Diploma thesis

Investigation of the role of tubulointerstitial changes on the long-term clinical outcome and therapeutic response in patients with ANCA-associated vasculitis

submitted by

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Statutory declaration

I declare on my honour that I have written the present work independently and without outside help, that I have not used sources other than those specified and that I have identified the passages, taken verbatim or in terms of content, as such.

Munich, 15.04.2022 *Johannes Mauritius Häfner eh*

Preamble

Das Leben ist schön!

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Glossary and Abbreviations

acute kidney injury	AKI
ANCA-associated vasculitis.	AAV
Anti-glomerular base membrane-antibody	anti-GBM
anti-neutrophil-cytoplasmatic antibodies	ANCA
B cell-activating factor	BAFF
B regulatory cells	B_{reg}
baseline	BL
Birmingham Vasculitis Activity Score	BVAS
B-lymphocyte stimulator	BLyS
Chapel Hill Consensus Conference	CHCC
cluster of differentiation	CD
c-reactive protein	CRP
Cyclophosphamide	CYC
cytoplasmatic-ANCA	c-ANCA
ear-nose-throat	ENT
end-stage renal disease	ESRD
enzyme-linked immunosorbent assay	ELISA
Eosinophilic Granulomatosis with Polyangiitis	EGPA
erythrocyte sedimentation rate	ESR
European Medicines Agency	EMA
focal necrotizing glomerulonephritis	FNGN
Glomerulonephritis	GN
Granulomatosis with Polyangiitis	GPA
high power field	hpf
indirect immunofluorescence	IIF
interquartile range	IQR
Interstitial fibrosis	IF
Interstitial fibrosis and tubular atrophy	IF/TA
lysosomal-associated membrane protein 2	LAMP-2
mannose-binding-lectin	MBL
Membrane attack complex	MAC
methotrexate	MTX
Microscopic polyangiitis	MPA
monocyte attracting protein	MCP-1
mycophenolate mofetil	MMF
myeloperoxidase	MPO
neutrophil extracellular traps	NET
perinuclear ANCA	p-ANCA
periodic acid-Schiff	PAS
Plasma exchange	PLEX
Proteinase 3	PR3
rapid progressive Glomerulonephritis	RPGN
reactive oxygen species	ROS
regulatory T-cells	T_{reg}
renal relapse	RR
renal replacement therapy	RRT
Rituximab	RTX
standard deviation	SD

Staphylococcus Aureus	SA
systemic lupus erythematosus	SLE
T-helper cells	T_{H1}
total interstitial inflammation	IT
tubular atrophy	TA
tumor necrosis factor	TNF

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Zusammenfassung

Einleitung: Mehrere Ansätze mit unterschiedlichen serologischen und klinischen Markern haben Widersprüchlichkeiten und unterschiedliche Zuverlässigkeit bei der Vorhersage des Krankheitsverlaufs und Therapieansprechens bei ANCA-assoziierter Vaskulitis (AAV) gezeigt. In dieser Studie wollen wir den Einfluss von tubulointerstitiellen Veränderungen und die Rolle von $CD3^+$-, $CD20^+$- und $CD138^+$-Zellen bei AAV-Patientinnen und Patienten auf den klinischen Verlauf und ihr Potenzial bei der Vorhersage des Krankheitsverlaufs aufzeigen.

Material und Methoden: Vierzig AAV-Patientinnen und Patienten, die zwischen 1.1.2014 und 1.11.2018 an der Abteilung für Nephrologie der Medizinischen Universität Graz behandelt wurden, wurden eingeschlossen und 36 Monate lang nachbeobachtet. Nierenbiopsien wurden nach der Banff-Klassifikation analysiert, um die tubulointerstitiellen Veränderungen zu beschreiben und um die Patientinnen und Patienten in Gruppen ohne/mit geringer (i0/i1) und mit mittelgradiger/schwerer interstitieller Entzündungszellinfiltration (i2/i3) einzuteilen. Darüber hinaus wurden immunhistochemische Färbungen durchgeführt, um die Patientinnen und Patienten basierend auf ihrer CD3-, CD20- und CD138-positiven Zellexpression zu unterteilen. Wir verglichen die Ergebnisse zwischen Patientinnen und Patienten, die älter und jünger als 60 Jahre alt waren. Der primäre Endpunkt war das Auftreten eines renalen Relaps (RR), während die sekundären Endpunkte die Einleitung einer Nierenersatztherapie oder eine Nierentransplantation waren. Zusätzlich wurde der Verlauf der Nierenfunktion anhand des Albumin/Kreatinin-Quotienten (ACR) und der geschätzten glomerulären Filtrationsrate (eGFR) während der Nachbeobachtungszeit zwischen den verschiedenen Gruppen verglichen.

Ergebnisse: Es gab keinen signifikanten Unterschied des primären Endpunkts zwischen den beschriebenen Gruppen. Die i2/i3-Gruppe hatte eine schlechtere ACR und eGFR im Vergleich zur i0/i1 Gruppe, wobei der Unterschied in den mittleren ACR-Werten zwischen den Gruppen i0/i1 und i2/i3 im Monat 36 der Nachbeobachtungszeit eine Signifikanz von $p = 0,016$ erreichte. Es wurde keine Korrelation zwischen $CD3^+$-, $CD20^+$- und $CD138^+$-Zellen und den Endpunkten bzw. dem Verlauf von eGFR und ACR beobachtet, aber der Großteil (N = 21; 72,4

%) der Patientinnen und Patienten wies eine hohe Infiltration von interstitiellen $CD138^{+}$-Plasmazellen in den Nierenbiopsien auf. Gegenüber den über 60-jährigen, hatten die Patientinnen und Patienten unter 60 Jahren eine signifikant höhere Rate an Nierentransplantationen ($p = 0,024$) und signifikant schlechtere mittlere ACR-Werte während des 24. bis 36. Monats ($p = 0,025$, $p = 0,042$ bzw. $p = 0,035$).

Diskussion: Unsere Ergebnisse stimmen mit bereits bestehenden Daten über die Korrelation zwischen tubulointerstitiellen Veränderungen und einem schlechteren Nierenoutcome überein. Patientinnen und Patienten mit ausgedehnten tubulointerstitiellen Veränderungen könnten ein höheres Risiko für eine schlechtere Nierenfunktion im Verlauf haben. Unsere Daten zeigen einen signifikanten Anteil an CD138+-Plasmazellen in menschlichen Nierenbiopsien von Patientinnen und Patienten mit AAV, was zusätzliche Einblicke in die renalen B-Zell-Cluster bei Patientinnen und Patienten mit AAV liefert.

Abstract

Background: Several approaches using different serological and clinical markers have shown inconsistencies and varying reliabilities in predicting treatment outcome in ANCA-associated vasculitis (AAV).

This study tries to demonstrate the impact of tubulointerstitial alterations and the role of $CD3^+$-, $CD20^+$- and $CD138^+$-cells in AAV-patients on the clinical course and their potential in predicting disease outcome.

Methods: Forty AAV-patients, treated between 1.1.2014 and 1.11.2018 at the Division of Nephrology of the Medical University of Graz were included and followed for 36 months. Kidney biopsies using the Banff classification were analyzed to describe the tubulointerstitial changes and divide the patients into groups with no/low or medium/severe tubulointerstitial immune cell (i0/i1 and i2/i3). Moreover, immunohistochemistry analysis was performed to subclassify the patients, based on their CD3-, CD20- and CD138 positive cell expression. Outcome between patients older and younger than 60 years old was compared. Primary endpoint was relapse rate (RR), while secondary endpoints were initiation of renal replacement therapy (RRT), such as dialysis or kidney transplantation. Additionally, the course of kidney function using albumin/creatinine ration (ACR) and estimated glomerular filtration rate (eGFR) during the follow-up time were compared between the different groups.

Results: For the primary endpoint there was no significant difference observable between the mentioned groups. The course of mean ACR and mean eGFR between the i0/i1 and i2/i3 group showed descriptively worse outcome in the i2/i3 group and there was a significant difference in mean ACR levels between groups i0/i1 and i2/i3 observable during month 36 of follow up (p=0.016), favouring group i0/i1.

No correlation between $CD3^+$-, $CD20^+$- and $CD138^+$-cells and renal outcome, measured by endpoints and course of eGFR and ACR, ACR was observed, but N=21 (72.4%) patients had a high renal infiltration of interstitial CD138+ plasma cells.

Patients younger than 60 years had a significantly higher rate of kidney transplantations (p=0.024) and significantly worse mean ACR rates during month 24 until month 36 in comparison to over 60 year olds (p=0.025, p=0.042, p=0.035, respectively).

Conclusions: Our findings go along with already existing data on the correlation between tubulointerstitial alterations and worse kidney outcome. Patients with extended tubulointerstitial changes might be at higher risk for worse renal outcome. Our data show a significant proportion of $CD138^{+}$-plasma cells in human kidney specimens of patients with AAV, providing additional insights on the renal B-cell clusters in patients with AAV.

1 Introduction

1.1 Glomerulonephritis

Glomerulonephritis (GN) is an umbrella term for a series of immune-mediated diseases, which cause intraglomerular inflammation and cellular proliferation. (1) Antibodies, immune complex deposits, complement activation, infiltration of inflammatory cells and scarring/sclerosis can be found in glomerulonephritides.
To understand glomerulonephritides, each entity of these diseases has to be seen individually. It is important to differentiate between glomerulonephritides and glomerulopathies. The latter is described as non-inflammatory, has no immunologic glomerular lesions and can be caused by hereditary or metabolic nephropathies among others (2). This diploma thesis focuses on the pathological and clinical course of glomerulonephritides with main focus on anti-neutrophil-cytoplasmatic antibodies (ANCA)-associated vasculitis.

GNs can be divided into nephritic and nephrotic syndromes according to their clinical presentation. The classical diagnostic and clinical characteristics of the different entities are shown in Table 1. (1,3)

Nephritic syndrome	**Nephrotic syndrome**
Haematuria (acanthocytes) Proteinuria (>1,5g/d) Facultative symptoms are: - Oedema - Hypertension - Low renal function - Oliguria	higher permeability of the glomerular capillaries: - proteinuria of >3,5g/d after exceeding hepatic compensation: - Hypalbuminaemia - Hyperlipoproteinemia - Oedema - Hypercoagulability

Table 1: Differences between nephritic and nephrotic syndrome

1.1.1 Classification of GN by their pathophysiology

The GN can be further subclassified by its underlying pathophysiology. (4) The Mayo Clinic/Renal Pathology Society Consensus Report on Pathologic

Classification, Diagnosis, and Reporting of GN from 2015 defined five different classes of GN:

1. Immune-complex GN:
   The immune-complex GN shows polyclonal immunoglobulin deposits and holds three different diseases: (1)
   - IgA-nephropathy: a disease with deposits of IgA1 and immune complexes, with complement activation. It's the most common form of idiopathic GN
   - Infection-related GN: with deposits of antibody-antigen complexes or C3-complements, which occur after different infections. In the past, these infections were most commonly caused by beta haemolysing Streptococcus of group B. Nowadays, the focus is on different pathogens such as staphylococcus or Gram-negative bacteria.
   - Lupus nephritis: with deposits of mesangial and subendothelial immune complexes. This disease is an organ manifestation of systemic lupus erythematosus (SLE), which occurs in over 60% of all SLE patients. (5)
2. Pauci-immune GN: (4)
   The pauci-immune GN shows no or very few immune deposits, while 80-90% of patients are positive for ANCA.
3. Anti-glomerular base membrane-antibody GN (anti-GBM):
   The disease shows "linear deposits of Ig, most often IgG and frequently, C3 along the GBM" (4).
4. Monoclonal Ig GN with "monotypic Ig deposits in the glomeruli and/or along tubular basement membranes" (4) can be associated with monoclonal gammopathy/paraproteinemia.
5. C3-glomerulopathy: with "dominant C3 deposits in the glomeruli with minimal or no Ig deposits" (4)

1.1.2 Rapid progressive Glomerulonephritis

Under the glomerulonephritides, the rapid progressive glomerulonephritis (RPGN) is one of the most aggressive forms of GN. It shows a nephritic disease course and a rapid progressive aggravation of renal dysfunction within weeks or months. (6)

1.1.2.1 Subclassification

There are three underlying pathophysiologies, which can cause RPGN. (1)

1. Type 1 (around 10% of the cases) is also called the anti-GBM-disease. It shows antibodies against the GBM. In 30% of the cases, patients with anti-GBM-antibodies show also myeloperoxidase (MPO)-ANCA in their serological probes. In the histopathological examination, IgG and C_3-complement depositions are detectable at the glomerular base membrane. Sixty five percent of all cases also involve the lungs, which is called “Goodpasture syndrome”. Due to similar structures in the alveolar and glomerular base membrane, a combination of RPGN and pulmonary haemorrhage (mainly characterized by haemoptysis, wet rales, and lung opacity) is possible.
2. Type 2 (approximately 40% of the cases): This type represents the immune-complex RPGN. The histopathological findings show granular deposits in the glomerular base membrane, for example in so-called “humps”. It often occurs after infection, together with a systemic disease, such as SLE, or IgA nephropathy.
3. Type 3 (approximately 50% of all cases) represents ANCA-associated vasculitides (AAV). Their characteristic is a histopathologic finding without any deposits of immune globulins or complement in the GBM. The disease is associated with MPO- and Proteinase 3 (PR3)-ANCA. The different subcategories and features of AAV or ANCA-associated GN will be discussed extensively later in this thesis.

1.1.2.2 Histopathology

The histopathological picture of RPGN shows glomeruli with necrosis and fibrin secretions in and outside of capillary loops. The proliferation of cells in the Bowman’s capsule space can fill it completely or partially, and later manifest as a crescent, which is the typical histopathological finding in RPGN. The crescent consists of fibroblasts, macrophages, podocytes, and parietal epithelial cells and its intensified formation leads to the rupture of the GBM. With the increasing amount of matrix and collagen in the crescent, it changes into a fibro cellular and eventually into a fibrous crescent. (2,7,8)

It has been shown that anti-GBM antibody or immune complexes can cause a signal cascade in podocytes, which results in podocyte dedifferentiation. (9)
The induction and inflammatory process of RPGN, caused by AAV, will be explained extensively in chapter 1.4.

1.1.2.3 Clinical presentation and diagnosis

The most typical clinical presentation of RPGN is a severe nephritic syndrome characterized by elevated plasma creatinine concentration (often over 3mg/dL) indicating an acute kidney injury (AKI), abnormal urinalysis with mild to moderate proteinuria and glomerular hematuria.
RPGN can also show general symptoms such as fatigue, and loss of weight. Hypertension often occurs in patients with advanced RPGN as a result of the AKI. Serological testing might show elevated c-reactive protein (CRP) values and erythrocyte sedimentation rate (ESR). In addition, serological markers are also crucial for the diagnosis of RPGN, including circulating anti-GBM-antibodies (type 1), immune complexes (for example IgA) (type 2) and ANCA autoantibodies among others. (1,10)
Additional clinical examinations, such as kidney ultrasound can be a useful additional tool in the complex process of diagnosing RPGN. The acute onset of RPGN leads to proteinaceous casts and inflammation, which reflect soundwaves and show an increased echogenicity, similar to a chronic kidney disease. In most cases the kidney size in RPGN is normal. (11)
For a proper diagnosis and therapeutic choice of RPGN and all glomerulonephritides a kidney biopsy should be performed. (6)

1.2 AAV nomenclature

The International Chapel Hill Consensus Conference (CHCC) Nomenclature of Vasculitides of 2012 categorized the AAV as a small vessel vasculitis. (12) Alongside the AAV there are several other small vessel vasculitides, including anti-GBM, cryoglobulinemic vasculitis, IgA Vasculitis and hypocomplementemic urticarial vasculitis among others. An overview of the nomenclature can be seen in Table 2.

Large Vessel Vasculitis	Takayasu Arteritis
	Giant cell arteritis
Medium Vessel Vasculitis	Polyarteritis nodosa
	Kawasaki disease
Small vessel vasculitis (SVV)	AAV - Microsopic Polyangiitis - Granulomatosis with Polyangiitis - Eosinophilic Granulomatosis with Polyangiitis
	Immune complex SVV - anti-glomerular-basement membrane disease - cryoglobulinemic vasculitis - IgA vasculitis - anti-c1q vasculitis
Variable vessel vasculitis	
Single-organ vasculitis	
Vasculitis associated with systemic disease	

Table 2: copied and modified from the 2012 CHCC (12)

As stated in the introduction of the CHCC, its nomenclature cannot be used as a diagnostic or classification system. It does not describe any clinical or histopathological criteria that have to be observed in a patient in order to diagnose a specific vasculitis. It merely dictates which name should be used to describe the patient's disease.

The 2012 CHCC divides the AAV into three subgroups which can be distinguished by their clinicopathologic features as follows:

- Microscopic polyangiitis (MPA)

  Few or no immune deposits are present. Necrotizing GN, arteritis in small and medium vessels and pulmonary capillaritis mostly occur. Any other inflammation apart from vessel-centred inflammation is absent, including granulomatous inflammations.
- Granulomatosis with Polyangiitis (GPA)

  GPA includes necrotizing granulomatous inflammation mostly found in the respiratory tract which affects small to medium vessels. Ocular and pulmonary involvement is often reported. Necrotizing GN occurs very often.
- Eosinophilic granulomatosis with polyangiitis (EGPA)

  EGPA is associated with eosinophil infiltrations, necrotizing and granulomatous inflammation and involves mainly small to medium vessels. It often affects the respiratory tract (e.g. nasal polyps) and is also often

associated with asthma bronchiale and eosinophilia. It is quite common that EGPA patients have no renal involvement and ANCA positivity in EGPA seems to be dependent on renal involvement and its severity. (13) Both granulomatous and non-granulomatous inflammation may be present in EGPA patients. Non-granulomatous eosinophil-rich involvement of lungs, myocardium and gastrointestinal tract often occurs. (12)

1.3 Epidemiology and predisposing factors of AAV

AAV epidemiology data exists for specific and regional populations, f.e. Berti et. al. (14) proved that the incidence and prevalence rate for AAV in Minnesota are 2.0/100,000 and 35/100,000, respectively. On the other hand, in the UK the incidence rate was reported to be around 1.2 per 100,000 inhabitants in the population ≥ 18 years old. (15) In other countries with European ancestry the incidence of AAV is around 2 per 100,000. (16) However, there is no general multicentred study to specify an exact incidence and prevalence rate. (14)

Differing GPA and MPA incidences depending on the geographic location could be shown in Europe. In Spain, MPA had a higher annual incidence rate than in North Norway, while GPA was more often seen in North Norway. (17) Berti et. al. found similar incidence rates for GPA and MPA (1.3 and 1.6 / 100,000, respectively) in Olmsted county / Minnesota, a region with mostly north-European predecessors. (18) Additionally, Watts et al. reported, an annual incidence rate of 1.13 and 0.59/100,000 inhabitants for GPA and MPA, in the population of the catchment area of the Norfolk and Norwich University Hospitals in England. (19) Moreover, different incidence rates were reported between Caucasian (of northern-Europe descent) and non-Caucasian populations. (16) As an example, the incidence rate of the European population in New Zealand is twice as high as in the Maori or Asian population in New Zealand. (20) In Chinese patients, who were diagnosed in Bejing, had a higher incidence of MPA than of GPA (21). In a comparison between the incidences of AAV in the UK and Japan the average annual incidence rate in the UK for overall AAV was reported to be around 2.18 / 100,000, which is less than in the Japanese population (2.26 / 100,000). The incidences for GPA and MPA in the UK were 1.43/100,000 and 0.65/100,000 and 0.21/100,000 and 1.82/100,000 respectively in Japan. (22)

In summary, the average incidence of AAV in white Caucasians is approximately 2/100,000, while MPA and GPA numbers appear to differ according to geographic latitude and/or different ancestry.

