Improving clinical management in ANCA-associated vasculitis

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A.A.E. de Joode

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Voor mijn vader

en op de verjaardag van mijn moeder

... We can't pretend we haven't been told. We've all heard the proverbs, heard the philosophers, heard our grandparents warning us about wasted time, heard the poets urging us to seize the day.

Still, sometimes we have to see for ourselves. We have to make our own mistakes.

We have to learn our own lessons until we finally understand for ourselves, what Benjamin Franklin meant:

' That knowing is better than wondering. That waking is better than sleeping. And that even the biggest failure, even the worst, most intractable mistake, beats the hell out of never trying'...

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General introduction.

INTRODUCTION IN VASCULITIS

The systemic vasculitides are rare inflammatory diseases characterized by inflammation of blood vessel walls, possible in any part of the body (1-6). There are several different types of vasculitis. There is an infectious vasculitis where invasion and proliferation of pathogens in vessel walls results directly in inflammation. Another kind is secondary vasculitis, referring to vasculitis that is associated with divers aetiologies like reaction to drugs or chemicals or often occurring as a consequence of other illnesses such as cancer or systemic diseases like rheumatoid arthritis or lupus vasculitis (2,4,6).
Less common are the primary systemic vasculitides, i.e. idiopathic forms, not known to be caused by direct vessel wall invasion by pathogens or related to obvious reasons.
During the Chapel Hill Consensus Conference on the nomenclature of systemic vasculitis in 1994, revised in 2012, an attempt was made to distinguish the primary vasculitides according to vessel wall size into large-vessel-, medium sized-, and small vessel vasculitis (6). The extent of the vasculitis may account for partial stenosis of larger vessels but may also lead to complete stenosis of medium and small arteries, veins and capillaries; the other way around, it may also lead to minor or serious bleeding complications (6). This is important for diagnosis, treatment, but also for the prognosis. Because of the possibility of damage to vessel walls throughout the whole body, the primary systemic vasculitides can affect various organs and the different forms of vasculitis can cause different damage: some are relatively mild and require no treatment, while the other type of disease can cause severe and serious illnesses or even death if not diagnosed promptly and treated appropriately (6).
The large- and medium sized vessel vasculitis are beyond the scope of this thesis; all of the following is focused on small vessel vasculitis.

The small vessel vasculitides can be divided into those associated with antineutrophil cytoplasmic antibody (ANCA) and those not associated with ANCA (6).
ANCA are auto-antibodies that recognize neutrophil and monocyte constituents, the main targets being proteinase 3 (PR3) and myeloperoxidase (MPO); it is estimated that PR3-ANCA and MPO-ANCA are found in >90% of patients with ANCA-associated vasculitis (AAV) (7-10).
Strong in vivo evidence that at least MPO-ANCA is pathogenic was achieved with the development of the first mouse model in which injection of MPO-ANCA induced glomerulonephritis and vasculitis comparable to human disease (11,12). However, this is not reproduced in models that use PR3-ANCA as antigen. Maybe this is one of the reasons that there are meaningful clinical differences, for instance in disease spectrum, between patients with PR3-AAV and with MPO-AAV (6,7,13). In general, patients with PR3-ANCA have more widespread extrarenal organ involvement and more active lesions at time of

presentation, compared to MPO-ANCA who have more chronic lesions. Another important difference is the higher relapse rate in PR3-ANCA positive patients (7,14-16).

Patients suffering from ANCA-associated vasculitis may present with a rapid clinical decline due to life threatening progressive loss of renal or respiratory function, but as mentioned before, the organs that can be affected are widespread. Since the disease can affect various organs, the symptoms can easily be confused with other illnesses (5). Because of this potential rapid deterioration, once suspected, a prompt diagnosis of AAV may lead to instalment (or well based abstention in case of a high probability of a differential diagnosis) of appropriate and effective therapy and maybe could reverse a potential fatal outcome.

The disease can usually be controlled by use of steroids and immunosuppressive drugs, although there is no definite cure for AAV (17,18). Due to the frequent occurrence of relapses, long term maintenance therapy is often required. Induction treatment as well as maintenance therapy are accompanied by serious side effects and although the combined treatment modalities have turned these progressive and often fatal diseases into chronic conditions, mortality and morbidity are substantial, either due to disease itself or the toxicity of prolonged courses of immunosuppressive treatment (17,19).

AIMS AND OUTLINE OF THE THESIS.

This thesis focuses on improving outcome by methods to recognize and correctly diagnose the disease as soon as AAV is suspected. Furthermore, an attempt has been made to identify parameters for prognosis and relapse risk. Knowing these parameters, a proposal for tailor-made therapy is defined to potentially reduce the well-known toxicity of induction treatment, long-term maintenance therapy and especially the re-instalment of aggressive treatment for relapses.
Hopefully, this proposal will enable treating physicians to make a well-funded choice in immunosuppression, leading to a better quality of life and longer life expectancy for individualized ANCA-associated vasculitis patients.

Part 1 of this thesis focuses on tools for prompt diagnosis, with emphasis on the diagnostic performance of two rapid ANCA- and anti-GBM test methods, Dotblot and Phadia ELiA (chapter 2). Furthermore, in chapter 3, predictors for patient and renal survival at diagnosis and after induction therapy in ANCA-associated vasculitis with and without renal involvement are evaluated. In chapter 4, we describe a stepwise immunosuppressive approach, i.e adding plasmapheresis relatively late after diagnosis, in 26 AAV-patients to standard induction therapy in 50 comparable controls. In chapter 5, characteristics of stabilization and remission of renal involvement are described.

Part 2 starts with a full review of all aspects on maintenance therapy with emphasis on relapses and risk factors. Recommendations for choice of agent and duration of maintenance therapy are made for different patient groups and a flow chart for maintenance therapy is added (chapter 6).
In the next two chapters, azathioprine as maintenance agent is evaluated in a national study as well as in a large international cohort. Chapter 7 describes the results of a prospective multicentre clinical trial ('AZA-ANCA' trial), set up to evaluate efficacy and safety of extended azathioprine maintenance therapy in patients with PR3-AAV. The choice for extension of maintenance therapy was based on a retrospective national study which showed that patients with PR3-ANCA who remained c-ANCA positive during treatment, were significantly more prone for relapse during long-term follow up.
In chapter 8, we studied whether in AAV-patients the duration of azathioprine maintenance therapy influenced relapse rate during long-term follow up. For this chapter, we were enabled by European Vasculitis Study Group (EUVAS) and French Vasculitis Study Group (FVSG) to study and evaluate the treatment characteristics and results of 6 international and large studies on AAV-patients.

Finally, in chapter 9, the results of our studies are summarized and they are put into perspective. This is also the place for speculation on future developments.

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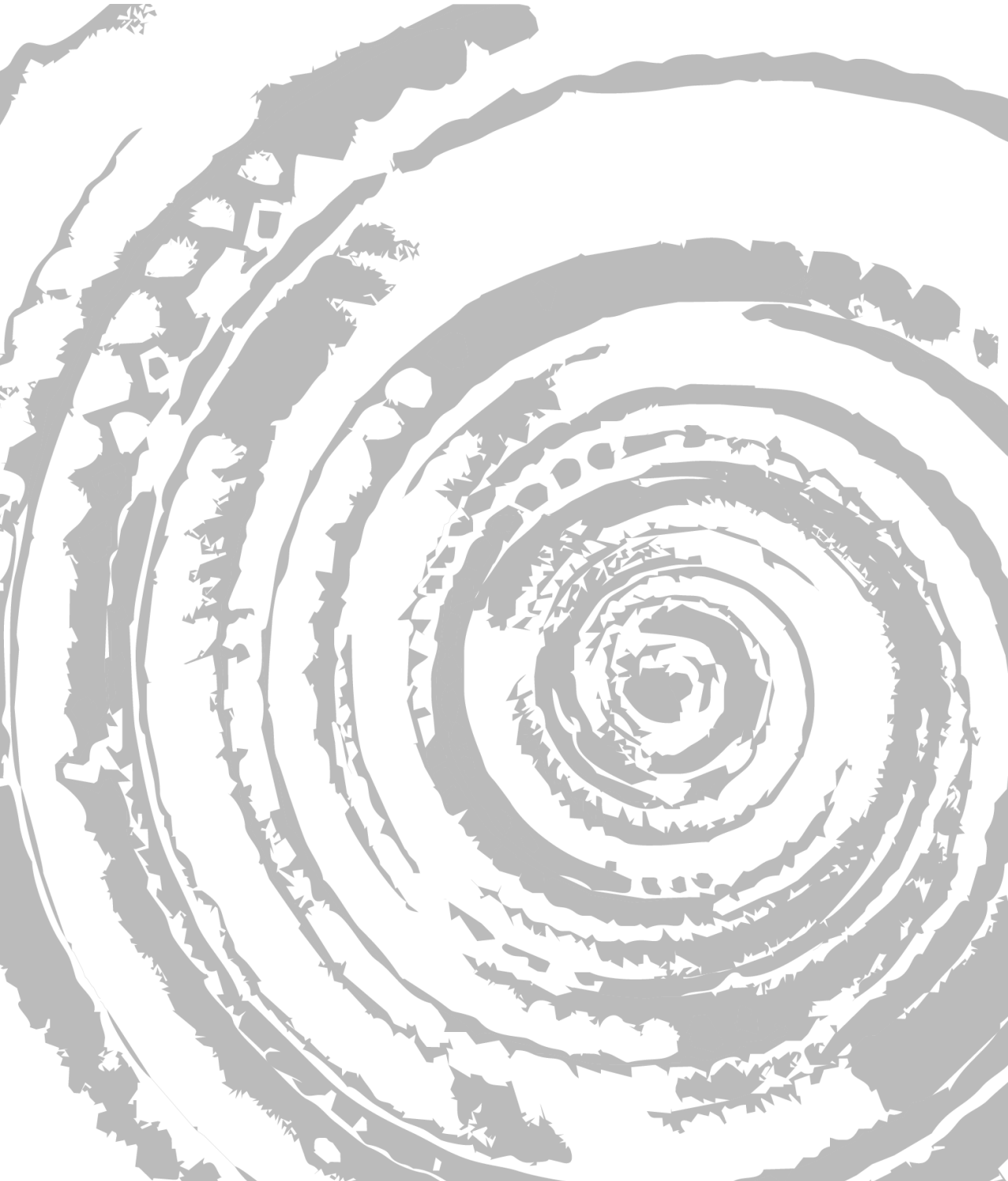

PART 1

2

Performance of two strategies for urgent ANCA and anti-GBM analysis in vasculitis.

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ABSTRACT

Background

In anti-neutrophil cytoplasmic antibodies (ANCA) associated small vessel vasculitis (AAV), rapid testing for ANCA and anti-glomerular basement membrane (GBM) antibodies may be beneficial for therapeutic purpose.

Objective

We analysed the diagnostic performance of two rapid ANCA and anti-GBM test methods in 260 patients with suspected AAV.

Methods

Between January 2004 and November 2010, we analysed 260 samples by qualitative Dotblot (Biomedical Diagnostics); retrospective analysis followed with directly coated highly sensitive automated Phadia ELiA and ELiA anti-GBM.

Results were related to the final clinical diagnosis and compared with routine capture ELISA.

Results

Seventy-four patients had a final diagnosis of AAV (n=62) or anti-GBM disease (n=12). Both Dotblot and ELiA detected all 12 cases of anti-GBM disease; 2 false positive results were found.

Dotblot detected ANCA in 56 of 62 AAV-patients (sens 90%, NPV 97%), and showed 5 false positives (spec 97%, PPV 90%). The Phadia ELiA anti-PR3^{s} or anti-MPO^{s} was positive in 57 of 62 AAV patients (sens 92%, NPV 97%), and had 5 false positives (spec 97%, PPV 88%). Routine capture ELISA was equally accurate (sens 94%, spec 97%, PPV 88%, NPV 98%).

Conclusion

The Dotblot and Phadia ELiA on anti-GBM, anti-PR3s and anti-MPOs performed excellent; results were almost identical to routine ELISA. When suspicion of AAV or anti-GBM disease is high and diagnosis is urgently needed, both tests are very powerful for rapid serological diagnosis. Further studies have to confirm the test performances in samples routinely presented for ANCA-testing and in follow-up of positive patients.

INTRODUCTION

In anti-neutrophil cytoplasmic antibody (ANCA) associated small vessel vasculitis (AAV), patients may present with a rapid clinical decline due to life threatening progressive loss of renal or respiratory function. Because of this possible rapid deterioration, patients who are suspected of AAV may benefit from rapid testing for ANCA and anti-glomerular basement membrane (GBM) antibodies to start immunosuppressive therapy as soon as a diagnosis is serologically supported and to differentiate these diseases from other conditions.

The classical method to detect presence of ANCA is indirect immunofluorescence (IIF) on ethanol-fixed neutrophils which may show a C-ANCA (granular cytoplasmatic) pattern or P-ANCA (perinuclear staining) pattern.[1-3] In patients with small vessel vasculitis (AAV), these patterns are usually associated with antibodies against proteinase 3 (PR3) and myeloperoxidase (MPO) which are strongly associated with the presence of granulomatosis with poly-angiitis (GPA) or microscopic poly-angiitis (MPA).[3-5] However, C-ANCA and P-ANCA as detected by IIF are not equivalent to the presence of anti-PR3 and anti-MPO antibodies respectively, as ANCA with different or unknown antigenetic specificities do occur in other diseases as well.[2,4,8] The diagnosis of ANCA-associated vasculitis is of course made by clinical symptoms and biopsy, so presence of C-ANCA or P-ANCA only gives a clue to the diagnosis. [5] Therefore, it has been agreed that after a positive result on IIF, one should confirm these ANCA with antigen specific enzyme linked immunosorbent assay (direct and/or capture ELISA) to improve sensitivity and especially specificity.[3-7,9] Recently, studies on different strategies have been published: screening with ELISA and confirming positive results with IIF could be as valuable and accurate as the other way around and because of the specificity of newer antigen-specific serological tests, there even has been doubt whether testing for ANCA with IIF is still necessary at all.[4,5] Since early treatment can prevent adverse outcome in systemic small vessel vasculitis, a drawback of both IIF and ELISA (or a combination of tests) is the time needed for obtaining results. Although ELISA has the advantage of being automated and requires less technical experience, the time needed to obtain a result still can be substantial due to various logistical reasons.[4,10]

Routinely, in our laboratory ANCA are tested by IIF followed by an in house antigen-specific capture ELISA.[6] IIF is performed twice a week and the antigen-specific capture ELISA once a week; the results are thus usually not available on the day the sample is taken from the patient. For rapid detection of ANCA, we therefore use a qualitative Dotblot detecting PR3-ANCA, MPO-ANCA and anti-GBM (Biomedical Diagnostics), which has the results available within 2 hours. We prospectively analysed the diagnostic performance of our qualitive Dotblot for rapid assessment of the presence of PR3-ANCA, MPO-ANCA and anti-GBM in a cohort of patients with suspected AAV. We compared the results of this rapid qualitative

test with the final clinical diagnosis and with our standard IIF (C- or P-ANCA), capture ANCA-ELISA, and anti-GBM direct ELISA.[7, 8] In addition, we retrospectively analysed the performance of the novel quantitative Phadia high sensitive ELiA anti-PR3^{s} and anti- MPO^{s} anchor tests which have recently become available for rapid detection of ANCA and anti-GBM antibodies. Also these data were compared to the final clinical diagnosis and our routine serological analysis with IIF and antigen-specific capture ELISA.

METHODS

Patients

Consecutive samples sent to our Laboratory for urgent analysis of ANCA and anti-GBM between January 2004 and November 2010 were included in this study. The requests came from seven different clinical centres, including our own university hospital. The study population included 260 serum samples, taken from 260 patients who were suspected for AVV or anti-GBM related pulmonal-renal syndrome based on various clinical grounds (Table 1). The physician requesting the urgent analysis had to contact the coordinator of the laboratory to discuss the need for urgent determination. We recorded the reasons for these requests. A few weeks later, the physician requesting the urgent ANCA and anti-GBM determination was contacted and asked to provide the diagnosis that had been made. The final clinical diagnosis was based on biopsy results and other additional test results and clinical symptoms and signs. Different forms of small vessel vasculitis were classified using the Chapel Hill Classification criteria with the modification by Watts et all.[12] Finally, at the time of the analysis of the stored samples by the Phadia ELiA the physicians were asked to re-assess the diagnosis at the time the sample for ANCA and anti-GBM was taken and to see whether during follow up a diagnosis of AAV or anti-GBM had been made.

Methods

At the time of request, all samples were tested immediately after arrival at the laboratory by commercially available Dotblot for PR3-ANCA, MPO-ANCA and anti-GBM (MBG Dot, Biomedical Diagnostics, Antwerp, Belgium) performed according to the manufacturer's instructions.[2] Results were available within 2 hours after receipt of the patient's blood sample and reported back by telephone.

In the following days results were confirmed with our standard in house combination of IIF on ethanol-fixed neutrophil slides as described earlier and capture ELISA for anti-PR3 and anti-MPO (all human native antigens PR3 and MPO antigen) and remaining samples were stored (-20˚C).[11,13-16,18] Anti-GBM antibodies were measured in an external diagnostic laboratory (Sanquin, Amsterdam, The Netherlands) using an in house direct ELISA.[17]

Retrospectively, all stored samples were tested with the novel anchor coated highly sensitive (hs) Phadia ELiA (Thermo Fisher Scientific/Phadia, Freiburg, Germany) using human native antigens, performed on a Phadia250 analyser. Reference values were as recommended by the manufacturer. This ELiA-test method was calibrated on international standards available for MPO-and PR3-ANCA and results are in IU/ml. In addition, all samples were tested for ELiA anti-GBM on the Phadia250 analyser (human recombinant antigen).
A Cohen's kappa was used to compare interrater agreement for Dotblot and ELiA data with routine ELISA. For calculation of sensitivity, specificity and likelihood ratios, we compared the serological results obtained by the different methods to the final clinical diagnosis. In case of double positivity for ANCA and anti-GBM antibodies, the best fitting test result of the two in view of the final diagnosis was considered true positive and the other result as false negative.

RESULTS

Patients, reasons for request and diagnoses.
Two hundred sixty patients from 7 different centres were suspected for AAV or anti-GBM, based on one or more clinical findings. Median age of patients enrolled was 64.9 years, interquartile range 45.3-73.6. The following clinical symptoms and signs were the main reasons for the requests for rapid testing: in renal involvement, main cause was loss of or decline in kidney function combined with haematuria, or suspicion of renal disease combined with systemic complaints like myalgia, tiredness or fever. For pulmonary involvement, main reason was respiratory insufficiency, most of the time combined with lesions on chest X-ray or pulmonary CT. Second reason was haemoptysis combined with dyspnoea and coughing and/or systemic complaints.
In addition to specific signs or symptoms suggestive of vasculitic organ involvement, in many patients a presumed non-infectious systemic inflammatory syndrome was the reason for request of rapid testing. Table 1 shows numbers and summary of reasons of request for rapid testing.
Seventy-four patients (28.5%) had a final diagnosis of anti-GBM-disease and AAV. Twelve of these patients had anti-GBM disease, 35 patients were diagnosed with GPA, 21 patients with MPA, 4 with RLV and 2 patients with eosinophilic granulomatosis with poly-angiitis. In one hundred eighty-six patients no diagnosis of AAV and anti-GBM disease was made. The final diagnosis in this latter group varied from specific solitary organ diseases like minimal change disease, Alports syndrome or alveolar haemosiderosis to sepsis and hematologic cancer or solid tumours (Table 2). During follow up, no diagnosis of AAV or anti-GBM-disease in any of these patients was made.

Table 1 Main reasons for rapid testing according to organ involvement: clinical indications for testing ANCA and anti-GBM (most requests were based on combination of symptoms)

Renal	N= 200
Systemic symptoms	N= 126
Pulmonary	N= 94
Neurological	N=29
ENT	N=26
Abdominal	N= 18
Dermal/soft tissue	N= 24
Otherwise (f.i biochemical abnormality)	N=16

ANCA= anti-neutrophil cytoplasmic antibody
Anti-GBM= anti-glomerular basement membrane

Table 2 Clinical outcome other than AAV: definite diagnosis (specified in supplementary material)

Renal	N= 62
Connective tissue disorders	N= 9
Urological	N= 2
Pulmonary	N=19
Infectious disorders	N= 26
Carcinoma	N= 19
Cardial	N= 9
Neurological	N= 2
Unknown	N= 38

AAV=ANCA-associated vasculitis.

Results of Dotblot, IIF, ELISA and Phadia ELiA

With the qualitative Dotblot test, six patients in whom a diagnosis of AAV was made, tested negative for anti-PR3 and anti-MPO antibodies (8%). The final diagnosis was GPA in 2, MPA in 3 and RLV in 1 patient. In the 186 patients in whom no diagnosis of AAV or anti-GBM disease was made, Dotblot was positive in 5 for ANCA (3%): 2 patients were positive for anti-PR3 and 3 patients for anti-MPO. These patients were diagnosed with sarcoidosis (n=2), IgA-nephropathy (n=1), endocarditis (n=1) and renal failure without a final diagnosis (n=1). In addition, one patient with anti-GBM disease was also positive for anti-MPO by the Dotblot test and as this patient did not show findings compatible with additional AAV,

Table 3 Clinical outcomes compared to outcomes of all test methods

	Dotblot	ELISA	IIF	Phadia hs	Phadia anti-GBM
GPA (n=35)	33	33	32	33	
MPA (n=21)	18	20	21	19	
RLV (n=4)	3	3	3	3	
CSS (n=2)	2	2	2	2	
Anti-GBM (n=12)	12	12			12
Others (n=186)	5	6	41	5	

GPA= granulomatisis with poly-angiitis.
MPA= microscopic poly-angiitis
RLV= renal limited vasculitis
CSS= Churg Strauss Syndrome
IIF= indirect immunofluorescence

Table 4 Sensitivity, specificity, positive and negative predictive value for all methods of testing in 260 serum samples of patients suspected for AAV.

	Dotblot	ELISA	IIF	ELiA anti-PR3	ELiA anti-MPO
Sensitivity	90%	94%	94%	93%	92%
Specificity	97%	97%	78%	97%	98%
PPV	90%	88%	59%	86%	87%
NPV	97%	98%	97%	98%	97%

AAV= ANCA-associated vasculitis

it was also deemed to be a false-positive (Table 3 and 4). This resulted in a sensitivity of 90%, specificity of 97%, positive predictive value (PPV) of 90% and negative predictive value (NPV) of 97% for the diagnosis of AAV for ANCA detected by the Dotblot test (Table 4). The likelihood ratio for a positive test was 33.6, for a negative test 0.099.

Dotblot detected all 12 cases of anti-GBM-disease. In one patient, anti-MPO antibodies were also positive (see above). In two patients with MPA, Dotblot showed double-positivity for MPO-ANCA and anti-GBM while no evidence for renal anti-GBM disease was found on renal biopsy, resulting in two false positives. Sensitivity for anti-GBM disease was therefore

100%, specificity 99%, positive predictive value 86% and negative predictive value 100%. Likelihood ratio for positive test 124, for a negative test 0.0. Interrater agreement between Dotblot and ELISA by Cohen's kappa was 0.89 for anti-PR3, 0.85 for anti-MPO and 0.92 for anti-GBM.

Routine standard IIF for ANCA and capture ELISA for anti-PR3 and anti-MPO antibodies showed three and four false negative results, respectively, in patients in whom a final diagnosis of AAV was made. Three of the four patients that were negative in capture ELISA were also negative in Dotblot: these patients were diagnosed as GPA (n=2) and RLV (n=1). IIF was also negative in two of these three patients; the last false negative result in both IIF and capture ELISA was found in a patient who was diagnosed with GPA but was positive for anti-PR3 in the Dotblot (Table 3 and 4)

Capture ELISA tested false positive in 8 patients (4 anti-PR3, 4 anti-MPO): five of these patients were also positive in Dotblot (two with sarcoidosis, one renal failure without final diagnosis, one endocarditis, one anti-GBM disease). The other three patients were diagnosed with tubulo-interstitial nephritis (n=2) and anti-GBM disease (n=1), respectively. For capture PR3- and MPO-ELISA, the sensitivity was 94%, specificity 97%, PPV 88%, and NPV 98% (Table 5).

Although we found a high sensitivity of 94% for IIF, specificity was only 78% (false positive P-ANCA in 18 patients, atypical ANCA in 23 patients, no false positive C-ANCA), resulting in a PPV of 59% and a NPV of 97% (Table 5). Likelihood ratios for positive test were 29 and 4.2 for capture ELISA and IIF respectively, for a negative test 0.067 and 0.083 respectively.

The direct ELISA for anti-GBM was positive in all twelve patients with anti-GBM-disease. In two of these patients, anti-MPO antibodies were also found by capture ELISA (see discussion earlier). In one of the same patients with MPA in whom Dotblot detected anti-GBM antibodies a positive anti-GBM by ELISA was found. This results in a likelihood ratio for a positive test of 248, for a negative test of 0.0.

The retrospective results of the highly sensitive Phadia ELiA test method showed five false negative results; these patients were diagnosed with GPA (n=2), MPA (n=2), RLV (n=1). These were the same patients who were negative in the Dotblot and three that were also negative by capture ELISA and IIF.

The Phadia ELiA hs anti-PR3^{s} and hs anti-MPO^{s} showed 5 false positive results (3 and 4 respectively): again, these were the same patients who were false positive as tested by ELISA and Dotblot (diagnosed with sarcoidosis, endocarditis, tubulo-interstitial nephritis and anti-GBM disease).

All twelve patients with anti-GBM-disease tested positive by Phadia ELiA. In three of these patients, Phadia found a double positivity for anti-MPO antibodies also. In two patients with MPA (the same as were mentioned at results of Dotblot), Phadia was positive for MPO-ANCA and anti-GBM as well. Sensitivity, specificity, PPV and NPV for Phadia ELiA hs anti-PR3^{s} and hs anti-MPO are given in Table 5. The likelihood ratio for positive ANCA was

34.2, for anti-GBM 124; for a negative test, it was 0.083 and 0.0 respectively. Interrater agreement between Phadia and ELISA by Cohen's kappa was 0.88 for anti-PR3, 0.85 for anti-MPO and 0.93 for anti-GBM.

DISCUSSION

ANCA and anti-GBM tests are used to support or dismiss a diagnosis of primary anti-neutrophil cytoplasmic antibody associated systemic small vessel vasculitis, and anti-GBM pulmonary renal syndrome, respectively. Patients with these diseases often present with acute severe disease manifestations and may exhibit rapidly progressive and life threatening organ failure. A rapid diagnosis and instalment of effective therapy may be very important for optimal outcome in these circumstances. In the international consensus statement in 1999, it was agreed that for ANCA testing in "new" patients, screening should be with IIF and confirmation by ELISAs that detect ANCA specific for PR3 or MPO. [3-7,9] In common practice like in our laboratory, a drawback for these methods is the time needed for obtaining results, which is especially important in acute situations. Rapid serologic assays identifying patients who may benefit from more targeted diagnostic procedures and early immunosuppressive treatment can be helpful in these circumstances, given that these rapid assays have sufficient sensitivity and especially specificity. Different assays, including Dotblot or line blot, rapid ELISAs and assays on automated random access analysers are commercially available[10,19,20].

We have tested the performance of two systems available for rapid testing in a clinically highly relevant context, i.e. consecutive patients in whom the physician in charge asked for urgent determination of the presence of ANCA. First of all, our results show that physicians are capable of defining on clinical grounds a sample of patients with a high a priori chance of ANCA associated small vessel vasculitis with a prevalence of nearly 24%, despite an annual incidence of this diseases of only 10-20 per million.[21] In a previous study on 1434 new patient samples routinely send to our laboratory from roughly the same hospitals as the current study, only 51 samples (3.5%) were positive for anti-PR3 and anti-MPO antibodies. [8] Second, our study indicates that both a qualitative Dotblot and a novel quantitative Phadia ELiA anchor ANCA tests perform excellently with high sensitivity and specificity in this population highly suspected of having systemic small vessel vasculitis. The clinical utility of both ELiA and Dotblot was concordant with our standard capture-ELISA and clearly superior to IIF, especially with regard to specificity. It can be concluded that both the Dotblot and the Phadia ELiA both perform excellently in an acute setting with a substantial a priori prevalence of AAV and can be used for diagnostic purposes. Although a negative ANCA-IIF has a high negative predictive value, lack of specificity makes this test, outside screening, only of limited value in this setting. [4,13] However, recently, automated reading

of IIF by pattern recognition software has demonstrated high diagnostic performance for the assessment of ANCA and subjective interpretation or poor laboratory reproducibility seems to be accurate and at least comparable to visual scoring. This may shed a new light on the promising debate about the role of IIF in future ANCA-diagnostics [10,22]

Both Dotblot and ELiA detected all 12 cases of anti-GBM disease, as did the standard direct ELISA. Since specificity (100%) and positive predictive value (86-92%) were high, we considered both ELiA and Dotblot of high clinical use for urgent testing in suspected anti-GBM disease. Our findings of double serum positivity are in concordance with prior literature, since it is known that up to 30% of patients with anti-GBM disease have serum positivity for MPO-ANCA as well.[2,23] As none of the MPO-ANCA/anti-GBM double positive patients as detected by the different serological tests in the 12 patients with anti-GBM disease displayed additional proof of AAV, these cases were considered false positive with respect to the detection of MPO-ANCA. Likewise, MPO-ANCA/anti-GBM double positive patients with a final diagnosis of AAV without features of additional anti-GBM disease were considered anti-GBM false positives, resulting in a specificity and positive predictive value of less than 100% for anti-GBM testing.

Although we included only 260 serum samples, the 74 patients diagnosed with AAV and anti-GBM disease represents a rather large population of PR3-ANCA and MPO-ANCA positive patients, although the prevalence of anti-GBM disease is low due to its rare nature. Still, because of the high sensitivity and specificity demonstrated by the serological tests, our conclusion that rapid serological screening is valuable applies not only for the diagnosis of GPA and MPA, but also for anti-GBM disease. There are more commercially available PR3-ANCA, MPO-ANCA and anti-GBM assays than tested in this study. All these assays may show differences in sensitivity and specificity and therefore, interpretation of test results is dependent on the test system used. International standards for PR3-ANCA and MPO-ANCA are available and the ELiA test method is calibrated on these standards.

A potential problem of comparing our serological data to the final clinical diagnosis is circular reasoning, i.e. the serological results as detected by the Dotblot test were taken into consideration by the clinicians to make a diagnosis. This is especially important since accurate clinical diagnostics are critically important in determining the analytical and clinical importance of a serological test. To rule out as much as possible the possibility that reporting positive or negative test results have led to incorrect diagnoses, we have again contacted all physicians months and sometimes years after serological testing and requested them to review the patient chart and data and to verify and confirm the diagnosis. No cases were encountered where during follow up the diagnosis of AAV or anti-GBM was overturned, nor where any patients initially judged not to have AAV or anti-GBM disease who were later on diagnosed with AAV or anti-GBM disease found.

