From the Department of Clinical Science,
Intervention and Technology – CLINTEC
Division of Renal Medicine
Karolinska Institutet, Stockholm, Sweden

INFLAMMATORY BIOMARKERS IN ANCA-ASSOCIATED VASCULITIS

Ásta Dögg Jónasdóttir

Karolinska Institutet

Stockholm 2022

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2022

ISBN 978-91 -8016 -838 -0

Cover illustration: by Una Lorenzen. Pentraxin-3, modified from Wang Z, Wang X, Zou H et al (2020) The Basic Characteristics of the Pentraxin Family and Their Functions in Tumor Progression. Front. Immunol. 11:1757. doi: 10.3389/fimmu.2020.01757. Copyright © 2020 Wang, Wang, Zou, Dai, Feng, Zhang, Xiao, Liu and Cheng. Creative Commons Attribution License (CC BY).

Inflammatory biomarkers in ANCA-associated vasculitis

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Ásta Dögg Jónasdóttir

The thesis will be defended in public at 4U Solen, Alfred Nobels Allé 8 (ANA8), Campus Flemingsberg, Karolinska Institutet on Friday the 25th of November 2022 at 9.00 am.

Principal Supervisor:
Professor Annette Bruchfeld, MD, PhD
Karolinska Institutet
Department of Clinical Science, Intervention and Technology - CLINTEC
Division of Renal Medicine
and
Linköping University
Department of Health, Medicine and Caring Sciences

Co-supervisor(s):
Professor Iva Gunnarsson, MD, PhD
Karolinska Institutet
Department of Medicine, Solna
Division of Rheumatology

Associate Professor Aleksandra Antovic, MD, PhD
Karolinska Institutet
Department of Medicine, Solna
Division of Rheumatology

Opponent:
Professor emeritus Knut Aasarød, MD, PhD
Norwegian University of Science and Technology (NTNU)
Department of Clinical and Molecular Medicine

Examination Board:
Professor Maria Svensson, MD, PhD
Uppsala University
Department of Medical Sciences
Renal Medicine

Associate Professor Thomas Hellmark, PhD
Lund University
Department of Clinical Sciences
Division of Nephrology

Associate Professor Lotta Ljung, MD, PhD
Umeå University
Department of Public Health and Clinical Medicine
and
Karolinska Institutet
Department of Medicine, Solna
Division of Clinical Epidemiology

To Sóllilja, Jónas Jökull and Snæfríður

POPULAR SCIENCE SUMMARY OF THE THESIS

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are relatively rare and serious autoimmune diseases causing inflammation in small blood vessels. They are characterized by the presence of autoantibodies called ANCA. The incidence varies between 4.6 to 33 cases/million per year and the prevalence between 30 to 421 per million. The clinical manifestations vary, from a limited local form to multi-organ involvement with serious organ failure. The kidneys are affected in 70-90% of patients and the disease can lead to kidney failure. If AAV is left untreated, the death rate is high, around 80% in the first year after diagnosis. The treatment consists of corticosteroids and other immunosuppressives to regulate the immune response and thereby suppress the disease activity. The treatment is often associated with side effects such as infections, diabetes, osteoporosis, cancer, and infertility. The risk of a disease relapse is high, about 50% of patients experience a disease relapse within the first five years after diagnosis. Unfortunately, there are no reliable methods to predict the risk of serious disease or the relapse risk in the individual patient.

The aim of this thesis was to identify and investigate biological molecules (biomarkers) that could be useful for monitoring disease activity in AAV.

In study 1, we investigated three different biomarkers that are expressed on extracellular vesicles. Extracellular vesicles are small particles released from various cells. Their composition, action and effect depend on the type of cells and the conditions they derive from. We found that concentrations of extracellular vesicles expressing the molecule Pentraxin-3 (PTX3) and high mobility group box 1 (HMGB1) were higher in AAV patients with active compared to inactive disease and that their concentrations correlated with disease activity.

In study 2, we investigated PTX3, a marker of inflammation. PTX3 has been found to be elevated in patients with autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). We found that the levels of PTX3 in blood and urine were higher in AAV patients with active disease compared to at a follow-up where most patients had inactive disease. We also found that the levels correlated with disease activity.

In study 3, we investigated the protein TNF-related weak inducer of aptoptosis (TWEAK) by measuring levels in blood and urine from patients at disease diagnosis and follow-up. We found that the levels of TWEAK in urine were higher in patients with active AAV compared to at a follow-up where most patients had inactive disease, and that the urinary levels correlated to

disease activity. When performing immunohistochemical staining on kidney biopsies, we saw stronger staining of TWEAK in patients with active AAV compared to controls.

In study 4, we investigated extracellular vesicles expressing markers that can affect blood coagulation. We found that the concentration of extracellular vesicles that express the molecules tissue factor (TF) and citrullinated histone-3 (H3Cit) were higher in patients with active compared to inactive disease and that the concentration of extracellular vesicles expressing TF correlated to disease activity in patients with AAV. We also investigated whether these extracellular vesicles affected the generation of thrombin, a protein important for blood coagulation. Our results show that the thrombin generation was increased in patients with AAV compared to controls and that extracellular vesicles may influence the thrombin generation.

In conclusion, in this thesis we have investigated different potential biomarkers in AAV. These biomarkers may be involved in both the development of the disease and the complications seen in AAV. They can potentially be used to assess the AAV disease activity and possible identify patients at risk of occurrence of a disease relapse.

ABSTRACT

Background: Antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) is a heterogenous group of relatively rare and serious small vessel vasculitides, characterized by the presence of proteinase 3 (PR3) ANCA or myeloperoxidase (MPO) ANCA. The disease manifestations vary widely, from a localized form to a serious disease with multi-organ failure. When untreated the mortality rate is high, around 80% in the first year from diagnosis. Improvements in treatment have resulted in better prognosis but disease relapses, comorbidities and treatment side effects remain a challenge.

Aims: the aims of this thesis were the following: 1) to identify potential non-invasive biomarkers that could aid in the evaluation of disease activity in AAV, and 2) to explore the role of these potential biomarkers in the pathogenesis of AAV.

Material and methods: Patients from a well characterized cohort of AAV patients were included in the studies. For comparison control samples from population-based cohorts were used. Biomarkers were measured in urine and/or plasma/serum. Analysis of extracellular vesicles (EVs) was performed with flow cytometry. Thrombin generation was evaluated with a modified Calibrated Automated Thrombogram (CAT) assay after addition of EV-enriched pellets. The disease activity was assessed with the Birmingham Vasculitis Activity Score (BVAS). Immunohistochemical staining was carried out on kidney biopsies and the well-established Berden score was used for histopathological classification.

Study I: MPO-positive extracellular vesicles (MPO^+EVs) expressing pentraxin-3 (PTX3), high mobility group box 1 (HMGB1) protein, and tumor necrosis factor-like weak inducer of apoptosis (TWEAK) were analysed in 46 AAV patient, (whereof 23 patients had an active disease) and 23 controls. Serum HMGB1, PTX3 and TWEAK levels were also measured. Levels of MPO^+EVs expressing all investigated biomarkers were significantly higher in patients compared to controls. Levels of MPO+EVs expressing PTX3 and HMGB1 were significantly higher in active compared to inactive disease and correlated with BVAS. Moreover, serum PTX3 levels were higher in patients with active compared to inactive disease and correlated with BVAS but the same was not found for TWEAK or HMGB1.

Study II: Plasma PTX3 levels were measured in 79 patients with active AAV at baseline and 6-month follow-up as well as in 23 controls. Urinary PTX3 levels were measured in 34 of the patients. The plasma and urinary PTX3 levels were significantly higher at baseline compared to follow-up and correlated with BVAS at baseline. Both the plasma and urinary PTX3 levels

correlated to albuminuria and the estimated glomerular filtration rate. Moreover, patients with kidney involvement had higher plasma and urinary PTX3 levels compared to those without.

Study III: Serum TWEAK levels were measured in 74 patients with active AAV at baseline and 6-month follow-up as well as in 20 controls. Urinary TWEAK levels were measured in 69 patients. Urinary TWEAK levels were higher at baseline compared to follow-up and correlated with BVAS at baseline. Patients with kidney involvement had higher urinary levels compared to those without and a correlation between urinary TWEAK and albuminuria was found. Serum TWEAK levels were higher in patients at inclusion compared to follow-up but did not correlate with BVAS. A significant difference in serum TWEAK levels in AAV patients compared to controls was not seen. Immunohistochemical staining of kidney tissue from AAV patients showed a clear expression of TWEAK and a weaker staining for fibroblast growth factor-inducible 14 (Fn14) (TWEAKs receptor).

Study IV: MPO-positive extracellular vesicles (MPO^+EVs) expressing tissue factor (TF), citrullinated histone-3 (H3Cit) and plasminogen (Plg) were analysed in 46 patients with AAV (whereof 23 had active disease) and 23 controls. Concentrations of MPO^+EVs expressing TF and H3Cit were significantly higher in patients with active compared to inactive disease and controls. Concentrations of MPO^+EVs expressing Plg were higher in the total AAV group compared to controls but there was no difference between active and inactive patients. Concentrations of MPO^+EVs expressing TF correlated with disease activity. Thrombin generation was augmented in AAV patients compared to controls and a correlation between MPO^+EVs expressing TF and H3Cit and the parameters of thrombin generation was found.

Conclusions: Our findings indicate that circulating and urinary PTX3, urinary TWEAK as well as EVs expressing PTX3, HMGB1 and TF could be useful biomarkers in AAV. Thrombin generation is augmented in patients with AAV and is associated with concentrations of MPO^+EVs expressing TF and H3Cit. These findings suggest that these biomarkers may have a role in the pathogenesis of the hypercoagulability seen in AAV.

LIST OF SCIENTIFIC PAPERS

I. Manojlovic M, Juto A, **JONASDOTTIR A**, Colic J, Vojinovic J, Nordin A, Bruchfeld A, Gunnarsson I, Mobarrez F, Antovic A.
Microparticles expressing myeloperoxidase as potential biomarkers in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV)
Journal of Molecular Medicine. 2020; 98:1279-1286

II. **JONASDOTTIR AD**, Antovic A, Qureshi AR, Nordin A, Malmström V, Gunnarsson I, Bruchfeld A.
Pentraxin-3 – a potential biomarker in ANCA-associated vasculitis
Scandinavian Journal of Rheumatology. 2022; Apr 6:1-9.
doi: 10.1080/03009742.2022.2045790. Epub ahead of print

III. **JONASDOTTIR AD**, Schwartz A, Söderberg M, Wernerson A, Qureshi AR, Antovic A, Gunnarsson I, Bruchfeld A.
Urinary TWEAK reflects disease activity in ANCA-associated vasculitis
Manuscript

IV. **JONASDOTTIR AD**, Manojlovic M, Vojinovic J, Nordin A, Bruchfeld A, Gunnarsson I, Mobarrez F*, Antovic A*.
Increased thrombin generation correlates with circulating extracelluar vesicles exposing tissue factor and citrullinated histone-3 in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis
Manuscript

**Equal contribution*

CONTENTS

LIST OF ABBREVIATIONS

AAV	ANCA Associated Vasculitis
ANCA	Anti-neutrophil cytoplasmic antibodies
ACR	American College of Rheumatology
AZA	Azathioprine
AUC	Area under the curve
BAFF	B-cell activating factor
BVAS	Birmingham Vasculitis Activity Score
CAT	Calibrated automated thrombogram
C-ANCA	Cytoplasmic ANCA
CHCC	Chapel Hill Consensus Conference
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRP	C-reactive protein
CVD	Cardiovascular disease
CYC	Cyclophosphamide
DCVAS	Diagnostic and Classification Criteria for Vasculitis study
eGFR	Estimated glomerular filtration rate
EGPA	Eosinophilic granulomatosis with polyangiitis
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency´s
ENT	Ear, nose, and throat
ESR	Erythrocyte sedimentation rate
ETP	Endogenous thrombin potential
EULAR	The European League Against Rheumatism
EUVAS	The European Vasculitis Society
EV	Extracellular vesicles
Fn14	Fibroblast growth factor-inducible molecule 14
GC	Glucocorticoid
GPA	Granulomatosis with polyangiitis
GN	Glomerulonephritis

H3Cit	Citrullinated histone-3
HMGB1	High mobility group box 1
HLA	Human leukocyte antigen
IgAN	Immunoglobulin A nephropathy
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IL	Interleukin
ISEV	International Society for Extracellular Vesicles
iv	Intravenous
LAMP-2	Lysosomal membrane protein 2
LN	Lupus nephritis
LM	Lund Malmö
MAC	The membrane attack complex
MAF	Multidimensional Assessment of Fatigue
MBL	Mannose-binding lectin
MCD	Minimal change disease
MHC II	Major histocompatibility complex II
MMF	Mycophenolate mofetil
MN	Membranous nephropathy
MPA	Microscopic polyangiitis
MTX	Methotrexate
MPO	Myeloperoxidase
NET	Neutrophil extracellular trap
NEV	Neutrophil-derived extracellular vesicles
NF- ƙβ	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK cells	Natural killer cells
NPP	Normal pool plasma
OMERACT	Outcomes Measures in Rheumatology
QoL	Quality of life
P-ANCA	Perinuclear ANCA

PAD4	Peptidylarginine deiminase 4
PBMC	Peripheral blood mononuclear cells
PLEX	Plasma exchange
Plg	Plasminogen
PPP	Platelet poor plasma
PR3	Proteinase-3
PTX3	Pentraxin-3
RA	Rheumatoid arthritis
RCT	Randomized controlled trial
ROC	Receiver operating characteristics
ROS	Reactive oxygen species
RT	Room temperature
RTX	Rituximab
SAP	Serum amyloid P component
SLE	Systemic lupus erythematosus
sTWEAK	Serum TWEAK
S. aureus	Staphylococcus aureus
SSc	Systemic sclerosis
TF	Tissue factor
Th17	T helper 17
TNF	Tumor necrosis factor
tPA	Tissue plasminogen activator
Tregs	Regulatory T cells
TWEAK	TNF-like weak inducer of apoptosis
uPTX3	Urinary PTX3
uPTX3/Cr	Urine PTX3-to-creatinine ratio
uTWEAK	Urinary TWEAK
uTWEAK/Cr	Urine TWEAK-to-creatinine ratio
VDI	Vasculitis Damage Index
VTE	Venous thromboembolism

1 INTRODUCTION

1.1 ANCA-ASSOCIATED VASCULITIS

Systemic vasculitides are heterogeneous diseases that involve inflammation of blood vessel walls which can lead to necrosis and organ damage. Several attempts to standardize the classification and nomenclature of vasculitides based on disease criteria have been pursued. The International Chapel Hill Consensus Conference (CHCC) nomenclature is the most used definition where vasculitides are subdivided into groups based on a combination of findings. Non-infectious vasculitides are categorized by the type of vessels involved, large, medium and small vessel vasculitis (1) (figure 1). Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) is a group of necrotizing vasculitides that predominantly affect small and medium sized vessels with few or no immunoglobulin or complement deposits (pauci-immune). According to the CHCC nomenclature AAV are further divided into clinical variants or diseases, i.e. granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA). These conditions are associated with ANCAs that are specific for proteinase 3 (PR3-ANCA) or myelo-peroxidase (MPO-ANCA) (1, 2). MPO-ANCA is typically, but not exclusively, seen in MPA and in 40% of EGPA cases, whereas and PR3-ANCA is mostly seen in GPA. A granulomatous inflammation with necrosis is seen in GPA and EGPA in contrast to MPA which is characterized by non-granulomatous necrotizing inflammation (1).

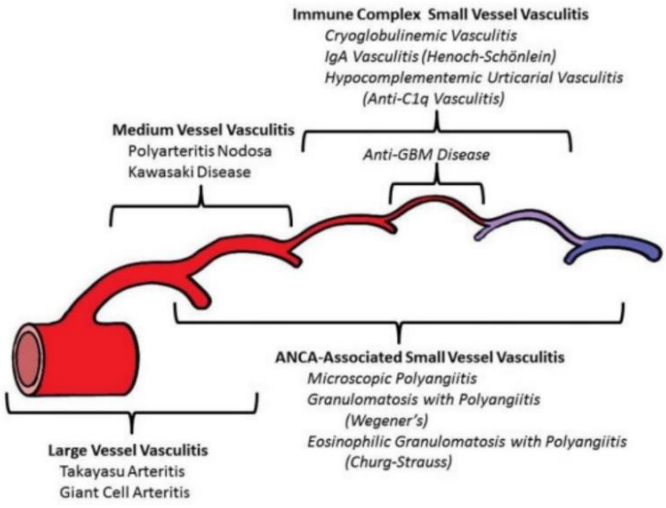


Figure 1. *Distribution of vessel involvement by large vessel vasculitis, medium vessel vasculitis, and small vessel vasculitis. Reproduced from (1) with permission from John Wiley and sons.*

1.2 CLASSIFICATION CRITERIA

There has been a lack of validated diagnostic criteria for AAV although classification criteria have been used to standardize inclusion of cohorts of patients in research settings. The classification of American College of Rheumatology (ACR) was introduced in 1990 (3). These criteria have limitations due to the absence of ANCA testing and what is more the lack of MPA

criteria since it was not a widely recognized condition at that time. In 2007 the European Medicines Agency (EMA) published an algorithm for categorizing patients with ANCA associated vasculitis in epidemiologic studies (4). Recently, the ACR/EMA published a new revised classification criteria for GPA, MPA, and EGPA based on the multinational Diagnostic and Classification Criteria for Vasculitis study (DCVAS) with more weight being put on ANCA positivity (5-7). The classification into different phenotypes has been debated and it has been suggested that a classification based on ANCA serotypes would be more appropriate as the disease course, genetic markers, and biomarkers biology correlate more strongly to ANCA serotypes than the proposed clinical phenotypes (8, 9).

1.3 EPIDEMIOLOGY

AAV are relatively rare conditions, the incidence varies between 4.6 to 33 cases per million/year and the prevalence between 30 to 421 per million (10, 11). The incidence of the different AAV subgroups is associated with geographical variation, with GPA being more common in Australia and northern Europe than MPA which is seen more in Asia and southern Europe (12-16). Although AAV can present in any age, the incidence increases with age and AAV is slightly more common in males than in females (11). In southern Sweden, the incidence has been shown to be about 21 cases per million/year with increasing incidence over 75 years of age (17). The differences in the registered prevalence and incidence might partly be due to difference in definitions and classification criteria but also to increased awareness of the disease and improved case identification.

1.4 CLINICAL MANIFESTATIONS

The clinical presentation varies widely, from a limited form restricted to the upper airway tract to a severe disease with multi-organ failure. Nearly every organ system of the body can be affected. Patients with AAV commonly present with prodromal symptoms and signs early in the course of the disease such as fatigue, myalgias, arthralgias, weight loss and fever. Upper respiratory tract involvement such as sinusitis, otitis, epistaxis, ulcerations, nasal deformity, and septal perforation are common and usually seen in GPA but not MPA. Lung manifestations, ranging from focal pulmonary infiltrates to alveolar hemorrhage, can occur in both GPA and MPA. Kidney involvement occurs in approximately 90% of patients with MPA,

70% of patients with GPA and can lead to kidney failure (18). Other GPA and MPA manifestations include neuropathy, especially mononeuritis multiplex, cutaneous manifestations, sensorineural and/or conductive hearing loss, gastrointestinal ulcerations, and ophthalmic manifestations such as conjunctivitis, scleritis, and uveitis. EGPA typically presents with symptoms of allergic rhinitis, asthma, and eosinophilia. Cardiac and neurological manifestations are more common in EGPA but kidney involvement less so compared to MPA/GPA (2, 19, 20). Summary of the potential organ involvement and the clinical manifestations of AAV is shown in figure 2 and table 1.

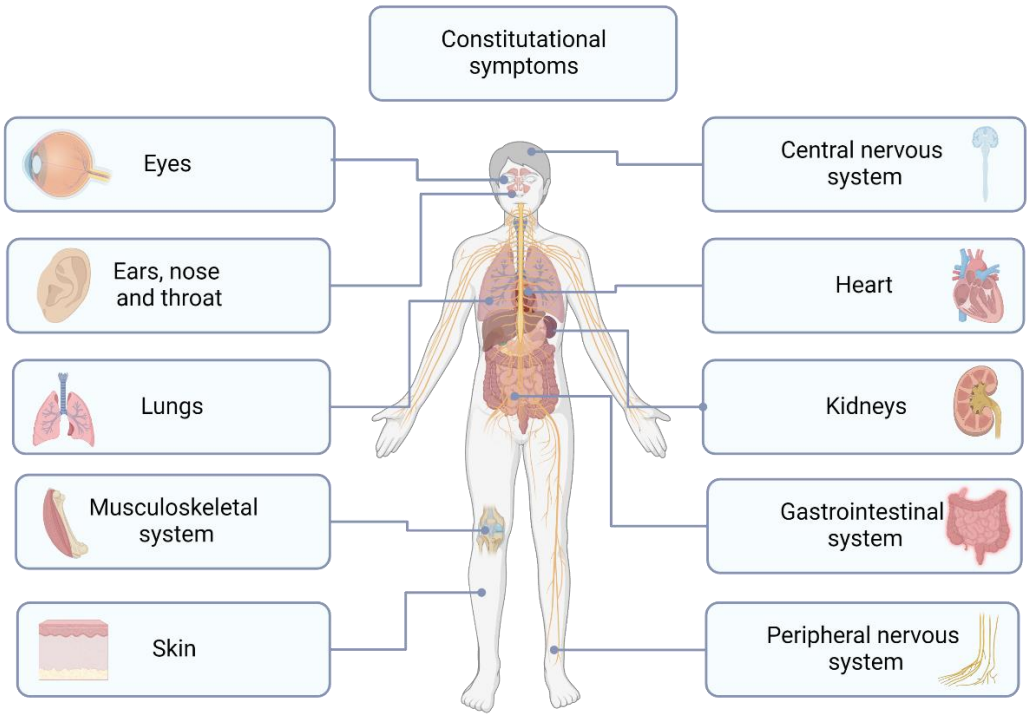


Figure 2. *Schematic presentation of the organ involvement in ANCA-associated vasculitis. Created with Biorender.com*

1.5 DIAGNOSIS

The diagnosis of AAV can be cumbersome as the symptoms are often non-specific, especially in the prodromal phase that can last from weeks to years before diagnosis contributing to a delay in diagnosis and referrals to adequate care (21, 22). A case-control study from the United Kingdom demonstrated that AAV patients had increased contact with the health care in the year before diagnosis, especially during the last 3 months, and about 50% of the patients had one or more contact with secondary care givers before diagnosis (23). As untreated AAV carries a high mortality and morbidity it is of great importance to promptly identify these patients. Delay in diagnosis has been associated with an increased risk of mortality and kidney failure (24).

Table 1. Potential clinical manifestations of ANCA-associated vasculitis

SYSTEM	MANIFESTATIONS
Constitutional symptoms	Fever, weight loss, anorexia, fatigue
Kidneys	Hematuria, proteinuria, acute kidney injury/failure, crescentic necrotizing glomerulonephritis
Lungs	Cough, dyspnea, stridor, wheeze, infiltrates, pulmonary hemorrhage, pulmonary fibrosis, nodules/cavitation (GPA), pleuritis, asthma (EGPA)
Ear, nose and throat	Rhinitis, sinusitis, bloody nasal discharge, nasal crusting, nasal ulceration, otitis media, sensorineural hearing loss, conductive hearing loss, nasal perforation (GPA), saddle nose (GPA), subglottic stenosis, tracheal stenosis
Skin/mucosal	Palpable purpura, ulcers, urticaria, leukocytoclastic vasculitis, digital infarcts, oral ulcers
Nerve	Peripheral neuropathy (typically mononeuritis multiplex), headache, seizures, cerebrovascular infarcts, spinal cord lesions
Musculoskeletal	Myalgia, arthralgia, arthritis
Gastrointestinal	Gastrointestinal bleeding, diarrhea, nausea, abdominal pain, bowel ischemia, hepatitis (rare), pancreatitis (rare)
Eyes	Scleritis, uveitis, keratitis, retinal vasculitis, retinal hemorrhages, proptosis, and orbital granulomatous masses (GPA)
Heart	Myocarditis (most common in EGPA), heart block, endocarditis, pericarditis, pericardial effusions, valvular heart disease, cardiomyopathy

GPA: granulomatosis with polyangiitis, EGPA: eosinophilic granulomatosis with polyangiitis. Information from (2, 25-27).

The diagnosis of AAV is based on clinical findings, supported by serology and histopathology. As patients often have more than one organ manifestation a systemic approach is needed (2, 19). ANCA is present in approximately 90% of patients with GPA and MPA (28, 29). Previously, the main method for detection of ANCA was indirect immunofluorescence (IF) of ethanol-fixed neutrophils (figure 3). Antibodies against PR3 shows a diffuse staining in the cytoplasm (cytoplasmic ANCA, C-ANCA) whereas antibodies against MPO display a perinuclear staining pattern (perinuclear ANCA, P-ANCA) (30) (figure 3). The recommended

diagnostic screening method at present is however high-quality immunoassays, methods highly sensitive and specific for the detection of MPO- and PR3-ANCA. A positive ANCA serology strongly supports the diagnosis of AAV, but false-negative and false-positive results can be seen (31). The gold standard for a definite diagnosis of AAV are characteristic histopathological changes on biopsies (32). Kidney biopsies provide the best diagnostic yield, histopathological changes have been shown to be present in up to 91.5% of GPA patients with kidney manifestations whereas lung and ear, nose and throat (ENT) biopsies have lower sensitivity (33-35).

1.6 PATHOGENESIS

1.6.1 Risk factors and genetic factors

The pathogenesis and etiology of AAV is complex and not fully understood but may involve environmental as well as genetic factors.

Several environmental factors have been associated with AAV development. Exposure to various drugs including propylthiouracil, cocaine/levamisole and hydralazine have been shown to be associated with AAV (36). Silica dust exposure has also been associated with increased risk of AAV development (37). There has been much interest in infectious agents as potential triggers in AAV (38). Chronic nasal carriage of staphylococcus aureus (S. aureus) seems to be a risk factor for relapses in patients with GPA (39) and prophylactic treatment with co-trimoxazole has been shown to decrease relapses in a prospective study of GPA patients (40). A recently published study revealed that patients with active GPA more often have positive nasal cultures for S. aureus compared to patients with inactive disease and controls. The same study found increased abundance of S. aureus in nasal microbiota in patients with active GPA compared to controls using shotgun metagenomic analysis (41).

Several genes, including the major histocompatibility complex II (MHC II), have been associated with AAV. The human leukocyte antigen (HLA)-DP region was shown to have the strongest association with PR3 ANCA and HLA-DQ with MPO ANCA. Furthermore, there is an association with PRNT and SERPINA1 in PR3 positive AAV. Interestingly there seems to be a stronger genetic association with the type of ANCA (PR3 vs MPO) than the clinical phenotype (GPA vs MPA) (42, 43). This may partly explain the geographical variation in AAV subtypes although other factors such as UV radiation and latitude have been found to be associated with ANCA serotypes (16).

1.6.2 The role of ANCA

ANCAs are autoantibodies directed against primary granule constituents in the cytoplasm of neutrophils and monocytes (44). ANCA and the association to small-vessel vasculitis was initially described in 1982 (45).

There is some debate about the pathogenicity of ANCAs although animal studies have strongly indicated their pathogenic role, mainly in MPA models. Injection with MPO-ANCA or splenocytes from immunized MPO-deficient mice have been shown to cause focal necrotizing crescentic glomerulonephritis (GN) in mice (46) and MPO-ANCA has been found to be pathogenic in a rat model (47). Moreover, MPO deficient mice transplanted with MPO positive bone marrow develop necrotizing crescentic GN (48). A case report describing a maternal transfer of MPO-ANCA to a neonate leading to a pulmonary hemorrhage, proteinuria and hematuria further suggests a causative role of ANCA (49). The development of a PR3-ANCA animal model has been less straightforward and therefore the role of PR3-ANCA in the pathogenesis of AAV is less studied (50).

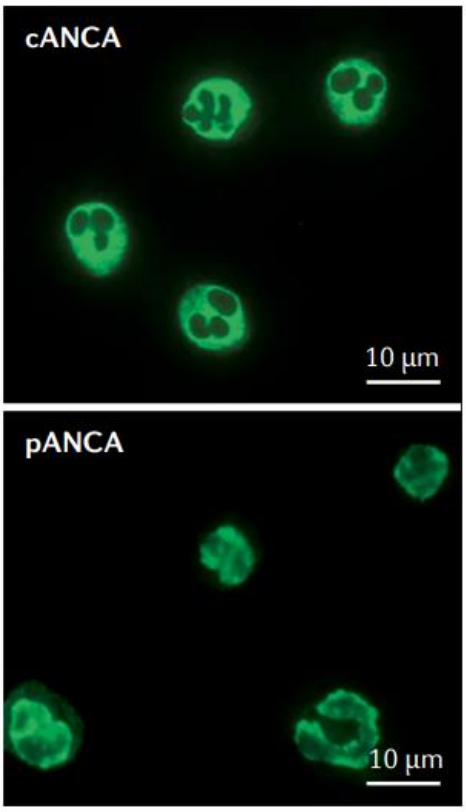


Figure 3 *Patterns of antineutrophil cytoplasmic antibodies (ANCA) by indirect immunofluorescence. Cytoplasmic ANCA (C-ANCA) and perinuclear ANCA (P-ANCA). Reprinted by permission from Springer Nature Customer Service Centre GmbH. (2).*

There is however not a clear correlation between ANCA titers in AAV patients and disease activity although persistently positive ANCA and a rise in titers have been shown to be associated with relapses (51), particularly in serious disease with lung and kidney involvement (52, 53). Furthermore, not all patients with a typical clinical presentation and pathological findings, such as pauci-immune crescent GN, are ANCA positive and ANCA has been also been demonstrated to be present in healthy individuals (54). This has raised speculations that there might be other ANCA antigens involved in the pathogenesis of AAV than PR3 and MPO antigens (55). One such antigen is lysosomal membrane protein 2 (LAMP-2). It has been shown that LAMP-2 cross-reacts with FimH, a bacterial adhesin, and immunization with FimH leads to LAMP-2 ANCA production and a focal necrotizing GN (56). Whether LAMP-2 is involved in the pathogenesis of AAV in humans is however still not clear.

1.6.3 Priming and activation of neutrophils

Priming of neutrophils by cytokines and a direct action of ANCA on primed neutrophils are believed to be core elements of the pathogenesis of AAV (57). An animal model of MPO AAV has shown that neutrophil depletion prior to transfer of anti-MPO antibodies prevents disease development of necrotizing and crescentic GN (58). Priming is believed to be triggered by for instance infectious agents and the associated cytokine release from dendritic cells, T-cells, and macrophages. Priming of neutrophils leads to translocation of ANCA antigens to the neutrophil cell surface to which ANCA bind. The Fc regions of ANCAs also engage with Fcγ receptors on neutrophil surfaces. This binding starts a cascade that leads to activation of the neutrophils. Activated neutrophils produce cytokines, undergo respiratory burst, degranulation and NETosis, a form of cell death with the release of neutrophil extracellular traps (NETs) leading to vascular endothelial injury (57, 59-63). NETs are made of chromatin fibers and granule proteins and contain various peptides, including MPO and PR3 (62). NETs function is believed to trap and kill microbial invaders but can also cause tissue damage in autoinflammatory conditions (59, 62, 64). NETs are present in inflammatory lesions in kidney biopsies from patients with active AAV and elevated NET fragments in serum are present in active disease (62). The serum DNAse 1 activity which degrades NETs has been shown to be reduced in AAV (65). The release of reactive oxygen species (ROS) and formation of NETs by activated neutrophils lead to endothelial damage (60) and upon induction with ANCA NETs have been shown to trigger activation of the alternative complement pathway (66). NETs are also believed to be involved in loss of tolerance to specific antigens, such as PR3 and MPO, as it has been demonstrated that NETs can transfer cytoplasmic antigens from neutrophils to dendritic cells and thereby trigger further immune responses (67).

