Consequences of disease and treatment in ANCA-associated vasculitis

Tuin, Janneke

Chapter eight

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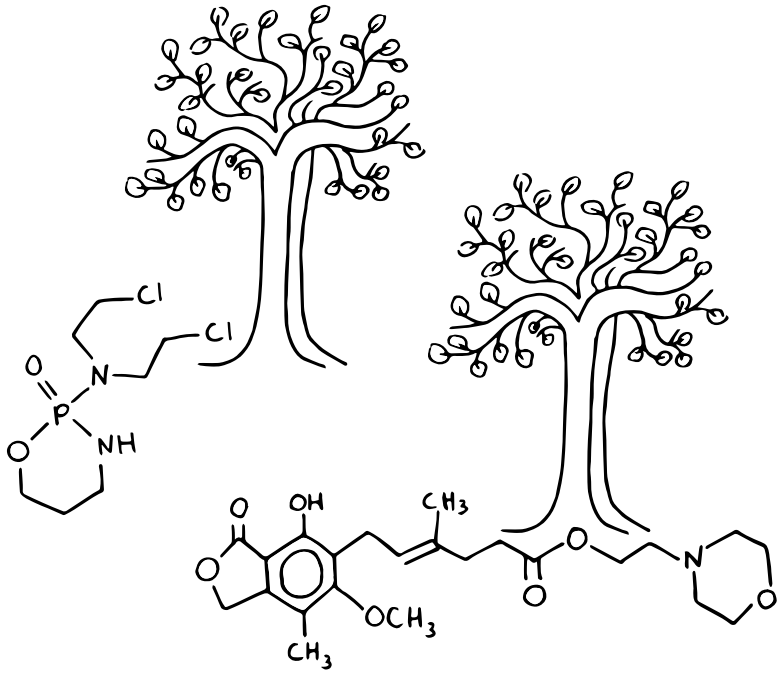

Mycophenolate mofetil versus cyclophosphamide for the induction of remission in non-life-threatening relapses of antineutrophil cytoplasmic antibody-associated vasculitis: a randomized controlled trial

Janneke Tuin MD[1], Patricia. M. Stassen MD PhD[2], Daria I. Bogdan[1], Jan Broekroelofs[3] MD PhD, Pieter van Paassen MD PhD[4], Jan Willem Cohen Tervaert[4], Jan-Stephan F. Sanders MD PhD[1], Coen A. Stegeman MD PhD[1]

Department of Internal Medicine, Division of [1]Nephrology, University of Groningen, University Medical Center Groningen, The Netherlands. Department of Internal Medicine, Division of [2]Acute medicine, Maastricht University Medical Center. Department of Internal Medicine, Division of [3]Nephrology, Medisch Centrum Leeuwarden. Department of Internal Medicine[4], Maastricht University Medical Center.

Abstract

Introduction. Cyclophosphamide (CYC) has been the mainstay of treatment of ANCA-associated vasculitis (AAV). However, CYC has unfavorable side-effects and alternatives are needed. Evidence suggests that mycophenolate mofetil (MMF) can induce sustained remission in non-life-threatening disease. The purpose of this study is to compare the efficacy and safety of MMF versus CYC for the induction treatment of non-life-threatening relapses of PR3 and MPO-AAV.

Methods. We conducted a multi-center randomized controlled trial. Patients with a first or second relapse of AAV were randomized to induction treatment with CYC or MMF, both in combination with glucocorticoids. Maintenance therapy consisted of azathioprine in both arms. Primary outcome was remission at six months and disease free survival at 2 and 4 years.

Results. Eighty-four patients were enrolled of whom 41 received MMF and 43 CYC. Eighty-nine per cent of patients were PR3-ANCA-positive. At six months, 66% of MMF treated patients versus 81% of CYC treated patients were in remission ($\chi 2$ (1) = 2.62, p= 0.11). There was a significant interaction between treatment and disease severity at study entry on successful remission induction. The hazard ratio for failure of MMF therapy compared to CYC in the highest tertile (BVAS>19) was 7.79 (95% CI 1.6- 48.3). Disease-free survival at 24 and 48 months was 61% and 39% for CYC, and 46% and 34% for MMF, respectively (logrank test, p= 0.26).

Conclusion. MMF is a viable alternative to CYC for the treatment of non-life-threatening relapses of AAV, with the exception of patients with a higher BVAS (> 19), as the efficacy of MMF in this subgroup seems significantly lower.