The demographic trends of glomerular diseases in the south-eastern USA were investigated by O'Shaughnessy et. al. and observed a predominance of lupus nephritis and IgA Nephropathy in patients who were < 60 years old, while AAV was more often diagnosed in > 60-year-old individuals. (23) The age incidence peak of MPA, GPA and EGPA lies between 65-75 years and male individuals are slightly more often affected. (24)

The main environmental influences in the aetiology of AAV are still unknown. (16) Nevertheless, there are some environmental and genetic factors, which can have an impact on AAV. According to Yashiro et al. and Takeuchi et al., who both published data after two large earthquakes in Japan, environmental dust may cause an increased incidence in AAV. (25,26) On the other hand Webber et al. showed that the dust produced by the collapsing World Trade Centre in 2001 did not have an impact on AAV incidence. (27) Another publication suggests an influence of silica on the development of AAV. (28) Bronchiectasis and autoimmune diseases such as type 1 diabetes, inflammatory bowel disease and rheumatoid arthritis may have an association with GPA. (16,29)

Interestingly, Watts et al. found a cyclical pattern for GPA which repeats every 7,6 years. For the population in the catchment area of the Norfolk and Norwich University Hospital the peak of this pattern appeared in 2005 with an incidence of 2.83, while the lowest incidence of 2.2 per 100,000 inhabitants occurred in 2002. (19)

Genetic association for AAV has been identified, especially in those with MPO-ANCA with further evidence for a genetic influence on the pathogenesis. Lyons et. al. identified an association of the gene locus SERPINA1 and "GPA, but not MPA when each subtype was compared with controls." PRTN3 is also suggested to be associated with GPA.

Moreover, the authors identified single-nucleotide polymorphisms in HLA-DP, SERPINA 1 (encoding α-antitrypsin, the enzyme for inhibiting PR3) and PRTN3 (encoding the PR3 molecule) associated with the development of PR3. The significance of the association shown between genetics and disease was observed to be higher in PR3-ANCA than in the clinically diagnosed GPA. (30) In addition,

HLA-DPB1*0401 is a GPA susceptibility allele and HLA-DPB1*0401 population allele frequencies may help explain variations in GPA incidence described in the literature. (31)

Interestingly, to our knowledge no gene locus association could be found until now for MPA. However, an affiliation with HLA-DQ polymorphisms could be identified in patients with MPO-AAV. These data deliver further evidence for the thesis, that MPO- and PR3-ANCA are two different diseases showing different MHC and gene loci. (30,32).

1.4 Pathophysiology of AAV

In the pathophysiology of AAV, ANCA antigens and ANCA autoantibodies play a major role. They are suspected to cause and influence the inflammation process and disease activity. The role of ANCA antibodies is not yet completely understood in the inflammation process of the small vessels seen in patients with AAV.

1.4.1 ANCA antigens

There are two known autoantigens, which seems to play a major role in the pathophysiology and clinical course of AAV.

One is the antigen PR3, which is produced and stored in neutrophils in very high levels but can also be found in monocytes and macrophages in lower levels. The PR3 antigen is “a 29kDa serine protease” and is part of the neutrophil-derived protease family stored in azurophilic granules, together with other “family-members”, such as neutrophil elastase, cathepsin G and azurocidin. All the representatives of the neutrophil-derived protease family are stored as an inactive proform. They can be activated by the “removal of an amino-terminal dipeptide by the lysosomal cysteine exopeptidase dipeptidyl peptidase I.” (33)

The other important antigen in AAV pathophysiology is MPO, which is a 147kDa homodimeric protein and consists of several monomerswhich are held together by a disulfide bridge. These monomers consist of a heavy glycosylated and a light chain. The glycosylated structure of the heavy chain is important for MPOs enzymatic activity. (34)

MPO-ANCA plays a role in the corporal defence against pathogens and has bactericidal properties, as it converts chloride anions and hydrogen peroxidase into hypochlorous acid. Hypochlorous acid acts as a bactericidal toxin.

MPO are present in neutrophils and are stored in their azurophilic granules. By release, MPOs help phagosomes to degrade opsonized pathogens. (35)

1.4.2 Pathogenic influence of ANCA

In the 1980s and early 1990, several publications showed a link between GPA and other forms of vasculitis and the appearance of ANCA. (36,37) Tervaert et al. described firstly the occurrence of autoantibodies directed against myeloid lysosomal enzymes in patients with necrotizing arteritis suggesting them as a useful adjunct for the classification of this group of patients. (38)

The discovery of ANCAs in the serum of AAV patients led to extended research about these antibodies.

1.4.2.1 Evidence for the pathogenicity of ANCA

It is hypothesized that ANCA antibodies are the causal pathogenic factor for AAV (39), and evidence on the correlation between ANCA-serum titres and disease activity and/or therapy response exists.

In in vitro experiments both PR3 and MPO could activate primed neutrophils leading them to release toxic oxygen radicals, lytic and proinflammatory enzymes, complement alternative pathway–activating factors, and neutrophil extracellular traps (NETs). (39–41)

By injecting MPO-ANCA into mice, AAV could be induced, but the same experiment did not show the same result for PR3-ANCA. (42) In details, Xiao et al. tested the effects of injecting purified anti-MPO IgG in wild-type mice and mice without functionable B- and T- cells ($Rag2^{-/-}$ mice). Both animal groups developed pauci-immune glomerular necrosis and crescent formation, which shows strong evidence for a direct pathogenic role for MPO-ANCA IgG in human GN and vasculitis. (43)

1.4.2.2 Evidence against the pathogenicity of ANCA

Despite the extended evidence on the pathogenicity of ANCA antibodies, there are still doubts and phenomena, which need to be discussed. Some patients with an AAV disease phenotype are proven ANCA-negative, while other AAV-patients maintain remission, although their serum ANCA titers are highly positive. (44)

1.4.2.3 ANCA antibodies in other autoimmune disorders

Apart from AAV, several other autoimmune diseases show ANCA positivity, including inflammatory bowel diseases, such as ulcerative colitis or Morbus Crohn.

Roozendaal et al. concluded that ANCAs are useful markers for ulcerative Colitis and colonic localization in Crohn's disease. (45,46)

ANCA positivity can also be observed in patients with SLE. (47) Choi et al. found PR3-ANCA positivity in patients with endocarditis. From this aspect a misdiagnosis of endocarditis as AAV would lead to inappropriate and delayed treatment of endocarditis. (48) Also patients with cystic fibrosis can show ANCA-positivity. (49)

According to Levy et al. in patients with anti-GBM disease 32% of the blood samples were tested positive for ANCA, while 5% of all ANCA-positive serum samples were also positive for anti-GBM antibodies. The occurrence of both antibodies is called: “double-positive-disease”. (50)

There are other epitopes, besides PR3 and MPO that were reported as autoantigens for ANCAs. As an example, elastase-specific ANCAs were found in patients with drug-induced AAV. (51,52), while Kain et al. detected ANCAs in patients with pauci-immune focal necrotizing glomerulonephritis (FNGN) specific for lysosomal-associated membrane protein 2 (LAMP-2). Interestingly, even patients who showed PR3-ANCA or MPO-ANCA positivity or absence of those ANCA subtypes were tested positive for LAMP-2. (53) These results however, could not have been replicated in other studies. (39,54,55)

In a non-pathogenic context, MPO and PR3-ANCA positivity can also occur in healthy individuals. Those antibodies show less pathogenicity, have lower titres than pathogenic ANCAs (56,57) and are called natural or non-pathogenic-autoantibodies. (39)

1.4.3 The pathophysiological process

The pathological process occurs in several different steps as listed below. An overview of the exact immunological procedures can be seen in Figure 1 and is extensively discussed below.

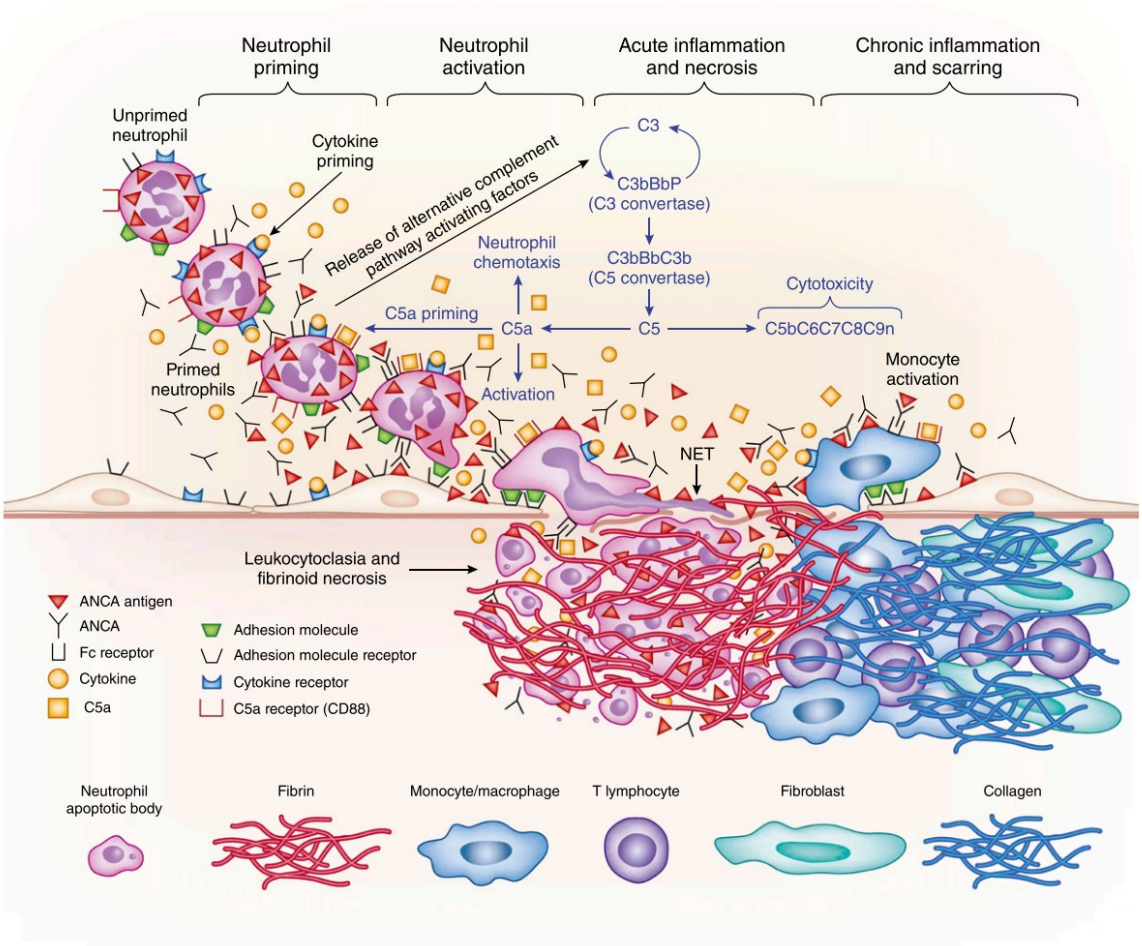


Figure 1: Pathogenesis of vascular lesions in AAV and GN, with the kind permission of J.C. Jennette et al. CJASN 2017 (44)

1.4.3.1 Loss of tolerance against MPO and PR3

In every autoimmune process there is a target antigen that the immune system can not recognize as its own. In the case of AAV, autoimmunity develops towards antigens of MPO or PR3. (58) As these autoantibodies are contributors of a healthy immune system, triggers for a loss of tolerance against those antigens can be supposed.

However, the main mechanisms for the loss of tolerance are still not fully understood. Several minor connections and mechanisms for the onset of autoimmunity in AAV have been found.

The tolerance towards PR3 and MPO antigens is regulated by autoimmune regulator-induced thymic MPO expression. (58,59)

In addition, tolerance for MPO and PR3 can be lost by the occurrence of dendritic cells that are loaded with NETs which indicates that the inflammation process of AAV itself can enhance a loss of tolerance towards PR3 and MPO. (60) ANCAs on

apoptotic neutrophils with PR3 on their surface “triggered secretion of inflammatory cytokines, including granulocyte CSF and chemokines.” (61)

1.4.3.2 Priming of neutrophils

Priming is the act of transferring ANCA-antigens from the inside of neutrophils to the surface. (44) That is a crucial part before the activation of neutrophils and the initiation of the pathological process can take place. Without the surfacing of ANCA-antigens, ANCAs can not bind to the their antigens. (39) Among others, priming can be accomplished by the cytokine tumor necrosis factor (TNF) (62), bacterial lipopolysaccharide (63), and C5a from the complement cascade. (64) Also a concurrent viral infection of the respiratory tract has been showed to be associated with the priming of neutrophils. (44)

1.4.3.3 Activation of neutrophils

The ability of PR3- and MPO-ANCA to activate neutrophils was first shown 30 years ago. (62) Shortly, the $F(ab')_2$ ANCA fragments bind to neutrophil surface antigens (39,65) leading to a release of inflammation mediators causing endothelial cell damage. (66)

Moreover, the binding of ANCA-IgG and ANCA-antigen forms an immune complex which engages Fcγ-receptors. This engagement results in an enhanced neutrophil inflammation response. (67,68)

Because of ANCA antigens on the monocytical surface, monocytes can be activated by ANCAs. (69,70)

1.4.3.4 The role of neutrophils and monocytes in AAV pathogenesis

After their activation, neutrophils induce tissue and endothelial damage using several inflammatory reactions, as degranulation of destructive enzymes, production of reactive oxygen species (ROS) and release of NETs. (62,71)

The release of NETs is also called NETosis and is a result of neutrophil apoptosis. (72) It is an important factor in endothelial injury and necrosis. (73,74) NETs are built up by chromatin fibres with a diameter of 15-17nm and include DNA and histones, as their major components. The NETs are interfused by antimicrobial peptides and enzymes such as neutrophil elastase, cathepsin G and MPO from

neutrophil granules. (75) They are an important contributor to counterattack invading microorganisms causing their immobilisation and determination. Due to their ability to neutralize microorganisms and to play a part in the autoimmune process, Kaplan et al. described them as double-edged swords. (76) Induction of NETosis can happen spontaneously. In comparison to healthy individuals with AAV showed more spontaneous NETosis, while there is also evidence of an ANCA triggered induction of NETosis. Nevertheless, for a full understanding of the exact mechanism of NETosis induction, further investigations are required. (77)

In AAV patients, NETs could be detected in the glomeruli in kidney biopsies and suggested to be declared as the cause of neutrophils necrosis in inflammatory sites. (73) They could also be found in skin lesions and thrombi from AAV patients. (41) In addition, several publications confirmed the appearance of NETs in the circulation of AAV patients. (78,79) In accordance, further evidence shows their pathogenicity and proves their ability to activate dendritic cells and autoreactive B-cells and induce endothelial damage. (66,80) Moreover, Wang et al. showed the ability of NETs to activate alternative complement pathways (81), which is another very important factor in the pathogenesis of AAV.

Together with neutrophils, monocytes also play a crucial role in the pathogenesis of AAV. Among other features, they are able to release proinflammatory cytokines, IL-8 and monocyte attracting protein (MCP-1). (69,70) IL-8 attracts and activates neutrophils at the inflammation site and can enhance the pathologic process, while MCP-1 attracts monocytes and macrophages. It “could participate in the transition of ANCA-induced vascular injury from predominantly neutrophil-rich inflammation to predominantly monocyte and/or macrophage-rich inflammation, including granulomatous inflammation.” (39) Importantly, monocytes store ANCA antigens in their peroxidase-positive lysosomes. (82)

1.4.3.4.1 *Neutrophil adherence to endothelium*

Another important factor in AAV pathophysiology is the neutrophil adherence to endothelium.

ANCAs promote adherence, induction and migration of neutrophils through the endothelium. Moreover, B2 integrins, selectins and neutrophil chemokines are believed to play a major role in ANCA-induced neutrophil recruitment. (58,83,84)

Activated neutrophils release ANCAs which bind to the nearby endothelium. MPO keeps its enzymic function and interacts with hydrogen peroxide to detach endothelial cells from their substratum. (85)

The complex of ANCA autoantibodies and antigens, bound to the Fcγ-receptor of neutrophils, is suggested to bind to endothelial cells, resulting in a connection between neutrophil and endothelial cells. (39) The antigens bound to the endothelium in the glomeruli, can be detected by autoreactive T-cells and further injury can be mediated. (86)

1.4.3.5 The role of complement activation

As complement activation plays a very important role in AAV pathogenicity, the basics of the complement system will be explained in the next section.

1.4.3.5.1 *Basics of the complement system and activation*

The complement system consists of more than 20 proteins which are solved in serum or cell-bound. One of its most important purposes is the opsonization of pathogens and antigens through a process of marking an unwanted microorganism attracting phagocytes to eliminate them.

Another important feature of the complement system is the formation of a membrane attack complex (MAC), which leads to apoptosis of the attacked cell. On the other hand, parts of the complement system can activate a local inflammation process. (87) The different ways activating the complement system are explained below.

1.4.3.5.1.1 Alternative pathway

The reaction of the alternative pathway is initiated by a C3-molecule which spontaneously splits into the fragments C3a and C3b. The bigger C3b part is very reactive and is quickly deactivated by the surrounding water which prevents damage to cellular structures. In the presence of microbial surfaces, the C3b molecule persists and can bind to Bb which originates from factor B. Factor B is then split into Ba and Bb by factor D, which is a serine protease.

The union of C3b and Bb results in the formation of the molecule C3Bb which can split C3 molecules (C3-convertase). After that, C3 splits into C3a and C3b.The latter accumulates on the microbial surface to serve as an opsonin for phagocytosis. The C3Bb is an unstable form and binds properdin to gain stability.

To form C5-convertase, C3Bb unifies with C3b to form C3bBbC3b (=C5-convertase). (87) The activation of the alternative pathway is shown in Figure 2.

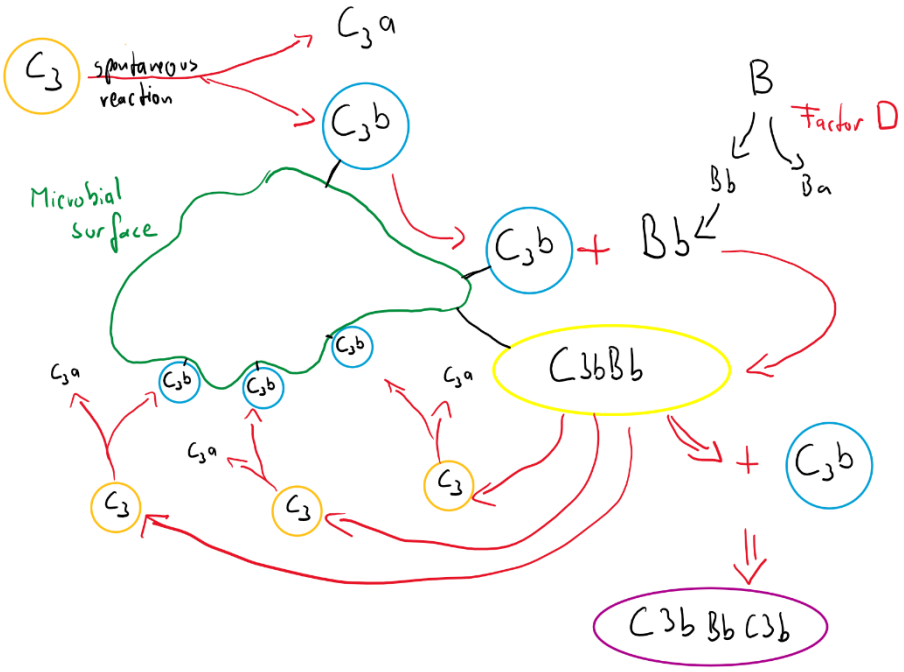


Figure 2: The different components and the procedure of the alternative pathway

1.4.3.5.1.2 Classical complement pathway

The reaction of the classical complement pathway is activated by the binding of antibodies to their referring antigens. Due to this connection, a binding site (Fc-region) for complement factor C1 is uncovered which in turn activates factor C1. C1 is a compound of the subunits C1qr2s2 (one C1q, 2x C1r and 2x C1s). C1q then connects to the Fc-region of the certain antibody. Only when two IgG-molecules or one IgM-molecule connect to C1q the complement pathway can be initiated. The binding activates C1r conversing it autoproteolytically into the activated serine proteinase C1r., which then activates C1s, converting it into an activated serine protease. The activated C1s becomes the catalizator of a reaction leading to the production of C4a and C4b from C4 and C2a and C2b from C2.

