclophosphamide and 26 in the azathioprine cohort. Actuarial survival was not different between the CYC and AZA group and 5 year mortality did not differ significantly (figure 2). Table 4 shows causes of death in the cyclophosphamide and azathioprine cohort. In both cohorts infectious diseases were a primary cause of death (10 CYC; 14 AZA). In the cyclophosphamide group two patients died of active vasculitis, in the azathioprine group none. Of 12 patients the cause of death was unknown (7 CYC, 5 AZA). In total 7 patients died of a malignancy (6 CYC, 1 AZA). As aforementioned, patients in the cyclophosphamide group tended to be older and were followed for a longer period of time in comparison to the azathioprine group.



Time	0	24	48	60	72	96	120
Cyc (N)	113	104	96	47	83	74	53
Aza (N)	134	105	86	26	53	36	24

Figure 2. Survival of cyclophosphamide and azathioprine cohort

VDI

VDI at 6, 12, 24, 36, and 60 months after diagnosis was compared between the cyclophosphamide and azathioprine group. After correction for multiple comparisons VDI-

scores did not differ between the cyclophosphamide group as compared to the azathioprine group. Both critical and treatment related damage items were absent in most patients, and did not differ between the cyclophosphamide group and the azathioprine group. Finally, in all patients there was a significant increase in VDI after first (n=110, p<0.0001), second (n=51, p<0.0001), and third relapse (n=19, p=0.03).

Table 4. Causes of death

n	Cyc	n	Aza
10	infectious disease	14	infectious disease
	pneumonia (5), mediastinitis, septicaemia (2),		pneumonia (8), septicaemia (4), diverticulitis,
	endocarditis and Aspergillus infection		meningo-encephalitis
6	malignancy	1	malignancy
	B-CLL, sarcoma, unknown primary,		unknown primary
	adenoid-, colonic, and pancreatic cancer		
3	cerebrovascular disease	2	pulmonary embolism
7	cardiac disease	2	cardiac disease
6	renal insufficiency	1	respiratory insufficiency following aspiration
2	pulmonary haemorrhage	1	tension pneumothorax after subclavian vein cannulation
2	cerebral vasculitis		
1	aortic dissection		
1	diffuse intravascular coagulation		
2	no specific cause		
7	unknown cause	5	unknown cause

Leukopenia and infections

In the first two years after diagnosis 26 patients in the cyclophosphamide cohort (23%) and 29 patients (22%) in the azathioprine group experienced at least one leukopenic episode (Leukocytes <4.0). Most of these episodes occurred within 6 months after diagnosis (71%, n=39). Twenty-four of these thirty-nine leukopenic episodes were associated with the occurrence of infections (12 CYC, 12 AZA). Leukopenia within 6 months after diagnosis was significantly associated with the occurrence of infection (RR 3.53; 95% CI 1.97-6.35; p<0.001).

Most infectious episodes occurred within 6 (100), and 24 months after diagnosis (130). Characteristics of infectious episodes in the first two years after diagnosis are depicted in table 5. In total 100 infections (49 CYC, 51 AZA) occurred within 6 months after diagnosis in 77 patietns (37 patients in CYC and 40 patients in the AZA (p=0.62)). From 6 months after diagnosis, when patients in the AZA group were switched to azathioprine, the number of patients experiencing an infectious episode did not differ between the cyclophosphamide and the azathioprine group (17 versus 11, p=0.14) (table 5).

Malignancies

Malignancies are depicted in table 6. At diagnosis 15 patients had a history of malignancy which was considered to be cured (8 CYC, 7 AZA). In the cyclophosphamide group one patient eventually died of recurrence of malignancy present at diagnosis of AAV (adenocarcinoma), the patient with T cell lymphoma died of a secondary radiation sarcoma. None of the patients in the azathioprine group died of their previously diagnosed malignancies, or had progressive disease.