Introduction

Antineutrophil cytoplasmic antibody-associated vasculitides (AAV) are a group of autoimmune diseases, which are associated with the presence of circulating antineutrophil cytoplasmic antibodies (ANCA). Three types of AAV can be distinguished: (1) microscopic polyangiitis, (2) granulomatosis with polyangiitis, and (3) eosinophilic granulomatosis with polyangiitis [1]. These chronic multisystem pathologies can be life-threatening due to amongst others acute renal failure or pulmonary hemorrhage. Therefore most AAV patients require significant immunosuppression during remission induction and long-term maintenance treatment. The introduction of the alkylating agent cyclophosphamide (CYC) in the 1960's improved survival substantially; however, at the expense of serious acute and long-term side effects, such as bone marrow depression, opportunistic infections, gonadal failure, haemorrhagic cystitis and bladder cancer [2]. These pressing issues called for alternatives with lower toxicity with at least-equal potency.

Literature suggests that mycophenolate mofetil (MMF), a non-alkylating pro-drug which selectively inhibits lymphocyte proliferation, can safely induce sustained remission in AAV [3-7]. Instead of damaging DNA, mycophenolate mofetil inhibits the enzyme inosine monophosphate dehydrogenase and thereby reduced the guanine triphosphate synthesis, which is necessary for lymphocyte proliferation [8]. Comparative studies for induction of remission with mycophenolate mofetil are limited. This prompted us to test the efficacy and safety of mycophenolate mofetil compared to cyclophosphamide for the induction of remission in non-life-threatening relapses, including both proteinase 3 (PR3) and myeloperoxidase (MPO)-ANCA associated vasculitis. We report the results of our multi-center randomized controlled trial.

Patients & methods

Study design and patients

This multi-center randomized trial was conducted between 2005 and 2014 at eight centers in The Netherlands. The study protocol was reviewed and approved by the local IRB of every participating center. The study was registered on www.clinicaltrials.gov with identifier: NCT00103792.

Patients were classified as GPA or MPA according to criteria adopted from the Chapel Hill Consensus Conference Nomenclature [1]. Consecutive patients with a first or second non-life-threatening relapse of PR3-ANCA or MPO-ANCA positive ANCA-associated vasculitis were eligible for study inclusion. The diagnosis was preferably histological proven. All patients had to provide written informed consent to participate. Patients were not eligible for study inclusion if any of the following criteria was present: life-threatening disease, defined as serious alveolar bleeding or (threatening) respiratory insufficiency, serum creatinine >500µmol/l, or new dialysis dependence; ongoing high-dose maintenance therapy at the time of the relapse, more specifically cyclophosphamide ≥100mg/day and prednisolone ≥25mg/day; known intolerance to cyclophosphamide, mycophenolate mofetil or azathioprine; pregnancy; desire to have children or inadequate contraception; inability to provide informed consent; age <18 years.

Enrollment and randomization

Participants were recruited from the outpatient clinic from the participating centers. Patients

were enrolled by the treating physician. Patients were randomized to receive open-label oral cyclophosphamide or mycophenolate mofetil in a 1:1 ratio as induction treatment. Randomization took place centrally in the UMCG and was performed with the use of permuted blocks of 4 or 6. Patients were stratified according to ANCA-specificity (PR3 or MPO) and whether this was a the first or second relapse.

Treatment protocol

Group A received induction treatment with oral cyclophosphamide 2 mg/kg/d (age >65 1.5 mg/kg/d) for 6 months. Group B received induction treatment with mycophenolate mofetil 1000 mg twice daily for 6 months. Both treatment groups received prednisolone in a tapering regime during induction treatment. All patients received initially prednisolone 1 mg/kg/d (max 60 mg) until six weeks after start of treatment. Thereafter, prednisolone was tapered with 10 mg every two weeks until a daily dose of 30 mg/d, subsequently with 5 mg every two week until 15 mg daily and with 2.5 mg every four weeks until discontinuation of prednisolone. With this regimen, by 6 months prednisolone should be 10 mg daily and should be discontinued by 9,5 months after study entry.
All patients were switched to maintenance therapy with azathioprine 1.5 mg/kg/d after six months, provided that the patient was in stable remission for at least three months. If not, switch of therapy could be postponed to a maximum of nine months after start of therapy. When remission was not attained within 6 months, therapy was considered to have failed. Azathioprine maintenance therapy was tapered after 12 months and completely withdrawn 2 years after study entry.
If the following events occurred, dosages had to be adjusted as it follows: leukocytopenia (<4,0x109/L) prompted for a 25% reduction of cyclophosphamide, mycophenolate mofetil and azathioprine; if the leukocyte count dropped below 3.0x109/L, medication had to be stopped temporarily until leukocyte counts normalized, and treatment had to be resumed with at least 25% decrease. The treatment protocol was stopped after 4 years of follow-up, or if remission was not achieved within 6 months, a relapse developed, the patient experienced unacceptable side-effects or withdrawal of informed consent.

Concomitant medication

Both groups received additional therapy, entailing prophylaxis against Pneumocystis pneumonia (co-trimoxazole for 6 months), osteoporosis prophylaxis, candida prophylaxis (until prednisolone dose reached ≤15 mg/day), and prophylaxis of dyspeptic symptoms (as required).