As a further step, C2a and C4b connect and emerge as C4b2a, which is the C3 proteinase of the classical pathway. This C3 proteinase enables the conversion from C3 into C3a, which can activate a local inflammation reaction. The component C3b can bind to microbial surfaces and can be detected by phagocytic cells such as neutrophils, macrophages, B- and T-cells.

Finally, the C3b molecules unifies with the C4b2a complex and builds the C4b2a3b-complex, which is the C5-convertase of the classical complement pathway. (87) The activation of the classical complement pathway is shown in Figure 3.

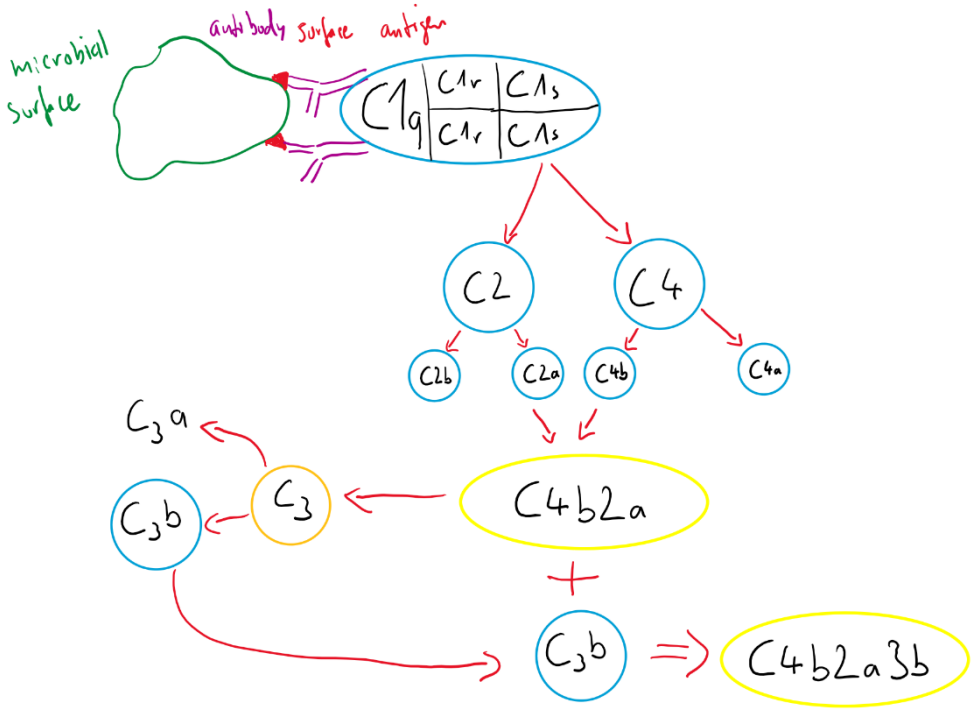


Figure 3: The different components and the procedure of the classical pathway

1.4.3.5.1.3 Lectin pathway

Another way to activate the complement system is the lectin pathway. Activation via Lectin starts with the binding of mannose-binding-lectin (MBL) to mannose structures on microbial surfaces. Mannose has similar structures to C1q and can activate C1r and therefore also C1s. Mannose is also able to bind to an MBL-associated serine protease that splits C4.

The rest of the lectin-activated complement pathway follows the already described patterns. (87)

1.4.3.5.1.4 Synthesis of the MAC

The two C5-convertases C4b2a3b from the classical complement pathway together with C3bBbC3b from the alternative complement pathway can split C5 into C5a and C5b. After C5a is released, it connects to the microbial surface. The remaining complement components (C6, C7, C8, and C9) then connect to C5b and form the structure of MAC, which creates a channel into the cell and affects an inflow of water and calcium ions inducing cell lysis and cell death. (87) The synthesis of the MAC is shown in Figure 4.

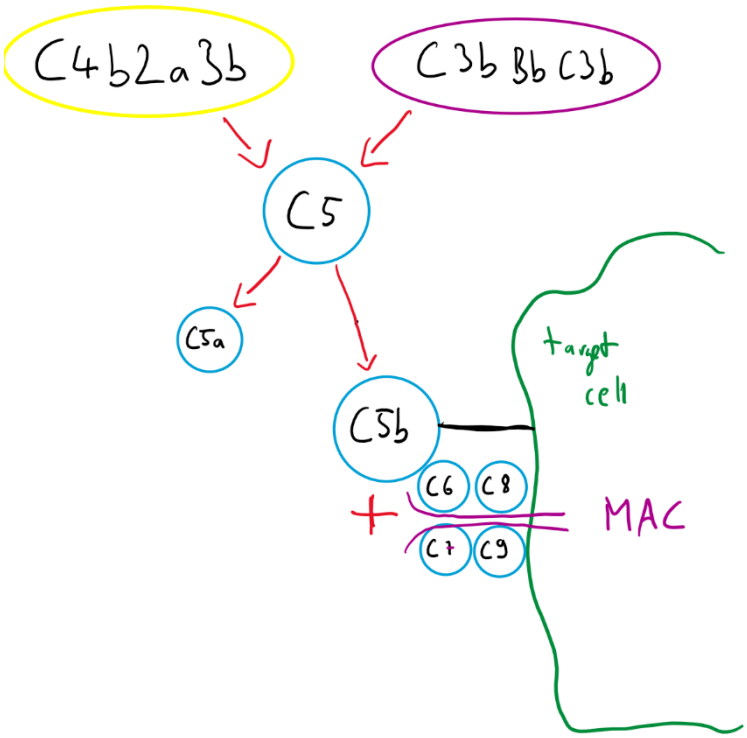


Figure 4: Synthesis of the MAC

1.4.3.5.2 *Complement activation in AAV*

Due to immune complement paucity seen in glomerular AAV-lesions, the role of complement system in the pathophysiology of AAV was long believed to be of a minor importance. (12,58)

However, data from the last decades highlighted the crucial pathophysiological role of the complement system in AAV leading to new therapeutic approaches.

In the last decade, Xia et al. showed that the injection of MPO IgG into wild-type and of anti-MPO splenocytes into immune-deficient mice leads to crescentic GN. However, most importantly the evolvement of GN could be blocked by complement depletion. C4 knockout mice showed crescentic GN similar to that of the wild-type mice, while C5 and factor B knockout mice developed no disease.

After this observation, PR3-ANCA and MPO-ANCA-IgG antibodies from AAV patients with healthy controls were taken and incubated with neutrophils. While ANCA-IgG from the healthy controls showed no effect, the ANCAs from patients with AAV caused complement activation.

As C4 is needed for the activation of both classical and lectin-binding pathways, these findings allowed the assumption that ANCAs caused a release of components which in turn activate the alternative complement pathway.

There is no certainty about the component activating the alternative complement pathway. (88) A study investigating complement factors showed higher urinary levels of Bb, C3a, C5a, and soluble C5b-9 in individuals in an active stage of AAV than patients in remission. It also showed a correlation between the density of Bb in glomeruli and the proportion of total crescents, the extent of interstitial infiltrate, interstitial fibrosis and tubular atrophy (IF/TA), whereas it showed an inverse correlation with the proportion of normal glomeruli. The presence of Bb in urine correlated with serum levels and correlated inversely with the proportion of normal glomeruli. (89)

As already mentioned above, C5a has the ability to prime neutrophils, (64) but besides of it, it can also activate neutrophils via its receptor C5aR (CD88). C5a is a very important factor for chemotaxis for neutrophils and monocytes. (44,90)

The role of C5a as a therapeutic target in the inflammatory process of AAV has been shown using new treatment agents such as eculizumab and avacopan.

Eculizumab, a humanized anti-C5 monoclonal antibody, was used “off-label” in a patient with acute renal failure due to an AAV and the patient’s renal function could almost fully recover. (91)

Avacopan (CCX168) blocks human C5aR. In mouse models, its therapeutic effect in anti-MPO-induced necrotizing and crescentic GNwas shown. The results led to clinical studies evaluating its effectiveness in human subjects. (92,93)

1.4.3.6 B-cells

B-cells also form a very important part of AAV pathophysiology producing ANCAs and $CD38^{+}$ activated B-cells show a positive correlation with disease activity. (94)

Different subsets of B-cells have been found to be associated with the disease course of AAV. (66)

CD5 is a cluster of differentiation (CD) which can be found on B regulatory cells (B_{reg}), their main function being to release IL-10, which in turn seems to have an improved immune response in autoimmunity animal models. (95)

Bunch et al. could discover low levels of $CD5^{+}$ B-cells in patients with active AAV, while during remission the levels normalized again. Patients who had low CD5+ cell levels and low maintenance immunosuppression showed earlier relapses than patients with normal $CD5^{+}$ cell levels and similar maintenance immunosuppression. (97,98)

In addition, regulatory B-cells have been reported to decrease the differentiation of native T cells into regulatory T-cells (T_{reg}), decrease the appearance of T_{H1} (T-helper cells), $T_{H17,}$ and ANCA producing B cells. (66)

B cell survival is regulated by a B-lymphocyte stimulator also called B cell-activating factor (BLyS and BAFF, respectively) and a proliferation-inducing ligand. Both factors are released by neutrophils activated via ANCAs. (66) BLyS was investigated thoroughly as it consistently showed higher serum levels in AAV patients than in the healthy controls. (99) In accordance, a further study could report a correlation between BLyS and GPA disease activity. Untreated GPA patients showed high levels of BLyS, while treated GPA patients had similar BLyS levels when compared to the healthy control group. (100) As a consequence, the authors suggested BLyS as a possible drug target, especially in constantly relapsing patients. Howevere, a phase III trial of the anti-BLyS-treatment belimumab added to azathioprine and oral glucocorticoids maintenance therapy failed to show a reduced relapse rate in AAV patients. (66)

Culton et al. showed a two to four-fold higher expression of CD19 on memory B cells in AAV and SLE patients than in healthy controls. (101) Prendecki et al. assumed, that this subset of B-cells may represent autoreactive B-cells (66) and may assume that SLE and AAV show a similar mechanism for loss of B-tolerance. (101)

1.4.3.6.1 *Plasma cell marker CD138*

CD138 is also called syndecan-1 and belongs to the syndecan family which is responsible for cell-matrix and cell-cell interactions and is almost exclusively expressed by plasma-cell and epithelial cells. Syndecans consist of transmembrane heparan sulfate proteoglycans. This is why renal tubular epithelial cells become unspecifically stained in comparison to the normal $CD138^+$ cells.

The syndecan-1 cells can bind to ligands which are responsible for the development of inflammation. They play a role in the recruitment of leucocytes in setting up chemokine gradients and they help to rebuild extracellular structures during the restoration of injured tissues.

Several studies have shown that $CD138^+$ cells play a role in various diseases, such as interglomerular basement membrane nephritis, Crohn's disease, allergic lung inflammation, allergic contact dermatitis, etc. (102)

In biopsies of AAV-patients CD138 positive cells were only rarely found in scattered B-cell distribution and nodular distribution patterns. There were no CD138 positive cells in higher organized patterns. (103) Berden showed the presence of CD138 positive cells in mostly mildly affected patients (i0, i1 and g0, g1), subclassified by BANFF score. They found more TIN and higher counts of CD3 and CD138 positive cells in AAV-patients with MPO-ANCA, than in PR3-ANCA. (104)

1.4.3.7 T-cells

In the pathophysiology of AAV, T_{reg}s play a significant role. (105)

T_{reg}s have immunosuppressive abilities, mostly due to their production of IL-10 and TGF-β. Due to their ability to control the immune response, they are an important component in the prevention of autoimmune diseases. (87)

Morgan et al. examined the T-cell count in GPA patients and found an increased fraction of $CD4^+$- and $CD25^+$-cells, but a decreased fraction of Foxp3 cells. In this study the patients with more rapid disease remission were associated with higher $CD4^+$- and Foxp3- cell proportions, while low levels of T_{reg}s were related to a higher rate of GPA disease relapse. (105)

Free et al. found an increased T_{reg} cell population in AAV patients with active disease, however these cells had a decreased suppressive function. Also, a higher frequency of CD4 positive cells which did not react to the T_{reg} suppressive signals were found. (106)

Further evidence shows a possible involvement of the TH17 axis in the pathogenesis of AAV. Th17 cells produce IL-17, which is an important mediator for the formation of autoimmune diseases. IL-17 shows a wide range of effects such as the release of pro-inflammatory cytokines (TNF-α, IL-6, and IL-8), up-regulation of adhesion and MHC molecules and recruitment of monocytes and neutrophils. All these effects are typically found during the acute phase of AAV. Moreover, Th17 maintenance is critically dependent on IL-23. Nogueira et al. found elevated IL-17A and IL-23 serum levels in acute AAV patients. IL-23 serum levels correlated with higher disease levels and with increased ANCA levels, while some recovered patients retained increased IL-17A and Il-23 serum levels which may contribute to the high relapse rates. (107) In addition, MPO-ANCA-activated neutrophils produce IL-17A and IL-23 which trigger the development of Th17-mediated autoimmunity. (108)

In an animal study, IL-17A deficient mice were almost completely protected from MPO-ANCA-induced disease. (66,109)
The passive transfer of anti-MPO AAV could be accomplished by using CD4 positive T-cells. It proves that injury can be induced via humoral and cell-mediated immune mechanisms. (110)
Regarding CD8 positive T-cell, in an animal model T cell depletion in the effector phase of disease attenuated injury in murine anti–MPO GN. (111) A different study confirmed the important part of T-cells in disease injury and could show the reaction of T-cells towards MPO-antigens in glomeruli resulting in T-cell inducing “delayed-type hypersensitivity-like necrotizing glomerular lesions”. (86) In both studies pathogenic epitopes for MPO-specific $CD8^+$ and $CD4^+$ T-cells were found.

1.4.3.8 Granulomatosis

The occurrence of granulomatosis is mainly seen in GPA. Like many other topics there are still uncertainties about granulomatosis formation. Jenette and Falk suggest that granulomatosis is an innate inflammatory response to an acute extravascular inflammation that is initiated by ANCA-induced neutrophil activation. (39) In an early study, Fienberg et al., reviewed lung biopsies of 35 patients with GPA granulomatosis. In this study, a state of micronecrosis that shows neutrophils and the development of the pathognomonic organized palisading granuloma was found, which resembles the early stage of granulomatosis. (112) In addition to neutrophils, the early manifestation of granulomatosis also shows some multinucleated giant cells. Later on, the appearance of multinucleated cells at the margin of the central necrotic area can be observed. The surrounding is interfused by dendritic cells, T lymphocytes, B lymphocytes, and plasma cells forming a follicular structure of ectopic lymphoid tissue. (39)

1.5 AAV Diagnosis

There are several aspects, which have to be taken into account when diagnosing AAV. To name a few, histopathological aspects, which defines specific alterations in the context of an AAV, the clinical appearance, as well as the serological markers for AAV.

1.5.1 Clinical appearance

The early presentation of vasculitis has very unspecific symptoms, such as weight loss, fatigue, myalgia, or fever. These symptoms can persist for several months leading to frequent misdiagnosis of vasculitis as infection, tumor-like lesions, arthralgia, or depression among others. (113,114)

Several organ systems can be affected by AAV leading to various clinical symptoms as listed below. (114)

- Ear, Nose, and Throat (ENT):
  AAV (i.e. those with GPA and EGPA) can show necrotizing or granulomatous inflammation and causes several symptoms in the ear-nose-throat (ENT) area such as chronic rhinitis, laryngitis, or sinusitis.
- Respiratory tract / pulmonary involvement
  Necrotizing, granulomatous inflammation or pulmonary capillaritis in the respiratory tract can occur. These inflammations may lead to shortness of breath, cough, pulmonary haemorrhage with haemoptysis, and cavitating lung nodules.
- Ophthalmologic involvement
  AAV with an ophthalmologic involvement presents granulomatous orbital/retroorbital masses, optic neuritis, retinal vasculitis, or anterior segment inflammation.
- Skin
  AAV can result in purpural or petechial rash with necrotizing dermal vasculitis.
- Nervous system
  The AAV-associated lesions in the vasa nervorum might lead to mononeuritis multiplex in the peripheral nervous system.
- Renal involvement
  Kidneys affected by AAV show mainly RPGN. The symptoms of RPGN are listed in chapter 1.1.2.3.

1.5.1.1 Clinical characteristics of GPA and MPA

Mahr et al. analyzed the differences in the clinical phenotypes of GPA and MPA patients representing data from several countries in Europe and Mexico.

The mean age of GPA patients was 55,2 and 61,8 for MPA patients, respectively. There was a slight male predominance for both GPA and MPA (54,3% and 51,6%, respectively). GPA patients showed predominantly PR3-ANCA positivity (78,5%), while in those with MPA an MPO-ANCA preponderance (61,4%) was seen. (115) On the contrary, another study investigating MPO-positive GPA patients in China, found that 60,7% of GPA patients were positive for MPO-ANCA. (116) GPA patients mainly suffered from ENT involvement (84,3%) and MPA patients frequently showed kidney involvement. (115) Other authors showed that almost all patients with an early systemic or limited GPA can be tested positively for ANCA (117), whereas patients with locoregional GPA (a mild and regional form of ENT involvement) do not always show ANCA positivity. (118)

Importantly, relapse rates were higher in the GPA group (46,5%) than in the MPA group (19,5%), while the mortality rate was significantly higher in the MPA group (29,6%) compared to the GPA group (13,9%). (115)

The numbers are summarized in Table 3.

Comparison	**Granulomatosis with Polyangiitis**	**Microscopic Polyangiitis**
ANCA	Most often PR3-ANCA (78,5%) (different in China)	Both can be found, but MPO is more frequent (61,4%)
Mean age at diagnosis (yrs)	55,2	61,8
Male sex (%)	54,3	51,6
Involvement - Kidneys (%) - Lung (%) - ENT (%)	 76,5 66,7 84,3	 97,1 46,9 21,3
Events - Relapse (%) - Death (%)	 46,5 13,9	 19,5 29,6

Table 3: The comparison between GPA and MPA; sources: (115,116)

1.5.1.2 Clinical characteristics of EGPA

Patients with EGPA show several differences in clinical presentation as compared to those with GPA and MPA.

In early onset, EGPA patients may have clinically manifested asthma bronchiale long before eosinophilia can be detected. Even after an EGPA treatment asthma can persist and if so, is usually difficult to control.

In this context, renal involvement is less frequent than in GPA and MPA (26,7% vs 76,5% and 97,1%, respectively). (13,115) Eosinophilic myocarditis, causing cardiomyopathy is one of the diseases mainly caused by EGPA.

