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ANCA-associated glomerulonephritis • Sanjeevan Sriskandarajah

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Prognostic factors and outcome in a Norwegian cohort

Sanjeevan Sriskandarajah

Thesis for the Degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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UNIVERSITY OF BERGEN

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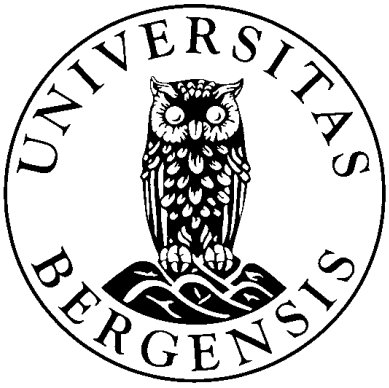
Scientific environment



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"Science never solves a problem without creating ten more".

– George Bernard Shaw

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Abstract

Objective. The objective of this dissertation is to (I) explore the temporal survival and identify prognostic factors in patients with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis with renal affection, (II) validate a recently proposed histopathological classification model, and (III) quantify the incidence of malignancy in this patient group.

Methods. Patients with biopsy-proven pauci-immune crescentic glomerulonephritis and positive ANCA serology were included from the Norwegian Kidney Biopsy Registry from (I/III) 1988 through 2012 and (II) 1991 through 2012. Using the unique 11-digit personal number, end-stage renal failure and death were identified by record-linkage with the Norwegian Renal Registry and the Norwegian Population Registry. The cause of death was obtained from the Cause of Death Registry, Statistics Norway. (II) All biopsies were scored according to the histopathological classification model (focal, mixed, crescentic, and sclerotic class) by an experienced nephropathologist. A receiver operator characteristic curve was calculated to test the performance of the classification model. (III) The incidence of cancer was identified by cross-linkage with the Norwegian Cancer Registry.

Results. (I) Four hundred and fifty-five patients were included. One hundred and twenty-four (27%) developed end-stage renal disease, and 165 (36%) deaths occurred. Independent negative predictors were low baseline estimated-glomerular filtration rate < 15 ml/min per 1.73 m^2, older age (> 60 years) and baseline serum albumin < 30 g/L. Patients diagnosed between 2003 and 2012 had better baseline renal function than those diagnosed between 1988 and 2002 (27 versus 37 ml/min per 1.73 m^2) and more favourable end-stage renal disease-free survival (1-year risk, 19% versus 13%; 10-year risk, 37% versus 26%). Compared to the general population, the risk of mortality was 2.8-fold higher (95% confidence interval, 2.4 to 3.3).

(II) The histopathologic classification model was validated in 250 patients. The model could successfully predict renal survival, with the focal and sclerotic classes having the best and worst outcome (hazard ratio, 9.65; 95% confidence interval, 2.4 to 39.2), respectively. The area under the curve in the receiver operator characteristic curve was calculated to be 0.72 (95% confidence interval, 0.7 to 0.8). (III) Four hundred and nineteen patients were included in the study cohort. The standardised incidence ratio for the development of at least one cancer in this patient cohort compared to the general population was 1.09 (95% confidence interval, 0.8 to 1.5). There were large number of cases of non-melanoma skin cancers and bone marrow malignancies, with a standardised incidence ratio of 3.40 (95% confidence interval, 1.6 to 7.1) and 3.52 (95% confidence interval, 1.3 to 9.4), respectively.

Conclusion. (I) Although the prognosis improved in patients with ANCA-associated glomerulonephritis, mortality remains high compared to the general population. Improvement in prognosis might be due to earlier diagnosis before irreversible organ damage occurs. (II) The histopathological classification model is of average quality as a predictor of end-stage renal disease. Patients with kidney biopsies classified as sclerotic class are particularly at risk of developing end-stage renal disease. (III) The risk of malignancy was not significantly increased in this patient cohort compared to the general population. Non-melanoma skin cancer was the most frequent malignancy, and a major contributor to the observed overall cancer occurrence in this patient population.

List of Abbreviations

AAV: ANCA-associated vasculitis

ACR: American College of Rheumatology

ANCA: Antineutrophil cytoplasmic antibody

ANCA-GN: ANCA-associated GN

AUC: Area under curve

BVAS: Birmingham Vasculitis Activity Score

C-ANCA: Cytoplasmic-ANCA

CHCC: Chapel Hill Consensus Conference

CYC: Cyclophosphamide

eGFR: Estimated glomerular filtration rate

EGPA: Eosinophilic granulomatosis with polyangiitis

ELISA: Enzyme-linked immunosorbent assay

EMEA: European Medicines Agency

ENT: Ear, nose, and throat

ESRD: End-stage renal disease

GN: Glomerulonephritis

GPA: Granulomatosis with polyangiitis

HLA: Human leukocytes antigen

HR: Hazard ratio

IIF: Indirect immunofluorescence

IV: Intravenous

MPA: Microscopic polyangiitis

MPO: Myeloperoxidase

NCR: Norwegian Cancer Registry

NKBR: Norwegian Kidney Biopsy Registry

NMSC: Non-melanoma skin cancer

P-ANCA: Perinuclear-ANCA

PR3: Proteinase 3

SIR: Standardised incidence ratio

SMR: Standardised mortality ratio

ROC: Receiver operator characteristic

RRT: Renal replacement therapy

List of publications

I. Sriskandarajah S, Aasarød K, Skrede S, Knoop T, Reisæter AV, Bjørneklett R. **Improved Prognosis in Norwegian Patients with Glomerulonephritis Associated with Anti-Neutrophil Cytoplasmic Antibodies.** Nephrology Dialysis Transplantation 2015; 30 Suppl 1: i67-75

II. Bjørneklett R, Sriskandarajah S, Leif Bostad. **Prognostic Value of Histological Classification of ANCA Associated Glomerulonephritis.** Clinical Journal of the American Society of Nephrology 2016 Dec 7; 11(12): 2159-2167

III. Sriskandarajah S, Bostad L, Myklebust TÅ, Møller B, Skrede S, Bjørneklett R. **Cancer in ANCA-associated Glomerulonephritis: A Registry-based Cohort Study** *(Submitted)*

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1. Introduction

1.1. Background

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a primary systemic autoimmune disease, characterised by necrotising inflammation affecting small to medium size blood vessels in the body. It is a clinical and pathological syndrome consisting primarily of granulomatosis with polyangiitis (GPA) (formerly known as Wegener's granulomatosis (WG)) and microscopic polyangiitis (MPA), and less commonly, eosinophilic granulomatosis with polyangiitis (EGPA) (formerly known as Churg-Strauss syndrome). The cause is still unknown, but most patients have circulating autoantibodies (ANCAs) in their blood sera. These autoantibodies are pathogenic and directed towards neutrophil granulocytes and monocytes, and activate these by binding to the surface-near antigens, myeloperoxidase (MPO) and serine protease 3 (PR3). Clinical symptoms may vary and affect many organs, but left untreated, lead to rapid onset of renal loss and respiratory failure. The kidney is the most prevalent target of injury, resulting in a rapid progressive glomerulonephritis (GN). Introduction of immunosuppressive therapy in the 1960s and ANCA serology in the 1980s has revolutionised the diagnostic process and improved the prognosis over the last 5 decades. AAV is currently a chronic disease with a remitting-relapsing course. The mortality rate is still higher than the general population, and treatment-related adverse effects are a major concern (1-3).

Due to the rare nature of the disease, data on prognosis is scarce in the literature. Long-term trends in mortality and morbidity and identifying prognostic parameters of outcome are targets for this thesis.

1.2. Classification

Historically, Kussmaul and Maier reported the first case study of vasculitis in 1866 (4). They observed nodular inflammation in small- and medium-sized blood vessels in a patient that presented with nonspecific signs, such as fever, anorexia, myalgia, abdominal pain, paraesthesia, and oliguria. The condition was named periarteritis nodosa, which was renamed as polyarteritis nodosa. Over time, vasculitis targeting predominantly small- to medium-sized vessels was identified and separated from polyarteritis nodosa. These were highlighted by Godman and Churg in 1957 as WG, “microscopic form of periarteritis” (MPA), and Churg-Strauss syndrome (1, 5).

The ACR classification 1990

In 1990, the American College of Rheumatology (ACR) published the first standardised classification of vasculitis (6-8). The classification criteria consisted of 7 diagnoses: WG, polyarteritis nodosa, Churg-Strauss syndrome, hypersensitivity vasculitis, Henoch-Schönlein purpura, giant cell arteritis, and Takayasu arteritis. WG was diagnosed on the presence of two or more of the following criteria: 1) nasal or oral inflammation, 2) chest radiograph showing nodules, infiltrates, or cavities, 3) haematuria or red cell casts in urine sediment, and 4) granulomatous inflammation on biopsy. It is important to mention that the ACR classification tools were intended to distinguish patients in epidemiological research, and had little clinical diagnostic value. Additionally, the classification did not include ANCA serology as criteria, or contained any classification criteria for MPA.

The Chapel Hill Consensus Conference 1994 and 2012 definitions

The Chapel Hill Consensus Conference in 1994 aimed at defining the nomenclature of primary systemic vasculitis (9). The vasculitis was classified according to the smallest affected vessel, i.e. small vessel vasculitis affects predominantly intraparenchymal capillaries, venules, arterioles, and arteries. The nomenclature introduced MPA and surrogate markers for vasculitis (e.g. haematuria, proteinuria, and the presence of red cell casts in urine sediment are diagnostic markers for glomerulonephritis). Polyarteritis nodosa was distinguished from MPA by the absence of small vessel inflammation. Moreover, WG was restricted to vessels with granulomatous inflammation, while non-granulomatous lesions characterised MPA. The definitions were revised in 2012 with the inclusion of ANCA serology (10). The small vessel vasculitis was divided into ANCA-associated (GPA, MPA, EGPA, and single organ AAV) with paucity of immune deposits in the vessel wall, and immune deposition rich immune-complex mediated vasculitis. Importantly, it was emphasised that the definitions were not valid as classification or diagnostic criteria for vasculitis. The 2012 definition of AAV is depicted in Table 1.

Table 1. The Chapel Hill Consensus Conference 2012 definition of AAV (10)	
General	Necrotising vasculitis, few or no immune deposits, affects predominantly small vessels, associated with ANCA (MPO-, PR3-, or negative ANCA)
MPA	Necrotising vasculitis of small and medium vessels may occur. Necrotising GN is very common, pulmonary capillaritis often occurs. Granulomatous inflammation is absent.
GPA	Necrotising granulomatous inflammation usually involving upper and lower respiratory tract. Necrotising GN is frequent.
EGPA	Eosinophil-rich and necrotising granulomatous inflammation usually involving the respiratory tract and associated with asthma and

	eosinophilia. ANCAs are more common when GN is present.