1.6.4 The complement system

Previously the complement system was not believed to play an important role in AAV as complements are typically not found in staining of lesions and circulating levels are not reduced in AAV. This is in contrast to some immune complex mediated glomerular diseases and systemic lupus erythematosus (SLE) (68). However, in recent years, accumulating evidence has revealed that activation of the complement system, particularly the alternative pathway, plays a pivotal role in the pathogenesis of AAV.

An anti-MPO AAV animal model study demonstrated that mice deficient in C5 or factor B did not develop the disease when injected with anti-MPO IgG. Moreover, mice deficient in C4, a central component of the classical and lectin pathways, were not protected from disease development (69). Treatment with C5 inhibiting antibodies prior to administration of anti-MPO

IgG thwarts disease development in mice and MPO-deficient mice immunized with MPO, irradiated and then transplanted with bone-marrow from C5a-/- mice as well as C5aR knockout mice are protected from the disease development (70-72). Murine studies have furthermore demonstrated that C5a receptor 1 is required for induction of MPO-ANCA and neutrophil recruitment and activation in the kidney (73). Defects in Factor H, a major regulator of complement activation, have been shown to be present in patients with AAV and are associated with activation of neutrophils (74). Moreover, MPO has been demonstrated to be capable of activating the alternative complement pathway and to reduce the regulatory activity of Factor H (75, 76).

Although there is sparse complement deposition in the glomeruli in AAV, some studies have revealed C3 and C3c deposition in kidney biopsies (77-79) and furthermore found that the presence of these depositions were associated with more severe kidney injury (78) and worse overall and kidney survival prognosis (79). Another study on kidney biopsies from AAV patients found that the membrane attack complex (MAC), C3d , factor B and factor P were present in active lesions in patients with AAV but not C4d or mannose-binding lectin (MBL) (80). Similar findings were seen in another study on kidney biopsies from AAV patients where depositions of C3c, C3d and C5-9 were present in glomeruli. The same study demonstrated higher urinary C3a, C5a, C5b-9 and Bb levels in AAV patients with active disease compared to in remission and healthy controls (81). Another study reported elevated plasma levels of circulating C3a, C5a, soluble C5b-9 and Bb in patients with active disease compared to patients in remission but there was no difference in C4d levels (82). In a study of 76 AAV patients, 5% had low levels of C3 and/or C4. The overall and kidney prognosis was found to be worse for patients with hypocomplementemia and all patients with hypocomplementemia had kidney involvement (83). The complement involvement may not be limited to the alternative pathway, the results of a recent study indicate a role of immune-complex mediated activation of the classical pathway as well in the pathogenesis of AAV (84).

Schreiber et al showed in vitro that ANCA mediated activation of neutrophils induced activation of the complement cascade and a production of C5a in serum, which in turn resulted in priming of neutrophils and ANCA-induced respiratory burst (71). A later study demonstrated that by providing a scaffold for activation of the alternative complement pathway NETs contribute to endothelial injury (60). These findings suggest that neutrophil activation promotes activation of the alternative complement pathway leading to the generation of C5a which is believed to impact the priming of neutrophils. This causes a circle of amplification that contributes to the pathogenesis of AAV (85).

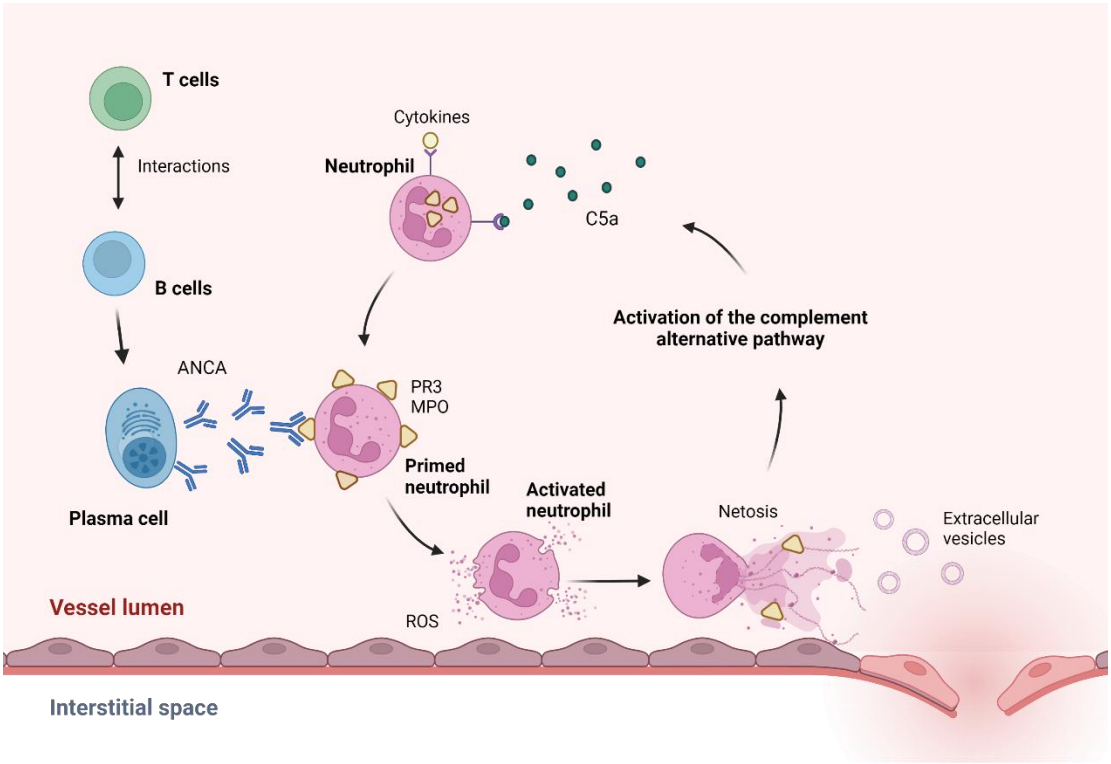


Figure 4. *Shematic presentation of pathways in the pathogenesis of AAV. Cytokines and C5a prime neutrophils. Primed neutrophils express PR3 and MPO which ANCA bind to. This binding leads to activation of the neutrophils with production of cytokines, respiratory burst, and release of neutrophil extracellular traps (NETs). During NETosis extracellular vesicles are released. This process results in vascular endothelial injury. Adapted from "Endothelial Barrier Inflammation and Leak (Layout)", by Biorender.com (2022). Retrieved from https://app.biorender.com/biorender-templates*

1.6.5 T-cells

Cellular immunity is also implicated in disease development in AAV. As noted above PR3 ANCA has been strongly associated with HLA-DP (42). $CD4^+$ T-cells promote the production of ANCA after presentation of PR3 and MPO (57). Numbers of CD4+ effector memory T cells are decreased in blood and elevated in urine in active AAV (86) and infiltration of both CD4+ and CD8+ T cells is seen in affected tissue (87, 88). Although there is conflicting evidence on the role of regulatory T-cells (Tregs) in AAV it is believed that impairment in their function could contribute to the loss of tolerance (89-91). Decrease in the number of Tregs have been associated with disease activity in AAV (92) and $CD4^+$T cells showing resistance to Treg cell suppression have been found in AAV patients (93). Levels of T helper 17 (Th17) cells, cells characterized by production of interleukin-17 (IL-17), have been demonstrated to be increased in patients with AAV. Levels of IL-17 and IL-23, a cytokine that stimulates the release of IL-17 from Th-17 cells, have furthermore been shown to be elevated in patients with active AAV (94). Moreover, IL-17 deficient mice developed less severe anti-MPO necrotizing GN (95). In patients treated with Rituximab (RTX), T-cell mediated tubulo-interstitial inflammation has been shown to predict worse kidney outcome (96).

1.6.6 B-cells

B-cells are believed to be of vital importance in the pathogenesis of AAV. B-cells produce ANCA and have become an important and effective therapy target. RTX, an anti-CD20 depleting antibody, causes B-cell depletion in the periphery and is today one of the first-line treatments in AAV (32). Animal studies have indicated that regulatory B-cells are important in suppressing immune responses in autoimmunity (97). The B cell survival factor B cell-activating factor (BAFF) has been found to be elevated in active AAV (98). In vitro studies have demonstrated increased expression of BAFF on the membrane surface of neutrophils following ANCA mediated activation and that BAFF release from ANCA-activated neutrophils promotes B-cell survival (99). A difference in B-cell subsets in AAV patients with higher C19 expression compared to controls may be important for autoreactive B-cell activation (100). Other studies have revealed a decrease in regulatory B-cells in AAV (101). Recently, Wang et al demonstrated elevated counts of a subset of circulating B-cells in active disease and a correlation with kidney injury (102). Furthermore, activated B cells have been shown to be present in granulomatous lesions in AAV (103). Unpublished but reported data demonstrates that although RTX treatment causes near-completion of plasma B-cells counts subsets of B-cells in tissues are not affected (104). This may explain, at least partly, the risk of relapse in RTX treated patients.

A schematic presentation of the pathways involved in the pathogenesis of AAV is shown in figure 4.

1.7 TREATMENT

The treatment consists of immunosuppressive therapy given in two phases, a remission induction phase that lasts for 3 to 6 months and a maintenance phase to prevent relapses (32).

1.7.1 Remission induction treatment

The goal with induction treatment in AAV is to reach a clinical remission.

1.7.1.1 Glucocorticoids

Glucocorticoids (GCs) have traditionally been a central part of AAV treatment although there are no trials that have assessed the effect of GCs alone. Patients with severe manifestations such as alveolar hemorrhage and rapidly progressive GN can be given intravenous (iv) GC pulses although the data on the benefits is sparse and it has moreover been shown to be associated with an increased risk of infections and diabetes mellitus (105). In the recently published PEXIVAS trial patients were randomized to a standard- or low-dose GC treatment.

A low-dose treatment was noninferior to a standard-dose when it came to mortality and kidney failure and there was a reduction in severe infections during the first year of follow-up in the low-dose group (106).

1.7.1.2 Cyclophosphamide

Cyclophosphamide (CYC) has been the standard treatment since the 1970s. Initially it was given orally in daily doses. There were however concerns of side effects and cumulative dose especially with repeated treatment courses. Later the CYCLOPS trial showed that iv CYC given in pulses was as effective at achieving remission as oral CYC therapy (107). There was a lower total dose of CYC and decreased risk of complications. In a long-term follow-up study, there were however more relapses in the iv pulsed group especially in those with PR3-ANCA positive disease (108).

1.7.1.3 Rituximab

RTX is a chimeric monoclonal mouse antibody directed against the CD20 antigen found on the surface of B-lymphocytes. Treatment with RTX leads to B cell depletion (109). Two randomized controlled trials (RCTs) have compared RTX to CYC, RAVE and RITUXIVAS (110, 111). Both showed RTX to be noninferior to CYC. Furthermore, RTX seems to be more effective in relapsing disease (111).

RTX in combination with CYC has been used as an induction treatment and seems to have a favorable outcome in severe AAV (112, 113).

1.7.1.4 Methotrexate and mycophenolate mofetil

Methotrexate (MTX) can be considered in patients with non-organ threatening disease and normal kidney function as an alternative to CYC and RTX (32). A RCT comparing MTX and daily oral CYC showed noninferiority in remission rates at 6 months. However, MTX was less effective in patients with pulmonary manifestations and more serious disease and long-term follow-up revealed higher relapse rate in patients receiving MTX (114).

The MYCYC RCT compared mycophenolate mofetil (MMF) to iv CYC treatment and found noninferiority in achieving remission although relapses were more common in the MMF group. (115). MMF treatment could be an alternative in patients with milder disease without life-threatening organ manifestations, especially in patients with MPO-ANCA positive disease (116).

1.7.1.5 Plasma exchange

Plasma exchange (PLEX) has traditionally been recommended in patient with rapidly progressive GN and with diffuse alveolar hemorrhage. However, the results of the PEXIVAS trial showed that PLEX did not lead to reduction of mortality or kidney failure (106). A recently published meta-analysis did not show any effect of PLEX on mortality but a reduced risk of kidney failure as well as an increased risk of serious infections at 12 months follow-up (117).

1.7.1.6 Complement inhibition

There has been a growing interest in the targeting the complement system with treatment while reducing the burden of GCs. A recently published RCT, the ADVOCATE trial, compared the use of prednisolone versus avacopan, a C5a receptor inhibitor, in combination with either CYC or RTX for induction therapy. The results demonstrated that avacopan was noninferior to prednisolone when it came to remission at 26 weeks and superior to prednisolone with respect to remission at 52 weeks. Additionally, patients treated with avacopan reported better kidney function, improved health related quality of life (QoL) and fewer infections (118).

1.7.2 Remission maintenance treatment

The second phase of AAV treatment is a remission maintenance treatment with the aim to avoid relapses in the disease.

Previously oral CYC in combination with GCs was used for remission maintenance. The CYCAZAREM trial compared oral CYC treatment with azathioprine (AZA) and showed a similar relapse rate in the groups (119). Another trial compared MMF and AZA and found higher relapse rate in the MMF group. Severe adverse events were similar in both groups. Thus, MMF is no longer considered a first line maintenance therapy unless AZA intolerance is present (120). There has been increasing focus on RTX as remission maintenance treatment in recent years. The MAINRITSAN trial compared RTX with AZA and demonstrated the superiority of RTX with significantly fewer relapses (121). All the above-mentioned studies were done on patients that had received CYC as induction treatment. Recently, the results of the RITAZAREM trial were presented where treatment with AZA was compared to RTX following an remission induction treatment with RTX for relapsing AAV. The results show that RTX was superior to AZA in maintaining remission (122).

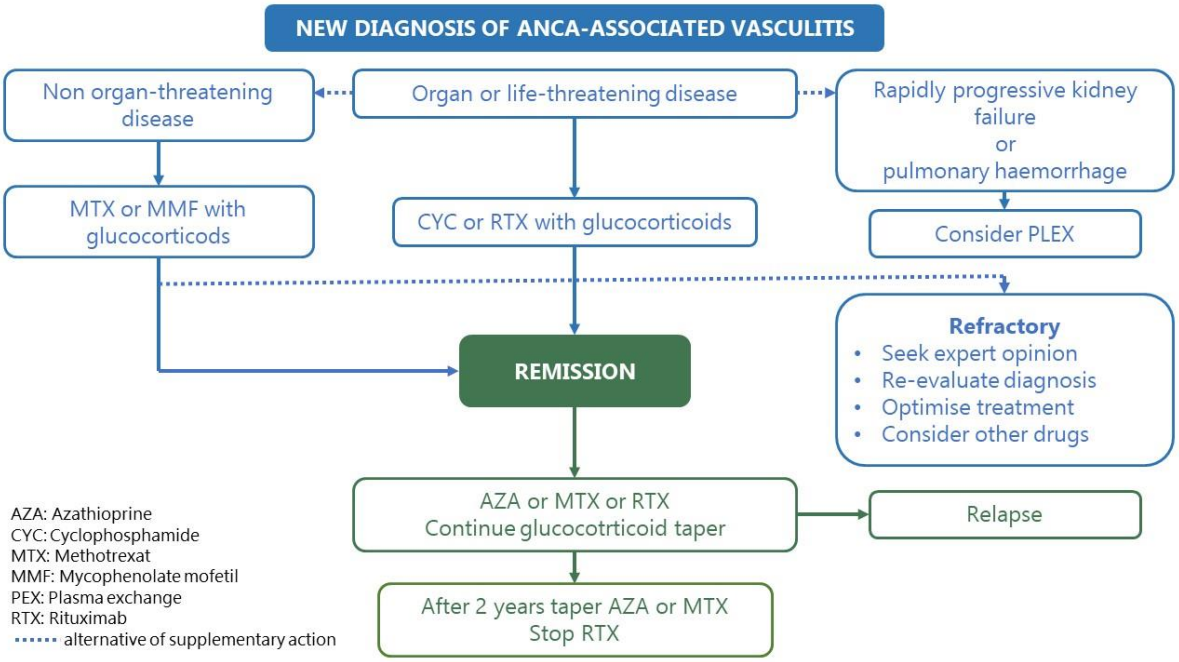


Figure 5. *Algorithm to describe the management of ANCA-associated vasculitis. Dashed lines indicate alternative or supplementary action to consider. Reproduced from (32) with permission from BMJ Publishing Group Ltd.*

The optimal duration of the remission maintenance treatment is unclear. One trial compared two- to four-year AZA and GC treatment duration. The results showed a significant decrease in relapses in the long-treatment group as well as better kidney outcome, but an increased risk of infection (123).

In 2016, the European League Against Rheumatism (EULAR), the European Renal Association, and the European Vasculitis Society (EUVAS) published an updated management recommendation for AAV (32). An algorithm to describe the management guidelines is shown in figure 5.

1.8 PROGNOSIS

Untreated AAV carries a 1-year mortality of around 80% with about 5 months median survival time (124). The outcome for AAV has however improved dramatically over time with modern treatment. In a systematic review the 5-year survival rate for MPA, GPA and EGPA was 45–76%, 74–91% and 60–97%, respectively (125). Nevertheless, current therapies are associated with adverse effects and these in combination with the severity and morbidity of the disease impact the prognosis (126, 127). The main cause of death in the first year after diagnosis are infections and active vasculitis with around 60% of deaths being related to therapy. Later the most common causes of mortality are cardiovascular disease (CVD), infections, and malignancy (127, 128). Advanced kidney disease, high disease activity and older age at presentation are all predictors of poor outcome (129).

Treatment is generally effective, but relapses are common with over 50% of patients relapsing within 5 years while some may have refractory disease (130-132). Risk factors that have been associated with increased risk for a disease relapse include PR3-ANCA positive disease, GPA phenotype, cardiovascular, pulmonary and ENT involvement, withdrawal of GCs, lower cumulative CYC dose and persistence of ANCA positivity (131, 133-136). Patients with kidney involvement (creatinine >200 µmol/l at diagnosis) seem to have a lower relapse risk (133) although sclerotic changes on kidney biopsy have been associated with increased risk for relapse in the long term (137). Relapse with kidney involvement has been associated with a loss in kidney function and an increased risk of kidney failure (138, 139). Long term damage in AAV is not only associated with disease features but also with treatment. The duration of GC use has been associated with increased long-term damage in AAV as well as disease severity at onset, age and number of relapses (140).

1.9 QUALITY OF LIFE

With advances in treatment AAV has become more of a chronic disease with a substantial risk of relapse. Although the prognosis has improved patients report a poorer physical and mental QoL compared to the general population even when in remission (141). Patients commonly experience symptoms such as fatigue, decreased energy and pain and rate them as severe manifestations (142). Reduced QoL is not only caused by disease manifestations or the high risk of relapse but also treatment related side-effects (143).

1.10 COMORBIDITIES

With modern treatment and improved diagnosis AAV has become a lifelong chronic disease with increased risk for comorbid conditions such as infections, CVD, venous thrombosis, and malignancies.

The use of immunosuppressive treatment increases the infection risk and today infections are the leading cause of morbidity and mortality in the first year after diagnosis of AAV (127, 128). Prophylactic treatment against pneumocystis jirovecii is recommended during induction treatment (32). Routine vaccination is recommended for all AAV patients including annual vaccination for influenza and pneumococcal infection, but live-attenuated vaccines should be avoided in patients on immunosuppressive therapy (144). Vaccination for Covid19 is recommended and patients with AAV should receive early booster doses due to risk of reduced vaccine response with ongoing immunosuppressive therapy (145). Treatment choices have also been influenced by the Covid19 pandemic due to concerns of reduced response to vaccination, particularly in relation to RTX treatment (146). The risk of severe SARS-Cov-2 infection and

Covid19 related mortality is increased in patients with AAV particularly in patients on high doses of immunosuppressive treatment and with impaired kidney function (147, 148).

Patients with AAV have an increased risk of CVD (149-151).The reason for this is probably multifactorial, the use of GCs, inflammatory state and impaired kidney function are all risk factors for the development of CVD (152-154).

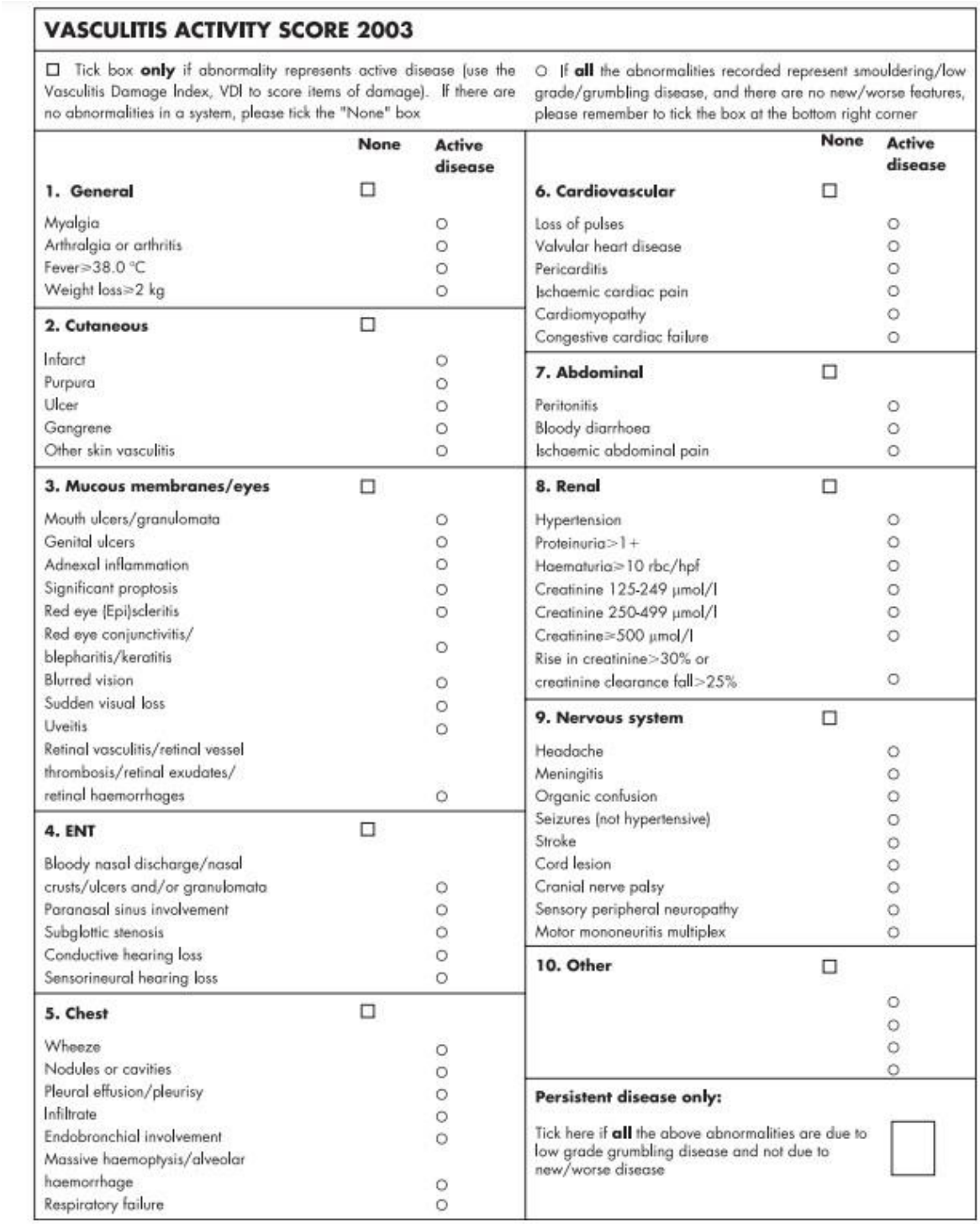
Malignancies are more frequent in AAV patients compared to the general population. Treatment with CYC increases the risk of urine bladder cancer, leukemia and non-melanoma skin carcinoma (155). AZA treatment is also known to be associated with increased risk of non-melanoma skin cancers (156).

Patients with AAV have an increased risk of developing venous thromboembolism (VTE) compared to the general population and even to patients with other auto-immune inflammatory diseases such as SLE and rheumatoid arthritis (RA) (149, 157, 158). Among the factors that have been associated with an increased risk of VTE are heart (159), gastrointestinal and cutaneous involvement (160), lung hemorrhage (159), MPO-ANCA positivity, increased disease activity and kidney involvement (161).

1.11 MONITORING, ASSESSMENT OF DISEASE ACTIVITY AND DAMAGE

The aim of monitoring patients in AAV is an early identification of a disease relapse as well as assessing treatment related toxicity. Clinical evaluation and regular testing (blood and urine) are the mainstay of monitoring. Non-specific inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are generally of limited value (162-165) and as noted previously, ANCA titers are not reliable disease activity markers (51, 166-168). Urine analysis is used for monitoring kidney disease activity although non-specific, haematuria may be an indicator of kidney flare but can also reflect chronic glomerular injury (169, 170). Although the degree of proteinuria in active disease is typically associated with kidney outcome (171, 172) it can be related to irreversible damage and glomerular scarring. Tissue biopsies can be useful when organ-specific disease is suspected such as kidney biopsies in case of persistent abnormal findings on urine analysis. However, repeat biopsies to monitor kidney disease are not routinely performed and carry a risk of complications (173).

Additionally, treatment specific follow-up include monitoring for myelosuppression, particularly leukopenia, after CYC treatment and late-onset neutropenia as well as hypogammaglobulinemia in patients treated with RTX, which can affect treatment decision (2, 25, 174, 175).



VASCULITIS ACTIVITY SCORE 2003

☐ Tick box **only** if abnormality represents active disease (use the Vasculitis Damage Index, VDI to score items of damage). If there are no abnormalities in a system, please tick the "None" box

○ If **all** the abnormalities recorded represent smouldering/low grade/grumbling disease, and there are no new/worse features, please remember to tick the box at the bottom right corner

	None	Active disease
1. General	☐	
Myalgia		○
Arthralgia or arthritis		○
Fever≥38.0 °C		○
Weight loss≥2 kg		○
2. Cutaneous	☐	
Infarct		○
Purpura		○
Ulcer		○
Gangrene		○
Other skin vasculitis		○
3. Mucous membranes/eyes	☐	
Mouth ulcers/granulomata		○
Genital ulcers		○
Adnexal inflammation		○
Significant proptosis		○
Red eye (Epi)scleritis		○
Red eye conjunctivitis/ blepharitis/keratitis		○
Blurred vision		○
Sudden visual loss		○
Uveitis		○
Retinal vasculitis/retinal vessel thrombosis/retinal exudates/ retinal haemorrhages		○
4. ENT	☐	
Bloody nasal discharge/nasal crusts/ulcers and/or granulomata		○
Paranasal sinus involvement		○
Subglottic stenosis		○
Conductive hearing loss		○
Sensorineural hearing loss		○
5. Chest	☐	
Wheeze		○
Nodules or cavities		○
Pleural effusion/pleurisy		○
Infiltrate		○
Endobronchial involvement		○
Massive haemoptysis/alveolar haemorrhage		○
Respiratory failure		○

	None	Active disease
6. Cardiovascular	☐	
Loss of pulses		○
Valvular heart disease		○
Pericarditis		○
Ischaemic cardiac pain		○
Cardiomyopathy		○
Congestive cardiac failure		○
7. Abdominal	☐	
Peritonitis		○
Bloody diarrhoea		○
Ischaemic abdominal pain		○
8. Renal	☐	
Hypertension		○
Proteinuria>1+		○
Haematuria≥10 rbc/hpf		○
Creatinine 125-249 μmol/l		○
Creatinine 250-499 μmol/l		○
Creatinine≥500 μmol/l		○
Rise in creatinine>30% or creatinine clearance fall>25%		○
9. Nervous system	☐	
Headache		○
Meningitis		○
Organic confusion		○
Seizures (not hypertensive)		○
Stroke		○
Cord lesion		○
Cranial nerve palsy		○
Sensory peripheral neuropathy		○
Motor mononeuritis multiplex		○
10. Other	☐	
		○
		○
		○
		○

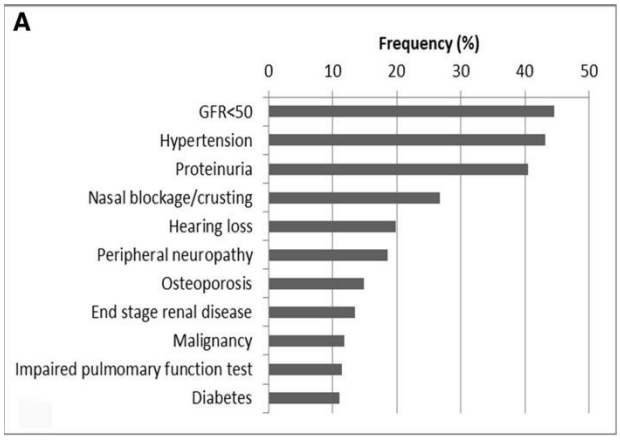
Persistent disease only:

Tick here if **all** the above abnormalities are due to low grade grumbling disease and not due to new/worse disease ☐

***Figure 6**. Birmingham Vasculitis Activity Score (BVAS), version 3. Reproduced from (177) with permission from BMJ Publishing Group Ltd.*

For the purpose of clinical studies, EULAR and the Outcomes Measures in Rheumatology (OMERACT) groups has recommended using a disease activity score list such as BVAS, for assessment of disease activity (176). BVAS is a list with 56 items divided into ten categories, one with general features, eight organ-specific and one open (figure 6). Manifestations are only scored when they or of new-onset or worsening. Remission is defined as a score of 0 (177, 178). The Vascular Damage Index (VDI) is used for standardized evaluation of damage. The

index contains 11 systems, 10 organ-specific and one for other damage. The VDI not only quantifies disease related damage but also damage due to treatment (179). As shown in figure 7 patients with AAV commonly experience not only disease related damage but even damage caused by treatment (140).



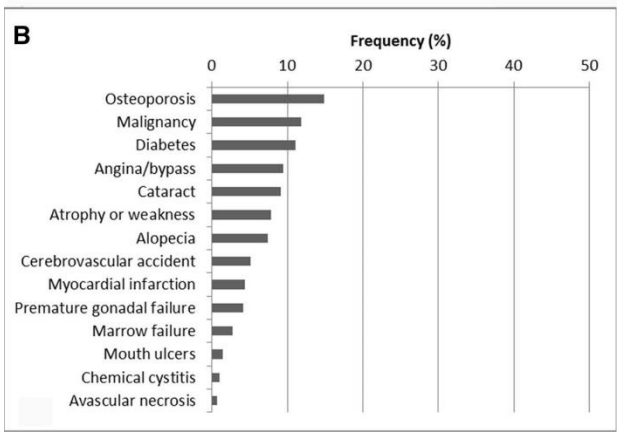


Figure 7*. Frequency of VDI items of damage at a long-term follow-up from four European Vasculitis Study Group trials. A) Frequency of the most common VDI items of damage at a long-term follow-up. B) Frequency of the 15 treatment-related damage items at a long-term follow-up. VDI: Vasculitis Damage Index; GFR: glomerular filtration rate. Reproduced from (140) by permission of Oxford University Press.*

Histopathological scoring systems have been developed for ANCA associated vasculitis. The Berden classification describes histopathological changes that are divided into four categories, focal, crescentic, mixed, and sclerotic (180). The categories have been shown to predict outcome with sclerotic lesions being associated with worse outcome and focal changes with a more favorable outcome (181). Another clinicopathological score to predict kidney outcome was developed by Brix et al. Beside glomerular change, the score includes interstitial and tubular changes as well as estimated glomerular filtration rate (eGFR) at diagnosis (182).

1.12 SPECIFIC BACKGROUND OF THE THESIS STUDIES

In this section, the biomarkers investigated in the studies included in the thesis are presented.

1.12.1 Biomarkers

The definition of a biomarker is a "defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions". Biomarkers can be molecular, histologic, radiographic, digital, or physiologic characteristics. There are various benefits of biomarkers

including disease diagnosis, monitoring, predicting prognosis and response to treatment (183) (figure 8).