Outcomes and definitions

Primary end point was remission at six months and disease free survival at 2 and 4 years. Secondary end points were time to induction of remission, cumulative organ damage, renal function and adverse events.
Remission was defined as the absence of manifestations attributable to active disease (BVAS=0) and CRP<10mg/dl. Induction therapy was considered to have failed when remission was not attained within six months or when remission was not sustained throughout the first six months. A relapse was defined as the reoccurrence or new appearance of organ involvement attributable to active vasculitis and requiring increase in or reintroduction of immunosuppression. A major relapse was

defined as the reoccurrence or new onset of potentially organ- or life threatening disease activity that cannot be treated with an increase of glucocorticoids alone and requires further escalation of therapy (i.e. the administration of cyclophosphamide). All other relapses were classified as “minor”. A leukocyte count lower than 4,0x109/l was defined as leukocytopenia and a lymphocyte count below 0,8x109/l as lymphocytopenia. Infection was defined as disease requiring antibacterial or antiviral treatment, with or without the need for hospital admission; i.e. the term does not apply to a presumably viral respiratory tract infections that did not require medical treatment.

Disease assessments

Patient evaluation was conducted upon start of treatment, once every four weeks during the first 6 months, and then once every 3 months for up to 4 years. Physical examination entailed measuring blood pressure and weight. Routine laboratory included: a full blood count, C-reactive protein, estimated sedimentation rate, urea and ANCA titers. In addition, 24hour urine analysis was performed to monitor creatinine clearance and proteinuria. Spot urine was collected for analysis of hematuria and erythrocyte cylinders. Chest X-rays or CT scans were obtained when clinical indicated. Disease activity was assessed using the Birmingham Vasculitis Activity Score (BVAS) [9]. In addition, the Vasculitis Damage Index (VDI) was used to quantify the cumulative organ damage [10]. Adverse events were graded according to National Cancer Institute’s NCI Common Terminology Criteria for Adverse Events version 4.0.

Statistical analyses

SPSS version 23.0 (SPSS IBM, New York, U.S.A.) was used for data analysis. Data are shown as mean ± standard deviation (SD) for normal distributed data and as median and interquartile range (IQR) for nonnormal distributed data. Comparisons between groups were tested using the Student’s t-test and Mann Whitney-U as appropriate. Correlations were analyzed with Spearman’s rho correlation coefficient or Pearson’s correlation coefficient as appropriate.

The primary analysis was tested based on an intention-to-treat method. Patients who dropped out of the study for any reason before 6 months were considered having treatment failure.

Disease free survival was estimated by Kaplan-Meier actuarial survival curves with log-rank testing to compare groups. To investigate treatment efficacy for remission induction a multivariable analysis was performed using Cox proportional hazards models adjusting for the baseline covariates age at inclusion, ANCA specificity, BVAS at study entry and renal function at study entry. Statistical significance was defined as a two-sided $p< 0.05$.

Based upon our previous study we expected successful induction of remission in 90% of patients treated with cyclophosphamide. We considered mycophenolate mofetil to be similar effective when the relative difference in remission rate would be less than 25%. Randomization of 90 patients was required to achieve a power of 0.8 with an α of 0.05.

Results

Patients

Eighty-four patients with relapsing PR3 and MPO-AAV were enrolled in this multi-center randomized

controlled trial between 2005 and 2014. A list of patient inclusion by participating center is shown in supplement 1. A flowchart of all randomized and analyzed patients is shown in Figure 1. The target inclusion was 90 patients based on the power calculation. Enrollment proceeded slower than anticipated and it was decided to discontinue the study prematurely in August 2014. At that time, 84 patients were enrolled. Forty-one patients were randomized to mycophenolate mofetil and 43 patients received cyclophosphamide. Demographic and clinical characteristics were similar

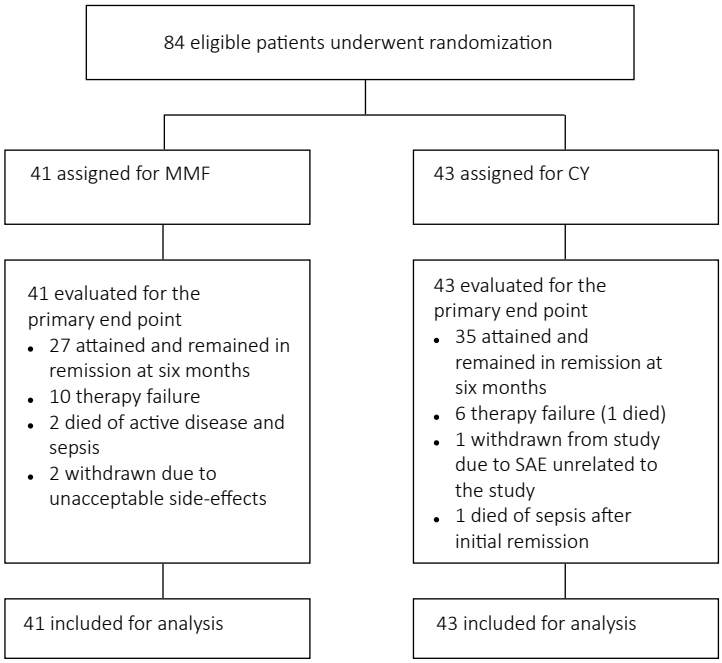

Figure 1. Flowchart of randomized and analyzed patients.