The European Medicines Agency (EMEA) algorithm 2007

Due to the absence of classification criteria of MPA in the ACR criteria and the CHCC 1994 definitions, in 2007, the EMEA developed a stepwise consensus algorithm for classifying AAV and polyarteritis nodosa. The purpose was to incorporate the ACR criteria and the CHCC 1994 definitions into a harmonised and standardised system to be utilised in epidemiological studies (11). The ACR criteria was first preferred for classifying Churg-Strauss Syndrome and WG, whereas CHCC 1994 defined MPA after the exclusion of the previous conditions.

1.3. Epidemiology

Incidence and prevalence

The overall incidence of AAV in Europe has been estimated to be about 6-23 cases per million population (12-15). In a recent biopsy-based study of Swedish patients with ANCA-associated GN (ANCA-GN), the annual incidence was estimated to be 13.2 cases per million population (16). In comparison, in Norwegian patients with GPA, the annual incidence rate was estimated to be 9.3 cases per million population (17). The incidence rate is higher in GPA than MPA, 11.3 versus 5.9 cases per million population per year, but this varies in the literature. EGPA is the least frequent clinical phenotype; its highest annual incidence rate was estimated to be 2.7 cases per million population in the UK (18).

Longitudinal epidemiology studies have shown an increased incidence of AAV since the early 1980s. Reports from the United Kingdom have observed a combined annual incidence of GPA and MPA of 1.5 in 1980-1986 to 6.1 cases per million population by the end of 1989 (19, 20). This finding

was comparable with a Swedish study by Knight *et al.,* who reported an increasing incidence from 3 to 8 cases per million population per year (21). This trend might reflect an actual increase in incidence, but recent papers have shown a stable rate over the last decade (15, 22-27). Alternatively, the increase in the incidence might be due to the introduction of ANCA serology and more awareness of the disease among healthcare workers, which led to an increased case capture.

The prevalence of AAV in Europe is estimated to be 45-184 cases per million population (14, 15, 27, 28). The prevalence has also increased during the last few decades, proportionately to the incidence rate. In Norway, the prevalence of GPA has increased from 30.4 in 1988 to 95.1 cases per million population in 1998 (17). This trend might be a result of increased patient survival and improved case identification. Data on the prevalence of MPA is scarce but has been reported to range from 19.3 to 94 cases per million population (15, 20, 29). The prevalence of GPA is somewhat lower in southern Europe, reported to be 24 in France (30) and 42 cases per million population in Turkey (15), respectively. This observation strengthens the assumption of a north-south gradient where GPA is more prevalent in Scandinavian countries. The age-specific peak incidence is in the age groups 55-64 (22) and 65-74 years (20). AAV affects both genders equally and most patients are Caucasian. Several studies from Europe and Asia have shown that AAV was twice as prevalent among Europeans than non-Europeans (13, 30, 31).

Geographical and seasonal variations

Watts and colleagues described a difference in incidence between GPA and MPA in northern and southern Europe. The incidence of GPA was higher in the northern region, while MPA was more frequent in southern Europe, which is called a north-south gradient (12). Comparable observation was also

seen in New Zealand, with a 2-3 times higher incidence of GPA in southern latitudes compared to northernmost latitudes (31). Possible explanations for the north-south gradient might be due to different UV radiation exposure or genetic variations between ethnicities. Conversely, this gradient was not observed in other studies from the southern hemisphere, showing a comparable incidence rate of GPA to reports from northern Norway (24, 32). Similar results have also been evident in a Swedish report, with an incidence rate of MPA similar to rates from southern Europe (26). Moreover, MPA and MPO-ANCA are more common in Asian countries, like Japan and China, compared to Europe (33).

Studies have also suggested seasonal and periodic fluctuations in onset of AAV, but contradictory results have been reported on this topic. Incidence peaking every 3-4 years and more symptoms occurring during dark winter periods might reflect environmental risk factors such as viral infections, i.e. influenza, while disease onset clustering around spring and summer periods is suggested to be associated with allergic inflammation. Pertinently, studies from France (34) and Japan (35) showed that symptoms developed predominantly during summer periods. In contrast, other papers could not demonstrate any seasonal fluctuation in disease onset (17, 26, 36).

1.4. Aetiology

Genetic Aspects

The cause of AAV remains unknown but is likely multifactorial, involving both environmental and genetic factors. Altered gene expression might lead to aberrant protein synthesis and/or function. Results from recent genetic studies support the role of a dysregulated inflammatory process involving the major histocompatibility complex system, alpha-1-antitrypsin, and cytotoxic T lymphocyte associated antigen-4 in the pathogenesis of AAV. The two major clinical syndromes of AAV, GPA and MPA, have been recently described in genome-wide association studies as aetiologically distinct diseases. The correlation between candidate genes and disease were differentiated, particularly with PR3 and MPO disease subsets (37-40). Furthermore, in a large meta-analysis, investigators identified 33 genetic variants that were associated with AAV. A subdivision of AAV broken down by PR3 and MPO, had a stronger genetic correlation than with the clinical syndrome of GPA and MPA (41). Familial clustering of these risk alleles might also increase the susceptibility to develop AAV (42-45).

The human leukocyte antigen (HLA) system is crucial in disease aetiopathogenesis in AAV. These gene complexes encode antigen-presenting proteins on the surface of a cell to T-lymphocytes. Mutations of these gene complexes may lead to autoimmune inflammation that results in AAV. Different loci in this gene region were associated with distinct AAV subtypes (HLA-DP with PR3-ANCA and GPA, and HLA-DQ with MPO-ANCA and MPA). Another genetic association is with PR3-ANCA and the allele deficiency of the serpin A1 gene that encodes alpha-1-antitrypsin, which is an inhibitor of PR3 (37, 39, 46, 47). These observations underscore the distinct genetic background between the two ANCA antigens.

The genetic findings correlate well with epidemiologic studies. Ethnic differences have been observed, with the prevalence of AAV being higher

among Europeans than among non-Europeans (30, 31). An American study identified that African-Americans with PR3-ANCA-positive AAV were carriers of an HLA allelic variant of Caucasian descent rather than a variant of African descent (47). Furthermore, patients with positive PR3-ANCA show better disease control with rituximab than with cyclophosphamide (CYC), but have a higher cardiovascular risk and are more prone to develop relapse after transplantation (48-50). Lastly, extrarenal organ manifestation with granulomas is more common in PR3-ANCA (51). Additionally, MPO-ANCAs are associated with higher treatment resistance, end-stage renal disease (ESRD), and death (52, 53). A recent report has also postulated that a rise in PR3-ANCA during disease remission is indicative of relapse, particularly in patients with severe renal and lung involvement (54).

These findings imply that AAV consists of distinct disease entities and should be classified as MPO- and PR3-ANCA rather than GPA and MPO phenotypes when evaluating choice of treatment and conducting clinical trials in the future.

Environment

Environmental agents might elicit AAV in genetically susceptible individuals. This hypothesis is strengthened by geographical distribution in the occurrence of the disease. The frequency of MPA is 9 times higher in rural populations compared to urban areas (24), and GPA has been associated with farming and with contact with cattle and pigs (55). Moreover, the higher prevalence in the elderly population and no significant gender difference implicates cumulative environmental expositional triggers. The cyclic pattern and seasonal fluctuations of the occurrence of GPA have been associated with risk factors, such as influenza or atopy (20, 34). Furthermore, increased nasal carriage of *staphylococcus (S.) aureus* has been correlated with disease relapse, particularly in PR3-ANCA (28, 56). Indeed, nasal

carriage of *S. aureus* occurs in 63% of patients with WG compared to only 25% in the healthy population (57).

Several mechanisms have been proposed in the interaction between infection and vasculitis. First, an infectious milieu with proinflammatory cytokines and other mediators can prime neutrophils to increase the expression of PR3 on the cell surface. This might explain the frequent rate of relapse in PR3-ANCA AAV (58, 59). Second, pathogenic peptides can demonstrate molecular mimicry to the human peptide. During an infection, antibodies produced during an infection targeting foreign proteins might cross-react with self-peptides and cause autoimmunity. In support of this theory are studies that have shown immunization of mice with bacterial peptides induced the production of antibodies against human PR3 (60) and human lysosome-associated membrane protein-2 (61). Third, *S. aureus* strains that produce superantigens (Toxic Shock Syndrome Toxin-1) stimulate lymphocytes and are associated with an increased relapse rates in patients with WG (62). Lastly, Toll-like receptors bind infectious agents and activate the proinflammatory response. It has been observed that ligation with bacterial DNA resulted in ANCA production (56, 63). Chronic nasal colonization in patients with GPA might also be facilitated by dysfunctional cytokine expression and reduced secretion of Interleukin-8 (IL-8) in the nasal epithelium when stimulated by *S. aureus* (64). Also, these pathogens can resist phagocytosis and replicate intracellularly in neutrophil granulocytes (65).

Among other environmental risk factors, low ultraviolet radiation, silica, heavy metals, antithyroid and antihypertensive drug allergies, and cocaine have been proposed as triggers (66). Exposures to different levels of ultraviolet radiation might partly explain the latitudinal gradient of AAV, as suggested by some studies. An international ecological study in 2009 concluded that there was a latitudinal increased incidence of GPA and EGPA, but not of MPA (67). Whether this reflects a direct protective effect of ambient

ultraviolet radiation or an indirect effect through the effects on vitamin D synthesis is unclear. Inhalation of air pollutants, such as silica, has been associated with an increase in incidence of AAV, in particular MPO-ANCA (68). A recent meta-analysis stated that silica exposure doubled the risk of developing AAV (69). This correlation might also explain the increased prevalence in rural areas where there are a higher number of industrial worksites and more exposure to respirable dust. Both vitamin D deficiency and silica have been associated with other autoimmune conditions. The effect of tobacco smoking on AAV is not well studied and data are inconsistent (28).

These findings suggest that although environmental triggers play a key role in the pathogenesis of AAV, different genetic backgrounds might also determine the responses to these triggers.

1.5. Pathogenesis

Antineutrophil Cytoplasmic Antibodies

ANCA were first described in 1982 in patients with segmental necrotising glomerulonephritis and clinical symptoms of systemic vasculitis (70). Since then, clinical and experimental studies have described the pathogenic role of ANCA in GPA, MPA, EGPA, and renal-limited pauci-immune vasculitis, now grouped as AAV (71-75). ANCA are present in > 90% of GPA and MPA, and approximately 75% of EGPA with GN. These autoantibodies consist predominantly of immunoglobulin G. Their presence is specific to AAV and titres correlate well with disease activity. PR3-ANCA levels are usually not detectable in patients in remission, whereas they are significantly increased in active disease. The observed efficacy of immunosuppressive therapy targeting B-cells and plasma exchange in AAV is also in support of the pathogenicity of ANCA (76-78).