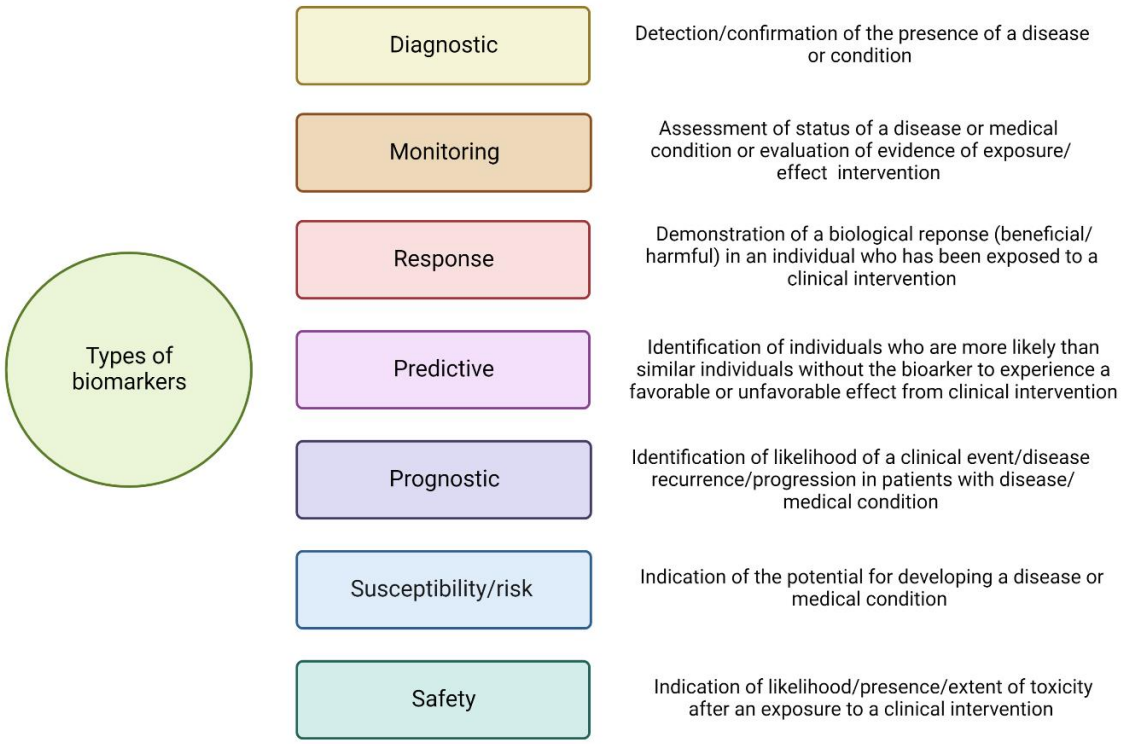


Figure 8*. Seven categories of biomarkers according to the classification by the FDA-NIH Biomarker Working Group (183). Created with Biorender.com.*

There are no reliable biomarkers available for evaluating disease activity, predicting flares, or guiding individual treatment in AAV. Relapses are common and require treatment modifications. It is essential to identify novel biomarkers as it would enable tailored individual treatment, thwart relapses, decrease the need for repeat biopsies as a diagnostic tool and potentially reduce adverse treatment effects.

1.12.2 Study II: Pentraxin 3

Pentraxins are a superfamily of multifunctional proteins. They are divided into short pentraxins such as CRP and serum amyloid P component (SAP) and long pentraxins such as Pentraxin-3 (PTX3) (184). In contrast to CRP and SAP, that are mainly produced in the liver in response to IL-6, PTX3 is produced locally by a variety of cells, including neutrophils vascular endothelial cells, dendritic cells, macrophages, monocytes and kidney epithelial cells in response to various inflammatory cytokines such as IL-1β and tumor necrosis factor α (TNFα). PTX3 is therefore believed to more closely reflect local inflammation in tissues compared to CRP (185, 186).

PTX3 has a complex oligomeric structure with two tetramers connected by covalent bonds composing octamers (187). PTX3 plays a multifunctional role in innate immunity and

inflammation (figure 9). PTX3 is a soluble pattern recognition receptor (188) and has the ability to recognize a number of different ligands, including pathogens, apoptotic cells, complement components and extracellular matrix proteins (185, 188) and is able to activate opsonization and promote phagocytosis (189). PTX3 contributes to both complement activation as well as regulation (190). It is known to interact with C1q, a recognition protein for the classical complement pathway, (191, 192), Ficolin-2, activator of the lectin pathway (193), and it has been shown to bind to factor H, a principal regulatory protein in the alternative pathway. Through this it has a modulating effect on regulation in tissue injury where it prevents an excessive inflammatory response (194). Moreover, neutrophils store vast amounts of PTX3 in granules which is released upon respiratory bursts and PTX3 can be found in NETs (195) and it even seems to be instrumental in the regulation of NETs (196). Additionally, PTX3 regulates inflammation and dampens neutrophil recruitment (197) and is believed to play a role in tissue remodeling, vascular damage and angiogenesis and may be an indicator of vascular inflammation (198, 199). PTX3 has been shown to be present in endothelial cells from skin biopsies in patients with small-vessel vasculitis (200).

Circulating PTX3 levels have previously been shown to be elevated in various autoimmune diseases including SLE (201-203), RA (204, 205) systemic sclerosis (SSc) (206, 207) and Takayasu arteritis (208). PTX3 has also been reported to be elevated in chronic kidney disease (CKD) (209) and to predict all-cause mortality in patients with CKD (210). Murine model studies have indicated that PTX3 may limit post ischemic acute and chronic kidney injury (211) and shown that PTX3 administration leads to diminished interstitial fibrosis in the kidney (212).

Previous studies have assessed PTX3 levels in a cross-sectional case control studies of AAV patients, demonstrating higher levels in patients with active compared to inactive disease and healthy controls (200, 213). Anti-PTX3 antibodies have also been detected in AAV patients (214, 215) but their role in the pathogenesis has not been demonstrated and the significance of these findings is not clear. Despite being a candidate biomarker in AAV, PTX3 has previously not been studied in a longitudinal cohort of AAV patients.

1.12.3 Study III: TNF-like weak inducer of apoptosis

TNF-like weak inducer of apoptosis (TWEAK) is a type II transmembrane glycoprotein of the TNF superfamily. TWEAK is cleaved by proteolysis and circulates in plasma in a soluble form

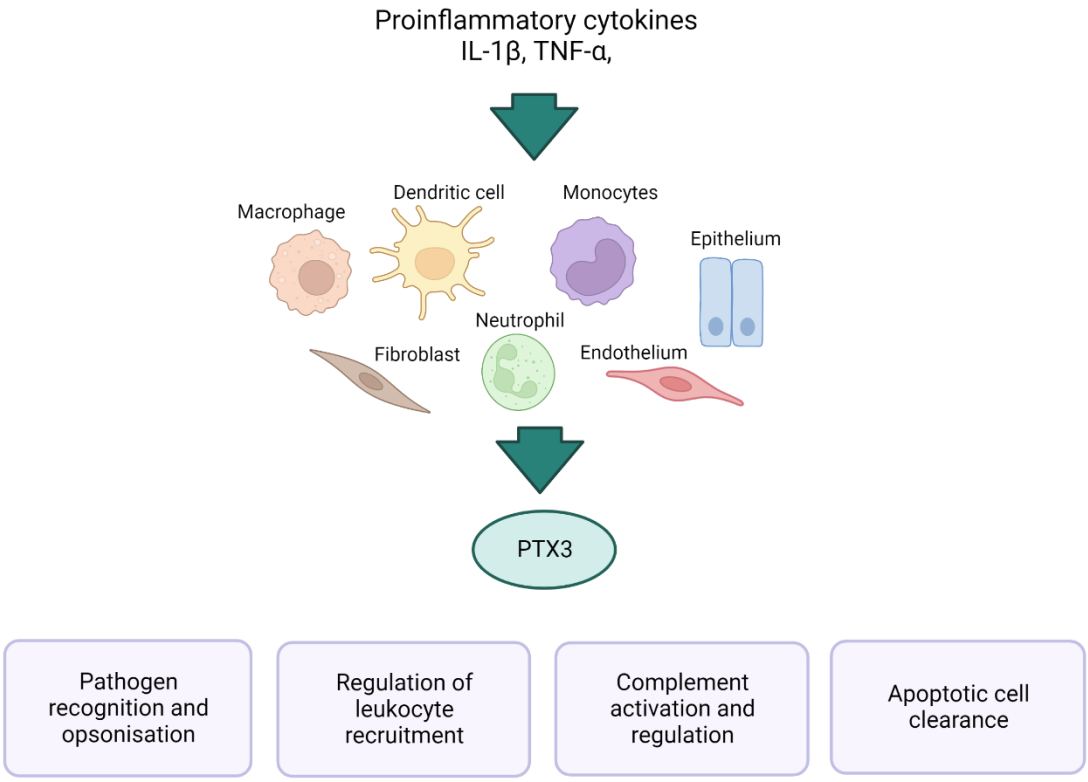


Figure 9. *Schematic presentation of the cellular sources and examples of the roles of pentraxin-3 (PTX3). Created with Biorender.com,*

in trimers. TWEAK expression has been shown in various inflammatory cells such as monocytes, dendritic cells, and natural killer (NK) cells (216-218) as well as in endothelial cells and tubular cells in the kidney (219, 220). TWEAK in its soluble and membranous form mediates several important biological effects by activating its receptor, fibroblast growth factor-inducible-14 (Fn14) (221, 222). Fn14 is expressed on various cells including, endothelial, epithelial cells (223, 224) as well as on podocytes, tubular cells and mesangial cells in the kidney (218, 219, 225, 226). The effects of TWEAK/Fn14 depends on cells and circumstances (227) and include upregulation of inflammatory cytokines and chemokines (228), induction of endothelial cell survival and proliferation (229), cell death by apoptosis and necrosis (230, 231) and induction of fibrogenesis (232) (figure 10). TWEAK has also shown to bind to the scavenger receptor CD163. Urinary soluble CD163 has been suggested to be a potential biomarker in ANCA-associated GN (233-235).

TWEAK/Fn14 is moreover a factor in the development of kidney injury (236). In the absence of kidney disease Fn14 expression is low but is upregulated after kidney injury and TWEAK significantly activates the inflammatory response under these conditions (237). TWEAK blockade has been shown to decrease the severity of disease in a murine experimental lupus nephritis (LN) model (238). Serum TWEAK (sTWEAK) decreases with progressive loss of

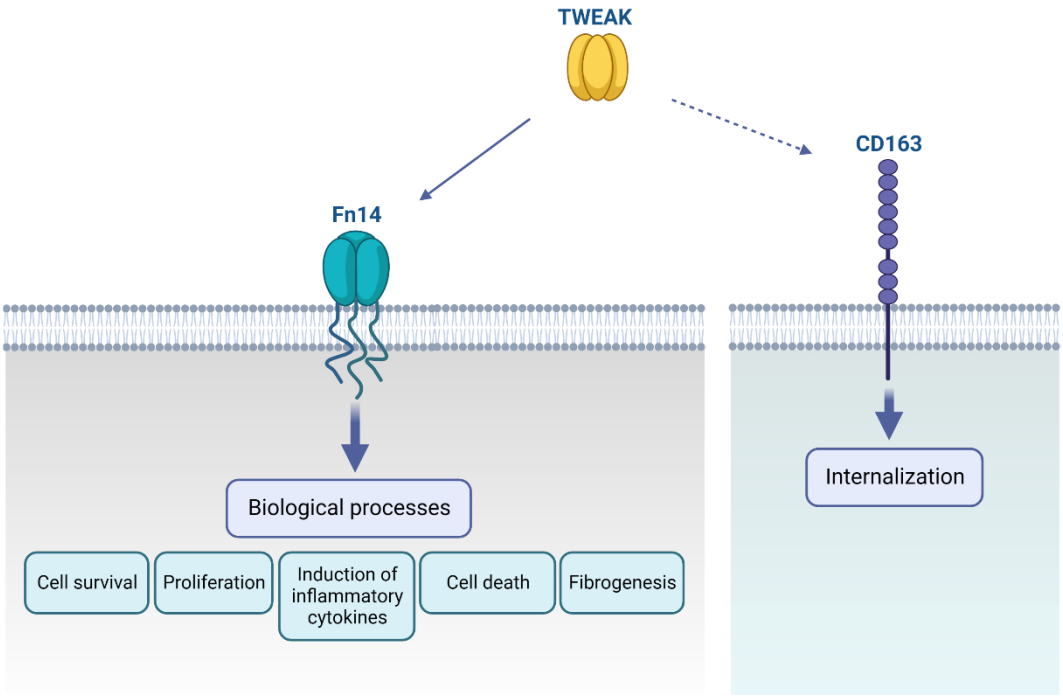


Figure 10. *A schematic representation of TWEAK and receptors. TWEAK binds to Fn14, expressed on various cells, to activate intracellular signaling and downstream cellular responses. The CD163 receptor, expressed exclusively on macrophages, binds to TWEAK and is believed to act as a scavenger receptor. Fn14: factor-inducible molecule 14, TWEAK: tumor necrosis factor-like weak inducer of apoptosis. Created with biorender.com.*

kidney function, increases after renal transplantation and is associated with worse outcome in chronic kidney disease (239, 240).

sTWEAK levels are increased in patients with certain autoimmune inflammatory diseases and have been found to correlate with disease activity in patients with RA (241), IgA vasculitis (242) and SLE (243, 244). Urinary TWEAK (uTWEAK) has been shown to be a potential biomarker in LN and IgA nephropathy (IgAN) (245-248). The role of TWEAK has not previously been investigated in a longitudinal cohort of AAV patients.

1.12.4 Study I and IV: Extracellular vesicles expressing biomarkers

Extracellular vesicles (EVs) are small membranous vesicles released by various cell types, including platelets, neutrophils, endothelial cells and monocytes (249, 250). EVs express membrane markers of the parent cells and their composition does not only depend on their origin but is also affected by the environment and process leading up to their formation (251). EVs have been shown to play a role in cell-to-cell communication and contribute to angiogenesis, thrombosis and inflammation (252, 253). Despite their role as pro-inflammatory components EVs can also exert an anti-inflammatory effect (254). EVs are divided into three subtypes based on their size, cellular origin, function, release pathway and biogenesis; 1) exosomes, derived from intracellular organelles, typically 30-150 nm in diameter, 2)

microvesicles, derived from the cell membrane, typically 100-1000 nm in diameter and 3) apoptotic bodies, derived from dying cells, typically 50-5000 nm in diameter (255) (figure 11). The International Society for Extracellular Vesicles (ISEV) have endorsed the term "extracellular vesicles" as the generic term to be applied, with specific terms only to be applied where there is evidence of the subcellular origin (256). In this text the term EVs will therefore be used.

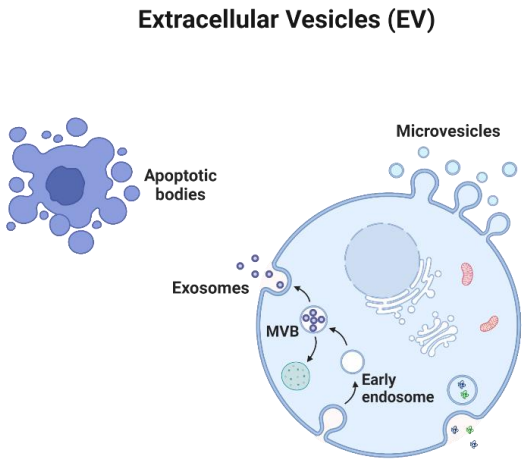


Figure 11. *Three subtypes of extracellular vesicles (EVs). Adapted from "Extracellular Vesicle Separation by Density Gradient Ultracentrifugation", by BioRender.com (2022). Retrieved from https://app.biorender-.com/biorender-templates.*

EVs have gained increasing interest as novel promising biomarkers in systemic inflammatory diseases (257) although their potential use in AAV is not yet delineated. As previously described ANCA-induced neutrophil activation plays a pivotal role in the pathogenesis of AAV (258, 259). Primed neutrophils undergo degranulation, respiratory burst and release of NETs (57). During this process EVs are released. Levels of circulating endothelial derived EVs have been shown to be elevated in patients with AAV and to correlate with disease activity (260, 261). Because of the well described role of neutrophils in the pathogenesis of AAV the focus has more recently been on neutrophil derived EVs (NEVs) in the context of AAV. ANCA have shown to induce EVs release from primed neutrophils in vitro, including EVs containing PR3 and MPO antigens (262). Pitanga et al showed that EVs derived from neutrophils contain active MPO and these EVs cause endothelial cell damage by the loss of morphology and membrane integrity (263). Previous studies have demonstrated elevated levels of leucocyte and neutrophil derived EVs in AAV patients (261, 264). In a previous study from our group MPO positive EVs (MPO^+EVs) expressing complement factors C3a and C5a were shown to be elevated in AAV patients compared to controls and levels also correlated with BVAS. The concentrations were also significantly higher with kidney involvement compared to patients without (265).

As noted previously is PTX3 found in neutrophils granules and released following activation of neutrophils and is found in NETs (195). TWEAK is also of interest as it plays a role in development of kidney injury and is found in increased levels in various systemic inflammatory diseases as described previously (236, 241, 243, 244). Another molecule of interest in AAV is

high mobility group box 1 (HMGB1) protein that is believed to contribute to priming of neutrophils, translocation of ANCA antigens to neutrophil cell surface and potentiate ANCA induced NET formation (266, 267). Elevated levels of HMGBI have been shown in AAV patients with kidney involvement compared to inactive kidney disease (268) and found to correlate with AAV disease activity (269).

A crucial step in NETs formation is the citrullination of histone-3 by the enzyme peptidyl-arginine deiminase 4 (PAD4) leading to chromatin condensation (270). Citrullinated histone 3 (H3Cit) is considered a specific biomarker for NETs and can be measured in plasma (271). The expression of H3Cit on EVs has been described in an human endotoxemia model (272). To our knowledge EVs expressing H3Cit have not been explored in systemic inflammatory diseases. NETs are known to contribute to thrombosis in inflammatory state by interacting with platelets and activation of the coagulation cascade (273). The presence of NETs have previously been reported in venous thrombi derived from patient with AAV (274).

Tissue factor (TF) is a principal initiator of physiological coagulation, the intravascular exposure of TF on the surface of blood cells and circulating EVs is a main trigger of thrombus formation (275). It has been demonstrated that EVs bearing TF can bind and transfer lipids and proteins to activated platelets (276). Following stimulation by ANCAs, NEVs and NETs express TF, and the expression of TF on NEVs has been found to be elevated in plasma from patients with AAV (277). Elevated EV TF activity has been associated with VTE risk in AAV patients (278). Moreover, it has been demonstrated that NEVs released by primed neutrophils in the presence of PR3-ANCA enhance thrombin generation (262).

Plasminogen (Plg), a major component of the fibrinolytic pathway is a precursor of the active enzyme plasmin that degrades fibrin (279). The presence of anti-Plg and anti-tissue plasminogen activator (tPA) antibodies have been demonstrated to be present in AAV patients (280-282). Anti-Plg antibodies have been shown to be associated with VTE (282), disease activity (281), kidney function impairment, glomerular necrosis and cellular crescents in patients with AAV (280). Beside its role in fibrinolysis, plasminogen contributes to various inflammatory regulatory processes (283).

The expression of the above-mentioned candidate biomarkers on circulating MPO positive EVs has not been investigated previously in patients with AAV

2 RESEARCH AIMS

The overall aim of this thesis was to identify potential non-invasive biomarkers that could be useful in monitoring disease activity in AAV and to evaluate the role of these potential biomarkers in the pathogenesis of AAV.

The specific objectives for the studies were as follows.

Study I

- To assess the expression of PTX3, HMGB1, and TWEAK on circulating MPO^+EVs in patients with AAV.
- To investigate the role of MPO^+EVs expressing PTX3, HMGB1, and TWEAK as potential biomarkers of inflammatory processes by comparing levels in AAV patients with active and inactive disease to healthy controls.

Study II

- To explore the role of plasma and urinary PTX3 as potential biomarkers in a longitudinal cohort of AAV patients.

Study III

- To investigate the role of serum and urinary TWEAK as potential biomarkers in a longitudinal cohort of AAV patients.
- To evaluate TWEAK and Fn14 expression in kidney biopsies from patients with AAV with kidney involvement compared to control kidney tissue.
- To investigate the association between TWEAK levels and histopathological scoring in patients with AAV.

Study IV

- To investigate the expression of H3Cit, TF and Plg on circulating MPO^+EVs in patients with active and inactive AAV compared to controls.
- To study the expression of MPO^+EVs expressing H3Cit, TF and Plg in relation to thrombin generation in patients with AAV.

3 MATERIALS AND METHODS

3.1 PATIENTS

3.1.1 The cohort

Since 2009 the Departments of Nephrology and Rheumatology at the Karolinska University Hospital in Stockholm have collaborated on a research project where patients with AAV have been included in a well phenotyped patient cohort (the "VASKA" cohort). A share of the patients has been included at diagnosis (new diagnosis or a disease relapse) and followed prospectively ("VASKA-long"). To date about 360 patients have been included, whereof about 230 have been included into a cross-sectional study ("VASKA-tvär") and about 130 newly diagnosed or relapsing patients have been included prospectively into the longitudinal cohort with up to 10 years follow-up. Patients are considered for inclusion if they have a diagnosis of AAV (GPA, MPA or EGPA), are over 18 years of age and have or have had a positive ANCA serology (PR3 or/and MPO). At inclusion clinical evaluation is performed. Blood and urine samples are collected, including peripheral blood mononuclear cells (PBMC) and genetic samples, and ANCA measurement performed. Disease activity is assessed with BVAS and organ damage with VDI. In addition, all patients fill out several questionnaires, including QoL, educational needs assessment tool, and Multidimensional Assessment of Fatigue (MAF). Inclusion of new patients is ongoing. The characteristics of the "VASKA" cohort are shown in figure 12.

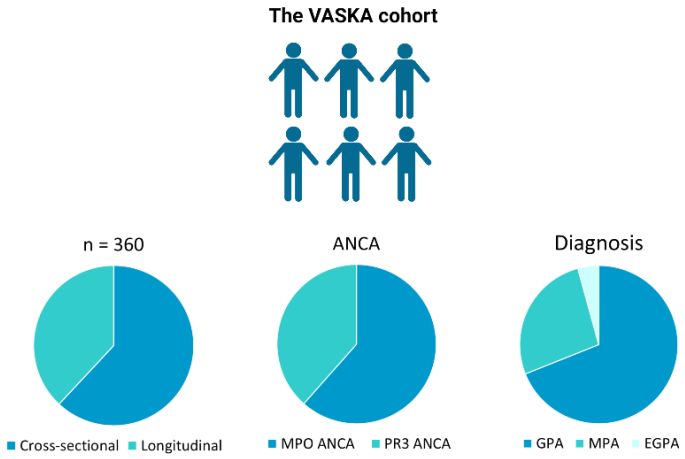


***Figure 12**. The "VASKA" cohort. Characteristics of the ANCA-associated vasculitis cohort at Karolinska University Hospital. ANCA: antineutrophil cytoplasmic antibodies; MPO: myeloperoxidase; PR3: proteinase-3, GPA: granulomatosis with polyangiitis, MPA: microscopic polyangiitis, EGPA: eosinophilic granulomatosis with polyangiitis. Created with Biorender.com.*

Study I and IV

A cross-sectional cohort of patients with active and inactive AAV were included in the studies. Inclusion criteria were age ≥18 years and the presence of MPO- and/or PR3 ANCA.

Study II and III

Patients with active AAV were included in the study. The patients were included at diagnosis, either with a new diagnosis or a disease relapse, and were followed prospectively for 6 months. The patients were ≥18 years of age and were all positive for MPO- and/or PR3 ANCA.

3.2 CONTROLS

Study I and IV

Age and sex-matched subjects from a population-based cohort were included for comparison. The controls have been described in a previous study (284).

Study II

Randomly selected controls from the Swedish Population Registry were included in for comparison. The controls have been described in a previous study (284).

Study III

Randomly selected controls from a population-based cohort in Stockholm were included for comparison. The controls have been described in previous studies (285, 286).

Treatment

The immunosuppressive treatments, remission induction treatment and remission maintenance treatment, patients received were recorded. The daily dose at inclusion was registered and the cumulative GC dose at inclusion was calculated. In **study II and III** the daily dose at follow-up was registered. In case of inclusion of patients at relapse, the cumulative GC dose was calculated from the diagnosis of relapse when higher doses as part of induction treatment were started. Lower doses (up to 10 mg) before the diagnosis of relapse were not included in the calculation of the cumulative dose.

3.3 ASSESSMENT OF DISEASE ACTIVITY AND KIDNEY INVOLVEMENT

Disease activity was assessed using BVAS version 3 (178) according to EULAR recommendations (32). The BVAS was recorded by an experienced nephrologist or rheumatologist for all patients at inclusion and at the follow-up in the longitudinal patient group. Disease remission was defined as a BVAS of 0. Disease relapse was defined as an increase in disease activity after remission, requiring change in treatment.

Kidney involvement was defined as kidney biopsy findings consistent with pauci-immune vasculitis or a clinical presentation with elevated creatinine levels and/or significant haematuria (defined as ≥ 2 dipstick urinalysis or ≥ 10 erythrocytes per high-power field on urinary sediment).

3.4 BLOOD AND URINE SAMPLING

Peripheral venous blood was collected. Serum and plasma were obtained, centrifugated within 4 hours, divided into aliquiots and stored frozen at -70°C for future analysis. Additionally, citrated plasma was collected, centrifugation was performed within 1 hour after sampling and samples were stored frozen at -70°C.

Morning urine samples were obtained and stored frozen at 70°C within 4 hours for future analysis.

3.5 LABORATORY MEASUREMENTS

In all studies routine laboratory analyses were carried out using standard methods at the Department of Clinical Chemistry at the Karolinska University Hospital, these included serum CRP, plasma creatinine, ESR, urine dip stick and urine sediment. Quantification of albuminuria was carried out by measurement of albumin-to-creatinine ratio (mg/mmol) in a morning urine sample using standard methods at the Department of Clinical Chemistry at Karolinska University Hospital. In **study II, III and IV** eGFR was calculated using the CKD Epidemiology Collaboration (CKD-EPI) equation (287) and in **study I** the Lund Malmö equation (LM revised) (288) was used.

3.6 ANCA TESTING

PR3- and MPO-ANCA titers were analysed using standard ELISA methods (direct ELiA, Euro diagnostic) or multiplex (BIORAD, BioPlex TM 2200) according to clinical routine at the Department of Clinical Immunology at Karolinska University Hospital.

3.7 PENTRAXIN-3, TWEAK, HMGB1

In **study I and II** serum/plasma and urine PTX3 levels were analysed using a commercially available ELISA kit from R&D Systems Europe Ltd (Abington, UK). The urinary pentraxin-3 to creatinine ratio (uPTX3/Cr) was calculated (ng/mmol) for normalization.

In **study I** serum TWEAK levels were determined using Human TWEAK ELISA kit (Thermo Scientific USA). In **study III** serum and urinary TWEAK levels were measured by TWEAK ELISA kit from BioScience (Hatfield, UK). Urinary TWEAK levels were normalized to urine creatinine concentrations measured in the same urine sample (ng/mmol).

In **study I** serum HMGB-1 levels were measured using the commercial Tecan HMGB1 ELISA Kit (Fisher Scientific, USA).

3.8 DETECTION OF EXTRACELLULAR VESICLES USING FLOW-CYTOMETRY

(The term microparticles was used in **paper I** but according to the IVES recommendation the term extracellular vesicles will be used in this thesis).

As described above peripheral blood was collected in vacutainer tubes (Becton Dickinson) containing trisodium citrate). Serum, respectively platelet poor plasma (PPP) was obtained within 60 min of sampling by centrifugation at 2000 g for 20 min at room temperature (RT) and stored frozen -70°C. PPP was thawed in a water bath at 37°C for approximately 5 min, followed by centrifugation of samples at 2000 g for 20 min at a room temperature (RT), in order to remove any cells or larger debris that might interfere with the analysis. The superanatant was centrifuged again at 13,000 g for 2 min in RT. Twenty µl of the superantant was incubated in dark for 20 min with 5 µl of monoclonal antibodies, anti-MPO-PE (Beckman Coulter, Brea, CA, USA) together with antibodies for pentraxin 3-Dylight 488 (anti-pentraxin 3, Abcam Cambrigde, UK), HMGB1-Dylight 488 (R&D Systems, MN, USA), and TWEAK-Dylight 633 (anti-TWEAK, LSBio.Inc., Seatttle, WA, USA) **(Study I)** and with antibodies for tissue factor (CD142,D, NJ, USA), citrulinated histone-3 (anti-H3it, Abcam, Cambridge, UK) and anti-plasminogen (Abcam, Cambridge, UK) (**Study IV**). Samples were fixed prior to analysis (Cellfix, BD, NJ, USA) after incubation. Measurement of EVs was performed by flow cytometry on a Beckman gallios instruemnt (Beckman Coutler, Brea, CA, USA) with the threshold set to forward scatter. The EVs gate was determined using Megamix plus beads (0.3-1.0 µm, Bioytex, Marseille). MPO^{+}EVs were defined as particles <1.0 µm in size and positive for anti-MPO PE. Conjugate isotype-matched immunoglobulins with no reactivity against

human antigens were used as negative controls (IgG PE, IgG Dylight 633, Dylight 488, and Dylight 755, Abcam, Cambrigde, UK). Results were presented as EVs/µl plasma, processeed from the 20 µl supernatant obainted after centrifugation. The intra- and interassay coefficient of variation for MPO^{+}MPs measurement were less than 9%.

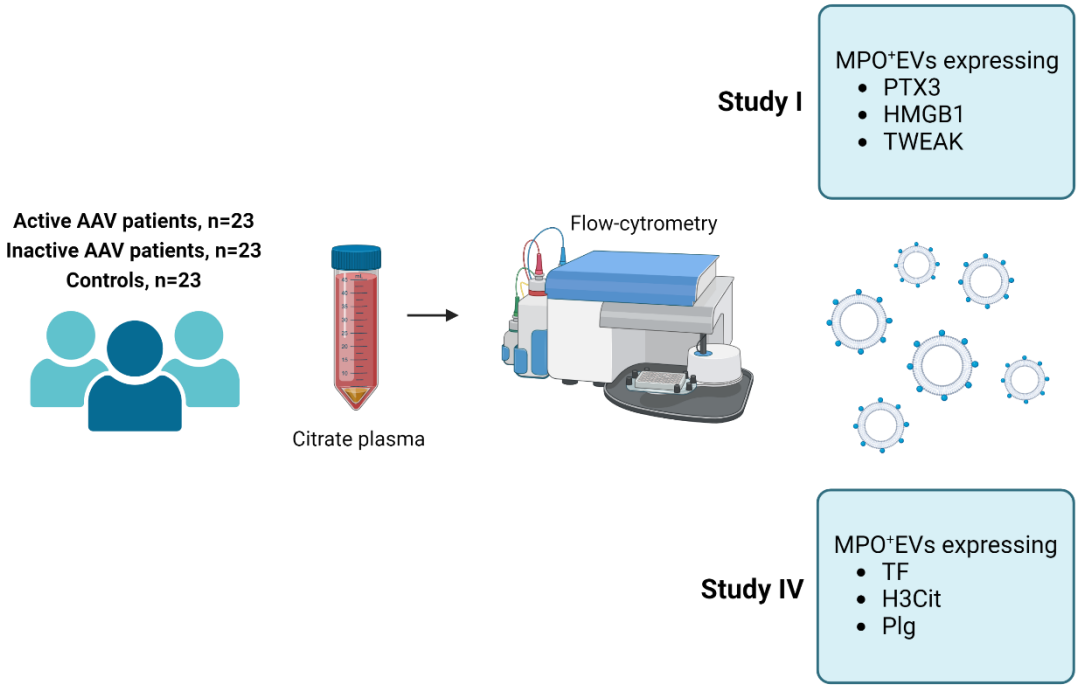


Figure 13. *The use of flow-cytometry in study I and IV. Flow-cytometry assay were performed on citrate samples for detection of MPO positive extracellular vesicles expressing PTX3, HMGB1, TWEAK (study 1) and TF, H3Cit and Plg (study IV). MPO: myeloperoxidase; EV: extracellular vesicle; PTX3: Pentraxin-3, HMGB1: high mobility group box 1; TWEAK: TNF-like weak inducer of apoptosis, TF: Tissue factor; H3Cit: Citrullinated histone-3, Plg: Plasminogen. Created with biorender.com.*

3.9 ACTIVITY ASSAY

In **study IV**, a modified method based on Calibrated Automated Thrombogram (CAT – assay) (289) was used to evaluate the pro-coagulant effect of EVs on thrombin generation. Measurements were carried out in transparent u-bottom 96-well plate on Thermo Scientific Flurosakan Ascent (Thermo Scientific). PPP was thawed in a water bath at 37°C for approximately 5 minutes, followed by two subsequent centrifugations, first at 2000g for 20 minutes and then at 20000g for 45 minutes, both at RT, to obtain an EVs-enriched pellet. The pellet was added to a previously centrifugated normal pool plasma (NPP) from healthy control (2000g for 30 min at RT), without the addition of TF or phospholipids that the original CAT method requires. Thrombin generation was measured during 60 minutes at 37°C. The experiment was performed with the pellet from all patients and all control samples. Thrombin generation curve (Thrombogram) was generated by measuring fluorescence each 20 seconds

and different parameters of thrombin generation were calculated by the analyzing software (Thrombinscope).