The majority of EGPA patients are ANCA negative, but in approximately 40% of the cases ANCA positivity exist (almost always MPO). In ANCA-positive EGPA patients renal and nerval involvement occurs more often. (114) Moreover, ANCA positivity seems to be associated with renal involvement and its severity. (119) On the other hand, cardiomyopathy, pulmonary infiltrates and localized EGPA are more common in ANCA-negative EGPA. (114,119)

1.5.1.3 Differences in clinical appearance and characteristics depending on ANCA specificity

There are doubts about the usefulness of the clinicopathologic classification of AAV suggested by the CHCC of 2012. In accordance, an ANCA-based subclassification has been proposed, which might provide the opportunity to better characterise AAV at individual and cohort level. (119)

Mohammad et al. conducted a study in southern Sweden with N=201 AAV patients, comparing the clinical data between PR3-and MPO-ANCA subgroups. Among these individuals, N=183 patients were tested positive for ANCA, while the mean age was 63,2 and 68,7 for the PR3-ANCA and MPO-ANCA groups, respectively. A male preponderance in the PR3-ANCA group and a female preponderance in the MPO-ANCA group (56,5%) (120) was observed and confirmed by an another study by Joode et al. (121) Nevertheless, other studies showed a male preponderance in both groups. (122).

According to studies by Mohammad et al. and Deshayes et al., both ENT and kidney involvement appeared more often in the PR3-ANCA- and MPO-ANCA-group, than vice versa. In the study from Mohammad et al. higher mortality rates in MPO-ANCA patients were observed, while Deshayes et al. could not confirm these findings. (120,122)

Deshayes et al. observed insignificantly more relapses in PR3-, than in MPO- ANCA positive individuals (122). Similarly, another study showed an almost two-fold higher relapse rate in PR3-ANCA, as compared to MPO-ANCA patients.
The comparison of the differences in organ involvement according to the discussed studies is summarized in Table 4.

Comparison	**PR3-ANCA**	**MPO-ANCA**
Mean age at diagnosis (yrs)	63,2 61*	68,7 63*
Male sex (%)	60,2 54*	43,5 57* 48#
Involvement		
- Kidneys (%)	68 66*	85 84*
- Lung (%)	72*	70*
- ENT (%)	59 66*	19 29*
Events		
- Relapse (%)	44*	30*
- Death (%)	37 24*	42 13*

*Table 4: The comparison between PR3- and MPO-ANCA; sources: without marks = (120); with *=(122); with #= (121)*

1.5.2 Antibodies / Serologic classification

The name AAV already indicates the influence of ANCAs causing and influencing this certain type of disease. (123) Today, ANCA is one of the most important serological tools to diagnose AAV. (124)
Already in the 1990s, ANCAs were found in patients but played no role in diagnosing small vessel vasculitides such as AAV. Over the years the major pathogenic role in the pathophysiology of AAV became evident and the detection of ANCA antibodies has been included into the diagnostic approach and classification criteria of patients with AAV. (124)

AAV patients are classified as “ANCA-negative” if they meet special criteria, as seronegativity for ANCA, rheumatoid arthritis and lupus, but still show all the necessary histopathologic criteria for AAV. (12)

1.5.2.1 ANCA testing techniques

1.5.2.1.1 *The traditional indirect immunofluorescence technique*

The traditional tool for detecting ANCAs is the indirect immunofluorescence (IIF) technique. For this technique, the patient’s serum must be incubated. During the procedure, fluorescein isothiocyanate conjugates with human anti-human IgG antibodies and is added to white blood cells or neutrophilic granulocytes which are fixed in alcohol.

When using a fluorescence microscope, the IIF method can distinguish between cytoplasmatic (c-ANCA) or perinuclear (p-ANCA) staining patterns.

C-ANCA is most often found in PR3-ANCA-patients and p-ANCA is associated with MPO-ANCA.

This type of ANCA testing is semiquantitative and labor-intensive and needs specialized and trained technicians. The technique can be executed automatically, but this automation process is not yet worldwide available. (119)

1.5.2.1.2 *Antigen-specific tests for MPO-ANCA and PR3-ANCA*

The second method for distinguishing between ANCA subtypes is by using antigen-specific tests for MPO- and Pr3-ANCA.

Therefore, an enzyme-linked immunosorbent assay (ELISA) is used which has been modified several times so that in the present form there are 3 existing generations of ELISA, each with different sensitivities and specificities for the different ANCA-subtypes.

The first generation of antigen-specific tests is used for detecting antigen-specific antibodies in many autoimmune diseases and is a direct non-competitive ELISA called direct ELISA. This special type of ELISAs has a solid antigen which is bound to microtiter plates. In order to stop the binding of unspecific antibodies the free protein-binding sites on the carrier get blocked. It is considered a true quantitative assay for ANCA-detection.

After the blockage of the protein-binding sites additional steps have to be implemented. They are the same in all three ELISA generations.

In the next two steps the test serum is added so possible ANCAs can bind to the antigens, presented in the different ELISA generations. (119) A second antibody, connected to protein is added to the ELISA and connects to the required antibody, in this case: ANCA. ELISA uses a protein-driven reaction. The protein linked to ANCA catalyzes a reaction that results in a change of color and can be measured by photometry. The degree of color change represents the amount of ANCA within the test serum. (87)

The second generation of ELISAs is called capture ELISA. In capture ELISAs, monoclonal antibodies specific for MPO or PR3 are placed on the microtiter plates and an antigen can be added which binds to the antibodies. From here the two already mentioned steps can be executed.

The capture ELISA has increased sensitivity and specificity for detecting PR3-ANCA compared to direct-ELISA. (125) For MPO-ANCA-detection the specificity was higher in capture ELISA, but had the same sensitivity compared to direct ELISA. (126) “Importantly, high PR3-ANCA levels as measured by a capture ELISA but not high PR3-ANCA levels as measured by a direct ELISA are associated with decreased patient survival in patients with AAV with renal involvement” (119)

The third generation of ANCA-ELISA is called anchor ELISA. In this technique, the ELISA carrier is coated with peptide linkers. Antigens are bound to these peptide linkers and from there the two already mentioned steps can be executed. Third-generation ELISAs show an increased sensitivity in detecting PR3-ANCA in comparison to previous generations of ELISAs. (127,128)

1.5.3 Histopathologic classification

A kidney biopsy helps to ensure the diagnosis of AAV.

1.5.3.1 Pauci-immune GN

The AAV histopathology mainly follows the above mentioned histopathology of RPGN detailed in chapter 1.1.2.2. Nevertheless, some differences are discussed below.

The characteristic kidney lesion of AAV is called “pauci-immune” focal necrotizing or /and crescentic GN. It is characterized by the paucity of immune complexes in segmental necrosis of glomeruli, hence “pauci-immune”. (114) The extent of the

inflammation can range from small and segmental lesions to extensive and large with circumferential crescents. (32) (Figure 5 and Figure 6)

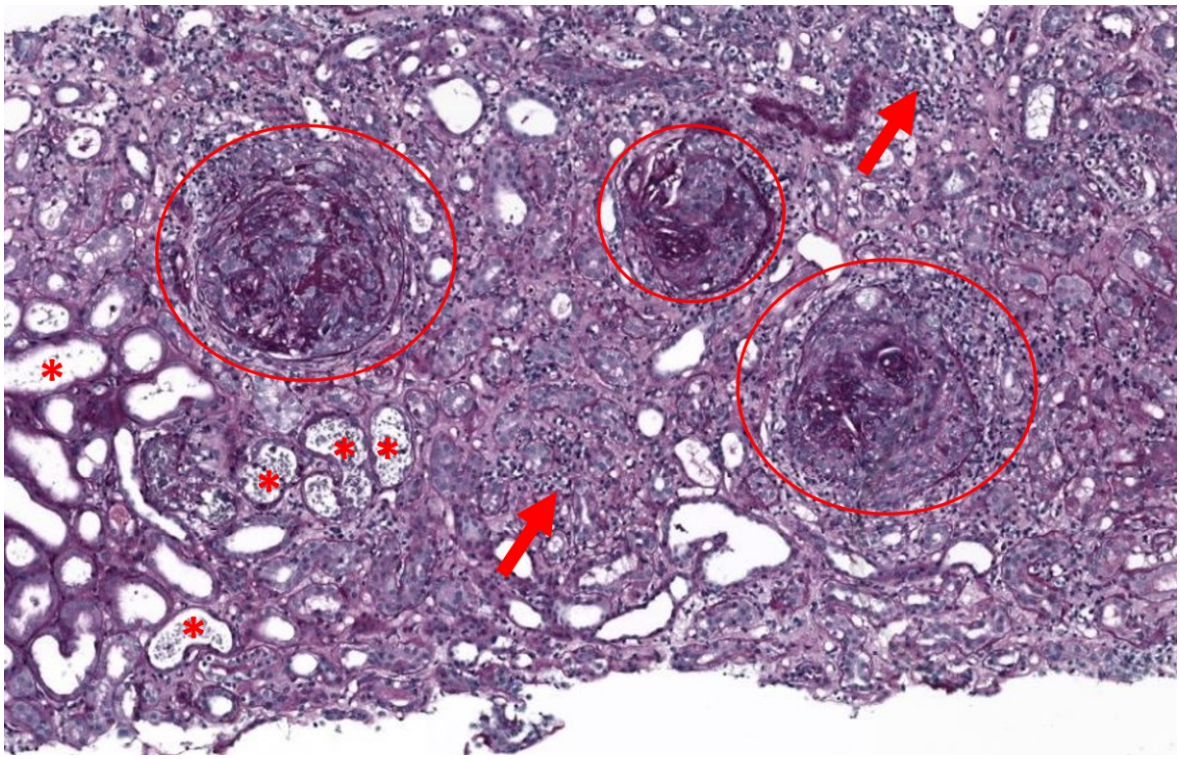


Figure 5: Overview of a PAS-stained kidney biopsy showing large cellular crescent occupying every glomerulum (as highlighted by the circles). Note the concomitant tubulo-interstitial nephritis (arrows) and signs of severe acute tubular injury with red blood cells within the tubular lumina (asteriks). 5x

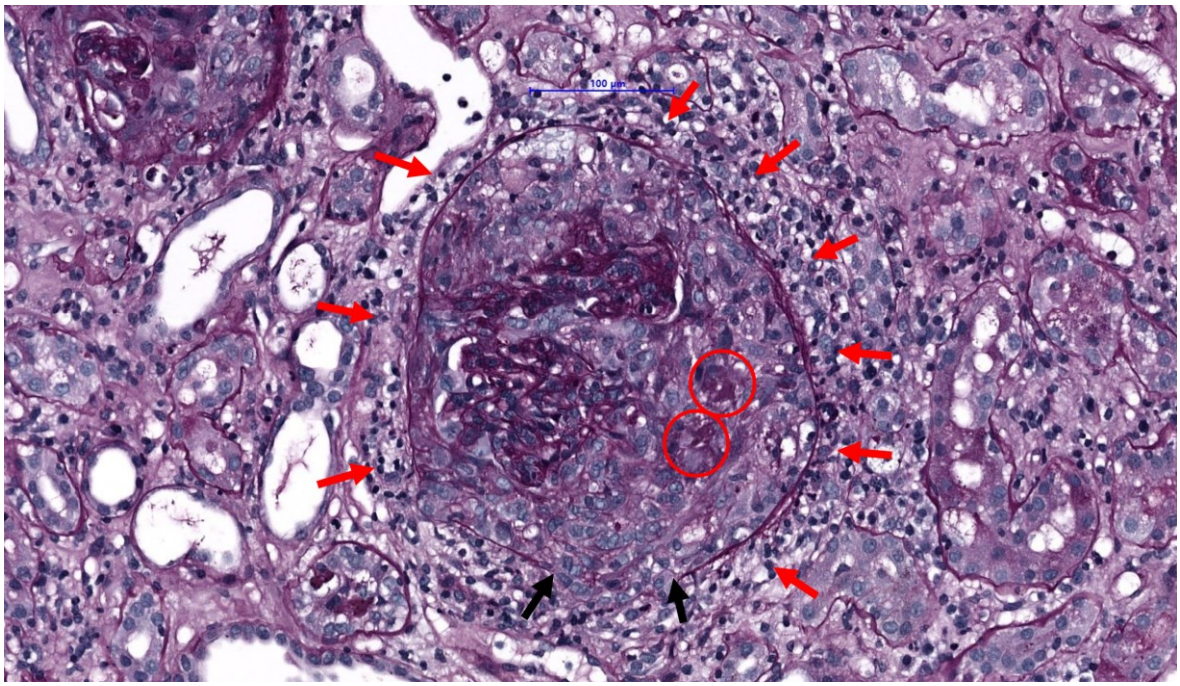


Figure 6: PAS-stained kidney biopsy showing a large cellular cresecent, focal fibrinoid necrosis (red circles) and disruption of the Bowman capsule (black arrow). Periglomerular interstitial lymphocytic infiltrate (red arrows). 20x

Due to the inflammatory process the glomeruli undergo sclerosis, hence glomerulosclerosis. It presents transmural scarring in which the elastic internal lamina is destroyed. Glomerulosclerosis can occur as segmental or global and its severity and extent reflects the evolving injury during time.

Glomerular damage is often coexistent with inflammation of small arteries and interstitial infiltrates around the necrotic glomeruli or blood vessels. The inflammation presents interstitial infiltrates and rarely multinucleated giants cells. (114)

1.5.3.2 AAV lesions in the respiratory tract

Granulomatosis in the upper and lower respiratory tract is predominantly associated with GPA. The center of the granulomatous inflammation very often holds necrotic tissue and granulocytic fragments. It is surrounded by “a palisade of epithelioid cells and in EGPA, by large numbers of eosinophils”. (114)

In contrary to renal granulomatous inflammation, there is an almost consistent presence of multinucleated giant cells in samples of the respiratory tract with granulomatosis. Multinucleated giant cells in respiratory tract biopsy samples, nasal swabs, and bronchoalveolar lavage of individuals suspect for AAV are strong indicators for GPA and EGPA.

Besides granulomatosis other histopathologic patterns can also occur. In case of lung involvement, neutrophilic capillaritis in the lungs is seen in all types of AAV. Alveolar hemorrhage with micro-abscesses, extravasation of erythrocytes, areas of necrosis and fibrin, can also be seen and is a life-threatening complication in patients with AAV.

Saddle nose deformity in GPA is caused by granulomatous inflammation, affecting soft tissue and cartilage and eventually destroying it. Also large ulcers and skinned epithelium can indicate a presentation of GPA.

The granulomatous inflammation and nasal involvement are not only a feature of GPA but also of EGPA. (114)

1.5.4 Birmingham Vasculitis Activity Score (BVAS)

The BVAS is an important score for the evaluation of vasculitis activity. It contains 8 categories referring to the affected tissue (cutaneous, mucous membranes/eyes, ENT, chest, cardiovascular, abdominal, nervous system and renal), one category referring to general symptoms (for example, myalgia, fever, or weight loss) and one

open category. The latter is mainly used as a tool for clinical research and measures disease activity, remission, relapse/flare and therapy response using a point system. A patient in remission scores zero points and, while ≥ 1 point represents an active disease. (114,129)

1.5.5 Classification systems

In 2009 during the Oxford classification of IgA nephropathy in 2009, the MEST-Score was introduced. It is a well-established tool for evaluating the clinical outcome of affected IgA patients. (130) Unfortunately there is no such well-developed and approved tool for predicting the clinical outcome in patients with AAV. This seems to be a widely recognized dilemma and several new classification systems have been proposed in order to better predict clinical outcome in AAV. (131)

Lionaki et al. compared three classification systems: the 2012 CHCC definitions (involving GPA, MPA and EGPA), the European Medicines Agency (EMA), which describes categories for GPA and MPA and the ANCA-based classification system. They concluded, that the ANCA-based classification system shows an association with the disease outcome and therapy response (i.e. in those with PR3-AAV), while the 2012 CHCC and EMA systems were not able to predict relapses in patients with AAV. (123)

Deshayes et al. made a different approach and retrospectively categorized AAV patients in a classification system, based on clinicopathologic classification (GPA,MPA, EGPA) and AAV-serotype. Here, serological AAV subclassification into PR3- and MPO-ANCA (analyzing the subgroups: anti-PR3 GPA, anti-MPO GPA, anti-PR3 MPA and anti-MPO MPA) were not associated with renal survival and relapse free rates.

Thus, the authors conclude that the conjunction of ANCA-seropositivity and the clinicopathologic subclassification (GPA, MPA and EGPA) plays a smaller role in the prediction of relapse and renal survival rates than assumed. (122)

In a comment from Mahr et al. the trial by Deshayes et al. was critizised mainly due to the relatively small sample size. In their conclusion, the authors point out the still existing dilemma of a missing “perfect” tool, which is capable of capturing the entity AAV in all its aspects and represents the outcome with a reliable prognostic value. In accordance, they propose yet another different approach for classifying AAV. With the new approach, the authors try to represent all the different aspects of AAV,

using a separation into non-severe AAV, with low risk of renal/life mortality and high risk for relapse, severe PR3-AAV, with intermediate risk of renal/life mortality and intermediate risk for relapse and severe MPO-AAV, with a high risk of renal/life mortality and low risk for relapse. This system contains clinical, histopathological and serological aspects, predicts the risk of renal relapse (RR) or life-threatening disease and is therefore relevant for therapy. (132) (see Table 5.)

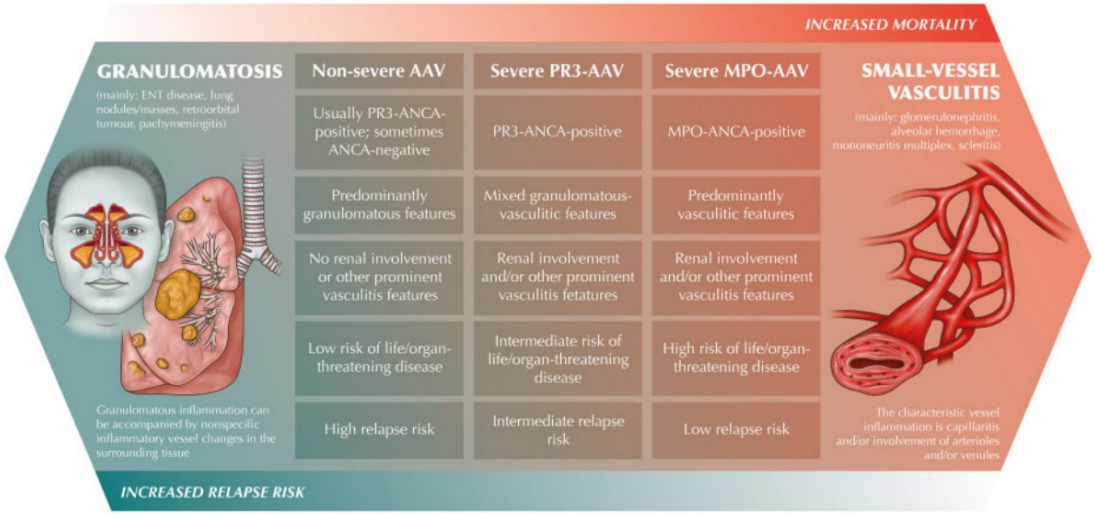


Table 5: Schematic subcategorization of AAV in three clinically relevant disease categories defined by clinical features and ANCA specificity. (132) with the permission of Alfred Mahr

1.6 Prognosis

1.6.1 Mortality and RR

Due to newly introduced therapies and specific ANCA testing, the 5-year survival rate of AAV has experienced a steady increase. Rhee et al. showed improvements in end-stage renal disease (ESRD)-free survival and decreasing death rates

According to a study from Steinberg et al. included all AAV cases in the entire USA from 1999 until 2017 in their study and found an age-adjusted mortality rate of 1.86 per one million persons. The AAV mortality decreased by almost 2% per year. (133) In addition, other studies also confirmed the decreasing trend of mortality in AAV-patients. (134) Nevertheless, relapse rates continued on a similar level between 1985 until 2009. (135) An occurrence of relapse is currently estimated to happen in around 50% of the cases during the first five years, after diagnosis. (114)

1.6.2 Histopathologic findings and disease outcome

In 2010 Berden et al. established a histopathological classification system, that could predict renal survival and mortality. They defined four histopathological findings, namely focal, crescentic, mixed and sclerotic, as categories for their classification system. Each of the categories had to be present in 50% or more of the Glomeruli to be counted as such. The best outcome was seen in the focal category, while the worst outcome on renal survival and mortality was described in the sclerotic category. (136)

In 2012 the same authors observed a significant correlation between TA, $CD3^+$ cell-mediated tubulitis and renal function (measured by eGFR) at month 12 of follow-up. At month 24 only tubular atrophy (TA) showed a predictive value for renal function. (104)

Huang et al. performed a validation study to the classification system of Berden et al. and found no prognostic difference between the mixed and the crescentic classification category. Thus, they proposed a different approach, by using the proportion of normal glomeruli or TA. (137)

Recently Bitton et al. published another approach investigating total interstitial inflammation (IT) and IF/TA among others concerning their predictive value. IT and IF/TA showed strong predictive abilities for ESRD and IT also for relapse . (131)

Several studies investigated the impact of different cell lines on outcome in AAV-patients.