Table 1. Baseline characteristics of all included patients at study entry.

Patient characteristic	All patients	CYC (n= 43)	MMF (n= 41)	p-value
Age (years)	60 (±12)	60 (±11)	60 (±13)	0.84
Sex (male)	57 (68%)	30 (70%)	27 (66%)	0.70
ANCA specificity (PR-3)	75 (89%)	38 (88%)	37 (90%)	0.78
First relapse	62 (74%)	31 (72%)	31 (76%)	0.71
Organ involvement at study entry				
ENT	40 (48%)	19 (44%)	21 (51%)	0.52
Pulmonary	42 (50%)	24 (56%)	18 (44%)	0.28
Renal	63 (75%)	32 (74%)	31 (76%)	0.90
BVAS	15 [13- 19]	16 [13-20]	15 [13- 19]	0.70
CRP (mg/L)	36 [12- 85]	40 [13- 125]	33 [10- 57]	0.24
Creat µmol/L	103 [85- 153]	100 [83- 160]	112 [86- 148]	0.45
VDI	1 [1-2]	1 [1-3]	1 [1-2]	0.73

Except where indicated otherwise, values are the number (%).

in patients randomized to cyclophosphamide and mycophenolate mofetil at time of randomization (Table 1). The majority of included patients were PR-3 ANCA positive (89%). Sixty-two percent had experienced their first relapse (74%) at time of inclusion. The median BVAS at study entry was 15 [IQR 13- 19]. Sixty-three patients had renal involvement (75%) and the median creatinine level was 103 µmol/L [IQR 85- 153].

Efficacy assessments

Remission induction

Twenty-seven of 41 patients (66%) treated with mycophenolate mofetil as compared to 35 of 43 patients (81%) treated with cyclphosphamide were in stable remission at six months ($\chi 2$ (1) = 2.62, p= 0.11). The relative risk for therapy failure with mycophenolate mofetil therapy was 1.8 [95% CI 0.9- 3.9]. Eight patients treated with cyclophosphamide were not in remission at six months due to progression of disease which required escalation of therapy (n= 6) and resulted in death in one patient, occurrence of an adverse event unrelated to the study for which the patient was withdrawn

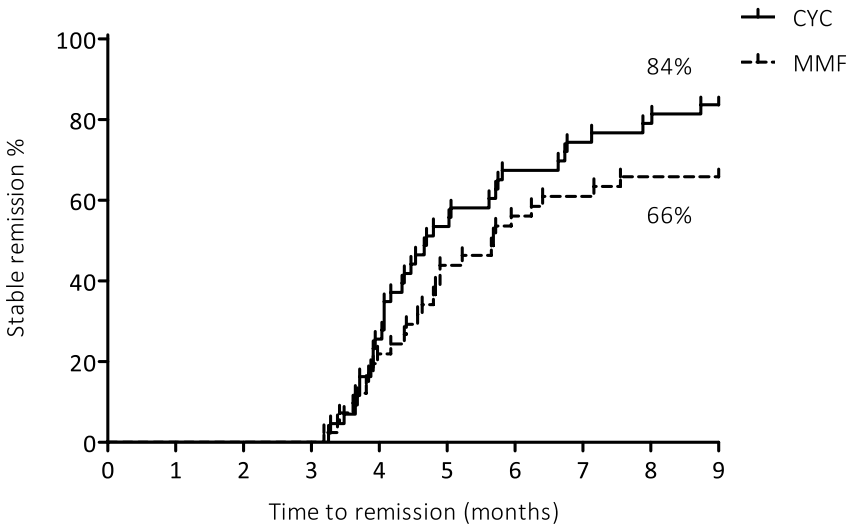

Figure 2. Time to stable remission according to treatment allocation.