The calculated area under the curve (AUC) represents the total amount of thrombin generated over time and is called endogenous thrombin potential (ETP). Time to start of thrombin generation is the lag time, the maximal concentration of thrombin generated is called peak thrombin and the time to maximal thrombin generation is called time to peak (figure 14).

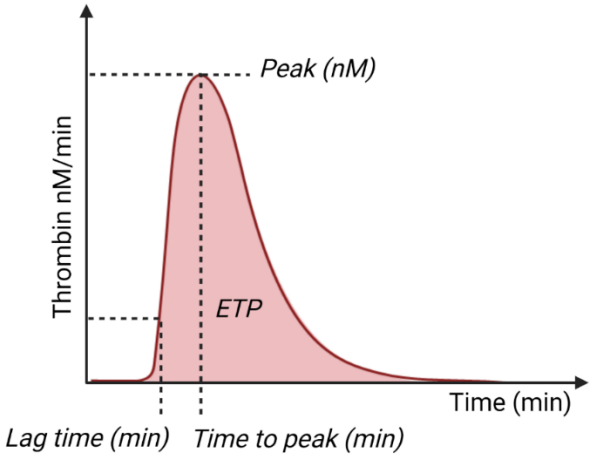


***Figure 14**. Schematic presentation of a thrombin generation curve and parameters. ETP: endogenous thrombin potential. Created with BioRender.com*

3.10 IMMUNOHISTOCHEMICAL STAINING

In **study III** kidney biopsies from AAV patients were stained for TWEAK and its receptor Fn14 using immunohistochemistry (IHC). Macroscopically unaffected tissue from nephrectomy, performed due to kidney cancer, was used as control. The biopsies and tissues were paraffin-embedded and sectioned into 1.5 µm thick sections at the Pathology Kidney Laboratory at Karolinska University Hospital in Huddinge, Stockholm. For IHC the avidin/biotin blocking kit (Vector Laboratories #SP2001) and DAB substrate kit, peroxidase (Vector Laboratories #SK-4100) were used according to the manufacturer's guidelines. Previous deparaffinization and hydration was performed through washing stapes of xylene and ethanol (100%, 95%, 70%) and MilliQ. Antigen-retrieval was achieved by microwaving the slides for 25 min in 10 mM citrate buffer (pH 6). Primary antibodies, anti-hum TWEAK R/Fn14 (R&D Systems, #AF1199) and anti-TWEAK (Bioworld technology, Inc., #BS2454), were used at 4°C over night and as secondary antibodies, horse anti-goat IgG antibody (H+L), Biotinylated (vector laboratories, #BA-9500-1.5) and goat anti-rabbit IgG, biotinylated (vector laboratories, #BA-1000-1.5), were used.

3.11 HISTOPATHOLOGICAL CLASSIFICATION SCORE

In **study III** a histopathological classification score for AAV (180) was used to score all available kidney biopsies. The Berden classification distinguishes four classes of patterns of kidney injury: focal, mixed, sclerotic, and crescentic (figure 15). A total of 39 biopsies were available for scoring. An experienced kidney pathologist, blinded to patient data, scored the biopsies.

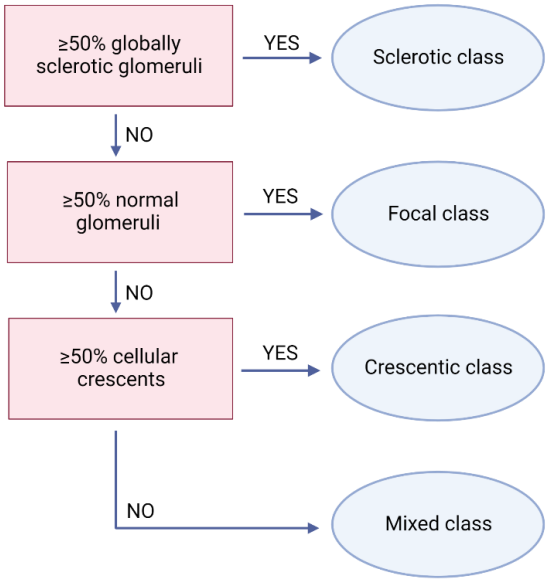


Figure 15. *Histopathologic classification flowchart. Used and adapted with permission from the American Society of Nephrology from (180); permission conveyed through Copyright Clearance Center, Inc. Created with Biorender.com.*

3.12 STATISTICAL ANALYSIS

Statistical analysis was performed with JMP software (version 14; SAS, Cary, NC, USA) and GraphPad Prism (version 4 and 9, GraphPad Software, Lacolla, CA, USA).

For assessing distribution of variables, the Shapiro-Wilk test was used. Normally distributed variables were presented as mean and standard deviation (SD). Non-normally distributed variables were presented as median and 25^{th} to 75^{th} percentiles and categorical variables as frequency and percentage. Independent samples *t* tests (parametric) and Mann-Whitney U/Wilcoxon rank-sum test (non-parametric) were used to assess the difference in variables between groups. Wilcoxon signed rank test (non-parametric) was used to compare difference in variables between time-points. For comparison of more than two groups a Kruskal-Wallis analysis of variance (ANOVA) (non-parametric) was used. Fischer´s exact test was used for comparison of categorical data. Correlation between variables was analysed using Pearson (parametric) or Spearman (non-parametric) correlation analysis depending on data type and distribution. To evaluate the prognostic value of investigated parameters in predicting disease activity in **study I**, receiver-operator characteristic (ROC) curve analysis was performed. ROC

curves are presented with respective AUC and 95% confidence intervals (CI). Similarly, a ROC curve analysis was performed in **study II** to assess the prognostic value of baseline PTX3 in predicting kidney function at 6 months. A *P* value <0.05 was regarded as statistically significant.

3.13 ETHICAL CONSIDERATIONS

Patients received oral and written information when they were offered participation in the cohort and were informed that they could withdraw their consent at any time. At inclusion and at follow-up a clinical evaluation was performed, and blood and urine samples were collected. Approval was also given for kidney tissue studies by the ethics committee. All data was anonymised, and no information could be traced to a particular individual. These procedures did not expose the patient for increased risk but could have led to more frequent visits to the hospital. To try to avoid inconvenience for the patients, study visits were scheduled on same days as planned clinical appointments for follow-up when possible. The aim of the studies included in this thesis was to identify biomarkers that could improve the diagnosis, aid in assessing disease activity, and as well as investigating their role in the pathogenesis. With modern treatment AAV have become chronic diseases with a relatively high disease relapse risk, requring a life-long follow-up. It is likely that future patients could benefit but the participiants could potentially also gain from the results of these research projects during the course of their disease duration.

3.14 ETHICS COMMITTEE APPROVALS

The studies included in the thesis were all conducted in accordance with the Declaration of Helsinki and study protocols were approved by the Regional Ethical Review Board and the Swedish Ethical Review Authority. A written informed consent was obtained from all participants at inclusion.

4 RESULTS

4.1 THE COHORT

A schematic presentation of the patients and controls included in the studies is shown in figure 16 and the baseline characteristics of the patients in the studies are shown in table 2.

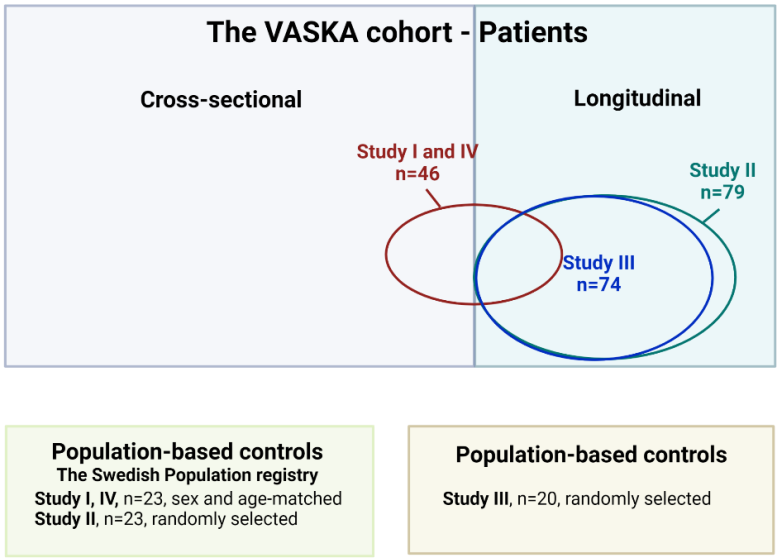


Figure 16. *Schematic presentation of patients and controls included in the studies. Each study is presented with a circle and the overlap between the study cohorts is demonstrated. Created with Biorender.com*

4.2 STUDY I AND IV

4.2.1 Patients and controls

In these cross-sectional studies forty-six patients with AAV were included in the study, 23 with active disease and 23 with inactive disease (in remission). Baseline characteristics of patients are shown in table 2. Of the patients with active disease 22 had a newly diagnosed disease (median disease duration 4 days, range 0-177 days). The median disease duration in the inactive disease patient group was 5.3 years (range 1.2-12 years). In the active disease group, all patients had a newly diagnose disease except one patient who had a relapse of a previously diagnosed disease. None of the patients included in the study had previously suffered from a cardiac event or venous thromboembolic disease (VTE), none had ongoing anticoagulant treatment or had received plasma exchange therapy at the time of sampling.

Twenty-three age and sex-matched subjects were included for comparison. The controls have been described in a previous study (284). Twelve were males and 11 females, mean age 64 years (range 49-81 years).

Table 2. Baseline characteristics of the patients included in studies I-IV.

	Study I and IV		**Study II**	**Study III**
	Cross sectional		**Longitudinal**	**Longitudinal**
	Active AAV	Inactive AAV		
	n=23	n=23	n=79	n=74
Sex, female, n (%)	10 (43.5)	11 (47.8)	36 (45.5)	32 (43.2)
Age, years (range)	63 (24-81)	65 (26-81)	58 (19-86)	64 (19-86)
ANCA[a]				
PR3, n (%)	10 (43.5)[b]	11 (47.8)[b]	48 (60.8)	47 (63.5)
MPO, n (%)	14 (60.9)[b]	13 (54.2)[b]	31 (39.2)	27 (36.5)
Diagnosis				
GPA, n (%)	10 (43.5)	13 (56.5)	54 (68.4)	53 (71.6)
MPA, n (%)	13 (56.5)	10 (43.5)	21 (26.6)	17 (23.0)
EGPA, n (%)	0 (0)	0 (0)	4 (5.0)	4 (5.4)

ANCA: antineutrophil cytoplasmic antibody, AAV: ANCA-associated vasculitis; F: female, PR3: proteinase-3, MPO: myeloperoxidase; GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis, BVAS: Birmingham Vasculitis Activity Score.
[a]ANCA specificity ever
[b]Two patients were positive for both PR3- and MPO-ANCA

4.2.2 Disease activity, laboratory results and treatment

The median BVAS was 14 in the subgroup of active AAV patients, as per definition all inactive patients had a BVAS of 0. CRP and ESR was significantly higher in the active AAV group compared to inactive group (*P* 0.002 and *P* 0.03, respectively). There was not a significant difference in plasma creatinine levels between patients with active and inactive disease.

In the active group all but two patients had received treatment at the time of sampling. All the patients who had received treatment were treated with GCs, five had received CYC, two were treated with MTX, one with MMF and one patient diagnosed with a disease relapse was on AZA. The median daily prednisolone dose was significantly higher in the active AAV group compared to the inactive group (median 60 mg vs 5 mg, P <0.001). None of the patients in the active AAV group had received RTX at the time of sampling.

Fifteen patients in the inactive group were treated with prednisolone at the time of sampling, six with AZA, five with MMF and four with MTX. Three of the patients in the inactive disease subgroup were not on any remission maintenance treatment at the time of sampling.

4.2.3 Expression of investigated biomarkers on myeloperoxidase positive extracellular vesicles.

In **study I** we found concentrations of MPO^+EVs and MPO+EVs expressing PTX3, HMGB1 and TWEAK to be significantly higher in the AAV patients compared to controls (all *P* <0.001). Concentrations of MPO^+EVs expressing PTX3 and HMGB1 were significantly higher in the active disease group compared to the inactive group (*P* 0.001, *P* 0.006, respectively). There was no difference in concentrations of MPO^+EVs expressing TWEAK between the active and the inactive group. Serum PTX3 levels were significantly higher in AAV patients compared to controls (1.45 vs 0.72 ng/ml, P <0.05) and in active patients compared to inactive patients (2.3 vs 1.0 ng/ml, *P* 0.001). There was no significant difference in HMGB1 or TWEAK levels when comparing all AAV patients to controls are in the active group compared to the inactive group. No difference was found in levels of investigated makers expressed on MPO^+EVs or in serum in patients with and without kidney involvement in the active group nor was there a difference when comparing PR3- and MPO positive patients. In the inactive disease subgroup, no difference was found in concentrations of the investigated markers on EVs or in serum comparing patients on GCs treatment vs not.

A correlation was found between BVAS and MPO^+EVs expressing PTX3 (*r*=0.56, *P* <0.001) and HMGB1 (*r*=0.36, *P* 0.01). A correlation between serum PTX3 and BVAS (*r*=0.69, *P* <0.001) was found but not between serum HMGB1 or TWEAK and BVAS. There was a correlation between serum PTX3 and $PTX3^+MPO^+$EVs (*r*=0.3, *P*<0.05) but no correlation was found between serum HMGB1 or TWEAK and MPO^+EVs expressing these markers. No

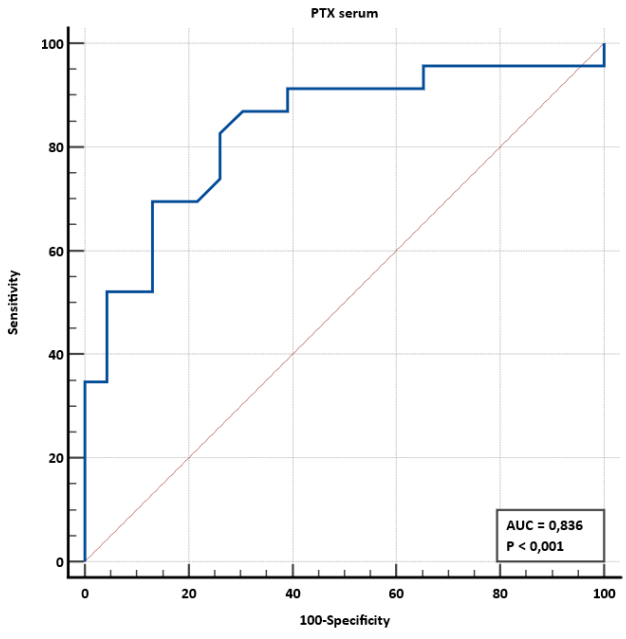


Figure 17. *ROC curve analysis for serum PTX3 levels in predicting active disease in patients with ANCA-associated Vasculitis. Previously published as supplementary material. Adapted and reproduced from paper I. Open access. AUC: Area under the curve.*

correlation was found between the daily prednisolone dose at the time of sampling and the levels of the investigated markers in the active patient group. A ROC analysis was performed to assess the validity of PTX3, $PTX3^+MPO^+$EVs and $HMGB1^+MPO^+$EVs in identifying active AAV. AUC were 0.8 (0.7-0.9) and 0.7 (0.6-0.9) and 0.7 (0.5-0.9) respectively (figure 17).

In **study IV**, significantly higher concentrations of MPO^+EVs expressing TF, H3Cit and Plg were found in AAV patients compared to controls (*P* <0.001 for all). Concentrations of TF^+MPO^+EVs and $H3Cit^+MPO^+$EVs were higher in active patients compared to inactive patients (*P* 0.017 and *P* 0.034, respectively) but no difference was found in Plg^+MPO^+EVs between the active and inactive group. Patients with inactive disease had higher concentrations of MPO^+EVs expressing TF, H3Cit and Plg compared to controls (*P* 0.004, *P* <0.001 and *P* <0.001, respectively). No difference was found in concentrations of MPO^+EVs expressing the investigated markers between patients with kidney involvement compared to those without in the active disease subgroup.

For all AAV patients, a significant correlation was found between TF^+MPO^+EVs levels and BVAS (ρ=0.43, *P* <0.003) but not between BVAS and MPO^+EVs expressing H3Cit or Plg. A significant correlation was also noted between TF^+MPO^+EVs and $H3Cit^+MPO^+$EVs (ρ=0.49, *P* 0.001).

In the active AAV group a significant correlation was found between age and TF^+ and $H3Cit^+MPO^+$EVs (ρ=0.59, *P* 0.003, ρ=0.46, *P* 0.03, respectively) and a negative correlation between age and Plg^+MPO^+EVs (ρ-0.42, 0.046). MPO^+EVs correlated with plasma creatinine and eGFR (ρ=0.57, *P* 0.005 and ρ=-0.52, *P* 0.01, respectively). No such correlation was found in the inactive group or the control group.

4.2.4 Thrombin generation variables

A modified CAT assay was performed to explore the capacity of EVs to generate thrombin. The lag time and time to peak were significantly shorter, the ETP and the peak of thrombin generation increased in NPP after addition of EVs from AAV patients compared to variables obtained in NPP after addition of EVs from the control subjects (*P* <0.001 for all). When compared separately to EVs extracted from controls, EVs extracted from plasma of patients with both active and inactive disease both initiated formation of higher ETP (*P* <0.001, *P* <0.001, respectively) and peak levels (*P* <0.001, *P* <0.001, respectively), as well as shorter lag time (*P*>0.001, *P* 0.002, respectively) and time to peak (*P* <0,001, *P* 0.003, respectively).

There was no difference in these thrombin generation variables with addition of NPP from patients with active compared to inactive disease.

4.2.5 Association between investigated parameters and the thrombin generation curve

For the whole AAV group, a significant correlation was found between the peak of thrombin generation and the concentrations of TF^+MPO^+EVs and $H3Cit^+MPO^+$EVs. Similarly, a significant correlation was seen between ETP and TF^+MPO^+EVs and $H3Cit^+MPO^+$EVs. A negative correlation was found between the Plg^+MPO^+EVs and time to peak and the lag time (table 3 and figure 18).

In the active AAV group a significant correlation was seen between $H3Cit^+MPO^+$EVs and the peak of thrombin generation (ρ=0.44, *P* 0.04) and time to peak (ρ=-0.45, *P* 0.03). A negative correlation was found between Plg^+MPO^+EVs and the peak of thrombin generation (ρ=-0.42, *P* 0.05) and in the inactive subgroup a negative correlation between Plg^+MPO^+EVs and peak of thrombin generation (ρ=-0.60, *P* 0.003) and the lag time (ρ=-0.62, *P* 0.002).

Table 3 Spearman rank correlation coefficient for the investigated markers on myeloperoxidase positive extracellular vesicles and parameters of thrombin generation in the whole ANCA-associated vasculitis group.

	TF^+MPO^+EVs		**$H3Cit^+MPO^+$EVs**		**Plg^+MPO^+EVs**	
	ρ	***P***	**ρ**	***P***	**ρ**	***P***
Lag time	0.003	0.98	-0.07	0.68	-0.41	***0.005***
Time to Peak	-0.03	0.85	-0.11	0.48	-0.33	***0.03***
Peak	0.33	***0.02***	0.44	***0.003***	-0.17	0.26
ETP	0.31	***0.04***	0.37	***0.01***	-0.07	0.65

TF^+MPO^+EVs: myeloperoxidase positive extracellular vesicles expressing TF; $H3Cit^+MPO^+$EVs: myeloperoxidase positive extracellular vesicles expressing citrullinated histone-3, Plg^+MPO^+EVs: myeloperoxidase positive extracellular vesicles expressing plasminogen; Peak: peak thrombin concentration; ETP: endogenous thrombin potential.

4.3 STUDY II

4.3.1 Patients and controls

In this study 79 patients from the longitudinal AAV cohort were included in the study. The patients were included at the time of diagnosis, and all had active disease. Seventy had a newly diagnosed disease and 9 had a relapse of a previously diagnosed AAV. All patients were followed prospectively for 6 months (median 195 days from follow-up). Baseline characteristics of the patients included are shown in table 2. For comparison, 23 randomly

selected controls from the Swedish Population Registry, described in a previous study (284), were included.

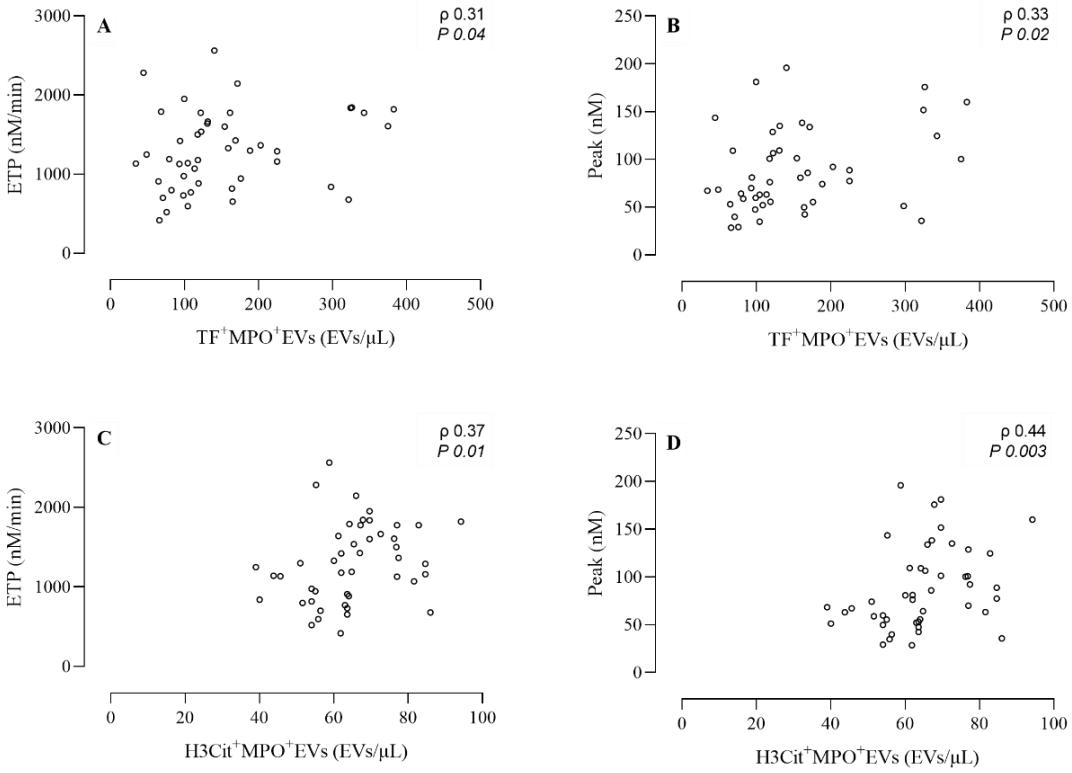


Figure 18*. Correlation between the investigated markers expressed on MPO positive extracellular vesicles (MPO+EVs) and the thrombin generation parameters in the total group of ANCA-associated vasculitis patient. Correlation analysis was performed using the Spearman rank correlation coefficient. Correlation between A) MPO⁺EVs expressing tissue factor (TF) and endogen thrombin potential (ETP), B) MPO⁺EVs expressing TF and peak thrombin concentration (Peak), C) MPO⁺EVs expressing citrullinated hiostone-3 (H3Cit) and ETP, and D) MPO⁺EVs expressing H3Cit and Peak. Previously unpublished.*

4.3.2 Treatment

Seventy-one of the patients were included and sampled after the onset of induction treatment (median 5 days, range 0-37 days). All these patients had received treatment with GCs, 34 of which had received iv GC treatment. Thirty-seven patients had received treatment with CYC (n=25, 31.6%), RTX (n=3, 3.8%), MTX (n=7, 8.9%) or MMF (n=2, 2,5%) at the time of inclusion. Only one patient had received more than one dose of CYC before inclusion. Two patients with localized ENT disease manifestations had only received high-dose GC treatment.

At follow-up 58 patients (73%) had been started on maintenance treatment with AZA, MMF, RTX or MTX in combination with a low dose of GCs.

4.3.3 Disease activity and phenotype.

Disease activity assessed using BVAS decreased from a median of 15 at inclusion to a median of 0 at the 6-month follow-up (*P* <0.001). Five patients had an active disease at the follow-up. All these patients had ENT manifestations and two had lung involvement. Forty-seven of the patients had kidney involvement at baseline, in 43 patients this was confirmed with a kidney biopsy and four patients were diagnosed clinically.

4.3.4 Laboratory results

4.3.4.1 Plasma PTX3 levels

Plasma PTX3 levels were significantly higher at baseline compared to at follow-up (median 2.85 vs 1.23 ng/mL, *P* <0.001). When comparing patients at inclusion and follow-up separately to controls, the levels were significantly higher in patients at inclusion vs controls (median 2.85 vs 0.75 ng/mL, *P* <0.0001) and patients at follow-up vs controls (1.23 vs 0.75 ng/mL, *P* 0.008). Plasma PTX3 levels correlated with BVAS at baseline (ρ=0.45, *P* <0.001). No difference in PTX3 levels at baseline between patients with PR3- and MPO-ANCA positive disease was found. When comparing patients in remission (BVAS 0) and patients with an active disease (n=5) at the follow-up no significant difference was found between plasma PTX3 levels. Plasma PTX3 levels were higher at baseline in patients with a newly diagnosed disease compared to patients with a diagnosed relapse (*P* 0.046) and were significantly higher in patients with kidney manifestations at baseline compared to those without (median 3.62 vs 1.47 ng/mL, *P* 0.001) (figure 20). Furthermore, a significant correlation was found between plasma PTX3 and creatinine, eGFR, and albuminuria at baseline (ρ=0.38, *P* <0.001; ρ=-0.41, *P* <0.001 and ρ=0,44, *P* <0.0001, respectively). Patients with haematuria had significantly higher PTX3 levels (median 3.98 vs 1.48 ng/mL, *P* 0.0002) at baseline but there were no correlations between CRP levels or ESR and plasma PTX3 levels.

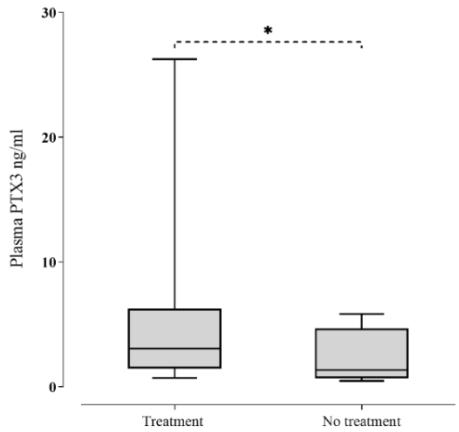


Figure 19. *Comparison of plasma pentraxin-3 (PTX3) levels in patients that had received treatment at inclusion compared to treatment naïve patients. * P<0.05. Previously unpublished*

Patients who had received induction treatment at inclusion (n=71) had a significantly higher plasma PTX3 levels compared to treatment naïve patients (n=8), 3.15 ng/mL vs 1.35 ng/mL, *P* 0.03 (figure 19). Furthermore, there was a significant correlation between the cumulative GCs dose in patients at baseline and plasma PTX3 levels (ρ=0.37, *P* <0.001) and similarly a significant correlation was found between the actual daily GC dose at sampling at baseline and plasma PTX3 levels (ρ=0.43, *P* <0.0001). No difference was found in plasma PTX3 levels when comparing patients that received CYC vs those who had not. Similarly, no difference was found between plasma PTX3 levels in patients that had received MTX at baseline compared to those who had not. As only two patients had received RTX and another two patients had received MMF at baseline no comparative analysis was performed for these groups.

4.3.4.2 Urinary PTX3 levels

Urine samples for PTX3 analysis were available in a subset of the patients, 34 patients at baseline and 33 at follow-up. Urinary PTX3-to-creatinine ratio (uPTX3/Cr) could be calculated in 28 patients at baseline and 31 at follow-up, 26 of whom were available at both time points for comparison over time. All but 3 of the patients were in remission (BVAS 0) at follow-up. Levels of uPTX3/Cr were significantly higher at baseline compared to follow-up (median 0.8 vs 0 ng/mmol, *P* 0.01) and a significant correlation was found between uPTX3/Cr and BVAS at baseline (ρ=0.49, *P* 0.008). At follow-up there was no difference between uPTX3/Cr levels in patients in remission vs those with active disease. No correlation between plasma PTX3 and uPTX3/Cr at baseline was found nor a difference in uPTX3/Cr levels between patients with PR3- and MPO-ANCA positive disease. uPTX3/Cr levels were significantly higher in patients with kidney involvement compared to those without (*P* 0.005) (figure 20) and a significant correlation was found between uPTX3/Cr and creatinine (ρ=0.47, *P* 0.01), eGFR (ρ=-0.42, *P* 0.025) and albuminuria (ρ=0.54, *P* 0.002) at baseline. When comparing patients who had received treatment at inclusion vs treatment naïve patients no difference was found in uPTX/Cr nor was there a significant correlation between the cumulative GC dose or current daily dose at baseline and uPTX3/Cr.

4.3.4.3 Predictive role of plasma PTX3

To assess whether PTX3 could be a potential prognostic factor for kidney function (eGFR) at the 6-month follow-up a ROC curve analysis was performed. The AUC was 0.62.

4.3.4.4 CRP and ESR levels

CRP was significantly higher at baseline than follow-up (9.0 mg/L vs 2.0 mg/L, *P* <0.001). There was no correlation between BVAS and CRP levels or ESR at baseline. CRP levels did not correlate to BVAS. No difference was found in CRP levels between patients with and without kidney involvement.

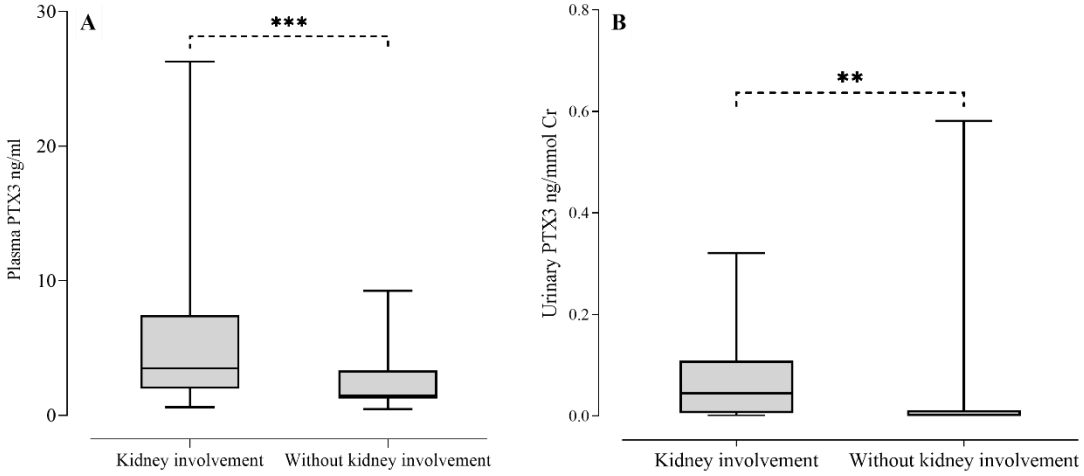


Figure 20. *Comparison of A) plasma Pentraxin-3 (PTX3) and B) urinary PTX3 levels between patients with and without kidney involvement. ** P <0.01, *** P<0.001. Previously unpublished.*

4.4 STUDY III

4.4.1 Patients and controls

Seventy-four patients from the longitudinal cohort were included in the study. The patients were included at diagnosis (a new diagnosis or a disease relapse) and followed prospectively for 6 months (median 194 days). Sixty-five (88%) had a newly diagnosed disease and nine (12%) had a diagnosis of a relapsing previously diagnosed AAV. Baseline characteristics of the patients included are shown in table 2.