In 1999 Bajema et al. showed the predictive value of diffuse interstitial infiltrates. It correlated with mean creatinine values at baseline (BL) and also during follow-up. (138)

Geetha et al. In their publication of 2017 they can not find any correlation between lymphocytic infiltrates (B- and T-cells) and BL renal function or long-term prognosis. (139)

A publication by Brix et al. from 2018 found a correlation between different lymphocytic classes and renal survival. The classes were formed using CD3 and CD20 numbers and their distribution in the pathohistological sample (no B-cells, scattered pattern, cell clusters of $CD3^+$ and $CD20^+$ and nodular aggregates with surrounding T-cells and B-cells in the middle). Interestingly they found no association between the degree of lymphocytic organisation and severity of IF/TA or the percentage of glomerulosclerosis. (140)

The already mentioned publication by Berden et al. from 2012 showed, that T-cell mediated tubulitis in AAV-patients treated with RTX is a negative predictor for renal outcome. Individuals with B-cell intraglomerular infiltration showed significant reduction in eGFR during follow-up. (104)

Bitton et al. observed a correlation between higher interstitial $CD20^+$-, $CD68^+$- and $CD136^+$-cell numbers and a higher risk for ESRD. (131)

Interestingly one publication found a correlation between the absence of interstitial infiltrates and an increased risk of relapse. (141)

In a risk score model, the percentage of IF/TA showed a predictive value, together with the percentage of normal glomeruli and BL eGFR. (142)

1.6.3 Clinical predictors of relapses

There are several factors, which can influence relapses and survival, such as infections, the occurrence of relapse during the first year and grade of renal at initiation (143). Also, the presence of renal haematuria is associated with RR. (144)

In patients in remission, the measurement of urinary soluble CD163 and urinary monocyte chemoattractant protein-1 seems to be a useful marker to identify subtle renal AAV flares. (145)

There is evidence of chronic nasal infection with Staphylococcus Aureus (SA) causing higher relapse rates in GPA patients, than in patients without SA carriage. (146) (147)

As already mentioned, the clinicopathologic and serological phenotype of AAV can be helpful to predict relapse risk. (148) Several other clinical factors might influence the disease outcome, such as “older age, severity of renal dysfunction, the presence of pulmonary haemorrhage and disease activity measured by BVAS.” (114)

1.7 Therapy and Management of AAV

Patients who are diagnosed with AAV especially those with RPGN need to be treated immediately using different immunosuppressive agents. (1)

The treatment of AAV can be divided into two phases, namely the remission induction and maintenance therapies. (114) In case of multi-organ involvement, such as in those with pulmonary-renal syndrome, additional therapy concepts, such as PLEX can be applied. (32)

1.7.1 Remission induction therapy in GPA and MPA

Remission induction therapy can be administered in a newly diagnosed or in a relapsed patient. The aim of this therapy is to reduce the acute and active inflammation caused by AAV.

As soon as there is suspicion for an ongoing AAV, induction therapy should be administered, without waiting for the result of the kidney biopsy, since delayed induction therapy is associated with worse outcomes. (114)

1.7.1.1 Glucocorticoids

In the 2016 EULAR/ERA-EDTA recommendations, the use of glucocorticoids in AAV remission induction therapy is recommended. (149) Glucocorticoids show a rapid effect on AAV (114) and are administered together with other immunosuppressive medication. (149) Initially, the application of 1mg/kg per day of prednisone followed by the tapering protocol from the PEXIVAS study is recommended. (114)

The PEXIVAS study by Walsh et al. could show similar outcomes for mortality and kidney survival with a reduced-dose regimen of glucocorticoids, compared to a standard-dose regimen. (150) This is of a great importance, since reducing glucocorticoids resembles the best possible way of reducing severe adverse events during remission induction therapy, such as severe infectious complications. As there are no established rules and guidelines for corticoid use in non-severe AAV cases, initial glucocorticoid doses may be reduced. (114)

In the recently published RITAZAREM study by Smith et al., the investigators observed the equivalent effect of different starting doses of glucocorticoids (1mg/kg/day vs 0,5mg/kg/day), administered together with rituximab (RTX) in relapsed AAV patients. (151)

1.7.1.2 Cyclophosphamide vs. RTX

In patients with severe renal involvement, it is common to use either Cyclophosphamide (CYC) or RTX together with GCs as induction therapy. Both therapy regimens are still standard for the treatment of AAV, but there is increasing evidence for better efficacy of RTX in induction therapy. (152) Unfortunately, RTX is worldwide not as available as CYC, as RTX is more expensive than CYC. For remission induction therapy with CYC two possible regimens exist. There is a pulsed intermittent application of CYC and a continuous oral dose every day. (114)

In 2009 de Groot et al. could show equal effectiveness for the pulsed application of CYC and the daily application of CYC. (153) In the pulsed IV regimen they employed 3 doses of 15mg/kg CYC every two weeks and continued with an application every three weeks for three to six months. In the daily oral regimen 2mg/kg per day were administered. (154) The big advantage of the pulsed regimen is the decreased cumulative dose of CYC and thereby the decreased rate of adverse advents, such as leukopenia. (154) However, according to an other study by Harper et al., the pulsed regimen of CYC had a higher risk for relapse than the daily oral regimen of CYC. (155)

The use of RTX in AAV was extensively investigated. In the RAVE study in 2010 by Stone et al. no inferiority for RTX remission induction therapy in comparison to CYC was showed. (156) The same outcome was seen by Specks et al. in 2013. (157) The RITUXVAS trial showed no superiority of RTX over CYC in sustaining remission and rates of adverse advents. (156) Importantly, Unizony et al. conducted a post hoc analysis of the RAVE trial, which showed the superiority of RTX, in comparison to CYC in PR3-AAV patients. (152)

Regimes using 375mg/m^2 RTX once a week for 4 weeks, (152,153,156,157) or two doses of 1000mg of RTX in a 2-week interval can be used. (114)

1.7.1.3 Remission induction therapy in non-severe courses of AAV

In non-severe AAV disease courses, mycophenolate mofetil (MMF) or methotrexate (MTX) can be considered. (114)

Notably, the MYCYC trial by Jones et al. showed no inferiority of MMF in terms of remission induction as compared to CYC. In this analysis, relapse rates were increased in the MMF group, compared to the CYC control group especially in PR3-ANCA patients. (158)

MTX is capable of replacing CYC in certain cases as remission induction therapy but has several limitations. The use of MTX for remission induction is recommended for patients with a non-severe disease course and without risk of organ damage, since it showed less effectiveness for remission induction in patients with extensive disease and lung involvement. It also should be avoided in patients with reduced kidney function (i.e. eGFR $<$ 30 ml/min/1.73m^2) and/or if active urine sediment is present. Furthermore, the MTX remission-induced arm showed a higher rate of relapses than the patients from the CYC control group. (159)

1.7.1.4 New therapeutic agents for remission induction

The discovery of the role of the alternative complement pathway in AAV pathophysiology has led to new therapeutic opportunities. Avacopan (CCX168) inhibits the C5a receptor and ameliorates the effects of C5a. In the CLEAR trial, Jayne et al. showed that avacopan could effectively replace high-dose glucocorticoid treatments during induction therapy with RTX or CYC in AAV patients. (93)

The ADVOCATE trial showed no inferiority and no superiority in terms of maintaining remission for avacopan over prednisone at week 26 of follow-up, but avacopan showed a higher remission rate during week 52 of follow-up. (160)

1.7.2 Maintenance therapy

Maintenance therapy has the goal to prevent relapses and maintain remission. On the other hand, it is important to minimize adverse events that can be caused by the long-term use immunosuppressive medication.

In 2014, the MAINRITSAN study was released, which showed the superiority of RTX in maintaining remission, in comparison to an azathioprine (AZA) regimen. The study compared the two different maintenance therapies in newly diagnosed and relapsed AAV patients, concerning relapse rates during a follow-up of 28 months. (161)

The MAINRITSAN 2 trial compared “individually tailored and fixed-schedule RTX regimens” and showed no significant differences between the two regimens in maintaining remission. In the individually tailored arm, the indication for RTX applications was decided on trimestral monitoring of biological parameters, such as ANCA titres and $CD19^{+}$ B-lymphocyte levels. The patients, who remained in remission and received this regimen needed lower RTX cumulative doses. (162) Importantly, prolonged maintenance therapy with RTX over 18 months lead to reduced relapse rates. (163)

The dosage of RTX in maintenance therapy may differ according to the used protocol from 500 to 1000mg every six months. (153,161,164)

MMF, MTX and AZA regimens can be used in a combination with or without GCs for maintenance therapy, predominantly after a CYC remission induction therapy. The optimal duration of maintenance therapy with those agents, however, is still not known. (114) A randomized-control trial compared the durations of AZA remission

therapy (2 vs. 4 years) and found comparable relapse rates in both groups. (165) AZA maintenance dosage depends on testing thiopurine methyltransferase before the start of therapy. If tested and within normal quantities, 2mg/kg per day can be administered, but the maximum dose should never exceed 200mg/day. Without thiopurine methyltransferase testing doses of 50mg/day can be initiated, gradually increasing up to 2mg/kg/day, but constant observation of therapy toleration is needed. (153)

The dosage of MTX in maintenance therapy begins at 15mg/week and can be increased every two to eight weeks by 5mg/week but should not exceed 25mg/week. (153,166,167)

The dosage of MMF in maintenance therapy is initiated with 2000mg daily and is reduced to 1500mg after 12 months and 1000mg per day after 18 months. (168) The maximal tolerated dose for patients with chronic renal failure is 2 grams daily. (169) MMF presented inferior, showing more RR, than AZA. (170)

1.7.3 Plasma exchange (PLEX)

There is evidence for the advantage of removing ANCA autoantibodies for controlling the acute and active inflammation caused by AAV. Jayne et al. showed in the MEPEX study that a PLEX could lead to a decreased risk of ESRD. (171) In the recent randomized controlled PEXIVAS trial by Walsh et al. was shown, that the application of PLEX did not reduce mortality or the rate of ESRD in patients with severe AAV. (150)

The review from Specks et al. underline the point of view of the PEXIVAS trial (172) arguing on the missing evidence for implementing PLEX in AAV-patients, even in AAV-patients with severe renal involvement. The authors oppose the view of Kronbichler et al., who criticize certain points of the PEXIVAS trial design (f.e. insufficient number of applied PLEX, no ANCA-level driven approach, limited validity in defining severity of alveolar haemorrhage based on oxygen saturation, numerous patients with higher fatality from smaller centers with less expertise). Kronbichler et al. especially emphasize the importance of PLEX in alveolar haemorrhage, which has been proven by several other smaller trials. (173)

1.7.4 EGPA treatment

The treatment of EGPA is similar to treatment of GPA and MPA. The extent of the treatment is individually decided, according to the disease severity of the affected

patient. Using the Five-Factor Score, the disease severity can be measured. A severe disease course is defined by “the presence of substantial renal involvement (severe proteinuria or impaired kidney function), cardiomyopathy, gastrointestinal involvement or central nervous system involvement” needing an intensive treatment with known agents used in the treatment of GPA and MPA. (114) Puéchal et al observed induction therapy with AZA and GCs in patients with non-severe AAV. This induction therapy did not result in decreased GC dose, nor did it decrease the incidence of asthma/rhinosinusitis exacerbations in the observed EGPA patients. (174)

Mepolizumab, an anti-IL-5 monoclonal antibody, is a new approach in the treatment of EGPA, which has the capability to decrease the count of eosinophils, to reduce the accumulative GC dose and the rate of RR in most patients. (175) RTX can decrease the cumulative dose of prednisolone, however asthma exacerbations and ENT RR remained high during this treatment. (176)

According to the British Society for Rheumatology, EGPA patients should be treated with RTX in a similar approach like patients with GPA and MPA. (177)

1.7.5 Monitoring AAV treatment

During AAV treatment the patients should be monitored continuously in an ambulatory setting. A new flare, adverse events and new onset of comorbid conditions should be recognized as soon as possible.

Assessing kidney function and disease activity, should be performed every time the patients attend an ambulatory check-up.

Importantly, low levels of IgG can occur after an RTX treatment, which can lead to therapy discontinuation or regimen change.

If other systems are involved (cardiac involvement, trachea-bronchial disease etc.), special check-ups should be considered. (114)

1.7.6 Side Effects of AAV treatment

Side effects are a crucial part of handling the treatment of AAV and “the greatest threat to patients with AAV in the first year of therapy is from adverse events rather than active vasculitis”. (178)

1.7.6.1 Side effects of Glucocorticoids

GCs have an extensive variety of adverse events, such as “infection, bone disease, dysglycemia, obesity, hypertension, psychosis, gastrointestinal bleeding, cataracts, and long-term risks of cardiovascular disease.” (179)

According to Geetha et al. recorded adverse events of GCs are “weight gain > 10 kg (29%), new-onset diabetes (8.2%), peptic ulcer disease (2.6%), fractures (2.5%), and avascular necrosis (0.4%); and during long-term follow-up, cataracts (25%), diabetes (38%), osteoporosis (38%), and hypertension (41%).” (32)

1.7.6.2 Side effects of CYC

There are several known adverse events of CYC. Among others, it can lead to leukopenia and correlating infections. (179)

Thus, a check-up with a complete count of blood cells every two weeks should be followed and the dose of CYC should be modified if the leukocyte count falls under $3,5 \times 10^9$ / L. (32)

During the metabolism process of CYC, acrolein is produced, which is a bladder toxic metabolite causing haemorrhagic cystitis. (179) Therefore, patients treated with intravenous CYC should be provided with mesna, which binds acrolein and, thereby, protects the bladder. (32)

CYC treatment has been shown to be associated with malignancies due to DNA damage during treatment. (179) If the cumulative dose of CYC exceeds 36 g, the incidence of malignant adverse events, such as “skin cancer, myeloid malignancies, and bladder cancer” is increased. (32)

Patients with CYC treatment are at risk of decreased fertility. (179) The dose of CYC is associated with primary ovarian failure among women and with permanent azoospermia among men. Preventive measures are an administration of gonadotropin-releasing hormone for ovarian suppression among women, but the effectiveness is discussed. For men, semen cryopreservation is recommended, especially for male patients whose CYC treatment exceeds a cumulative dose over 10g. (32)

1.7.6.3 Side effects of RTX

RTX is a monoclonal antibody, that depletes B-cells. This effect can be observed for 6-12 months after administration and the drug itself is traceable in the circulation for 3-6 months.

Infections during RTX therapy occur, which were observed with 7% and 18% in the RAVE and RITUXVAS trials, respectively. (179)

In addition, flares and reactivation of hepatitis B and C was reported in patients, who were treated with RTX. (32)

A very rare adverse advent of RTX is progressive multifocal leukoencephalopathy. It is an often fatal, neurologic disease, which is triggered by a reactivated John Cunningham polyomavirus. Most patients which were reported with multifocal leukoencephalopathy, were also treated with other medication and chemotherapy.

Hypogammaglobulinemia can be a late complication of RTX treatment, (179) occurring in approximately 50% of AAV-patients. In severe cases of recurring infections (~5%), intravenous immunoglobulin has to be administered, to prevent recurring infections. (32)

Late-onset neutropenia is another late complication of RTX therapy which mostly occurs 2-6 months after the last administration of RTX. In most cases, the disease course is asymptomatic and patients recover quickly. However, in affected patients with an additional infection, the administration of granulocyte colony-stimulating factor can be implemented. (179)

1.7.7 RRT in AAV patients

Unfortunately, there is still a percentage of 20% to 25% of AAV patients, who end up with ESRD and need for RRT.

Patients on RRT show decreased relapse. In patients without extrarenal manifestation, especially MPO-ANCA serotypes, withdrawing the immunosuppressive medication is being discussed. (32)

1.7.8 Kidney transplantation in AAV patients

In AAV patients with kidney failure, “kidney transplantation is the treatment of choice”. Relapse rates remain very low, with the use of modern immunosuppressive medication, such as MMF and tacrolimus. (180)

2 Material and Methods

2.1 Patients' Characteristics and study design

This is a retrospective single-center cohort study. A total of 94 AAV patients, who were treated at the Division of Nephrology of the Medical University of Graz from 1 January 2004 to 1 November 2018 were evaluated.

Patients were involved, if they met the inclusion criteria as followed:

- newly diagnosed AAV with biopsy proven RPGN (according to 2012 CHCC criteria) (12)
- age ≥ 18 years at time of renal biopsy
- follow-up time at least 24 months
- time of diagnosis: 1. January 2004 – 1. November 2018

Patients were excluded, if the clinical follow-up was performed in an external hospital. Figure 7 shows the flow-chart of the evaluation process.