Table 5. Infectious episodes

0-6 months	**CYC**	**Leukopenia admission**		**AZA**	**Leukopenia admission**		
Viral	24	10	16	15	4	8	
CMV	12	9	11	10	4	7	
varicella zoster	5	0	2	5	0	1	
Fungal	7	7	3	3	0	3	
Bacterial	12	3	10	27	6	18	
PCP	2	2	2	1	1	1	
Unknown	4	1	1	5	1	3	
Patients, n (%)	37 (33%)	12	18	40 (30%)	12	23	p=0.62
6-24 months	**CYC**	**Leukopenia admission**		**AZA**	**Leukopenia admission**		
Viral	7	1	4	1	0	0	
CMV	2	1	2	0			
varicella zoster	5	0	2	1	0	0	
Fungal	2	0	0	1	0	0	
bacterial	4	0	4	5	0	5	
PCP	1	0	1	0			
Unknown	5	0	2		1	3	
Patients, n (%)	17 (16%)	1	11	11 (9%)	1	6	p=0.14

Table 6. Malignancies

n	Cyc	n	Aza
before diagnosis			
2	Prostate cancer	2	Breast cancer
2	Lymphoma (T cell (died), NHL)	2	Urothelial cell carcinoma
1	Planocellular carcinoma lung	1	Colonic cancer
1	Adenocarcinoma (died)	1	Melanoma
1	Paraganglioma	1	Basocellular carcinoma
1	Meningeoma		
after diagnosis			
4	Basocellular carcinoma	2	Basocellular carcinoma (+ recurrence)
2	Prostate cancer	2	Planocellular carcinoma
2	Breast cancer	1	Urothelial cell carcinoma (+ recurrence)
1	Planocellular carcinoma	1	Liver metastases with unknown primary (died)
1	Sarcoma (died)		
1	Colonic cancer (died)		
1	Pancreatic cancer (died),		
1	Unknown primary with liver metastases (died)		
1	B cell lymphoma (died) and urothelial cell carcinoma		

In the CYC group 15 malignancies developed in 14 patients during 1046 person years of follow-up. One patient developed a B cell lymphoma, and subsequently a urothelial cell carcinoma. Five patients died of active cancer (B cell lymphoma, colonic cancer, pancreatic cancer, sarcoma, unknow primary). In the azathioprine group, during 762 person years follow-up, 6 patients developed a malignancy. One of these patients died of active cancer (unknown primary). At 5 years after diagnosis the incidence of malignancy did not differ significantly between the cyclophosphamide and azathioprine group (RR 1.34; 95% CI 0.30-5.91; p=0.69)

DISCUSSION

Our retrospective single center study shows azathioprine maintenance is not inferior to cyclophosphamide maintenance in sustaining remission of AAV during long-term follow-up. In 2003 we found a trend towards an increased relapse rate during five years follow-up in the azathioprine group (88 CYC vs 48 AZA; RR 1.51; 95% CI, 0.92 to 2.68; p=0.099) [7]. Now, with extended follow-up and more patients in both cohorts no difference appeared between the two treatment strategies. Relapse rates in the cyclophosphamide group at 18, 36 and 60 months were 85%, 68%, and 54%, and in the azathioprine group 89%, 65%, and 44%, respectively. So far, in two randomized controlled trials azathioprine maintenance has been compared with cyclophosphamide (CYCAZAREM) or MTX (WEGENT), respectively. In the CYCAZAREM study, at 18 months relapse-free survival was 86.3% in the cyclophosphamide group and 84.5% in the azathioprine group, respectively [2]. In the WEGENT study, which compared adverse events of azathioprine and MTX, after 18 months relapse-free survival was 88.9%, and after 36 months 64.1% in the azathioprine group [14]. Thus the results of our study are comparable with the randomized controlled trials that have addressed maintenance therapy up to 18 and 36 months after diagnosis. Additionally, our retrospective study is unique in the number of patients included, the length of follow-up, the low number of patients lost to follow-up and the small number of physicians taking care of the patients over the years.