Finally, it should be mentioned that this study is only relevant for the rapid requests for serologic diagnosis of AAV and anti-GBM disease in new patients presenting with symptoms

suggestive of AAV. Both the positive and negative predictive value of a laboratory test for an uncommon disease vary tremendously among patients with different clinical manifestations and for patient populations with different a priori prevalence of the disease in question.[3,8,21] It is of utmost importance that assays used in clinical practice are validated for their performance in relevant samples and that interpretation of the serological results is done with acknowledgement of the possible differences in disease prevalence. This means that in a population with high pretest-probability for AAV or anti-GBM disease, both rapid tests and the routine tests studied here perform excellently and can be used as a diagnostic tool. However, in a population with an AAV prevalence of 1%-2% or less and for anti-GBM disease even lower, the predictive positive value will be less as even a high specificity in these circumstances will lead to a numerical increase in false positives. The negative predictive value, however, will remain largely intact due to the high sensitivity as indicated by the extreme negative likelihood ratios for a negative test result. Therefore, a clinician has to be critically aware of who is being tested as well as to have insight in the significance of the test results. This will highly improve understanding and interpreting test results and therefore improve treatment of patients suspected for AAV.[8,24]

In conclusion, we show that in a cohort of patients highly suspected for AAV and anti-GBM disease both a qualitative Dotblot assay and a quantitative automated high sensitive ELiA system for the serological detection of anti-PR3, anti-MPO and anti-GBM antibodies have a high positive and negative predictive value and are comparable to standard ELISA systems. Therefore, these rapid serological assays should enable clinicians to make or dismiss a well-funded clinical diagnosis of AAV and anti-GBM disease and lead to rapid institution of appropriate therapy in case of a positive result and further diagnostic evaluation in case of a negative result, respectively.

SUMMARY

We analysed diagnostic performance of two rapid test methods for ANCA and anti-GBM determination in a relevant patient group presenting with suspected small-vessel vasculitis (n=260). Both test methods provided excellent positive and negative predictive value.

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3

Renal survival in proteinase 3 and myeloperoxidase anti-neutrophil cytoplasmic antibody (ANCA) associated systemic vasculitis.

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SUMMARY

Background and objective

This study evaluated predictors for patient and renal survival in patients with ANCA associated vasculitis (AAV) with and without renal involvement.

Design, setting, participants and measurements

There were 273 consecutive AAV patients from January 1990 until December 2007, who were followed until death, loss to follow up or December 2010. Based on organ-involvement, patients were divided into renal (n=212) and nonrenal groups (n=61). The primary endpoint was ESRD requiring for renal replacement therapy (RRT) or renal transplantation or death.

Results

Patient survival was significantly better in nonrenal group compared with the renal group (hazard ratio, 0.55; 95% confidence interval, 0.33-0.92; P=0.02).

In the renal group, renal survival was significantly worse in MPO-ANCA-positive patients (n=65) compared to PR3-ANCA- positive patients (n= 138) (hazard ratio, 2.1, 95% confidence interval, 1.11-3.8; P= 0.01). Of 48 patients who needed RRT at diagnosis, 11 patients (23%) died within 6 months and 14 patients (29%) did not regain renal function.

Of all 23 patients who regained renal function after RRT, 7 patients (30%) were temporarily dialysis independent and needed dialysis later (range 13-63 months). Five patients had a renal relapse in the 6 months before restart of RRT.

Of all 203 PR3-ANCA- positive patients and MPO-ANCA- positive patients with renal involvement, 12 patients (6%) developed ESRD during follow-up. These patients were classified as CKD stage 4 or 5 after initial treatment and eight patients had a renal relapse before becoming dialysis dependent.

Conclusions

AAV-patients with renal involvement who needed RRT had the worst survival probability. In multivariate analysis, the only major determinants for long-term renal survival were renal function at 6 months and renal relapses.

INTRODUCTION

Systemic small vessel vasculitis and necrotizing GN with little or no immune deposits (pauci-immune) is frequently associated with the presence of ANCA against proteinase 3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA)(1-3). It is estimated that PR3-ANCA or MPO-ANCA are found in > 90% of patients with ANCA-associated vasculitis (AAV), with a specificity as high as 98% for PR3-ANCA and of 91% for MPO-ANCA (2,4,5).
Both types of antibodies are associated with granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and renal limited vasculitis (1-3). It is recognized that there are meaningful clinical differences between patients with PR3- AAV and MPO-AAV and it has been suggested that PR3-AAV and MPO-AAV are distinct diseases since PR3-ANCA-positive patients and MPO-ANCA- positive patients demonstrate a different disease-spectrum (2,6,7). In general, patients with PR3-ANCA have more widespread extrarenal organ involvement and more active renal lesions at time of diagnosis compared with MPO-ANCA positive patients who have more chronic lesions (2,8,9). Another important difference is the higher relapse rate found in PR3-ANCA positive AAV-patients, which may be a major prognostic factor for renal survival during long-term follow up (3).
In clinical practice, the differences between PR3-AAV and MPO-AAV could possibly be used for therapeutic diversity in future treatment, such as intensity of treatment or duration of maintenance therapy. Questions also remain about differences in prognosis between AAV without and with renal involvement, especially in those patients who need renal replacement therapy (RRT).
To answer these questions, we retrospectively studied our cohort of patients with systemic AAV to determine the differences between PR3-ANCA-positive patients and MPO-ANCA-positive patients and to evaluate other determinants for renal outcome (renal function at 6 months, relapse, hypertension and proteinuria) during long-term follow up.

MATERIALS AND METHODS

We included all 273 consecutive patients diagnosed and treated with systemic AAV at our centre between January 1990 and December 2007. Patients were followed until death, loss to follow up or December 2010. Median duration of follow up for all patients was 88 months (interquartile range [IQR], 47.5-144.5 months). Thirty-two (12%) patients were lost to follow-up, mainly because of transfer of care to other hospitals. In our cohort, 155 patients (64%) were alive at December 2010.
First, we divided all 273 AAV-patients into groups with (n=212) and without (n= 61) renal involvement at diagnosis and compared clinical characteristics and outcome.

Then, we divided all patients with renal involvement according to ANCA-specificity, i.e. PR3-ANCA (n=138), MPO-ANCA (n=65) and PR3-ANCA- negative patients and MPO-ANCA-negative patients (n=9). In these groups, we compared clinical characteristics and patient and renal outcome according to ANCA-specificity as well as the influence of relapses on renal function.
Finally, we looked for differences in all patients with renal involvement who were in need of RRT at diagnosis (n=48) and compared outcomes with the renal-involvement group who did not need RRT (n=164).

Clinical diagnosis and ANCA-analysis
Based on clinical characteristics, patients were suspected of having AAV and tested for ANCA. When a positive ANCA was found in indirect immunofluorescence (IIF), its specificity was confirmed by antigen specific ELISA, as previously described (10).
When clinically indicated, biopsies were performed. Disease severity at diagnosis and relapse was scored using the Birmingham Vasculitis Activity Score (BVAS)(11).

Definitions
Renal involvement was based on clinical data (active urinary sediment, proteinuria, impaired renal function) or by biopsy.
Need for dialysis at baseline was defined as RRT within 6 weeks after diagnosis.
Relapse was defined as recurrence or new appearance of organ involvement attributable to active vasculitis and requiring increase in or reintroduction of immunosuppression. Renal relapse was defined as a rise in or appearance of new proteinuria and haematuria, a rise in serum creatinine (mg/dl) > 10% with active urinary sediment or renal biopsy showing disease activity. During follow up, renal function was assessed as estimated GFR (eGFR)(ml/min per 1.73m^2) using the Modification of Diet in Renal Disease-formula.

Treatment
Induction treatment consisted of daily oral cyclophosphamide (2 mg/kg, and adjusted for age > 65 years to 1.5 mg/kg) and prednisolone (1 mg/kg; maximal dosage of 60 mg/day). The patients with severe renal involvement (serum creatinine > 5,66, dialysis dependency at diagnosis or progressive disease during the first weeks) received additional therapy with plasma exchange (three times per week for three weeks). Doses of cyclophosphamide were adjusted to maintain the white blood cell count above 4 x 10*9/L. After 4-6 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 2-4 weeks.
During the period 1990-1996, once remission was achieved, maintenance therapy consisted of oral cyclophosphamide with a daily dose tapered by 25 mg every 3 months (n=117). From 1996 onward, patients were switched to azathioprine maintenance therapy

(1.5-2 mg/kg body weight daily) after 3 months of stable remission (n=156). From 1 year after diagnosis azathioprine was tapered by 25 mg every 3 months.

Statistical analysis.

Data were analysed using SPSS16 and GraphPad Prism (version 5.01) software. Values are given as mean ± SD and median (range). Groups were compared using the unpaired t-test or chi-squared test. For paired data, a paired t-test was used. Relapse-free survival was assessed with actuarial survival curves, calculated using Kaplan-Meier estimates for survival distribution. Differences between groups in survival were analysed with the log-rank test. Multivariate backward stepwise Cox proportional hazard analysis with time to death, time to start or restart RRT and relapse as the time-dependent variables was performed to determine the significance of different risk factors in patient and renal outcomes. Other co-variates in our multivariable model were age, sex, ANCA specificity, dialysis at diagnosis, nonrenal relapse, hypertension and proteinuria at 6 months and eGFR at 6 months. A two-sided P-value < 0.05 was considered statistically significant. Decline in renal function was estimated as the slope (ml/min per 1.73m^2) per year of the individual linear regression line of eGFR over time until relapse or end of follow-up. Frequency of relapse was expressed as number of relapses per patient-year.

RESULTS

Patient Baseline Clinical Characteristics

Patients were divided according to presence (n=212) or absence (n=61) of renal involvement at diagnosis (Table 1). Compared with patients without renal involvement at diagnosis, patients in the renal involvement group were older (P= 0.01), and had higher levels of baseline C-reactive protein RP (P= 0.001) and BVAS (P < 0.001). A higher creatinine level at baseline was also found in the renal group.

Within the renal involvement group, we divided the cohorts to differences in ANCA specificity (Table 2). Patients in the PR3 group were more often men (66%) compared with the MPO-group (48%) (P= 0.01). MPO-ANCA- positive patients were older (P= 0.02), and had higher levels of creatinine (P < 0.001) at baseline. Of patients with MPO-AAV, 31% needed dialysis at diagnosis versus 20% in PR3-group (P=0.10). PR3-ANCA- positive patients more often had ear, nose and throat involvement (p < 0.001) and had higher C-reactive protein and BVAS at baseline (p<0.001).

Patient survival

During follow-up, 87 patients (32%) died; cumulative estimated patient survival rates at 1, 5 and 10 years were 90%, 83% and 74% respectively.

Table 1 Clinical characteristics at baseline of 273 patients with AAV: differences between patients without (NR) and with renal involvement (R).

	NR	R	p-value
Number	61	212	
Age (mean)	52 (14)	58 (16)	0.01*
Sex (n)	32	126	0.94
Diagnosis			
GPA (n)	55	132	< 0.0001*
MPA (n)	6	52	0.01*
Renal limited vasc	0	28	
Comorbidity			
Hypertension	1	4	0.90
DM	1	15	0.11
CVD	6	20	0.34
AID**	1	9	0.34
Malignancy	4	15	0.89
ENT (n)	52	124	0.0001*
Pulm (n)	26	40	0.0001*
BVAS med (range)	13 (7-27)	23 (7-48)	< 0.0001*
Creat mean (SD)	0.94 (0.20)	3.63 (3.42)	< 0.0001*
CRP mean (SD)	7.6 (8.3)	10.8 (9.0)	0.001*
Proteinuria (gr/24 hr)	0.09	1.7	< 0.0001*
RRT	0	49***	
Plasmapheresis	0	48	

Abbreviations:
GPA=granulomatosis with polyangiitis, MPA=microscopic polyangiitis, DM=diabetes mellitus, CVD=cardiovascular disease, AID= auto-immune diseases, ENT=ear, nose and throat, BVAS=Birmingham Vasculitis Activity Score, Creat=Creatinine (mg/dL), CRP=C-reactive protein, RRT=renal replacement therapy.

** Concomitant auto-immune diseases:

NR	R	
Morbus Crohn	Morbus Sjogren	1
	Sarcoidosis	1
	Psoriasis	1
	Hypothyroidism	4
	Hyperthyroidism	2

***: 48 PR3- and MPO-positive patients, one PR3- and MPO-ANCA-negative patient.

Table 2 Clinical characteristics at baseline of 212 AAV-patients with renal involvement: differences between PR3-ANCA positive patients, MPO-ANCA positive patients and PR3- and MPO-ANCA negative patients (=ANCA neg) (statistical analysis for PR3 vs MPO).

	PR3	MPO	p-value	ANCA neg
Number	138	65		9
Age (mean)	56 (16)	61 (15)	0.02*	60 (15)
Sex (n)	91	31	0.01*	4
Comorbidity				
Hypertension	3	0	0.23	1
DM	8	7	0.21	0
CVD	10	8	0.24	2
AID**	3	5	0.06	1
Malignancy	12	2	0.14	1
Diagnosis				
GPA (n)	122	7		3
MPA (n)	13	38		1
Renal limited vasc	3	20		5
ENT (n)	106	15	< 0.0001*	3
Pulm (n)	31	8	0.08	1
BVAS med (range)	26 (7-48)	18 (10-32)	< 0.0001*	18 (12-25)
Creat mean (SD)	3.04 (2.84)	4.82 (4.22)	0.0008*	3.54 (2.75)
CRP mean (SD)	13 (9.5)	7.2 (6.5)	<0.0001*	4.3 (4.2)
Proteinuria (gr/24 hr)	1.6	2.4	0.004*	1.4
RRT (n)	28	20	0.10	1
Plasmapheresis (n)	33	15	0.90	0

Abbreviations:
PR3=proteinase 3, MPO=myeloperoxidase, ANCA= anti-neutrophil cytoplasmic antibody, DM=diabetes mellitus, CVD=cardiovascular disease, AID= auto-immune diseases, GPA=granulomatosis with polyangiitis, MPA=microscopic polyangiitis, ENT=ear, nose and throat, BVAS=Birmingham Vasculitis Activity Score, Creat=Creatinine (mg/dL), CRP=C-reactive protein, RRT=renal replacement therapy.

** Concomitant auto-immune diseases:

PR3		MPO		ANCA NEG	
Hypothyroidism	2	Hypothyroidism	2	Hyperthyroidism	1
Psoriasis	1	Hyperthyroidism	1		
		Sarcoidosis	1		
		Morbus Sjogren	1		

Patients without renal involvement showed higher survival rates compared with all patients with renal involvement (hazard ratio[HR], 0.55; 95% confidence interval [95% CI], 0.33-0.92; P= 0.02). The need for RRT at diagnosis accounted for this difference (HR, 0.27; 95% CI, 0.15-0.47; P< 0.001) (Figure 1), because mortality in patients with renal involvement who did not need RRT was not different compared with patients in the nonrenal involvement group (HR, 0.72; 95% CI, 0.38-1.46; P=0.32). In addition, patients who needed RRT were more prone for death due to infectious disease compared with patients who did not need RRT (P = 0.04).

We compared patient survival according to ANCA specificity in all patients with renal involvement (Figure 2). During the first 6 months after diagnosis, a survival disadvantage was found for patients with MPO-ANCA compared with PR3-ANCA (HR, 0.38; 95% CI, 0.22-0.65; P= 0.003). However, in multivariate analysis with inclusion of age at diagnosis, this survival difference was no longer significant (95% CI, 0.35-2.14; P= 0.76). As shown, during follow- up after 6 months, the survival curves are parallel in course.

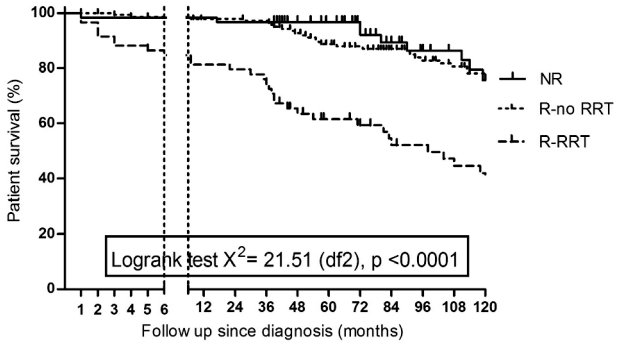
Renal survival

In the nonrenal group, no patient developed ESRD during follow up, despite occurrence of 17 renal relapses in 13 patients in this group (1 MPO-ANCA- positive patient and 12 PR3-ANCA- positive patients).

In the renal involvement group, renal survival was worse in MPO-ANCA positive patients, compared with PR3-ANCA-positive patients and ANCA- negative patients (HR, 2.1; 95% CI, 1.11-3.8; P=0.01) (Figure 3).

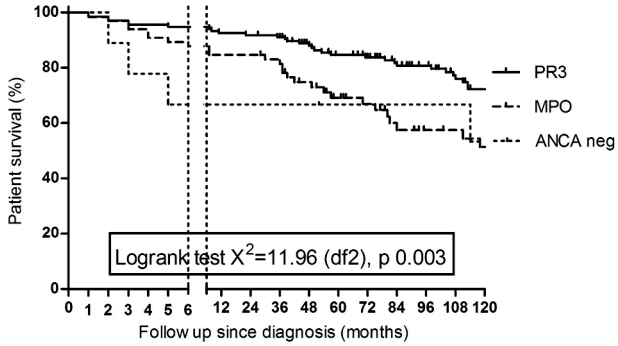
Forty-eight PR3-ANCA- positive patients and MPO-ANCA- positive patients needed RRT at diagnosis. Eleven patients (23%) died within 6 months (2 patients where independent of RRT at time of death) and 14 patients (29%) did not regain renal function at all. Seven patients (15%) regained renal function only temporarily and needed RRT later (range 13-63 months after diagnosis). Sixteen patients (33%) regained and maintained renal function. Of all 164 patients with renal involvement who were dialysis independent at diagnosis, 12 patients (7%) developed ESRD during follow-up and then needed RRT. Further details are given in Figure 4.

Of all PR3-ANCA- positive patients who became dialysis dependent during follow up (n=14), mean eGFR at 6 months was 27 ml/min per 1.73m^2. Thirteen patients experienced relapse in the 6 months before becoming dialysis dependent; only one patient, whose eGFR was 20 ml/min per 1.73m^2 at 6 months, showed a slow decline in renal function during follow up and reached ESRD 178 months after diagnosis. All five MPO-ANCA- positive patients who became dialysis dependent during follow up were classified as CKD stage 4 or 5 after initial treatment. They did not experience relapses, but showed a more rapid decline in renal function (range in slope -1.3 to -4.75 ml/min per year) compared with the mean slope of 0.06 ml/min per year in the other MPO-ANCA- positive patients.



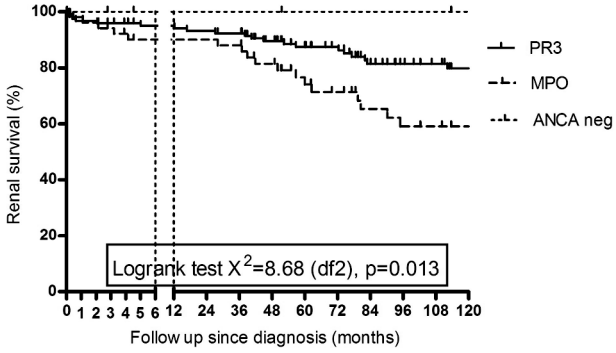
No at risk	0	6	12	60	96	120
NR	61	61	60	48	28	19
R-no RRT	164	142	138	102	77	65
R-RRT	48	39	39	19	11	6

Figure 1 Patient survival in ANCA-associated vasculitis without renal involvement (non-renal=NR) compared to patients with renal-involvement and renal replacement therapy (R-RRT) and renal involvement without renal replacement therapy (R-no RRT)



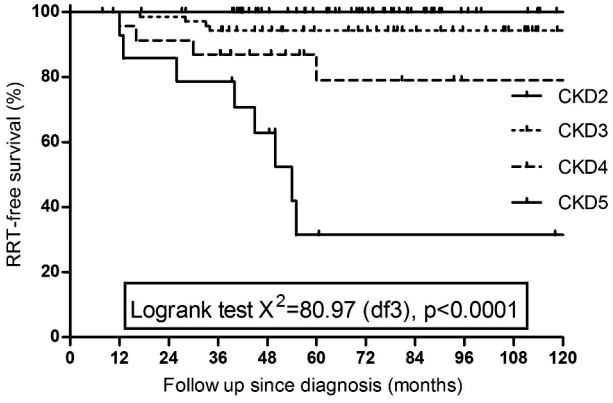
No at risk	0	6	12	60	96	120
PR3	138	128	123	98	75	59
MPO	65	57	56	35	22	18
ANCA neg	9	7	7	6	6	5

Figure 2 Differences in patient survival in PR3-ANCA, MPO-ANCA positive patients and ANCA negative patients (statistical analysis for PR3 versus MPO).



No at risk	0	3	6	12	60	96	120
PR3-ANCA	138	126	113	108	83	58	49
MPO-ANCA	65	54	48	44	30	20	17
ANCA neg	9	8	7	7	6	5	5

Figure 3 Renal survival according to ANCA-specificity in ANCA-associated vasculitis patients with renal involvement.



No at risk	6	12	60	96	120
CKD2	101	101	78	47	37
CKD3	63	60	47	32	22
CKD4	23	23	12	8	8
CKD5	14	14	4	3	2

Figure 4 Renal replacement therapy-free survival according to CKD-stage for all patients with renal involvement alive > 6 months after diagnosis.

These findings indicate that renal function after initial treatment is a major prognostic factor for reaching ESRD (P< 0.001), also shown in Figure 4. In multivariate analysis renal relapses were also significantly associated with the development of ESRD (P=0.02), whereas other covariates were not (Table 3).
Of all 212 AAV patients with renal involvement, 138 patients were PR3-ANCA positive and 65 patients were MPO-ANCA positive at diagnosis.
No statistical difference was found in number of patients who needed RRT at diagnosis between MPO-ANCA- positive patients (31%) versus PR3-ANCA- positive patients (20%) (P= 0.10), or the number of patients who recovered renal function (P=0.29). However, for patients who presented with CKD stage 4 or 5 at diagnosis, recovery of renal function in the first 6 months was less for MPO-ANCA- positive patients (median 8.5; IQR 1.75-15), compared with PR3-ANCA- positive patients (median 12.5, IQR 5-21) (data not shown, Supplemental Figures). For patients with eGFR > 30 ml/min per 1.73m2, recovery was not different. Overall, we found a difference in renal function at baseline and during follow- up (P< 0.001) between MPO-ANCA- positive patients and PR3-ANCA- positive patients with renal involvement (Figure 6). Within both groups, no differences were found comparing eGFR at 6 months and at 5 years. During follow up, 11% of MPO-positive patients had a decline in renal function of 1 CKD-stage, compared with 3% of PR3-positive patients. In those patients who did not experience a relapse, the slope of decline in renal function was -0.06 ml/min per year (SD 3.13) for MPO-ANCA positive patients, compared with a slope of -0.26 ml/min per year (SD 3.18) for PR3-positive patients. Therefore, we concluded that although MPO-ANCA-positive patients present with more advanced renal damage and show less recovery of eGFR after induction therapy, long-term outcome shows little tendency to deterioration to ESRD. Inclusion of patients without renal involvement did not substantially change this finding.

Table 3 Relative risk for renal failure for CKD stage and renal relapse in multivariate analysis.

CKD	RR (95% CI)	p-value
3	ref	
4	16.4 (4.5-59.7)	< 0.0001*
5	43.1 (11.7-158.6)	< 0.0001*
Renal relapse	3.27 (1.24-8.63)	0.01*
Mean arterial pressure (per mmHg)	0.99 (0.96-1.01)	p= 0.29
Proteinuria (per gr/24 hr)	1.14 (0.62-2.1)	p= 0.62

Variables in multivariate analysis: time to death, time to (re)start of renal replacement therapy, relapse as time-dependent variables. Other co-variates are age, sex, ANCA-specificity, dialysis at diagnosis, non-renal relapse, hypertension and proteinuria and eGFR at six months.

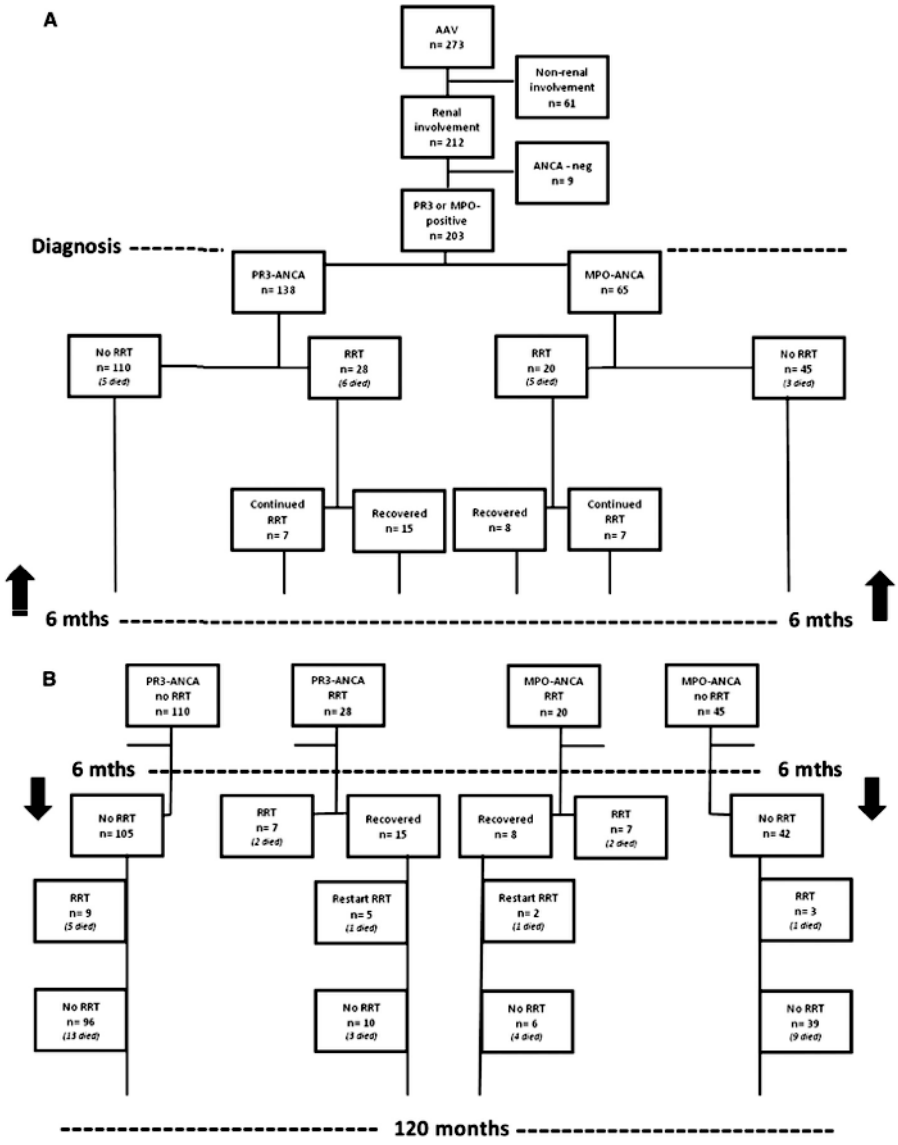


Figure 5A/B Flowchart of renal survival before and after six months of treatment in patients with PR3- and MPO-ANCA-associated vasculitis with renal involvement.

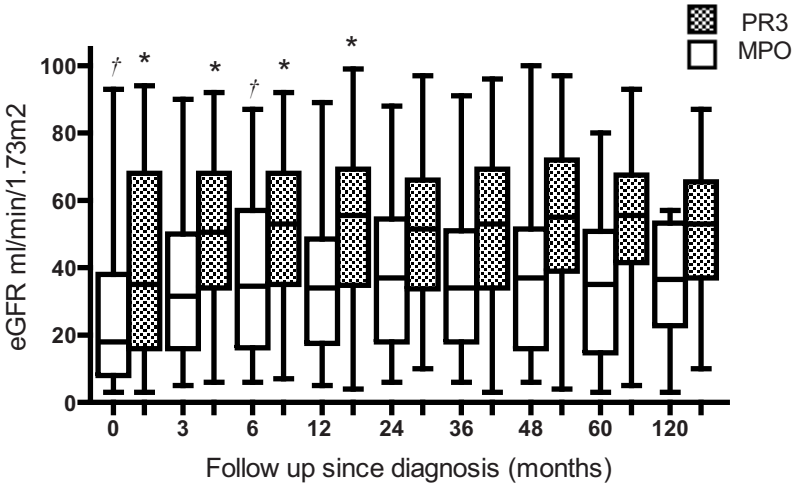


Figure 6 Course in eGFR (ml/min/1.73m2) during long term follow up of patients with PR3- and MPO-ANCA-associated vasculitis with renal involvement at diagnosis.

Relapses

During a follow up period from 6 months after diagnosis to 120 months, 223 relapses occurred, 105 of which were renal relapses (47%). Of 249 patients alive after the first 6 months of treatment, 124 patients (50%) did not experience a relapse and 60 (24%) patients experienced at least two relapse. Overall frequency of relapses per patient-years was 0.10; this frequency was 0.051 during the first year after diagnosis, 0.17 from 1 year to 5 years, and 0.056 after 5 years.

PR3-ANCA-positive patients experienced more relapses than MPO-ANCA-positive patients (P< 0.001, Supplemental Figures 4 and 5). We analysed the influence of these renal relapses on renal function by comparing renal function 1 year before and 1 year after relapse. We found that renal relapse caused a significant loss of eGFR of 8 ± 4 ml/min, (P< 0.001, Supplemental Figures). In addition, a difference in eGFR was found comparing those patients with renal relapses and those without at 120 months after diagnosis (P=0.01) (Supplemental Figure 7).

PR3- ANCA-negative and MPO-ANCA- negative patients

Nine PR3-ANCA-negative patients and MPO-ANCA-negative patients were diagnosed with vasculitis with renal involvement. Four patients died during follow-up, three of them within 6 months after diagnosis. One patient needed for RRT at diagnosis. His kidney function recovered during the first months of treatment but he died due to a severe infection. Of all

six patients who were alive > 6 months after diagnosis, no other patient developed ESRD despite five relapses (three of which were renal relapses) in three patients. During long-term follow- up, the slope of decline in kidney function was comparable with the ANCA-positive patients with renal involvement (data not shown).

DISCUSSION

Although induction therapy for AAV has become standardized currently, questions remain regarding possible individualized treatment strategies for AAV with and without renal involvement and potential differences between PR3-ANCA- positive patients and MPO-ANCA- positive patients (6). In this retrospective study, we evaluated both patient and renal survival in patients with AAV and aimed to report differences between the aforementioned groups.

Overall cumulative survival rates in our cohort were similar to previous studies, with 1-year patient survival of 90% and 10 year survival of 74% (1,2,4). Patients with renal involvement at diagnosis had worse survival outcomes. In addition, severity of renal involvement at diagnosis affected both patient and renal survival. In multivariate analysis a low eGFR at 6 months and renal relapses were associated with reaching ESRD. However, traditional risk factors like hypertension and proteinuria at diagnosis and 6 months had no significant prognostic value.