from the study (n= 1) and death due to sepsis after achieving stable remission (n= 1). Therapy failed to induce stable remission within 6 months in 14 patients treated with mycophenolate mofetil. In 10 patients disease progressed and of them 2 patients improved initially but experienced increasing disease activity within six months. Two patients died due to exacerbated disease combined with sepsis and two patients discontinued therapy due to unacceptable side-effects (Figure 1). All failures related to disease progression in the cyclophosphamide treated group occurred within 5

weeks. Failure related to disease progression in the mycophenolate mofetil group was observed in 8 patients within 5 weeks, one patient after 7 weeks, one patient after 12 weeks and two patients showed improvement initially but had renewed disease activity after 16 weeks of therapy.
Time to stable remission did not differ significantly between mycophenolate mofetil and cyclophosphamide treated patients, median time to remission was 7.3 weeks [IQR 3.7- 11.7] versus median 6.4 weeks [IQR4.0- 11.9], p= 0.85, respectively (Figure 2). One cyclophosphamide treated patient did attain stable remission, but died of sepsis before the primary end point was attained.
A logrank test showed that treatment with mycophenolate mofetil was not significantly associated with therapy failure within 6 months (HR 2.24 [95% CI 0.90- 5.55], p= 0.08) (Table 2, model 1).

Table 2. Cox proportional hazard analysis for treatment failure within 6 months.

	HR (95% CI)	p- value
Number of events	21	
Treatment (MMF)	2.24 (0.90- 5.55)	0.08
Treatment (MMF)*	2.27 (0.91- 5.63)	0.08
Treatment (MMF)**	3.34 (1.21- 9.24)	0.02

**Treatment (MMF), adjusted for age. ** Treatment (MMF), adjusted for age, ANCA specificity, BVAS at study entry, eGFR at study entry.*

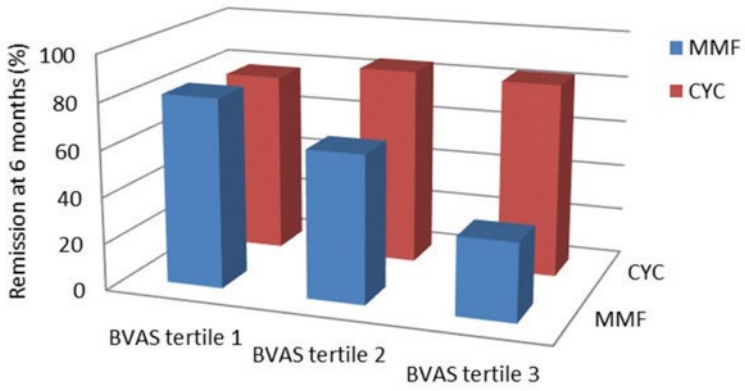

Figure 3. Interaction between treatment and disease severity at study entry and percentages of sustained remission at 6 months. BVAS tertile 1: BVAS< 14; BVAS tertile 2: BVAS 14- 19; BVAS tertile 3: BVAS > 19.

In multivariable Cox proportional hazard analysis, after adjustment for age, ANCA specificity, BVAS and GFR at study entry treatment with mycophenolate mofetil was significantly association with therapy failure within 6 months (HR 3.34 [95% CI 1.21- 9.24], p= 0.02) (Table 2, model 3). There was a significant interaction between BVAS and treatment (p= 0.03). Patients treated with mycophenolate mofetil and high disease severity were less likely to attain remission compared to

patients with cyclophosphamide. The hazard ratio for failure of mycophenolate mofetil therapy compared to cyclophosphamide in the highest tertile (BVAS>19) was 7.8 (95% CI 1.6- 48.3). The efficacy of cyclophosphamide appeared to be unrelated of disease severity at study entry (Figure 3).

Relapse

Thirteen of 27 mycophenolate mofetil treated patients in remission at six months (48%) compared to sixteen of 35 cyclophosphamide treated patients in remission at six months (46%) developed a relapse during follow-up (Logrank test, p= 0.95, HR 1.03 [95% CI 0.49- 2.14], Figure 4). The median C-reactive protein at time of relapse was 32 mg/L [IQR 18- 74] for cyclophosphamide treated patients and 27 mg/L [IQR 11- 100] for mycophenolate mofetil treated patients (Mann-Whitney U, p= 0.98). The median BVAS at time of relapse was 11 [IQR 5- 16] for cyclophosphamide treated patients and 12 [IQR 8- 12] for mycophenolate mofetil treated patients (Mann-Whitney U, p= 0.91). For all patients, disease-free survival at 24 and 48 months was 61% and 39% for cyclophosphamide, and 43% and 32% for mycophenolate mofetil, respectively (24 months logrank test, p= 0.10, HR 1.70 [95% CI 0.91- 3.20]; 48 months logrank test, p= 0.17, HR 1.49 [95% CI 0.84- 2.62]) (Supplementary figure 1). Three cyclophosphamide treated patients and 1 mycophenolate mofetil treated patient were still in follow-up at 24 to 32 months and 28 months respectively.