For comparison, sTWEAK levels were measured in 20 randomly selected controls from a population-based cohort, 6 were females, 14 males, median age 64 years (range 59-70). The controls have been described in previous studies (285, 286).

4.4.2 Treatment

For 67 patients (90.5%) induction treatment had been started at the time of sampling. Median time from initiation was 5 days (range 0-37 days). All these patients had received treatment with GCs, 29 had received intravenous GC treatment. The median cumulative dose of GC at baseline was 525 mg. Twenty-one patients had received CYC of which three patients had

received 2 CYC doses, three RTX, six MTX, and two had received MMF. At follow-up, remission maintenance treatment with AZA, MMF, MTX or RTX had been initiated in 55 of the patients.

4.4.3 Disease activity and phenotype

At inclusion, the disease activity assessed using BVAS was 15 at inclusion and decreased to a median of 0 at the 6-month follow-up (*P* <0.0001). Five patients had an active disease at the follow-up. Forty-two (55.8%) of the patients had kidney involvement at baseline, in which 39 patients confirmed with a kidney biopsy and whereas three patients were diagnosed clinically.

4.4.4 Laboratory results

4.4.4.1 Urinary TWEAK

Urine samples for measurement of TWEAK-to-Creatinine ratio (uTWEAK/Cr) were available in 69 of the patients. The uTWEAK/Cr levels were higher at baseline compared to at the follow-up (median 7.21 pg/mmol vs 4.94 pg/mmol, *P* 0.001). Patients that had received treatment at baseline had higher levels of uTWEAK/Cr compared to the patients who had not (*P* 0.03). When comparing patients in remission (n=69) with patients with active disease (n=5) at follow-up no significant difference was found in uTWEAK/Cr levels. There was no significant difference in uTWEAK/Cr levels in patients with a new diagnosis compared to a disease relapse. Patients with PR3-ANCA positive disease had higher uTWEAK/Cr than patients with MPO-ANCA positive disease (5.6 vs 3.9 ng/mmol, *P* 0.05), although the difference was not statistically significant. Patients with kidney involvement had higher uTWEAK/Cr levels at baseline compared with patients without (median 8.49 pg/mL vs 6.34 pg/mL, *P* 0.03).

A correlation was found between uTWEAK/Cr levels and BVAS at baseline (ρ=0.33, *P* 0.006) as well as a correlation between albuminuria and uTWEAK/Cr (ρ 0.28, *P* 0.022). No correlation was found between creatinine and uTWEAK/Cr and there was not a significant difference in uTWEAK/Cr levels in patients with and without haematuria at baseline or at follow-up. Furthermore, no correlation was found between uTWEAK/Cr and sTWEAK levels.

4.4.4.2 Serum TWEAK levels

sTWEAK levels were significantly higher in AAV patients at inclusion compared to follow-up (median 465.6 pg/ml vs 436.1 pg/ml, *P* 0.009) but there was not a significant difference in sTWEAK levels between patients at inclusion or follow-up when compared to controls (P 0.67 and P 0.054, respectively) (figure 21). When comparing subgroups, no difference was found in

sTWEAK levels between patients with a newly diagnosed disease compared to a disease relapse or in patients that were in remission compared to those who had an active disease at the follow-up. There was no difference in sTWEAK levels comparing patients with and without kidney involvement.

No correlation was found between sTWEAK and BVAS at inclusion (ρ=-0.06, *P* 0.60). A significant correlation was found between sTWEAK levels and creatinine (ρ=-0.326, *P* 0.005) and eGFR (ρ=0.313, *P* 0.007) at baseline but no correlation between sTWEAK levels and albuminuria was seen.

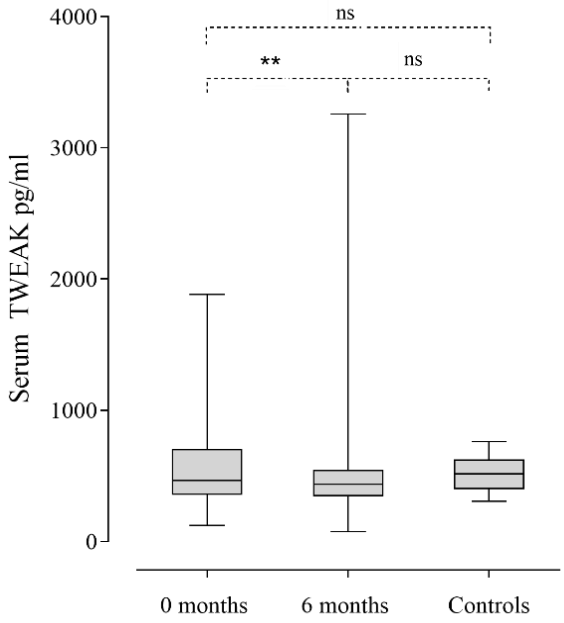


Figure 21. *Comparison of serum TWEAK levels in patients with ANCA-associated vasculitis at baseline (0 months) and follow-up (6 months) and controls. Wilcoxon signed-rank test used for comparison between timepoints and Wilcoxon rank sum test used for comparison between patients and controls. **P<0.01. ns: not significant. Previously unpublished.*

4.4.4.3 CRP and ESR levels

CRP and ESR levels were significantly higher in patients at inclusion compared to follow-up (median 10 vs 2 mg/l, *P* <0.001 and 35 vs 12 mm, P 0.001, respectively) but there was not a significant correlation between BVAS and CRP or ESR levels at baseline nor a correlation between CRP or ESR levels and TWEAK levels.

4.4.4.4 Immunohistochemical staining

Two kidney biopsies from patients with active AAV were stained using IHC. One biopsy had about 90% glomerular crescent/necrosis and the other more than 50% glomerular crescents. A biopsy from a nephrectomised patient was stained as a control. The staining showed a clear expression of TWEAK in the kidney biopsies from AAV patients when compared to the control kidney tissue. A high TWEAK expression was found in both tubular and glomerular areas. In the glomeruli TWEAK accumulation was found in the formed crescents and the podocyte foot

processes. Moreover, TWEAK expression was seen in circular shapes of the endothelial cells lining the capillary walls. TWEAK expression was found both in the proximal and distal tubules. Staining for Fn14, TWEAKs receptor, showed a slightly increased expression, mainly in the tissue from the patient with severe glomerular crescent/necrosis (around 90%). The control tissue showed faint podocyte and endothelial glomerular staining and upregulated expression of Fn14 in the podocyte foot processes (in a linear staining pattern) and increased Fn14 expression in the glomerular endothelial cells.

Histopathological classification score

Kidney biopsies were available for 39 of the patients with kidney involvement. One patient was excluded as there were no histopathological findings consistent with AAV on the biopsy nor an elevation of plasma creatinine or significant haematuria. The patient was therefore not considered to have kidney involvement. When scored by an experienced kidney pathologist, 21 of the biopsies were classified as focal, nine as mixed, three as sclerotic and five as crescentic. When urinary and sTWEAK levels were compared between these groups no significant differences were found.

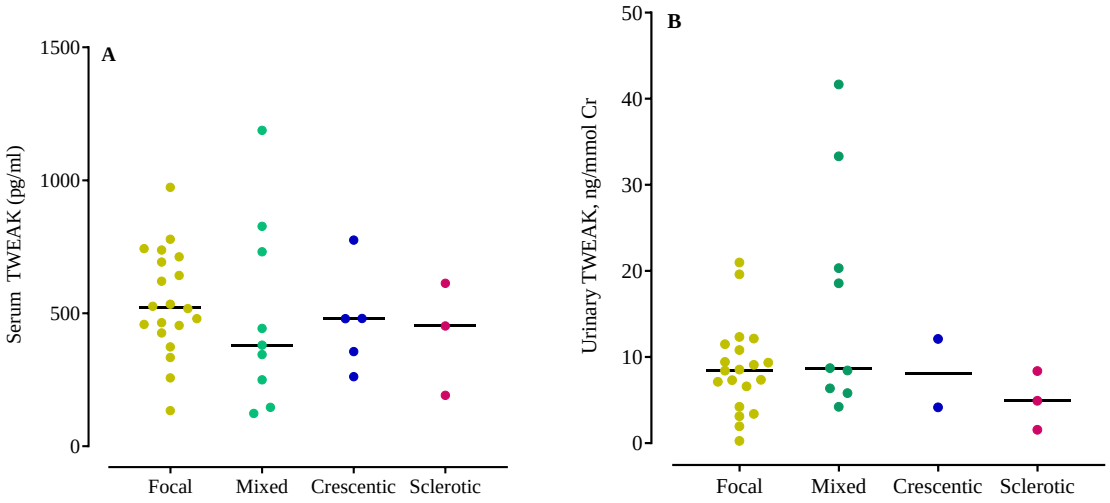


Figure 22. *a) Serum TWEAK levels in different histopathologic groups. b) Urinary TWEAK levels in different histopathologic groups. Previously unpublished.*

5 DISCUSSION

AAV are a group of serious and potentially organ- and life-threatening heterogenous diseases. Although increased understanding of AAV has led to improved diagnosis and treatment, challenges remain. Relapses are common, the optimal treatment duration is not defined, and treatment with immunosuppressives carries risk of adverse effects such as infections, malignancies, osteoporosis, diabetes mellitus and infertility with on impact on patient´s QoL. No fully reliable methods exist to assess disease activity, evaluate treatment response, and predict relapses. It is therefore important to identify novel biomarkers to assist and improve evaluation of the disease course as well as enable individualized treatment. BVAS is the standardized tool for disease activity assessment in clinical AAV research, combining laboratory and clinical findings. It is however cumbersome to use in clinical practice, as it is time-consuming and appropriate training is needed for proper use. Biopsies are the gold standard for diagnosis but are invasive and carry a risk of complications.

In the studies presented in this thesis we aimed to identify non-invasive novel biomarkers and to better understand their link to pathogenic mechanisms in AAV. The pathogenesis of AAV is complex and although our understanding of the pathophysiological processes has increased in the recent years much remains elucidated.

5.1 THE ROLE OF PENTRAXIN-3 IN AAV

We investigated PTX3 as a potential biomarker in study **I and II**. In **study I**, a cross sectional study, we found that circulating PTX3 levels were significantly higher in patients with active disease compared to population-based controls and that the levels were higher in active compared to inactive disease. **Study II** was a longitudinal study where AAV patients were prospectively followed from diagnosis (new disease or a disease relapse) to a 6-month follow-up. Our main findings were that plasma PTX3 levels were higher in patients with active disease at baseline compared to follow-up, where majority of the patients had inactive disease, and compared to levels in population-based controls. uPTX3 levels were also higher in patients at inclusion compared to at the follow-up. We found a correlation between disease activity (assessed with BVAS) and circulating PTX3 in **study I** and similarly, both urinary and plasma PTX3 levels correlated with BVAS in **study II**. In **study I**, a ROC analysis was performed to assess the validity of plasma PTX3 levels in identifying active AAV disease where the AUC was 0.83 which suggest that PTX3 could be a useful disease activity marker in AAV.

Our results are in line with the results of previous cross-sectional studies on AAV patients that have shown higher plasma PTX3 levels in active compared to inactive disease (200) and a correlation between plasma PTX3 and BVAS (213). Interestingly, circulating PTX3 levels have been reported to be higher in AAV patients compared to patients with SLE, RA and CREST (calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasises) syndrome (200). Elevated circulating PTX3 levels have been demonstrated in active SLE and Takayasu arteritis (208, 290, 291) and a correlation with disease activity has been reported (201-203, 213). Moreover, circulating PTX3 has also been shown to be elevated in patients with other conditions such as sepsis, systemic inflammatory response syndrome and myocardial infarction (292-295). uPTX3 levels have been studied in LN and have been found to be higher in active compared to inactive disease (290). Thus, PTX3 does not seem to be a disease specific marker for AAV but a potential useful biomarker in various inflammatory diseases.

CRP is a commonly used biomarker in the assessment of disease activity in systemic inflammatory diseases in absence of other more dependable markers. Although patients with active disease had higher CRP levels than patients at the follow-up in **study II** and compared to patients in remission in **study I**, we did not find a significant correlation between CRP and BVAS at baseline in **study II**. Moreover, there was no significant correlation between PTX3 and CRP. These findings are in line with results of other studies that have investigated the association between CRP and PTX3 in AAV (200) and other systemic inflammatory diseases such as SLE, ankylosing spondylitis, and SSc (201, 207, 296-298). However, a significant correlation was found between PTX3 and CRP levels in a cross-sectional study of 63 AAV patients (213) and a study of children with active IgA vasculitis (299). Our results indicate that PTX3 is a more reliable biomarker than CRP in relation to assessment of disease activity in AAV. CRP is produced by hepatocytes in response to inflammatory signals, most prominently IL-6, whereas PTX3 is produced locally in tissues in response to various cytokines (185). PTX3 might therefore be a better marker of localized inflammatory activity and more easily pick up emerging inflammation than CRP. PTX3 has previously been shown to have much shorter time to peak than CRP following myocardial infarction, supporting PTX3 role as an early marker of tissue injury (292).

We found no correlation between plasma PTX3 and uPTX3 levels in **study II**. Urine samples for measurement of uPTX3/Cr were only available in a subset of the patients thus making it difficult to draw firm conclusions from these results. To our knowledge the association between urinary and plasma PTX3 in AAV has not been studied previously. A significant correlation

between plasma and uPTX3 levels has however been reported in LN (290). Further studies are needed to better delineate the relationship between urinary and plasma PTX3 in AAV.

In **study II**, levels of plasma and urinary PTX3 were higher in patients with kidney involvement compared to those without. Moreover, urinary and plasma PTX3 correlated with eGFR as well as albuminuria. The association between uPTX3 levels and kidney involvement suggests that uPTX3 reflects local production caused by inflammation in the kidneys. Elevated uPTX3 levels have been found in patients with urinary tract infections and increased serum PTX3 levels in patients with kidney parenchymal scars caused by pyelonephritis (300, 301). Expression of PTX3 in kidney tissue has been found in glomerular mesangial cells, endothelial cells and infiltrating inflammatory cells in IgAN (302) and in tubulointerstitial areas in LN (290). Previously a histopathological staining of kidney tissue in a patient with MPO-ANCA positive disease has been described. A prominent PTX3 expression was seen in interstitial inflammatory cells and peritubular capillaries (303). Therefore, elevated local PTX3 production in the kidney and the urinary tract seems to be independent of systemic PTX3 levels and thus reflect local inflammatory process. The results of animal and in vivo studies indicate that PTX3 has an regulatory effect on tissue inflammation in acute kidney injury and limits the development of chronic kidney injury (211, 212, 304). These findings support the role of PTX3 in the pathogenesis of acute inflammatory kidney disease. One possible explanation for the correlation between plasma PTX3 and kidney function might be a retention caused by decreased glomerular filtration due to PTX3 size (42 kDA, multimers 440 kDa) (191). Whether the elevated plasma PTX3 levels in kidney disease reflect local injury and inflammation in the kidney or impaired glomerular filtration remains therefore unclear.

Although we found a correlation between plasma PTX3 and BVAS and higher PTX3 levels in patients with kidney involvement, plasma PTX3 at baseline does not seem have a predictive value for kidney function during short-term follow-up.

In **study I**, there was no difference in plasma PTX3 between patients treated with ongoing GCs treatment compared to those without. As most of the patients had received treatment prior to inclusion in **study II** we investigated the effect of treatment on plasma and uPTX3 levels. We found that patients who had received treatment at baseline had higher plasma PTX3 levels. This may be explained by more severe disease in this group of patients. At baseline, a correlation between plasma PTX3 levels and both the daily and the cumulative GC dose was found. Comparable results have been found in SLE patients (202). In contrast to our findings, Fazzini et al found higher serum PTX3 levels in treatment naïve AAV patients compared to

those on treatment (most commonly prednisolone and AZA) (200) and a study on Takayasu´s arteritis showed no correlation between plasma PTX3 levels and the current prednisolone dose (291). The correlation between higher cumulative GC dose and PTX3 may demonstrate an association between plasma PTX3 levels and disease activity, as higher doses of GCs are more commonly administrated in more severe disease. However, elevated levels could also potentially be explained by a direct effect of GCs on PTX3 production. Circulating PTX3 levels have been reported to be higher in patients with Cushing syndrome and intravenous GC treatment has been shown to increase serum PTX3 levels in healthy subjects (305). A negative correlation was found between CRP and the cumulative GC dose at baseline, indicating that CRP levels are more affected by GC treatment.

5.2 THE ROLE OF TWEAK IN AAV

In study **III**, we investigated the role of urinary and sTWEAK in a longitudinal cohort of AAV patients. uTWEAK levels were higher at baseline compared to 6-month follow-up and a significant correlation was found between uTWEAK and BVAS at baseline. This is the first study where TWEAK has been investigated in a longitudinal cohort of AAV patients. uTWEAK has previously been studied in SLE, particularly LN, where uTWEAK levels have been found to be higher in active compared inactive disease (246-248, 306-308) and to be associated with disease activity (246-248, 306, 309). Moreover, uTWEAK levels have been found to decrease significantly after induction treatment in a longitudinal study of LN patients (310) and to be a potential predictive biomarker for remission in LN patients (311). In one of these studies, 14 AAV patients with active kidney involvement were included as disease controls. The patients with AAV were significantly older and had worse kidney function compared to the active LN group. No difference was found between uTWEAK levels in patients with AAV and active LN (309). uTWEAK as a biomarker has also been studied in other glomerular diseases. A study of IgAN demonstrated higher uTWEAK in IgAN compared to healthy controls. Moreover, higher uTWEAK levels were found in a glomerular disease control group, consisting of patients with LN, membranous nephropathy (MN), focal segmental glomerulosclerosis and minimal change disease (MCD), when compared to healthy controls. There were no significant differences in levels between IgAN patients and the other glomerular diseases (245). In **study III**, we found a significant correlation between albuminuria and uTWEAK at baseline. Most studies on uTWEAK in LN patients have shown no correlation between proteinuria and uTWEAK levels (309-311) while it has been reported in others (248) These discrepant findings suggest that different processes lie behind increased proteinuria and elevated uTWEAK levels and that uTWEAK does not only reflect damaged glomerular

filtration barrier but may also be a marker of local kidney inflammation. However, Sasaki et al found a significant correlation between uTWEAK levels and proteinuria in patients with MN and MCD (245), diseases not characterized by infiltrative inflammation. Further studies are needed with comparison of uTWEAK levels in AAV with kidney manifestations and podocytopathies to better delineate this association. In **study III**, we showed that uTWEAK levels were higher at baseline in patients with kidney manifestations of AAV compared to those without although no correlation between kidney function (eGFR or plasma creatinine) and uTWEAK levels was found. Fn14 and TWEAK have been shown to be upregulated in experimental acute kidney injury (219, 312) and blockade of TWEAK and Fn14 has been demonstrated to diminish kidney injury in animal models and *in vitro* studies (238, 313, 314). However, TWEAK also induces proliferation in tubular cells and may potentially contribute to recovery in acute kidney injury (312). Interestingly, Schwartz et al studied uTWEAK as a biomarker in LN and compared to controls with non-inflammatory kidney disease. Although levels were elevated in LN patients, uTWEAK was not found to be higher in controls with kidney disease due to diabetes nephropathy and hypertension compared to controls without kidney disease (246). This indicates that uTWEAK reflects inflammatory activity in the kidney and is not only a marker of kidney tissue damage.

As most of the patients in the cohort had received treatment at the time of sampling at baseline, we investigated the treatment effect on uTWEAK levels. We found a significant, although weak, correlation between the cumulative GCs dose at baseline and uTWEAK levels. Studies of patients with LN have not demonstrated a correlation between a cumulative GC dose and uTWEAK (311) or difference in levels in patients treated with GCs vs those not on treatment (306). In **study III**, we found elevated uTWEAK levels in patients who had received treatment at sampling at baseline compared to patients who were treatment naïve. Similarly, uTWEAK levels were higher in patients treated with CYC at the time of sampling vs those who had not received CYC. These findings are likely explained by an increased disease activity and thereby a choice of preferred therapy in these patients. Our results indicate that uTWEAK levels may be affected by GCs and other immunosuppression treatment although the pathogenesis behind this is unclear.

In **study I**, we measured sTWEAK levels in active and inactive AAV patients and compared to population-based controls. There were no significant differences in sTWEAK levels when comparing patients with active to inactive disease or when comparing patients with AAV to controls. In **study III**, a significantly higher sTWEAK levels were found in patients at baseline compared to follow-up. However, there was no difference in serum levels when comparing

patients to controls. sTWEAK levels have been found to be lower in SLE patients compared to healthy controls (246) and higher in active disease compared to inactive (309). A study of LN including 31 AAV disease controls showed no difference in sTWEAK levels when comparing AAV and LN (309). sTWEAK has been shown to be elevated in other systemic inflammatory diseases such as psoriatic arthritis, SSc and RA, when compared to controls (241, 315, 316). Interestingly, sTWEAK are decreased in diabetes mellitus and kidney failure but have shown to increase after kidney transplantation (240, 317, 318). In study **III**, we found a correlation between sTWEAK and eGFR and negative correlation to creatinine. This association between sTWEAK and kidney function could be due to increased uptake by Fn14 which is upregulated in tissue injury (237). Another potential explanation is an upregulation of CD163, TWEAKs scavenger receptor (319). CD163 is a transmembrane protein expressed on the surface of macrophages (320) and in-vitro studies have demonstrated that CD163-expressing macrophages can bind and internalize TWEAK (321) which potentially could lead to lower sTWEAK levels. It has furthermore been shown that CD163 is upregulated in kidney tissue in acute crescentic GN in AAV (322). Interestingly, urinary CD163 has been shown to be a promising potential biomarker in ANCA-associated GN (235, 323, 324). However, urinary CD163 has also been shown to be elevated in LN and therefore does not seem to be disease specific (325, 326). Further studies are needed to investigate the relationship between TWEAK and CD163 in AAV.

In **study III**, no correlation between serum and urinary TWEAK levels was seen. Similarly, Sazaki et al found no correlation between serum and urinary TWEAK levels in patients with IgAN (245). These findings may be explained, at least partly, by upregulation of TWEAK in acute inflammation in the kidney inflammation AAV.

In **study III**, using IHC we found increased expression TWEAK and fainter staining for Fn14 in kidney tissue from AAV patients. TWEAK expression was noted in tubular areas, glomerular crescents, and podocytes as well as in the capillary walls. TWEAK staining in glomeruli has previously been reported in AAV patients (245). The increased expression of TWEAK on podocytes in AAV patients may indicate that the TWEAK/Fn14 pathway could be involved in the pathogenesis of proteinuria in AAV. Although proteinuria is a frequent finding in ANCA-associated GN (327) the involvement of podocytes in the developement of proteinuria in AAV has not been well delineated. Recently, studies on podocyte morphology in AAV patient have shown increased podocyte foot process width in AAV patients (328) and this in turn correlates with proteinuria (329).

We did not find differences in TWEAK levels across the histopathologic classification groups. However, due to a limited number of kidney biopsies investigated no firm conclusions can be drawn from these results.

5.3 THE ROLE OF CIRCULATING MPO$^+$EVS EXPRESSING INFLAMMATORY MARKERS IN AAV

In **study I**, we investigated inflammatory markers expressed on MPO$^+$EVs. The EVs expressing MPO can be assumed to be mostly of neutrophilic origin as MPO is mainly stored in neutrophilic granules, although found in monocytes in much smaller quantities (330). We found that concentrations of MPO$^+$EVs and MPO$^+$EVs expressing PTX3, TWEAK and HMGB1 in plasma were higher in patients with AAV compared to population-based controls. Concentrations of MPO$^+$EVs expressing HMGB1 and PTX3 were significantly higher in the active AAV group compared to the inactive group and a significant correlation was found between BVAS and MPO$^+$EVs expressing HMGB1 as well as PTX3.

EVs are released from various cells, including neutrophils, platelets, monocytes, and endothelial cells. EVs can fuse with target cells transferring biomolecules from the parenting cell and mediate long-distance cell-to-cell communication such as inflammation, coagulation and endothelial dysfunction (331, 332). Levels of EVs derived from endothelial cells and platelets have been shown to be elevated in AAV (261, 333). As neutrophils play a pivotal role in the pathogenesis there has been increasing interest in the effect of EVs of neutrophilic origin in AAV. Daniel et al demonstrated increased levels of NEVs in patients with active compared to inactive vasculitis (the majority with AAV) (264). Similar findings were shown in another study of patients with AAV. The leucocytic and endothelial derived EV count was higher in active AAV compared to patients in remission/partial remission and healthy controls and correlated with disease activity assessed by BVAS (261).

There is increasing evidence for the inflammatory mediation induced by receptors, proteins and mRNAs expressed on NEVs in AAV. In vitro studies have shown that NEVs cause release of cytokines and activation of endothelial cells (262, 334). Hong et al showed that ANCA activated primed neutrophils release EVs expressing PR3 and MPO and that NEVs activate endothelial cells causing ROS production and a release of cytokines (262). NEVs bearing B1-kinin receptor have been reported in AAV patients AAV and in vitro EVs transfer of B1-kinin-receptors to kidney cells induce calcium influx after stimulation (335). Surmiak et al recently demonstrated that EVs in plasma from patients with GPA cause dsDNA release from neutrophils and ROS production (336). Furthermore, a recently published study showed that

after stimulation (including ANCA mediated) NEVs carry miRNAs that are taken up by endothelial cells and induce vascular damage (337). The effect of EVs expressing MPO on endothelial and epithelial cells has also been investigated. MPO associated with activated human NEVs cause ROS mediated injury to vascular endothelial cells in vitro (263) and NEV expressing MPO inhibit migration and proliferation of intestinal epithelial cells, limiting wound healing (338).

Our results show that $PTX3^{+}MPO^{+}$EVs concentrations correlated with disease activity and were significantly elevated in acute AAV and in the total AAV group compared to controls. Furthermore, a correlation to serum PTX3 was found. This further supports PTX3 contribution to inflammatory conditions in active AAV and its role as a potential biomarker in AAV. As PTX3 is released in NETs during NETosis (57), increased expression of PTX3 on NEVs may be a reflection of amplified neutrophil mediated inflammation and NETosis in AAV. Surprisingly, concentrations of MPO^{+}EV expressing PTX3 did not differ between patients with and without kidney involvement in **study** I although we found plasma PTX3 levels to be higher in patients with kidney manifestations in **study II**. These discrepant results could be due to the smaller patient sample included in **study I**. One proposed explanation for the elevated plasma PTX3 levels is impaired glomerular filtration in kidney injury. However, this should also affect levels of EVs as it is unlikely that EVs of inflammatory cell origin pass an intact glomerular filtration barrier because of their size. Although EVs have been found in urine, they are predominantly of urogenital cellular origin (339). As stated previously, PTX3 is a multifunctional protein with diverse roles in immunity and inflammation. The effect and the role of EVs expressing PTX3 in the pathogenesis of AAV ought to be studied further in animal models and larger prospective studies of AAV patients.

Concentrations of $TWEAK^{+}MPO^{+}$EVs were significantly higher in AAV patients compared to controls but no difference was seen between active and inactive patient and no significant correlation to disease activity was found. This is consistent with the results of our study on sTWEAK levels in AAV patients (**study I and III**). Neither sTWEAK levels nor TWEAK expressed on NEVs did reflect disease activity in AAV.

Serum levels of HMGB1 measured with ELISA did not differ between AAV patients and controls or between the active AAV subgroup and the inactive AAV group. Previously, plasma HMGB-1 levels (measured with ELISA) have been reported to be higher in active AAV compared to patients in remission and healthy controls and to correlate with BVAS (269). Elevated serum levels measured with Western blot have also been shown in AAV patients with

kidney involvement compared to patients in remission (268). In contrast to these findings, no difference was reported between HMGB1 where levels AAV patients and healthy controls were measured with ELISA (340). This discrepancy in findings may be explained by the difference in methods used (341). In **study I**, no difference in serum HMBG1 levels was detected in AAV patients compared to controls or in active compared to inactive disease. In contrast to serum levels, concentrations of MPO^+EV expressing HMGB1 were higher in AAV patients compared to controls and there was also a significant difference in levels between the active and the inactive AAV group. Furthermore, $HMGB1^+MPO^+$EV concentrations correlated significantly with disease activity. Pisetsky has previously reported release of EVs and HMGB1 from stimulated macrophages in vitro and later a release of platelet and monocyte derived EVs expressing HMGB1 in patients after lipopolysaccharide administration. A possible explanation for our findings is that measurement of HMGB1 expressed on EVs is a more sensitive measurement as HMGB1 is primarily released on EVs, as suggested by Pisetsky (342). Supporting this is the lack of correlation between serum HMGB1 and MPO^+EVs expressing HMGB1 in **study I**. MPO^+EVs expressing HMGB1 may therefore be a potential disease activity biomarker in AAV.

5.4 THE ROLE OF CIRCULATING MPO^+EVS EXPRESSING MARKERS INVOLVED IN COAGULATION IN AAV

In this study we found that concentrations of MPO^+EVs and EVs expressing all the investigated markers, TF, H3Cit and Plg were higher in the total AAV patient group compared to controls and MPO^+EV expressing TF and H3Cit were elevated in active compared to inactive disease. Thrombin generation explored using a modified CAT assay was increased in patients with AAV compared to controls, but there was no difference in thrombin generation comparing inactive and active patients. A correlation between thrombin generation parameters and MPO^+EVs expressing H3Cit, TF and Plg was found.

There has been considerable interest in the procoagulant role of EV. In **study IV**, we used a modified thrombin generation assay to explore the effect of EVs on thrombin generation. Thrombin generation was measured after addition of EVs enriched pellet obtained from plasma samples of AAV patients and control subject instead of TF and phospholipids as in the original CAT method (343). Similar modified thrombin generation assays have previously been described. Bidot et al demonstrated enhanced EV mediated thrombin generation in patients with thrombosis compared to healthy controls. The EV mediated thrombin generation was even more increased in patients with recurrent thrombosis compared to patients with history of a single thrombotic event (344). Later Eleftheriou et al investigated EV mediated thrombin

generation in children with vasculitis (five with active and four with inactive AAV) using similar modified assay. An increase in EV mediated thrombin generation was found in active compared to inactive disease (345). In **study IV**, we found an augmented thrombin generation in the whole AAV patient group compared to controls using the modified CAT assay. This indicates that the procoagulant abilities of EVs contribute to the hypercoagulability seen in AAV.

TF is a key initiator of blood coagulation cascade leading to thrombin generation (275). Concentrations of NEVs bearing TF have been shown to be elevated in AAV (277) and EVs expressing TF have been demonstrated to bind and transfer proteins and lipids to activated platelets (276). Kambas et al showed that neutrophils from patients with active AAV release TF-expressing EVs and NETs. Additionally, they found higher levels of EVs expressing TF in AAV patients with active compared to inactive disease and a correlation between levels of NEVs expressing TF and BVAS (277). Huang et al later demonstrated that C5-primed neutrophils activated by ANCA released EVs and NETs expressing TF and furthermore that NETs containing TF had thrombin generation capacity (346). In a prospective study of AAV patients increased EV TF activity was associated with increased risk of VTE, even in remission (278). All these findings support the role of EVs expressing TF in the mechanisms behind hypercoagulability in AAV. The results of **study IV** further support these previous findings as NEVs expressing TF were not only elevated in active disease and correlated to disease activity, but also correlated to parameters of thrombin generation and may therefore contribute to the procoagulant state seen in AAV.