Animal models showed that when mice were injected with anti-MPO immunoglobulin, they developed crescentic glomerulonephritis and systemic vasculitis similar to that of humans (79). The pathogenicity of ANCA was further described in a case report from 2005 of a newborn that presented with pulmonary-renal syndrome after placental transmission of MPO-ANCA (80). In vitro studies have shown that ANCA IgG can activate neutrophils that are primed by proinflammatory cytokines. Priming causes the cells to release MPO and PR3 onto the cell surface and into neutrophil extracellular traps. It has been postulated that ANCA binds to these surface autoantigens on neutrophils and monocytes, resulting in respiratory bursts, degranulation of toxic agents, apoptosis, and necrosis that ultimately result in endothelial damage and extracapillary granulomas. These inflammatory agents activate the alternative complement pathway C5a, which in turn attracts more neutrophils and primes them. This creates an inflammatory loop that amplifies the disease process. The neutrophils also extrude extracellular traps containing chromatin fibrous material that are decorated with MPO and PR3.

These traps might further facilitate the inflammatory response and activate the coagulation cascade, resulting in fibrinoid necrosis. Over time, the neutrophil-dense necrotic inflammation is replaced with invasion of macrophages, monocytes, and T-cells. Breakage of the endothelial cells also causes leakage of serum in the interstitial space and activation of epithelial cells. This process is observed in the glomeruli as the characteristic crescent formation. Activation of fibroblasts will lead to the production of collagen IV and the final stage of chronic inflammation, irreversible scarring (76, 77, 81).

This chain reaction leads to the clinical outcome of small-vessel vasculitis with alveolar lung haemorrhage, glomerulonephritis, peripheral neuropathy, gastrointestinal ulceration and haemorrhage, and muscle arteritis causing myalgia (72).

Although there is evidence of the aetiopathological effect of ANCA and its epitopes in AAV, “natural” ANCA are detectable in serum of healthy individuals. These autoantibodies have different epitopes than pathogenic ANCA, and have both lower titres and lower affinity to neutrophils. Epitope diversity may also be a possible explanation for patients with AAV, who are ANCA-negative, according to routine enzyme-linked immunosorbent assays (ELISA). It is hypothesised that exogenous stimuli, such as infection, atopy, or silica can alter epitope specificity, and cause a pathogenic transformation (82, 83).

Complement factors

Until recently the role of complement factors had in the pathogenesis of AAV, has been incompletely described, partly due to their absence in kidney biopsies (1). However, experimental and biopsy data have recently illuminated their key role, particularly in the alternative pathway in AAV (77). Complement factors have been observed in both serum (84) and urine (85) in

patients with AAV. Furthermore, groups have also discovered that inhibiting complement C5 in the complement pathway protected against glomerular damage (86, 87). C5a acts as a chemoattractant and primer of neutrophils. Activated neutrophils secrete mediators that in turn amplify the complement pathway, causing a positive feedback loop. Attenuation of GN by blocking factor B and the presence of properdin in biopsies of patients with AAV indicate that the alternative pathway is pivotal in the pathogenesis of AAV (88). Low baseline serum complement C3 is also associated with an increased risk of ESRD and death (89, 90).

B-cell activating factor

B-cell activating factor promotes B-cell survival and immunoglobulin synthesis. Autoreactive B-cells are particularly dependent on these factors for maturation. Elevated levels of this factor have been shown in rheumatologic diseases, including WG, when compared to healthy controls (91, 92). Researchers are currently investigating therapeutic agents (belimumab and blisibimod) targeting this factor (93).

1.6. Clinical Characteristics

Organ damage

AAV is pathologically characterised by an inflammatory reaction in the blood vessels with fibrinoid necrosis and granulomatous lesions. Despite having similar pathophysiology, there are certain clinical differences between GPA, MPA, and EGPA. Upper respiratory manifestations are more common in GPA than MPA. Granulomas are less frequent on histologic examination of MPA (94, 95). In addition to granulomas, EGPA is characterised by the presence of asthma and hypereosinophilia, and less frequently, circulating ANCAs (96).

Patients with AAV usually experience prodromal “flu-like” symptoms with fever, headaches, anorexia, malaise, weight loss, and myalgia or arthralgia. The prodromal symptoms might last for several weeks or months. Other presentations might be local vasculitis with as a skin rash and tender nodules, scleritis, bloody rhinitis, or arthritis. Ear, nose, and throat (ENT) involvement such as hearing loss, otalgia, recurrent sinusitis and otitis media, saddle nose, or nasal crusting may be the initial clinical manifestation. The upper respiratory tract is involved in most patients with GPA (70-100%), but only in 35% of patients with MPA (1, 97). In a recent report among 414 patients enrolled in clinical trials, 45% presented with ENT involvement (77% of patients with GPA and only 23% of patients with MPA) (98).

Lung affection with alveolar haemorrhage and GN are hallmark lesions of AAV and important causes of morbidity and mortality. GN occurs in about 70-80% of all cases (53, 98, 99), ranging between 11-77% in GPA, 79-100% in MPA, and 12-67% in EGPA (100-103). The occurrence of alveolar haemorrhage is observed to be 7-45% in GPA, 10-30% in MPA, whereas it is rare in EGPA (104). Hirayama and colleagues reported in their study of 1147 Japanese patients with ANCA-GN, most of them with MPO-ANCA positive, that 52% had lung involvement of which 15% had severe lung haemorrhage

(105). About 50% of patients have cutaneous manifestations, such as rash and tender nodules. Disease manifestations of the nervous system and the eyes are also common (2, 106, 107).

Table 2. Clinical manifestations in AAV	
Organ	Clinical manifestation
General	Fever, malaise, fatigue, anorexia, weight loss, neck ache, headache, polymyalgia, polyarthralgia
Cardiovascular	Pericarditis, pericardial effusion, occlusive vascular disease, cardiomyopathy, congestive heart failure
ENT	Hearing loss, otalgia, otitis media, nasal crusting, epistaxis, mucosal inflammation, nasal bridge collapse, recurrent sinusitis, mouth ulcers
Eye	Scleritis, episcleritis, keratitis, uveitis, retinal changes
Gastrointestinal	Mesenteric vasculitis, bowl ischaemia, peritonitis
Kidney	Haematuria, proteinuria, cellular casts in urine cytology, rapid renal impairment
Nervous system	Cranial nerve palsies, sensory peripheral neuropathy, mononeuritis multiplex, seizures, meningitis
Respiratory	Tracheal stenosis, cough, stridor, wheezing, dyspnoea, haemoptysis, lung nodules, pleuritis and effusion, pulmonary haemorrhage, respiratory failure
Skin	Tender nodules, livedo reticularis, leucocytoclastic angiitis,

	digital infarcts, cutaneous ulcers and purpura, genital ulcers

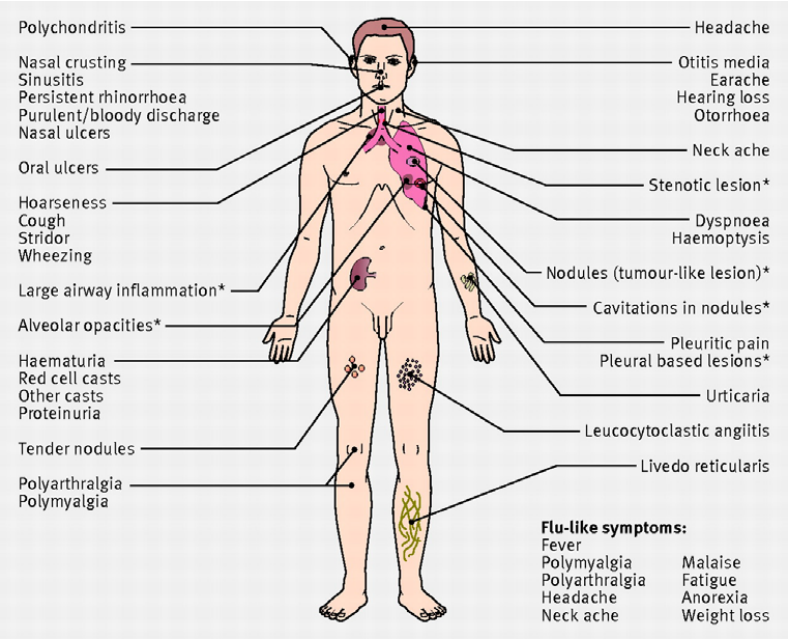


Figure 1. Organ manifestations in ANCA-associated vasculitis. Reproduced from [Diagnosis and management of ANCA associated vasculitis, Annelies Berden et al., vol.nr: 344, page numbers: e26, copyright notice year: 2012] with permission from BMJ Publishing Group Ltd (2).

1.7. Diagnosis

Early detection

The signs and symptoms of AAV might vary and therefore delay the diagnosis for months, resulting in irreversible organ damage. Thus, the clinician must be vigilant and consider testing patients with unexplained chronic inflammatory disease, particularly in the airways and the kidneys. Kidney affection typically presents with haematuria and proteinuria, but patients might not perceive it until symptoms of uraemia are present. Early urine dipstick testing and microscopic urine sediment analysis will reveal erythrocytes, granular casts, and erythrocyte casts compatible with glomerulonephritis. A blood test should also be performed in the initial evaluation, which might reveal increased serum creatinine, elevated C-reactive protein and erythrocyte sedimentation rate, and decreased leucocytes and platelets. Although erythrocyte sedimentation rate and C-reactive protein are non-specific markers of inflammation, they have high sensitivity for active vasculitis and are helpful in ruling out the condition (108). A normochromic-normocytic anaemia may also be indicative of a systemic inflammatory disease (1, 2, 97).

Patients with antibiotic-refractory cough and haemoptysis should have a chest X-ray to reveal lung nodules/granulomas or infiltrates. Cases with unexplained conjunctivitis, retroorbital mass, or skin vasculitis with general symptoms should also be targeted for testing (97). Differential diagnoses such as infection, malignancy, or drug-induced vasculitis are also important to rule out (109).

The Birmingham Vasculitis Activity Score (BVAS) is a validated instrument for assessing disease activity in systemic vasculitis, especially GPA. It contains a list of 66 manifestations of vasculitis with nine organ-specific symptoms. The sum of the score from all organs reflects the disease

activity and severity, and ranges from 0-68. The BVAS can be used in new, persistent, or relapsed disease (110, 111).