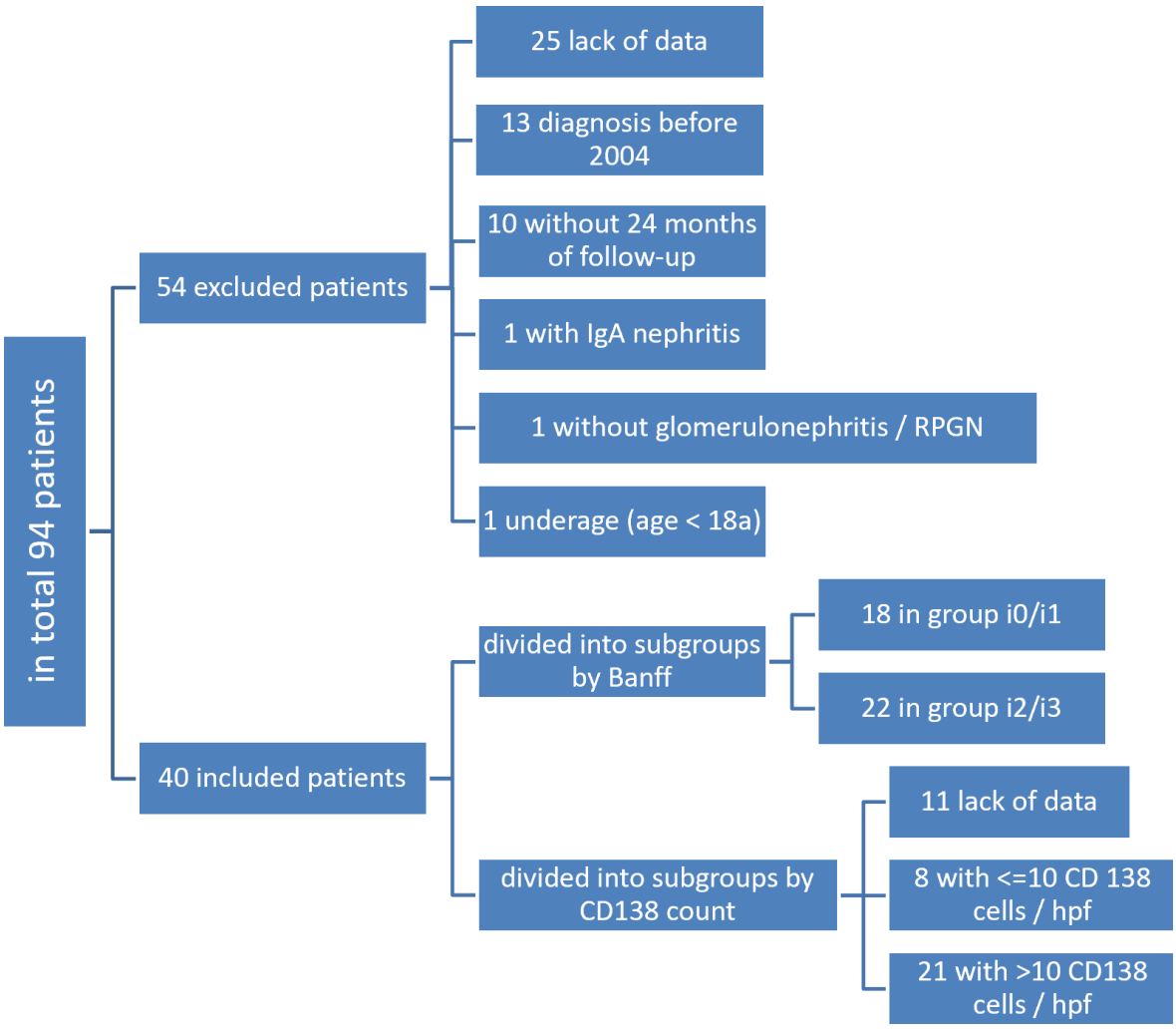


Figure 7: patient selection

Laboratory and clinical data, including serum creatinine, eGFR (defined by Epidemiology Collaboration formula), urea, serum albumin, ANCA titres, spot urine protein excretion (proteinuria, albuminuria, ACR), comorbidities (diabetes type I and II, arterial hypertension), organ manifestations (cardiovascular, nervous system, skin, ENT, lung,) were collected during predefined time points of data survey (on the day of the biopsy and at months 1, 3, 6, 12, 18, 24, 30 and 36 after the kidney biopsy). ANCA positivity was further classified as anti-PR3 and anti-MPO or both. In addition, occurrence of RRs, data on therapy regimen (including CYC, RTX, AZA, MTX, MMF) and dosage for induction and maintenance therapy, including accompanying therapy (GCs and inhibition of renin-angiotensin-aldosterone-system, PLEX) was recorded. The need of RRT at first presentation or during follow-up was also documented. The patient data were compiled retrospectively from the electronic data records of our center (MEDOCS system).
Patients were treated according to international (i.e. based on the RAVE, CYCLOPS and IMPROVE studies) and local guidelines. (156,168,170)

2.2 Pathological evaluations

For the comparison of the possible predictive value of tubulointerstitial lesions different approaches have been used. First, two groups were defined, using the Banff lesion score. Patients with a Banff lesion score i0 or i1 and those with a Banff lesion score i2 or i3 were compared.
Second, the patients were divided into two groups, according to plasma cell-rich CD138 findings in their kidney biopsies.

For the light microscopic examination, the kidney biopsy specimens were put in formalin and prepared according to the diagnostic work-up by Roufosse et al., cutting the paraffin block in “in several numbered level sections examined with hematoxylin-eosin, periodic acid-Schiff (PAS), trichrome-elastic and Jones or methenamine silver stains.” (181) After preparation, the samples were reviewed by a single pathologist, according to Banff lesion score. For defining cohort groups we used the Banff lesion score i (interstitial inflammation), for which only non-fibrotic and non-scarred areas of cortex must be used. (181) Areas not suitable for Banff lesion score i, are “fibrotic areas, the immediate subcapsular cortex, and the

adventitia around large veins and lymphatics". (182) The Banff score subclassifies according to the extent of inflammation in unscarred cortical parenchyma and is defined as i0 if less than 10%, i1 if 10 to 25%, i2 if 26-50% and i3 if more than 50% of unscarred cortical parenchyma is filled with interstitial infiltrates. (183)

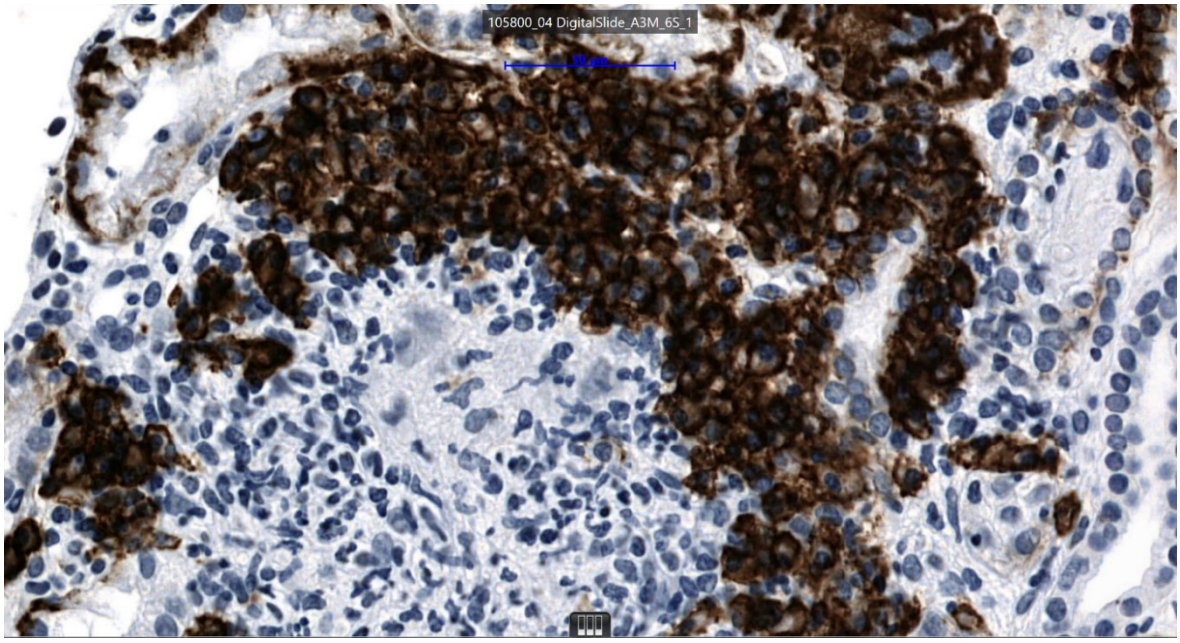


Figure 8: Example for a plasma-cell-rich sample; the renal tubular cells are unspecifically stained, they can not be added to the actual CD138⁺ cell count

The immunohistochemical stainings were performed for T-cells ($CD3^+$), B-cells ($CD20^+$) and plasma cells ($CD138^+$). Cells were stained with a CD3 monoclonal antibody (mAb) (Flex, Polyclonal Rabbit Anti-Human CD3, GA503), CD20 mAb (Flex, Monoclonal Mouse Anti-Human CD20cy, Clone L26, Code GA604) and a CD138 mAb (Flex, Monoclonal Mouse Anti-Human CD138, Clone MI15, Code GA642) by Dako Omnis according to standard Dako staining procedure, using the Dako Omnis instruments. There is unspecific CD138 staining in renal tubular epithelial cells, which can be seen in Figure 8. Those cells were not included in the positive CD138-cell count.

$CD3^+$-, $CD20^+$- and $CD138^+$-cells were counted, using an electronic light microscopy program (CaseCenter by 3DHISTECH). We counted positive cells per high power field (hpf) in an x40 magnification. As proposed by Cornell et al. plasma cell findings were defined as plasma cell-rich if more than 10 plasma cells/hpf can be found, using light microscopy (184), while 10 or fewer plasma cells/hpf were defined as "non-plasma cell-rich".

Outcome definitions

RR was defined and modified according to the study of Göçeroğlu et al. (141), as followed:

- Increase of creatinine of 30% in comparison to BL creatinine.
- Decrease of eGFR of 25% in comparison to BL eGFR.
- New onset of renal haematuria and / or proteinuria.
- New remission induction therapy.

2.3 Ethical Statement

The study protocol was approved by the institutional ethics committee of the Medical University of Graz, Austria (EK-numbers: 31-401 ex 18/19 and 27-069 ex 14/15).

No informed consent was obtained from the participants, as it was a retrospective cohort study of real-life data collected during patients' regular visits. There were no additional visits or interventions due to the study.

The study was conducted under the principles of the Declaration of Helsinki.

2.4 Study Outcome

The primary endpoint was renal RR. Secondary endpoints were the need for RRT during follow-up and kidney transplantation.

2.5 Statistical Analysis

Quantitative and continuous parameters were summarized as median and interquartile range for age and as mean values for ACR and eGFR. Categorical variables were expressed in absolute and relative numbers and missing data were not imputed. For a comparison of disease course between the groups the differences in quantitative parameters (eGFR and ACR) were compared using Mann-Whitney-U-Tests. The statistical analyses for categorical variables, including end points (RR, the need for RRT and kidney transplantation), groups (defined by BANFF, age over and under 60 and CD138 expression), sex, ANCA specificity, organ manifestations and comorbidities were performed by using chi^2-tests and Fisher's exact test.

Univariate logistic regression models to test for associated predictors between non-metrical endpoints and $CD3^+$, $CD20^+$ and $CD138^+$ cells was used.

For the statistical comparison between the infiltration of different immune cells ($CD3^+$, $CD20^+$ and $CD138^+$) and quantitatively scaled variables the Spearman correlation was used.

Analyses were conducted using SPSS Version 26 (IBM Corp., Armonk, NY, USA). Exact P-values are reported with a two-sided P-value of 0.05 or less considered statistically significant.

3 Results

3.1 Patients' characteristics at BL

Forty patients with AAV were included in the data analysis. Clinical data for groups i0/i1, i2/i3 and CD138=<10 and CD138>10 are summarized in Table 6.

patients	total	i0/i1	i2/i3	p-value	CD138=<10	CD138>10	p-value
total	40	18	22		8	21	
age at diagnosis (years)	57 [46-65]	61.5 [49-65]	53 [46 - 62]		58 [52-71]	59 [47-65]	
Female sex	21 (52.5)	9 (50)	12 (54.5)	0.775	4 (50)	10 (47.6)	0.909
ANCA specificity and diagnosis							
PR3-ANCA	18 (45.0)	9 (50)	9 (40.9)	0.565	3 (37.5)	9 (42.9)	0.793
MPO-ANCA	20 (50)	8 (44.4)	12 (54.5)	0.565	5 (62.5)	12 (57.1)	0.793
Anti-GBM	2 (5)	2 (11.1)	0	0.196	0	2 (9.5)	1.000
GPA	21 (52.5)	10 (55.6)	11 (50)	0.726	4 (50)	11 (52.4)	1.000
MPA	16 (40)	6 (33.3)	10 (45.5)	0.436	4 (50)	8 (38.1)	0.683
EGPA	2 (5)	1 (5.6)	1 (4.5)	1.000	0	2 (9.5)	1.000
ANCA neg. pauci-immune GN	1 (2.5)	1 (5.6)	0	0.450	0	0	0
Involved systems							
Lung involvement	26 (65)	13(72,2)	13 (59.1)	0.386	6 (75)	12 (57.1)	0.671
Pulmonary-renal syndrome	10 (25)	4 (22.2)	6 (27.3)	1.000	1 (12.5)	5 (23.8)	0.647
ENT-involvement	19 (47.5)	6 (33.3)	13 (59.1)	0.105	2 (25)	11 (52.4)	0.238
Skin involvement	2 (5)	1 (5.6)	1 (4.5)	1.000	0	1 (4.8)	1.000
Involvement nerval system	9 (22.5)	5 (27.8)	4 (18.2)	0.705	1 (12.5)	7 (33.3)	0.381
Cardiovascular involvement	1 (2.5)	1 (5.6)	0	0.405	1 (12.5)	0	0.276
Comorbidities							
Arterial hypertension	30 (75)	13(72,2)	17 (77.3)	0.714	8 (100)	14 (66.7)	0.142
Diabetes Mellitus I	0	0	0	0	0	0	0
Diabetes Mellitus II	4 (10)	1 (5.6)	3 (13.6)	0.258	0	3 (14.3)	0.540
Need for RRT during diagnosis	13 (32.5)	5 (27.8)	8 (36.4)	0.564	2 (25)	7 (33.3)	1.000

Table 6: Clinical data of all included patients at BL.
Values are expressed as median, with interquartile range (IQR) [Q1 and Q3], or numbers (percentage).

Median age of all included AAV-patients was 57 years (19-81 years, mean 55.5, standard deviation (SD) ±13.28). Twenty-two patients were younger than 60 years and 18 patients were older than 60 years at the time of biopsy. Characteristics in patients older than 60 and younger than 60 years old are summarized in Table 7 and the age distribution of all included patients can be seen in Figure 9.

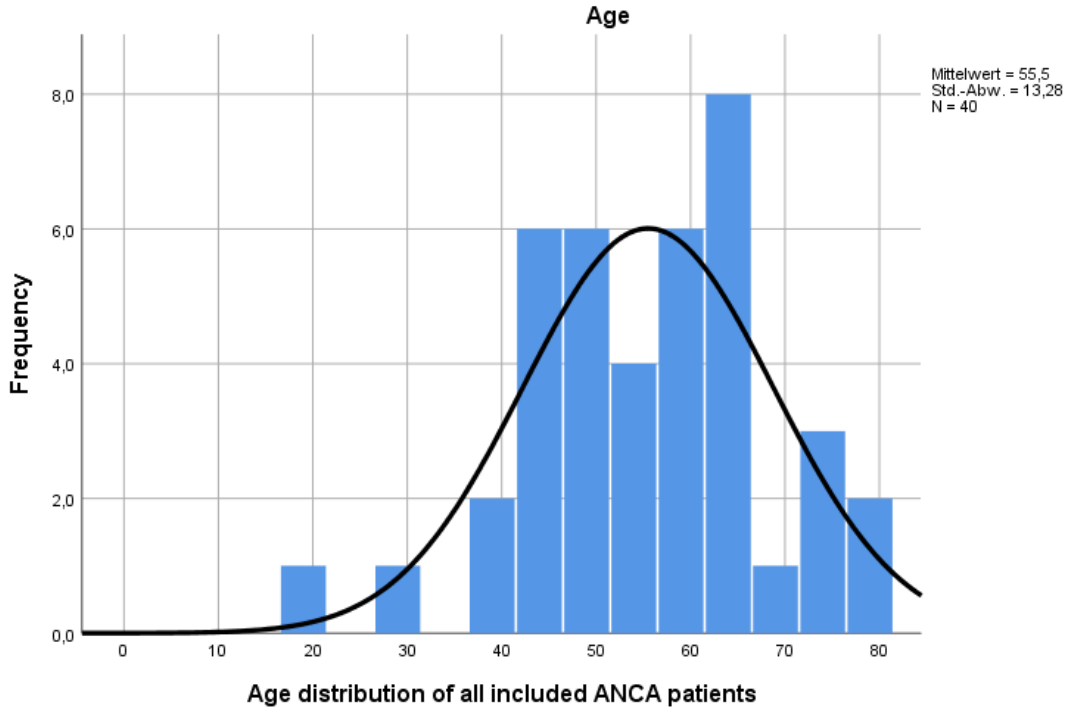


Figure 9: age distribution of all included AAV patients. Mean = 55.5, SD= ±13.28, n=40

Eighteen (45%) patients in total were PR3-ANCA positive, while 20 (50%) were MPO-ANCA positive. Two (5%) patients were positive for anti-GBM and MPO-ANCA, and therefore fulfilled the requirements for a double-positivity-disease. Twenty-one (52.5%) patients had GPA, 16 (40%) had MPA and 2 (5%) had EGPA. Only one (2.5%) patient had ANCA negative pauci-immune GN.

Among all included patients 25 (62.5%) patients showed lung involvement and 10 (25%) had a pulmonary-renal syndrome. Nineteen patients (47.5%) were affected by an ENT involvement, 2 (5%) had an involvement of the skin, 9 (22.5%) of the nerval system and one (2.5%) of the cardiovascular system.

The most common comorbidity was arterial hypertension with 30 (75%) affected patients during time of diagnosis, no patients had diabetes mellitus type I, while 4 (10%) suffered from diabetes mellitus type II. Thirteen (32.5%) patients needed RRT at the time of diagnosis.

patients	total	Under 60	Over 60	p-value
total	40	22 (55)	18 (45)	
Female sex	21 (52,5)	11 (50)	10 (55,6)	0.726
ANCA specificity and diagnosis				
PR3-ANCA	18 (45)	11 (50)	7 (38,9)	0.482
MPO-ANCA	20 (50)	10 (45.5)	10 (55.6)	0.525
Anti-GBM	2 (5)	0	2 (11,1)	0.196
GPA	21 (52,5)	12 (54,5)	9 (50)	0.775
MPA	16 (40)	8 (36,4)	8 (44,4)	0.604

EGPA	2 (5)	1 (4,5)	1 (5,6)	1.000
ANCA neg. pauci-immune GN	1 (2,5)	1 (4,5)	0	1.000
Involved systems				
Lung involvement	26 (65)	12 (54,5)	14 (77,8)	0.186
Pulmonary-renal syndrome	10 (25)	3 (13,6)	7 (38,9)	0.140
ENT-involvement	19 (47,5)	13 (59,1)	6 (33,3)	0.105
Skin involvement	2 (5)	2 (9,1)	0	0.492
Involvement nerval system	9 (22,5)	6 (27,3)	3 (16,7)	0.138
Cardiovascular involvement	1 (2,5)	1 (4,5)	0	1.000
Comorbidities				
Arterial hypertension	30 (75)	15 (68,2)	15 (83,3)	0.464
Diabetes Mellitus I	0	0	0	0
Diabetes Mellitus II	4 (10)	3 (13,6)	1 (5,6)	0.613
Need for renal replacement therapy during diagnosis	13 (32,5)	5 (22,7)	8 (44,4)	0.145

Table 7: Distribution of clinical parameters, (pre-)existing conditions in the groups over and under 60. Values are expressed as numbers (percentage).

The group i0/i1 consisted of 18 patients, 9 (50%) of them were female and 9 were male. The median age was 61.5 years (37-81 years, mean 58.4 and SD ±12.11). The group i2/i3 consisted of 22 patients with 12 (54.5%) females and 10 (45.5%) males. The median age was 53 years (19-74 years, mean 53.1 and SD±13.99). There were more patients under the age of 60 in the i2/i3 group (15 (68.2%) vs. 7 (38.9%) p=0.064). There was no significance in the listed parameters and grade of Banff lesion score i.

In total 8 patients were in the CD138=<10 group, 4 of them were male and 4 (50%) were female. The median age was 58 years (37-74 years, mean 58.6 and SD ±12.25).

Twenty-one patients showed CD138>10 positive cells, 10 (47.6%) of them were female and 11 (52.4%) were male. The median age was 59 years (19-81 years, mean 55.7 and SD ±13.97). The distribution of the listed parameters relating to the CD138 counts were not significant.