NETs are known to play a significant role in the mechanism of thrombosis in inflammatory state by interaction with the coagulation cascade and platelets (273). As previously noted, H3Cit is a specific biomarker for NETs (271). Mural model studies have demonstrated interaction between H3Cit and von Willebrand factor contributing to growth and stabilization of VTE (347). Interestingly, the presence of NETs in a venous thrombus derived from a patients with AAV has been reported (274). NEVs expressing H3Cit have not previously been investigated in AAV. Not only did we find elevated levels of $H3Cit^{+}MPO^{+}$EVs in AAV compared to controls in **study IV** but also higher levels in active disease compared to remission. However, we did not find a correlation between $H3Cit^{+}MPO^{+}$EVs and BVAS. A correlation between concentrations of NEVs expressing H3Cit with parameters of thrombin generation both in the whole group of AAV patients but also in the active disease subgroup was found. These results indicate that NEVs expressing components of NETs contribute to the procoagulant state in AAV patients, particularly in active disease.

Plasminogen (Plg) is a key component of the fibrinolytic system (279). The presence of Plg antibodies has been reported in AAV patients and have been found to delay the conversion of Plg to plasmin in vitro and to be associated with VTE in AAV patients (280-282). Furthermore, an association was found between levels of Plg antibodies and histologic lesions in the kidney and impaired kidney function (280). Beside its role in fibrinolysis, Plg exerts diverse inflammatory effects such as complement interaction, immune cell recruitment and resolution of inflammation (348). EV expressing Plg have not been measured previously in AAV. In **study IV**, while levels of $Plg^{+}MPO^{+}EV$ did not correlate to disease activity and were not significantly elevated in active disease, the levels were higher in patients compared to controls. The elevated concentrations of $MPO^{+}EVs$ expressing plasminogen in AAV patients may potentially be explained by the interaction between the fibrinolytic system, coagulation factors and complements in inflammation (283).

Interestingly, the levels of MPO EVs expressing all the investigated markers were significantly higher in patients with AAV, even in inactive disease, compared to controls. This suggest that NEVs expressing factors involved in coagulation may contribute to the increased risk of VTE seen in patients with AAV, even in remission (283).

5.5 GENERAL DISCUSSION

One of the clinical challenges in caring for AAV patients is the lack of a reliable and validated biomarkers to aid in the assessment of disease activity. Previous studies in other inflammatory diseases have demonstrated elevated PTX3 and TWEAK levels in active disease. These markers thus do not seem to be disease specific but may also contribute to inflammatory processes in AAV. Our novel results indicate that PTX3 and TWEAK may potentially have a role as biomarkers in monitoring disease activity in AAV. NEVs expressing TF, PTX3 and HMGB1 were shown to be potential biomarkers for assessment of disease activity but may also be involved in the disease pathogenesis. Furthermore, NEVs expressing factors involved in coagulation may contribute to the increased risk of thrombosis in AAV.

The studies presented in this thesis show that although most of the investigated biomarkers are lower in inactive compared to active disease, they are higher in patients in remission compared to controls. These findings indicate that despite improved treatment there may be an ongoing inflammatory activity in patients considered to be in remission. AAV patients, even in remission, commonly have decreased QoL with prominent symptoms such as fatigue, reduced energy levels, sleep disturbances and pain for which the mechanism is poorly understood and where treatment options are limited (141, 142). Although the reason for this is likely to be

multifactorial, an ongoing inflammatory process may be a contributing factor to those symptoms. Furthermore, this inflammatory activity may play a role in development of disease complications such as increased risk of CVD and VTE.

5.6 LIMITATIONS

All patients included in the studies came from the AAV cohort at the Karolinska University Hospital and were studied cross-sectionally or followed prospectively over time from diagnosis. Patients with a broad range of disease manifestations are included in the cohort, ranging from localized ENT manifestations to serious disease with multi-organ involvement. Treatment decisions were made by the treating physician. At the inclusion in the studies, CYC was the most common remission induction treatment used in systemic disease. RTX had not yet become a standard induction treatment option and therefore the effect of RTX on these biomarkers could not be assessed, nor compared to CYC or other treatment.

5.6.1 Study I and IV

The limitation of these studies is the relatively low number of both patients and controls included. Another limitation is the cross-sectional design and lack of disease controls.

Moreover, all but one patient in the active disease group had received treatment before sampling. We addressed this by investigating the association between the investigated markers and the treatment patients had received.

In **study IV**, the modified method based on a CAT-assay was used to assess thrombin generation. We did not extract specific EVs from the pellet and the EVs may therefore be derived from various cells and not specifically of neutrophilic origin. The effect of EVs derived from other cells cannot be ruled out. When measuring levels of investigated biomarkers, the majority of MPO^+EVs were assumed to be of neutrophilic origin since the MPO is primarily found in neutrophil granules, although also expressed on monocytes.

5.6.2 Study II and III

One limitation of the study was that most patients had received treatment before the time of sampling at baseline. As AAV are a group of serious and potentially life-threatening diseases, recruitment of patients before treatment onset is challenging. As in the other studies we attempted to address this limitation by evaluating the effect of treatment on levels of the investigated biomarkers.

Another limitation is the relatively short follow-up. Longer follow-up duration would have made evaluation of the role of PTX3 (**study II**) and TWEAK (**study III**) as potential predictors of disease relapse more suitable.

Thirdly, in **study II and III** urine samples for measurement of the investigated biomarkers were not available for all the study subjects and no urine samples from controls were available for comparison.

Finally, the lack of disease controls in these studies is a limitation.

6 CONCLUSIONS

In the studies presented in this thesis plasma and urinary PTX3 as well as urinary TWEAK were identified as potential biomarkers in AAV. These biomarkers were increased in active AAV and reflected disease activity in contrast to the commonly used inflammatory marker CRP. Although PTX3 and uTWEAK are not disease specific, we believe that they could aid in the monitoring of disease activity in AAV patients. As the markers were found to be elevated in AAV patients with kidney involvement and correlated with albuminuria novel research questions arise on their more specific role in the pathogenesis of kidney inflammation in AAV.

Concentrations of MPO positive EVs expressing PTX3, HMGB1 and TF were elevated in active disease compared to remission and reflected disease activity. This indicates that they may have a role in the pathogenesis and exacerbation of AAV and may potentially serve as biomarkers in the diagnosis and monitoring of AAV.

We found that circulating PTX3 levels and concentrations of MPO^+EVs expressing HMGB1, TWEAK, H3Cit, TF and Plg were higher in patients in remission compared to controls indicating that even during remission AAV patients have persistent inflammation compared to healthy subjects. This could be an important aspect in the increased risk of complications such as cardiovascular and thromboembolic events in AAV patients.

Finally, we found that patients with AAV had augmented thrombin generation compared to controls. As the concentrations of MPO^+EVs expressing TF and H3Cit correlated with parameters of thrombin generation our results indicate that functional procoagulant properties of EVs depend at least partly on MPO^+EVs expressing these factors. These results suggest that NEVs may contribute to the vascular injury and hypercoagulability seen in AAV patients.

7 POINTS OF PERSPECTIVE AND FUTURE CONSIDERATIONS

Even though our understanding of the pathogenesis of AAV has increased during recent decades and modern immunosuppressive treatment has changed the landscape in bringing about improved prognosis and decreased mortality rate challenges remain. Diagnosis and monitoring of disease activity, treatment related side-effects and the lack of individualized treatment options further contribute to these challenges. To date there are no known reliable biomarkers to evaluate disease activity in AAV and studies identifying better diagnostic and monitoring tools are needed. Non-invasive tests that reliably reflect disease activity and predict relapses would diminish the need for invasive procedures such as kidney biopsies to establish a relapse diagnosis. In this thesis, we have identified several potential biomarkers that could potentially aid in evaluation of AAV patients. Moreover, there has long been an unmet need of personalized treatment in AAV. Further evaluation of potential biomarkers may contribute to improvements in adapting individual therapy options for AAV with the long-term goal to reduce the risk of organ damage, especially kidney failure. Another important aspect would be a better assessment of risk and timely treatment of potential complications, such as identifying patients that would benefit from prophylactic anticoagulation due to an increased VTE risk.

Although our findings suggest that PTX3 might be a potential candidate biomarker in AAV much remains unclear. Due to the short-term follow-up in our study its role in detecting relapse could not be evaluated and thus the role of PTX3 in detecting flares requires longitudinal studies with follow-up of longer duration or prospective randomized trials. Furthermore, to clarify any impact of PTX3 in kidney injury investigations of PTX3 mechanistic role in vasculitis animal models as well as studies of PTX3 expression in kidney tissue from patients from AAV are still needed.

Similarly, although our findings suggest that urinary TWEAK may be a useful biomarker in AAV, the role in predicting relapsing disease remains to be evaluated. It would furthermore be informative to investigate the role of urinary TWEAK and its scavenger receptor CD163 in AAV. Urinary CD163 had been shown to be a promising novel non-invasive biomarker in ANCA-associated GN. Future studies evaluating both urinary TWEAK and CD163 could improve our knowledge of the role of TWEAK as a potential biomarker and in the pathogenesis of AAV. IHC staining on two kidney tissue samples demonstrated increased expression of TWEAK as well as a detectable staining for Fn14 in active AAV patients with crescentic GN. Prospective studies including tissue samples from a larger set of patients with a range of

histopathological presentations as well as from patients in remission would add to the knowledge on TWEAKs role in kidney injury in AAV.

EVs expressing inflammatory markers and markers of coagulation reflect disease activity and EVs expressing H3Cit and TF seem to be associated with augmented thrombin generation. However, our studies are relatively small and performed in a cross-sectional manner, thus need to be replicated in larger longitudinal studies. Although we found a correlation between NEVs expressing TF and H3Cit and parameters of thrombin generation the clinical implications of this have not been investigated. Studies of the association between NEVs expressing these markers and thromboembolic disease in a longitudinal cohort of AAV patients are needed. Moreover, we did not extract specific EVs from the pellet used in the modified CAT assay and therefore the EVs can be of various origin. More sophisticated isolation of EVs and investigation of the effect on thrombin generation could contribute to our understanding of how different EVs contribute to hypercoagulability in active and inactive AAV.

8 ACKNOWLEDGEMENTS

Firstly, I want to express my gratitude to all the patients who have participated in our studies. Without their engagement and willingness to take part in research this work would not have been possible.

I wish to thank everyone who have supported and helped me in so many ways during my PhD studies.

Special thanks to,

Annette Bruchfeld, my main supervisor. You are such an inspiration and a great role model for women in science. Thank you for all your support, encouragement, guidance, and our inspiring discussions. Thank you for always taking the time for me even when your schedule was beyond busy and for getting me back on track when needed. I greatly admire your devotion to research and your clinical skills; it really has been a privilege to work with you. I hope this is just the beginning of our collaboration.

Iva Gunnarsson, my co-supervisor. You really are an inspiring scientist and a great supervisor. Thank you for all your support along the way, wisdom, encouragement, insightful comments, and motivating discussions. Thank you for always being available and for keeping me on track.

Aleksandra Antovic, my co-supervisor. Thank you for the opportunity to work with you on this fascinating research. Thank you for your guidance and our fruitful discussions.

Sunna Snædal, my mentor during this PhD journey, dear friend, roommate, life-coach par excellence, and another great role model for women in medicine. Thank you for your endless support, words of wisdoms, pep talks, and laughs. I could not have chosen a better mentor.

Abdul Rashid (Tony) Qureshi for guidance with the statistics. For always making time for me when I got stuck and needed advice on how to proceed.

Professor **Peter Stenvinkel** for the support, positive attitude, and for all the help after my move to Iceland.

The former and current heads of the Nephrology Unit at Karolinska University hospital. **Olof Heimbürger** for hiring me and thereby making this possible and for all the guidance, wisdom, and help throughout the years. **Peter Barany** for being such an inspiring clinician and researcher. **Sara Lind af Hageby**, **Helena Rydell** and **Mats Söderhäll**, thank you for the opportunity for combining research and clinical work during my years at Karolinska and for all the understanding and help after my move to Iceland.

To all my **co-workers** and **co-authors** of these manuscripts. Special thanks to **Magnus Söderberg, Annika Wernerson** and **Angelina Schwartz** for their work on histopathologic

classification and immunohistochemical staining of biopsies, and to **Fariborz Mobarrez** for his work on extracellular vesicle analysis.

The **VASKA research group** at Karolinska, I am so very grateful for having had the opportunity to be part of your fantastic group. Thank you for the motivation, inspiration, and all the insightful comments on my work throughout the years.

I also want to thank all the researcher and clinicians who have contributed to the VASKA cohort.

To the former and current team **of nurses at the Research Unit** at Karolinska Huddinge for the cooperation. It was such a pleasure to work with you.

Björn Anderstam, Ann-Christin Bragfors-Helin and **Monica Eriksson** at the Renal Laboratory, Karolinska Instititutet, CLINTEC for their support and help with the laboratory analysis.

Nida Soutari at the Clinical Chemistry and Blood Coagulation laboratory, CMM, Karolinska Institutet for teaching me about laboratory methods.

My wonderful **colleagues at the Nephrology Department at Karolinska**. For all the friendship, guidance, motivation, and fun through my "Stockholm years". I really miss you all!

Linn Berg, administrator at CLINTEC, thank you for all the help and for always responding so promptly to my (not so few) questions and emails.

Eva Johansson, administrator at the Nephrology Unit, for always keeping an open door and for being an administrative genius.

The **kidney journal club**. Thank you for all our motivating sessions through the years. A special thanks to **Anna Witasp** for not only making the JC possible but also for all the invaluable advice and wisdom.

Margrét Birna, the head of the Nephrology Department at Landspitali, for all the understanding and the opportunity to combine research and clinical work after my move to Iceland.

My fantastic colleagues at the Nephrology Unit at Landspítali, **Fjölnir, Helga, Hrefna, Ólafur Skúli, Ragnar** and **Runólfur**, for all the encouragement and motivation on a daily basis and for stepping in when I was away for research.

Agneta Månsson-Broberg and **Karin Sendek**, my boss and co-worker at the course in Clinical Medicine at Karolinska Institutet. Thank you for all your support and encouragement.

I would also like to express my gratitude to,

My Anna (**Anna Levin**), although you have not had an official role in my PhD journey you have contributed so immensely to this thesis with your endless support, advice, and ideas. Thank you for always having a solution to my problems (that usually involves a lot of extra work for you), and for being my best friend. Now we can talk about something other than my thesis, I promise! I admire your dedication, curiosity, and thoroughness, you really are a great scientist. Thank you for everything!

Jessica Smolander, my dear friend. Thank you for all your invaluable support, help and advice and thank you for arranging my Stockholm life during these last months.

Helga Ágústa, my M.Sc. supervisor, for getting me started in research and for being a dear friend. I hope we can do some research together in the future.

My fantastic "spena" friends, **Ásthildur, Sunna, Gyða, Auður** and **Nanna** and special thanks to my friend and amazing artist **Una** for making the beautiful cover illustration. Thank you all for your encouragement, support and for reminding me of all the fantastic things awaiting.

My friends **Berglind**, **Elín**, **Guðný**, **Sigrún**, **Halla, Þóra, Katarina** and **Markus**, for your friendship and support.

My friend and neighbour **Birna,** for the help with corrective reading and for taking me out for regular runs when this work felt overwhelming.

My little (big) sister **Silla**, thank you for all your incredible help and support and for always believing in me. You are the best! Many thanks to my beloved cousins, **Rakel** and **Rebekka**, for giving me inspiration and for making life so much more fun.

My mother, **Bergþóra**, you are my biggest role model, with your intelligence, warmth, and kindness. Thank you for always being there for me and believing in me.

And finally, my children, **Sóllilja**, **Jónas Jökull** and **Snæfríður**, for all the patience and understanding during these last months and for constantly reminding me that life is wonderful and my **Þórður**, my beloved husband and best friend. Without your support I could never have completed this work. You are my rock!

This work was supported by the regional agreement on medical training and clinical research (ALF) between Karolinska Institutet and Region Stockholm, the Swedish Society of Nephrology, the Swedish Kidney Foundation, King Gustaf the V-80 years foundation, the Swedish Rheumatism Association and the Landspitali University Hospital Research Fund.

9 REFERENCES

1. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum. 2013;65(1):1-11.

2. Kitching AR, Anders HJ, Basu N, Brouwer E, Gordon J, Jayne DR, et al. ANCA-associated vasculitis. Nat Rev Dis Primers. 2020;6(1):71.

3. Fries JF, Hunder GG, Bloch DA, Michel BA, Arend WP, Calabrese LH, et al. The American College of Rheumatology 1990 criteria for the classification of vasculitis. Summary. Arthritis Rheum. 1990;33(8):1135-6.

4. Watts R, Lane S, Hanslik T, Hauser T, Hellmich B, Koldingsnes W, et al. Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. Ann Rheum Dis. 2007;66(2):222-7.

5. Suppiah R, Robson JC, Grayson PC, Ponte C, Craven A, Khalid S, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for microscopic polyangiitis. Ann Rheum Dis. 2022;81(3):321-6.

6. Robson JC, Grayson PC, Ponte C, Suppiah R, Craven A, Judge A, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for granulomatosis with polyangiitis. Ann Rheum Dis. 2022;81(3):315-20.

7. Grayson PC, Ponte C, Suppiah R, Robson JC, Craven A, Judge A, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology Classification Criteria for Eosinophilic Granulomatosis with Polyangiitis. Ann Rheum Dis. 2022;81(3):309-14.

8. Kronbichler A, Bond M, Dejaco C**.** Classification criteria for ANCA-associated vasculitis: one size does not fit all! Rheumatology (Oxford). 2022.

9. Windpessl M, Bettac EL, Gauckler P, Shin JI, Geetha D, Kronbichler A**.** ANCA Status or Clinical Phenotype - What Counts More? Curr Rheumatol Rep. 2021;23(6):37.

10. Berti A, Cornec D, Crowson CS, Specks U, Matteson EL**.** The Epidemiology of Antineutrophil Cytoplasmic Autoantibody-Associated Vasculitis in Olmsted County, Minnesota: A Twenty-Year US Population-Based Study. Arthritis Rheumatol. 2017;69(12):2338-50.

11. Mohammad AJ**.** An update on the epidemiology of ANCA-associated vasculitis. Rheumatology (Oxford). 2020;59(Supplement_3):iii42-iii50.

12. Watts RA, Gonzalez-Gay MA, Lane SE, Garcia-Porrua C, Bentham G, Scott DG**.** Geoepidemiology of systemic vasculitis: comparison of the incidence in two regions of Europe. Ann Rheum Dis. 2001;60(2):170-2.

13. Fujimoto S, Watts RA, Kobayashi S, Suzuki K, Jayne DR, Scott DG, et al. Comparison of the epidemiology of anti-neutrophil cytoplasmic antibody-associated vasculitis between Japan and the U.K. Rheumatology (Oxford). 2011;50(10):1916-20.

14. Ormerod AS, Cook MC. Epidemiology of primary systemic vasculitis in the Australian Capital Territory and south-eastern New South Wales. Intern Med J. 2008;38(11):816-23.

15. Pearce FA, Craven A, Merkel PA, Luqmani RA, Watts RA. Global ethnic and geographic differences in the clinical presentations of anti-neutrophil cytoplasm antibody-associated vasculitis. Rheumatology (Oxford). 2017;56(11):1962-9.

16. Weiner M, Bjorneklett R, Hruskova Z, Mackinnon B, Poulton CJ, Sindelar L, et al. Proteinase-3 and myeloperoxidase serotype in relation to demographic factors and geographic distribution in anti-neutrophil cytoplasmic antibody-associated glomerulonephritis. Nephrol Dial Transplant. 2019;34(2):301-8.

17. Mohammad AJ, Jacobsson LT, Westman KW, Sturfelt G, Segelmark M. Incidence and survival rates in Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome and polyarteritis nodosa. Rheumatology (Oxford). 2009;48(12):1560-5.

18. Sinico RA, Di Toma L, Radice A. Renal involvement in anti-neutrophil cytoplasmic autoantibody associated vasculitis. Autoimmun Rev. 2013;12(4):477-82.

19. Yates M, Watts R. ANCA-associated vasculitis. Clin Med (Lond). 2017;17(1):60-4.

20. Falk RJ, Hogan S, Carey TS, Jennette JC. Clinical course of anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and systemic vasculitis. The Glomerular Disease Collaborative Network. Ann Intern Med. 1990;113(9):656-63.

21. Yacyshyn E, Johnson A, Rode M, Pagnoux C. Patient-driven online survey on the clinical manifestations and diagnostic delay of granulomatosis with polyangiitis. Joint Bone Spine. 2016;83(5):599-600.

22. Poulton CJ, Nachman PH, Hu Y, McGregor JG, Jennette JC, Falk RJ, et al. Pathways to renal biopsy and diagnosis among patients with ANCA small-vessel vasculitis. Clin Exp Rheumatol. 2013;31(1 Suppl 75):S32-7.

23. Pearce FA, Hubbard RB, Grainge MJ, Watts RA, Abhishek A, Lanyon PC. Can granulomatosis with polyangiitis be diagnosed earlier in primary care? A case-control study. QJM. 2018;111(1):39-45.

24. Houben E, Groenland SL, van der Heijden JW, Voskuyl AE, Doodeman HJ, Penne EL. Relation between duration of the prodromal phase and renal damage in ANCA-associated vasculitis. BMC Nephrol. 2017;18(1):378.

25. Geetha D, Jefferson JA. ANCA-Associated Vasculitis: Core Curriculum 2020. Am J Kidney Dis. 2020;75(1):124-37.

26. Jennette JC, Falk RJ. Small-vessel vasculitis. N Engl J Med. 1997;337(21):1512-23.

27. Lutalo PM, D'Cruz DP. Diagnosis and classification of granulomatosis with polyangiitis (aka Wegener's granulomatosis). J Autoimmun. 2014;48-49:94-8.

28. Guillevin L, Durand-Gasselin B, Cevallos R, Gayraud M, Lhote F, Callard P, et al. Microscopic polyangiitis: clinical and laboratory findings in eighty-five patients. Arthritis Rheum. 1999;42(3):421-30.

29. Finkielman JD, Lee AS, Hummel AM, Viss MA, Jacob GL, Homburger HA, et al. ANCA are detectable in nearly all patients with active severe Wegener's granulomatosis. Am J Med. 2007;120(7):643 e9-14.

30. Savige J, Gillis D, Benson E, Davies D, Esnault V, Falk RJ, et al. International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). Am J Clin Pathol. 1999;111(4):507-13.

31. Bossuyt X, Cohen Tervaert JW, Arimura Y, Blockmans D, Flores-Suarez LF, Guillevin L, et al. Position paper: Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. Nat Rev Rheumatol. 2017;13(11):683-92.

32. Yates M, Watts RA, Bajema IM, Cid MC, Crestani B, Hauser T, et al. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. Ann Rheum Dis. 2016;75(9):1583-94.

33. Aasarod K, Bostad L, Hammerstrom J, Jorstad S, Iversen BM**.** Renal histopathology and clinical course in 94 patients with Wegener's granulomatosis. Nephrol Dial Transplant. 2001;16(5):953-60.

34. Devaney KO, Travis WD, Hoffman G, Leavitt R, Lebovics R, Fauci AS**.** Interpretation of head and neck biopsies in Wegener's granulomatosis. A pathologic study of 126 biopsies in 70 patients. Am J Surg Pathol. 1990;14(6):555-64.

35. Schnabel A, Holl-Ulrich K, Dalhoff K, Reuter M, Gross WL**.** Efficacy of transbronchial biopsy in pulmonary vaculitides. Eur Respir J. 1997;10(12):2738-43.

36. Grau RG**.** Drug-Induced Vasculitis: New Insights and a Changing Lineup of Suspects. Curr Rheumatol Rep. 2015;17(12):71.

37. Gomez-Puerta JA, Gedmintas L, Costenbader KH**.** The association between silica exposure and development of ANCA-associated vasculitis: systematic review and meta-analysis. Autoimmun Rev. 2013;12(12):1129-35.

38. Pinching AJ, Rees AJ, Pussell BA, Lockwood CM, Mitchison RS, Peters DK**.** Relapses in Wegener's granulomatosis: the role of infection. Br Med J. 1980;281(6244):836-8.

39. Stegeman CA, Tervaert JW, Sluiter WJ, Manson WL, de Jong PE, Kallenberg CG**.** Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. Ann Intern Med. 1994;120(1):12-7.

40. Zycinska K, Wardyn KA, Zielonka TM, Krupa R, Lukas W**.** Co-trimoxazole and prevention of relapses of PR3-ANCA positive vasculitis with pulmonary involvement. Eur J Med Res. 2009;14 Suppl 4:265-7.

41. Wagner J, Harrison EM, Martinez Del Pero M, Blane B, Mayer G, Leierer J, et al. The composition and functional protein subsystems of the human nasal microbiome in granulomatosis with polyangiitis: a pilot study. Microbiome. 2019;7(1):137.

42. Lyons PA, Rayner TF, Trivedi S, Holle JU, Watts RA, Jayne DR, et al. Genetically distinct subsets within ANCA-associated vasculitis. N Engl J Med. 2012;367(3):214-23.

43. Merkel PA, Xie G, Monach PA, Ji X, Ciavatta DJ, Byun J, et al. Identification of Functional and Expression Polymorphisms Associated With Risk for Antineutrophil Cytoplasmic Autoantibody-Associated Vasculitis. Arthritis Rheumatol. 2017;69(5):1054-66.

44. van der Woude FJ, Rasmussen N, Lobatto S, Wiik A, Permin H, van Es LA, et al. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet. 1985;1(8426):425-9.

45. Davies DJ, Moran JE, Niall JF, Ryan GB**.** Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? Br Med J (Clin Res Ed). 1982;285(6342):606.

46. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. J Clin Invest. 2002;110(7):955-63.

47. Little MA, Smyth CL, Yadav R, Ambrose L, Cook HT, Nourshargh S, et al. Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. Blood. 2005;106(6):2050-8.

48. Schreiber A, Xiao H, Falk RJ, Jennette JC**.** Bone marrow-derived cells are sufficient and necessary targets to mediate glomerulonephritis and vasculitis induced by anti-myeloperoxidase antibodies. J Am Soc Nephrol. 2006;17(12):3355-64.

49. Bansal PJ, Tobin MC**.** Neonatal microscopic polyangiitis secondary to transfer of maternal myeloperoxidase-antineutrophil cytoplasmic antibody resulting in neonatal pulmonary hemorrhage and renal involvement. Ann Allergy Asthma Immunol. 2004;93(4):398-401.

50. Little MA**.** L7. Animal models of PR3-ANCA vasculitis: approaches and controversies. Presse Med. 2013;42(4 Pt 2):512-5.

51. Tomasson G, Grayson PC, Mahr AD, Lavalley M, Merkel PA**.** Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis--a meta-analysis. Rheumatology (Oxford). 2012;51(1):100-9.

52. Fussner LA, Hummel AM, Schroeder DR, Silva F, Cartin-Ceba R, Snyder MR, et al. Factors Determining the Clinical Utility of Serial Measurements of Antineutrophil Cytoplasmic Antibodies Targeting Proteinase 3. Arthritis Rheumatol. 2016;68(7):1700-10.

53. Kemna MJ, Damoiseaux J, Austen J, Winkens B, Peters J, van Paassen P, et al. ANCA as a predictor of relapse: useful in patients with renal involvement but not in patients with nonrenal disease. J Am Soc Nephrol. 2015;26(3):537-42.

54. Cui Z, Zhao MH, Segelmark M, Hellmark T**.** Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. Kidney Int. 2010;78(6):590-7.

55. Hutton HL, Holdsworth SR, Kitching AR**.** ANCA-Associated Vasculitis: Pathogenesis, Models, and Preclinical Testing. Semin Nephrol. 2017;37(5):418-35.

56. Kain R, Exner M, Brandes R, Ziebermayr R, Cunningham D, Alderson CA, et al. Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. Nat Med. 2008;14(10):1088-96.

57. Nakazawa D, Masuda S, Tomaru U, Ishizu A**.** Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. Nat Rev Rheumatol. 2019;15(2):91-101.

58. Xiao H, Heeringa P, Liu Z, Huugen D, Hu P, Maeda N, et al. The role of neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibodies. Am J Pathol. 2005;167(1):39-45.

59. Kessenbrock K, Krumbholz M, Schonermarck U, Back W, Gross WL, Werb Z, et al. Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med. 2009;15(6):623-5.

60. Schreiber A, Rousselle A, Becker JU, von Massenhausen A, Linkermann A, Kettritz R**.** Necroptosis controls NET generation and mediates complement activation, endothelial damage, and autoimmune vasculitis. Proc Natl Acad Sci U S A. 2017;114(45):E9618-E25.

61. Kettritz R**.** How anti-neutrophil cytoplasmic autoantibodies activate neutrophils. Clin Exp Immunol. 2012;169(3):220-8.

62. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, et al. Novel cell death program leads to neutrophil extracellular traps. J Cell Biol. 2007;176(2):231-41.

63. Porges AJ, Redecha PB, Kimberly WT, Csernok E, Gross WL, Kimberly RP**.** Anti-neutrophil cytoplasmic antibodies engage and activate human neutrophils via Fc gamma RIIa. J Immunol. 1994;153(3):1271-80.

64. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303(5663):1532-5.

65. Nakazawa D, Shida H, Tomaru U, Yoshida M, Nishio S, Atsumi T, et al. Enhanced formation and disordered regulation of NETs in myeloperoxidase-ANCA-associated microscopic polyangiitis. J Am Soc Nephrol. 2014;25(5):990-7.

66. Wang H, Wang C, Zhao MH, Chen M**.** Neutrophil extracellular traps can activate alternative complement pathways. Clin Exp Immunol. 2015;181(3):518-27.

67. Sangaletti S, Tripodo C, Chiodoni C, Guarnotta C, Cappetti B, Casalini P, et al. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. Blood. 2012;120(15):3007-18.

68. Trouw LA, Seelen MA, Daha MR**.** Complement and renal disease. Mol Immunol. 2003;40(2-4):125-34.

69. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC**.** Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. Am J Pathol. 2007;170(1):52-64.

70. Huugen D, van Esch A, Xiao H, Peutz-Kootstra CJ, Buurman WA, Tervaert JW, et al. Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. Kidney Int. 2007;71(7):646-54.

71. Schreiber A, Xiao H, Jennette JC, Schneider W, Luft FC, Kettritz R**.** C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. J Am Soc Nephrol. 2009;20(2):289-98.

72. Xiao H, Dairaghi DJ, Powers JP, Ertl LS, Baumgart T, Wang Y, et al. C5a receptor (CD88) blockade protects against MPO-ANCA GN. J Am Soc Nephrol. 2014;25(2):225-31.

73. Dick J, Gan PY, Ford SL, Odobasic D, Alikhan MA, Loosen SH, et al. C5a receptor 1 promotes autoimmunity, neutrophil dysfunction and injury in experimental anti-myeloperoxidase glomerulonephritis. Kidney Int. 2018;93(3):615-25.

74. Chen SF, Wang FM, Li ZY, Yu F, Chen M, Zhao MH**.** Complement Factor H Inhibits Anti-Neutrophil Cytoplasmic Autoantibody-Induced Neutrophil Activation by Interacting With Neutrophils. Front Immunol. 2018;9:559.

75. Chen SF, Wang FM, Li ZY, Yu F, Chen M, Zhao MH**.** Myeloperoxidase influences the complement regulatory activity of complement factor H. Rheumatology (Oxford). 2018;57(12):2213-24.

76. O'Flynn J, Dixon KO, Faber Krol MC, Daha MR, van Kooten C**.** Myeloperoxidase directs properdin-mediated complement activation. J Innate Immun. 2014;6(4):417-25.

77. Haas M, Eustace JA**.** Immune complex deposits in ANCA-associated crescentic glomerulonephritis: a study of 126 cases. Kidney Int. 2004;65(6):2145-52.

78. Chen M, Xing GQ, Yu F, Liu G, Zhao MH**.** Complement deposition in renal histopathology of patients with ANCA-associated pauci-immune glomerulonephritis. Nephrol Dial Transplant. 2009;24(4):1247-52.

79. Oba R, Kanzaki G, Sasaki T, Okabayashi Y, Haruhara K, Okabe M, et al. Long-Term Renal Survival in Antineutrophil Cytoplasmic Antibody-Associated Glomerulonephritis With Complement C3 Deposition. Kidney Int Rep. 2021;6(10):2661-70.