Serology

With the introduction of the indirect immunofluorescence (IIF), ANCA became a sensitive serologic marker for AAV. In GPA, ANCA produce a diffuse granular cytoplasmic staining pattern in ethanol-fixed neutrophils (C-ANCA) (112), whereas it produce a perinuclear-staining pattern in MPA and EGPA (P-ANCA) (113). The primary target antigens for P- and C-ANCA were identified as MPO and PR3, respectively. When preparing with ethanol, MPO would dissolve from cytoplasmic granules and attach to the nucleus, resulting in a perinuclear-staining pattern, whereas PR3 would not dissolve and therefore yield a cytoplasmic staining pattern. The ELISA test, using purified MPO and PR3 for ANCA detection, was developed and made commercially available in 1990 (72, 114).

Most laboratories use the IIF as a screening method to identify ANCA, and if positive, then they perform a confirmatory ELISA test (115). IIF has higher sensitivity, but ELISA is more specific. The combined sensitivity and specificity of these two tests have been estimated to be 82% and 99%, respectively (116, 117). According to four large international randomised trials, ANCAs are positive in 90-95% of patients with active GPA or MPA (78, 118-120). In GPA, PR3-ANCAs are detected in 40-90% of patients, whereas MPO-ANCAs are identified in less than 10% of cases. MPO-ANCAs are detected in 65-90% of patients with MPA and in 30-40% of patients with EGPA (121).

In summary, GPA has a classic cytoplasmic pattern in IIF and is associated with PR3-ANCA, while MPO-ANCA and the perinuclear pattern on IIF is more common in MPA and EGPA.

Patients with symptoms of AAV should be referred to the nearest specialist for care, especially when presenting with organ failure. An ANCA assay can be requested in all hospitals and is a tool for diagnosis and a marker of disease activity. ANCA serology helps to distinguish between disease entities, and predict response to therapy and disease control (2, 109, 122). Nevertheless, the result must be evaluated based on the clinical findings. A negative test does not exclude the diagnosis, as some patients have an ANCA-negative disease. Possible explanations for a negative test might be epitope diversity or ANCA specificity to other antigens (e.g. anti-elastase and cathepsin G). One study has shown that among ANCA-negative cases, a blood serum factor binds to specific MPO-epitopes-ANCA and prevents them from being detected by routine tests. These immunoglobulins were detected only after they were purified (123-125). If the clinical suspicion is high, despite a negative ANCA test, the assay should be repeated after a few weeks. One must also keep in mind that ANCA are not specific for AAV as it is detected in other inflammatory conditions, such as inflammatory bowel disease, infection, rheumatoid arthritis, connective tissue disease, and secondary to drug reactions (126). Thus, it is not advised to use it as a screening tool without a clear indication. On the other hand, a high ANCA titre combined with multiorgan affection is indicative of AAV (127).

In Table 3, McLaren and colleagues have proposed a list of clinical syndromes associated with systemic vasculitis where the ANCA assay is recommended (121, 128).

Table 3. Clinical indications for ANCA testing
Chronic necrotising disease of the upper airways
Cavitating pulmonary nodules
Suspected Churg-Strauss syndrome

Pulmonary-renal syndrome, pulmonary haemorrhage
Subglottic stenosis
Refractory sinusitis or otitis
Rapidly progressive glomerulonephritis
Cutaneous vasculitis accompanied by systemic symptoms
Mononeuritis multiplex or peripheral neuropathy

Novel markers. A new and promising diagnostic biomarker of AAV, is anti-pentraxin-3 autoantibodies. Pentraxin 3 has similarities with PR3 and MPO autoantigens, and is stored in neutrophil granules. A study showed that these autoantibodies were present in 56 of 150 AAV patients compared to only 12 out of 227 patients with IgA nephropathy. Also, it had a distinct staining pattern on IIF compared to classic C-ANCA and P-ANCA. Higher levels of autoantibodies were also detected during active disease than under quiescence (129). Lysosome-associated membrane protein-2 antibodies are also potential biomarkers that are present during active disease and not detectable during remission. Interestingly, these autoantibodies have also been found in patients, who are ANCA-negative (130). Elevated complement factors have been observed in the blood and urine during active disease compared with that during remission. Activated complement factor B was also inversely correlated with the percentage of normal glomeruli in the biopsy (84, 85). Standardised assays need to be developed and validated in large cohorts before these tests can be implemented in routine care.

Renal biopsy

A biopsy is the gold standard in the diagnosis of ANCA-GN. The inflammation in the glomerular capillary can cause disruptions of the

glomerular basal membrane. The influx of inflammatory mediators and fibrin causes fibrinoid necrosis and causes leukocytes to produce cellular crescent formation in the Bowman's space. The lesions might be acute with cellular/fibrocellular crescents or fibrous in more developed stages, with the invasion of collagen-producing fibroblasts. The proportion of affected glomeruli might vary from focal and segmental lesions to diffuse and global injury. Interstitial inflammation can also be apparent with nephritis, tubulitis, and tubular atrophy. The GN might also be accompanied by granulomas and interstitial vasculitis (131-133).

The crescentic and necrotising inflammation in light microscopy is indicative of a rapid and progressive GN. In addition to ANCA-GN, possible differential diagnoses include immune complex-mediated diseases (e.g. lupus nephritis, anti-glomerular membrane disease, IgA nephritis and postinfectious GN). Immunofluorescence staining of the biopsy is the next step to differentiate these conditions. In ANCA-GN, there is usually a low level of immunoglobulin pattern, a so-called "pauci-immune" appearance, characterised by < 2 on a scale from 0 to 4 (76). Conversely, a granular staining pattern is suggestive of immune complex-mediated disease, whereas a linear immunoglobulin G staining pattern indicates anti-glomerular membrane disease.

The renal biopsy has been reported to influence and guide therapy decisions in 82% of all cases (134). The rate of biopsy might vary, but Scandinavian groups have documented a biopsy rate ranging from 68 to 87% in patients with ANCA-GN (16, 131).

Complications of renal biopsy are rare. Macroscopic haematuria and need for erythrocyte transfusion occurs in about 2-3 % and 1% of all biopsies, respectively. Perinephric haematoma is more prevalent and appears in 12% in all cases, but only approximately 0.5% of all patients are in need of angiographic intervention to stop the bleeding. Risk of bleeding is associated

with older age, systolic blood pressure, poor renal function, and small hospital size where the procedure was performed. Lastly, in a large meta-analysis, only two deaths were observed following 8971 biopsies (135, 136).

Although renal biopsy is a relatively safe procedure, the need for this practice in patients, who are ANCA-positive, with typical clinical symptoms, is debatable. Another controversy with tissue sampling is its utility as a longitudinal prognostic marker; The procedures are invasive and not desirable for repeated assessments of disease control (108).

1.8. Treatment and adverse effects

The aim of therapy in ANCA-GN is the induction of remission and resolution of symptoms, such as haematuria, proteinuria, and improved serum creatinine. Response to treatment varies; some patients experience refractory disease with declining renal function despite high doses of immunosuppression, whereas other patients experience complete remission, with or without any relapses. Judicious caretaking and tailoring therapy is prudent for optimal outcome.

Since autoantibodies play a major role in the pathogenesis of AAV, B-cell depletion is an important target in therapy. In the early 1950s and 1960s, steroid monotherapy was introduced as a treatment for AAV. Prior to this, no standardised therapy was described. Untreated patients had a poor outcome and over 90% died after two years. Most patients died of renal failure and respiratory dysfunction (137). Since the 1970s, the addition of CYC led to a dramatic improvement in prognosis. With immunosuppression, survival rate rose to 90% during the first year after diagnosis and 75% achieved complete remission. However, CYC is associated with an increased risk of infections and malignancy. During recent years, several landmark clinical trials aiming at reducing the exposure to CYC and investigate alternative and more effective regimens, have resulted in a crucial improvement of therapy (97, 100, 138).

Induction therapy

Glucocorticoids are the cornerstone in therapy and usually administered as intravenous (IV) methylprednisolone 1 g for three consecutive days. Thereafter treatment with oral prednisone of 1 mg/kg up to 80 mg per day for 4 weeks follows, before gradually tapering, e.g. 10% every 1 to 2 weeks, over 3-5 months (139). Long-term steroid use is associated with adverse effects, especially infections, osteoporosis, hypertension, cataracts, skin atrophy, gastrointestinal bleeding, cardiovascular disease, and diabetes. Although

some suggest discontinuing low-dose steroids after 6 months, optimal duration of therapy remains controversial (140-146).

CYC or rituximab combined with steroids is recommended as first line treatment in inducing remission in patients with severe AAV. In non-organ-threatening disease, methotrexate (118, 147) or mycophenolate mofetil (148) can be considered. Methotrexate is equally efficient as CYC in remission induction, but less effective in preventing relapses (149). Plasma exchange is considered as a rescue therapy in patients presenting with lung haemorrhage and/or serum creatinine > 500 µmol/L (78, 139). Although this procedure improves renal survival, the overall mortality is unchanged. Long-term benefit of plasma exchange needs to be studied further (150). In a clinical setting, patients with severe organ failure receive both plasma exchange and IV glucocorticoids simultaneously (151).

CYC is a cytotoxic agent that inhibits DNA replication by alkylating guanidine nucleotides (152). It is administered as either pulse IV (15 mg/kg every 14 days for 1 month, then every 3 weeks) or as continuous daily treatment by oral route, usually at doses of 2 mg/kg. The dose may be adjusted depending on leukocyte count, age, and renal function. The duration of treatment depends on patient response, but is recommended from 3 to 6 months. Although both routes are equally effective in achieving remission, the cumulative dose of CYC is higher with oral administration and is associated with a lower rate of relapses (119, 153). However, the IV route is associated with fewer bladder-related adverse effects and favoured over oral in the latest therapy guidelines (139). CYC is associated with severe adverse effects, such as haemorrhagic cystitis, infertility, leucopoenia or neutropaenia, alopecia, bone marrow toxicity and malignancy (97, 119, 138, 154, 155).

Rituximab is an anti-CD20 monoclonal antibody that targets B-cells and induces apoptosis and depletion of the cell population for up to 12 months

(156). It has been proven non-inferior to CYC in two randomised clinical trials in remission induction, combined with steroids. In addition, rituximab had better disease control, particularly in PR3-ANCA AAV (157, 158). In these studies, patients received infusions of 375 mg/m^2 once a week for 4 weeks. The frequency of adverse effects, such as infection, was comparable with rituximab and CYC. Owing to the high cost, rituximab should be considered in patients who plan to get pregnant, or have relapsing/refractory disease. Vigilant monitoring with blood tests is advised since rituximab is associated with hypogammaglobulinemia and opportunistic infections (156, 159, 160).