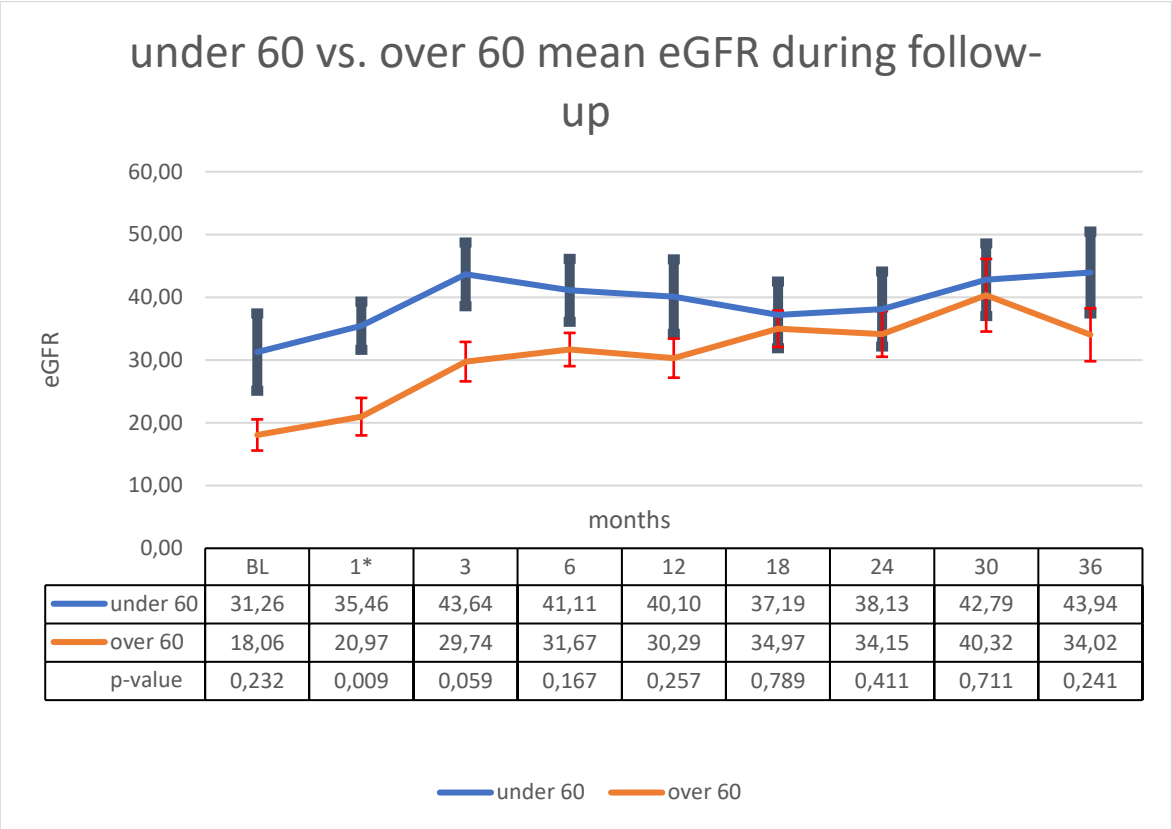
The different treatment regimens and the differences in therapy between the groups can be seen in Table 8.

patients		total	i0/i1	i2/i3	CD138=<1	CD138>10
total		40	18	22	8	21
Induction therapy with:						
CYC	- **total** - **Pulsed** - **Oral** - **change**	37 (92,5) 27 (67,5) 9 (22,5) 1 (2,5)	18 (100) 10 (55,6) 7 (38,9) 1 (5,6)	19 (86,4) 17 (77,3) 2 (9,1) 0	8 (100) 5 (62,5) 2 (25) 1 (12,5)	20 (95,2) 14 (66,7) 6 (28,6) 0
RTX		15 (37,5)	7 (38,9)	8 (36,4)	2 (25)	7 (33,3)
Change in RTX therapy		11 (27,5)	7 (38,9)	6 (27,3)	2 (25)	4 (19)
Maintenance therapy with: - **MMF** - **RTX** - **AZA** - **Change in therapy** - **No therapy**		 16 (40) 18 (45) 16 (40) 12 (30) 2 (5)	 7 (38,9) 8 (44,4) 8 (44,4) 6 (33,3) 0	 9 (40,9) 10 (45,5) 8 (36,4) 6 (27,3) 2 (9,1)	 2 (25) 3 (37,5) 6 (75) 4 (50) 1 (12,5)	 12 (57,1) 8 (38,1) 7 (33,3) 6 (28,6) 1 (4,8)
GC therapy at: - **Time of biopsy (%)** - **24 months** - **36 months**		 29 (72,5) 35 (87,5) 32 (80)	 17 (94,4) 16 (88,9) 14 (77,8)	 12 (54,5) 19 (86,4) 18 (81,8)	 4 (50) 6 (75) 6 (75)	 17 (81) 19 (90,5) 17 (81)
Plasma exchange		21 (52,5)	10 (55,6)	11 (50)	5 (62,5)	10 (47,6)

Table 8: treatment differences in all patients and the different groups

3.2 Differences in mean ACR and mean eGFR course

The mean eGFR was significantly improved in the patients younger than 60 starting from first month of follow-up, followed by a similar course until month 36. Figure 10 shows the eGFR course of the patients younger and older than 60 years old.

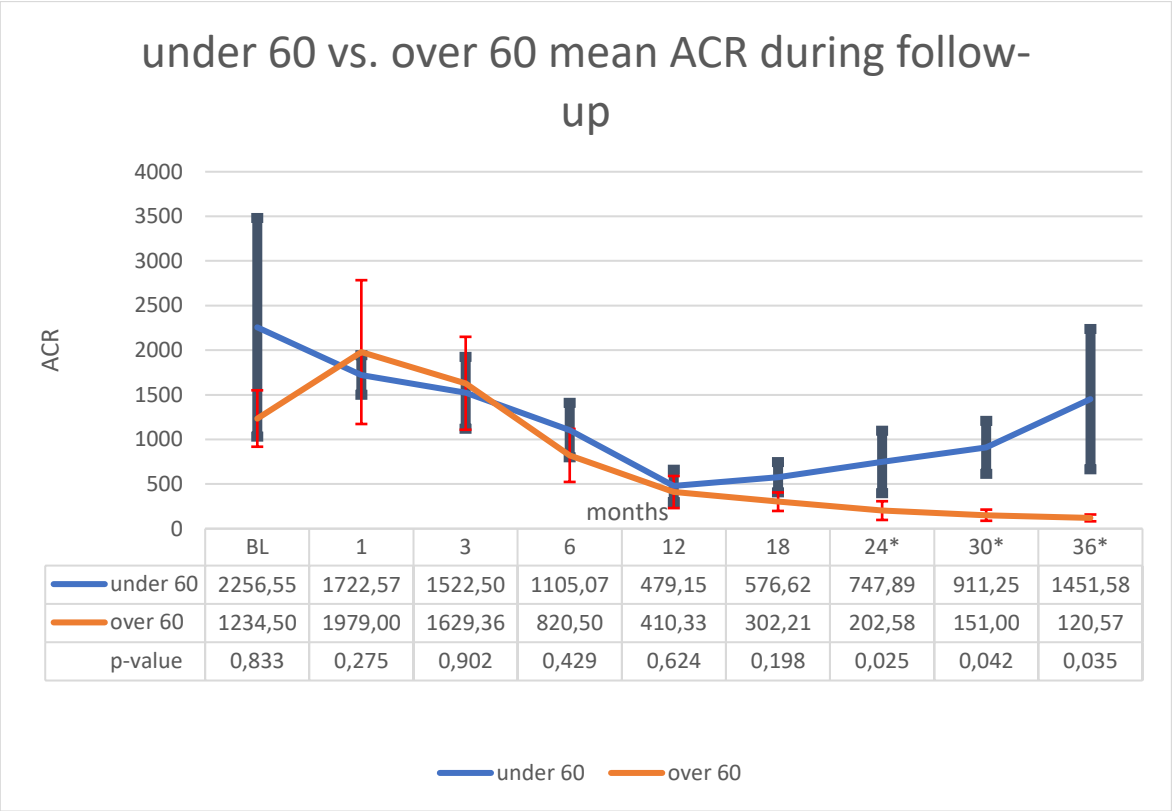


	BL	1*	3	6	12	18	24	30	36
under 60	31,26	35,46	43,64	41,11	40,10	37,19	38,13	42,79	43,94
over 60	18,06	20,97	29,74	31,67	30,29	34,97	34,15	40,32	34,02
p-value	0,232	0,009	0,059	0,167	0,257	0,789	0,411	0,711	0,241

Figure 10: Course of the clinical parameter eGFR; all values are mean and in ml/min/1,73 m2 or show p-value; the whiskers show standard error

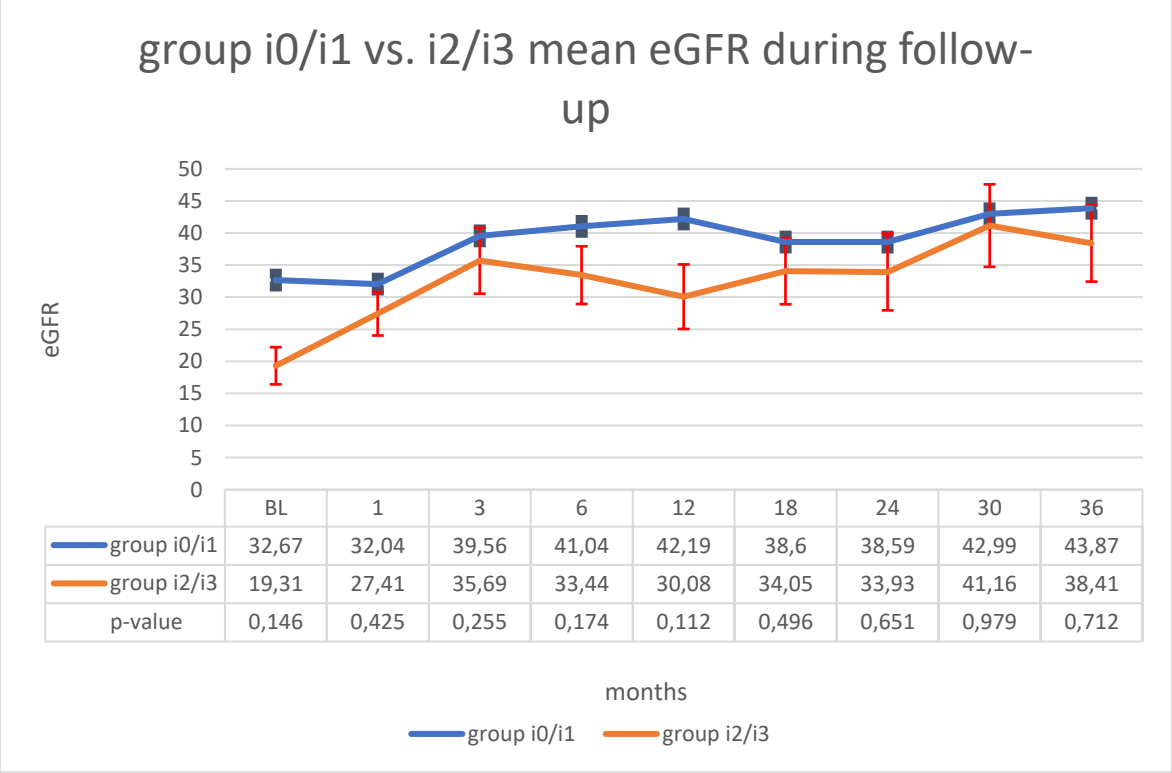
Mean ACR was significantly worse in patients under 60 during months 24 until 36. At earlier time points no significant differences were found. (Figure 11)

No statistically significant differences in eGFR were seen during the follow up between the groups i0/i1 vs. i2/i3, but i0/i1 patients tended to have a higher eGFR compared to the i2/i3 patient group. The eGFR values at the predefined time-points during the follow-up are seen in Figure 12.



	BL	1	3	6	12	18	24*	30*	36*
under 60	2256,55	1722,57	1522,50	1105,07	479,15	576,62	747,89	911,25	1451,58
over 60	1234,50	1979,00	1629,36	820,50	410,33	302,21	202,58	151,00	120,57
p-value	0,833	0,275	0,902	0,429	0,624	0,198	0,025	0,042	0,035

Figure 11: Course of the clinical parameter ACR; all values are in mg/g Crea; the whiskers show standard error

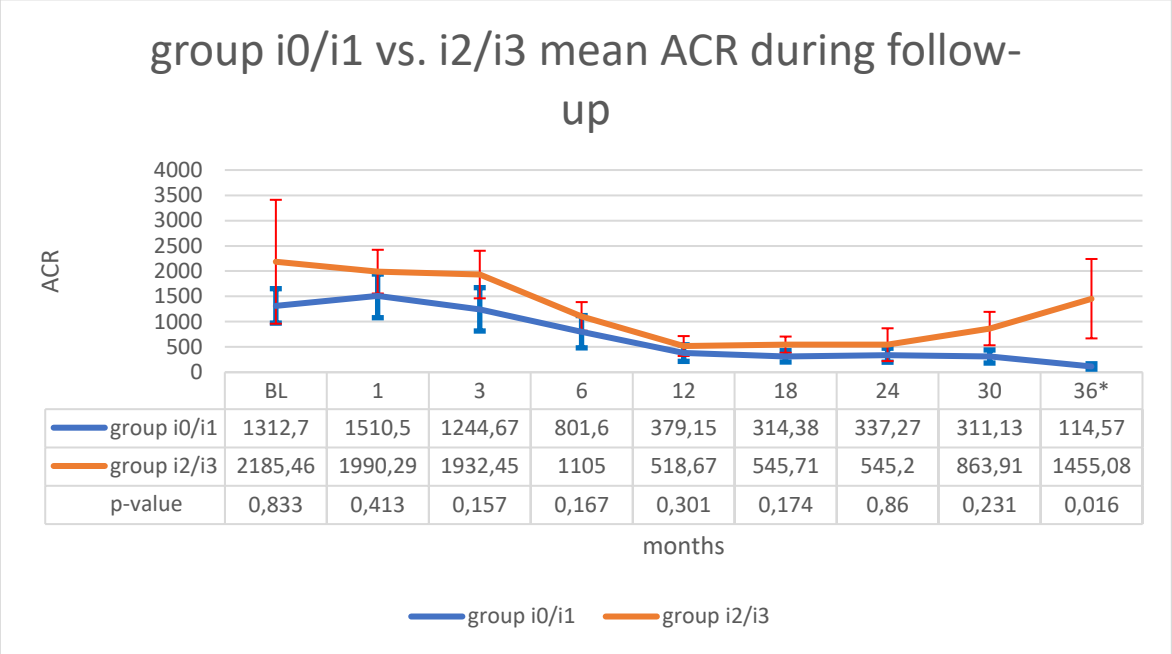


	BL	1	3	6	12	18	24	30	36
group i0/i1	32,67	32,04	39,56	41,04	42,19	38,6	38,59	42,99	43,87
group i2/i3	19,31	27,41	35,69	33,44	30,08	34,05	33,93	41,16	38,41
p-value	0,146	0,425	0,255	0,174	0,112	0,496	0,651	0,979	0,712

Figure 12: Course of the clinical parameter eGFR; all values are mean and in ml/min/1,73 m2; the whiskers show standard error

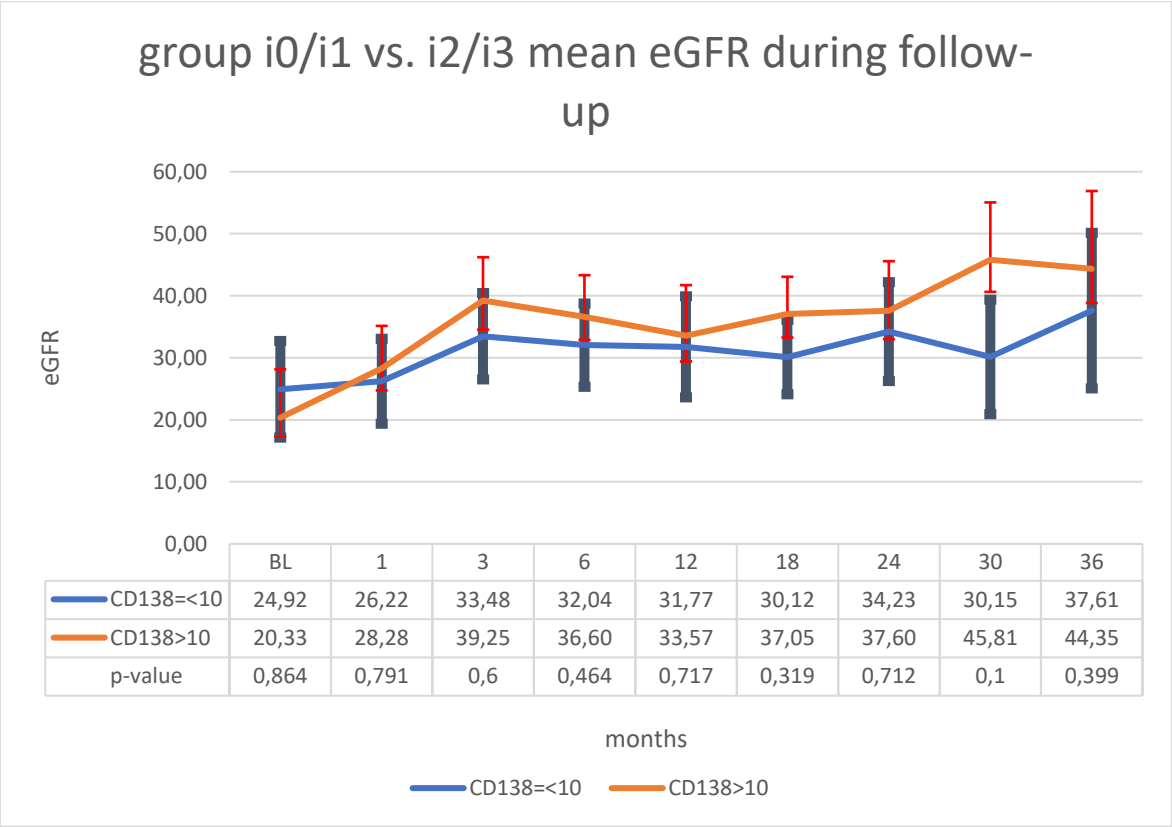
During the entire follow up the mean ACR of group i0/i1 stayed on a lower level than the mean ACR of group i2/i3and reached a statistically significant difference at month 36, that can be seen in Figure 13.