80. Xing GQ, Chen M, Liu G, Heeringa P, Zhang JJ, Zheng X, et al. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. J Clin Immunol. 2009;29(3):282-91.

81. Gou SJ, Yuan J, Wang C, Zhao MH, Chen M**.** Alternative complement pathway activation products in urine and kidneys of patients with ANCA-associated GN. Clin J Am Soc Nephrol. 2013;8(11):1884-91.

82. Gou SJ, Yuan J, Chen M, Yu F, Zhao MH**.** Circulating complement activation in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. Kidney Int. 2013;83(1):129-37.

83. Deshayes S, Aouba A, Khoy K, Mariotte D, Lobbedez T, Martin Silva N**.** Hypocomplementemia is associated with worse renal survival in ANCA-positive granulomatosis with polyangiitis and microscopic polyangiitis. PLoS One. 2018;13(4):e0195680.

84. Kojima T, Inoue D, Wajima T, Uchida T, Yamada M, Ohsawa I, et al. Circulating immune-complexes and complement activation through the classical pathway in myeloperoxidase-ANCA-associated glomerulonephritis. Ren Fail. 2022;44(1):714-23.

85. Chen M, Jayne DRW, Zhao MH**.** Complement in ANCA-associated vasculitis: mechanisms and implications for management. Nat Rev Nephrol. 2017;13(6):359-67.

86. Abdulahad WH, Kallenberg CG, Limburg PC, Stegeman CA**.** Urinary CD4+ effector memory T cells reflect renal disease activity in antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2009;60(9):2830-8.

87. Weidner S, Carl M, Riess R, Rupprecht HD**.** Histologic analysis of renal leukocyte infiltration in antineutrophil cytoplasmic antibody-associated vasculitis: importance of monocyte and neutrophil infiltration in tissue damage. Arthritis Rheum. 2004;50(11):3651-7.

88. Wilde B, Thewissen M, Damoiseaux J, van Paassen P, Witzke O, Tervaert JW**.** T cells in ANCA-associated vasculitis: what can we learn from lesional versus circulating T cells? Arthritis Res Ther. 2010;12(1):204.

89. Ghali JR, Wang YM, Holdsworth SR, Kitching AR**.** Regulatory T cells in immune-mediated renal disease. Nephrology (Carlton). 2016;21(2):86-96.

90. Jennette JC, Falk RJ**.** B cell-mediated pathogenesis of ANCA-mediated vasculitis. Semin Immunopathol. 2014;36(3):327-38.

91. Wang Y, Zhang S, Zhang N, Feng M, Liang Z, Zhao X, et al. Reduced activated regulatory T cells and imbalance of Th17/activated Treg cells marks renal involvement in ANCA-associated vasculitis. Mol Immunol. 2020;118:19-29.

92. Zabinska M, Koscielska-Kasprzak K, Krajewska J, Bartoszek D, Augustyniak-Bartosik H, Krajewska M**.** Immune Cells Profiling in ANCA-Associated Vasculitis Patients-Relation to Disease Activity. Cells. 2021;10(7).

93. Free ME, Bunch DO, McGregor JA, Jones BE, Berg EA, Hogan SL, et al. Patients with antineutrophil cytoplasmic antibody-associated vasculitis have defective Treg cell function exacerbated by the presence of a suppression-resistant effector cell population. Arthritis Rheum. 2013;65(7):1922-33.

94. Nogueira E, Hamour S, Sawant D, Henderson S, Mansfield N, Chavele KM, et al. Serum IL-17 and IL-23 levels and autoantigen-specific Th17 cells are elevated in patients with ANCA-associated vasculitis. Nephrol Dial Transplant. 2010;25(7):2209-17.

95. Gan PY, Steinmetz OM, Tan DS, O'Sullivan KM, Ooi JD, Iwakura Y, et al. Th17 cells promote autoimmune anti-myeloperoxidase glomerulonephritis. J Am Soc Nephrol. 2010;21(6):925-31.

96. Berden AE, Jones RB, Erasmus DD, Walsh M, Noel LH, Ferrario F, et al. Tubular lesions predict renal outcome in antineutrophil cytoplasmic antibody-associated glomerulonephritis after rituximab therapy. J Am Soc Nephrol. 2012;23(2):313-21.

97. Mauri C**.** Regulation of immunity and autoimmunity by B cells. Curr Opin Immunol. 2010;22(6):761-7.

98. Nagai M, Hirayama K, Ebihara I, Shimohata H, Kobayashi M, Koyama A**.** Serum levels of BAFF and APRIL in myeloperoxidase anti-neutrophil cytoplasmic autoantibody-associated renal vasculitis: association with disease activity. Nephron Clin Pract. 2011;118(4):c339-45.

99. Holden NJ, Williams JM, Morgan MD, Challa A, Gordon J, Pepper RJ, et al. ANCA-stimulated neutrophils release BLyS and promote B cell survival: a clinically relevant cellular process. Ann Rheum Dis. 2011;70(12):2229-33.

100. Culton DA, Nicholas MW, Bunch DO, Zhen QL, Kepler TB, Dooley MA, et al. Similar CD19 dysregulation in two autoantibody-associated autoimmune diseases suggests a shared mechanism of B-cell tolerance loss. J Clin Immunol. 2007;27(1):53-68.

101. Wilde B, Thewissen M, Damoiseaux J, Knippenberg S, Hilhorst M, van Paassen P, et al. Regulatory B cells in ANCA-associated vasculitis. Ann Rheum Dis. 2013;72(8):1416-9.

102. Wang C, Li ZY, Gong Y, You R, Ming H, Zhao, et al. Increased frequency of IgD-CD27(hi)CD38(hi) B cells and its association with the renal involvement in ANCA-associated vasculitis. Arthritis Res Ther. 2022;24(1):109.

103. Zhao Y, Odell E, Choong LM, Barone F, Fields P, Wilkins B, et al. Granulomatosis with polyangiitis involves sustained mucosal inflammation that is rich in B-cell survival factors and autoantigen. Rheumatology (Oxford). 2012;51(9):1580-6.

104. McClure M CM, Lee J, Tuong K, Ferdinand J, Gopaluni S, Jones R, Jayne D, Clatworthy M**.** Single-cell transcriptome profiling of rituximab-resistant tissue B cells in ANCA-associated vasculitis. Proceedings of the 20th International Vasculitis and ANCA workshop 3-6th April 2022. 20th International Vasculitis and ANCA conference (Vasculitis2022), 2022. Dublin, Ireland.

105. Chanouzas D, McGregor JAG, Nightingale P, Salama AD, Szpirt WM, Basu N, et al. Intravenous pulse methylprednisolone for induction of remission in severe ANCA associated Vasculitis: a multi-center retrospective cohort study. BMC Nephrol. 2019;20(1):58.

106. Walsh M, Merkel PA, Jayne DRW**.** Plasma Exchange and Glucocorticoids in Severe ANCA-Associated Vasculitis. Reply. N Engl J Med. 2020;382(22):2169.

107. de Groot K, Harper L, Jayne DR, Flores Suarez LF, Gregorini G, Gross WL, et al. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. Ann Intern Med. 2009;150(10):670-80.

108. Harper L, Morgan MD, Walsh M, Hoglund P, Westman K, Flossmann O, et al. Pulse versus daily oral cyclophosphamide for induction of remission in ANCA-associated vasculitis: long-term follow-up. Ann Rheum Dis. 2012;71(6):955-60.

109. Onrust SV, Lamb HM, Balfour JA**.** Rituximab. Drugs. 1999;58(1):79-88; discussion 9-90.

110. Jones RB, Tervaert JW, Hauser T, Luqmani R, Morgan MD, Peh CA, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. N Engl J Med. 2010;363(3):211-20.

111. Stone JH, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med. 2010;363(3):221-32.

112. Gulati K, Edwards H, Prendecki M, Cairns TD, Condon M, Galliford J, et al. Combination treatment with rituximab, low-dose cyclophosphamide and plasma exchange for severe antineutrophil cytoplasmic antibody-associated vasculitis. Kidney Int. 2021;100(6):1316-24.

113. McAdoo SP, Medjeral-Thomas N, Gopaluni S, Tanna A, Mansfield N, Galliford J, et al. Long-term follow-up of a combined rituximab and cyclophosphamide regimen in renal anti-neutrophil cytoplasm antibody-associated vasculitis. Nephrol Dial Transplant. 2019;34(1):63-73.

114. De Groot K, Rasmussen N, Bacon PA, Tervaert JW, Feighery C, Gregorini G, et al. Randomized trial of cyclophosphamide versus methotrexate for induction of

remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2005;52(8):2461-9.

115. Jones RB, Hiemstra TF, Ballarin J, Blockmans DE, Brogan P, Bruchfeld A, et al. Mycophenolate mofetil versus cyclophosphamide for remission induction in ANCA-associated vasculitis: a randomised, non-inferiority trial. Ann Rheum Dis. 2019;78(3):399-405.

116. Kuzuya K, Morita T, Kumanogoh A**.** Efficacy of mycophenolate mofetil as a remission induction therapy in antineutrophil cytoplasmic antibody: associated vasculitis-a meta-analysis. RMD Open. 2020;6(1).

117. Walsh M, Collister D, Zeng L, Merkel PA, Pusey CD, Guyatt G, et al. The effects of plasma exchange in patients with ANCA-associated vasculitis: an updated systematic review and meta-analysis. BMJ. 2022;376:e064604.

118. Jayne DRW, Merkel PA, Schall TJ, Bekker P, Group AS**.** Avacopan for the Treatment of ANCA-Associated Vasculitis. N Engl J Med. 2021;384(7):599-609.

119. Jayne D, Rasmussen N, Andrassy K, Bacon P, Tervaert JW, Dadoniene J, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. N Engl J Med. 2003;349(1):36-44.

120. Hiemstra TF, Walsh M, Mahr A, Savage CO, de Groot K, Harper L, et al. Mycophenolate mofetil vs azathioprine for remission maintenance in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized controlled trial. JAMA. 2010;304(21):2381-8.

121. Guillevin L, Pagnoux C, Karras A, Khouatra C, Aumaitre O, Cohen P, et al. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. N Engl J Med. 2014;371(19):1771-80.

122. Smith RJ, D; Merkel PA on behalf of RITAZAREM Investigators**.** OP0026 A RANDOMIZED, CONTROLLED TRIAL OF RITUXIMAB VERSUS AZATHIOPRINE AFTER INDUCTION OF REMISSION WITH RITUXIMAB FOR PATIENTS WITH ANCA-ASSOCIATED VASCULITIS AND RELAPSING DISEASE. Annals of the Rheumatic Diseases. 2020;79:19-20.

123. Karras A, Pagnoux C, Haubitz M, Groot K, Puechal X, Tervaert JWC, et al. Randomised controlled trial of prolonged treatment in the remission phase of ANCA-associated vasculitis. Ann Rheum Dis. 2017;76(10):1662-8.

124. Walton EW**.** Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). Br Med J. 1958;2(5091):265-70.

125. Mukhtyar C, Flossmann O, Hellmich B, Bacon P, Cid M, Cohen-Tervaert JW, et al. Outcomes from studies of antineutrophil cytoplasm antibody associated vasculitis: a systematic review by the European League Against Rheumatism systemic vasculitis task force. Ann Rheum Dis. 2008;67(7):1004-10.

126. Zand L, Specks U, Sethi S, Fervenza FC**.** Treatment of ANCA-associated vasculitis: new therapies and a look at old entities. Adv Chronic Kidney Dis. 2014;21(2):182-93.

127. Little MA, Nightingale P, Verburgh CA, Hauser T, De Groot K, Savage C, et al. Early mortality in systemic vasculitis: relative contribution of adverse events and active vasculitis. Ann Rheum Dis. 2010;69(6):1036-43.

128. Robson J, Doll H, Suppiah R, Flossmann O, Harper L, Hoglund P, et al. Damage in the anca-associated vasculitides: long-term data from the European vasculitis study group (EUVAS) therapeutic trials. Ann Rheum Dis. 2015;74(1):177-84.

129. Flossmann O, Berden A, de Groot K, Hagen C, Harper L, Heijl C, et al. Long-term patient survival in ANCA-associated vasculitis. Ann Rheum Dis. 2011;70(3):488-94.

130. Westman KW, Bygren PG, Olsson H, Ranstam J, Wieslander J**.** Relapse rate, renal survival, and cancer morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis with renal involvement. J Am Soc Nephrol. 1998;9(5):842-52.

131. Hogan SL, Falk RJ, Chin H, Cai J, Jennette CE, Jennette JC, et al. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. Ann Intern Med. 2005;143(9):621-31.

132. Hruskova Z, Casian AL, Konopasek P, Svobodova B, Frausova D, Lanska V, et al. Long-term outcome of severe alveolar haemorrhage in ANCA-associated vasculitis: a retrospective cohort study. Scand J Rheumatol. 2013;42(3):211-4.

133. Walsh M, Flossmann O, Berden A, Westman K, Hoglund P, Stegeman C, et al. Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2012;64(2):542-8.

134. Hara A, Wada T, Sada KE, Amano K, Dobashi H, Harigai M, et al. Risk Factors for Relapse of Antineutrophil Cytoplasmic Antibody-associated Vasculitis in Japan: A Nationwide, Prospective Cohort Study. J Rheumatol. 2018;45(4):521-8.

135. Terrier B, Pagnoux C, Perrodeau E, Karras A, Khouatra C, Aumaitre O, et al. Long-term efficacy of remission-maintenance regimens for ANCA-associated vasculitides. Ann Rheum Dis. 2018;77(8):1150-6.

136. Koldingsnes W, Nossent JC**.** Baseline features and initial treatment as predictors of remission and relapse in Wegener's granulomatosis. J Rheumatol. 2003;30(1):80-8.

137. Goceroglu A, Berden AE, Fiocco M, Flossmann O, Westman KW, Ferrario F, et al. ANCA-Associated Glomerulonephritis: Risk Factors for Renal Relapse. PLoS One. 2016;11(12):e0165402.

138. Slot MC, Tervaert JW, Franssen CF, Stegeman CA**.** Renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement. Kidney Int. 2003;63(2):670-7.

139. de Joode AA, Sanders JS, Stegeman CA**.** Renal survival in proteinase 3 and myeloperoxidase ANCA-associated systemic vasculitis. Clin J Am Soc Nephrol. 2013;8(10):1709-17.

140. Robson J, Doll H, Suppiah R, Flossmann O, Harper L, Hoglund P, et al. Glucocorticoid treatment and damage in the anti-neutrophil cytoplasm antibody-associated vasculitides: long-term data from the European Vasculitis Study Group trials. Rheumatology (Oxford). 2015;54(3):471-81.

141. Basu N, McClean A, Harper L, Amft EN, Dhaun N, Luqmani RA, et al. The characterisation and determinants of quality of life in ANCA associated vasculitis. Ann Rheum Dis. 2014;73(1):207-11.

142. Herlyn K, Hellmich B, Seo P, Merkel PA. Patient-reported outcome assessment in vasculitis may provide important data and a unique perspective. Arthritis Care Res (Hoboken). 2010;62(11):1639-45.

143. Robson JC, Dawson J, Cronholm PF, Ashdown S, Easley E, Kellom KS, et al. Patient perceptions of glucocorticoids in anti-neutrophil cytoplasmic antibody-associated vasculitis. Rheumatol Int. 2018;38(4):675-82.

144. Furer V, Rondaan C, Heijstek MW, Agmon-Levin N, van Assen S, Bijl M, et al. 2019 update of EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. Ann Rheum Dis. 2020;79(1):39-52.

145. Stevens KI, Frangou E, Shin JIL, Anders HJ, Bruchfeld A, Schonermarck U, et al. Perspective on COVID-19 vaccination in patients with immune-mediated kidney diseases: consensus statements from the ERA-IWG and EUVAS. Nephrol Dial Transplant. 2022;37(8):1400-10.

146. Bruchfeld A, Kronbichler A, Alberici F, Fervenza FC, Jayne DRW, Segelmark M, et al. COVID-19 and ANCA-associated vasculitis: recommendations for vaccine preparedness and the use of rituximab. Nephrol Dial Transplant. 2021;36(9):1758-60.

147. Antovic A, Bruchfeld A, Ekland J, Lovstrom B, Hugelius A, Borjesson O, et al. Risks and treatment related aspects of COVID-19 infection in patients with ANCA-associated vasculitis. Scand J Rheumatol. 2022:1-6.

148. Kronbichler A, Geetha D, Smith RM, Egan AC, Bajema IM, Schonermarck U, et al. The COVID-19 pandemic and ANCA-associated vasculitis - reports from the EUVAS meeting and EUVAS education forum. Autoimmun Rev. 2021;20(12):102986.

149. Berti A, Matteson EL, Crowson CS, Specks U, Cornec D. Risk of Cardiovascular Disease and Venous Thromboembolism Among Patients With Incident ANCA-Associated Vasculitis: A 20-Year Population-Based Cohort Study. Mayo Clin Proc. 2018;93(5):597-606.

150. Avina-Zubieta JA, Mai A, Amiri N, Dehghan N, Ann Tan J, Sayre EC, et al. Risk of Myocardial Infarction and Stroke in Patients With Granulomatosis With Polyangiitis (Wegener's): A Population-Based Study. Arthritis Rheumatol. 2016;68(11):2752-9.

151. Faurschou M, Mellemkjaer L, Sorensen IJ, Svalgaard Thomsen B, Dreyer L, Baslund B. Increased morbidity from ischemic heart disease in patients with Wegener's granulomatosis. Arthritis Rheum. 2009;60(4):1187-92.

152. Alfaddagh A, Martin SS, Leucker TM, Michos ED, Blaha MJ, Lowenstein CJ, et al. Inflammation and cardiovascular disease: From mechanisms to therapeutics. Am J Prev Cardiol. 2020;4:100130.

153. Pujades-Rodriguez M, Morgan AW, Cubbon RM, Wu J. Dose-dependent oral glucocorticoid cardiovascular risks in people with immune-mediated inflammatory diseases: A population-based cohort study. PLoS Med. 2020;17(12):e1003432.

154. Jankowski J, Floege J, Fliser D, Bohm M, Marx N. Cardiovascular Disease in Chronic Kidney Disease: Pathophysiological Insights and Therapeutic Options. Circulation. 2021;143(11):1157-72.

155. Jefferson JA. Complications of Immunosuppression in Glomerular Disease. Clin J Am Soc Nephrol. 2018;13(8):1264-75.

156. Maddox JS, Soltani K**.** Risk of nonmelanoma skin cancer with azathioprine use. Inflamm Bowel Dis. 2008;14(10):1425-31.

157. Merkel PA, Lo GH, Holbrook JT, Tibbs AK, Allen NB, Davis JC, Jr., et al. Brief communication: high incidence of venous thrombotic events among patients with Wegener granulomatosis: the Wegener's Clinical Occurrence of Thrombosis (WeCLOT) Study. Ann Intern Med. 2005;142(8):620-6.

158. Tomasson G, Monach PA, Merkel PA**.** Thromboembolic disease in vasculitis. Curr Opin Rheumatol. 2009;21(1):41-6.

159. Kronbichler A, Leierer J, Shin JI, Merkel PA, Spiera R, Seo P, et al. Association of Pulmonary Hemorrhage, Positive Proteinase 3, and Urinary Red Blood Cell Casts With Venous Thromboembolism in Antineutrophil Cytoplasmic Antibody-Associated Vasculitis. Arthritis Rheumatol. 2019;71(11):1888-93.

160. Kronbichler A, Leierer J, Leierer G, Mayer G, Casian A, Hoglund P, et al. Clinical associations with venous thromboembolism in anti-neutrophil cytoplasm antibody-associated vasculitides. Rheumatology (Oxford). 2017;56(5):704-8.

161. Hansrivijit P, Trongtorsak A, Gadhiya KP, Lnu K, Dimech CT, Thongprayoon C, et al. Incidence and risk factors of venous thromboembolism in ANCA-associated vasculitis: a metaanalysis and metaregression. Clin Rheumatol. 2021;40(7):2843-53.

162. Kalsch AI, Csernok E, Munch D, Birck R, Yard BA, Gross W, et al. Use of highly sensitive C-reactive protein for followup of Wegener's granulomatosis. J Rheumatol. 2010;37(11):2319-25.

163. Hind CR, Winearls CG, Lockwood CM, Rees AJ, Pepys MB**.** Objective monitoring of activity in Wegener's granulomatosis by measurement of serum C-reactive protein concentration. Clin Nephrol. 1984;21(6):341-5.

164. Sproson EL, Jones NS, Al-Deiri M, Lanyon P**.** Lessons learnt in the management of Wegener's Granulomatosis: long-term follow-up of 60 patients. Rhinology. 2007;45(1):63-7.

165. Monach PA, Warner RL, Tomasson G, Specks U, Stone JH, Ding L, et al. Serum proteins reflecting inflammation, injury and repair as biomarkers of disease activity in ANCA-associated vasculitis. Ann Rheum Dis. 2013;72(8):1342-50.

166. Finkielman JD, Merkel PA, Schroeder D, Hoffman GS, Spiera R, St Clair EW, et al. Antiproteinase 3 antineutrophil cytoplasmic antibodies and disease activity in Wegener granulomatosis. Ann Intern Med. 2007;147(9):611-9.

167. Girard T, Mahr A, Noel LH, Cordier JF, Lesavre P, Andre MH, et al. Are antineutrophil cytoplasmic antibodies a marker predictive of relapse in Wegener's granulomatosis? A prospective study. Rheumatology (Oxford). 2001;40(2):147-51.

168. Boomsma MM, Stegeman CA, van der Leij MJ, Oost W, Hermans J, Kallenberg CG, et al. Prediction of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibody levels: a prospective study. Arthritis Rheum. 2000;43(9):2025-33.

169. Lv L, Chang DY, Li ZY, Chen M, Hu Z, Zhao MH**.** Persistent hematuria in patients with antineutrophil cytoplasmic antibody-associated vasculitis during clinical remission: chronic glomerular lesion or low-grade active renal vasculitis? BMC Nephrol. 2017;18(1):354.

170. Geetha D, Seo P, Ellis C, Kuperman M, Levine SM**.** Persistent or new onset microscopic hematuria in patients with small vessel vasculitis in remission: findings on renal biopsy. J Rheumatol. 2012;39(7):1413-7.

171. Neumann I, Kain R, Regele H, Soleiman A, Kandutsch S, Meisl FT**.** Histological and clinical predictors of early and late renal outcome in ANCA-associated vasculitis. Nephrol Dial Transplant. 2005;20(1):96-104.

172. Cordova-Sanchez BM, Mejia-Vilet JM, Morales-Buenrostro LE, Loyola-Rodriguez G, Uribe-Uribe NO, Correa-Rotter R**.** Clinical presentation and outcome prediction of clinical, serological, and histopathological classification schemes in ANCA-associated vasculitis with renal involvement. Clin Rheumatol. 2016;35(7):1805-16.

173. Kajawo S, Ekrikpo U, Moloi MW, Noubiap JJ, Osman MA, Okpechi-Samuel US, et al. A Systematic Review of Complications Associated With Percutaneous Native Kidney Biopsies in Adults in Low- and Middle-Income Countries. Kidney Int Rep. 2021;6(1):78-90.

174. Knight A, Sundstrom Y, Borjesson O, Bruchfeld A, Malmstrom V, Gunnarsson I**.** Late-onset neutropenia after rituximab in ANCA-associated vasculitis. Scand J Rheumatol. 2016;45(5):404-7.

175. Besada E, Koldingsnes W, Nossent JC**.** Serum immunoglobulin levels and risk factors for hypogammaglobulinaemia during long-term maintenance therapy with rituximab in patients with granulomatosis with polyangiitis. Rheumatology (Oxford). 2014;53(10):1818-24.

176. Merkel PA, Aydin SZ, Boers M, Direskeneli H, Herlyn K, Seo P, et al. The OMERACT core set of outcome measures for use in clinical trials of ANCA-associated vasculitis. J Rheumatol. 2011;38(7):1480-6.

177. Flossmann O, Bacon P, de Groot K, Jayne D, Rasmussen N, Seo P, et al. Development of comprehensive disease assessment in systemic vasculitis. Postgrad Med J. 2008;84(989):143-52.

178. Mukhtyar C, Lee R, Brown D, Carruthers D, Dasgupta B, Dubey S, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). Ann Rheum Dis. 2009;68(12):1827-32.

179. Exley AR, Bacon PA, Luqmani RA, Kitas GD, Gordon C, Savage CO, et al. Development and initial validation of the Vasculitis Damage Index for the standardized clinical assessment of damage in the systemic vasculitides. Arthritis Rheum. 1997;40(2):371-80.

180. Berden AE, Ferrario F, Hagen EC, Jayne DR, Jennette JC, Joh K, et al. Histopathologic classification of ANCA-associated glomerulonephritis. J Am Soc Nephrol. 2010;21(10):1628-36.

181. van Daalen EE, Wester Trejo MAC, Goceroglu A, Ferrario F, Joh K, Noel LH, et al. Developments in the Histopathological Classification of ANCA-Associated Glomerulonephritis. Clin J Am Soc Nephrol. 2020.

182. Brix SR, Noriega M, Tennstedt P, Vettorazzi E, Busch M, Nitschke M, et al. Development and validation of a renal risk score in ANCA-associated glomerulonephritis. Kidney Int. 2018;94(6):1177-88.

183. . BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring (MD); 2016.

184. Bottazzi B, Garlanda C, Salvatori G, Jeannin P, Manfredi A, Mantovani A**.** Pentraxins as a key component of innate immunity. Curr Opin Immunol. 2006;18(1):10-5.

185. Mantovani A, Garlanda C, Doni A, Bottazzi B**.** Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. J Clin Immunol. 2008;28(1):1-13.

186. Deban L, Jaillon S, Garlanda C, Bottazzi B, Mantovani A**.** Pentraxins in innate immunity: lessons from PTX3. Cell Tissue Res. 2011;343(1):237-49.

187. Inforzato A, Rivieccio V, Morreale AP, Bastone A, Salustri A, Scarchilli L, et al. Structural characterization of PTX3 disulfide bond network and its multimeric status in cumulus matrix organization. J Biol Chem. 2008;283(15):10147-61.

188. Mantovani A, Garlanda C, Bottazzi B**.** Pentraxin 3, a non-redundant soluble pattern recognition receptor involved in innate immunity. Vaccine. 2003;21 Suppl 2:S43-7.

189. Lu J, Mold C, Du Clos TW, Sun PD**.** Pentraxins and Fc Receptor-Mediated Immune Responses. Front Immunol. 2018;9:2607.

190. Ma YJ, Garred P**.** Pentraxins in Complement Activation and Regulation. Front Immunol. 2018;9:3046.

191. Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, et al. Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. J Biol Chem. 1997;272(52):32817-23.

192. Nauta AJ, Bottazzi B, Mantovani A, Salvatori G, Kishore U, Schwaeble WJ, et al. Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. Eur J Immunol. 2003;33(2):465-73.

193. Ma YJ, Doni A, Hummelshoj T, Honore C, Bastone A, Mantovani A, et al. Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. J Biol Chem. 2009;284(41):28263-75.

194. Deban L, Jarva H, Lehtinen MJ, Bottazzi B, Bastone A, Doni A, et al. Binding of the long pentraxin PTX3 to factor H: interacting domains and function in the regulation of complement activation. J Immunol. 2008;181(12):8433-40.

195. Jaillon S, Peri G, Delneste Y, Fremaux I, Doni A, Moalli F, et al. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. J Exp Med. 2007;204(4):793-804.

196. Daigo K, Takamatsu Y, Hamakubo T**.** The Protective Effect against Extracellular Histones Afforded by Long-Pentraxin PTX3 as a Regulator of NETs. Front Immunol. 2016;7:344.

197. Deban L, Russo RC, Sironi M, Moalli F, Scanziani M, Zambelli V, et al. Regulation of leukocyte recruitment by the long pentraxin PTX3. Nat Immunol. 2010;11(4):328-34.

198. Inforzato A, Jaillon S, Moalli F, Barbati E, Bonavita E, Bottazzi B, et al. The long pentraxin PTX3 at the crossroads between innate immunity and tissue remodelling. Tissue Antigens. 2011;77(4):271-82.

199. Presta M, Camozzi M, Salvatori G, Rusnati M**.** Role of the soluble pattern recognition receptor PTX3 in vascular biology. J Cell Mol Med. 2007;11(4):723-38.

200. Fazzini F, Peri G, Doni A, Dell'Antonio G, Dal Cin E, Bozzolo E, et al. PTX3 in small-vessel vasculitides: an independent indicator of disease activity produced at sites of inflammation. Arthritis Rheum. 2001;44(12):2841-50.

201. Assandri R, Monari M, Colombo A, Dossi A, Montanelli A**.** Pentraxin 3 Plasma Levels and Disease Activity in Systemic Lupus Erythematosus. Autoimmune Dis. 2015;2015:354014.

202. Shimada Y, Asanuma YF, Yokota K, Yoshida Y, Kajiyama H, Sato K, et al. Pentraxin 3 is associated with disease activity but not atherosclerosis in patients with systemic lupus erythematosus. Mod Rheumatol. 2014;24(1):78-85.

203. Cieslik P, Hrycek A**.** Pentraxin 3 as a biomarker of local inflammatory response to vascular injury in systemic lupus erythematosus. Autoimmunity. 2015;48(4):242-50.

204. Klimek E, Skalska A, Kwasny-Krochin B, Surdacki A, Sulicka J, Korkosz M, et al. Differential associations of inflammatory and endothelial biomarkers with disease activity in rheumatoid arthritis of short duration. Mediators Inflamm. 2014;2014:681635.

205. Sharma A, Khan R, Gupta N, Sharma A, Zaheer MS, Abbas M, et al. Acute phase reactant, Pentraxin 3, as a novel marker for the diagnosis of rheumatoid arthritis. Clin Chim Acta. 2018;480:65-70.

206. Iwata Y, Yoshizaki A, Ogawa F, Komura K, Hara T, Muroi E, et al. Increased serum pentraxin 3 in patients with systemic sclerosis. J Rheumatol. 2009;36(5):976-83.

207. Shirai Y, Okazaki Y, Inoue Y, Tamura Y, Yasuoka H, Takeuchi T, et al. Elevated levels of pentraxin 3 in systemic sclerosis: associations with vascular manifestations and defective vasculogenesis. Arthritis Rheumatol. 2015;67(2):498-507.

208. Dagna L, Salvo F, Tiraboschi M, Bozzolo EP, Franchini S, Doglioni C, et al. Pentraxin-3 as a marker of disease activity in Takayasu arteritis. Ann Intern Med. 2011;155(7):425-33.

209. Anderson CE, Hamm LL, Batuman G, Kumbala DR, Chen CS, Kallu SG, et al. The association of angiogenic factors and chronic kidney disease. BMC Nephrol. 2018;19(1):117.

210. Tong M, Carrero JJ, Qureshi AR, Anderstam B, Heimburger O, Barany P, et al. Plasma pentraxin 3 in patients with chronic kidney disease: associations with renal function, protein-energy wasting, cardiovascular disease, and mortality. Clin J Am Soc Nephrol. 2007;2(5):889-97.

211. Lech M, Rommele C, Grobmayr R, Eka Susanti H, Kulkarni OP, Wang S, et al. Endogenous and exogenous pentraxin-3 limits postischemic acute and chronic kidney injury. Kidney Int. 2013;83(4):647-61.

212. Xiao Y, Yang N, Zhang Q, Wang Y, Yang S, Liu Z**.** Pentraxin 3 inhibits acute renal injury-induced interstitial fibrosis through suppression of IL-6/Stat3 pathway. Inflammation. 2014;37(5):1895-901.

213. Ramirez GA, Rovere-Querini P, Blasi M, Sartorelli S, Di Chio MC, Baldini M, et al. PTX3 Intercepts Vascular Inflammation in Systemic Immune-Mediated Diseases. Front Immunol. 2019;10:1135.

214. Simon A, Subra JF, Guilpain P, Jeannin P, Pignon P, Blanchard S, et al. Detection of Anti-Pentraxin-3 Autoantibodies in ANCA-Associated Vasculitis. PLoS One. 2016;11(1):e0147091.