Maintenance therapy

The optimal duration of maintenance therapy remains unknown. It is guided by both the clinical and the serologic response to treatment. Consensus guidelines recommend continued treatment for at least 24 months. Prolonged maintenance therapy was associated with fewer relapses as opposed to early cessation (109, 139, 146). Thus, the duration of therapy should be tailored based on a global assessment of the patient (161, 162). Routine examination should be conducted every 1 to 3 months with clinical assessment, blood, and urine analyses. Longitudinal ANCA measurements might help to identify patients at risk of disease flares, who could benefit from intensifying monitoring. However, persistent ANCA or an altering titre is an insufficient reason to change therapy (54, 163, 164).

The therapeutic armamentarium includes azathioprine (120, 165), methotrexate (166, 167), rituximab (168, 169), mycophenolate mofetil (170), and leflunomide (171). Azathioprine (2 mg/kg/day) and methotrexate (20-25 mg/kg/week) are equally effective, but azathioprine is preferred in patients with renal insufficiency (166). Mycophenolate mofetil (2 g/day) was associated with more frequent relapses compared to azathioprine, and should be restricted to patients who are intolerant of azathioprine (170). Leflunomide (20 mg/day) is an alternative to azathioprine and methotrexate, but its role in

therapy of AAV needs to be elucidated further (171). The role of rituximab in maintenance therapy is under investigation and lacks consensus. Choosing the optimal relapse-preventing protocol after induction infusion poses a challenge and needs to be refined. Current protocols recommend rituximab-based regimens for refractory or relapsing disease, particularly in PR3-ANCA AAV (139, 169, 172-175).

Prophylactic co-trimoxazole in high doses (320/1600 mg/day), in addition to standard therapy might further reduce the risk of relapse (176). More importantly, low-dose co-trimoxazole is recommended to prevent *Pneumocystis jiroveci* pneumonia (109).

Transplantation

Patients in a period of quiescence can be referred for a renal transplantation. Both patient and renal survival was better in AAV-grafts than in a matched non-diabetes cohort (177). Furthermore, graft and patient survival have been estimated to be 100% after 1 year, and 79% and 67% after 10 years (178). A recent Dutch study showed that 11 of 110 patients with ANCA-GN relapsed within the first 5 years after transplantation. Disease recurrence was independently associated with allograft loss (179).

Novel agents

Currently, the B-cell activating factor inhibitors belimumab and blisibimod are under investigation in clinical trials. Compared to rituximab, these drugs are more selective and have more favourable safety profiles (93, 109). Preliminary results from a recent clinical trial showed that the addition of CCX16 (a C5aR inhibitor) to standard therapy, successfully replaced steroids in remission induction of AAV (180). Trials with etanercept, a tumour necrosis factor (TNF) alpha inhibitor, have been unsuccessful and associated with increased risk of infection and cancer (181).

1.9. Prognosis and Outcome

Patient Outcome

Although the clinical outcome has improved over time, mortality and morbidity remain high. The survival rate varies with study design, definition of end-points, age distribution, disease severity, and the length of follow-up. Compared to the general population, patients with AAV have an increased mortality rate with an estimated standardised mortality ratio (SMR) ranging from 1.6-3.6 (3, 182-184). In tables 4 and 5, results from studies offering data on patient survival, renal survival and SMR in patients with AAV are summarized.

In a Norwegian cohort of patients with WG, SMR was calculated to be 3.8 (107), which is better than what was described in an American cohort of patients with WG (SMR 4.6) (185).

Survival within the first year after diagnosis has been reported between 71% and 99% and between 66% and 90% during 5 years of follow-up (3, 53, 106, 107, 182-184, 186-192). In a follow-up study of patients with AAV enrolled in clinical trials, a 1- and 5-year cumulative survival of 88% and 78% was reported, respectively (3). Five-year patient survival for AAV phenotypes ranged from 74-91% in GPA, 45-76% in MPA, and 60-97% in EGPA, respectively (193). One- and 5-year survival rates have been reported to be more favourable for patients with PR3-ANCA-positive disease (87% and 72%) compared to patients with MPO-ANCA-positive disease (86% and 66%) (53).

Patients with AAV who present with severe disease have a poor prognosis. In a retrospective cohort study of 36 PR3-ANCA and 17 MPO-ANCA positive cases, patients with lung haemorrhage had 83% and 58% survival rates were seen at 3 and 49 months of follow-up, respectively (194). In these patients, 98% also had renal vasculitis and 53% a need for RRT. In

another study, Hirayama *et al.* (1088 patients with MPO-ANCA and 114 patients with PR3-ANCA) described a 5-year survival rate of 42% in patients with AAV with lung haemorrhage compared to 73% in those without lung involvement (105). In a French study, patients admitted to an intensive care unit in northern France due to an acute manifestation of small-vessel vasculitis, which included AAV and anti-glomerular basement membrane disease, had a 90-day mortality rate of 18%. The primary cause for admission was respiratory failure, and most of the deaths were related to disease flare and infection (195). The 1-year survival rate in patients with ESRD is reported to be in the range 64-83%, and 28% were alive after 5 years of follow-up (196).

During the first year, therapy-related adverse events, such as infections and active vasculitis, were the primary causes of deaths (3, 183, 187, 197, 198). Cardiovascular diseases and neoplasms are common causes of deaths after the first year of follow-up. In long-term follow-up of patients enrolled in clinical trials, disease-related renal dysfunction (reduced eGFR, hypertension, and proteinuria), otolaryngological damage (hearing loss, nasal crusting), and treatment-related damages (hypertension, osteoporosis, diabetes, and malignancy) increased over time (3, 141, 199).

Renal Outcome

ESRD occurs in 14-28% of all patients with AAV (53, 184, 196, 199). In a Norwegian cohort of patients with GPA, 10 out of 53 patients developed ESRD, with 1-, 5-, and 10-year renal survival estimated to be 93%, 86%, and 77%, respectively (186). In comparison, a recent retrospective American study showed an overall renal survival of 67%. In this study, the cumulative renal survival at 1- and 5-years was estimated to be 75% and 54%, respectively (191). Therefore, ESRD remains a major concern in this patient group. Patients with ESRD are more susceptible to severe infection-related

death (20% versus 8%) than in non-AAV controls (200). Older patients, in particular, have poorer renal function and are more susceptible to the toxic effects of treatment, with accelerated progression to ESRD. Among elderly patients > 75 years with GPA and MPA, a multicentre study reported 1-year and overall renal survival to be 75% and 72%, respectively (189). However, a similar trend has also been described in paediatric cohorts. In a nationwide study of young patients with AAV younger than 18 years, progression to ESRD was 74% and 70% after 1- and 5-years of follow-up, respectively. Nonetheless, the mortality rate was only 6% during follow-up, which is less severe compared to that in adult cohorts (201).

It has been suggested that patients with MPO-ANCA have a higher risk of developing ESRD than patients with PR3-ANCA (52, 53, 101, 202), but ANCA specificity had no significant prognostic value in a recent study by Rhee *et al.* (191). In addition, in patients with AAV and ESRD, MPA phenotype might indicate poorer renal allograft survival and higher mortality rate (203).

Patients with severe renal dysfunction at diagnosis may not respond to conventional treatment and are at risk of developing ESRD and/or death. In a recent clinical trial, investigators included 137 patients with newly diagnosed AAV and a serum creatinine > 500 μmol/L. Adjuvant IV methylprednisolone was compared to plasma exchange, in addition to oral CYC and corticosteroids. During a median follow-up of 4 years, 86 (63%) developed the composite end-point of ESRD and/or death. Thirty-three developed ESRD in the steroid group compared to 23 in the plasma exchange group ($p = 0.08$). The most common cause of death was severe infection (150).

Patients on RRT have a median survival rate of 5 years. Cumulative survival rates at 3 months, 1, 3, and 5 years of follow-up are estimated to 96%, 85%, 68%, and 53%, respectively. Cardiovascular and infections were the most common causes of death in patients and AAV on chronic dialysis. In

one study, 45 of 425 patients with AAV who were in need of RRT at diagnosis regained their renal function, and 19 remained dialysis-free during the study period (200). Aasarød and co-workers reported in their study that almost 50% of patients who were on dialysis at diagnosis benefited from therapy and regained their renal function (107). These findings emphasise the Kidney Disease Improving Global Outcome guidelines statement that immunosuppressive treatment should be continued for at least 3 months in patients with ESRD.

Relapse

Despite numerous trials and advances in treatment, AAV remains an incurable disease, and over time, 50% experience relapse (97, 101, 107, 161). Among patients with AAV enrolled in clinical trials, 38% relapsed during 1804 patient-years at risk. PR3-ANCA and cardiovascular involvement increased the risk of relapse, while an initial serum creatinine level > 200 μmol/L was associated with lower relapse rate (161). ESRD and death might act as competing risk factors for relapse, and patients with better renal function might live long enough to develop relapses. Also, uraemia can cause dysfunction in the immune system that might hinder a relapse (161).

In a retrospective cohort study from the United States, 35% of patients with AAV relapsed. In this study, interestingly, no temporal improvement in renal relapse rate was observed. Also, duration of CYC or ANCA subtype had no prognostic impact on relapse (191). This is in contrast to previous studies (52, 188, 202, 204), which have shown a 2-fold increased risk of relapse in patients with positive PR3-ANCA compared to patients with MPO-ANCA. In recent Chinese cohorts of patients with AAV, patients with PR3-ANCA had a 30% increased risk of relapse compared to patients who were MPO-ANCA-positive (205, 206). In young patients, a paediatric cohort study of patients

with AAV showed that 43% relapsed after a median follow-up period of 5 years (201).

In a randomised clinical trial conducted by Sanders and colleagues, with patients with positive C-ANCA in quiescence, extended azathioprine maintenance therapy had a limited effect on relapse-free survival compared to standard therapy. Moreover, positive C-ANCA serology at remission was not associated with a large number of cases with relapses (163).

Risk factors

Older age, dialysis-dependency, low serum albumin, higher serum creatinine, lower BVAS score, and lung involvement at baseline are associated with higher mortality. Poor renal function at diagnosis and severe scarring (global sclerosis) of the glomeruli has been associated with ESRD (3, 106, 107, 133, 186, 188, 189, 191, 207, 208). Conversely, PR3-ANCA subtype is associated with better renal survival (53, 202). The results are conflicting regarding differences in mortality between ANCA specificity, and recent studies have not found any significant differences (3, 52, 53, 202). Novel discoveries in genetic differences between PR3-ANCA and MPO-ANCA with distinct treatment response and outcome might indicate a need for a new ANCA type classification. This might simplify future clinical trials and guide therapy (37, 48, 53, 202, 203, 209, 210). Also mentioned previously, serum C3 is an independent predictor of long-term renal and patient survival (89, 90).

Lionaki and colleagues described in their retrospective study of AAV that patients who progressed to ESRD received shorter duration of immunosuppression with CYC, compared to patients who did not develop ESRD (52). Upper respiratory involvement is associated with better patient

outcome (98, 183, 211). Studies have also demonstrated an improved prognosis in patients with AAV diagnosed in recent times, compared to that of previous decades. More awareness of this disease entity and earlier diagnosis with less pronounced kidney failure has been postulated as an important contributor to this trend (182, 183, 188, 192, 208).