Patients who needed RRT had worse survival rates, were prone for remaining dependent of RRT and showed a 30% risk for loss of renal function and becoming RRT-dependent if renal function improved after initial treatment. Renal survival was 100% in patients without renal involvement at diagnosis.

In our cohort renal relapse was associated with a median decline of 8 ml/min in renal function, comparable with previously reported data of Slot el al (3). This decline in renal function involves patients with PR3-AAV, in particular because these patients are especially at risk for relapse of disease (2,4,5). However, during long-term follow-up only a small percentage of patients (3%) moved downwards in CKD stage. The fact that almost all PR3-ANCA- positive patients (13 of 14) who became dialysis dependent during follow- up had a relapse before reaching ESRD highlights our finding that relapse is a significant predictor for renal function during follow up.

MPO-ANCA- positive patients presented with worse renal function and higher levels of proteinuria, probably due to advanced chronic damage at presentation. Those patients who presented with CKD stage 4 or 5 also showed less recovery of renal function. Renal survival was thus significantly worse (HR 2.1, 95% CI 1.11-3.8, p=0.01), compared with PR3-ANCA- positive patients. The majority of MPO-positive patients had a marginal decline in renal function during follow up (slope -0.06 ml/min, SD 3,13); however patients who

became dialysis dependent had somewhat steeper slopes (especially a mean eGFR of only 14.5 ml/min per 1.73m^2 at 6 months after diagnosis). Because MPO-ANCA- positive patients seldom experience relapses, this underscores our finding that renal survival is also predicted by a marginal or low eGFR at 6 months after diagnosis.

Our study has several limitations. First, this is a retrospective analysis of a single centre cohort, encompassing > 2 decades of clinical observation. Renal biopsies were not undertaken in all patients and information on comorbidities prior to presentation is scarce. Although induction therapy was standardized, maintenance regimes and treatment of traditional risk factors like proteinuria and hypertension at baseline and at 6 months were not. Our population consists mainly of Caucasians and geographically, it includes more PR3-postive than MPO-positive patients. However, to our knowledge, this is one of the largest series of long-term follow- up in AAV especially focused on differences between PR3-ANCA-positive patients and MPO-ANCA- positive patients.

CONCLUSION

PR3-AAV and MPO-AAV present as different entities at diagnosis because MPO-ANCA-positive patients are older and have higher serum creatinine at baseline. Both factors are of prognostic value for patient survival as well as renal survival in AAV (12). During follow up, PR3-ANCA- positive patients show a good recovery in renal function during the first 6 months and although relapses occur frequently, they seldom reach ESRD. In contrast, MPO-ANCA- positive patients present with worse renal function and have less recovery of renal function, independent of hypertension or proteinuria. Therefore, although relapses are scarce, they still result in ESRD.

Thus, although presentation and disease course are different, renal function at diagnosis and especially the regained renal function after initial treatment at 6 months are major predictors for renal survival in both groups, next to renal relapses. In particular traditional risk factors as hypertension or proteinuria at 6 months seemed not to be associated with reaching ESRD and becoming dialysis dependent.

On the basis of our observations, it seems to be important to regain as much renal function as possible after diagnosis has been made. Therefore, one could argue to expand treatment options when response to standard initial treatment is limited, for instance by adding plasmapheresis.

Supplementary material provided, showing

- Table 4: Causes of death during long term follow up in ANCA-associated vasculitis patients without renal involvement (NR) versus renal involvement (R) and in renal involvement with renal replacement therapy (RRT) versus without (no RRT).
- Figure 7: Differences in development of proteinuria between PR3- and MPO-ANCA associated vasculitis patients during long term follow up.
- Figure 8: course in eGFR (ml/min/1.73m2) in first six months after treatment of patients with PR3- and MPO-ANCA-associated vasculitis with renal involvement at diagnosis.
- Figure 9. Number and characteristics of relapses
- Figure 10: Relapse free survival according to ANCA specificity
- Figure 11: Delta eGFR (ml/min/1.73m^2) per patient per renal relapse
- Figure 12: Delta eGFR (ml/min/1.73m^2) during long-term follow up: difference between renal relapse versus no renal relapse.
- Figure 13: Recovery in eGFR (ml/min/1.73m2) in first six months of follow up: difference between MPO and PR3 for CKD stage IV and V.

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SUPPLEMENTARY MATERIAL

Table 4 Causes of death during long term follow up in ANCA-associated vasculitis patients without renal involvement (NR) versus renal involvement (R) and in renal involvement with renal replacement therapy (RRT) versus without (no RRT).

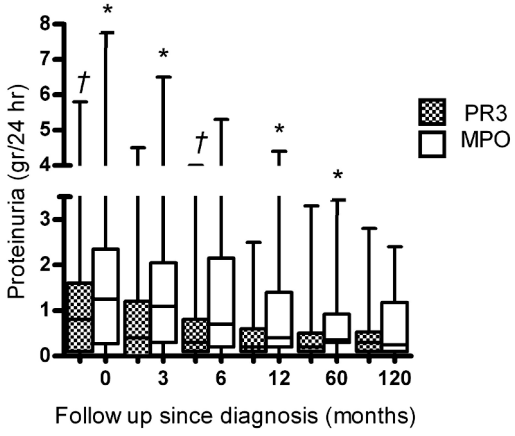
	Non renal	Renal	RRT	no RRT
Number	61	212	61	151
Number of death	11	76	34	42
Active vasculitis	0	4	2	2
Infectious	2	27*	13	14**
Cardiovascular	1	11	5	6
Malignancy	2	6	4	2
Others (incl ESRD)	1	10	5	5
Unknown	5	18	5	13

* p=0.05

** p= 0.04

Abbreviations:
ESRD=end stage renal disease.

Figure 7 Differences in development of proteinuria between PR3- and MPO-ANCA associated vasculitis patients during long term follow up.



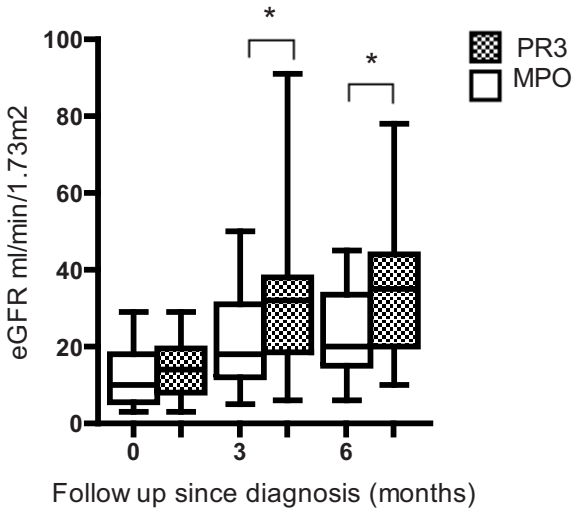


Figure 8 Course in eGFR (ml/min/1.73m2) in first six months after treatment of patients with PR3- and MPO-ANCA-associated vasculitis with renal involvement at diagnosis.

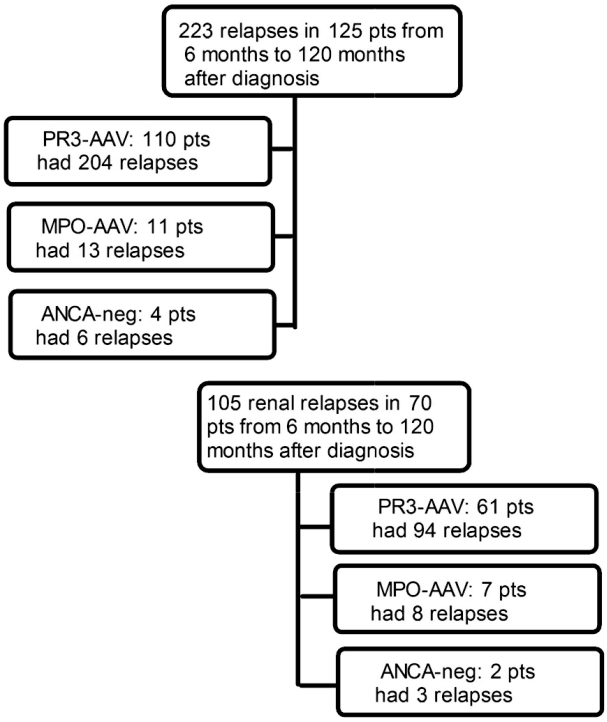
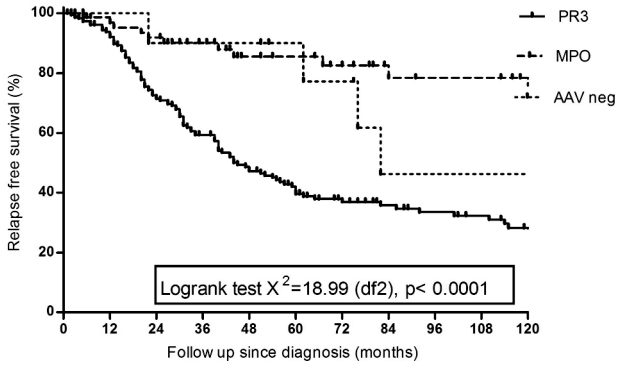


Figure 9 Number and characteristics of relapses.



No at risk 0	6	12	60	96	120	
PR3	138	123	114	36	20	13
MPO	65	56	53	31	18	15
ANCA neg 48	37	34	16	8	3	

Figure 10 Relapse free survival according to ANCA specificity

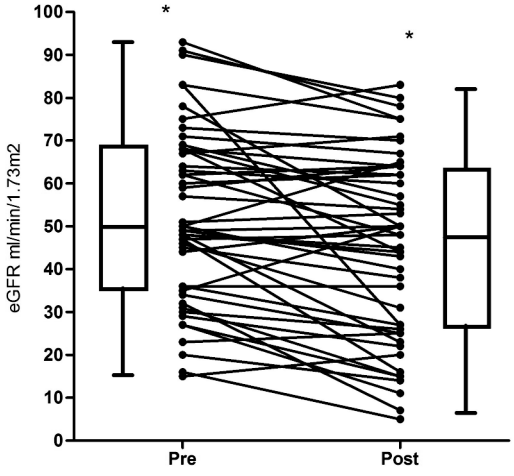


Figure 11 Delta eGFR per patient per renal relapse

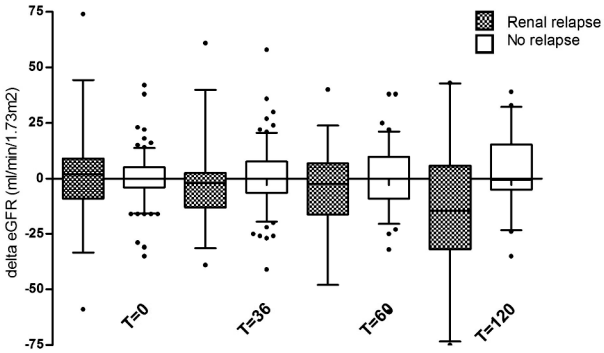


Figure 12 Delta eGFR (ml/min/1.73m^2) during long term follow up: difference between renal relapse versus no renal relapse.

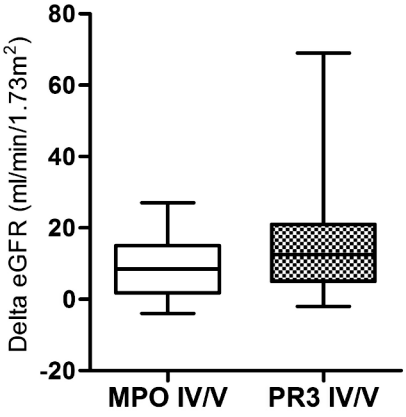


Figure 13 Recovery in eGFR (ml/min/1.73m2) in first six months of follow up: difference between MPO and PR3 for CKD stage IV and V.

4

Plasmapheresis rescue therapy in progressive systemic ANCA-associated vasculitis: single-centre results of stepwise escalation of immunosuppression.

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ABSTRACT

Objective

we evaluated 26 patients with antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) with progressive disease despite treatment with cyclophosphamide and steroids treated with additional plasmapheresis and compared outcome with 50 matched-disease controls.

Methods

patients diagnosed with AAV and treated with cyclophosphamide from January 1990 until December 2009 (n= 272) were included when plasmapheresis was not started at diagnosis but added for progressive disease during initial standard therapy (n=26). We selected controls equal for age, Birmingham vasculitis activity score and creatinine at diagnosis. Primary endpoint was estimated glomerular filtration rate (eGFR) or death.

Results

plasmapheresis was added 18 days (range 5 to 41) after start of therapy. In 11 patients, a rise in serum creatinine > 30% led to plasmapheresis; insufficient response to induction (n=11), progressive pulmonary disease (n=3) or progressive necrotic lesions (n=1) were other indications. In the plasmapheresis group, six patients needed renal replacement therapy (RRT), and three controls. Five years after diagnosis, four patients had died in the plasmapheresis group against eight controls (P=0.94).

At baseline, mean eGFR was 44 ml/min/1.73m2 in plasmapheresis group versus 43 ml/min/1.73m2 in controls. At start of plasmapheresis, eGFR was 26 ml/min/1.73 m2 (P=0.003), at 6 months mean eGFR had significantly improved to 44 ml/min/1.73m2 (P=0.0003), comparable to eGFR in controls, 48 ml/min/1.73m2. During long-term follow-up there was no difference in renal function between the groups.

Conclusion

AAV patients with progressive disease despite standard induction therapy in whom plasmapheresis was added, had significant improvement in renal function and similar long-term outcome in both renal survival and patient survival as matched disease controls.

INTRODUCTION

Antineutrophil cytoplasmic autoantibody (ANCA) associated vasculitides (AAV) are rare, potentially fatal diseases, usually with multi-organ involvement. Nowadays, therapy with cyclophosphamide and prednisolone results in disease remission in 80-90% of patients; however, those patients who present with advanced renal failure or pulmonary haemorrhage have significantly worse outcomes (1-3). Thus, in these patients stepwise introduction of more aggressive immunosuppressive induction therapy might be beneficial.
As ANCA are nowadays deemed pathogenic, their removal by plasmapheresis is rational (1-6). Elimination of circulating ANCA may stop the pathological process until corticosteroids and cyclophosphamide suppress inflammation and autoantibody production (4-6). In addition, removal of other pro-inflammatory factors could contribute to and augment the clinical effects of plasmapheresis (1-4,6-8).
Although plasmapheresis has been shown to be of benefit in patients with rapidly progressive glomerulonephritis caused by anti-glomerular basement membrane (GBM)-antibodies and in vasculitis patients with pulmonary haemorrhage, evidence for the use of plasmapheresis in AAV is limited (1,3,5,8). Based on previous studies, present recommendations for plasmapheresis or plasma exchange in vasculitis are limited to severe renal disease with creatinine > 566 umol/d (although one study suggested to start earlier with creatinine levels > 2.85 mg/dl based on positive effect on preservation of renal function), dialysis dependency and pulmonary haemorrhage or disease refractory to traditional therapy (1,3-8).
In our hospital, plasmapheresis is also used as rescue therapy in AAV patients when response to initial induction therapy is insufficient and when clinical condition or renal function worsens due to on-going vasculitis disease activity. We retrospectively evaluated a single-centre cohort of AAV patients undergoing plasmapheresis for so-called 'extended criteria' to evaluate outcome of these patients.

PATIENTS, MATERIALS AND METHODS

Patients

Between January 1990 and December 2009, 272 newly diagnosed patients with AAV started induction therapy with cyclophosphamide and prednisolone. In 22 patients, plasmapheresis was added to initial induction treatment as these patients presented with severe renal failure (dialysis dependency and/or serum creatinine > 566 umol/dl) or pulmonary haemorrhage. In 26 of the remaining 250 patients, plasmapheresis was added as rescue therapy following an unsatisfactory response to initial induction therapy with cyclophosphamide and prednisolone with persistent vasculitic activity and progressive clinical signs or symptoms. From the remaining cohort (n=224), we selected two controls

that were equal in age (range ± 5 years), Birmingham vasculitis score (BVAS; range ± 5) and serum creatinine (range ± 50) at diagnosis for every patient who received plasmapheresis as rescue therapy. Two plasmapheresis rescue patients could only be matched to one instead of two controls (due to age at diagnosis and limited number of patients in that age range), resulting in a control group of only 50 instead of 52 patients.

Patients data and follow-up

When at diagnosis, a positive ANCA was found in indirect immunofluorescence (IIF), its specificity was confirmed by antigen-specific enzyme-linked immune-absorbent assay (ELISA) (9). Different forms of AABV were classified using the Chapel Hill Classification criteria with the modification of Watts et al (10-12). After diagnosis, all patients were followed intensively with frequent evaluation of disease activity, complications and other events, whether admitted to the hospital or as outpatient. Baseline data included renal function (serum creatinine, creatinine clearance, proteinuria, urinary sediment), C-reactive protein (CRP), ANCA and assessment of BVAS. Monitoring of therapy response included also these latter measurements which were performed at least weekly for the first 6-8 weeks. Data extracted from the patients records included the baseline data (start of therapy), the data at the moment of start of added plasmapheresis (and the matching time point in the matched controls) and every 3 months during the first year and yearly thereafter.

Renal function was assessed as eGFR (ml/min/1.73m2) using the modification of diet in renal disease (MDRD formula).

During follow-up, complications possibly related to therapy were recorded. Relapses of vasculitic disease activity were assessed as time of relapse was defined as the moment institution of renewed/intensified immunosuppressive therapy for active vasculitis disease. All patients were followed until death (n=24), loss of follow up (n=2) or June 30th 2012 (n=246).

Treatment: immunosuppressive protocol and plasmapheresis.

Induction treatment consisted of oral cyclophosphamide (2 mg/kg, adjusted to 1.5 mg/kg in case of age > 65 years or signs of bone marrow insufficiency) and prednisolone (1 mg/kg; maximum dose 60 mg/day). In both the groups, five patients with severe disease at diagnosis were also treated with intravenous high-dose methylprednisolone (1000 mg) during three consecutive days. Doses of cyclophosphamide were adjusted to maintain the white blood cell count above 4 x 10*9/L. Biopsies were only performed when diagnosis was doubtful or when no response to treatment was observed. After 4-6 weeks of stable remission, in both groups the prednisolone dose was tapered by 10 mg every 2 weeks until the dose reached 30 mg and thereafter by 5 mg every 2-4 weeks.

Plasmapheresis (45 ml/kg/day), filtration method, was performed against albumin 5% every other day for nine sessions in 3 weeks in total (6,13). As fresh frozen plasma is far

more expensive, has potential transmission/contamination risks and no proven advantage, it was only used instead of albumin 5% to restore clotting factors in case of bleeding complications or planned invasive procedures. When no response was observed and a (second) renal biopsy showed on-going active disease, another nine plasma exchanges in 3 weeks followed.
During 1990-1996, maintenance therapy consisted of oral cyclophosphamide with tapering of the daily dose by 25 mg every 3 months starting after 3 months of stable remission on cyclophosphamide (n=117). From 1996, patients were switched to azathioprine maintenance therapy (1.5-2 mg/kg body weight daily) after 3 months of stable remission on cyclophosphamide (n=165). From 1 year after diagnosis, azathioprine was tapered by 25 mg every 3 months.

Statistical analysis.
Groups were matched in 2: 1 manner. Data were analysed using SPSS16 and Graph Pad Prism version 5.01. Values are given as median and range. Groups were compared using unpaired t-test or chi-squared test. For paired data, a paired Student's t-test was used. Relapse-free survival was assessed with actuarial survival curves, calculated using Kaplan-Meier estimates for survival distribution. Differences between groups in survival were analyzed with log-rank test. A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Clinical characteristics and renal course in plasmapheresis group.
Mean time to start plasmapheresis as rescue therapy after diagnosis was 18 days (range 5-41); most patients underwent nine plasmapheresis sessions (range 4-18). Of the 26 patients who started plasmapheresis, three patients had to be treated because of worsened pulmonary symptoms, one patient started plasmapheresis for worsening symptoms of necrotic acra and the remaining 22 patients started because of insufficient response or deterioration in renal function. In 15 of these patients, a renal biopsy showed on-going vasculitis-activity, while in seven patients a renal biopsy could not be performed due to contraindications; six of these 22 patients needed renal replacement therapy (RRT) when renal function deteriorated.
Mean eGFR in all the 26 patients decreased from 44 ml/min/1.73m2 at time of diagnosis and start of standard induction therapy to 26.4. ml/min/1.73m2 ($P= 0.003$). After starting plasmapheresis, eGFR improved to 43.3 ml/min/1.73m2 at 6 months after diagnosis and 48.4 ml/min/1.73 m2 at 12 months after diagnosis. During long-term follow-up, eGFR did not decline: 5 and 10 years after diagnosis mean eGFR was 60 and 54 ml/min/1.73 m2, respectively.

Of the six patients who started RRT, five patients became dialysis-independent within 3 months after diagnosis following addition of plasmapheresis rescue therapy and only one continued RRT. During long-term follow-up, two additional patients developed end stage renal disease (ESRD); one of them had been treated with RRT during the first month after diagnosis.
Two patients died within 4 months after diagnosis and treatment with plasmapheresis, both due to septic condition and respiratory insufficiency caused by pulmonary aspergillosis and pneumocystis jirovecii pneumonia (as an exception to our local protocol, no prophylaxis had been initiated because of a known allergy to co-trimoxazole).

Plasmapheresis versus controls

Baseline

Clinical characteristics of patients in plasmapheresis group and controls are given in Table 1.
Only mean CRP levels at baseline differed significantly between both groups, 143 vs 105 mg/l (P=0.045). Granulomatosis with polyangiitis and microscopic polyangiitis were equally distributed over the groups. Twenty-one patients (81%) in plasmapheresis group were proteinase 3 (PR3)-ANCA positive against 33 controls (66%).

Mortality

During long-term follow up, 27 patients (36 %) died, 6 (23 %) in the plasmapheresis group and 21 (42 %) in the controls (P=0.10). Mortality in the plasmapheresis group at 1, 5 and 10 years was 92%, 80% and 72% respectively and 88%, 83% and 67% in the controls ([HR 0.81; 95%CI 0.31-2.00; P=0.66] for death during long-term follow-up, Fig 1). No difference in causes of death were found between the groups (Supporting Information). Patients in the plasmapheresis group had no higher risk for dying of infectious diseases (P=0.49).

Renal function

At diagnosis mean eGFR in both groups did not differ, 44 ml/min/1.73m2 (range 12-122) in plasmapheresis group and 43 ml/min/1.73m2 (range 8-110) in controls (Fig.2). In the plasmapheresis group, mean eGFR significantly deteriorated from baseline to time of start plasmapheresis to 26.4 ml/min/1.73m2 (range 9-110) while in the same period, eGFR in controls had slightly improved (P=0.0004).
After treatment expansion with plasmapheresis, renal function improved significantly in the plasmapheresis-group and became comparable to renal function in controls (Fig. 2). Also long-term renal function remained comparable in both groups.
Six patients in the plasmapheresis group as well as three controls had to start RRT. During long-term follow-up, two patients in the plasmapheresis group reached ESRD, while only one control started RRT.

Table 1 clinical characteristics at baseline: differences between 26 patients treated with plasmapheresis and 50 controls (C).

	Plasmapheresis	Controls	p-value
Number (n)	26	50	
Age mean (range	56 (26-81)	56 (20-78(	0.95
Sex (m): nr (%)	16 (62)	28 (56)	0.64
Diagnosis			
GPA: n	20	31	0.73
MPA/NCGN: n	5/1	13/6	0.43/0.24
ANCA-specificity			
PR3: n	21	33	0.18
MPO: n	5	14	0.40
ANCA neg: n	0	3	0.20
ENT: n (%)	15 (58)	43 (86)	0.37
Pulm: n (%)	13 (50)	27(54)	0.74
Ren: n (%)	26 (100)	47 (94)	0.20
Creat: mean (range)	224 (54-431)	221 (40-483)	0.52
Creat < 125: nr (%)	10 (38%)	19 (38%)	0.92
Creat 125-299: nr (%)	7 (27%)	15 (30%)	0.83
Creat >300: nr (%)	9 (35%)	16 (32%)	0.77
BVAS: mean (range)	20 (12-32)	22 (15-30)	0.83
CRP mean (range	143 (29-312)	205 (8-307)	0.045

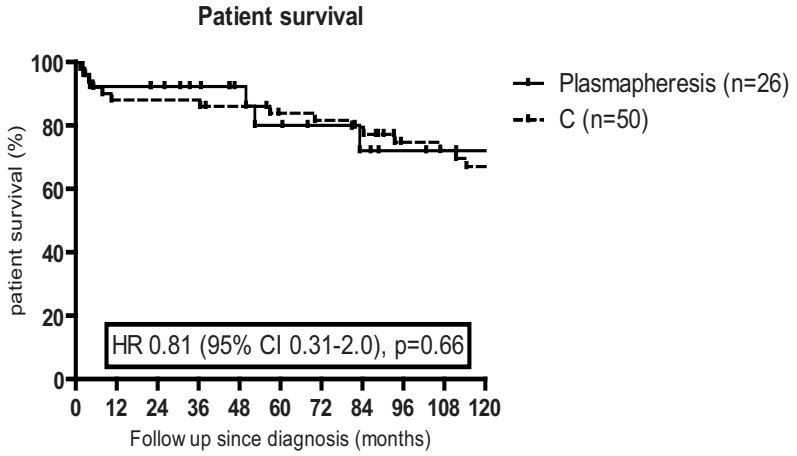
GPA: granulomatosis with poly-angiitis, MPA"microscopic poly-angiitis, NCGN: necrotizing and crescentic glomerulonephritis. PR3: proteinase 3, MPO, myeloperoxidase, ENT: ear nose and throat, Pulm: pulmonary, Ren: renal, Creat: serum Creatinine.

Relapses, Course in ANCA-titre and CRP

Fifteen patients (58%) in plasmapheresis group and 20 patients (40%) in controls experienced a relapse (P=0.009; HR, 2.9; 95% CI, 1.3-6.4) (Fig 3).

At baseline, 100% of patients in plasmapheresis group and 94% in controls were ANCA positive. Significantly more patients became ANCA-negative in plasmapheresis group compared to controls at 3 and 6 months (P<0.0001)(Fig 4). Beyond 6 months, ANCA titres became and remained positive in >50% of patients, irrespective of former treatment.

As CRP is regarded as a marker for active disease, its course was evaluated during and after the start of plasmapheresis. In both groups, the highest CRP levels were found at baseline. In the controls, levels of CRP declined and remained stable during follow-up, while in the plasmapheresis group, CRP decreased to a mean value of 43 mg/l at start of



No at risk	0	6	12	60	96	120
Plasmapheresis	26	24	24	13	7	5
C	50	46	44	38	29	26

Figure 1 Patient survival in months during long term follow up.

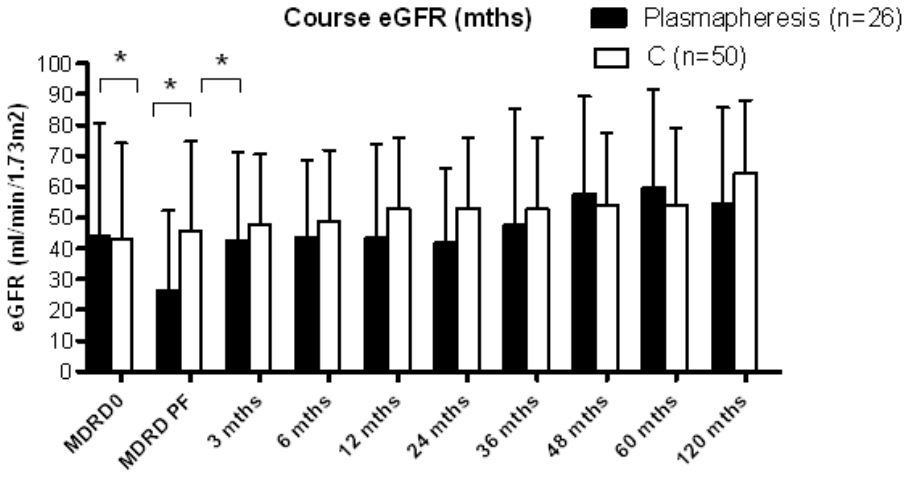
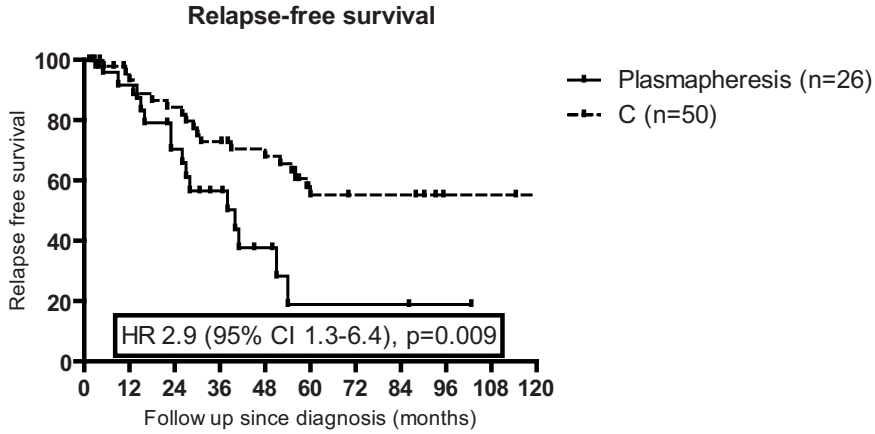


Figure 2 Renal function in MDRD during long term follow up.



No at risk	0	6	12	60	96	120
Plasmapheresis	26	23	22	2	1	0
C	50	45	42	21	15	14

Figure 3 relapse-free survival in months during long term follow up.

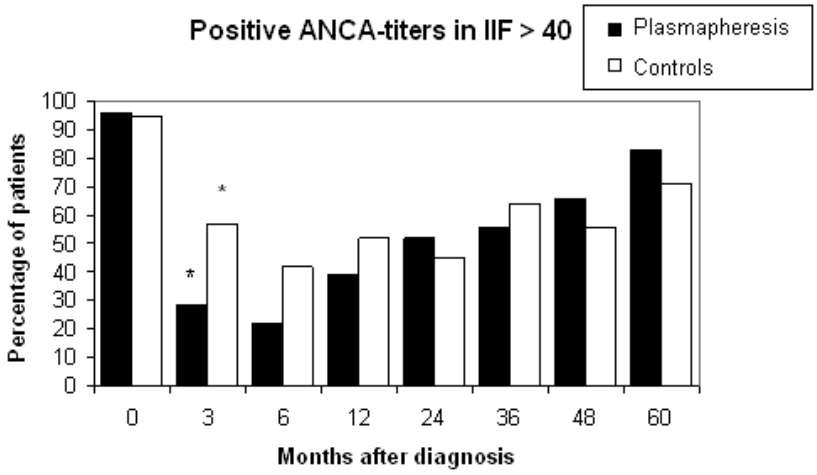


Figure 4 Percentage of patients with positive ANCA titres in IIF after diagnosis (months)

plasmapheresis and fluctuated at low levels over the following weeks. Given the course of clinical parameters and renal function, this may have been related to infectious episodes and not due to active vasculitis.

Adverse events: infectious diseases and others.