215. Padoan R, Gatto M, Ghirardello A, Tonello M, Franco C, Felicetti M, et al. IgG anti-Pentraxin 3 antibodies are a novel biomarker of ANCA-associated vasculitis and better identify patients with eosinophilic granulomatosis with polyangiitis. J Autoimmun. 2021;124:102725.

216. Michaelson JS, Wisniacki N, Burkly LC, Putterman C**.** Role of TWEAK in lupus nephritis: a bench-to-bedside review. J Autoimmun. 2012;39(3):130-42.

217. Kim SH, Kang YJ, Kim WJ, Woo DK, Lee Y, Kim DI, et al. TWEAK can induce pro-inflammatory cytokines and matrix metalloproteinase-9 in macrophages. Circ J. 2004;68(4):396-9.

218. Maecker H, Varfolomeev E, Kischkel F, Lawrence D, LeBlanc H, Lee W, et al. TWEAK attenuates the transition from innate to adaptive immunity. Cell. 2005;123(5):931-44.

219. Justo P, Sanz AB, Sanchez-Nino MD, Winkles JA, Lorz C, Egido J, et al. Cytokine cooperation in renal tubular cell injury: the role of TWEAK. Kidney Int. 2006;70(10):1750-8.

220. Donohue PJ, Richards CM, Brown SA, Hanscom HN, Buschman J, Thangada S, et al. TWEAK is an endothelial cell growth and chemotactic factor that also potentiates FGF-2 and VEGF-A mitogenic activity. Arterioscler Thromb Vasc Biol. 2003;23(4):594-600.

221. Brown SA, Ghosh A, Winkles JA**.** Full-length, membrane-anchored TWEAK can function as a juxtacrine signaling molecule and activate the NF-kappaB pathway. J Biol Chem. 2010;285(23):17432-41.

222. Roos C, Wicovsky A, Muller N, Salzmann S, Rosenthal T, Kalthoff H, et al. Soluble and transmembrane TNF-like weak inducer of apoptosis differentially activate the classical and noncanonical NF-kappa B pathway. J Immunol. 2010;185(3):1593-605.

223. Michaelson JS, Cho S, Browning B, Zheng TS, Lincecum JM, Wang MZ, et al. Tweak induces mammary epithelial branching morphogenesis. Oncogene. 2005;24(16):2613-24.

224. Wiley SR, Cassiano L, Lofton T, Davis-Smith T, Winkles JA, Lindner V, et al. A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. Immunity. 2001;15(5):837-46.

225. Sanchez-Nino MD, Poveda J, Sanz AB, Mezzano S, Carrasco S, Fernandez-Fernandez B, et al. Fn14 in podocytes and proteinuric kidney disease. Biochim Biophys Acta. 2013;1832(12):2232-43.

226. Campbell S, Burkly LC, Gao HX, Berman JW, Su L, Browning B, et al. Proinflammatory effects of TWEAK/Fn14 interactions in glomerular mesangial cells. J Immunol. 2006;176(3):1889-98.

227. Burkly LC, Michaelson JS, Hahm K, Jakubowski A, Zheng TS**.** TWEAKing tissue remodeling by a multifunctional cytokine: role of TWEAK/Fn14 pathway in health and disease. Cytokine. 2007;40(1):1-16.

228. Chicheportiche Y, Chicheportiche R, Sizing I, Thompson J, Benjamin CB, Ambrose C, et al. Proinflammatory activity of TWEAK on human dermal fibroblasts and synoviocytes: blocking and enhancing effects of anti-TWEAK monoclonal antibodies. Arthritis Res. 2002;4(2):126-33.

229. Jakubowski A, Browning B, Lukashev M, Sizing I, Thompson JS, Benjamin CD, et al. Dual role for TWEAK in angiogenic regulation. J Cell Sci. 2002;115(Pt 2):267-74.

230. Nakayama M, Ishidoh K, Kayagaki N, Kojima Y, Yamaguchi N, Nakano H, et al. Multiple pathways of TWEAK-induced cell death. J Immunol. 2002;168(2):734-43.

231. Nakayama M, Ishidoh K, Kojima Y, Harada N, Kominami E, Okumura K, et al. Fibroblast growth factor-inducible 14 mediates multiple pathways of TWEAK-induced cell death. J Immunol. 2003;170(1):341-8.

232. Gomez IG, Roach AM, Nakagawa N, Amatucci A, Johnson BG, Dunn K, et al. TWEAK-Fn14 Signaling Activates Myofibroblasts to Drive Progression of Fibrotic Kidney Disease. J Am Soc Nephrol. 2016;27(12):3639-52.

233. Yilmaz MI, Carrero JJ, Ortiz A, Martin-Ventura JL, Sonmez A, Saglam M, et al. Soluble TWEAK plasma levels as a novel biomarker of endothelial function in patients with chronic kidney disease. Clin J Am Soc Nephrol. 2009;4(11):1716-23.

234. Ruiz-Ortega M, Ortiz A, Ramos AM**.** Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and kidney disease. Curr Opin Nephrol Hypertens. 2014;23(1):93-100.

235. Moran SM, Scott J, Clarkson MR, Conlon N, Dunne J, Griffin MD, et al. The Clinical Application of Urine Soluble CD163 in ANCA-Associated Vasculitis. J Am Soc Nephrol. 2021;32(11):2920-32.

236. Sanz AB, Izquierdo MC, Sanchez-Nino MD, Ucero AC, Egido J, Ruiz-Ortega M, et al. TWEAK and the progression of renal disease: clinical translation. Nephrol Dial Transplant. 2014;29 Suppl 1:i54-i62.

237. Sanz AB, Sanchez-Nino MD, Ortiz A**.** TWEAK, a multifunctional cytokine in kidney injury. Kidney Int. 2011;80(7):708-18.

238. Zhao Z, Burkly LC, Campbell S, Schwartz N, Molano A, Choudhury A, et al. TWEAK/Fn14 interactions are instrumental in the pathogenesis of nephritis in the chronic graft-versus-host model of systemic lupus erythematosus. J Immunol. 2007;179(11):7949-58.

239. Carrero JJ, Ortiz A, Qureshi AR, Martin-Ventura JL, Barany P, Heimburger O, et al. Additive effects of soluble TWEAK and inflammation on mortality in hemodialysis patients. Clin J Am Soc Nephrol. 2009;4(1):110-8.

240. Yilmaz MI, Sonmez A, Saglam M, Yaman H, Unal HU, Gok M, et al. Soluble TWEAK plasma levels increase after renal transplantation and associate with the improvement of endothelial function. Eur J Clin Invest. 2013;43(12):1250-7.

241. Park MC, Chung SJ, Park YB, Lee SK**.** Relationship of serum TWEAK level to cytokine level, disease activity, and response to anti-TNF treatment in patients with rheumatoid arthritis. Scand J Rheumatol. 2008;37(3):173-8.

242. Chen T, Guo ZP, Li MM, Li JY, Jiao XY, Zhang YH, et al. Tumour necrosis factor-like weak inducer of apoptosis (TWEAK), an important mediator of endothelial

inflammation, is associated with the pathogenesis of Henoch-Schonlein purpura. Clin Exp Immunol. 2011;166(1):64-71.

243. Choe JY, Kim SK**.** Serum TWEAK as a biomarker for disease activity of systemic lupus erythematosus. Inflamm Res. 2016;65(6):479-88.

244. Wang C, Chen LL, Pan HF, Leng RX, Qin WZ, Ye DQ**.** Expression of human tumor necrosis factor-like weak inducer of apoptosis in patients with systemic lupus erythematosus. Clin Rheumatol. 2012;31(2):335-9.

245. Sasaki Y, Shimizu Y, Suzuki Y, Horikoshi S, Tomino Y**.** TWEAK/Fn14 system and crescent formation in IgA nephropathy. BMC Nephrol. 2015;16:27.

246. Schwartz N, Rubinstein T, Burkly LC, Collins CE, Blanco I, Su L, et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. Arthritis Res Ther. 2009;11(5):R143.

247. Xuejing Z, Jiazhen T, Jun L, Xiangqing X, Shuguang Y, Fuyou L**.** Urinary TWEAK level as a marker of lupus nephritis activity in 46 cases. J Biomed Biotechnol. 2012;2012:359647.

248. El-Shehaby A, Darweesh H, El-Khatib M, Momtaz M, Marzouk S, El-Shaarawy N, et al. Correlations of urinary biomarkers, TNF-like weak inducer of apoptosis (TWEAK), osteoprotegerin (OPG), monocyte chemoattractant protein-1 (MCP-1), and IL-8 with lupus nephritis. J Clin Immunol. 2011;31(5):848-56.

249. Morel O, Toti F, Hugel B, Freyssinet JM**.** Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. Curr Opin Hematol. 2004;11(3):156-64.

250. Bazzan E, Tine M, Casara A, Biondini D, Semenzato U, Cocconcelli E, et al. Critical Review of the Evolution of Extracellular Vesicles' Knowledge: From 1946 to Today. Int J Mol Sci. 2021;22(12).

251. Mause SF, Weber C**.** Microparticles: protagonists of a novel communication network for intercellular information exchange. Circ Res. 2010;107(9):1047-57.

252. Distler JH, Huber LC, Gay S, Distler O, Pisetsky DS**.** Microparticles as mediators of cellular cross-talk in inflammatory disease. Autoimmunity. 2006;39(8):683-90.

253. Reich N, Beyer C, Gelse K, Akhmetshina A, Dees C, Zwerina J, et al. Microparticles stimulate angiogenesis by inducing ELR(+) CXC-chemokines in synovial fibroblasts. J Cell Mol Med. 2011;15(4):756-62.

254. Gasser O, Schifferli JA**.** Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. Blood. 2004;104(8):2543-8.

255. Doyle LM, Wang MZ**.** Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells. 2019;8(7).

256. Thery C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 2018;7(1):1535750.

257. Xu K, Liu Q, Wu K, Liu L, Zhao M, Yang H, et al. Extracellular vesicles as potential biomarkers and therapeutic approaches in autoimmune diseases. J Transl Med. 2020;18(1):432.

258. Schonermarck U, Csernok E, Gross WL**.** Pathogenesis of anti-neutrophil cytoplasmic antibody-associated vasculitis: challenges and solutions 2014. Nephrol Dial Transplant. 2015;30 Suppl 1:i46-52.

259. Jennette JC, Falk RJ**.** Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. Nat Rev Rheumatol. 2014;10(8):463-73.

260. Brogan PA, Shah V, Brachet C, Harnden A, Mant D, Klein N, et al. Endothelial and platelet microparticles in vasculitis of the young. Arthritis Rheum. 2004;50(3):927-36.

261. Erdbruegger U, Grossheim M, Hertel B, Wyss K, Kirsch T, Woywodt A, et al. Diagnostic role of endothelial microparticles in vasculitis. Rheumatology (Oxford). 2008;47(12):1820-5.

262. Hong Y, Eleftheriou D, Hussain AA, Price-Kuehne FE, Savage CO, Jayne D, et al. Anti-neutrophil cytoplasmic antibodies stimulate release of neutrophil microparticles. J Am Soc Nephrol. 2012;23(1):49-62.

263. Pitanga TN, de Aragao Franca L, Rocha VC, Meirelles T, Borges VM, Goncalves MS, et al. Neutrophil-derived microparticles induce myeloperoxidase-mediated damage of vascular endothelial cells. BMC Cell Biol. 2014;15:21.

264. Daniel L, Fakhouri F, Joly D, Mouthon L, Nusbaum P, Grunfeld JP, et al. Increase of circulating neutrophil and platelet microparticles during acute vasculitis and hemodialysis. Kidney Int. 2006;69(8):1416-23.

265. Antovic A, Mobarrez F, Manojlovic M, Soutari N, De Porta Baggemar V, Nordin A, et al. Microparticles Expressing Myeloperoxidase and Complement C3a and C5a as Markers of Renal Involvement in Antineutrophil Cytoplasmic Antibody-associated Vasculitis. J Rheumatol. 2020;47(5):714-21.

266. Wang C, Wang H, Chang DY, Hao J, Zhao MH, Chen M**.** High mobility group box 1 contributes to anti-neutrophil cytoplasmic antibody-induced neutrophils activation through receptor for advanced glycation end products (RAGE) and Toll-like receptor 4. Arthritis Res Ther. 2015;17:64.

267. Ma YH, Ma TT, Wang C, Wang H, Chang DY, Chen M, et al. High-mobility group box 1 potentiates antineutrophil cytoplasmic antibody-inducing neutrophil extracellular traps formation. Arthritis Res Ther. 2016;18:2.

268. Bruchfeld A, Wendt M, Bratt J, Qureshi AR, Chavan S, Tracey KJ, et al. High-mobility group box-1 protein (HMGB1) is increased in antineutrophilic cytoplasmatic antibody (ANCA)-associated vasculitis with renal manifestations. Mol Med. 2011;17(1-2):29-35.

269. Wang C, Gou SJ, Chang DY, Yu F, Zhao MH, Chen M**.** Association of circulating level of high mobility group box 1 with disease activity in antineutrophil cytoplasmic autoantibody-associated vasculitis. Arthritis Care Res (Hoboken). 2013;65(11):1828-34.

270. Leshner M, Wang S, Lewis C, Zheng H, Chen XA, Santy L, et al. PAD4 mediated histone hypercitrullination induces heterochromatin decondensation and chromatin unfolding to form neutrophil extracellular trap-like structures. Front Immunol. 2012;3:307.

271. Thalin C, Daleskog M, Goransson SP, Schatzberg D, Lasselin J, Laska AC, et al. Validation of an enzyme-linked immunosorbent assay for the quantification of

citrullinated histone H3 as a marker for neutrophil extracellular traps in human plasma. Immunol Res. 2017;65(3):706-12.

272. Paues Goranson S, Thalin C, Lundstrom A, Hallstrom L, Lasselin J, Wallen H, et al. Circulating H3Cit is elevated in a human model of endotoxemia and can be detected bound to microvesicles. Sci Rep. 2018;8(1):12641.

273. Kapoor S, Opneja A, Nayak L**.** The role of neutrophils in thrombosis. Thromb Res. 2018;170:87-96.

274. Nakazawa D, Tomaru U, Yamamoto C, Jodo S, Ishizu A**.** Abundant neutrophil extracellular traps in thrombus of patient with microscopic polyangiitis. Front Immunol. 2012;3:333.

275. Bogdanov VY, Balasubramanian V, Hathcock J, Vele O, Lieb M, Nemerson Y**.** Alternatively spliced human tissue factor: a circulating, soluble, thrombogenic protein. Nat Med. 2003;9(4):458-62.

276. Del Conde I, Shrimpton CN, Thiagarajan P, Lopez JA**.** Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. Blood. 2005;106(5):1604-11.

277. Kambas K, Chrysanthopoulou A, Vassilopoulos D, Apostolidou E, Skendros P, Girod A, et al. Tissue factor expression in neutrophil extracellular traps and neutrophil derived microparticles in antineutrophil cytoplasmic antibody associated vasculitis may promote thromboinflammation and the thrombophilic state associated with the disease. Ann Rheum Dis. 2014;73(10):1854-63.

278. Mendoza CE, Brant EJ, McDermott ML, Froment A, Hu Y, Hogan SL, et al. Elevated Microparticle Tissue Factor Activity Differentiates Patients With Venous Thromboembolism in Anti-neutrophil Cytoplasmic Autoantibody Vasculitis. Kidney Int Rep. 2019;4(11):1617-29.

279. Chapin JC, Hajjar KA**.** Fibrinolysis and the control of blood coagulation. Blood Rev. 2015;29(1):17-24.

280. Berden AE, Nolan SL, Morris HL, Bertina RM, Erasmus DD, Hagen EC, et al. Anti-plasminogen antibodies compromise fibrinolysis and associate with renal histology in ANCA-associated vasculitis. J Am Soc Nephrol. 2010;21(12):2169-79.

281. Hao J, Wang C, Gou SJ, Zhao MH, Chen M**.** The association between anti-plasminogen antibodies and disease activity in ANCA-associated vasculitis. Rheumatology (Oxford). 2014;53(2):300-6.

282. Bautz DJ, Preston GA, Lionaki S, Hewins P, Wolberg AS, Yang JJ, et al. Antibodies with dual reactivity to plasminogen and complementary PR3 in PR3-ANCA vasculitis. J Am Soc Nephrol. 2008;19(12):2421-9.

283. Heissig B, Salama Y, Takahashi S, Osada T, Hattori K**.** The multifaceted role of plasminogen in inflammation. Cell Signal. 2020;75:109761.

284. Nordin A, Jensen-Urstad K, Bjornadal L, Pettersson S, Larsson A, Svenungsson E**.** Ischemic arterial events and atherosclerosis in patients with systemic sclerosis: a population-based case-control study. Arthritis Res Ther. 2013;15(4):R87.

285. Stenvinkel P, Heimburger O, Paultre F, Diczfalusy U, Wang T, Berglund L, et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney Int. 1999;55(5):1899-911.

286. Bruchfeld A, Carrero JJ, Qureshi AR, Lindholm B, Barany P, Heimburger O, et al. Elevated serum macrophage migration inhibitory factor (MIF) concentrations in chronic kidney disease (CKD) are associated with markers of oxidative stress and endothelial activation. Mol Med. 2009;15(3-4):70-5.

287. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

288. Nyman U, Grubb A, Larsson A, Hansson LO, Flodin M, Nordin G, et al. The revised Lund-Malmo GFR estimating equation outperforms MDRD and CKD-EPI across GFR, age and BMI intervals in a large Swedish population. Clin Chem Lab Med. 2014;52(6):815-24.

289. Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenvoord R, et al. Calibrated automated thrombin generation measurement in clotting plasma. Pathophysiol Haemost Thromb. 2003;33(1):4-15.

290. Pang Y, Tan Y, Li Y, Zhang J, Guo Y, Guo Z, et al. Pentraxin 3 Is Closely Associated With Tubulointerstitial Injury in Lupus Nephritis: A Large Multicenter Cross-Sectional Study. Medicine (Baltimore). 2016;95(3):e2520.

291. Ishihara T, Haraguchi G, Kamiishi T, Tezuka D, Inagaki H, Isobe M**.** Sensitive assessment of activity of Takayasu's arteritis by pentraxin3, a new biomarker. J Am Coll Cardiol. 2011;57(16):1712-3.

292. Peri G, Introna M, Corradi D, Iacuitti G, Signorini S, Avanzini F, et al. PTX3, A prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. Circulation. 2000;102(6):636-41.

293. Matsui S, Ishii J, Kitagawa F, Kuno A, Hattori K, Ishikawa M, et al. Pentraxin 3 in unstable angina and non-ST-segment elevation myocardial infarction. Atherosclerosis. 2010;210(1):220-5.

294. Bastrup-Birk S, Skjoedt MO, Munthe-Fog L, Strom JJ, Ma YJ, Garred P**.** Pentraxin-3 serum levels are associated with disease severity and mortality in patients with systemic inflammatory response syndrome. PLoS One. 2013;8(9):e73119.

295. Chen H, Li T, Yan S, Liu M, Liu K, Zhang H, et al. Pentraxin-3 Is a Strong Biomarker of Sepsis Severity Identification and Predictor of 90-Day Mortality in Intensive Care Units via Sepsis 3.0 Definitions. Diagnostics (Basel). 2021;11(10).

296. Deniz T, Kizilgul M, Uzunlulu M, Oguz A, Isbilen B**.** Levels of pentraxin 3 and relationship with disease activity in patients with ankylosing spondylitis. Acta Reumatol Port. 2014;39(2):137-42.

297. Nisihara R, Skare TL, Zeni JO, Rasera H, Lidani K, Messias-Reason I**.** Plasma levels of pentraxin 3 in patients with spondyloarthritis. Biomarkers. 2018;23(1):14-7.

298. Skare TL, Nisihara R, Ramos GP, Utiyama SR, Messias-Reason I**.** Pentraxin-3 levels in systemic lupus erythematosus: Association with cumulative damage but not with disease activity. Joint Bone Spine. 2015;82(6):466-7.

299. Ge W, Wang HL, Sun RP**.** Pentraxin 3 as a novel early biomarker for the prediction of Henoch-Schonlein purpura nephritis in children. Eur J Pediatr. 2014;173(2):213-8.

300. Jaillon S, Moalli F, Ragnarsdottir B, Bonavita E, Puthia M, Riva F, et al. The humoral pattern recognition molecule PTX3 is a key component of innate immunity against urinary tract infection. Immunity. 2014;40(4):621-32.

301. Becerir T, Yuksel S, Evrengul H, Ergin A, Enli Y**.** Urinary excretion of pentraxin-3 correlates with the presence of renal scar following acute pyelonephritis in children. Int Urol Nephrol. 2019;51(4):571-7.

302. Bussolati B, Peri G, Salvidio G, Verzola D, Mantovani A, Camussi G**.** The long pentraxin PTX3 is synthesized in IgA glomerulonephritis and activates mesangial cells. J Immunol. 2003;170(3):1466-72.

303. Ishida R, Nakai K, Fujii H, Goto S, Hara S, Imai N, et al. Elevated Expression of Pentraxin 3 in Anti-neutrophil Cytoplasmic Antibody-associated Glomerulonephritis with Normal Serum C-reactive Protein. Intern Med. 2015;54(11):1369-73.

304. Lee HH, Kim SY, Na JC, Yoon YE, Han WK**.** Exogenous pentraxin-3 inhibits the reactive oxygen species-mitochondrial and apoptosis pathway in acute kidney injury. PLoS One. 2018;13(4):e0195758.

305. Doni A, Mantovani G, Porta C, Tuckermann J, Reichardt HM, Kleiman A, et al. Cell-specific regulation of PTX3 by glucocorticoid hormones in hematopoietic and nonhematopoietic cells. J Biol Chem. 2008;283(44):29983-92.

306. Schwartz N, Su L, Burkly LC, Mackay M, Aranow C, Kollaros M, et al. Urinary TWEAK and the activity of lupus nephritis. J Autoimmun. 2006;27(4):242-50.

307. Salem MN, Taha HA, Abd El-Fattah El-Feqi M, Eesa NN, Mohamed RA**.** Urinary TNF-like weak inducer of apoptosis (TWEAK) as a biomarker of lupus nephritis. Z Rheumatol. 2018;77(1):71-7.

308. Landolt-Marticorena C, Prokopec SD, Morrison S, Noamani B, Bonilla D, Reich H, et al. A discrete cluster of urinary biomarkers discriminates between active systemic lupus erythematosus patients with and without glomerulonephritis. Arthritis Res Ther. 2016;18(1):218.

309. Mirioglu S, Cinar S, Yazici H, Ozluk Y, Kilicaslan I, Gul A, et al. Serum and urine TNF-like weak inducer of apoptosis, monocyte chemoattractant protein-1 and neutrophil gelatinase-associated lipocalin as biomarkers of disease activity in patients with systemic lupus erythematosus. Lupus. 2020;29(4):379-88.

310. Moloi MW, Rusch JA, Omar F, Ekrikpo U, Dandara C, Bello AK, et al. Urinary MCP-1 and TWEAK as non-invasive markers of disease activity and treatment response in patients with lupus nephritis in South Africa. Int Urol Nephrol. 2021;53(9):1865-73.

311. Suttichet TB, Kittanamongkolchai W, Phromjeen C, Anutrakulchai S, Panaput T, Ingsathit A, et al. Urine TWEAK level as a biomarker for early response to treatment in active lupus nephritis: a prospective multicentre study. Lupus Sci Med. 2019;6(1):e000298.

312. Hotta K, Sho M, Yamato I, Shimada K, Harada H, Akahori T, et al. Direct targeting of fibroblast growth factor-inducible 14 protein protects against renal ischemia reperfusion injury. Kidney Int. 2011;79(2):179-88.

313. Sanz AB, Justo P, Sanchez-Nino MD, Blanco-Colio LM, Winkles JA, Kreztler M, et al. The cytokine TWEAK modulates renal tubulointerstitial inflammation. J Am Soc Nephrol. 2008;19(4):695-703.

314. Xia Y, Campbell SR, Broder A, Herlitz L, Abadi M, Wu P, et al. Inhibition of the TWEAK/Fn14 pathway attenuates renal disease in nephrotoxic serum nephritis. Clin Immunol. 2012;145(2):108-21.

315. Xia L, Shen H, Xiao W, Lu J**.** Increased serum TWEAK levels in Psoriatic arthritis: relationship with disease activity and matrix metalloproteinase-3 serum levels. Cytokine. 2011;53(3):289-91.

316. Yanaba K, Yoshizaki A, Muroi E, Hara T, Ogawa F, Usui A, et al. Elevated circulating TWEAK levels in systemic sclerosis: association with lower frequency of pulmonary fibrosis. J Rheumatol. 2009;36(8):1657-62.

317. Llaurado G, Gonzalez-Clemente JM, Maymo-Masip E, Subias D, Vendrell J, Chacon MR**.** Serum levels of TWEAK and scavenger receptor CD163 in type 1 diabetes mellitus: relationship with cardiovascular risk factors. a case-control study. PLoS One. 2012;7(8):e43919.

318. Kralisch S, Ziegelmeier M, Bachmann A, Seeger J, Lossner U, Bluher M, et al. Serum levels of the atherosclerosis biomarker sTWEAK are decreased in type 2 diabetes and end-stage renal disease. Atherosclerosis. 2008;199(2):440-4.

319. Bover LC, Cardo-Vila M, Kuniyasu A, Sun J, Rangel R, Takeya M, et al. A previously unrecognized protein-protein interaction between TWEAK and CD163: potential biological implications. J Immunol. 2007;178(12):8183-94.

320. Fabriek BO, Dijkstra CD, van den Berg TK**.** The macrophage scavenger receptor CD163. Immunobiology. 2005;210(2-4):153-60.

321. Moreno JA, Munoz-Garcia B, Martin-Ventura JL, Madrigal-Matute J, Orbe J, Paramo JA, et al. The CD163-expressing macrophages recognize and internalize TWEAK: potential consequences in atherosclerosis. Atherosclerosis. 2009;207(1):103-10.

322. Li J, Liu CH, Xu DL, Gao B**.** Significance of CD163-Positive Macrophages in Proliferative Glomerulonephritis. Am J Med Sci. 2015;350(5):387-92.

323. Aendekerk JP, Timmermans S, Busch MH, Potjewijd J, Heeringa P, Damoiseaux J, et al. Urinary Soluble CD163 and Disease Activity in Biopsy-Proven ANCA-Associated Glomerulonephritis. Clin J Am Soc Nephrol. 2020;15(12):1740-8.

324. Villacorta J, Lucientes L, Goicoechea E, Acevedo M, Cavero T, Sanchez-Camara L, et al. Urinary soluble CD163 as a biomarker of disease activity and relapse in antineutrophil cytoplasm antibody-associated glomerulonephritis. Clin Kidney J. 2021;14(1):212-9.

325. Mejia-Vilet JM, Zhang XL, Cruz C, Cano-Verduzco ML, Shapiro JP, Nagaraja HN, et al. Urinary Soluble CD163: a Novel Noninvasive Biomarker of Activity for Lupus Nephritis. J Am Soc Nephrol. 2020;31(6):1335-47.

326. Huang YJ, Lin CH, Yang HY, Luo SF, Kuo CF**.** Urine Soluble CD163 Is a Promising Biomarker for the Diagnosis and Evaluation of Lupus Nephritis. Front Immunol. 2022;13:935700.

327. Hilhorst M, Wilde B, van Breda Vriesman P, van Paassen P, Cohen Tervaert JW, Limburg Renal R**.** Estimating renal survival using the ANCA-associated GN classification. J Am Soc Nephrol. 2013;24(9):1371-5.

328. Zou R, Wang SX, Liu G, Yu F, Chen M, Zhao MH. Podocyte Detachment Is Associated with Renal Prognosis in ANCA-Associated Glomerulonephritis: A Retrospective Cohort Study. Medicine (Baltimore). 2016;95(15):e3294.

329. van Daalen EE, Neeskens P, Zandbergen M, Harper L, Karras A, Vaglio A, et al. Podocytes and Proteinuria in ANCA-Associated Glomerulonephritis: A Case-Control Study. Front Immunol. 2019;10:1405.

330. van der Veen BS, de Winther MP, Heeringa P. Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease. Antioxid Redox Signal. 2009;11(11):2899-937.

331. Rother N, Yanginlar C, Pieterse E, Hilbrands L, van der Vlag J. Microparticles in Autoimmunity: Cause or Consequence of Disease? Front Immunol. 2022;13:822995.

332. Grange C, Bussolati B. Extracellular vesicles in kidney disease. Nat Rev Nephrol. 2022;18(8):499-513.

333. Brogan PA, Dillon MJ. Endothelial microparticles and the diagnosis of the vasculitides. Intern Med. 2004;43(12):1115-9.

334. Mesri M, Altieri DC. Endothelial cell activation by leukocyte microparticles. J Immunol. 1998;161(8):4382-7.

335. Kahn R, Mossberg M, Stahl AL, Johansson K, Lopatko Lindman I, Heijl C, et al. Microvesicle transfer of kinin B1-receptors is a novel inflammatory mechanism in vasculitis. Kidney Int. 2017;91(1):96-105.

336. Surmiak M, Gielicz A, Stojkov D, Szatanek R, Wawrzycka-Adamczyk K, Yousefi S, et al. LTB4 and 5-oxo-ETE from extracellular vesicles stimulate neutrophils in granulomatosis with polyangiitis. J Lipid Res. 2020;61(1):1-9.

337. Glemain A, Neel M, Neel A, Andre-Gregoire G, Gavard J, Martinet B, et al. Neutrophil-derived extracellular vesicles induce endothelial inflammation and damage through the transfer of miRNAs. J Autoimmun. 2022;129:102826.

338. Slater TW, Finkielsztein A, Mascarenhas LA, Mehl LC, Butin-Israeli V, Sumagin R. Neutrophil Microparticles Deliver Active Myeloperoxidase to Injured Mucosa To Inhibit Epithelial Wound Healing. J Immunol. 2017;198(7):2886-97.

339. Erdbrugger U, Blijdorp CJ, Bijnsdorp IV, Borras FE, Burger D, Bussolati B, et al. Urinary extracellular vesicles: A position paper by the Urine Task Force of the International Society for Extracellular Vesicles. J Extracell Vesicles. 2021;10(7):e12093.

340. de Souza A, Westra J, Bijzet J, Limburg PC, Stegeman CA, Bijl M, et al. Is serum HMGB1 a biomarker in ANCA-associated vasculitis? Arthritis Res Ther. 2013;15(5):R104.

341. Urbonaviciute V, Furnrohr BG, Weber C, Haslbeck M, Wilhelm S, Herrmann M, et al. Factors masking HMGB1 in human serum and plasma. J Leukoc Biol. 2007;81(1):67-74.

342. Pisetsky DS. The expression of HMGB1 on microparticles released during cell activation and cell death in vitro and in vivo. Mol Med. 2014;20:158-63.

343. Tripodi A. Thrombin Generation Assay and Its Application in the Clinical Laboratory. Clin Chem. 2016;62(5):699-707.

344. Bidot L, Jy W, Bidot C, Jr., Jimenez JJ, Fontana V, Horstman LL, et al. Microparticle-mediated thrombin generation assay: increased activity in patients with recurrent thrombosis. J Thromb Haemost. 2008;6(6):913-9.

345. Eleftheriou D, Hong Y, Klein NJ, Brogan PA**.** Thromboembolic disease in systemic vasculitis is associated with enhanced microparticle-mediated thrombin generation. J Thromb Haemost. 2011;9(9):1864-7.

346. Huang YM, Wang H, Wang C, Chen M, Zhao MH**.** Promotion of hypercoagulability in antineutrophil cytoplasmic antibody-associated vasculitis by C5a-induced tissue factor-expressing microparticles and neutrophil extracellular traps. Arthritis Rheumatol. 2015;67(10):2780-90.

347. Brill A, Fuchs TA, Savchenko AS, Thomas GM, Martinod K, De Meyer SF, et al. Neutrophil extracellular traps promote deep vein thrombosis in mice. J Thromb Haemost. 2012;10(1):136-44.

348. Baker SK, Strickland S**.** A critical role for plasminogen in inflammation. J Exp Med. 2020;217(4).