Obviously, a randomized controlled trial is superior to a cohort study. A number of limitations should be addressed when considering the results of the current study. First, possible identified confounders in this study include age, affected organ systems at diagnosis, additional treatment at diagnosis, and length of steroid treatment. Patients in the cyclophosphamide group tended to be older, more frequently had ENT involvement, and received steroids for a longer period during follow-up. We can not explain the age difference between the two groups, however this difference did not reach statistical significance. Secondly, more patients in the cyclophosphamide group had ENT involvement. This difference was not related to a difference in ANCA-status or diagnosis. Neither did disease severity as assessed by BVAS differ at diagnosis. In a previous study form the USA ENT involvement was associated with increased relapse risk, in contrast to a large French cohort of patients with ANCA-associated vasculitis in which it was not [3, 15]. Most relevant when comparing relapse rate in the two cohorts are additional differences in treatment regimens. Patients in the azathioprine group were more often treated with plasma exchange and methylprednisolone at diagnosis. Some patients in the azathioprine cohort were included

in the MEPEX study [16]. This study demonstrated that plasma exchange when compared with methylpednisolone increased the rate of renal recovery at one year after diagnosis in ANCA-associated vasculitis that presented with renal failure. It is unknown what the impact of plasma exchange and methylprednisolone therapy is on long-term outcome and relapse-rate. Further analysis of subgroups and long-term MEPEX data might provide more data. Furthermore, long-term steroid therapy differed between the two groups. Although steroid dosages did not differ at 3 and 12 months after diagnosis, more patients in the cyclophosphamide group were treated with long-term low dose steroid therapy. To what extent this strategy prevented relapses cannot be ascertained. Increased exposure to steroids would explain a reduced relapse rate, more infectious complications and more treatment related damage in the cyclophosphamide group. Obviously, these differences are of concern when interpreting the results of our study. Ideally long-term follow-up of a randomized study would exclude these factors. Regarding vasculitis and treatment related damage the VDI has been established as a reliable instrument. We did not find differences in VDI between the cyclophosphamide and azathioprine group. Also when comparing critical damage and treatment related damage items no differences were detected between the two cohorts. Thus, although the cyclophosphamide cohort was exposed to both cyclophosphamide and prednisolone for a longer period this did not result in differences in treatment related damage items of the VDI score.

The majority of infections occurred within 6 months after diagnosis, when both groups were treated with cyclophosphamide and most leukopenic episodes occurred. After six months the incidence of infectious complications diminished in both groups, and did not differ significantly between the groups. Also, in the CYCAZAREM study incidence of leukopenia and infection did not differ between the cyclophosphamide and azathioprine group [2]. In the CYCAZAREM study, during 18 months follow-up, 33 infections were documented in 144 patients, 17 (55%) were associated with concurrent leukopenia. In our study we documented considerably more infectious episodes within 24 months (136 in 247 patients versus 33 in 144 patients). This difference was mainly attributable to the first 3 months after diagnosis, when in the CYCAZAREM study only 7 infectious episodes were reported, whereas in our cohort 81 infectious episodes were documented. In the WEGENT study, after induction with cyclophosphamide, during a mean follow-up of 29 months 19 infectious episodes were reported in 63 patients on azathioprine maintenance and only 2 patients on azathioprine maintenance experienced neutropenia [14]. Thus, our data on infectious complications on azathioprine maintenance are comparable with data from CYCAZAREM and WEGENT. However, our study shows that the risk of

infectious complications is mainly related to induction therapy with the combination of cyclophosphamide and high-dose corticosteroids. Addition of more intensive therapy with plasma exchange and methylprednisolone will further augment this risk. Previously, in the MEPEX trial in 137 patients, 61 infectious complications were documented of which 37 life-threatening [16]. Eventually, 19 patients died within one year of an infectious complication.

Only 3 patients in our cohort experienced *Pneumocysitis Jirovecii* infection, stressing the effectiveness of prophylactic co-trimoxazole treatment. This compares favourably with earlier studies [17]. In the cyclophosphamide group more fungal infections were detected. In addition to differences in immunosuppressive therapy more rigid use of anti-fungal agents in later years might explain this difference. However, in the azathioprine group more bacterial infections were documented within 6 months after diagnosis. This difference might be explained by the more intensive immunosuppressive regimens with methylprednisolone and plasma exchange that were offered to patients diagnosed after 1997.

A variety of malignancies developed during follow-up of both cohorts. The incidence of malignancies did not differ between the cyclophosphamide and azathioprine group at 5 years after diagnosis. Only one patient in the cyclophosphamide group developed a urothelial cell carcinoma. Also, only one of 15 patients with a history of malignancy had recurrent disease.

Finally, mortality did not differ significantly between the groups. In both cohorts infectious diseases were major causes of death. A variety of other causes was identified, with only 2 patients in the cyclophosphamide group dying of active vasculitis.