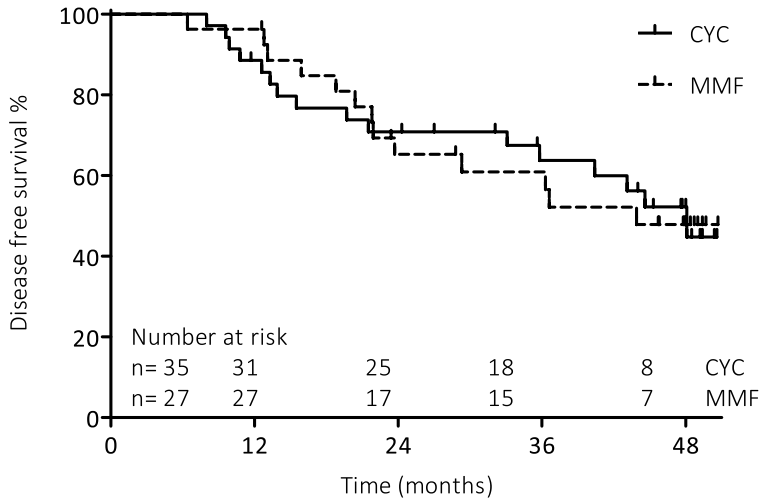

Figure 4. Disease free survival in patients in remission at six months.

Protocol violation

Not all patients received the maintenance therapy according to protocol. One patient randomized to cyclophosphamide induction therapy was azathioprine intolerant and switched back to cyclophosphamide in a tapering regimen. One patient treated with cyclophosphamide induction therapy received methotrexate maintenance therapy after a prolonged period of leucopenia on

cyclophosphamide. One patient treated with cyclophosphamide induction therapy was erroneously switched to mycophenolate mofetil maintenance after induction of remission. Three patients who were treated with mycophenolate mofetil induction therapy were azathioprine intolerant and were switched back on mycophenolate mofetil as maintenance therapy. Three patients were erroneously not switched from induction to maintenance therapy and continued on mycophenolate mofetil. After exclusion of these patients disease free survival remained not significantly different (data not shown).

Renal function

Thirty-two patients treated with cyclophosphamide (74%) compared to 31 patients treated with mycophenolate mofetil (76%) had active renal involvement at study entry. In this subgroup, the estimated glomerular filtration rate (eGFR) was similar for both treatment groups at baseline (mean eGFR mycophenolate mofetil group 49.2 (±23.4) ml/min/1.73m2 versus cyclophosphamide group 53.1 (±26.9) ml/min/1.73m2, p= 0.55). eGFR increased with 3.8 ml/min/1.73m2 in the mycophenolate mofetil treated group versus 5.8 ml/min/1.73m2 in the cyclophosphamide treated group from baseline to 6 months. End stage renal disease (ESRD) developed in 1 patients during follow-up.

Organ damage

At study entry, the majority of relapsing patients already had damage. The VDI at study entry of mycophenolate mofetil and cyclophosphamide treated patients were 1.6 (SD1.2) and 1.8 (SD1.6) (p= 0.50), respectively. Twenty-one patients in the cyclophosphamide group had no increase in the VDI from study entry to 6 months and 18 had no increase until 12 months. Twenty-two patients in the mycophenolate mofetil group had no increase in VDI from baseline to 6 months and 18 had no increase until 12 months. The mean VDI at 6 months for cyclophosphamide treated patients was 2.2 (SD 1.7) and for mycophenolate mofetil treated patients 2.1 (SD 2.0)(p= 0.72). At 12 months the mean VDI for cyclophosphamide patients was 2.3 (SD 1.8) and for mycophenolate mofetil patients 2.3 (2.1) (p= 0.94).

Adverse events

A total of 102 adverse events occurred during this study, excluding leukopenia, trombocytopenia and anemia (see Table 3). Four patients died during remission induction as described above. Two patients were withdrawn from the study, one mycophenolate mofetil treated patient because of a lung carcinoma and one cyclophosphamide treated patient because of a pancreas carcinoma of which both patients subsequently died.

Three malignancies in each treatment group developed. One pancreas carcinoma (see above), one esophagus carcinoma and one bladder carcinoma developed in the cyclophosphamide treated group. The patient who developed a bladder carcinoma experienced chemical cystitis during induction of remission and had experienced a course of hemorrhagic cystitis before study entry. One lung carcinoma (see above), one squamous cell carcinoma of the skin and melanoma developed in the mycophenolate mofetil treated group. All these patients were exposed to cyclophosphamide and glucocorticoids previous to study inclusion (see supplementary table 2).

Seven out of 34 (21%) mycophenolate mofetil treated patients versus 6 out of 40 (15%)

Table 3. Adverse events.