All adverse events are given in Table II. Nearly all infectious events occurred within the first month after starting treatment (35 periods, bacterial n=16, viral n=19). No significant difference for bacterial infections was found between the groups (P=0.62), while a trend was found toward more overall viral infections in the plasmapheresis group (P=0.07). For CMV-infections alone, the difference was significant (P=0.03).

Leucopenia was the most prevalent other adverse event in plasmapheresis group which was both statistically significant for mild (< 4.0* 10E9/L) leucopenic episodes (P=0.010) as well as severe (<1.5*10E9/L; P=0.001) compared to controls.

Table 2 Adverse events within 3 months after diagnosis for plasmapheresis- and control-groups.

	Plasmapheresis	Controls	p-value
Number (n)	26	50	
Infectious events	15	17	0.053
Bacterial: n (%)	6 (24)	9 (19)	0.62
Viral: n (%)	9 (36)	8 (17)	0.07
CMV: n (%)	6 (24)	3 (6)	0.03
Leucopenia: n (%)	21 (84)	25 (53)	0.0096*
Severe (< 1.5): n (%)	8 (32)	2 (4)	0.0012*
Diabetes mellitus: n (%)	3 (12)	4 (9)	0.63
Gastro-intestinal: n (%)	5 (20)	1 (2)	0.009*
Thrombosis: n (%)	4 (16)	4 (9)	0.034*
Haemorrhages: n (%)	1 (4)	3 (6)	0.067
Others: n (%)	6 (24)	2 (4)	0.011*

DISCUSSION

Plasmapheresis has been shown to be of benefit in AAV with renal failure and in patients presenting with alveolar haemorrhage (1-8). Two recent studies from Denmark reported better short-term and long-term renal survival in AAV patients who were treated with plasmapheresis added to standard immunosuppressive therapy at diagnosis (7,14).
Our study aimed to proof benefit of addition of plasmapheresis in patients who did not qualify for plasmapheresis therapy at diagnosis but deteriorated clinically while being treated with standard immunosuppressive treatment: until today, only anecdotal evidence existed for this stepwise approach (7).
In our cohort, plasmapheresis was started on average 18 days after diagnosis and start of standard therapy and this addition changed the course of disease. Renal function improved in almost all patients after starting plasmapheresis, was comparable to the controls at 6 months after diagnosis and followed the same course during long-term follow- up, even when added up to 5 weeks after diagnosis and initial treatment. Within 3 months after diagnosis, five of six patients who started RRT were independent of RRT. During long-term follow- up, only two patients reached ESRD.
Surprisingly, relapse-free survival was higher comparing the plasmapheresis group to controls, while maintenance therapy did not differ between the groups. Maybe this was caused by over-representation of PR3-positive patients, well known for higher relapse rates, in the plasmapheresis group (15). However, because we only studied a relative small number of patients, further studies need to be done to verify whether the population of patients selected for plasmapheresis indeed experience a higher relapse rate.

In the plasmapheresis-group, ANCA-levels decreased faster than in the controls, but increased again after 6 months. One could assume a rebound effect of increased production of auto-antibodies after removal, but this should be prevented by cyclophosphamide and prednisolone. Maybe the initial worsening course of disease in the plasmapheresis group suggests a more aggressive phenotype, with faster decline of ANCA after start plasmapheresis but a rebound of ANCA production after the end of this therapy and also more relapses. If this is true, increasing the total burden or lengthen the course of immunosuppressive therapy would maybe result in less frequent relapses.
One could probably attribute part of the adverse events (i.e. infections or haemorrhages) to the immunosuppressive regime and plasmapheresis itself. However, the combination of both therapies was not at the expense of more infectious adverse events. Although a trend was found toward more viral infections in the plasmapheresis group, especially in CMV-infections (P=0.03) during the first month of treatment, no significant differences were found between plasmapheresis group and controls. Other serious adverse events

did occur: more episodes of leucopenia were found in the plasmapheresis group, which more often led to an adjustment in immunosuppressive therapy.
Our study has limitations. It is a retrospective analysis, encompassing more than two decades of clinical observation in which treatment regimens and start of plasmapheresis were not yet fully standardized. In our hospital, biopsies are not taken routinely and only performed when diagnosis is unclear. Data on plasmapheresis and course of clinical symptoms or laboratory parameters may ne underreported.
We compared the plasmapheresis group to controls, in which the patients were broadly equal in age, creatinine and BVAS at diagnosis. These groups were not randomized and had a different initial disease course. Furthermore, the groups are small and in the subgroups even smaller. Therefore, we do recognize that comparing these groups has some major limitations. Nevertheless, this is the one of the first studies to address the significance of plasmapheresis in AAV especially without renal failure, added in the course of disease when response to standard induction therapy is lacking.
Future randomized studies should identify whether patients with AAV would benefit from adding plasmapheresis without renal failure or alveolar haemorrhage, especially since a recent report on long-term follow-up showed no beneficial effect of plasma exchange added as induction therapy compared to intravenous methylprednisolone (19).
On the basis of our finding, one could await initial response for at least 2-3 weeks, but optimal timing of adding plasmapheresis should be studied further. Although current treatment regimens are recommended for those with severe renal involvement, it is also still unclear whether all recommendations can be translated to patients who are dialysis dependent, as lessening toxicity to reduce infectious complications especially seems important in these patients (17,18). At last further studies could be aimed, next to plasmapheresis, at alternative treatment regimens like rituximab, which in prior studies has already been used successfully in refractory disease (19,20).

CONCLUSION

Our study showed a significant improvement in renal function after adding plasmapheresis as rescue therapy, which was not at the expense of higher mortality or increase of serious infectious episodes. Overall, no significant difference was found in development of ESRD during long-term follow-up after diagnosis. Therefore, next to present recommendations, adding plasmapheresis, even after 1 month of initial induction therapy, should be considered in those patients who show insufficient response to standard initial induction therapy.

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5

Microscopic haematuria in ANCA-associated vasculitis with glomerulonephritis during treatment and remission.

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Submitted

ABSTRACT

Background

The ANCA-associated small vessel vasculitides (AAV) are prone to cycles of relapse and remission (1). Renal involvement manifests as glomerulonephritis with microscopic haematuria, red blood cell casts, proteinuria and a variable decrease in renal function (1-3). Remission of renal vasculitis is defined as stabilization in serum creatinine (Creat) and resolution of haematuria while controversy exists about the persistence of haematuria (during apparent disease-remission) since it may indicate smouldering disease-activity or should be considered as a renal flare (1).

Objective

To clarify the course of haematuria after diagnosis and induction therapy and its possible predictive value of long term renal function.

Methods

96 Consecutive AAV-patients with renal involvement diagnosed and treated with systemic AAV between 1st of January 2000 to 31th December 2007 were followed for 60 months. Collected data were Creat, CRP (mg/ml), eGFR ml/min/1.73m2, creatinine-excretion in collected 24h urine (Ucreat/24 h), proteinuria (Uprot), ratio of proteinuria/ creatinine in 24h urine (Uprot/creat) and haematuria. Data were analysed for the complete study population and compared for MPO-ANCA and PR3-ANCA.

Results

At twelve months after diagnosis, haematuria was no longer detectable in 92% of all patients. In the PR3-ANCA group, haematuria disappeared after 13 months, while in the MPO-ANCA group haematuria persisted in 19% of the patients. On average, haematuria disappeared almost simultaneously with stabilisation of the renal function.

Conclusion

Haematuria persists for many months after diagnosis and disappears usually simultaneously with stabilisation of kidney function.

There was no relation between persistence of haematuria for over 12 months and renal function during follow up.

INTRODUCTION

The small-vessel vasculitides consist of a heterogeneous group of uncommon systemic multi-organ disorders that are prone to cycles of remission and relapse (1). These vasculitides include granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with poly-angiitis (eGPA) (1). These diseases are strongly associated with the presence of antineutrophil cytoplasmic autoantibodies (ANCA): more than 90% of patients have ANCA against proteinase 3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA) (1,4). Renal involvement is the most common severe manifestation in patients with ANCA-associated vasculitis (AAV), manifesting as glomerulonephritis with microscopic haematuria, red blood cell casts, proteinuria and a variable level of renal failure (1-3).
Since AAV is a chronic disease with disease-free periods and relapses, therapy usually starts with induction therapy based on cyclophosphamide and corticosteroids, inducing remission in weeks to months in most patients (5,6). Remission of renal vasculitis is defined as remission of inflammation with subsequent stabilisation in serum creatinine; thoughts about the resolution of haematuria and/or proteinuria are less clear (1). It is suggested that the persistence of haematuria in AAV patients (in apparent remission) reflects a kind of smouldering disease-activity or should be considered as a renal flare that might benefit from increasing immunosuppressive therapy (1).
However, controversy about the meaning or the time course of haematuria in patients with ANCA-associated vasculitis exists. The aim of this study was, therefore, to clarify the course of haematuria after diagnosis and induction therapy and its possible predictive value on longterm renal function. Since PR3- and MPO-ANCA associated vasculitides have distinct clinical and histopathological differences, comparison was made between these different entities.

METHODS

Study population

We studied 96 consecutive patients newly diagnosed with systemic AAV and renal involvement and treated in the period 1st of January 2000 to 31th December 2007 within our hospital. Patients were followed on regular outpatient clinic visits for at least 18 months following start of therapy ; thereafter follow up was completed 60 months after diagnosis, until death or loss to follow-up.
Due to insufficient follow-up data on urinary parameters in the first 18 months three patients were removed from the analysis leaving 93 patients in the final analysis.

Study design and definitions

During routine follow up, data were collected at different moments during the disease period, i.e. 0, 2 and 4 weeks and from month 2-18 at each visit on the outpatient clinic (at least every three months). Collected data were serum creatinine, CRP mg/l and 24 hour urine collection for creatinine and protein (Ucreat mmol/24 hr, Uprot gr/24 h). The Uprot/creat was calculated with the formula: proteinuria/creatinine-excretion, gr/24 h, in collected 24h urine. In addition, a freshly voided urine specimen was checked for presence of haem, and if positive, microscopic analysis of the urinary sediment was performed to quantify haematuria. A number less than 5 erythrocytes per high powered field (corresponds to approximately 25/ul) was regarded as non-significant or negative while 5 or more per field was regarded as significant haematuria. Malignancy of urogenital tract was ruled out in all cases. Remission of *haematuria* was defined as a normal value, it means a negative stick or < 5 erythrocytes/hu, for at least 3 consecutive month or a longer lasting course with one single abnormal value. The first of these 3 normal values was defined as the 'month of remission'.

Serum creatinine was used to define a clinical stable *renal function*. Renal function was considered stable at the lowest serum creatinine value and a stable serum creatinine for 3 consecutive months combined with stabilised clinical characteristics.

Proteinuria was assessed as the ratio of urinary protein (g/l) and creatinine (mmol/l). Remission of Uprot/creat (g/mmol) was defined as the lowest value during follow up followed by at least 3 months of stable values.

Long term persistence of haematuria, proteinuria and unstable serum creatinine were defined as not reaching the definition for remission at 12 months.

The course of the haematuria within the whole study population, as well as the differences in haematuria course for MPO-ANCA and PR3-ANCA patients were analysed. The same was done for the course of serum creatinine and the ratio of Uprot/Creat. Third, the moments of remission between serum creatinine and haematuria as well as serum creatinine and the ratio Uprot/Creat were compared. Finally, a comparison was made for the long term renal function. Primary outcome of this study was the course of haematuria during treatment; secondary outcomes were renal function (serum creatinine), proteinuria and change in renal function during follow up.

Statistical analyses

Descriptive statistics were generated and analysed using SPSS20. Outcomes were analysed with Mann-Whitney U test or Chi-square test. The course of haematuria, Creat and the Uprot/Creat were analysed with Kaplan Meier curves and the Log Rank test was used for comparison of groups.

RESULTS

Baseline characteristics

Fifty-six patients were PR3-ANCA positive (58%), 37 were MPO- ANCA-positive (39%), one was HNE-positive and two patients were ANCA negative.

Baseline characteristics were compared for PR3-ANCA and MPO-ANCA positive patients (table 1). Of all the patients, 65 were male (68%). Median age was 63 (range 51.3-72.5) years. In 38 patients (40%) the diagnosis was renal biopsy-proven. Twenty-four patients (26%) died within the 60 months of follow-up after diagnosis of which 15 (16%) during the first 18 months after diagnosis. Significant differences between PR3-ANCA positive and MPO-ANCA positive patients as expected, were found regarding sex, age, serum creatinine, eGFR, clearance, CRP, Ucreat/24h and Uprot/Creat.

Haematuria

At baseline haematuria was present in all patients. At 1, 3, 6, 12 and 18 months of follow up, haematuria was present in 58%, 28%, 22%, 10% and 6% of the patients respectively. Haematuria had disappeared in 72% and in 92% after 6 and 12 months respectively.

When comparing PR3-ANCA and MPO-ANCA at 6 and 12 months, haematuria had disappeared in 80% and 98% for PR3-ANCA and 57% and 81% in MPO-ANCA (log rank p = 0.005). After 13 months, in all PR3-ANCA positive patients haematuria was no longer detectable, while it persisted in 19% of the MPO-ANCA positive patients. (Fig 1)

Table 1 Baseline patient characteristics [a]

	MPO-ANCA *(n= 37)*	**PR3-ANCA** *(n= 56)*	**p-value**
Sex (M/F)	19/18	44/12	0.006[b]
Age (years)	66 (56-75)	60 (45-70)	0.03[b]
Haematuria (%)	100	100	0.72
Creatinine (µmol/l)	257 (144-626)	119 (100-328)	0.007[b]
eGFR (ml/min/1,73m^2)	17 (7-34)	56 (17-69)	0.001[b]
Clearance	20 (7-43)	59 (13-85)	0.002[b]
CRP (mg/l)	48 (14-120)	120 (50-169)	0.001[b]
UCreat (mmol/24u)	7.4 (5.2-10.2)	9.9 (5.7-12.8)	0.05[b]
Proteinuria (g/24u)	1.00 (0.3 -1.8)	0.8 (0.5-1.4)	0.50
Ratio Uprot/Creat (g/mmol)	0.19 (0.05-0.42)	0.09 (0.06 -0.16)	0.027[b]

[a] median (IQR) unless otherwise specified

[b] Statistically significant

Serum creatinine

Within 6 and 12 months after diagnosis, serum creatinine stabilized in 84% and 94% respectively. Comparing PR3-ANCA and MPO-ANCA at 6 and 12 months, serum creatinine was stable in 86% and 96% for PR3-ANCA and 80% and 88% in MPO-ANCA respectively (log rank p = 0.90).(Fig 2)

Haematuria and serum creatinine

The mean difference between a stable kidney function and the disappearance of haematuria was 0.036 months (SD 2.79). For the PR3-ANCA group this was 0.42 months (SD 2.72) and for MPO-ANCA -0.98 months (SD 2.80). This indicates that, after diagnosis, on average the haematuria disappearance and stabilisation of serum creatinine coincide in time.

A crosstab was generated to compare the patients with haematuria and unstable serum creatinine for more than 12 months. No correlation between these two parameters was found (Fisher's Exact p= 1.000); therefore no further prediction can be made based on the findings of the first 12-18 months after diagnosis.

Haematuria nor the persistence after 6 and 12 months were predictors for renal function over time nor for relapse until 60 months after diagnosis, not even when separately analysed for ANCA-specificity.

Proteinuria

After a follow-up period of 6 and 12 months from diagnosis, the Uprot/creat reached stabilisation in 35% and 74% of the patients respectively. Comparing PR3-ANCA and MPO-ANCA at 6 and 12 months, Uprot/creat was stable in 34% and 72% for PR3-ANCA and 36% and 79% in MPO-ANCA respectively (log rank p = 0.88). (Fig 3)

Proteinuria and serum creatinine

The mean difference between a stable kidney function and the stable Uprot/creat ratio was -6.1 months (SD 4.93) for the total study population. This indicates that serum creatinine stabilises on average about 6 months earlier than proteinuria does. There was no difference between the PR3-ANCA group (-6.0 months (SD 4.9)) and the MPO-ANCA group (-6.4 months, SD 5.0).

Patients survival, relapse-free survival and long term renal function

During long term follow up, survival rates were 88% at 12 months and 75% at 60 months. There was no difference in patient survival between PR3 and MPO-ANCA positive patients, p=0.36 (Fig 4)

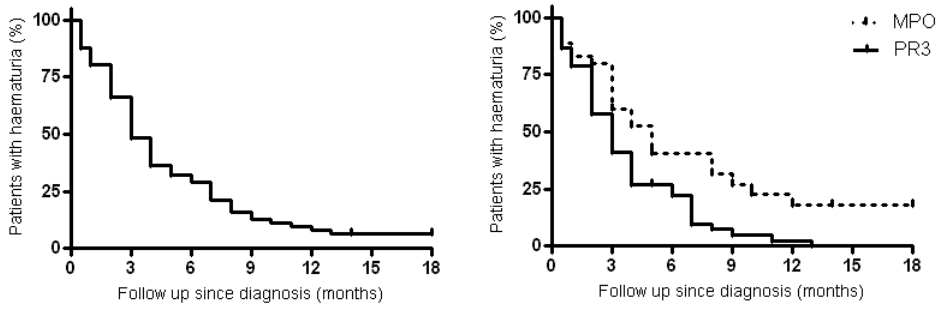

Figure 1A Survival curve of haematuria for complete study population,
1B PR3- versus MPO-ANCA positive patients ((Log rank p=0.003).

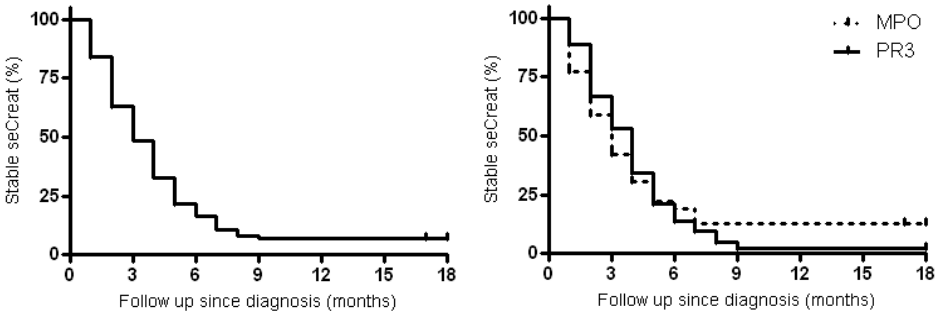

Figure 2A Stabilisation of serum creatinine for complete study population
2B PR3- versus MPO-ANCA positive patients.

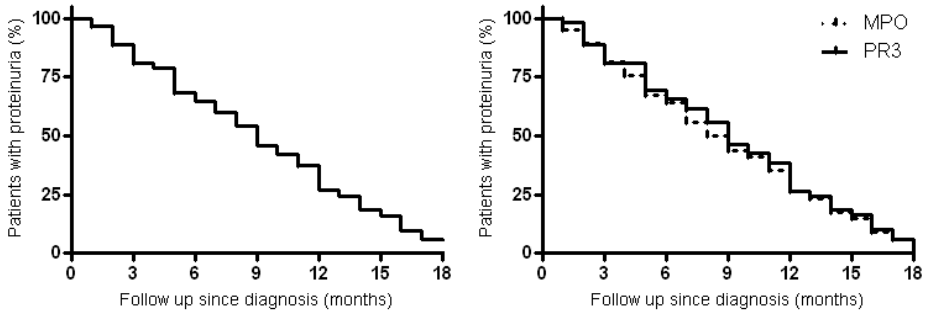

Figure 3A Course of Uprot/Creat in collected 24h urine for complete study population
3B PR3- versus MPO-ANCA positive patients

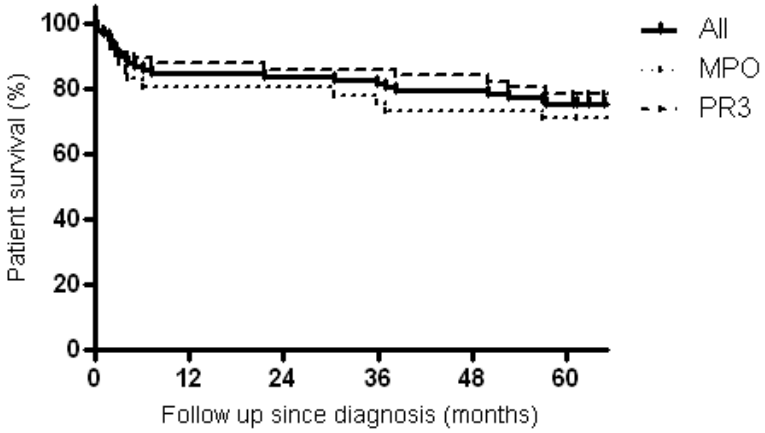

Figure 4 Patient survival curve for complete study population and PR3- versus MPO-ANCA positive patients (logrank p=0.36)

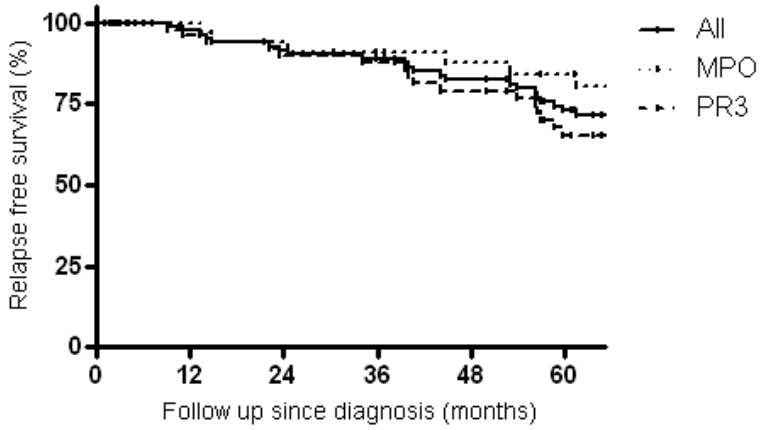

Figure 5 Relapse free survival curve for complete study populationand PR3- versus MPO-ANCA positive patients (logrank p=0.12)

At 60 months, a third of all patients had experienced a relapse: as expected, these relapses happened more often in the PR3-ANCA positive patients, 16% in MPO-patients (n=6) and 30% in PR3-patients (n=17), with a trend towards significance, p = 0.12. (Fig 5)
During long term follow up, overall renal function did not change significantly over time without difference for ANCA-specificity nor experiencing relapses (Fig 6)

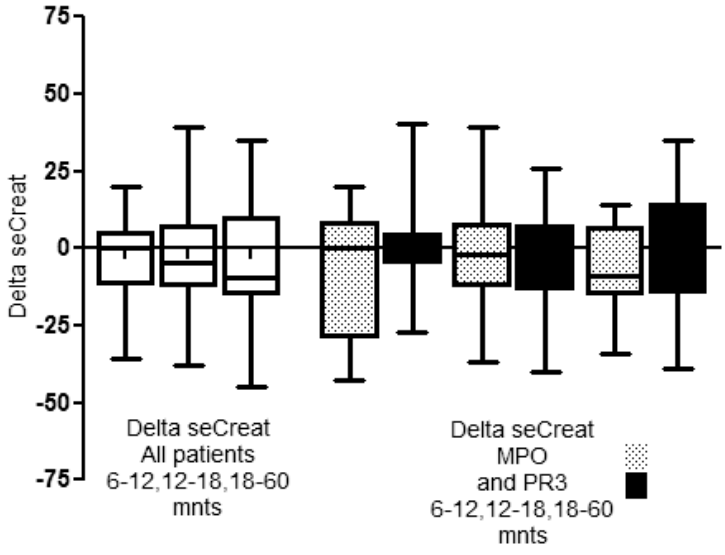

Figure 6 Change in serum creatinine comparing 6-12, 12-18 and 18-60 months after diagnosis, complete study population and PR3- versus MPO-ANCA positive patients

DISCUSSION

In this retrospective study of 96 patients with pauci-imune glomerulonephritis it was questioned whether microscopic haematuria in patients with renal vasculitis in clinical remission could represent chronic glomerular injury from prior episodes of disease-activity or may represent new glomerular pathology (1). We found that in general, haematuria disappears between three and six months after diagnosis and start of induction therapy and that the disappearance coincides in time with stabilisation of renal function. Nevertheless, as reflected by persisting proteinuria, microscopic injury may persists for a longer period. During follow up, persistence of haematuria is not associated with worsening renal function nor predictive for relapse.

In prior reports, Magrey et al suggested that a subset of patients with GPA and glomerulonephritis may have persistent microscopic haematuria from glomerular injury and not from active disease, stating that patients with GPA and glomerulonephritis probably achieved enduring remission, allowing withdrawal of medication, despite continued microsopic haematuria with or without RBC casts (3). Franssen et al. described resolution of haematuria within four months after start of treatment in a large cohort study of MPO-ANCA postive patients. None of the treated patients who developed chronic renal failure had haematuria at any time during follow-up but in addition each of the relapses encountered during follow up was associated with recurrence of haematuria (7). In our cohort of 96 patients with systemic AAV and renal involvement, after follow-up of 6 months, haematuria had disappeared in 72% of the whole study population. However, only in the PR3-ANCA positive patients, haematuria became negative at follow-up of 13 months, while in the MPO-ANCA positive patients, haematuria persistent in a small group (19%) of patients. There was no association with the persistence of haematuria and decline in renal function or relapse in this patients.
However, this difference between PR3-ANCA and MPO-ANCA-positive patients was significant, p=0.003, which again may underscore the conclusion that PR3-AAV and MPO-AAV are distinct diseases. On average the haematuria disappeared almost simultaneously with stabilisation of the renal function and only a small minority had on-going haematuria 6 months after diagnosis. The proteinuria persisted for about 6 months longer compared to stabilisation of the renal function. On this grounds, one can conclude that the disappearance of haematuria can act as a marker for stabilisation of renal function and absence of on-going inflammatory disease activity. Proteinuria therefore cannot be used as a marker for quiescence of renal disease. During long term follow up, haematuria or the persistence appeared not to be of predictive value for renal function nor for relapses and can certainly not act as a guidance for therapy

There are some considerations: a number of missing values during follow up could have influenced the analyses, for instance in defining date of remission in these particular patients. In addition, it is debatable whether the study population is representative for the population of patients with systemic AAV since in our tertiary referral centre mostly complex patients are treated who sometimes did not immediately responded to standard induction therapy. This can have had influence on outcomes. Nevertheless, considering the fact that even complex patients are also included in these analyses, we think that our findings on haematuria, remission and predictive value could be generalizable.
In conclusion, we found that haematuria probably acts as a sensitive marker for absence of inflammatory glomerular disease activity in most patients with systemic AAV and renal involvement. It's disappearance coincide with stabilisation of renal function and remission of the disease in almost all patients. However, if it persists, it is not predictive for worsening

renal function nor for relapse. This means that one should monitor these patients closely, but there is not enough evidence that supports escalation of immunosuppressive medication based on persistence of haematuria alone. Furthermore, since microscopic injury as indicated by proteinuria persists for longer duration, proteinuria does not seem to be a reliable marker for renal disease remission.

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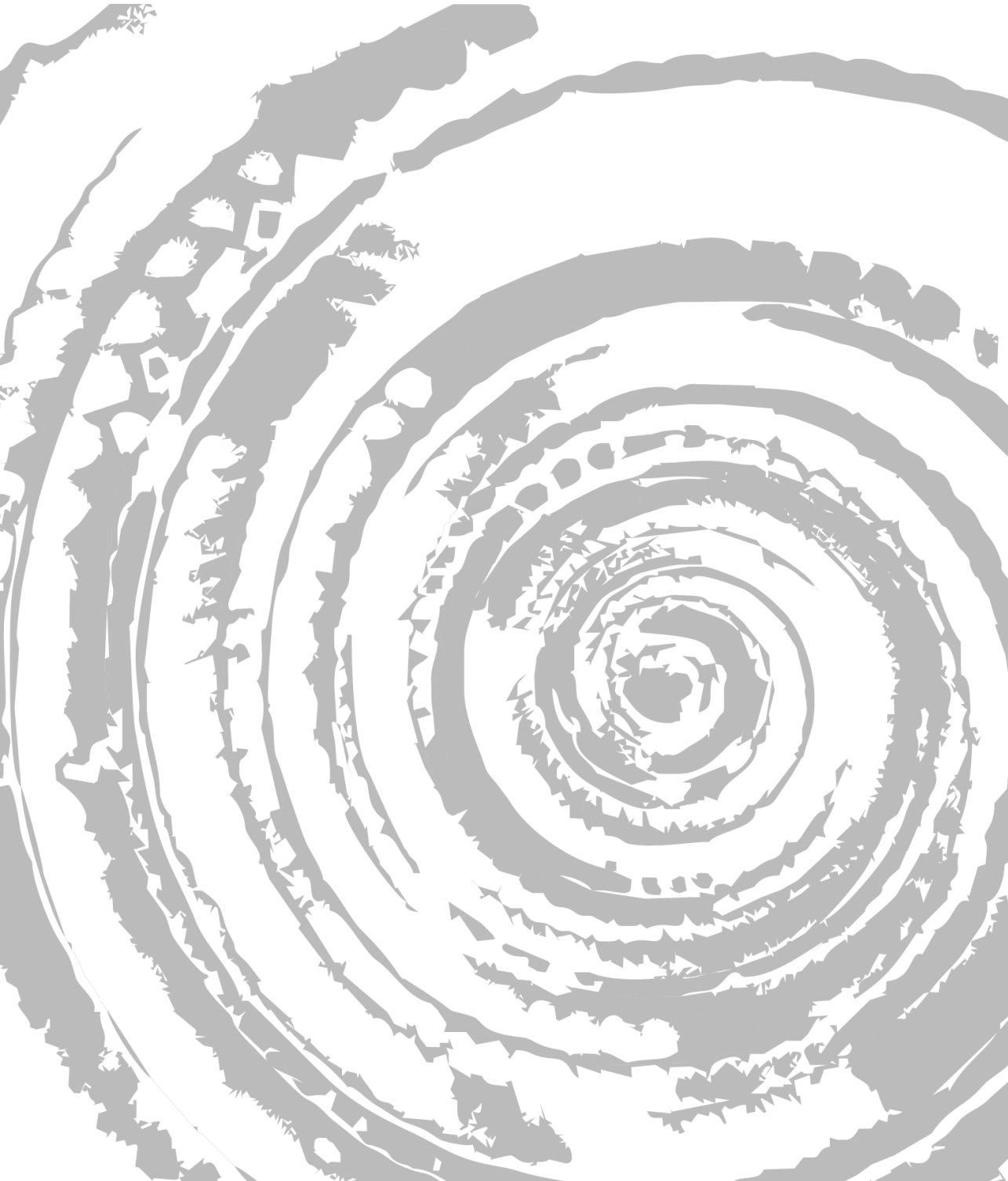

PART 2

6

Maintenance therapy in ANCA-associated vasculitis: who needs what and for how long?