When comparing the groups CD138 =<10 vs CD138>10 no significant difference between mean ACR, nor mean eGFR course during follow-up was found. (Figure 14 and Figure 15)



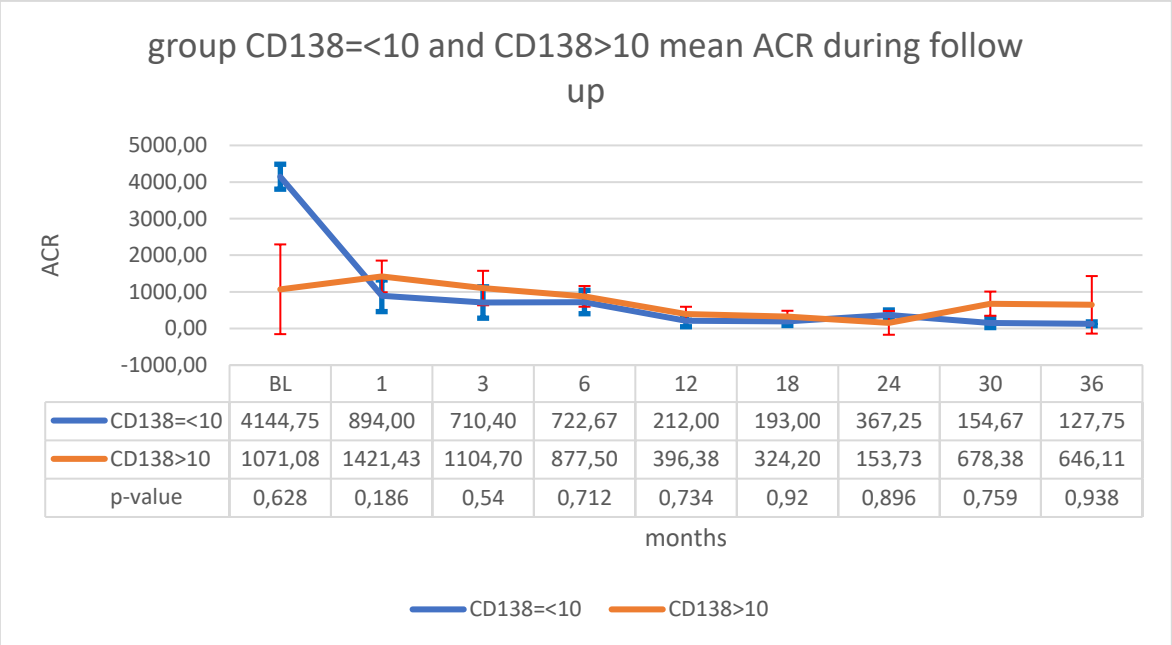
	BL	1	3	6	12	18	24	30	36*
group i0/i1	1312,7	1510,5	1244,67	801,6	379,15	314,38	337,27	311,13	114,57
group i2/i3	2185,46	1990,29	1932,45	1105	518,67	545,71	545,2	863,91	1455,08
p-value	0,833	0,413	0,157	0,167	0,301	0,174	0,86	0,231	0,016

Figure 13: Course of the clinical parameter ACR; all values are in mg/g Crea; the whiskers show standard error



	BL	1	3	6	12	18	24	30	36
CD138=<10	24,92	26,22	33,48	32,04	31,77	30,12	34,23	30,15	37,61
CD138>10	20,33	28,28	39,25	36,60	33,57	37,05	37,60	45,81	44,35
p-value	0,864	0,791	0,6	0,464	0,717	0,319	0,712	0,1	0,399

Figure 14: Course of the clinical parameter eGFR; all values are in mg/g Crea; the whiskers show standard error



	BL	1	3	6	12	18	24	30	36
CD138=<10	4144,75	894,00	710,40	722,67	212,00	193,00	367,25	154,67	127,75
CD138>10	1071,08	1421,43	1104,70	877,50	396,38	324,20	153,73	678,38	646,11
p-value	0,628	0,186	0,54	0,712	0,734	0,92	0,896	0,759	0,938

Figure 15: Course of the clinical parameter ACR; all values are in mg/g Crea; the whiskers show standard error

3.3 Endpoints

patients	total	i0/i1	i2/i3	p-value	CD138=<10	CD138>10	p-value
total	40	18	22		8	21	
Need for RRT during follow-up (%)	8 (20)	2 (11.1)	6 (27.3)	0.258	1 (12.5)	5 (23.8)	0.647
Kidney transplantation (%)	6 (15)	1 (5.6)	5 (22.7)	0.197	1 (12.5)	4 (19)	1.000
Renal Relapse (%)	8 (20)	2 (11.1)	6 (27.3)	0.258	1 (12.5)	3 (14.3)	1.000

Table 9: endpoints in all included AAV-patients and compared between the different groups

In total 8 (20%) patients needed RRT during follow-up, 6 (15%) were subjected to a kidney transplantation and 8 (20%) experienced a RR, as showed in Table 9.

There was no significant difference in the rate of RR between the groups over and under 60, nor in the rate of the need for RRT during follow-up (p-value=0.258, respectively).

All kidney transplantations were performed in the group with patients under the age of 60 (p=0.024). (Table 10)

patients	total	Under 60	Over 60	p-value
total	40	22	18	
Need for RRT during follow-up (%)	8 (20)	6 (27.3)	2 (11.1)	0.258
Kidney transplantation (%)	6 (15)	6 (27.3)	0	0.024
Renal Relapse (%)	8 (20)	6 (27.3)	2 (11.1)	0.258

Table 10: endpoints in all included AAV-patients and compared between the groups under and over 60 years

There was no significant difference in the predefined endpoints between the groups i0/i1 and i2/i3. In the i0/i1 group 2 (11.1%) patients needed RRT during follow-up, 1 (5.6%) was subjected to a kidney transplantation and 2 (11.1%) suffered from a RR. In group i2/i3 6 (27.3%) patients needed RRT during follow-up, 5 (22.7%) had a kidney transplantation and 6 (27.3%) had RR. (Table 9)

Similarly, there was also no significant difference between the groups CD138=<10 and CD138>10. In every endpoint one patient of the non-plasma-cell-rich group was reported (12.5% in all endpoints).

In the plasma-cell-rich group, 5 (23.8) patients needed RRT during follow-up, 4 (19%) had a kidney transplantation and 3 (14.3%) had a RR. (Table 9)

3.4 Correlation between $CD3^+$, $CD20^+$ and $CD138^+$ renal cell infiltration and eGFR and ACR

There was no significant correlation between the cell lines CD3, CD20 and CD138 and eGFR and ACR at BL and month 36 (end of follow-up). (Table 11) The graphic representation of the correlation between CD138 and ACR at BL and CD138 and ACR at month 36 can be seen below (Figure 16 and Figure 17).

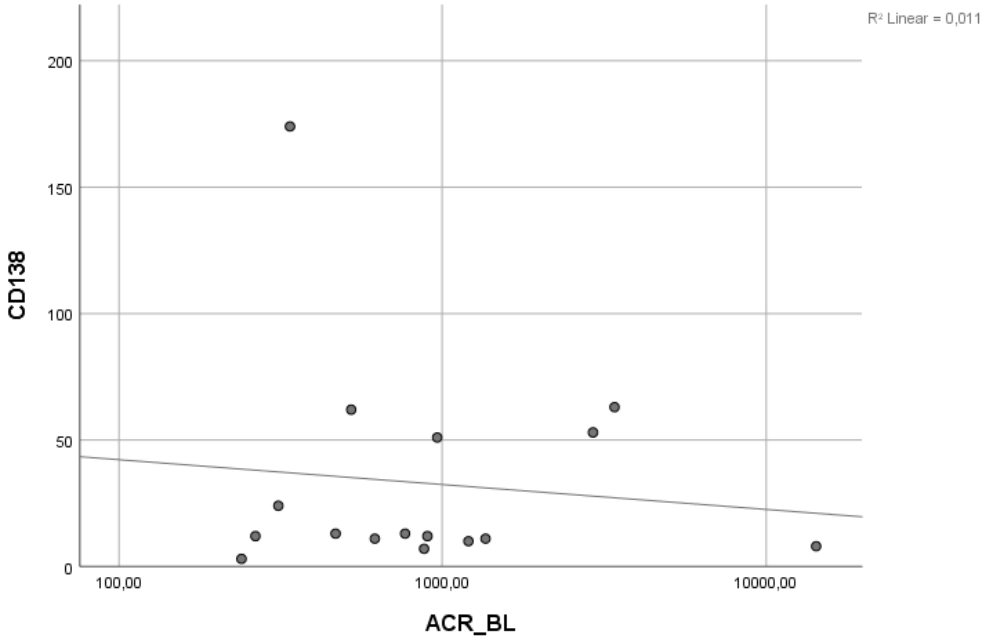


Figure 16: Scatter plot of the correlation between CD138 and ACR at baseline

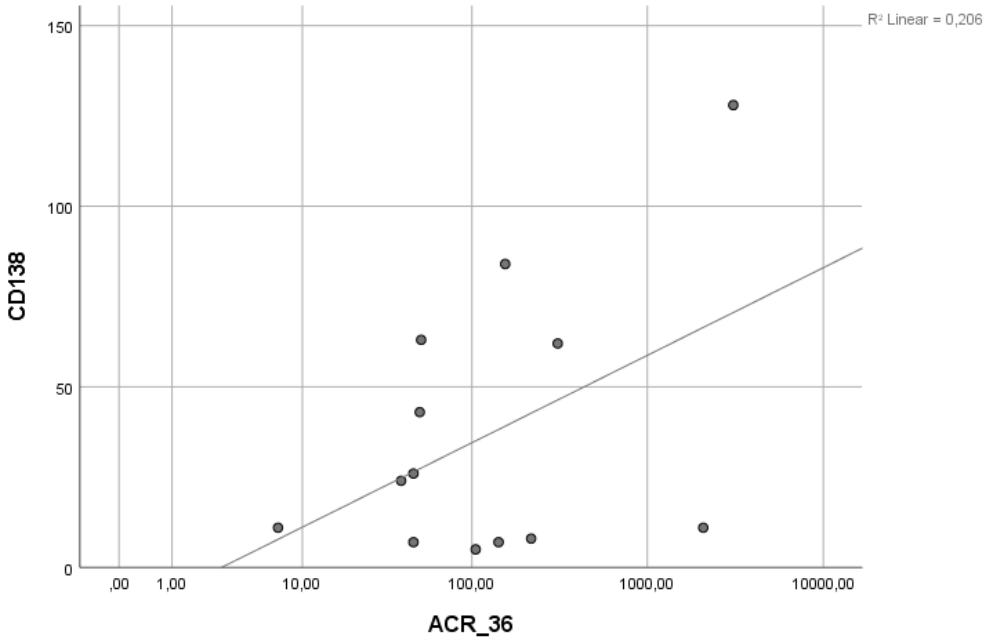


Figure 17: Scatter plot of the correlation between CD138 and ACR at month 36

p-values	eGFR Baseline	eGFR month 36	ACR Baseline	ACR month 36
CD3	0.514	0.116	0.389	0.303
CD20	0.967	0.424	0.875	0.390
CD138	0.292	0.560	0.978	0.359

Table 11: p-values of the correlation between CD3, CD20 and CD138 and the development of eGFR and ACR at BL and month 36

3.5 Endpoints and CD3⁺, CD20⁺ and CD138⁺ cell infiltration

A univariate logistic regression models was used to test for associated predictors between non-metrical endpoints and CD3⁺, CD20⁺ and CD138⁺ renal cell infiltration. There was no significant correlation detectable. (Table 12)

		odds ratio	p	95% Confidence interval	
				Lower value	Upper value
Need for renal replacement therapy	CD3	0.996	0.837	0.962	1.032
	CD20	1.019	0.620	0.945	1.100
	CD138	1.012	0.333	0.988	1.036
Kidney transplantation	CD3	1.018	0.340	0.981	1.057
	CD20	0.980	0.664	0.895	1.073
	CD138	1.002	0.862	0.977	1.028
Renal Relapse	CD3	1.001	0.972	0.958	1.045
	CD20	1.028	0.538	0.941	1.124
	CD138	1.006	0.676	0.976	1.037

Table 12: correlations between non-metrical endpoints and CD3+-, CD20+- and CD138+-cells.

3.6 Correlation between CD3⁺, CD20⁺ and CD138⁺ cell infiltration

There was significant correlation between CD3 and CD20 positive cells (rs (26) = .73, p<.001). and CD3 and CD138 positive cells (rs (26) = .59, p=.001). There was also a statistically significant correlation between CD20 and CD138 positive cells (rs (27) = .45, p<.016 - (Table 13).

		CD3	CD20	CD138
CD3	Sig. (2-tailed)		<0.001	0.001
	N		28	28
CD20	Sig. (2-tailed)	<0.001		0.016
	N	28		29
CD138	Sig. (2-tailed)	0.001	0.016	
	N	28	29	

Table 13: Overview of the correlations between CD3+-, CD20+- and CD138+-cells.

4 Discussion

As it has been discussed extensively in the introduction, there is a need for prognostic parameters to estimate disease outcome, especially RR and survival in patients with AAV. The use of the CHCC terminology, including GPA, MPA, EGPA and ANCA-negative GN showed overlapping features and inconsistencies in predicting RR and outcome. (115,122,123).

Our main findings show significant differences in mean ACR between the i0/i1 and i2/i3 group at 36 months and between patients older and younger than 60 years old from month 24 until month 36 of follow-up. More kidney transplantation in the group of patients aged over 60-years were observed. There were no significant differences between $CD3^+$-, $CD20^+$-, and $CD138^+$-cells and the endpoints (RR, the need for RRT and kidney transplantation) and no significant differences between the groups based on $CD138^+$ cell count and clinical course.

In our study, the median age of all included AAV patients was 57 years. Bitton et al. showed an older age distribution in their included patients: 63 years [IQR: 54-73]. (131) Scott et al. reported the age incidence peak of MPA, GPA and EGPA between 65 and 75. (24) Therefore, our patient cohort seems to be younger than comparable cohorts from other publications. It has to be taken into account, that the majority of the patients from our cohort were diagnosed after 2010, so it's possible that a biased median age is reported.

Interestingly, the proportion of extended interstitial changes tended to be higher in AAV patients in the group of patients under 60 years, than in the over 60-year-old group. Berden et al. observed no correlation between the BANFF classification and age. (104) There is no comparable data, which shows the age distribution (over and under 60 years) in patients, who were sub-grouped according to the BANFF classification.

During Follow-up mean ACR course between groups <60 vs. >60 keep similar levels until month 18, when mean ACRs begin to diverge. Under 60-year-old's mean ACR worsens, while mean ACR of the over 60-year-olds steadily improves, becoming significant from month 24 until month 36.

All kidney transplantations were performed in the patients younger than 60, which shows a significant distribution. It is questionable, whether this data is reliable, as some patients refused treatment, which has not been considered in this study.

The two groups i0/i1 and i2/i3 showed significant differences in mean ACR during follow-up, using Mann-Whitney-U-Tests. The differences in mean eGFRs between the BANFF groups performed descriptively better in group i0/i1 but were not significant at any point. Descriptively, the distribution of endpoints (need for RRT during follow-up, kidney transplantation and RR) between the groups i0/i1 and i2/i3 showed better outcome for group i0/i1, but they were never significant at any investigated time-point. The already mentioned trials on histopathological alterations predicting outcome, support our findings.

Bajema et al. reported significant correlations between serum creatinine values at BL and 1 year after biopsy and tubulointerstitial alterations, like diffuse interstitial infiltrates (mainly neutrophils), IF, TA and tubular intraepithelial infiltrates. (138) Berden et al. observed a significant correlation on eGFR extended TA and diffuse interstitial fibrosis (IF) during follow-up. $CD3^+$ cell dependent tubulitis and TA were reported as independent predictors for eGFR at 12 months of follow-up, but at month 24 of follow-up only TA remained as independent predictor. Patients with positive MPO-ANCA serology showed more severe tubulointerstitial inflammation and "…higher $CD20^+$ tubulitis scores, as well as higher $CD3^+$ and $CD138^+$ interstitial inflammation scores, than those patients who had a positive test for PR3-ANCA." (104) In the multivariable analysis of Brix et al. eGFR at BL and IF/TA showed significant values for predicting outcome. (142,142)

Also, in proteinuric glomerulopathies IF showed an association with decreased eGFR levels. (185)

Bitton et al. reported IF/TA >25%, TI>25% and ATN >=25% with significant prognostic value for ESRD and for RR the two independent factors TI>25% and a longer course of maintenance therapy were found. (131)

The courses of mean ACR remained similar during the first 30 months of follow up, however reached a statistically significant difference at month 36.

The observed phenomenon brings up the question, which factors could influence such a divergence in mean ACR between the groups i0/i1 and i2/i3?

The subclassification into i0/i1 and i2/i3 could serve as a decision tool, whether the AAV-patient needs an adapted maintenance therapy regimen. However, further investigation is needed, to evaluate if tubulointerstitial changes in AAV patients can help to define a patient collective, that would profit from prolonged maintenance therapy.

As already mentioned earlier in this thesis, Charles et al. showed, that patients with a prolonged RTX-maintenance therapy show less RR. (163) An analysis which adds TIN as a factor for prolonged maintenance therapy could give new insights in AAV therapy regimens.

The already mentioned MAINRITSAN 2 trial, shows equality between tailored and fixed-schedule RTX regimens for maintaining remission. The advantage in tailored RTX-regimens are the reduced accumulated RTX-doses. The patients in the tailored arm of the MAINRITSAN 2 study received RTX based on trimestrial monitoring of clinical parameters, as ANCA titres, and $CD19^+$ B-lymphocyte levels. (162) This concept could be used for patients with extended TIN.

We observed similar courses of mean ACRs between the groups over 60 vs. under 60 and i0/i1 vs. i2/i3.

The already mentioned diverging mean ACRs between the groups over and under 60 and the groups i0/i1 and i2/i3 hve to be investigated more thoroughly with larger sample sizes. Otherwise, the observed influence on clinical course, estimated with mean ACR, cannot be securely assigned to one of the groups. To our knowledge, there is no comparable data available.

Further investigation with larger sample sizes is needed to evaluate the impact of the subclassification according to tubulointerstitial changes on RR rate, kidney transplantation and the need for RRT during follow-up.

There was no significant difference in mean eGFR and ACR between the groups CD138=<10 and CD138>10. Similarly, there was no significant correlation regarding the predefined endpoints (need for RRT during follow-up, kidney transplantation and RR) between the two groups CD138=<10 and CD138>10.

Moreover, there was no correlation between $CD3^+$, $CD20^+$ and $CD138^+$ renal infiltrating cells and the mean eGFR and ACR neither at base line, nor at month 36. There was no significant associated predictor between different renal infiltrating immune cells and endpoints (need for RRT during follow-up, kidney transplantation and RR).

Our results go along with the findings of Geetha et al. The investigated $CD3^+$ and $CD20^+$ cells showed no consistent prognostic value regarding eGFR at base line and treatment response. (139) Brix et al. found an association between renal lymphocytic organisation and renal survival. (140) Berden et al. showed a significant correlation between T-cell mediated tubulitis and renal outcome and B-cell

intraglomerular infiltration and impaired kidney function (reduced eGFR during follow-up). (104)

Bitton et al. found three independent risk factors for ESRD, including lower eGFR at BL, IF/TA >25% and higher grades of interstitial lymphocytic organization. A higher CD3/CD20 ratio and the presence of RORγt-positive cells were identified as independent risk factors for RR. (131)

Twenty-one (72%) of the 29 individuals, in whose biopsies immunohistochemistry was performed, showed high CD138 positive cell expression, which opposes the already existing data. (186) Steinmetz et al. found only few CD138 positive cells in scattered B-cell distribution and nodular distribution patterns in kidney biopsies from AAV-patients. Higher organized B-cell patterns showed no CD138 positive cells at all. (103) Berden et al. could find higher CD138 positive cell counts in AAV-patients with MPO-ANCA and showed CD138 positive cells especially in mildly affected AAV patients.

There are several limitations, which have to be taken into account when interpreting the data. Most importantly, our study has a relatively small sample size. As this study is retrospective and single centered, there are missing data and additionally some of our patients refused continuous therapy, which might have influenced the outcome and the interpretation of our data. Moreover, patients with ESRD, without any therapy were included. These patients could never experience a RR, as they never reached remission.

Conclusion:

Our findings go along with already existing data on the correlation between tubulointerstitial alterations and worse kidney outcome. Patients with extended tubulointerstitial changes might be at higher risk for worse renal outcome. Our data show a significant proportion of $CD138^{+}$ plasma cells in human kidney specimens of patients with AAV, providing additional insights on the renal B-cell clusters in patients with AAV.

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Appendix – project plan

Appendix - Questionnaire