Table 4. Cohort studies on patient and renal survival in AAV

Authors (reference)	Year	No. of patients	Diagnosis	1-/5-year renal survival	1-/5-year patient survival
Aasarod (107)	2000	108	WG	91%*/75%	93%*/74%
Reinhold-Keller (106)	2000	155	WG	NR	99%/NR
Koldingsnes (186)	2002	56	WG	93%/86%	93%/79%
Slot (187)	2003	85	PR3-GN	NR	80%/73%
Booth (184)	2003	246	AAV-GN	NR	84%/76%
Eriksson (182)	2009	95	WG/MPA	94%/89%*	93%/84%*
Takala (183)	2010	492	WG	NR	83%/74%
Flossmann (3)	2011	535	AAV	NR	88%/78%
Hilhorst (188)	2013	181	AAV-GN	67%/54%	77%/66%
Mohammad (53)	2014	201	AAV	84%/77%	86%/69%
Andreiana (190)	2015	75	AAV	93%/64%	88%/67%
Weiner[a] (189)	2015	151	AAV	75%/NR	71%/NR

Wallace (192)	2016	465	GPA	NR	91%/78%*
Rhee (191)	2016	554	AAV-GN	75%/54%	91%/72%
*Calculated estimate; [a]Elderly population ≥ 75 years					

Table 5. Standardised mortality ratios (SMR) in patients with AAV

Authors (reference)	Year	N	SMR	95% CI	Predictors of outcome
Aasarod (107)	2000	108	3.8	2.6-5.6	Age at inclusion, low serum albumin
Booth (184)	2003	246	3.6	1.6-7.1	Age > 60 years, ESRD, initial creatinine > 200 umol/L, and sepsis
Lane (212)	2005	99	4.8	2.9-6.6	Age > 65 years
Eriksson (182) [a]Cohort 1 [b]Cohort 2	2009	95	 [c]2.5 [c]1.6	 0.9-5.5 0.6-3.2	Age, creatinine, ESRD, relapse, diagnosis before 1997
Takala (183)	2010	492	3.4	3.0-3.9	Older age and elevated creatinine at diagnosis
Holle (208)	2011	445	1.6	1.1-2.1	
Flossmann (3)	2011	535	2.6	2.2-3.1	eGFR < 15 ml/min per 1.73 m^2, older age, high BVAS score, low Hb and higher white cells count
Schirmer (213)	2016	144	1.4	0.9-2.1	Fibrosing interstitial lung disease, age at diagnosis, peripheral nerve system involvement

[a]Early cohort; [b]Late cohort; [c]5-year survival ratio

1.10. Malignancy

The association between immunosuppression and cancer has been known for several decades (214). Accordingly, several researchers have investigated the risk of cancer in patients with AAV undergoing immunosuppressive therapy. A brief summary of studies and results on the subject are presented in table 6.

There is a well-documented correlation between cancer and chronic systemic autoimmune diseases, such as rheumatoid arthritis (215), inflammatory bowel disease (216, 217), celiac disease (218), systemic lupus erythematosus (219), and sarcoidosis (220). Studies on patients who were treated with CYC for other malignant diseases had a dose-dependent increased risk of bladder and haematologic cancers (221, 222). Furthermore, there is a well-known association between immunosuppressive therapy and the occurrence of skin cancer in renal recipients (223). Thus, one of the objectives of the international renal research community was to investigate how to refine therapy to reduce the risk of malignancy.

Possible pathogenic pathways of malignancy in autoimmune diseases include putative interactions with treatment drugs, viral exposure, and traditional lifestyle risk factors (224, 225). The alkylating properties of CYC may cause mutations in the p53 tumour suppressor gene, and increase the risk of malignancies. Haemorrhagic cystitis, an adverse effect of CYC, was previously reported to precede bladder cancer, but this trend has not been verified in newer data. Hence, it is probably not a good marker for intensifying cancer surveillance. However, patients who have been exposed to CYC and later develop haematuria should be referred for a prompt cystoscopy (155).

The toxic and mutagenic properties attributed to immunosuppressive therapy, especially CYC, motivated researchers to find alternative treatment regimens (97, 138). The increased risk was most pronounced for urothelial carcinoma, squamous cell skin cancer, leukaemia, and lymphoma. The

excess risk depends on both the intensity and the duration of therapy. The bladder is particularly exposed to CYC toxicity due to renal excretion of the metabolites. There is a 7- to 11-fold increase in the risk of bladder cancer for at least a year after exposure to CYC (101, 155). According to Knight *et al*., the risk of bladder cancer doubled for every 10-gram increment in cumulative CYC dose and the risk was particularly high when cumulative doses exceeded 25 grams (155). In another study, by Faurschou *et al*., the risk for malignancies only increased in patients treated with high cumulative doses exceeding 36 grams of CYC. Thirty-six grams corresponded to 100 mg CYC per day for at least a year. There was also a long latency, up to 20 years, in the occurrence of malignancy after cessation of treatment (226, 227).

The introduction of sequential regimens, alternative immunosuppressive drugs, and lessons from landmark clinical trials heralded a new CYC sparing era. Compared to oral CYC, IV has less than 50% of the cumulative dose, and is less harmful for the urinary bladder. The decline in bladder cancer might reflect this trend; both Hoffman (97) and Talar-Williams (138) found a 30-fold increased risk in their American cohort; in contrast, there is only a 1- to 5-fold increased risk in recent studies. This might be due to the fact that 35% of all patients in this cohort received > 100 g CYC, while it was only 8% in a recent Danish cohort (226).

Early studies (Table 6) reflect older treatment regimens and might not correlate to present therapy related malignancies. Recent studies show a non-significant increase in cancer when excluding non-melanoma skin cancer (NMSC), which was the most prevalent type of cancer (228, 229). NMSC seems to be a major contributor to the reported standardised incidence ratio (SIR) for cancer, while the more severe bladder and haematologic cancers were rarer. NMSCs are the most common malignancy in transplant recipients. Azathioprine in particular is associated with sensitising the skin to the mutagenic effect of ultraviolet radiation. Ultraviolet radiation results in

decreased epidermal Langerhans cells (antigen presenting cells), which ultimately lead to dysfunctional local immunosurveillance (230, 231).

Cancer plays a major role in the morbidity of this patient group, but is a less prominent cause for mortality. Nonetheless, standard long-term care should include comprehensive haematologic, dermatologic, and urologic examinations. The potential risk of cancer with use of other therapeutic agents in AAV is still unclear and remains to be explored.

Table 6. A selection of studies on cancer incidence in AAV

Author (reference)	Westman (101)	Knight (232)	Faurshou (226)	Holle (208)	Heijl (228)	Rahmattulla (229)
Country	Sweden	Sweden	Denmark	Germany	Europe	Netherlands
Study design	Monocentre	Nationwide	Nationwide	Monocentre	Multicentre	Monocentre
AAV phenotypes studied	GPA/MPA	GPA	GPA	GPA	GPA/MPA	PGA/MPA
Follow-up period	1971-93	1969-1994	1973-1999	1966-2005	1995-2007	1991-2013
Patients (n)	123	1065	293	445	535	138
Mean/median obs. period (years)	4.6	NR	6.0	5.9	5.0	8.0
Cum. obs. period (person-years)	944	5708	2121	2572	2650	1339
Mean/median age (years)	61.8	NR	59.0	51.7	57.7	59.3
Cancer, all sites (n) and SIR (95% CI)	15 (1.6, 0.9-2.7)	110 (2.0, 1.7-2.5)	50 (2.1, 1.5-2.7)	18 (0.8, 0.5-1.4)	50 (1.58, 1.2-2.1)	85 (2.2, 1.6-2.9)
Bladder (n) and SIR (95% CI)	3 (4.8, 1-13.9)	14 (4.8, 2.6-8.1)	5 (3.6, 1.2-8.3)	4	4 (2.4, 0.7-6.1)	1 (1.4, 0.0-8.0)

Leukaemia (n) and SIR (95% CI)		7 (5.7, 2.3-12)	3 (5.9, 1.2-17)	3*	2 (3.25, 0.4-11.8)	
NMSC (n) and SIR (95% CI)	5 (10.4, 3.4-24.3)	18 (7.3, 4.4-12)	19 (4.7, 2.8-7.3)	3	15 (2.8, 1.2-4.6)	61 (4.2, 2.8-6.2)
Lymphoma (n) and SIR (95% CI)	1 (3.7, 0.1-20.5)	8 (4.2, 1.8-8.3)		3*	1 (1.1, 0.0-6.2)	1 (1.4, 0.0-7.6)

*Leukaemia and lymphoma combined

1.11. A Histopathologic Classification Model

In 2010, an international vasculitis group consisting of nephrologists and pathologists proposed a histologic classification model for ANCA-GN. The histopathological lesions in the glomeruli were divided into four classes: focal, crescentic, mixed, and sclerotic. The classification depends on the predominant change (≥ 50%) of the glomeruli. The focal class depends on ≥ 50% normal glomeruli, whereas the crescentic and sclerotic class have a predominance of cellular crescents and globally sclerotic glomeruli (≥ 50%). The biopsy is classified as mixed when no predominant lesions are present. The classification model was validated in 100 biopsies of patients with AAV, who were included in clinical trials. Two experienced renal pathologists, blinded to the clinical data, scored the biopsies. The model could successfully predict renal survival with ascending prognosis categories of sclerotic, mixed, crescentic, and focal classes, respectively (133).

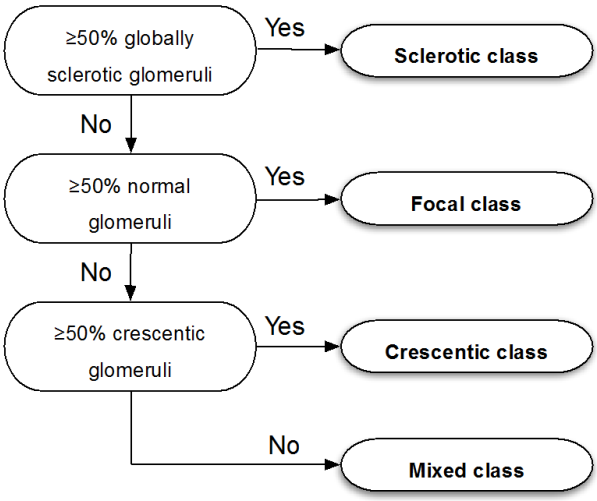
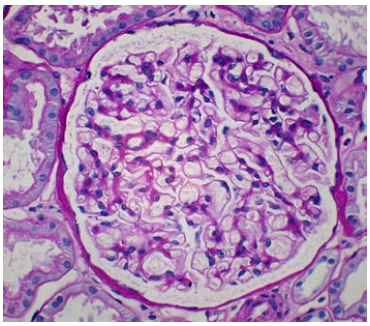
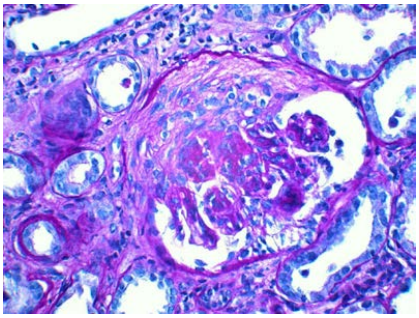


Figure 2. Histopathologic classification flowchart. Adopted from Berden AE, et al. (133).