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ABSTRACT

Anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides (AAV) are severe chronic auto-immune diseases in which the small vessels are inflamed. Nowadays, in the majority of patients disease can be brought into remission with cyclophosphamide and corticosteroids. However, depending on disease characteristics, patients with AAV have a risk of 29-60% to experience relapses of disease within 5 years despite maintenance therapy with less toxic agents, such as azathioprine, methotrexate or mycophenolate mofetil. More recently, rituximab has been found effective in both induction and maintenance of remission in AAV. This review discusses the different aspects of maintenance therapy in AAV based on reported cohorts and studies, including the different agents, therapy duration, efficacy or lack thereof and future directions. Finally, recommendations are made who to treat and for how long.

INTRODUCTION

The last quarter of the previous century has seen a tremendous development in the understanding and treatment of the diseases that we now commonly denote as anti-cytoplasmic antibody associated (ANCA) vasculitis. Following the observation of neutrophil-specific antibodies in association with segmental necrotizing glomerulonephritis in the early 1980s (1), van der Woude et al. described autoantibodies producing a diffuse granular cytoplasmic staining of neutrophils, so-called C-ANCA, in patients with Wegener's granulomatosis (2). A few years later, perinuclear staining of alcohol-fixed neutrophils (P-ANCA) was described predominantly in patients with microscopic poly-angiitis and renal limited small vessel vasculitis (3). Many subsequent studies at first focused on the pathogenesis of ANCA-associated diseases and reported that ANCA could activate neutrophils and monocytes resulting in a release of inflammatory constituents, suggesting a direct pathogenic role (4), and finally proved the pathogenic potential of these antibodies in a myeloperoxidase(MPO)-ANCA animal model (5)

Since then, finding a better treatment paradigm became a dominant focus of different studies. Due to the introduction of cyclophosphamide combined with high dose steroids in the 1960's (6), these diseases changed from progressive and often fatal to modifiable and treatable and thereby became more chronic conditions with unpredictable periods of relapse in about 25-40% of patients (6,7). Still, mortality and morbidity are substantial due to either disease or toxicity of prolonged courses of immunosuppressive treatment.

Many studies have addressed this toxicity–problem: avoidance of prolonged cyclophosphamide by a staged approach, limiting induction treatment to 3-6 months and followed by a longer period of less-toxic therapy with azathioprine to maintain disease remission, proved to be successful (7). By this scheme, remission was and is achieved in over 90% of patients (8).

Although initial disease control is obtained, the efficacy of these therapies on subsequent relapse is still not clear (9-11). This review focuses on maintenance therapy in ANCA associated vasculitis in order to answer the question who needs what maintenance therapy and for how long.

Relapses- definition

As remission is a clinical definition (defined by absence of manifestations of vasculitic disease activity) and not a pathophysiological one, so is relapse with a large variation in severity (9). It can be defined as new or worsened manifestation of ANCA-associated vasculitis after a period of partial or complete remission requiring a change in therapy which could be classified as minor and major, according to the absence or presence of threatened vital organ function (11-13).

Noteworthy, relapses can sometimes be triggered or preceded by and coincide with infection or malignancy, but more important, these important clinical phenomena can mimic relapse, so attention must be paid for a thorough differential diagnosis.

Relapses- prediction of risk

Because about 30-50% of patients experience a relapse within five years after diagnosis (12) ,the ability to predict risk of relapse and thus reserve potentially toxic therapy for those who are most likely to benefit, would be highly desirable (14).

A lot of studies addressed this topic and provided evidence that the risk of relapse is influenced by both disease and patient characteristics and also choice of therapy.

Patients with granulomatosis with poly-angiitis (GPA) have a relapse risk within 5 years of up to 60% versus 29% in microscopic poly-angiitis (MPA) (9). The presence of proteinase-3 (PR3)-ANCA (12,15), respiratory tract disease (in particular alveolar haemorrhage) and prior relapse have a significantly higher likelihood of relapses with increased risk of ~ 1.7 fold for each risk factor (16,17). In contrast, patients with higher serum creatinine levels and those who remain dependent of renal replacement therapy are at substantially lower risk of relapse of up to 60% less (12,18,19).

The presence of a positive ANCA at the time of remission increases the risk of relapse > 2 fold (13). The value of serial measurement of ANCA titre during follow up is still controversial (20,21) . Rises in ANCA titre occur prior to relapse in many patients but sensitivity and specificity of these rises are too low to guide therapy in individual patients (19,20) .

It has been suggested that relapse risk is more likely related to the organs that are affected than to the overall severity or aggressiveness of the vasculitis. This has been proven for respiratory tract involvement. For cardiovascular involvement this association is less clear and, but since cardiovascular involvement is uncommon and difficult to diagnose, it may have been underestimated in earlier studies (12).

Several genetic factors have been associated with AAV; only very few have been identified as related to disease course or to risk factors for relapse. Human leucocyte antigen (HLA)-DR antigens were thought to be weakly associated with increased relapse rate but it was unclear whether this was due to the class II antigens itself or due to linkage with polymorphism located close to class II genes (22). In a large genome-wide association study, both major-histocompatibility-complex (MHC) and non-MHC were found to be genetically associated with the antigenic specificity of ANCA. On this ground, GPA and MPA are supposed to be genetically distinct which may explain the different clinical spectrum and outcomes (23).

Previously, patients treated for only a few months were found to be likely to relapse, which led to recommendation of continuing immunosuppressive therapy (14). Recently, based on long-term follow-up data of a randomized trial, it has been suggested that a longer

duration of cyclophosphamide for induction remission was related to less relapses, a finding also reported by an older observational study (24,25)
Initial use of methotrexate (MTX) or intravenous cyclophosphamide (HR, 0.50; 95% CI, 0.26-0.93; P=0.029) has been associated with higher relapse rates than oral cyclophosphamide (26). There are some conflicting data on plasma exchange: the recently published long-term data of the methylprednisolone versus plasma exchange (MEPEX) showed a reduced number of relapses in the plasma exchange-group compared with high-dose prednisolone, 14.4% vs 20.5% (27); however, in a smaller retrospective study on plasmapheresis rescue therapy, an unexpected higher number of relapses was found (58%) during follow- up of 10 years (28). Early withdrawal of glucocorticoids is associated with higher relapse rate compared with long-term steroid regimes (43% versus 14%) as was found in a large meta-analysis of 13 studies with over 900 patients (29). Addition of sulfamethoxazole-trimethoprim (co-trimoxazole) to other immunosuppressive agents during remission, however, was associated with overall reduced relapse rates (30).
There might be a relation between infection- induced activation of neutrophils and monocytes/macrophages and subsequent development of disease activity since target antigens of ANCA are expressed and released by these cells (1,14). Several cases of subsequent events and also seasonal variations in incidence of disease, suggesting possible viral influences, have been described. It has even been hypothesized that the site of infection might even determine the location of active vasculitis (21). At last, nasal carriage of Staphylococcus aureus has been pointed out as a significant risk factor (RR 7.2) for the occurrence of relapse in GPA(31).

Relapses- consequences
Severe relapses could cause death or at least extensive organ damage as measured by vasculitis damage index (VDI), for instance due to stenotic/occlusive or aneurysmal changes of vessels, diffuse alveolar haemorrhage or subglottic stenosis (20,31). Over time, in a large cohort of patients, severe damage occurred in > 50% of patients and was mostly due to renal and cardiac involvement; however, the strongest predictors for cumulative organ damage were baseline organ damage and elevated erythrocyte sedimentation rate (ESR) (25).
In renal involvement, every renal relapse is associated with a decrease in estimated glomerular filtration rate (eGFR in ml/min per 1.73m2) of 8-12 ml/min (34). Additionally, patients who have a renal relapse with overt and active glomerulonephritis are also 4.7 times more likely to progress to end-stage renal disease, compared with those who do not experience a relapse, independent of other risk factors such as age, ANCA specificity or initial kidney function (16).

Relapsing ear, nose and throat disease-activity may lead to progressive subglottic and tracheal stenosis independent of therapy. These patients require frequent surgical intervention, and the clinician should remain vigilant for progression of disease (35).
Next to life- or organ- threatening events, the price for retreatment or prolonged courses of immunosuppression to prevent or cure relapse could be high due to side effects of these toxic treatment regimes. In the short-term, cyclophosphamide can induce haemorrhagic cystitis but most important, prolonged courses of leucopenia and associated infections are important contributors to morbidity (10). In the long-term, the risk of malignancy (in particular bladder cancer, leukaemia and lymphoma) increases with cumulative cyclophosphamide dose above 36 g, although a safe dose has not been determined (34). Furthermore, the risk of infertility (> 50% of women in childbearing age) should be taken into account when deciding on treatment of relapses (37).
Azathioprine, MTX and low dose glucocorticoids are usually the mainstay of long term maintenance therapy, next to mycophenolate mofetil (MMF). Most reported side events are gastro-intestinal complaints (abdominal pain, diarrhoea) and (viral) infections, but also mucositis and hepatotoxic effect have been described (10,36). Definitely, the use of glucocorticoids is associated with a wide range of metabolic, gastro-intestinal and psychiatric adverse events (10); the prolonged treatment with moderate to high doses of glucocorticoids significantly contribute to diffuse cumulative organ damage of any significance, for instance considerable weight gain, bone diseases and fractures (10,25).

Relapses- impact on quality of life (QOL)

ANCA associated vasculitis patients are exposed to long-term toxic therapies, suffer from accumulated organ damage and at risk for potentially life-threatening relapses. However, quality of life (QOL) is reported to be similar to other chronic conditions and of all this aforementioned threats, fatigue was the most important contributor and major determinant to poor QOL (37,38). QOL measurements also reported sleeping problems, perhaps due to uncertainty and fear about the relapsing nature of the disease, and neurological manifestations (often resulting in pain, sensory disturbance and functional disability) as important in defining QOL, next to old age and high current prednisolone dose (37,38). These factors can partially be associated with poor disease control and therefore can be regarded as potentially modifiable; however, the actual impact of relapses on QOL has not been reported.

Relapses- prevention

Because the consequences of retreatment of relapses are huge in terms of probable increase in both morbidity and mortality, a lot of studies addressed the issue of prevention of relapse. However, to demonstrate the efficacy of a medication, a study must include

a population at risk of relapse that is followed over a sufficiently long period of time. For a disease with a annual incidence in Northern Europe of 11-16 cases per million and a prevalence of 177 cases per million (10,35) , this is a difficult goal and there are only a few randomized trials comparing different medications (Table 1). What evidence has been delivered?

In 1990, Cohen Tervaert et al proved the need for maintenance therapy in order to prevent relapse by comparing pre-emptive immunosuppressive therapy based on rising ANCA-titres to patients who were only treated if there was a clinical relapse. Those who were treated pre-emptively experienced significantly less relapses, $P < 0.05$. Of note, those patients who were treated based on clinical relapse received a higher cumulative cyclophosphamide dose, than those who were treated pre-emptively (14).

In 2003, the randomized trial of cyclophosphamide versus azathioprine during remission in ANCA positive systemic vasculitis (CYCAZEREM) demonstrated in 155 patients that withdrawal of cyclophosphamide after induction of remission and substitution of azathioprine was safe and did not increase relapse rates. Duration of azathioprine-use was ~ 12 months (from +/- 6 to 18 months after initial therapy). Patients with severe renal involvement and serum creatinine > 500 umol/l were excluded from this trial (7).

Based on a few small studies and the known superior effect in treatment of systemic lupus erythematosus (SLE), MMF was studied as alternative to azathioprine in maintenance therapy in AAV (36)(Figure 1). Treatment duration was 42 months. Surprisingly, MMF was less effective because relapses were more common in the MMF group compared to azathioprine ($P= 0.03$); adverse events did not differ. The authors stated that MMF should not be considered as first line for maintenance therapy, although in might be considered in refractory cases of AAV (36).

Co-trimoxazole (800 mg sulfamethoxazole and 160 mg trimethoprim) was given twice daily for 24 months during and after treatment with cyclophosphamide and prednisolone and especially reduced the number of relapses involving the upper airways ($P= 0.03$). Although 20% of patients stopped treatment prematurely because of side effects, these were generally mild (30).

Due to the known effect in rheumatoid arthritis (RA) and based on smaller studies, MTX was also compared with azathioprine in maintenance therapy, especially addressing safety-issues during 15 months after diagnosis (39,40). Although in a smaller study, the number of renal relapse was high under methotrexate maintenance therapy (41), in this randomized trial the drug appeared to be similar to azathioprine in maintaining remission but also for the toxicity profile. However, although no differences were found in severe and less severe adverse events, the authors warn to adjust the dose to renal function and advise to avoid it in patients with severe renal impairment (39).

Due to increased understanding in pathogenesis of AAV and also the known effects in other rheumatic diseases, tumour necrosis factor-α (TNF-α) inhibitors were also introduced in

Table 1 Overview of randomized clinical trials of maintenance therapy in AAV.

	Inclusion	Induction	Maintenance
Cohen Tervaert et al, 1990 N= 20	Biopsy proven WG, in partial or complete remission		Depending on significant rise in ANCA: CYC 1 mg/kg/day + pred 30 mg/day N=9 or no maintenance N=11.
Stegeman et al, 1994 N=81	WG in remission	Various	CYC + pred according to protocol + Co-trimoxazol 960 mg twice weekly N=41 Placebo N=40
CYCAZEREM Jayne et al, 2003 N=144	Newly diagnosed WG/MPA/RLGN seCreat < 500 umol/l.	Cyc oral 2 mg/kg Pred 1 mg/kg	Cyc 1.5 mg/kg/day + pred N= 73 AZA 2 mg/kg/day + pred N=71
WGET WGET research group, 2005 N= 180	Newly diagnosed and flares, BVAS > 3	Severe: CYC 2 mg/kg + pred 0.5-1 mg/kg Limited: MTX 0.25 mg/kg/wk + pred 0.5-1 mg/kg	MTX max 25 mg/wk AZA 2 mg/kg/day +/- Etanercept 25 mg twice weekly N=89 with ETA N=91 without
WEGENT Pagnoux et al, 2008. N=126	WG/MPA	CYC i.v 0.6 mg/0.6 x m2 first two weeks, thereafter 0.7 mg/m2 every three weeks. Pred 1 mg/kg/day	AZA 2.0 mg/kg/day N=63 MTX 0.3 mg/kg/wk N=63

	Duration	Relapse	AE's
		CYC/pred N=0 No treatment: N=9 (82%), of which 6 within 3 months after ANCA-rise.	Mild. No differences between groups. Death N=1 (no treatment).
	Treatment 24 months. FU 24 months	Patients in remission Co-trim: 82% Without Co-trim: 60% P 0.40 In patients with respiratory tract disease P 0.03	Co-trim: N=8, 20% (medication discontinued) Placebo: N=2, 5% Death N=1 (placebo)
	Treatment 18 months	CYC: N=10, 13.7% AZA: N=11, 15.5% P 0.65	CYC: N=7, 10% AZA: N=8, 11% P 0.94 Death N=1.
	Treatment 12 months + tapering, FU 27 months.	ETA: N=51, 57.3% Without ETA: N=52, 57.1% P 0.54	ETA: 56.2% Without ETA: 57.1% P 0.90 Death ETA: N=4, Without ETA: N=2
	Treatment 15 months FU 29+/- 13 months	AZA: N=23, 37% MTX: N=21, 33% P 0.71	AZA: N=29, 46% MTX: N=35, 56% P 0.29. Death N=1 (MTX)

Table 1 Continued

	Inclusion	Induction	Maintenance
Metzler et al, 2009 N= 20	Generalized WG in complete or partial remission	CYC Pred	Stepwise approach in use of Leflunomide: N=20 -100 mg for 3 days - 20 mg/day for 12 weeks - 100 mg for 2 days - 30 mg/day for 12 weeks If necessary: - 40 mg/day for 12 weeks Optional: continuation for two consecutive years
Metzler et al, 2007 N=54	Generalized WG Exclusion: leucopenia < 4 mmol/l and seCreat > 115 umol/l,	CYC 2 mg/kg/day Pred 1 mg/kg/day	MTX N= 28 - Wk 1-4: 7.5 mg/wk - Wk 5-8: 15 mg/wk - Wk 8: 20 mg/wk Leflunomide: N=26 -100 mg for 3 days - 20 mg/day for 4 weeks - > 4 weeks: 30 mg/day
IMPROVE Hiemstra et al, 2010 N=156	Newly diagnosed AAV (WG/MPA)	CYC 1.5-2 mg/kg/day Pred 1 mg/kg/day	AZA 2 mg/kg/day N=80 MMF 2000 mg/day N=76

treatment for ANCA-associated vasculitis (42). Etanercept or placebo was added to standard maintenance regimen with either cyclophosphamide or methotrexate and glucocorticoids during 12-15 months. No differences were found in number of flares, but six cases of solid tumours were detected in the etanercept group. It was hypothesized that the combination of cyclophosphamide and TNF-a inhibition heightens the risk of cancer. Therefore, this drug is no longer recommend and also other TNF-a inhibition is advised not to use (42).

Duration	Relapse	AE's
Treatment 52 weeks (1.75-2.5 years)	N=9 (45%)	N= 20, 8.3 AE's per patient.
Study was ended prematurely by the advisory board. Aimed treatment duration 24 months (12 patients in each arm) Median FU 21 months (1-24)	MTX: N=13 (46%) LEF: N=6 (23%) P 0.09	MTX: N=17 LEF: N= 34 (1.3 per patient) P= 0.09 Death: ..
Treatment mean 39 months (0.66-53.6) FU 42 months	AZA: N=30, 37% MMF: N=42, 55% P 0.03	AZA: N=13, 16% MMF N=8, 7.5% P 0.12 Death N=2 (1 AZA, 1 MTX)

Since in RA the use of leflunomide seems to approach the potency of MTX, this drug was also studied as maintenance agent in AAV, especially since it does not accumulate in renal failure and does not cause leucopoenia (43). Relapse risks were equal or even better compared with standard agents (a controlled trail comparing MTX versus leflunomide was terminated early because of major relapse rate in MTX group) but there was a adverse event rate reported of up to 8.3 per patient (43,44).

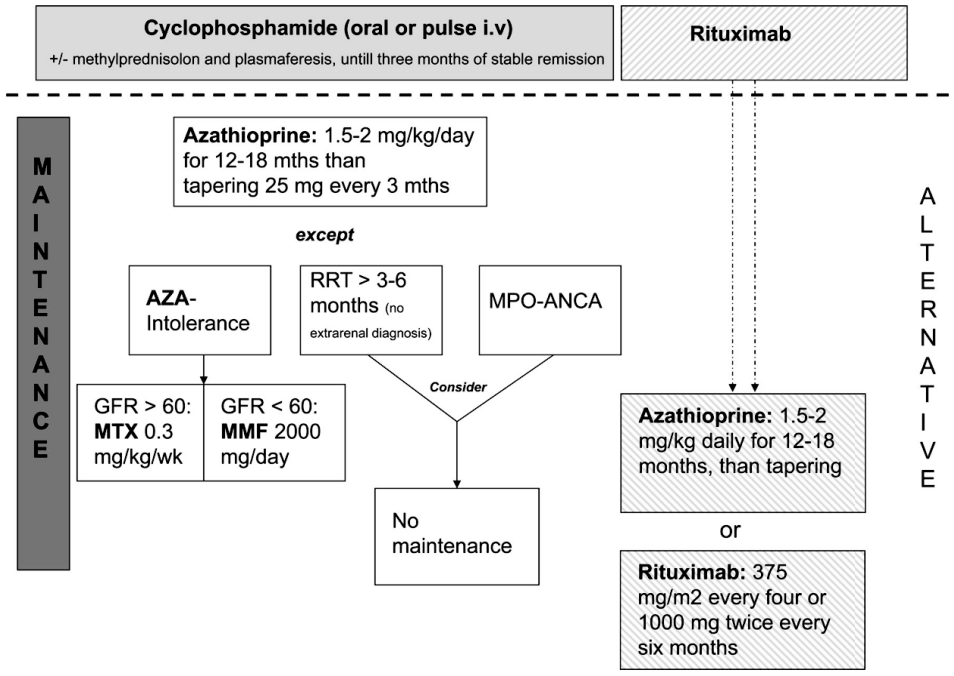

Figure 1 Proposed flow chart for maintenance treatment in AAV

Relapses- duration of maintenance therapy

The strategy of discontinuing maintenance therapy after 18-24 months was chosen as Committee consensus by analogy with the results of the aforementioned clinical trials (45). This duration may be somewhat arbitrary, especially when keeping in mind that in surveys on outcome reported median disease-free survival is 21.8 (range 9.8-27) months after diagnosis (46). Moreover, it is also known that most relapses happen during tapering or after discontinuing drugs.

The data of a large multi-centre study comparing short-term and long-term courses of azathioprine maintenance therapy are not yet published (REMAIN) but in a prospective study of 126 patients with PR3-ANCA- associated vasculitis, in a subgroup of patients who remained ANCA positive at time of remission (n=44) no difference in relapse rate was found for longer duration of azathioprine up to 48 months (47). This may raise questions about the effect of azathioprine maintenance effect at all. This same question about maintenance rose after the rituximab for induction-remission trail (RAVE). The rituximab-arm received no additional treatment but had the same outcomes on relapse rate at 18 months of follow up (48) (Table2).

Also, it has been suggested that a specific subset of patients who do not carry any risk factor for relapse (e.g. MPO-ANCA, no respiratory tract involvement) do no require any maintenance therapy at all (11). However, specific markers allowing reliable identification of low relapse risk have to be studied further to make clear statements about this topic.

Relapses- new developments

Although nowadays given a prominent role in induction therapy and refractory disease, present knowledge on rituximab- maintenance therapy is scarce but promising. After its first description in 2001 in ANCA-associated vasculitis, it was initially used for patients who were refractory to common immunosuppressants or relapsing under treatment (49). In two prospective randomized controlled studies, rituximab turned out to be as effective as cyclophosphamide at inducing remission (48,50,51), so both the Food and Drug Administration (2011) and the European Medicines Agency (2013) approved its use for the induction treatment of adults with GPA and MPA (49). The recently published prospective maintenance of remission using rituximab in systemic ANCA-associated vasculitis trial (MAINRITSAN) also demonstrates its efficacy in maintenance of remission (52).

Nevertheless, treatment regimens and dosages vary throughout the different studies and the most efficient scheme for both induction and maintenance has not been determined yet (52-54). One of the great advantages is the preservation of ovarian function; therefore its use can be considered in young women whose pregnancy wish is not yet fulfilled (38,51). Furthermore, a positive issue regarding the use of rituximab is the possibility to withdraw concomitant immunosuppressants and reduction of daily prednisolone-dose (54).

Safety issues are minimal but mainly consists of long-lasting severe infections (due to hypogammaglobulimia and loss of humoral immunity); guidelines recommend checking immunoglobulin levels before and after treatment (10,49,51). Complications of long-term outcome after rituximab, for instance late onset neutropenia (LON) has to be further studied, so close monitoring seems needed when rituximab is prescribed combined with other immunosuppressive therapies (49,54).

Another recent development is the use of intravenous abatacept, CTLA4 (cytotoxic T-lymphocyte associated protein 4 also known as CD 152) -IgG which is able to inhibit T-cell activation by blocking CD28 to its ligand, which may have impact on the GPA disease pathogenesis. In a small open label trial, this treatment was added in 20 patients with non-severe relapsing GPA and showed good results in both disease remission as prednisolone discontinuation and was well tolerated (55).

Another potential maintenance agent could be belimumab, an anti-BLyS (B-lymfocyte stimulator) monoclonal antibody, that inhibits B-cell activation. A large industry driven Phase III study is currently recruiting participant and aims to enrol 400 patients to evaluate efficacy and safety in remission maintenance by adding belimumab to azathioprine during approximately 3 years of follow up *(Clinical trials.gov. NCTO1663623)*

Table 2 Overview of trials in AAV and rituximab for maintenance therapy.

Besada et al, Norwegian GPA cohort study, 2013 N=35 Retrospective	GPA as registered in Nordnorsk Vaskulittregister.	Various prior to first RTX infusion. RTX 1gr i.v twice a weeks apart + methylprednisolone 125 mg.
Charles P et al, French vasculitis study group, 2014. N=80 Retrospective	AAV as defined by Chapel Hill nomenclature and Histological confirmation or ANCA detected. RTX as first-line, second- line or maintenance.	Induction with RTX: four different protocols, most frequently chosen 375 mg/m2 for 4 weeks. 25% concomitant immunosuppression.
Pendergraft WF et al, 2014. N=172.	Clinical/laboratory features with GPA/MPA or other forms of vasculitis together with PR3- or MPO-ANCA by ELISA	Various
Smith RM et al, 2012 N= 73.	GPA/MPA with refractory/ relapsing disease Exclusion: RTX as first line or FU < 6 months	Various

Pre-emptive RTX 1gr i.v twice a weeks apart or 1 gr bi-annually	FU since first RTX infusion: 47 months(2-88)	N=9 (23%), relapse rate of 6.6/100 patient years	Severe infections N=9 (26%) RTX discontinuation N=13 (37%) Death N=2.
Maintenance with RTX: 'several different regimes', N=64; most frequently chosen 375 mg/m2 every 6 months 13% concomitant immunosuppression	Median FU 18 months (IQR 12-37)	N=18 (23%) With RTX maintenance N=10 (20%) Without RTX maintenance therapy N=8 (44%) P 0.002	N=22 Infections N=12 (15%) Death N=5
Prior RTX: 1000 mg every 4 months. Other induction-protocol: RTX 1gr i.v twice a week apart, afterwards 1000 mg every 4 months	Median remission maintenance FU-time 2.1 years.	N=35 (20%), no statistical difference for prior treatment, for ANCA-specificity other ..	All AE's: N=121 Reguiring hospitalization: N=84 - infections: N=22 - disease-related: N=7 - Other: N=52 Death: N=10.
A: RTX 375 mg/m2 BSA 4 times or 1000 mg twice a week apart. Thereafter only at relapse. N=28 B: RTX 1000 mg twice a week apart, followed by 1 gram every 6 months for two years N=45 C: patients of group A, switching to routine retreatment N=19	Treatment FU A: 18 months (7-102) B: 40 months (4-61) C: 55 months (19-62) Long term FU	During two-years treatment A: N=19 (73%) B: N=5 (12%) C: N=2 (11%) During 4-years FU A: N=21 (81%) B: N=11 (26%) C: N= 10 (46%) P < 0.001 and P= 0.001	A: N=9 (28%), 16 events B: N=21 (47%), 45 events C: N= 7 (37%), 20 events P= 0.326 and P =0.984 Death: N=4 (groups?)

Table 2 Continued

Carvin-Ceba et al 2010 N= 53	GPA/MPA Refractory disease	Methylprednisolone Prednisolon 1 mg/kg/day RTX i.v 375 mg/m2 BSA during 4 weeks Or RTX i.v 1 gr twice every two weeks

RECOMMENDATIONS

Maintenance therapy: what to choose?

For patients who received standard cyclophosphamide for remission-induction, the use of azathioprine 2 mg/kg/day is most rationale, combined with low dose steroids, e.g. 10 mg/day (9). Duration should be close to 12 months, than tapering the dose by 25 mg every 3 months until discontinuation. Glucocorticoid doses can be reduced gradually after 6-18 months with the aim of discontinuing (based on individual patient responses) (20). Since azathioprine can cause considerable myelosuppression in patients who have low thiopurine methyltransferase (TPMT), the enzyme responsible for conversion of azathioprine to its active metabolites, routine testing is recommended in all guidelines (10,11,56)

Although associated with a higher relapse rate, mycophenolate mofetil (2000 mg/day) may be the best agent of choice in the case of allergy/intolerance for azathioprine (36). Methotrexate (0.3 mg/kg/wk, progressively increasing to 25 mg/wk) could be another alternative agent but dose should be adjusted to renal function and is not recommended in patients with GFR < 30 ml/min per 1.73 m2 (40).

Rituximab for maintenance therapy is still experimental but results are hopeful; in young women with future pregnancy wish, the use of rituximab maintenance should at least be considered (51,57,58). Treatment schemes vary from bi-annually 375 mg/m2 BSA to 1000 mg every four months or only at relapse. Until today, there are no randomized trials that prove a certain dose or one scheme superior to another. A dosage of 375 mg/m2 BSA two weeks apart every 6 months is reported most often.

There is no place for TNF-a blockers in maintenance therapy for ANCA-associated vasculitis

RTX without pred when reconstitution of B-lymfocytes - in patients who never were ANCA positive - combined with a rise in PR3-ANCA - without a rise in PR3-ANCA but with a history of relapse. N=53	FU since first RTX infusion: 4.4 years (2.7-6.2)	53 patients with > 2 courses of RTX: N=32, 60%.	Infection episodes N=30 Infusion related episodes N=16. Death N=2

(13). On the basis of what is yet known, there is also no support to continue potentially toxic immunosuppression after 24 months (47,59).
As suggested in kidney disease improving global outcomes guidelines, in patients who present with end- stage renal disease without any other systemic disease-manifestations and who continue the need for renal replacement therapy after 3 months of treatment, based on evident lower relapse rates, maintenance therapy could be abated (11,18,19).
Also, as patients with MPO-ANCA associated vasculitis are far less likely to relapse, there is no evidence for subsequent toxic maintenance therapy after the first episode of disease, and therefore it could be considered to continue only a low dose of glucocorticoids 5-10 mg/day for 12 months.
Difficulty in maintaining remission probably relates to the difference between true pathophysiological remission and the absence of clear evidence of disease activity. ANCA-titres should be determined at every outpatient visit and, although the association of rising ANCA-titres and relapse is still debated, suspicion is warranted when ANCA-titres change from negative to positive or rises substantially between visits. However, escalating therapy or reinstating immunosuppressive therapy based on ANCA-titres alone is not encouraged. Careful attention must be paid to all possible long-term side effects of these toxic immuno-suppressive agents, especially for the increased risk of myelosuppression, infec-tions and cancer (bladder cancer, leukaemia and lymphoma).

In case of relapse

It is known that relapses respond to therapy with a similar response rate as the initial disease (59). The severity of possible relapse should guide choice of therapy. In case of

a severe relapse, cyclophosphamide should be reintroduced, given that the cumulative dose is not exceeding 36 g. In patients who approach or do exceed this amount of drugs, rituximab 1000 mg twice is recommended every 4-6 months to avoid long-term toxicity of cyclophosphamide. Treatment should be combined with glucocorticoids and plasmapheresis when necessary.

In relapses that are not severe, re-instating or increasing doses of the provided maintenance agent (azathioprine, MMF or MTX) combined with glucocorticoids could lead to remission of disease activity. The addition of four cycles of intravenous immunoglobulins (0.5 g/kg/day) could be considered, but there is not enough evidence to recommend standard use for relapse in ANCA-associated vasculitis (60).

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7

Extended versus Standard Azathioprine Maintenance Therapy in Newly Diagnosed Proteinase-3 Anti-Neutrophil Cytoplasmic Antibody Associated Vasculitis Patients who Remain C-ANCA Positive after Induction of Remission: A Randomized Clinical Trial.

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ABSTRACT

Background

Cytoplasmic anti-neutrophil cytoplasmic antibody (C-ANCA) positivity at remission has been associated with an increased relapse rate in patients with proteinase-3 anti-neutrophil cytoplasmic antibody associated vasculitis (PR3-AAV) after a switch to azathioprine maintenance therapy. We therefore hypothesized that extended azathioprine maintenance therapy could reduce the incidence of relapse in this setting.