	Total (n= 84)	MMF (n= 41)	CYC (n= 43)
Adverse events (AE), n	102	47	55
AE grade 1 or 2, n	75	34	41
AE grade 3, n	19	8	11
AE grade 4, n	2	2	0
AE grade 5, n	6	3	3
Infections, n	46	19	27
Episodes of infections on induction treatment, n	19	8	11
≥ 1 infections during induction, n patients (%)	13	7/34 (21%)	6/40 (15%)
Grade ≥ 3, n	5	1	4
Treatment-related AE			
Chemical cystitis, n patients	1	0	1
Gonadal failure, n patients	1	0	1
Diabetes Mellitus, n patients	9	1	8
Malignancies	6	3	3
Intolerance MMF	3	3	0
Intolerance AZA	4	3	1
Leukopenia			
Episodes of leukopenia on induction treatment, n	15	3	2
≥ 1 episode of leukopenia during induction, n patients (%)	11 33	3/35 (9%) 15	8/40 (20%) 8
Episodes of leukopenia on maintenance treatment, n			
≥ 1 episode of leukopenia during maintenance, n patients (%)	19 (23%)	7 (17%)	12 (28%)
Anemia, maximum AE grade			
Grade 1 or 2, n patients	73	35	38
Grade 3	2	1	1
Trombocytopenia			
Grade 1, n patients	8	3	5
Grade 4*, n patients	1	0	1

*As part of a severe pancytopenia.

cyclophosphamide treated patients experienced at least one infection during induction treatment ($\chi 2$ (1) = 0.40, p= 0.53). The relative risk for infection during induction with cyclophosphamide was 0.7 [95% CI 0.3- 2.0]. One patient on mycophenolate mofetil experienced a severe generalized varicella zoster infection (AE grade 4) during an episode of severe pancytopenia. One patient in the cyclophosphamide group experienced a severe sepsis during an episode of leukopenia on cyclophosphamide induction and subsequently died (AE grade 5). Two patients experienced three grade 3 infections in the cyclophosphamide group during remission maintenance on azathioprine: one patient experienced both paronychia of a finger, which was complicated by osteomyelitis and

subsequent amputation of part of the finger and a recurrent infection of the hip after total hip replacement; one patient experienced an episode of cholangitis.
Three out of 35 (9%) of mycophenolate mofetil treated patients compared to 8 out of 40 (20%) of cyclophosphamide treated patients experienced at least one episode of leukopenia (neutrophil count <4,0x 10^9/L) during remission induction ($\chi 2$ (1) = 1.95, p= 0.16). The relative risk for neutropenia during induction with cyclophosphamide was 2.3 [95% CI 0.7- 8.1].

Discussion

We investigated whether mycophenolate mofetil combined with glucocorticoids could be an effective and safe alternative to cyclophosphamide for the induction of remission in patients with PR3 or MPO-ANCA positive vasculitis who experienced a non-life-threatening relapse. Mycophenolate mofetil induced sustained remission in a lower number of patients compared to cyclophosphamide, even though this difference did not reach statistical significance. Mycophenolate mofetil was less effective in patients with higher disease activity at study entry, whereas treatment efficacy of cyclophosphamide was unrelated to disease severity. After remission was attained a similar number of patients experienced a relapse during follow-up. No apparent difference between adverse events were observed.
These findings could be interpreted from various angles. Induction of remission did not differ statistically significant and, therefore, mycophenolate mofetil might be regarded as a safe alternative for cyclophosphamide. In addition, 66% of patients attained remission and experienced sustained remission for a similar time period compared to cyclophosphamide treated patients and were therefore not exposed to cyclophosphamide. Nonetheless, a difference of 15% was observed and it could be debated whether this difference is in fact not clinically relevant. Mycophenolate mofetil might therefore be a valuable option in non-life-threatening relapses of AAV in patients previously treated with cyclophosphamide or patients with a contraindications for cyclophosphamide or other agents.
Many studies have focused on alternatives for cyclophosphamide with at least the same efficacy and a less (severe) side-effects over the last decades. With the exception of rituximab many of these therapies have failed to show both similar induction success as well as similar maintenance of remission. Methotrexate was limited by a lower remission rate and methotrexate and pulse cyclophosphamide had both a tendency towards a higher relapse rate [11-13]. Tailoring therapy might improve outcome. The interaction we observed between disease severity and mycophenolate mofetil on remission induction suggest that mycophenolate mofetil might be a favorable option for patients with lower disease activity i.e BVAS <19, however this was not the primary end point of this stuy.
Our remission rate is a somewhat lower compared to other studies. Several studies did not assess remission at six months and in those studies early relapses were not considered treatment failure [5, 14]. Remission rates reported in studies including only or primarily MPO-ANCA positive patients yielded higher remission rates 76-79% [4, 6, 7]. The majority of our patients were PR3-ANCA positive and this might have influenced the remission rate. PR3 and MPO-ANCA-associated vasculitis are more and more regarded separate entities since recent developments have shown that PR3 and MPO-ANCA-associated vasculitis differ in organ involvement, genetic background and

prognosis [15]. At the same time as our study, the MYCYC trial initiated by the EUVAS study group was conducted. The MYCYC study included newly diagnosed patients and similar to our study, they were randomized to cyclophosphamide or mycophenolate mofetil therapy for the induction of remission. At this moment, the MYCYC trial was not yet fully published, but abstract publication in 2013 showed a similar remission rate of 66% in the mycophenolate mofetil treated group and a remission rate of 69% in the pulse i.v cyclophosphamide group [16]. Full-length publication of the MYCYC trial is eagerly awaited.