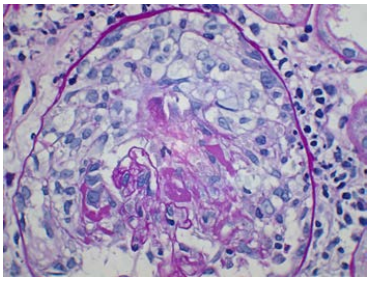
Normal



Crescentic



Crescentic



Sclerotic

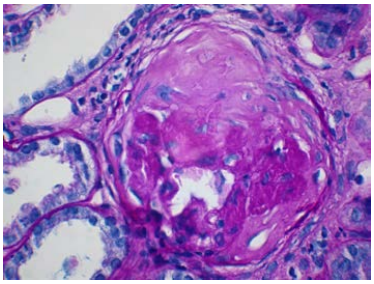


Figure 3. Examples of glomerular lesions: Normal glomerulus (upper left). Glomeruli with extracapillary proliferation and crescent formation (upper right and lower left). Global glomerulosclerosis with obliteration of the glomerulus (lower left). Glomeruli have been stained with periodic acid-Schiff. Courtesy of Leif Bostad

Until recently, twelve studies have validated the classification model (Table 7). The model is of predictive value for renal outcome, especially for focal and sclerotic classes. In the crescentic and the mixed classes, there are conflicting results in the literature. The discrepancy in outcome might be due to differences in ethnicity, ANCA specificity, and variations in treatment between the studies (132, 233, 234).

A high percentage of normal glomeruli in the biopsy specimen is a good predictor of favourable renal outcome. Patients with globally sclerotic glomeruli at baseline are associated with a poor response to treatment and are at an increased risk of developing ESRD. The percentage of crescentic glomeruli is associated with good renal recovery and these patients benefit

from therapy. These pathologic lesions are of prognostic value independently of the baseline eGFR. Tubulointerstitial inflammation and alterations might also be of prognostic value, but previous studies have shown inconsistent results. Moreover, an inclusion of this variable could further decrease the reliability of the classification scheme. Interestingly, compared to patients with PR3-ANCA, patients with positive MPO-ANCA have more chronic lesions at diagnosis, and poorer renal outcome (131, 234-236).

Table 7. Summary of studies validating the 2010 histologic classification model

Study	Time	Patients (n)	Class* (n)	1 year Renal Survival (%)	5 year Renal Survival (%)	ESRD or death (n (%))
Berden A.(133)	2010	100	16/55/16/13	93/84/69/50	93/76/61/50	ESRD: 1(7)/11(25)/ 6(46)/7(70)
Chang DY.(233)	2012	121	33/53/24/11	100/73/83/29	93/60/72/29	ESRD: 3(9)/15(28)/ 4(17)/8(73)
Muso E.(237)	2013	87	40/7/26/14	100/86/96/35	100/86/96/29	NR
Ellis CL. (238)	2013	76	20/18/27/11	90/78/81/73	NR	NR
Hilhorst M.(239)	2013	164	81/43/39/1	NR	91/64/69/-	NR
Unlu M.(240)	2013	141	31/69/29/12	NR	NR	ESRD: 4(13)/20(29)/ 10(34)/8(67)
Iwakiri T. (241)	2013	102	46/32/18/6	NR	NR	ESRD: 2(4.3)/9(28)/ 8(44)/4(67)
Ford S. (242)	2014	120	34/33/33/20	NR	NR	ESRD or death: 11(32)/14(42)/13(39)/ 16(80)
Togashi M. (243)	2014	54	17/8/19/10	2/22/11/33	NR	ESRD: 2(4)/9(28)/ 8(44)/4(67)
Nohr E. (236)	2014	67	15/25/20/7	100/92/70/71.4	NR	ESRD or death 1y: 1(6.7)/5(20)/8(40)/

						4(57)
Noone D. (244)	2014	40	13/20/2/5	77/55**/0	NR	ESRD: 0(0)/9(41)**/ 5(100)
Tanna A. (234)	2015	104	23/26/48/7	100/74/85/50	100/74/77/25	NR

*Class: Focal/Crescentic/Mixed/Sclerotic

**Mixed and Crescentic combined

1 Aims of the study

The overall aim of this thesis was to explore the prognosis and estimate potential prognostic risk factors in patients with ANCA-GN. To investigate this, our objectives were as follows:

- Paper I: To study the temporal survival and to identify prognostic factors in a retrospective cohort of patients with ANCA-GN.
- Paper II: To investigate the prognostic impact of a histopathologic classification in patients with renal biopsy-verified ANCA-GN.
- Paper III: To explore the occurrence of malignancies in patients with ANCA-GN.

2 Study Design

3.1 Participants

Inclusion criteria

In all papers, eligible patients had a kidney biopsy with pauci-immune glomerulonephritis and a positive ANCA serology. The inclusion of patients or cases was limited to the time periods of 1988-2012, 1991-2012, and 1988-2012 in papers I, II, and III.

Excluded cases

All patients with missing data or unknown ANCA serology were excluded. In paper II, cases with ≤ 2 glomeruli in the biopsy were excluded. In paper III, cases with known cancer prior to renal biopsy were excluded.

Observation period

In papers I and II, patients were observed to progression of ESRD, death, or end of follow-up. In paper III, patients were followed to the occurrence of cancer, death, or end of follow-up.

3.2. Data collection and quality registries

National quality registries contribute to monitoring and improving health care services. Systematic collection of treatment and outcome data help to assess the quality of medical care and identify areas that need to be improved (245). The Norwegian Directorate of Health, based on the need for health care improvement in this patient group and data coverage, is responsible for deciding whether a registry can be approved as a national quality registry. Annual registry reports are evaluated to monitor the quality of the data and coverage.

In all papers, cases were retrieved from the *Norwegian Kidney Biopsy Registry* (NKBR), which was established in 1988 by *Norsk Nyremedisinsk Forening, at Haukeland University Hospital, Bergen.* The registry has national coverage and consists of over 13,000 biopsies (non-neoplastic). The biopsy coverage is estimated to be 90%. In addition to morphologic data, reported by a pathology department, it also contains clinical, biochemical, and immunologic information at the time of biopsy, reported by the local nephrologist/physician. All biopsy specimens were also examined by an experienced nephropathologist at *Haukeland University Hospital*. Patients have to fill out a consent form before data can be registered in the NKBR. The registry was certified as a quality registry in 2012. Information about individual therapy is not registered in the NKBR.

Information on renal replacement therapy (RRT) and transplantation were collected from the *Norwegian Renal Registry*. Located at Oslo University Hospital, Rikshospitalet, all patients receiving RRT are registered from 1980 to the present date, and are reported from renal units in Norway. Data is reported on patients receiving RRT for chronic renal failure, while treatment of acute renal failure is not reported unless it turns out to be an irreversible failure. It is estimated to be a complete reporting based on annual crosschecks.

Deaths were retrieved from the *Norwegian Population Registry* where all deceased are compulsorily registered from death-certificates according to their unique 11-digit identification number. Causes of deaths were retrieved from the Cause of Death Registry, Statistics Norway. The registry was established in 1951, and information of the causes of deaths is obtained by standardised form, filled out by a physician. In 2014, the completeness of the registry was approximately 98% (http://statistikkbank.fhi.no/dar/). In our study, we categorised cause of deaths into active vasculitis, infection, cardiovascular disease, malignancy, or other causes.

In paper III, malignancies were identified from the *Norwegian Cancer Registry (NCR)*. With the exception of basal cell carcinoma of the skin, the registry has a near complete registration, estimated to be 99% (246). Since 1953, the registration of malignancies has been mandatory, and information is gathered from clinical and pathological reports and death certificates. The registry contains data of annual gender-specific incidence rates of cancer categorised by organ system, reported at 5-year intervals, stratified by age and time period.

In paper I, a standardised email survey was sent out to hospitals to collect information regarding maintenance treatment preferences and the availability of ANCA serology.

3.3 Definition of end-points and predicting factors

In all of the papers, ESRD was defined as commencement of renal replacement therapy with chronic dialysis or transplantation. Nephrotic-range proteinuria and low serum albumin were classified as ≥ 3 grams per 24 hours and ≤ 30 grams per litre, respectively. Hypertension was defined as ≥ 140/90 mmHg.
ANCA subgroups were categorised as C-ANCA/PR3-ANCA or P-ANCA/MPO-ANCA.

In paper I, the cohort was divided into an early and a late group diagnosed before or after December 31st, 2002. Furthermore, patients were also categorised into age groups (< 60 years, 60-74.9 years and ≥ 75 years) and mean eGFR either < or ≥ 15 ml/min per 1.73 m^2.

In paper II, all biopsies were evaluated and classified according to the histopathologic classification model (133); ≥ 50% of visible glomeruli with global sclerosis was sclerotic class, ≥ 50% normal glomeruli was focal, ≥ 50% crescentic formation was crescentic class, and all other were classified as mixed. In addition, we merged crescentic and mixed classes in the sub-analysis. Separate analyses were performed for cases with 3-9 and ≥ 10 glomeruli.

In paper III, the cohort was categorised into NMSC and non-NMSC subgroups. In the non-NMSC group, person-year at risk was calculated up to the occurrence of other cancer type, end of follow-up, or death. In patients, who have undergone transplants, the person-year at risk started from the date of kidney transplantation.

3.4. Statistical methods

General

Statistical analyses were calculated using the Statistical Package for the Social Sciences; SPSS for Mac and Windows, version 22 and 23, and STATA.

Continuous and categorical variables were compared with Mann-Whitney *U*-test, Chi square test (X^2), or Fisher's exact test. A two-tailed p-value of ≤ 0.05 and a confidence interval of 95% were considered statistically significant.