Methods

Patients newly diagnosed with PR3-AAV at 12 centers in The Netherlands during 2003-11 who received a standardized induction regimen consisting of oral cyclophosphamide and corticosteroids were enrolled (n=131). Patients were randomized to standard or extended azathioprine maintenance therapy when C-ANCA was positive at the time of stable remission. Standard maintenance treatment consisted of azathioprine (1.5-2.0 mg/kg) until one year after diagnosis and subsequent tapering to 25 mg every 3 months. Extended azathioprine maintenance therapy (1.5-2.0 mg/kg) was continued until 4 years after diagnosis and tapered thereafter. The primary endpoint was relapse-free survival at 4years after diagnosis.

Results

In patients with PR3-AAV who were C-ANCA positive at the time of stable remission, relapse-free survival at 4 years after diagnosis did not differ significantly between standard azathioprine (n= 24) and extended azathioprine (n= 21) maintenance therapy (P= 0.40). There was also no significant difference in relapse-free survival between patients receiving standard azathioprine (n= 106) versus extended azathioprine maintenance therapy (n= 21; P=0.94). In addition, there was no difference in relapse rate between patients with PR3-AAV who were C-ANCA positive (n= 45) at the time of remission versus patients who became C-ANCA negative at the time of remission (n= 82; P=0.62).

Conclusions

This randomized trial suggests that extended azathioprine maintenance therapy has only a limited effect on the prevention of relapse in patients with PR3-AAV at 4 years after diagnosis. Moreover, positive C-ANCA status at stable remission was not associated with an increased rate of relapse.

Trial registration

clinicaltrials.gov. Identifier NCT 00128895

INTRODUCTION

Granulomatosis with polyangiitis (GPA, previously called Wegener's granulomatosis) and microscopic polyangiitis (MPA) are small-vessel vasculitides frequently associated with antineutrophil cytoplasmic antibody (ANCA) (1). In the majority of patients with so-called ANCA-associated vasculitis (AAV), disease remission can be induced by treatment with cyclophosphamide and corticosteroids or, as shown more recently, with rituximab and corticosteroids. Higher cumulative doses of cyclophosphamide are associated with considerable toxicity, and therefore the current standard of care is a stepwise approach comprising cyclophosphamide induction therapy followed by a less toxic maintenance agent, preferably azathioprine or methotrexate, for at least 18 months after diagnosis (2-4). However, the optimal duration of this maintenance therapy is unknown and a substantial number of patients experience disease relapse within 5 years after diagnosis. These relapses are associated with considerable morbidity and mortality (5-7), and avoidance of relapse via patient-tailored maintenance therapy would be highly preferable.
Several studies have shown that patients positive for proteinase 3 (PR3)-ANCA are more prone to relapse during long-term follow-up compared with myeloperoxidase (MPO)-ANCA positive patients (8,(9). Additionally, in a retrospective study, we previously observed that in patients with PR3-ANCA associated vasculitis, positive cytoplasmic (C)-ANCA at the time of a switch to azathioprine following successful induction with cyclophosphamide is associated with a significantly increased risk for relapse during long-term follow-up (10).
Therefore, we hypothesized that in patients with PR3-AAV, positive C-ANCA after induction of stable remission is associated with an increased risk of relapse and that these patients may benefit from extended azathioprine maintenance therapy. Here, we report the results of a randomized multicentre clinical trial ('AZA-ANCA') to evaluate the efficacy and safety of extended azathioprine maintenance therapy in patients with PR3-AAV who remain C-ANCA positive after induction of remission by therapy including oral cyclophosphamide.

METHODS

The 'AZA-ANCA trial was conducted at 12 hospitals in The Netherlands between June 2003 and October 2014. The study protocol was reviewed and approved by the medical ethical committee of the University Medical Center Groningen'(UMCG) and the participating centres (no 2002/213).
Consecutive patients with newly diagnosed PR3-ANCA associated vasculitis who were treated with cyclophosphamide and prednisolone induction therapy were recruited. All participants were > 18 years of age and provided written informed consent.

Exclusion criteria were intolerance for azathioprine, or inability to give informed consent. Patients were withdrawn in the event of failure to control progressive disease using the induction protocol or for failure to achieve remission within 6 months after diagnosis.
Patients were enrolled between diagnosis and remission. After 3 months of remission, defined as 'stable remission', when therapy was switched to azathioprine, C-ANCA status was determined in a central laboratory at the UMCG by indirect immunofluorescence (IIF) (11). Patients who were C-ANCA positive (IIF ≥ 1: 40) were randomized in a 1:1 ratio to receive standard or extended azathioprine maintenance therapy. Patients who were C-ANCA negative at the time of stable remission were all treated according to the standard regimen with azathioprine maintenance therapy and decreasing doses of prednisolone.

Drug regimen and treatment protocol
All patients received oral cyclophosphamide (2 mg/kg) and prednisolone (1 mg/kg) for the induction of remission. Additionally, intravenous methylprednisolone pulses and/or plasmapheresis were permitted when indicated according to clinical judgement. Dosages of cyclophosphamide were adjusted to maintain the leukocyte count above 4x 10*9/liter. At 4-6 weeks after start of therapy, the daily prednisolone dosage was tapered by 10 mg every 2 weeks until 30 mg/day and thereafter decreased by 5 mg every 2-4 weeks. When the dose of prednisolone was 15 mg, it was tapered by 2.5 mg every 2 weeks. Steroids were stopped at week 28- 34 after diagnosis.
After remission had been sustained for 3 months ('stable remission'), patients were switched to azathioprine (1.5-2 mg/kg). The dose of azathioprine was adjusted to maintain the leukocyte count above 4x 10x 9/L. In the standard treatment arm, whether for C-ANCA negative patients or for C-ANCA positive patients randomized to receive standard azathioprine therapy, 1 year after diagnosis azathioprine was tapered by 25 mg every 3 months, resulting in a total treatment duration of approximately ~24-30 months. In the C-ANCA- positive group, patients randomized to the extended azathioprine arm continued to receive azathioprine at a dose of 1.5-2 mg/kg until 4 years after diagnosis, thereafter tapered by 25 mg every 3 months until discontinuation.
During treatment, all patients received prophylaxis against *Pneumocystis jirovecii* pneumonia (480 mg co-trimoxazol daily or 960 mg co-trimoxazol every other day). Prophylactic therapy against candidiasis and osteoporosis was given according to local practice.

Patient evaluation and outcomes
Patients were evaluated at the time of diagnosis, at the point of switch to azathioprine (i.e. at 'stable remission'), at 12 months and then every 3 months until the end of the study.
At each visit, disease activity was evaluated using the Birmingham Vasculitis Activity Score (BVAS) (12). Routine laboratory analysis included complete blood count, measurements of ESR, serum urea, creatinin, C-reactive protein (CRP), urinary sediment, and analysis of

24-h urinary protein. ANCA testing was performed locally at the time of diagnosis, during follow-up, and if relapse occurred. ANCA testing by IIF at the time of switch to azathioprine was performed centrally at the UMCG.
Remission was defined as a BVAS score of 0 and low stable CRP (< 10 mg/dl) (13). Relapse was defined as recurrence or the first appearance of one or more BVAS items attributable to active vasculitis (14).
The primary endpoint was relapse-free survival, defined as the time from remission to first relapse, at 4 years post-diagnosis. Secondary outcomes were the cumulative dosages of cyclophosphamide, prednisolone and azathioprine; cumulative organ damage; side effects due to study medication; and severity of relapses.

Statistical analysis

On the basis of our retrospective study, we anticipated that 50% of patients would be C-ANCA positive at the point of achieving stable remission (10). We calculated a predicted relapse rate of 80% at 4 years for patients with PR3-AAV who were C-ANCA positive at stable remission (10). The sample size was calculated based on the hypothesis that extended azathioprine maintenance therapy would reduce the 4year relapse risk by 30%, from 80% to 50%. Randomization of 90 C-ANCA- positive patients was required to achieve a power of 0.8 at a significance level of 0.05. Because we anticipated that 50% of enrolled patients would remain C-ANCA positive and thus be eligible for randomization, we aimed to include 180 patients with newly diagnosed PR3-AAV. Patients were randomized 1:1 to standard and extended azathioprine therapy. Closed envelopes with the randomized treatment duration were produced before inclusion of the first patient. Randomization was performed in blocks of four. Patients were stratified according to hospital, i.e., patients from the UMCG versus patients from other hospitals.
Analysis of the primary endpoint, the time from diagnosis until first relapse of disease, was based on the time to loss of follow-up, time to study end or time to 1 October 2014.
All analyses were performed with GraphPad Prism version 5.04. Groups were compared using Student's t-test or the $\chi 2$ test. For comparison of non-parametric data, the Mann-Whitney-U test or Kruskall-Wallis test were used. Relapse-free survival curves were assessed by Kaplan-Meier estimates for survival distribution. Differences between groups in survival after 12 months of therapy were analyzed using log-rank tests. The primary analysis was performed on an intention-to-treat basis. Additionally, a per-protocol analysis, limited to patients who received treatment in full accordance with the study protocol, was conducted. A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Between June 2003 and October 2011, 136 patients were enrolled in the study. Of these, five patients were excluded for various reasons (Figure 1). C-ANCA status was determined in 131 patients who achieved stable remission. Of these 131 patients, 86 patients (66%) were C-ANCA negative and therapy was switched to azathioprine with standard duration. Forty-five patients (34%) were C-ANCA positive at the time of stable remission and these patients were subsequently randomized. Of these 45 patients, 24 were randomized to standard duration of maintenance therapy and 21 patients were randomized to extended azathioprine maintenance therapy for 48 months after diagnosis.

Recruitment to trial was slower than anticipated, and C-ANCA positivity at stable remission (34%) was lower than expected. It was therefore concluded that the planned population of 90 C-ANCA- positive patients was not feasible within a reasonable time frame, and the decision was made in October 2011 to end enrolment prematurely, at which point 45 patients had been randomized.

Patient characteristics at baseline did not differ significantly between the three groups (Table 1). Most patients were diagnosed with GPA. Patients who were C-ANCA negative

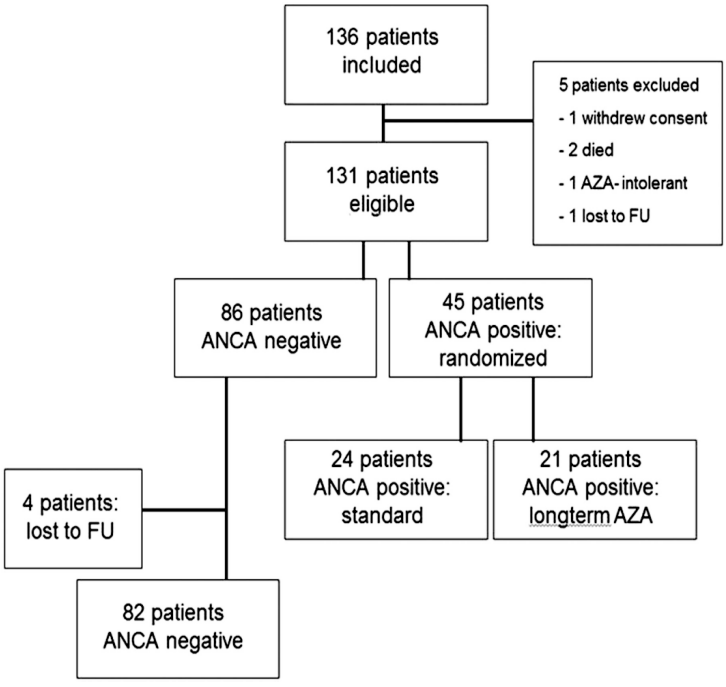

Figure 1 Flowchart of enrolment and randomization.

Table 1 Patient characteristics at diagnosis and at time of remission.

	C-ANCA negative	C-ANCA positive AZA standard	C-ANCA positive AZA extended	p
N	82	24	21	
Age (year)	55 (21-78)	53 (18-82)	56 (26-80)	0.36
Sex (men), n (%)	76 (62)	67 (16)	57 (12)	0.19
Diagnosis, n GPA/MPA/RL*	78/2/4	23/0/1	19/0/2	
Organ involvement:				
Renal, n (%)	62 (76)	16 (70)	16 (80)	0.49
Pulmonary, n (%)	45 (55)	16 (70)	13 (65)	0.66
ENT#, n (%)	62 (76)	20 (87)	11 (55)	0.12
BVAS	20 (8-33)	18 (8-33)	20 (9-39)	0.24
CRP (mg/l)	127 (2-350)	104 (2-286)	98 (1-347)	0.25
Creatinine at diagnosis (µmol/l)	199 (50-1008)	144 (57-572)	155 (65-799)	0.40
Methylprednisolone, n (%)	28 (34)	4 (17)	6 (29)	0.26
RRT**, n (%)	8 (10)	0	1 (5)	0.06
PPh##, n (%)	22 (27)	2 (8)	2 (10)	0.24
Time to remission months	4.8 (1.8-8.7)	4.4 (2.5-7.5)	4.4 (2.5-8.9)	0.08
Creatinine at remission (µmol/l)	130 (62-524)	109 (40-219)	102 (64-165)	0.47
Cyclophosphamide cumulative (gram), mean (SD)	17.7 (7.0)	14.7 (9.2)	13.2 (6.0)	0.10
Prednisolone cumulative (gram), mean (SD)	5.6 (1.9)	5.8 (2.0)	7.8 (3.5)	0.25
Treated per protocol, n (%)	67 (82)	22 (92)	17 (81)	0.48
Follow-up (months)	46.3 (25.8-52.9)	45.6 (24- 48.2)	45.2 (22-48.7)	0.37

Values are means and range unless indicated otherwise

* GPA/MPA/RL= granulomatosis with poly-angiitis/microscopic poly-angiitis/renal limited vasculitis.

#ENT = Ear, nose, throat

**RRT = Renal replacement therapy

##PPh= plasmapheresis

at the point of achieving stable remission were more likely to have renal insufficiency and require renal replacement therapy (P=0.06). There were no significant differences in the requirement for additional treatment with methylprednisolone or plasmaphereris. Cumulative cyclophosphamide and prednisolone dosages did not differ between the three groups. The mean time to stable remission was 4.6 months and was similar between the three groups. As expected, subsequent cumulative azathioprine dosages and durations were significantly higher in patients treated with extended azathioprine maintenance compared with patients given standard azathioprine maintenance therapy. In total, four patients were lost to follow-up during the study period.

Relapse-free survival

In total, 45 patients were C-ANCA positive at stable remission and were subsequently randomized, with 24 patients receiving standard-duration azathioprine maintenance therapy and 21 patients receiving extended azathioprine therapy The primary endpoint was relapse-free survival 4 years after diagnosis.

Of the 24 patients on standard azathioprine therapy, 11 patients (46%) experienced a relapse within 4 years after diagnosis, compared with 5 of the 21 patients on extended azathioprine maintenance (24%). Cumulative estimated relapse-free survival in the standard group was 88 and 51%, at 2 and 4 years after diagnosis, respectively, compared with78 and 72% in the extended treatment group {relative risk [RR] 0.65 [95% confidence interval (CI) 0.24-1.75]; P=0.40)(Figure 2A). The severity of relapses, as measured by BVAS, CRP and organ involvement, did not differ between the two groups (Table 2).

Among the patients who were C-ANCA negative at switch (n= 82), 33 patients (40%) experienced a relapse during follow-up within 4 years after diagnosis. For patients who received standard azathioprine maintenance, the cumulative estimated relapse-free survival at 2 and 4 years after diagnosis was 80 and 60%, respectively. Relapse risk, and the severity of relapses, did not differ significantly between the other two groups (Figure 2B and Table 2).

There was also no significant difference in relapse-free survival between all patients on standard azathioprine (n= 106) compared with patients treated with extended azathioprine maintenance therapy (n= 21; P=0.94) (supp mat, Figure S3A). Patients with PR3-AAV who were C-ANCA positive (n= 45) and patients who were C-ANCA negative (n= 86) at the time of remission did not differ significantly in terms of relapse rate (P=0.62) (supp mat, Figure S3B). C-ANCA positivity at switch was not significantly associated with the occurrence of relapse.

Per-protocol analysis

The per-protocol population excluded two patients in the C-ANCA- positive group randomized to standard therapy and four patients in the C-ANCA- positive group randomized to

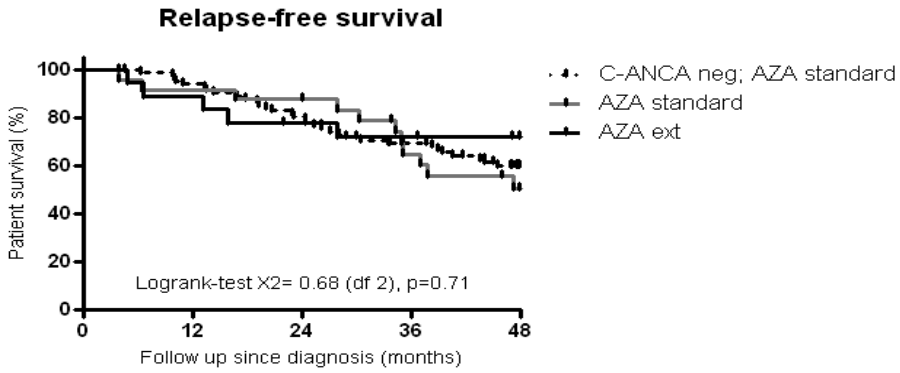

Figure 2A Relapse-free survival in C-ANCA positive patients randomized to standard and extended therapy.

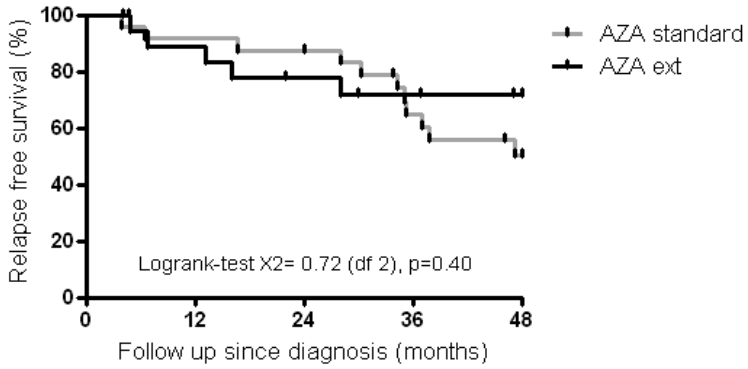

Figure 2B Relapse-free survival in C-ANCA negative patients at switch and C-ANCA positive patients randomized to standard and extended therapy.

extended azathioprine maintenance therapy. Azathioprine intolerance led to withdrawal of the study drug in four patients (three patients randomized to standard azathioprine and one patient randomized to extended azathioprine).

In the per-protocol analysis, relapse-free survival again did not differ significantly between C-ANCA- positive patients randomized to standard (n= 22) or extended azathioprine maintenance (n= 17) (P= 0.83). In the standard group, cumulative estimated relapse-free survival was 86 and 51%, at 2 and 4 years after diagnosis, respectively, compared to 71 and 63% in the extended group (RR 0.89; (95% CI 0.31-2.57); P= 0.83).

Table 2 Relapse characteristics.

	C-ANCA negative	C-ANCA positive AZA standard	C-ANCA positive AZA extended	p
Relapse, n (%)	33 (40)	11 (46)	5 (25)	0.28
Multiple relapses, n	4	2	2	
BVAS	12 (2-26)	14 (4-27))	9 (2-28)	0.30
CRP (mg/l)	46 (1-182)	70 (6-287)	95 (1-324)	0.62
Organ involvement:				
Renal, n (%)	15 (45)	8 (73)	2 (40)	0.26
Pulmonary, n (%)	5 (15)	3 (27)	1 (20)	0.66
ENT#, n (%)	15 (45)	7 (63)	1 (20)	0.26

Values are means and range unless indicated otherwise

#ENT = Ear, nose, throat

Death and adverse events

Eight patients died during the study. No deaths were directly attributable to study medication. Six of the patients who died were C-ANCA negative at remission and two were C-ANCA positive (both were randomized to extended azathioprine therapy). The causes of death were malignancy (n=4), infection (n=1), pulmonary fibrosis (n=1) and unknown (n=2). Survival was not significantly different between the three patient groups (P= 0.86).

The number of reported adverse events was not significantly different between the three groups (Table 3). In 24 patients in the C-ANCA- positive group treated with standard azathioprine, 27 adverse events were reported, compared with 19 adverse events in 21 C-ANCA positive patients treated with extended azathioprine (P=0.30).

Five severe infections were reported in both C-ANCA positive groups. Viral infective episodes were reported separately but were also not significantly different (P=0.67). In 13 C-ANCA negative patients, and in 3 patients in both the C-ANCA- positive groups, significant leukopenia led to a temporary dose reduction or withdrawal of azathioprine (P=0.91). Malignancy occurred in five patients in the C-ANCA- negative group (one bladder, two skin, two gastrointestinal), one patient in the C-ANCA- positive group treated with standard azathioprine therapy (breast cancer), and one patient in the extended azathioprine group (cholangiocarcinoma) (P= 0.92).

DISCUSSION

In this prospective, randomized study, we did not find a significant difference in relapse rate between standard-duration or extended-duration azathioprine maintenance therapy

Table 3 Adverse events.

	C-ANCA negative N (82)	C-ANCA positive AZA standard N (24)	C-ANCA positive AZA extended N (21)	p
Infection	10	5	5	0.32
CMV/HSV/HZV/Candida*	1/2/4/2	1/0/1/0	0/0/1/0	0.67
Diabetes Mellitus	8	3	3	0.81
Leucopenia	13	3	3	0.91
Thrombopenia	2	1	0	0.65
Cutaneous eruption	3	1	0	0.66
Eye involvement	3	1	0	0.66
ENT events#	0	1	0	0.12
Venous thrombosis	6	0	1	0.38
Gastrointestinal events	5	3	3	0.37
Liver toxicity	6	1	0	0.40
Polyneuropathy	1	0	0	0.76
Myopathy	1	0	1	0.40
Amaurosis fugax	1	0	0	0.76
Fractures	0	1	1	0.15
Non-STEMI	0	1	0	0.16
Pulmonary hypertension	1	0	0	0.76
Malignancy	5	1	1	0.92
Azathioprine intolerance	10	3	2	0.94
Death due to study drug	0	0	0	
Any adverse event	76	27	19	0.30

*CMV/HSV/HZV = cytomegalovirus/herpes simplex virus/herpes zoster virus

#ENT = Ear, nose, throat

in patients with PR3-AAV who were C-ANCA positive at the time of switch. A non-significant hazard ratioof 0.65 (95% CI 0.24-1.75; P= 0.40) with extended versus standard therapy was observed after 4 years of follow-up. Moreover, the severity of relapse did not differ between treatment groups. Analysis of relapse in the patients who were treated as per protocol did not change these findings. Furthermore, we were not able to confirm our previous finding that the relapse rate is increased in patients who were persistently C-ANCA positive.

A major limitation of this study was its premature termination, which meant that the trial was seriously underpowered. A posthoc power calculation showed that the study had only a 23% power to detect a significant between-group difference in the primary endpoint using a two-sided Fisher's Exact test. On the basis ofthe observed relapse rates, 89 patients

were required in each treatment arm to achieve 80% power, i.e. > 178 C-ANCA positive patients would have had to be randomized. Because 34% of patients were C-ANCA-positive at stable remission, at least 523 patients with PR3-AAV would have been required, Alternatively, the absolute difference in proportions would have had to be at least 0.38 to achieve 80% power, representing a 4-year relapse rate in the extended azathioprine maintenance group of < 8%. Because these patients were all PR3-ANCA positive patients, a well-known risk factor for relapse (8,(9), we do not believe this was realistic. Although our data suggest an effect of extended azathioprine maintenance therapy on prevention of relapse this cannot be confirmed due to the lack of power. The ongoing REMAIN study, which is also studying long-term azathioprine maintenance therapy in AAV, may help answer this question.

At baseline, we found no differences in disease severity between treatment groups. However, compared with our previous retrospective cohort of C-ANCA- positive patients (10), patients in the current study were older and had less ENT involvement. There was no difference in induction therapy because patients in both studies were treated with oral cyclophosphamide. As both age and renal failure are known to influence cyclophosphamide pharmacokinetics, the observed difference in age between the studies might have contributed to the difference in relapse rates, as might other patient and disease characteristics.

However, the most obvious reason for the difference between our retrospective and prospective studies is the size of the study populations: this study is considerably larger. Furthermore, this study has the important additional methodological advantage of prospective observation.

Since the CYCAZAREM study, azathioprine has been the maintenance agent of choice in AAV (2). The Kidney Disease: Improving Global Outcomes guideline advises a minimum duration of 18 months for azathioprine maintenance therapy (15). However, no evidence is available for longer duration of maintenance therapy. Because most relapses occur during tapering and discontinuation of immunosuppressive drugs, which typically takes place between months 12 and 24 after diagnosis, prolonging maintenance therapy in patients at highest risk for relapse is an attractive concept. PR3-ANCA positivity and renal function are major predictive factors for relapse in patients with AAV (8,16). However, although prolonging azathioprine maintenance therapy according to these risk factors seems rational, this study does not support this strategy, and other comparative data are lacking. Recent studies suggest that maintenance regimens with alternative agents, particularly rituximab, may be more effective than azathioprine maintenance therapy. The recently published MAINRITSAN study showed that after induction of remission with intravenous cyclophosphamide, rituximab was more effective in preventing relapse prevention than azathioprine after 2 of follow-up (17). Studies are required to confirm this finding, and to establish whether this is also true after induction therapy with rituximab.

The RAVE and the RITUXVAS trials have showed that induction of remission with rituximab without maintenance immunosuppression offered equal potency to induction with cyclophosphamide and subsequent azathioprine maintenance up to 18 months (18,(19).
Besides the fact that our study was underpowered, several other limitations should be acknowledged. Induction therapy comprised oral cyclophosphamide, so results should be interpreted cautiously for patients treated with intravenous cyclophosphamide. Previously, induction of remission with intravenous cyclophosphamide was found to be as effective as oral cyclophosphamide, and to be associated with fewer episodes of leukopenia, but it has also been associated with a higher subsequent relapse rate (20). In patients treated with intravenous cyclophosphamide, extended azathioprine maintenance might therefore be more effective than following induction therapy with oral cyclophosphamide. Finally, this study was not blinded. Because relapse is based on the BVAS, which includes several components that are relatively subjective, this could have introduced bias. However, in this study the severity of relapses did not differ between the treatment groups, making it is less likely that the unblinded design had an important influence on the results.
The overall relapse rate of 37% in our study is compatible with relapse rates in published studies of maintenance therapy with similar follow-up periods (3, 4). Therefore, in the light of the study limitations, we conclude that extended azathioprine maintenance therapy beyond 18 months has, if any, no more than a limited effect on the prevention of relapses in patients with PR3-AAV at 4 years after diagnosis.
Finally, adverse events were equally divided between the groups and comparable with earlier studies of azathioprine maintenance therapy (3,4). Long-term azathioprine therapy in our population was not associated with additional toxicity or infections, although potentially azathioprine-related toxicity could occur or only be recognized after longer follow-up.
In conclusion, this prospective study did not show that positive C-ANCA at the point of stable remission after induction therapy with oral cyclophosphamide is associated with a significantly increased risk of relapse in patients with PR3-AAV. Thus, the findings do not justify adoption of maintenance azathioprine therapy in this patient group. Furthermore, on the basis of our results we recommend a standard duration of azathioprine maintenance therapy (1.5-2.0 mg/kg/day for 1 year and subsequent tapering) after induction of stable remission with oral cyclophosphamide in patients with PR3-AAV.

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8

Long term azathioprine maintenance therapy in ANCA associated vasculitis: combined results of long term follow-up data.

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Submitted

ABSTRACT

Objectives

We studied whether in ANCA-associated vasculitis (AAV) patients, the duration of azathioprine maintenance therapy following cyclophosphamide induction therapy influenced relapse rate during long term follow up.

Methods

We included 380 newly diagnosed AAV-patients from six European multicenter studies with available long term follow up data and treated with azathioprine maintenance after successful remission induction with cyclophosphamide and high dose corticosteroids. The trials differed in inclusion criteria (disease severity) and in the duration of maintenance therapy. Patients were combined from all six studies and grouped according to the duration of azathioprine maintenance after remission induction: ≤18 months / ≤ 24 months /≤36 months /≤ 48 months / > 48 months. Primary outcome was relapse free survival at 60 months.

Results

380 patients, 58% male, median age at diagnosis 59.4 years (IQR 48.3-68.2) with GPA (n=236), MPA (n=132) or RLV (n=12) were included. Most patients, (n= 225, 59%) were anti-PR3 positive, 120 (32%) anti-MPO-positive and 35 (9%) ANCA negative. During follow up, 155 patients (40.8%) had a first relapse; 84 first relapses occurred during azathioprine-maintenance therapy (1 relapse per 117 patient months) and 71 after stopping azathioprine of which 49 occurred within 60 months after diagnosis (1 relapse/113 months) and 22 thereafter. During the first 12 months after stopping azathioprine, 20 relapses occurred (1 relapse/119 months). Twenty-nine relapses occurred between 12-60 months after discontinuation of azathioprine (1 relapse/186 months). Relapse free survival at 60 months after diagnosis was 65.3% for patients receiving azathioprine maintenance >18 months after diagnosis versus 55% for those who stopped maintenance ≤18 months (p 0.11). Relapse-free survival was furthermore associated with type of induction therapy (intravenous versus oral) and ANCA-specificity (PR3-ANCA versus MPO-ANCA/negative). Forty-nine patients (13%) died during long term follow up, resulting in a survival of 98%, 93% and 84% at 24 months, 60 months and 10 years after diagnosis respectively. Thirty-seven (10%) of all AAV-patients reached ESRD with a median follow up of 61.5 years.

Conclusions

Post-hoc analysis of combined trial data suggest that stopping azathioprine maintenance therapy does not lead to a significant increase in relapse rate and that azathioprine maintenance for more than 18 months after diagnosis does not significantly influence relapse free survival. ANCA specificity has more effect on relapse free survival than the duration of maintenance therapy and should be used to tailor therapy individually.

INTRODUCTION

Active anti-neutrophil cytoplasm auto-antibody (ANCA) associated vasculitis (AAV) can be successfully treated with cyclophosphamide or rituximab, combined with glucocorticoids. AAV is a chronic relapsing disease with substantial morbidity and mortality due to either disease itself or the toxicity of long term and repeated treatment (1-5). Untreated, AAV is almost invariably fatal (6). However, with the advent of immunosuppression, outcomes have improved considerably with 10-year survival reaching 60-90%. Disease remission is typically induced using cyclophosphamide and glucocorticoids over 3 to 6 months, allowing remission rates of 80-90% (4,7). Once remission has been achieved, immunosuppressive regimens are converted to less toxic maintenance treatments such as azathioprine or mycophenolate mofetil. However, disease relapses remain common, occurring in 50% of patients within 5 years (8). Relapses are associated with accrual of organ damage and morbidity.