Our study has severable notable strengths. Our study both considered induction of remission as well as disease free survival during an extensive follow-up period. In addition, this study was a randomized controlled trial with a fairly large number of patients. Our study had some important limitations as well. The study was discontinued prematurely and the calculated power for analysis of the primary end point was not reached.

We did not perform therapeutic drug monitoring of mycophenolate mofetil and the therapeutic doses are therefore not known. Previous studies have shown that AAV patients as well as healthy individuals exhibit a high interindividual variability with respect to the plasma concentrations of mycophenolic acid (MPA), the active metabolite of mycophenolate mofetil [17]. Recently, it was demonstrated MPA concentrations were associated with outcome in AAV [18]. Therapeutic drug monitoring might therefore be a valuable tool to improve therapy outcome.

In conclusion, mycophenolate mofetil is an effective treatment for the induction of remission in patients with a PR3 or MPO-ANCA positive vasculitis with a non-life-threatening relapse. Mycophenolate mofetil compared to oral cyclophosphamide led to a statistically non-significant lower number of patients achieving remission at 6 months and disease free survival at 4 years of follow-up was not significantly different. Mycophenolate mofetil is therefore a viable alternative to oral cyclophosphamide for the treatment of non-life-threatening relapses of AAV, with the exception of patients with a higher BVAS i.e. BVAS >19, as the efficacy of mycophenolate mofetil in this subgroup seems significantly lower. Tailoring therapy in combination with therapeutic drug monitoring might further improve outcome.

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COMPETING INTERESTS

The authors report no competing interests.

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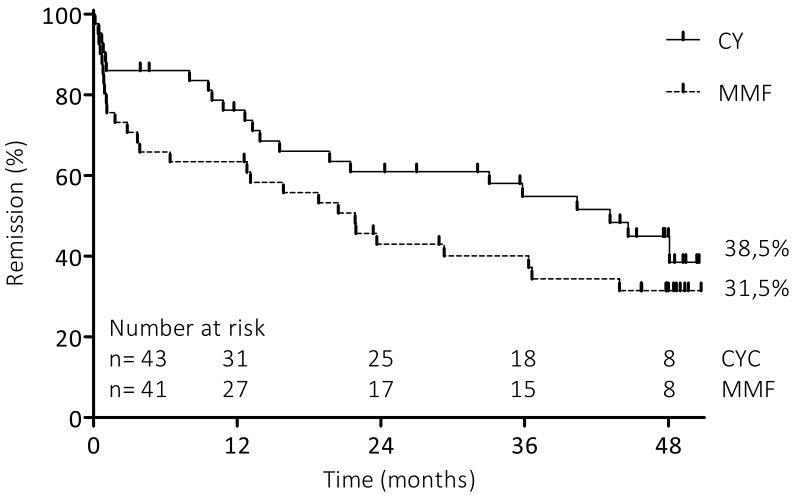
Supplementary material

Supplementary table 1. Inclusion sites.

Inclusion site	Number of included patients
University Medical Center Groningen	47
Maastricht University Medical Center	20
Medisch Centrum Leeuwarden	11
Scheper Ziekenhuis Emmen	2
Ziekenhuis St Jansdal Hardenberg	2
Isala Zwolle	1
Erasmus MC	1



Supplementary figure 1. Disease free survival from study entry to end of four year follow-up.

Supplementary table 2. Malignancies during study follow-up.

Malignancy during study	Time after study entry diagnosed	Previous treatment	Outcome, last information (date)
Randomization: CYC			
Pancreas carcinoma	4 months	Diagnosis: CYC and CS induction, AZA maintenance	Died
Esophagus carcinoma	2 months	Diagnosis: CYC and CS, 1 relapse CYC and CS	Treatment: surgery, recovered without sequelae (6-10-2016)
Bladder carcinoma	33 months	Diagnosis: CYC and CS	Recovered without sequelae (July 2016)
Randomization: MMF			
Lung carcinoma	12 months	Diagnosis: CYC and CS; First relapse: CYC and CS	Died
Squamous cell carcinoma	49 months	Diagnosis: CYC and CS; First relapse: CYC and CS	Recovered without sequelae
Melanoma	6 months	Unknown	Alive 9 years after inclusion