Our data confirm the success of a staged immunosuppressive approach. Starting with more aggressive therapy, i.e. cyclophosphamide with potential addition of methylprednisolone or plasma exchange, then down staging to azathioprine. However, in contrast to what we had expected, we did not find an advantage regarding treatment toxicity with azathioprine maintenance as compared to cyclophosphamide maintenance in the largest documented cohort of AAV patients. Therefore, preference for azathioprine over cyclophosphamide maintenance is not supported by head to head comparisons in ANCA-associated vasculitis.

In this study we also showed that both PR3-ANCA positive WG and MPA patients have a higher relapse risk than MPO-ANCA positive or ANCA negative patients. Previously, we showed that every relapse is associated with an irreversible loss of renal function [18]. Additionally, we found a significant increase of VDI after every consecutive relapse. Therefore, for a subgroup of PR3-ANCA positive patients prevention of relapses by adapting immunosuppressive therapy according to risk factors for relapse might be beneficial.

In conclusion, the past decades have shown major progress of evidence-based treatment strategies for AAV, leading to limited exposure of patients to cyclophosphamide. In our

retrospective study this reduction in cyclophosphamide duration by switching maintenance therapy to azathioprine is not associated with more relapses.

REFERENCES

1. Fauci AS, Wolff SM, Johnson JS. Effect of cyclophosphamide upon the immune response in Wegener's granulomatosis. N Engl J Med 1971; 285(27):1493-1496.
2. Jayne D, Rasmussen N, Andrassy K et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. N Engl J Med 2003; 349(1):36-44.
3. Pagnoux C, Hogan SL, Chin H et al. Predictors of treatment resistance and relapse in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis: Comparison of two independent cohorts. Arthritis Rheum 2008; 58(9):2908-2918.
4. Hoffman GS, Kerr GS, Leavitt RY et al. Wegener granulomatosis: an analysis of 158 patients. Ann Intern Med 1992; 116(6):488-498.
5. Nachman PH, Hogan SL, Jennette JC, Falk RJ. Treatment response and relapse in antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. J Am Soc Nephrol 1996; 7(1):33-39.
6. Sanders JS, Stassen PM, van Rossum AP, Kallenberg CG, Stegeman CA. Risk factors for relapse in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis: tools for treatment decisions? Clin Exp Rheumatol 2004; 22(6 Suppl 36):S94-101.
7. Sanders JS, Slot MC, Stegeman CA. Maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. N Engl J Med 2003; 349(21):2072-2073.
8. Reinhold-Keller E, Beuge N, Latza U et al. An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients. Arthritis Rheum 2000; 43(5):1021-1032.
9. Talar-Williams C, Hijazi YM, Walther MM et al. Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. Ann Intern Med 1996; 124(5):477-484.
10. Jennette JC, Falk RJ, Andrassy K et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Arthritis Rheum 1994; 37(2):187-192.
11. Luqmani RA, Bacon PA, Moots RJ et al. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. QJM 1994; 87(11):671-678.
12. Exley AR, Bacon PA, Luqmani RA et al. Development and initial validation of the Vasculitis Damage Index for the standardized clinical assessment of damage in the systemic vasculitides. Arthritis Rheum 1997; 40(2):371-380.
13. Stegeman CA, Cohen Tervaert JW, de Jong PE, Kallenberg CG. Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. N Engl J Med 1996; 335(1):16-20.
14. Pagnoux C, Mahr A, Hamidou MA et al. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. N Engl J Med 2008; 359(26):2790-2803.

15. Hogan SL, Falk RJ, Chin H et al. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. Ann Intern Med 2005; 143(9):621-631.
16. Jayne DR, Gaskin G, Rasmussen N et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. J Am Soc Nephrol 2007; 18(7):2180-2188.
17. Ognibene FP, Shelhamer JH, Hoffman GS et al. Pneumocystis carinii pneumonia: a major complication of immunosuppressive therapy in patients with Wegener's granulomatosis. Am J Respir Crit Care Med 1995; 151(3 Pt 1):795-799.
18. Slot MC, Cohen Tervaert JW, Franssen CF, Stegeman CA. Renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement. Kidney Int 2003; 63(2):670-677.