Randomized trials have compared various remission maintenance treatments including methotrexate, mycophenolate mofetil and azathioprine (4,5). Although these studies have established azathioprine as the superior maintenance therapy and methotrexate in non-renal patients, the optimal duration of maintenance therapy however is unknown. Current treatment guidelines recommend discontinuation of maintenance therapy after 18-24 months, since this was the typical maintenance treatment duration in previous clinical trials (4-6,9,10). Given this, it is striking that most relapses occur during or after withdrawal of maintenance therapy (11-13), consistent with the median disease-free survival of 21.8 (range 9.8-27) months after diagnosis (14-17). It is therefore reasonable to postulate that relapse rates may be reduced by extending the duration of maintenance therapy.
However, in a prospective study of 146 patients with PR3-ANCA associated vasculitis who remained ANCA-positive at the time of remission,, relapses rates were similar between those treated with azathioprine for the standard treatment duration (n=23) versus those receiving longer treatment duration (48 months, n=21) (18,19). In the rituximab for induction-remission trail (RAVE), the rituximab-arm received no additional maintenance treatment at all but had the same outcomes on relapse rate at 18 months of follow up compared with sequential cyclophosphamide and azathioprine (20). Both outcomes may thus raise questions about the influence on relapse rate and the overall efficacy of azathioprine 'maintenance'.
Using patient-level data from several landmark trials (4,5,7,10,21,22) with extended follow-up from the European (EUVAS) and French Vasculitis Study Groups (FVSG), we assessed the duration of maintenance therapy with azathioprine and its effect on relapses during long term follow up. In these trails, azathioprine was administrated during long term follow up for a period of 18-48 months, with follow up until 60 months and longer, thus allowing evaluation of the effect of treatment duration on relapse rate.-

METHODS

We included long term data from five EUVAS-trials and one FVSG trial (4,5,7,10,21,22). This resulted in individual data from 759 AAV patients, enrolled between 1995 and January 2009 from 70 hospitals in 15 countries. The trials were conducted according to the Helsinki declaration and subsequent amendments and had received ethical board approval in each participating country. All patients had newly diagnosed GPA, MPA or renal limited vasculitis at trial entry, according to Chapel Hill Consensus Conference definitions.

Data collection and assessment

In order to assess the relationship between azathioprine maintenance duration and relapse risk, we excluded from the analysis all patients who did not receive azathioprine as remission maintenance (n=225), those with less than 12 months follow-up data (n=134), and those experiencing relapse during the first 12 months (n=20) (Figure 1). The analysis cohort (n=380) were assigned to one of 6 bins based on the duration of azathioprine

Table 1 Main characteristics of original trials included

	Inclusion	Induction	Maintenance
CYCAZEREM Jayne et al, 2003 N=144	Newly diagnosed WG/ MPA/RLGN seCreat < 500 umol/l.	Cyc oral 2 mg/kg/day Pred 1 mg/kg/day	Cyc 1.5 mg/kg/day + pred N= 73 AZA 2 mg/kg/day + pred N=71
NORAM De Groot et al, 2005 N=100	Newly diagnosed AASV seCreat < 150 umol/l Without critical organ manifestations	Cyc oral 2 mg/kg N= 46 or MTX oral, 20-25 mg/ wk N = 49 + Pred 1 mg/kg/day	Cyc 1.5 mg/kg/day + pred N = 44 MTX oral, 20-25 mg/ wk N = 47

treatment: < 12 months, 12-18 months, 19-24, 25-36, 37-48 and 49-60 months.
Since the patients in the NORAM-trial received no azathioprine maintenance therapy after 12 months of induction therapy with cyclophosphamide, these patients were considered as a separate group and analyzed separately.

Statistical analysis

Summary data are presented as mean ± SD or median and interquartile range as appropriate. Between-group tests of proportions were carried out using the chi-square test. For comparison of non-parametric data, the Mann-Whitney U test or Kruskall Wallis-test with post-test for > two group comparison was used. The primary outcome of our analysis was relapse free survival and was assessed by Kaplan-Meier estimates for survival distribution. Survival estimates between groups were compared using the log-rank test. Univariate analysis was used to focus on the influence of discontinuation or continuation of

Duration	Relapse	AE's
Treatment and FU 18 months	CYC: N=10, 13.7%	CYC: N=7, 10%
	AZA: N=11, 15.5%	AZA: N=8, 11%
	P 0.65	P 0.94
		Death N=1.
Treatment 12 months	(major)	N= 51 (83 episodes)
	CYC:	CYC:
FU 18 months	N=9	Mild/moderate: 39 episodes
	20%	Severe to life threatening: 6 episodes
	MTX:	MTX:
	N=17	Mild/moderate: 29 episodes
	36%	Severe to life threatening: 9 episodes
	P 0.11	Death CYC N=2 (4%) MTX N=2 (4%)

Table 1 Continued

	Inclusion	Induction	Maintenance
MEPEX Jayne et al, 2007 N=137	Newly diagnosed AAV confirmed by renal biopsy and seCreat > 500 umol/l.	Plasma exchange N= 70 or Methylprednisolone 3000 mg N=67 and CYC oral 2.5 mg/kg/day +Pred 1 mg/kg/day	AZA 2 mg/kg/day after month 6
CYCLOPS De Groot et al, 2009. N=149	Newly diagnosed AAV with renal involvement	CYC i.v 15 mg/kg every 2-3 wks N=76 or CYC oral 2 mg/kg/day N=73 Both groups + Pred 1 mg/kg	AZA 2 mg/kg/day from 3 months after remission until month 18.
WEGENT Pagnoux et al, 2008. N=126	WG/MPA	CYC i.v 0.6 mg/0.6 x m2 first two weeks, thereafter 0.7 mg/m2 every three weeks. Pred 1 mg/kg/day	AZA 2.0 mg/kg/day N=63 MTX 0.3 mg/kg/wk N=63
IMPROVE Hiemstra et al, 2010 N=156	Newly diagnosed AAV (WG/MPA)	CYC 1.5-2 mg/kg/day + Pred 1 mg/kg/day	AZA 2 mg/kg/day N=80 MMF 2000 mg/day N=76

Duration	Relapse	AE's
Treatment and FU 12 months	N=2 (not mentioned in original article)	N=122 (244 episodes) - N=67 severe to life-threatening -PLEX N= 35 (50%) - Methyl N=32 (48%) P 0.80 Death PLEX N=19 (27%) Methyl N=16 (24%) P 0.68
Treatment and FU 18 months (0.25-18 months)	All patients N=19 (14.5%) CYC i.v N=13 (17%) CYC oral N=6 (8%) HR 2.01.	N= 114 (228 episodes) - N=85 mild to moderate - N=29 severe to life-threatening Death CYC i.v N=5 (7%) CYC oral N=9 (12%) P 0.79.
Treatment 15 months FU 29+/- 13 months	AZA: N=23, 37% MTX: N=21, 33% P 0.71	AZA: N=29, 46% MTX: N=35, 56% P 0.29. Death N=1 (MTX)
Treatment 39 months (0.66-53.6) FU 42 months	AZA: N=30, 37% MMF: N=42, 55% P 0.03	AZA: N=13, 16% MMF N=8, 7.5% P 0.12 Death N=2 (1 AZA, 1 MTX)

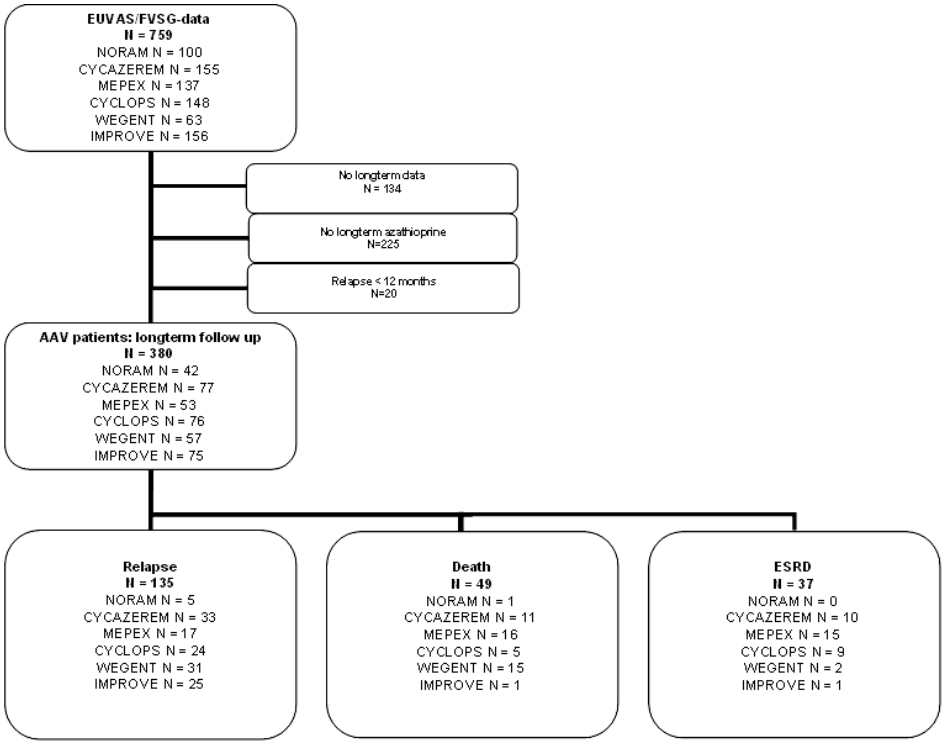

Figure 1A Flowchart on included studies and number of excluded data.

	Originally included	Original/ longterm Aza	Relapse < 12 mths	Included in analysis	Mean FU (mths)
NORAM	100	-	-	46	61,8
CYCAZEREM	155	71/ 77	4*	77	78,2
MEPEX	137	54	1**	53	44,3
CYCLOPS	149	76	2	76	46,4
WEGENT	159	63	6	57	97,8
IMPROVE	156	80	5	75	40,2

Figure 1B Flowchart on included studies and number of excluded data.

* 4 early relapses happened in patients who were not on long term azathioprine

** 2 relapses happened in patients who were not on long term azathioprine

azathioprine after 18, 24, 36, 48 and 60 months on relapse risks. Multivariate analysis using Cox-proportional hazards models, with withdrawal of azathioprine as time-dependent variable, was focused on differences between continuation of azathioprine, the period < 12 months after discontinuation of azathioprine- and the period > 12 months after discontinuation of azathioprine. Other co-variates in the multivariable model were age at presentation, sex, diagnosis (GPA versus MPA), route of cyclophosphamide administration (intravenous versus oral), duration of induction therapy, serum creatinine at presentation and BVAS at diagnosis. The proportional hazards assumptions were assessed using known predictors of relapse. For all comparisons, a two-sided p-value < 0.05 was considered statistically significant. Analyses were performed using SPSS22 and GraphPad Prism version 5.01.

RESULTS

Patients

All main characteristics and outcomes of the original clinical studies are summarized in table 1. Of all 759 AAV patients, eventually 380 patients were included in the analysis (Fig 1). For this patients, baseline characteristics for each trial cohort are shown in table 2. Trial cohorts differed in age, gender distribution and disease severity, as reflected in the baseline criteria (Table 2).

Overall, 221 (58%) patients were male. Median age at trial entry was 59.4 (48.3-68.2) years. Median serum creatinine was 142 (80-197) mmol/l and initial median BVAS was 19 (13-25). Two hundred thirty-six (62%) patients had GPA, while 225 (59%) of patients were anti-PR3 positive.

The protocol-defined duration of azathioprine therapy varied from <12 months to 42 months and left to the discretion of the physicians thereafter with the exception of IMPROVE, where maintenance was withdrawn after 42 months (4,5,7,10,21,22).

Relapses

During follow up, 155 patients (40.8%) had a first relapse; 84 first relapses occurred during azathioprine-maintenance therapy (1 relapse per 117 patient months) and 71 after discontinuation of azathioprine. Of this 71 relapses, 49 occurred within 60 months after diagnosis (1 relapse/113 months) and 22 thereafter. During the first 12 months after stopping azathioprine, 20 relapses occurred (1 relapse/119 months). Twenty-nine relapses occurred between 12-60 months after discontinuation of azathioprine (1 relapse/186 months). Relapse free survival at 60 months after diagnosis was 65.3% for patients receiving azathioprine maintenance >18 months after diagnosis versus 55.0% for those who stopped maintenance ≤18 months (p 0.11) (Fig 3A). At all later time points of

Table 2 Baseline characteristics of all included patients in the individual studies.

	CYCA-CEREM	NORAM	MEPEX	CYCLOPS	WEGENT	IMPROVE
N	77	42	53	76	56	75
Sex: M/F	36/41	20/22	37/16	42/34	31/25	55/20
Age: *median (range)*	56.8 (47.3-66.5)	53.5 (22-78)	64.8 (56.7-70.3)	60.7 (49.1-68.5)	59.3 (47.2-68.6)	61 (47.7-67.1)
GPA/MPA/RL	50/27/0	40/2/0	25/28/0	28/36/12	43/14/0	50/25/0
PR3/MPO/neg	48/17/12	33/5/4	30/18/5	34/41/1	36/17/4	44/22/9
BVAS: *median (range)*	18 (10-24)	15 (4-30)	20 (15-26)	20 (15-23)	24 (20-29)	19 (7-25)
seCreat: *median (range)*	146 (90-263)	84.5 (42-149)	724 (550-912)	162 (115-268)	105 (79-179)	177 (104-311)
Organ involvement: N (%)						
ENT	39 (51)*	42 (91)*	23 (53)	45 (59)	39 (70)	28 (37)
Pulm	44 (58)*	24 (51)*	18 (42)	45 (59)	44 (79)	36 (48)
Renal	71 (93)*	15 (32)*	43 (100)	75 (99)	45 (80)	42 (56)
Other	41 (53)	19 (41)*	22 (51)	42 (55)	30 (54)	20 (27)

discontinuing azathioprine maintenance therapy, comparing duration > and ≤ 24 months, > and ≤ 36 months and > and ≤ 48 months, a numerically small and not statistical significant increase in relapses was found (Fig 3B-D). Relapse-free survival was lower in patients who were treated with intravenous cyclophosphamide compared to oral treatment (p 0.042) and lower in patients with PR3-ANCA compared to MPO-ANCA (p 0.011).

In a multivariate Cox-proportional hazard model, relapse risk was associated with successful discontinuation (without relapse) of azathioprine during a period of > 12 months (p 0.027). This means that when no relapse occurs ≤ 12 months after withdrawal of azathioprine, the relapse risk seems to be decreased during long term follow. Relapse risk was associated with the route of administration of cyclophosphamide, intravenous versus oral, HR 1.2 (95% CI 1.015-1.432, p 0.023). Also PR3 versus other ANCA status, HR 1.32 (95% CI 1.1-1.57) p 0.002 and creatinine at diagnosis HR 0.99 (95% CI 0.998-1.000) p 0.032 were significant covariates (Table 3).

Patients in the NORAM-trail, who did not receive maintenance beyond 12 months after diagnosis, had a reduced 60 months actuarial relapse-free survival (39.9% versus 65.3%; p 0.001) (Fig 2B).

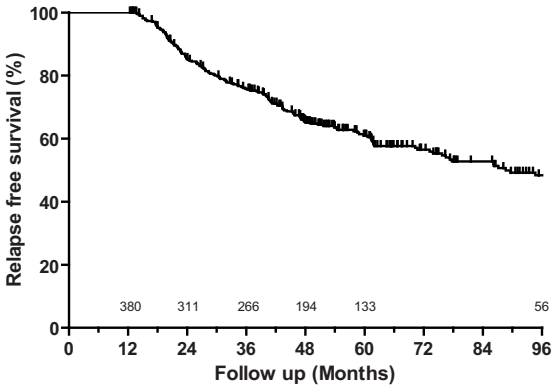

Figure 2A Relapse free survival during long term follow up, all patients.

Patients at risk (N=)

mths	12	24	36	48	60	96
N =	380	311	266	194	133	56

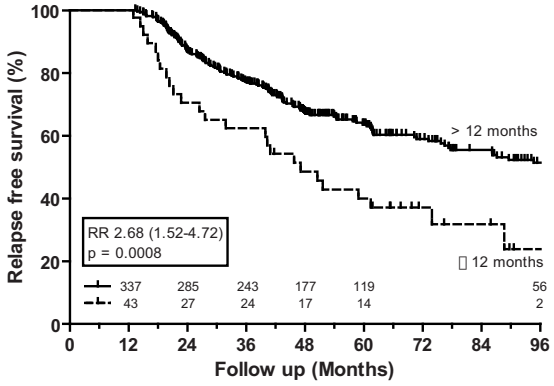

Figure 2B Relapse free survival during long term follow up,after withdrawal of azathioprine. P= 0.0008

Patients at risk (N=)

mths	12	24	36	48	60	96
> 12 mths	337	285	243	177	119	56
< 12 mths	43	27	24	17	14	2

Table 3 Multivariate Cox-proportional hazard analysis

	RR (95% CI)	p-value
Cyclophosphamide IV versus oral	1.206 (1.015-1.432)	0.023
PR3- versus other/none	1.316 (1.100-1.574)	0.002
Creatinine (diagnosis)	0.999 (0.998-1.000)	0.032
Azathioprine use (yes/no)	1.286 (0.870-1.901)	0.212
stopped < 12 months	1.183 (0.739-1.895)*	0.227
stopped > 12 months	0.570 (0.347-0.937)*	0.027

* as compared to current use

Table 4 Occurrence of at least one period of AE that can directly have been related to drug regimen

	NORAM > 6 mths	6-18 mths	19-24 mths	25-36 mths	37-48 mths	49-60 mths	P
Number of patients: n	44	46	78	58	74	81	
Infection: n (%)	2 (5)	20 (34)	19 (24)	19 (33)	27 (36)	19 (23)	0.07
CHD: n (%)	2 (5)	3 (7)	7 (9)	9 (16)	7 (9)	8 (10)	0.64
Malignancy: n (%)	4 (9)	-	7 (9)	6 (10)	8 (11)	9 (11)	0.24
Diabetes Mellitus: n (%)	2 (5)	5 (11)	7 (9)	6 (10)	11 (15)	6 (7)	0.63
Bone disorders: n (%)	2 (5)	4 (9)	5 (6)	5 (9)	4 (5)	3 (4)	0.73
Trombotic events: n (%)	1 (2)	2 (4)	2 (3)	6 (10)	6 (8)	7 (9)	0.35

Secondary outcomes: mortality and ESRD.

Of the selected patients who survived the first year without experiencing any relapse, 94 patients (13%) died during long term follow up, resulting in a survival at 12 months after diagnosis of 98%, of 93% at 60 months and 82% at 10 years after diagnosis. We did not find a difference in mortality between the groups with different azathioprine duration, i.e. < 12 months, 12-18 months, 19-24 months, 25-36 months, 36-48 months and 49-60 months. Causes of death were mainly cardiovascular and infectious (Table 5).

Thirty-seven patients (10%) reached ESRD (for distribution, see also Fig 1A).

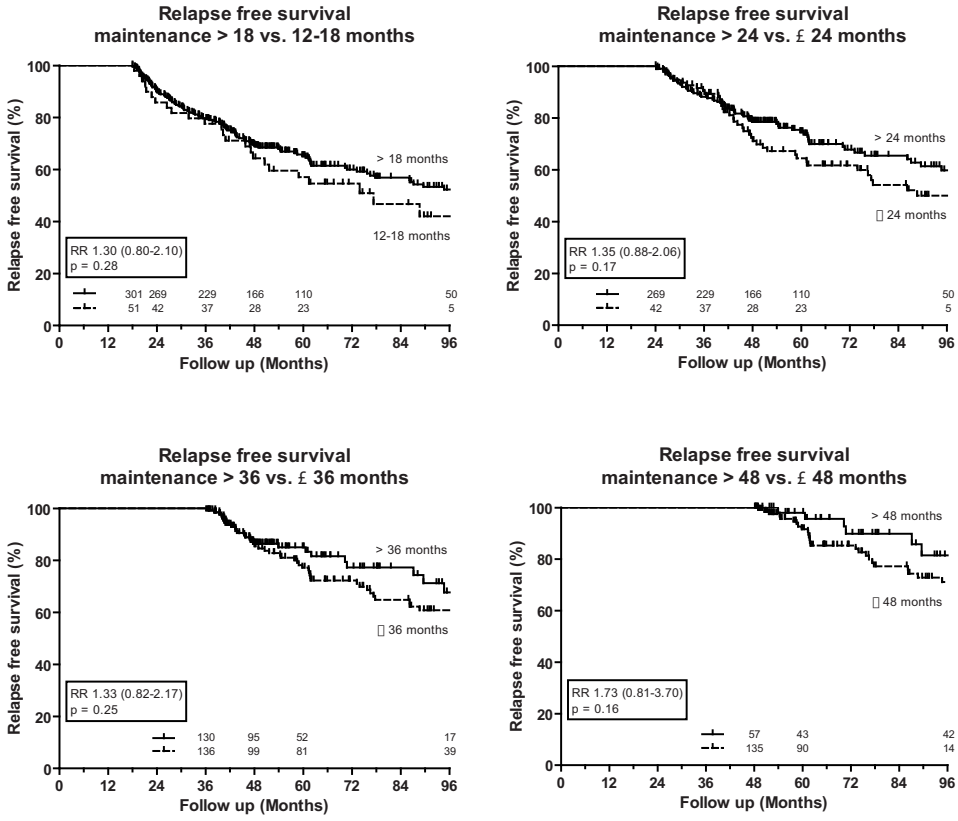

Figure 3 Relapse free survival curves for withdrawal after 18-48 months after diagnosis during longterm FU.

Adverse events

Serious adverse event that could have been related to immunosuppressive treatment were recorded during long term follow up. As depicted in Table 4, infection was the most documented adverse event but no difference in occurrence was found throughout the groups (p 0.07). Also, there was no difference for cardiovascular diseases (including cardiac events, stroke and revascularization-procedure, p 0.64), malignancy (p 0.24), diabetes mellitus (p 0.63), thrombotic events (p 0.35) and for bone disorders (p 0.73).

DISCUSSION

We have studied the relationship between duration of maintenance therapy and occurrence of relapses in an international cohort of 380 patients with AAV who were followed during and after termination of long term maintenance therapy. There was no significant difference in relapse-rate or relapse-free survival in patients who were treated beyond 12 months after diagnosis for different durations of treatment. Only patients in whom maintenance therapy was discontinued within 12 months after diagnosis were at higher risk of relapse. At all other analyzed time points from 18 months and beyond, a non-significant increase in relapse rate was observed in those patients who discontinued azathioprine maintenance therapy. Finally, in multivariate analysis, discontinuation of azathioprine maintenance therapy was associated with a slightly increased risk of relapses within 12 months. In addition intravenous administration of cyclophosphamide, anti-PR3 ANCA and higher serum creatinine at diagnosis were associated with relapse in multivariate analysis.
The concept of induction therapy at diagnosis followed by maintenance therapy to prevent relapse, described in 1983 by Fauci et al, has become the standard of care for AAV treatment (23). There is uncertainty as to the optimal duration of maintenance therapy after 18-24 months after diagnosis reported randomized trials has been relatively short (4,9).
After the findings of Slot et al, in which patients were followed 42 months, it seemed rational to treat patients at high risk for relapse- for instance those who remained ANCA-positive at time of remission- even longer than 18 months (18). However, concerns about maintenance and its duration do exist and controversy remains along with the proceeding of studies on maintenance agents and its duration. For instance, after rituximab-induction therapy, the occurrence of relapse at 18 months without any maintenance therapy was equal compared to cyclophosphamide induction followed by azathioprine maintenance therapy (20). Moreover, in a prospective study of 126 patients with PR3-ANCA associated vasculitis, the association of persistent ANCA-positivity and higher relapse-risk was not confirmed and longer azathioprine use was not associated with decrease in relapse rate (19). These findings may suggest, in line with our present finding, that the long term effect is perhaps more related to 'the first hit' of induction treatment than due to maintenance therapy. The results of a multi-center remission of maintenance-study comparing short and long term courses of azathioprine maintenance therapy (REMAIN) may contribute to this debate.
In contrast to that, the IMPROVE-study showed that maintenance therapy for more than 20 months with mycofenolate mofetil was less effective in prevention of relapse compared to azathioprine (5). Outcome in relapse rate was adjusted for a diversity of pre-specified factors that could have influenced relapse-risk like diagnostic subtype, route of cyclophosphamide administration and baseline serum creatinine level: by multivariate analysis, the HR for relapse associated with MMF-use was 1.80 (95% CI, 1.10-2.93, p 0.02).

This finding suggests that maintenance therapy does have positive effects on relapse rate and that this effect may depend on the agent of choice (5). Comparing azathioprine to methotrexate as maintenance agent did not demonstrate differences in relapse-rate (21). Recently, rituximab maintenance therapy following cyclophosphamide induction therapy was superior in reducing relapse rate ($P < 0.0001$) compared to azathioprine in the MAINRITSAN-trial (24). Also, the RITAZEREM-trial, which is currently still recruiting patients, will test whether rituximab is superior to azathioprine in the prevention of exacerbations in AAV patients with relapsing disease (25).

The aforementioned inconsistent findings on different maintenance agents and its duration does lead to uncertainty about the optimal duration of maintenance. It also prompts questions whether maintenance therapy itself is related to outcome during long term follow up, or whether baseline characteristics of patient, disease or induction-agents perhaps are more important determinants in disease-course during long term follow up. This hypothesis fits the recently published data of the long term follow up of the CYCAZEREM, suggesting a trend to more relapses in the shorter cyclophosphamide exposure group (26). It also fits our present study, in which relapse rate was not different in any of the azathioprine-duration groups. Another important issue of debate is the role of long term use of low-dose steroids in the prevention of relapse, which until now is not evaluated extensively. Since some claim that steroids improve disease control and in most trial protocols, the use of steroids often is left to the discretion of the threatening physician; this situation makes it harder to make a statement on the other immunosuppressive agents. However, we also do know that there is considerable difference in belief as in the use of steroids between doctors, centers and between countries there is a urgency to study the effects on steroids in the near future.
Next to doubts about necessity of maintenance, longer duration of immunosuppressive therapy could be related to an increase of for instance infectious or cardiovascular adverse events in those patients who are treated for > 18 months. In our study, these adverse effects were equally divided and not statistically different between the groups on various duration of maintenance. Causes of death mainly were cardiovascular causes and infection; whether these causes could have been related to either disease or treatment is unclear since other covariates like duration and dosage of induction therapy could have contributed. Due to the nature of the original trails, mean baseline serum creatinine was unequally divided between these groups, so conclusions cannot be drawn from our findings on ESRD.
The results of our study must be viewed in terms of their limitations. Although we were given the opportunity to compare long term follow up data of six landmark studies, these were largely extended follow up data that were reported beyond the scope of the original studies. Data analysis was performed only a selection of patients who survived the first year

after diagnosis without any relapse during this year. Unfortunately, we were also only able to compare long term outcomes by using clinical information collected at baseline; we are aware that the course of disease or the clinical situation could have influenced physicians' decisions on azathioprine duration. These follow up courses were not protocol determined and the reasons for continuing or changing management are not documented. Another major drawback is that we are not informed about steroids during follow up and this may be a confounder in our findings. Furthermore, data-collection may have not been optimal and for instance adverse events may have been underreported.
The comparison of heterogeneous trials which included different patient populations within the whole spectrum of AAV may lead to inconsistent findings. However, since this is also the population encountered in daily clinical practice, we think our findings can we applicable and are likely generalizable to patients with AAV in daily clinic.

In summary, we have found no evidence that extension of maintenance therapy with azathioprine maintenance therapy beyond 12-18 months after diagnosis is effective in relapse prevention. Intravenous administration of cyclophosphamide, PR3-ANCA and higher serum creatinine at diagnosis are significantly associated with relapse. Mortality was equally divided between the groups in our study and independent of duration of maintenance therapy, as were side effects that are known to be related to the long term treatment. Further research will hopefully elucidate the need for and benefits of maintenance therapy in ANCA-associated vasculitis in all the aspects of disease: the data of a large multi-center remission of maintenance-study comparing short and long term courses of azathioprine maintenance therapy are not yet published (REMAIN) but hopefully, the results or this large study will enable clinicians in daily practice to decide what maintenance agent to choose and for how long.

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... REMAIN...

9

Summary, general discussion, clinical implications and future perspectives.

SUMMARY

In ANCA-associated small vessel vasculitis, patients may present with life threatening rapid clinical decline due to single or multiple organ involvement, especially targeting kidney and lungs (1). The sooner the diagnosis is made, the sooner appropriate and efficacious therapy can be installed. In an ideal situation, patient-tailored therapy should be available, optimizing the risk/benefit ratio of treatment, especially since the burden of toxicity of the induction and maintenance agents of choice can be huge. In this view, duration of maintenance therapy is also important and both choices of specific agents as well as the duration of treatment may have implications for the risks of morbidity, mortality and the quality of life.
Following the initial treatment phase, there are several known risk factors for relapse and poor long term prognosis. In this thesis, we go into these factors to improve the clinical management and outcome of ANCA-associated vasculitis.

Part 1 of this thesis is dedicated to diagnosis, induction therapy and risk factors for long-term prognosis.
Chapter 1 provides a general introduction and a short overview of ANCA-associated vasculitis. The focus of chapter 2 is to enable clinicians to rapidly reject or confirm the diagnosis of AAV. In 260 serum samples of patients suspected of having AAV, we found that both a qualitative Dotblot-assay and quantitative automated highly sensitive Phadia EliA-system for anti-GBM, anti-PR3 and anti-MPO detection performed excellently as rapid serological test methods. Results are almost identical to the results of routine ELISA. In case of a positive result, based on high positive and negative predictive value, the clinician can make a well-funded choice for early aggressive, yet appropriate, treatment.
In chapter 3, we describe the results of our retrospective study of 273 systemic AAV-patients to determine the differences between PR3-ANCA and MPO-ANCA positive patients and other determinants for renal outcome. We found that these entities are different at presentation, as MPO-ANCA positive patients are older and have higher serum creatinine levels at baseline. For both groups, renal function at diagnosis and especially regained renal function after initial treatment at 6 months are major predictors for renal survival, next to renal relapses, since every renal relapse causes a loss in eGFR of 8 ± 4 ml/min/1.73m2. Although relapses are scarce in MPO-ANCA positive patients, presentation at diagnosis is more often with CKD stage IV and V, showing less renal recovery and thus these patients end up more frequently in ESRD compared to PR3-positive patients. Furthermore, patients who needed RRT at diagnosis had the worst survival probability, independent of ANCA-subtype.

The addition of plasmapheresis because of progressive or unresponsive disease after start of standard induction therapy was evaluated in chapter 4. Although plasmapheresis was added relatively late, i.e. a mean of 18 days (range 5-41) after start of standard induction therapy, we found a significant improvement in renal function and similar long-term outcome in both renal and patient survival as matched disease controls. The addition of plasmapheresis was not at the expense of more infectious adverse events.
As haematuria is a well-known phenomenon at presentation of AAV with renal involvement, we studied its course during induction treatment and remission in chapter 5. Haematuria is, as we found, present in all patients at diagnosis and disappears gradually in line with stabilisation of renal function, independent of ANCA-serology. In those patients with persistence of haematuria, it cannot be used as predictor for renal function or relapse during long term follow-up.

