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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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