Part 2 of this thesis focuses on maintenance therapy and suggestions for improving long-term outcome, with emphasis on reducing morbidity and increasing quality of life for individualized AAV-patients.
Chapter 6 consists of a full review on maintenance therapy. An overview of randomized clinical trials of maintenance therapy in AAV is given, next to an short synopsis of what is known about rituximab, the relatively newer agent for maintenance in AAV. Based on the outcomes of these studies, a proposal for patient- or disease tailored maintenance therapy is given at the end of the article.
In the next two chapters, two studies on azathioprine maintenance therapy are addressed. Chapter 7 describes a national randomized multi-center clinical trial on extended azathioprine maintenance therapy. Although in prior studies a C-ANCA titer that remained detectable in PR-3 ANCA positive patients was associated with an increased risk for relapse, the present study was unable to confirm this finding. Extended courses of azathioprine maintenance therapy did not reduce the incidence of relapses in patients with PR3-AAV and persisting positive C-ANCA titer compared to standard of care.
The findings on azathioprine maintenance therapy in an international survey on 6 large maintenance studies in chapter 8 confirm our national finding in chapter 7. Enabled by EUVAS and FVSG to compare outcome in this large and well-known studies in AAV, it was confirmed that longer duration of azathioprine maintenance treatment does not significantly influence relapse free survival.

GENERAL DISCUSSION AND CLINICAL IMPLICATIONS

On the English vasculitis-website 'vasculitis.org.uk: factsheet' it is stated " The key to successful treatment is *early recognition and early correct diagnosis*, followed by *prompt, appropriate and effective treatment*. This results in *better quality of life and longer life expectancy*".
There is no better way to outline the aims of this thesis.

> Early recognition and early diagnosis.
The classical method to detect presence of ANCA is indirect immunofluorescence (IIF) on ethanol-fixed neutrophils. These may show a C-ANCA (granular cytoplasmatic) pattern or a P-ANCA (perinuclear staining) pattern, usually associated with antibodies against proteinase 3 and myeloperoxidase respectively (1-3). However, as the definite diagnosis is made by clinical symptoms and biopsy and since ANCA (with different or unknown antigenetic specificities) do occur in other diseases or healthy persons as well, the presence of C-ANCA or P-ANCA is not specific enough to establish the diagnosis (2,4-8). To improve sensitivity and especially specificity, a positive IIF should be confirmed with antigen specific enzyme linked immunosorbent assay (ELISA); however both tests methods are very time-consuming, especially when the combination to connect optimal sensitivity with optimal specificity is used (3-7,9). Rapid serological detection of ANCA may ideally be life-saving, given that these methods have this sufficient sensitivity and especially specificity and may obviate the need for an indirect immunofluorescence test. Different assays, like qualitative Dotblot or line blots are commercially available for this rapid detection; a newer antigen-specific serological test for instance, is the quantitative novel anchor coated highly sensitive Phadia ELiA system (10-12).
In patients who are highly suspected of AAV, both rapid qualitative Dotblot assay and the quantitative Phadia proved to have very high positive and negative predictive values, identical and comparable to standard ELISA systems. These rapid test methods are therefore very useful in clinical situations where a high level of suspicion for AAV is present and rapid and important decisions about aggressive therapies are needed. In the future, the former agreement on sequential testing with IIF and ELISA will probably be changed; however, automated reading of IIF has also already been introduced, showing high diagnostic performance so all these new developments may feed the debate about optimal ANCA-diagnostics (10,13).

> Prompt, appropriate and effective treatment.
Once the serological test methods have given a clue to the diagnosis of ANCA-associated vasculitis, it is important to realize that there are meaningful clinical differences between PR3-AAV and MPO-AAV. In general, patients with PR3-ANCA have more widespread

extra-renal organ involvement and more active renal lesions at the time of diagnosis compared to MPO-ANCA, who have more chronic lesions (14-16). Patients with MPO-ANCA associated vasculitis do more often present with more compromised renal function and are or become more often in need for RRT; they also show less recovery of renal function (14,17,18). Patients with PR3-ANCA have higher relapse rates during follow up, which may cause decline in renal function (19,20).

All these factors may influence prognosis: patients with renal involvement, especially those who are in need of RRT at diagnosis, have worse renal and patient survival outcomes. Knowing this, the clinician can choose to adapt induction treatment or change his or her decisions about future maintenance therapy. In this thesis, for patients with AAV with renal involvement, we state that both renal function at baseline as well as regained renal function after initial treatment is a major predictor for renal survival during long-term follow-up. So it seems to be as important to withhold or limit treatment in those patients who will probably not recover, as to intensify immunosuppressive therapy when response to standard therapy is limited or insufficient in those patients who are supposed to recover. This argument also holds true for every (renal) relapse when treatment has to be adjusted.

A possible intensification of induction immunosuppressive therapy is to add plasmapheresis or plasma exchange, aiming for elimination of circulating ANCA. This removal has been shown of benefit for anti-glomerular basement membrane antibodies as well as in AAV-patients with pulmonary haemorrhage, but evidence is limited for at least this last indication (21-23). After the 'Randomized trial of plasma exchange or high dosage methylprednisolone as adjunctive therapy for severe renal vasculitis' (MEPEX), present indications are also severe renal disease with creatinine > 500 umol/l and dialysis dependency (21,23-26). However, although short-term results of this study appeared promising with a relative reduction of 50% for renal replacement therapy at 12 months, after 4 years there was no evidence of a net difference between the groups (27).

Plasmapheresis can also be considered for disease refractory to standard therapy. Patients who present with AAV often do not qualify for plasmapheresis at diagnosis based on the criteria above (21,23-26), but after start of induction treatment, clinical deterioration may occur and also renal function may initially worsen due to ongoing vasculitis disease activity despite starting therapy. Once these situations occur, the clinician at least has to consider a stepwise increment in immunosuppressive treatment as 'rescue therapy'. We found that following this policy, renal function and other vasculitic activity can improve to the same outcome as patients with comparable disease severity at the start of standard treatment that show a good response. This holds even true for addition of plasmapheresis on average 18 days after diagnosis and start of initial therapy which was found in our study.

While the removal of other beneficial ' plasma factors' could probably attribute to some adverse events, it also remains questionable whether patients with non severe renal dysfunction will benefit from or be harmed by plasmapheresis, since especially the patients

with severe renal dysfunction are the most likely to see measurable benefit (reducing the risk of ESRD). This is one of the key questions of a large international study, the 'Plasma exchange and glucocorticoid dosing in the treatment of antineutrophil cytoplasm antibody associated vasculitis study' (PEXIVAS), which has included patients with a broad range of kidney function, estimated GFR between 50 ml/min and requiring dialysis (28).
Nevertheless, in our cohort, the combination of therapies did not cause more serious infectious episodes or other important adverse events.

> Better quality of life and longer life expectancy.
With or without the addition of plasmapheresis or high doses of methylprednisolone, the mainstay of induction treatment is cyclophosphamide (orally or i.v.) combined with prednisolone. This therapy results in disease remission in 80-90% of patients; however, at least 25-40% of patients experience relapses (29,30), contributing to a substantial morbidity and mortality either due to disease itself or toxicity of prolonged or repeated courses of immunosuppressive treatment.
Many studies have identified risk factors for relapses and since a lot of knowledge has been gained about these risk factors, prevention of relapses by different maintenance regimes or agents has become a main issue of further research. On the other hand, the toxicity problem of aggressive induction therapy and long term use of maintenance agents is widely acknowledged and still remains a large clinical problem with tremendous impact on the quality of life of AAV-patients. Many studies have led to recommendations for less intense maintenance regimens in a combined effort to prevent the frequent relapses but also lessen toxicity. Finding clues for patient- or, perhaps even better, disease-tailored approaches is becoming more and more important. Are we any more near this goal than a couple of years ago?
For maintenance treatment, the best evidence still exists for the use of azathioprine after the CYCAZEREM study, in which withdrawal of cyclophosphamide at time of stable remission for 3 months and substitution with azathioprine proved safe and did not increase relapse rates (29). Although azathioprine usually is well tolerated, gastro-intestinal complaints and liver toxicity do occur, next to bone marrow depression and (viral) infections (31). Also a few patients do react with intolerance or present with a flu-like syndrome which in severe cases could lead to SIRS and multi organ failure. Ofcourse in these cases of intolerance, azathioprine should be discontinued and be replaced by another agent. The best alternative choices based on two large randomized and multi centres studies are methotrexate at creatinine clearance > 60 ml/min and mycophenolate mofetil at < creatinine clearance 60 ml/min (32-34).
Promising data are recently published for rituximab, a B-cell depleting agent, as induc-tion treatment in AAV, especially for young women with future pregnancy wish. An interes-

ting issue is that the patients who were given this new induction agent, remained on stable disease only using steroids as maintenance treatment (35). The "Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis' (MAINRITSAN), compared this two maintenance regimens and showed that rituximab -after induction with cyclophosphamide- was superior to azathioprine in maintaining remission after 28 months (36). Three retrospective cohort-studies also report succesfull outcomes with low relapse rates for those patients who were treated with rituximab maintenance therapy, whether interval-fixed or based on B-cell counts. In most of these patients, other immunosuppressive treatment were succefully discontinued (37-39). Since this agent has especially become agent of interest in the last years, unfortunately we were not able to extensively discuss it in chapter 6.

There is little clarity about the best duration of maintenance treatment. The KDIGO guideline advises a minimum duration of 18 months, among others based on the CYCAZEREM study (40). In our national randomized AZA-ANCA study, we did not find evidence that longer duration of azathioprine does positively influence relapse frequencies, even in patients who were most prone for relapse (like PR3-AAV or renal involvement). This finding was supported in the subsequent analysis of 6 large EUVAS/FVSG studies in which longer duration of azathioprine maintenance was also not associate with less relapses (29, 32-34). The coming 'prolonged remission-maintenance therapy in systemic vasculitis-study' (REMAIN) prospectively compares standard and long term azathioprine maintenance therapy during 24 and 48 months. Primary endpoint is relapse rate; secondary endpoints are, among others, adverse events: of this trial, the results are eagerly awaited ofcourse.

While up to now, longer duration of maintenance treatment is at least questionable, reducing the time on immunosuppressive therapy can be advantageous in restricted patient groups when the risk of side effects exceeds the benefits of continuing therapy. In patients positive for MPO-ANCA, who are at much lower risk for relapse, there is no evidence for subsequent toxic treatment beyond the first period of induction treatment and three months of stable disease. Therefore, it could be considered to continue only a low dose of glucocorticoids 5-10 mg/day for 12 months once stable remission for 3 months has been obtained on induction therapy (usually cyclophosphamide) (40-42). However, separate analyses for the different serological subtypes are lacking until now, even though there is growing evidence that these subtypes might represent different diseases. Also, based on evident low relapse rates, in patients who present with ESRD (without severe extra-renal manifestations) and who have to continue RRT after three months of induction therapy, maintenance treatment can be vigorously diminished (34-36).

Most of the relapses do occur during tapering and discontinuing immunosuppression and in surveys on outcome, a mean disease free survival of 21.8 months (range 9.8-27) is reported (43). Relapses usually similarly respond to therapy as the initial disease does

and the severity of the relapse should guide therapy. However, because of the high risk of toxicity especially in light of malignancy, the cumulative cyclophosphamide is important; in these cases, rituximab induction is recommended without difference in other outcomes like subsequent relapse rates (36,40,44).

FUTURE PERSPECTIVES

As described in the aforementioned, little by little we are getting closer to patient- and disease-tailored management but steps are small while the benefits for the individual patient can be huge. Some questions have been answered in the studies in this thesis and, based on currently available evidence, practical recommendations have been formulated regarding maintenance therapy in AAV. Still many questions remain unanswered and new questions arise continuously in daily clinic. Therefore, there are many opportunities for further research. Since ANCA-associated vasculitis is a rare disease, ideally national and international collaborations should be formed, at least to join expertise and daily experience, but also to share uncommon phenomena, to cooperate in the search for new therapies and to update or formulate new guidelines for treatment and follow up.

Recognition and diagnosis is made easier by rapid test methods like Dotblot and ELiA; already newer automated reading of IIF has become available. This will probably have consequences for the recommendation on screening for ANCA in the near future, but studies first have to prove the value of these tests alone or in combination in different clinical settings. Although the ELiA has proven its value in diagnostics at presentation of the disease, it will be interesting to study performances of its rapid quantification of PR3- and MPO-ANCA during follow up, f.i. as predictor of relapse in case of changes in ANCA-titer.

It is more and more recognized that there are meaningful differences between patients with PR3-ANCA and MPO-ANCA and this thesis also provides evidence that PR3-AAV and MPO-AAV are distinct diseases. Both major histocompatibility complex (MHC) and non-MHC loci are found to be genetically associated with the antigenetic specificity of ANCA, which may explain the different clinical spectrum and outcome (15). Further differences between the serological subtypes have to be analyzed and studied, for example in the variety of organs involved or active versus chronic lesions. Also, patients with relapsing disease or disease refractory to standard treatment remain important to study since the question remains whether these are a different phenotypes of AAV.

Although induction therapy is more or less standardized to cyclophosphamide and prednisolone, further randomized studies are already focusing on identifying which patients

would benefit from expanding therapy with plasmapheresis or from alternative treatment regimens with monoclonal antibodies among others. New agents, like abatacept, a CTLA4-IgG, or belimumab, an anti-BLys monoclonal antibody, are being tested, influencing and inhibiting T- and B-cell activation respectively (45,46). Reports about these new agents are awaited and will probably raise further questions.
The use of rituximab as induction- as well as maintenance agent has to be further investigated, especially focusing on the important issue whether it would be possible to abate cyclophosphamide at all. Studies has to focus on determining the most efficient treatment-scheme since this has not been determined yet (36). Its use in case of refractory disease can be further looked into, as well as its addition to cyclophosphamide or as replacement for plasmapheresis when response is lacking during the first induction phase. Other questions are whether one could do without other immunosuppressive maintenance therapy following rituximab induction and what is the time needed for gradually discontinuing. Ofcourse, also the (very) long term effect regarding safety and adverse events needs to be closely evaluated.
At last, while these new agents gain interest, the well known but sometimes overlooked agent prednisolone deserves new attention, especially since its effects are perhaps under-appreciated or perhaps overrated with more emphasis on the growing concerns about the adverse events of long term low-dose steroids.
Next to these topics, the current recommendations can be reconsidered for those who remain dialysis dependent during the course of disease, since lessening toxicity to reduce infectious complications especially seems important in these patients.

In conclusion, despite continuous advances in therapy, patients with AAV continue to have excess mortality and morbidity compared to the general population which persists during long-term follow up, in part related to toxicity of current therapies. Although the palette of therapeutic agents is being expanded, hopefully the future will bring clearer answers on which agent is best for what patient with different disease characteristics.
This will bring both clinician and patient to further improvement in clinical management.

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10

Nederlandse samenvatting.

INTRODUCTIE

Vasculitis, een ontsteking van de bloedvaten, is een zeldzame aandoening die alle bloedvaten van het menselijk lichaam kan treffen. Er zijn verschillende oorzaken voor deze aandoening: het kan optreden in aansluiting op een infectie of bijvoorbeeld kanker en het kan bijvoorbeeld veroorzaakt worden door een reactie op geneesmiddelen of andere giftige stoffen.
Een veel minder voorkomende vorm van deze bloedvatontsteking wordt veroorzaakt door een reactie van het lichaam zelf, als het eigen afweersysteem afweerstoffen (antistoffen) vormt tegen bepaalde lichaamseigen stoffen.
Normaal beschermt het afweersysteem het lichaam effectief tegen indringers van buitenaf, zoals bacteriën of virussen. Bescherming vindt onder andere plaats door de vorming van antistoffen, gemaakt als reactie tegen specifieke onderdelen van bijvoorbeeld virussen.
In de bedoelde vorm van vasculitis heeft het lichaam echter een verkeerd signaal opgevangen en maakt antistoffen tegen een specifieke groep witte bloedcellen. Deze antistoffen worden ANCA genoemd, anti-neutrofiele cytoplasmatische antilichamen. Deze ANCA's kunnen in het bloed onderzocht worden en maken de diagnose ANCA-geassocieerde vasculitis (letterlijk aan ANCA gerelateerde bloedvatontsteking) heel waarschijnlijk.
Er zijn verschillende soorten ANCA, gericht tegen verschillende eiwitten van de witte bloedcellen: de belangrijkste voor dit proefschrift zijn PR3-ANCA en MPO-ANCA, respectievelijk de eiwitten proteinase-3 en myeloperoxidase. Deze beide eiwitten zijn opgeslagen in een specifieke groep witte bloedcellen, de zogenaamde neutrofiele granulocyten. De neutrofiele granulocyten zijn onderdeel van het afweersysteem en van groot belang in de algemene reactie van het lichaam tegen met name bacteriën. Om de bacteriën te bestrijden, gebruikt de neutrofiele granulocyt de eiwitten PR3 en MPO. Door deze eiwitten worden de bacteriën gedood, maar ook de omgeving raakt beschadigd. Dit wordt over het algemeen als een ontsteking herkend, gekarakteriseerd door bijvoorbeeld roodheid, warmte, zwelling en pijn.
In het geval van vasculitis verzamelen deze witte bloedcellen zich in de bloedvatwanden, laten daar deze eiwitten, PR3 en MPO, vrijkomen en veroorzaken daarmee een beschadiging van de omgeving: de ontsteking van de bloedvaten. Het lichaam vangt nu een signaal op dat leidt tot aanmaak van de ANCA's, antistoffen tegen PR3 en MPO, in een poging de ontsteking te beperken. Het tegendeel echter gebeurt: door deze ANCA's wordt de ontstekingsreactie in de bloedvaten verder verhevigd.

In vasculitis kunnen bloedvaten door het hele lichaam betrokken zijn: dit proefschrift gaat vooral over de vormen van vasculitis waar de kleinste bloedvaten aangedaan zijn. Hierbij zijn vaak luchtwegen en nieren aangedaan. Dit is niet verwonderlijk aangezien het filterorgaan van de nier, genaamd de glomerulus, bestaat uit een kluwen van kleine tot

zeer kleine bloedvaatjes. Door de ontsteking die daarin ontstaat, raakt dit filterorgaan zodanig beschadigd dat het zijn functie niet meer voldoende kan uitoefenen. Hierdoor kan in heel korte tijd nierschade of nierinsufficiëntie ontstaan, maar geleidelijke achteruitgang is ook mogelijk.
Bij de longen veroorzaakt de ontsteking vaak vochtophoping, wat leidt tot benauwdheid, of longbloedingen, waarbij bloed opgehoest kan worden. Dit beide kan leiden tot moeizame ademhaling, zodanig dat de zuurstofvoorziening in het gedrang komt en beademing noodzakelijk kan worden.

Hoewel er inmiddels veel duidelijk is over het beloop van vasculitis, is nog altijd niet geheel duidelijk wat het eerste signaal is tot het ontstaan van deze aandoening. Aangezien de ANCA's voorkomen bij deze ziekte, lijkt het logisch te denken dat er een oorzakelijk verband zou kunnen bestaan. Voor ANCA's gericht tegen MPO is er inderdaad een oorzakelijke relatie aangetoond, echter voor PR3 is dit nog altijd niet gevonden.
Dat er een verschil is tussen PR3-ANCA en MPO-ANCA blijkt ook uit de verschillende ziekte-presentatie: patiënten waarbij PR3-ANCA wordt gevonden hebben vaak een uitgebreider ziekte waarbij meer organen zijn aangedaan met een vaak acuter begin. In het geval van MPO-ANCA is er vaak sprake van een sluimerende ziekte waarbij vaak al grotere schade bestaat als de patiënt zich aandient.

Hoewel tot diep in de twintigste eeuw vasculitis vaak een dodelijke afloop had, kan tegenwoordig de ziekte in de meeste gevallen in rustiger vaarwater gebracht worden met medicijnen die het afweersysteem sterk onderdrukken. Echter, deze medicijnen moeten vaak lang gebruikt worden om de ziekte onderdrukt te houden en een definitieve oplossing is er niet: hiermee is een chronische aandoening gecreëerd. In de meeste gevallen is er tijdens langdurige controle variatie in ziekteactiviteit d.w.z. dat er perioden zijn waarin patiënten nauwelijks klachten hebben maar dat er ook perioden zijn van hernieuwde opvlamming van de ziekte, een zogenaamd recidief of relapse. Deze recidieven van de ziekte zijn vergelijkbaar met het begin van ziekte, kunnen leiden tot nieuwe schade en behandeling bestaat dan ook uit het hervatten van de sterk onderdrukkende middelen van het afweersysteem.

De middelen die het afweersysteem onderdrukken zijn effectief maar hebben hun keerzijde: vooral verhoogde vatbaarheid voor infecties maar ook het eventueel ontstaan van verschillende vormen van kanker zijn bekende bijwerkingen. Uiteindelijk betekent dit dat zowel de ziekte, maar ook de medicatie zowel ziektelast maar zelfs ook overlijden kan veroorzaken. Tegenwoordig zijn de meeste studies er dan ook op gericht te zoeken naar geneesmiddelen met het grootste effect op de ziekte gecombineerd met het veroorzaken van de minste bijwerkingen. Een van de manieren om dit te bewerkstelligen is om tijdens

de ernstigste fase van de ziekte sterkere middelen te gebruiken en als de ziekte rustiger is, over te schakelen op mildere middelen. Daarnaast is men op zoek naar bepaalde kenmerken van de zowel ziekte of bepaalde patiënt-kenmerken die bepalend zouden kunnen zijn in de keuze welk middelen je nodig hebt en/of voor hoe lang. Hiermee zou een zogenaamde 'patient-tailored' keuze in medicatie gemaakt kunnen worden, dwz een behandelplan op maat.

HET PROEFSCHRIFT

Het eerste deel van het proefschrift is gewijd aan diagnostiek van vasculitis, op het bepalen van risicofactoren voor gunstiger en ongunstiger beloop van de ziekte en op behandeling in de eerste periode van de ziekte, vooral op uitbreiden van therapie als de ziekte niet rustig wil worden.
In hoofdstuk 2 wordt het onderzoek naar een tweetal sneltesten beschreven. Deze twee testmethoden worden vergeleken met de al bestaande en meest gebruikte onderzoeksmethoden en lijken betrouwbaar en effectief genoeg om met voldoende zekerheid te stellen dat de ziekte wel of niet bestaat. Dit is belangrijk omdat snel starten met behandeling bepalend kan zijn voor het verdere beloop. Ook het niet starten van therapie daarentegen, als er nog twijfel bestaat aan de diagnose, is belangrijk omdat de therapie ook nadelige bijwerkingen kan hebben.

Hoofdstuk 3 is gewijd aan het bepalen van mogelijke risicofactoren die een voorspellende waarde kunnen hebben voor het verdere beloop van de ziekte. In het meest gunstige geval zouden deze risicofactoren gebruikt kunnen worden bij de keuze voor de te gebruiken afweer onderdrukkende middelen, de doseringen en de duur; patiënten met weinig risicofactoren zouden minder lang behandeld kunnen worden met minder zware middelen dan patiënten met veel risicofactoren.
Het blijkt dat zowel de nierfunctie bij diagnose als op 6 maanden na eerste behandeling duidelijke voorspellende waarde heeft voor de nierfunctie op lange termijn. Daarnaast zijn opvlammingen van de ziekte, met name als daarbij ook de nier aangedaan is, risicovol voor de nierfunctie op langere termijn.
Patiënten met een MPO-ANCA hebben vaker een slechtere nierfunctie bij presentatie en hebben dan ook een groter risico op nierfalen. Tot slot blijken patiënten die tijdens behandeling van de ziekte moeten dialyseren vanwege nierfalen, de meest ongunstige prognose te hebben.

Het begin van deze ziekte is vaak acuut en ernstig; zodra de behandeling tijdig wordt gestart, kan snel effect optreden echter soms blijkt het moeilijk de ziekte rustig te krijgen

en is uitbreiding van de standaard behandeling nodig. Een van de methoden hiervoor is plasmaferese, een methode om bloedplasma van patiënten te vervangen door donor- of kunstmatig plasma. Met het verwijderen van het plasma worden ook de antistoffen verwijderd; de eigen bloedcellen worden weer teruggegeven. Meestal wordt deze plasmaferese al vroegtijdig in de behandeling toegevoegd bij de meest ernstig zieke patiënten. In ons ziekenhuis wordt deze behandeling soms ook gestart naast de standaard therapie als de ziekte niet rustig lijkt te worden. In hoofdstuk 4 worden de resultaten daarvan beschreven: 26 patiënten werden behandeld en vergeleken met vergelijkbare patiënten die de extra behandeling niet nodig hadden. De uitkomst wat nierfunctie en overleving bleek hetzelfde. De bijwerkingen van het toevoegen van een zware behandeling waren eveneens vergelijkbaar en niet toegenomen ten opzichte van de controle-patiënten. In hoofdstuk 5 worden klinische parameters van renale ziekteactiviteit, waaronder hematurie (d.w.z. rode bloedcellen in de urine) beschreven gedurende de eerste fase van behandeling als in stabiele situatie.

Deel 2 van het proefschrift is gericht op de onderhoudsbehandeling van vasculitis, d.w.z. de periode waarin de acute eerste fase van de ziekte is afgelopen maar er nog risico is op opvlammingen van de ziekte en ook optreden van bijwerkingen van de medicatie.
Op basis van het eerste deel van het proefschrift maar ook op basis van literatuuronderzoek worden daarnaast aanbevelingen gedaan welke patiënten meer en welke patiënten minder afweer onderdrukkende middelen zouden hoeven gebruiken en voor hoelang.
Hoofdstuk 6 is een overzicht van een aantal grote en toonaangevende onderzoeken die gedaan zijn om te bepalen hoelang vasculitis doorbehandeld moet worden. Aan het eind van dit hoofdstuk worden aanbevelingen gedaan over welke middelen in welke dosering en voor hoe lang. Hiermee hopen we een lans te breken voor behandeling op maat om daarmee zowel de ziekte optimaal te behandelen maar uiteraard overbehandeling te voorkomen en daarmee de bijwerkingen te minimaliseren.
In hoofdstuk 7 worden de resultaten beschreven van een grote Nederlandse studie, verricht om te onderzoeken wat de beste behandelduur van een bepaald onderhoudsmiddel, azathioprine, zou moeten zijn. In een eerder onderzoek was gebleken dat bepaalde patiënten met PR3-ANCA groter risico hadden om een opvlamming van de ziekte te krijgen en deze zouden baat kunnen hebben bij een langere behandelduur. In het beschreven onderzoek werd deze bevinding echter niet gedaan; er bleek daarnaast ook geen verschil in kortere en langere duur van onderhoudsbehandeling.
Hoofdstuk 8 bevestigt deze bevinding. In dit hoofdstuk worden de resultaten van 6 grote internationale studies beoordeeld waarin de nadruk opnieuw ligt op het vergelijken van de effecten van kortere of langere duur onderhoudsmedicatie. Er werd geen verschil gevonden in het optreden van ziekte-opvlamming. Daarnaast werd ook geen verschil gevonden in overlijden of het optreden van nierfalen tijdens langdurige follow up.

Uit deze twee hoofdstukken kan dus opgemaakt worden dat er voor een gemiddelde behandelduur van 18 maanden het meeste bewijs is geleverd, met het beste resultaat op de ziekte en de minste bijwerkingen. Het middel van keus is voor de meeste patiënten azathioprine.

CONCLUSIES EN OVERWEGINGEN VOOR TOEKOMSTIG ONDERZOEK.

Sinds de eerste beschrijving van ANCA's begin 1980 en de mogelijke associatie met de ziekte die nu ANCA-geassocieerde vasculitis heet, zijn de inzichten in de ontstaanswijze, de behandelingsmogelijkheden en de prognose bijzonder toegenomen. Echter, ondanks deze vooruitgang blijven patiënten met vasculitis een verhoogd sterfterisico hebben in vergelijking met leeftijdsgenoten van de algemene bevolking en daarnaast hebben zij een duidelijk verhoogde ziektelast. Dit lijkt zowel gerelateerd aan de ziekte als aan de medicatie waarmee de ziekte tot rust gebracht kan worden.
Er lijkt een periode te zijn ontstaan waarin verder onderzoek gericht moet worden op het vinden van patiënt-of ziekte gebonden factoren op grond waarvan een keuze gemaakt kan worden voor mildere of zwaardere en kortere of langere behandeling. Deze keuze zou ook gerelateerd kunnen worden aan eventuele prognostische en voorspellende factoren.
Wat betreft diagnose-mogelijkheden: hoe sneller de diagnose, des te sneller de start van de belangrijke therapie kan zijn. Nieuwe en snellere methoden zijn al op de markt en zullen nader getoetst moeten worden. Deze testmethoden zijn vooralsnog niet getoetst tijdens langdurige controle, maar zouden eventueel ook een rol kunnen spelen als voorspeller van opvlammingen van de ziekte. Ook dit zal onderwerp van onderzoek kunnen zijn.
Het verschil in ziekte-karakteristieken tussen patiënten positief voor PR3- en MPO- ANCA zal nader in kaart gebracht moeten worden. Wellicht heeft ook dit consequenties voor de keuze in therapie, o.a. voor therapie in de beginfase van de ziekte inclusief eventuele uitbreiding van therapie, maar ook ter overweging van onthouden van agressieve therapie met potentieel ernstige bijwerkingen als een ongunstige prognose heel waarschijnlijk lijkt op basis van een aantal aanwezige kenmerken.

Hoewel op basis van de huidige onderzoeken het onderhoudsmiddel azathioprine de beste keuze lijkt, worden nieuwe middelen op de markt gebracht die mogelijk even effectief zijn en minder bijwerkingen hebben. Een van die middelen is rituximab, een antilichaam dat met name ook gebruikt wordt in de eerste fase van de ziekte; hier zal ongetwijfeld nog veel nieuws over volgen. Daarnaast worden nieuwere middelen onderzocht die op verschillende manieren effect hebben op het afweersysteem. Ook van deze potentiele nieuwe middelen is de hoop dat het effect van de ziekte tenminste vergelijkbaar is met de huidige bestaande middelen, maar het bijwerkingenprofiel gunstiger. Hoewel deze

nieuwe middelen veel aandacht behoefen, zal daarnaast ook de huidige medicatie zoals bijvoorbeeld prednisolon, opnieuw onder de loep genomen moeten worden. Hierbij zal de aandacht vooral gericht moeten zijn op de balans tussen de veronderstelde positieve effecten en lange termijns complicaties. Bovendien zullen de huidige richtlijnen continue evaluatie en zonodig aanpassing aan recente ontwikkelingen behoeven.

Concluderend is er op een aantal vlakken in de diagnose, de behandeling en het voorkomen van opvlammingen nog winst te behalen. Hopelijk brengt vervolgonderzoek meer duidelijkheid in patiënt- en ziekte gebonden factoren op basis waarvan een gefundeerd en geïndividualiseerd behandelplan opgesteld kan worden waarmee de ziekte voldoende bestreden wordt en de bijwerkingen minimaal blijven. Dit zal de behandeling optimaliseren voor zowel de behandelende arts maar zeker ook voor de individuele patiënt.

Dankwoord.

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Dan min of meer in chronologische volgorde:
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“Still round the corner there may wait
A new road or a secret gate
And though I oft have passed them by
A day will come at last when I
Shall take the hidden paths that run
West of the Moon, East of the Sun.”