Mortality analysis

Comparisons of survival rates between study groups were calculated with the Kaplan-Meier method and the log-rank test. In paper I, a standardised mortality ratio (SMR) was calculated to compare the study cohort with the general population. The SMR was defined as the ratio between expected and observed deaths in the study population. The

expected number of deaths was the sum of the mortality risk in each person-year of follow-up in an age and gender matched general population, retrieved from Statistics Norway.

Standardised incidence ratio

In the last paper (III), a SIR was calculated to compare the occurrence of malignancy in the study population to a matched general population. The expected number of cancers is the sum of the risk in each person-year of follow-up of an age- and gender-matched general population. The confidence interval was calculated by assuming Poisson distribution of cancer frequency. Furthermore, subanalyses for the SIRs were calculated for time after diagnosis, gender, ANCA specificity, and kidney transplantation. In addition, a pooled-analysis was conducted by estimating expected and observed cancers from recent studies describing patients diagnosed with AAV after 1988 (227-229, 247), including results given in paper III.

Regression models

A Cox regression model was used in multivariate analysis to calculate the hazard ratio (HR) after adjustments for known confounders in all papers. In paper II, a logistic regression analysis was utilised to create a clinical classification model containing eGFR, age, gender, and ANCA subtype. Additionally, a separate model was calculated with combined clinical and pathologic parameters.

ROC Curve

In paper II, a receiver operator characteristic (ROC) curve was calculated to test the performance of the histopathologic classification scheme in predicting development of ESRD during the first year after diagnosis. The curve reflects the ratio of true positive and false positive rates. Calculation of the area under the curve (AUC) will range from 0.0 to 1.0, where 1.0 reflects a perfect predictor and 0.5 is equivalent to random

guessing. AUC was calculated first for the histologic model, and then for a clinical model with age, sex, ANCA subtype and eGFR. Lastly, AUC was calculated for a merged model including both histologic and clinical parameters.

3.5 Ethics

The studies were approved by the Regional Committee for Medical and Health Research Ethics (REC South-East, 2013/1083).

4 Results

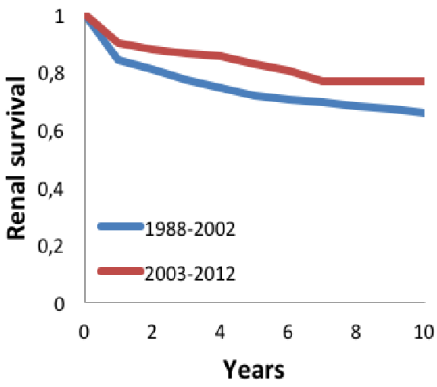
4.1 Paper I – The Prognosis in Patients with ANCA-GN

The cohort of 455 patients with ANCA-GN was followed for a mean 6.0 years (range, 0-23.4 years). During follow-up, 124 (27%) progressed to ESRD, 69 (15%) within the first year after diagnosis. Cumulative ESRD-free survival at 5, 10, and 15 years was 75%, 68%, and 58%, respectively. A total of 165 patients died during follow-up, 75 within the first year after diagnosis. The most common causes of deaths were cardiovascular disease (n = 58), infection (n = 43), and active vasculitis (n = 31).

Compared to the general population, there was a 2.8 fold increased risk of death in the study cohort, and a 10.8 fold higher mortality rate within the first year. In multivariate analysis, independent risk factors for ESRD were initial eGFR < 15 ml/min per 1.73 m^2 (HR 5.1, 95% CI; 3.5-7.4), male gender (HR 2.1, 1.4-3.1) P/MPO-ANCA (HR 1.8, 1.2-2.6), proteinuria ≥ 3 gram per 24 hours (HR 1.7, 1.1-2.4), and blood pressure ≥ 140/90 mmHg (1.9, 1.2-2.9). Independent risk factors for death within the first year of follow-up were eGFR <15 ml/min per 1.73 m^2 (HR 2.2, 95% CI; 1.4-3.5), age group 60-74.9 (HR 4.0, 1.8-8.1), and ≥ 75 years (HR 8.4, 3.8-18.6).

When comparing the time periods of 1988-2002 and 2003-2012, baseline characteristics show that the latter study group was significantly older (57.6 versus 61.5 years, p = 0.02), higher percentage of P-ANCA positive patients (76 (35%) versus 121 (51%), p < 0.001), lower rate of proteinuria at diagnosis (2.1 versus 1.6 gram per 24 hours, p = 0.01), and higher mean eGFR at diagnosis (27 versus 37 ml/min per 1.73 m^2, p < 0.001). Patients diagnosed before 2003 had a significantly higher risk of developing ESRD compared to those diagnosed between 2003 and 2012, but the result was not significant after adjustment for eGFR. Mortality within the

first year and in the age group ≥ 60 years old was significantly higher in 1988-2002 compared to the later period.



Cox Regression Model for ESRD

	N	Events	Adjusted HR
2003-2012	237	44	1.0
1988-2002	218	80	1.2 (0.8-1.7)

Figure 4. Kaplan-Meier plot showing renal survival in 455 patients with ANCA-GN categorised by the early (1988-2002) and late (2003-2012) study periods, p = 0.03.

Multiple Cox regression analysis comparing the risk of ESRD during total follow-up between the early and late study group in patients with ANCA-GN. HR (95% confidence interval), adjusted for age, gender, ANCA, and eGFR

4.2 Paper II – A Histopathologic classification of ANCA-GN

In this study, 250 cases with ≥ 10 glomeruli in the renal biopsy specimen were included and classified according to the histopathologic model into the categories focal (38%), mixed (24%), crescentic (28%), and sclerotic (9%). After a median follow-up time of 3.5 years, the primary end-point, ESRD, had occurred in 60 patients (24%).

The cumulative proportions of renal survival at 1 and 5 years were 96% and 90%, 86% and 75%, 81% and 69%, 56% and 51% for the focal, mixed, crescentic, and sclerotic class, respectively ($p < 0.01$). In multivariate analysis, the sclerotic class had a significantly worse prognosis than the focal (HR 9.65; 95% CI, 2.4-39.2) and mixed/crescentic classes (HR 3.3; 95% CI, 1.4-7.6), respectively. Interestingly, results were only significant within the first year after biopsy. No significant difference in mortality was observed between the histologic classes.

In ROC analysis, the AUC was calculated to be 0.72 (95% CI, 0.7-0.8), 0.79 (95% CI, 0.7-0.9), and 0.79 (95% CI, 0.7-0.9) for the histologic, clinical, and the combined classification model, respectively. Equivalent predictive results were demonstrated when applying the model in cases with 3-9 glomeruli.

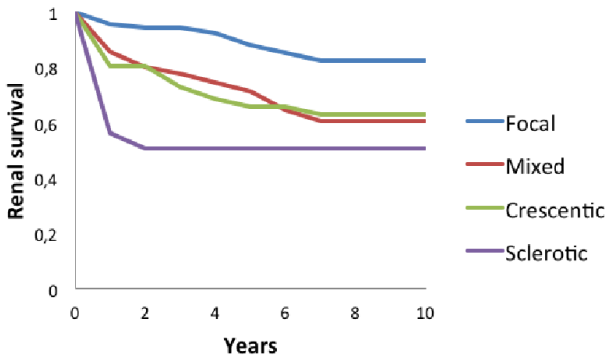


Figure 5. Kaplan-Meier plot showing the cumulative risk of developing end-stage renal disease in 250 patients with ANCA-GN, categorised according to the histopathological classification model (p < 0.001).

4.3 Paper III – The Risk of Cancer in Patients with ANCA-GN

Four hundred and nineteen patients were followed for a median length of 5.7 years (interquartile range, 2.8 to 11.3). Forty-one patients developed 46 cancers during a total of 3010 person years at risk. Patients who developed cancer were older (65 versus 61; P = 0.04), predominantly males (71% versus 53%; P = 0.03), and had higher overall mortality (25 (61%) versus 123 (33%); P < 0.001).

SIR for all cancers and non-NMSC was 1.09 (95% CI, 0.8 to 1.5) and 0.96 (95% CI, 0.7 to 1.3), respectively. The occurrence of cancer was highest 1 to 5 years after renal biopsy, with a SIR of 1.53 (95% CI, 1.0 to 2.3). The risk of cancer was significantly increased in NMSC, haematologic malignancies (two cases of chronic lymphocytic leukaemia and two cases of myelodysplastic syndrome), and among patients, who had undergone renal transplants, with a

SIR of 3.40 (95% CI, 1.6 to 7.1), 3.52 (95% CI, 1.3 to 9.4), and 2.12 (95% CI, 1.0 to 4.4), respectively. No significant risk increase was observed in subanalyses of gender and ANCA specificity.

The risk of cancer was assessed in a pooled-analysis of 1532 patients with 236 cancers. During 8801 patient years at risk, SIR was estimated to 1.72 (95% CI, 1.5 to 2.0) for all cancers and 1.21 (95% CI, 1.0 to 1.5) for non-NMSC, respectively.

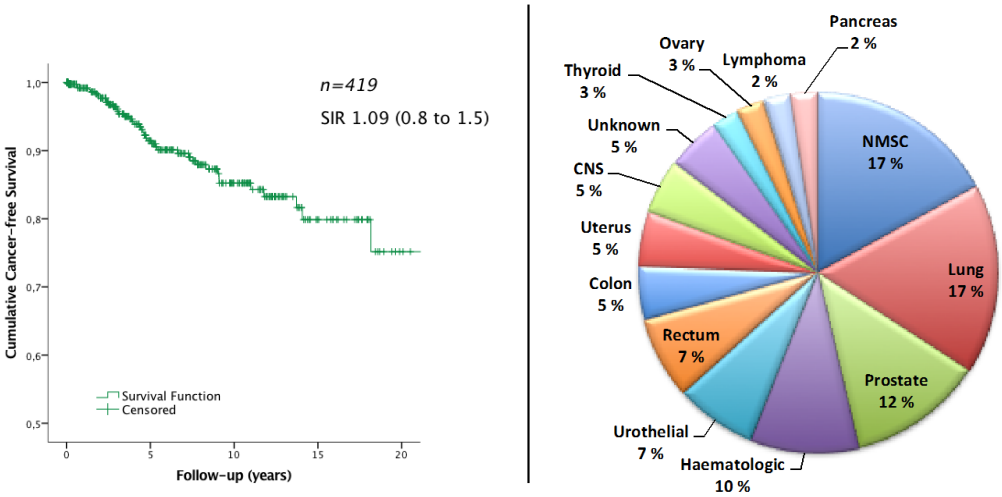


Figure 6. (Left) Kaplan Meier plot showing the cumulative cancer-free survival for 419 patients with ANCA-GN. (Right) Primary cancer occurrence in patients with ANCA-GN. NMSC (7 cases), lung (7), prostate (5), haematologic (4), urinary bladder and ureter (3), rectum (3), colon (2), uterus (2), central nervous system (2), unknown primary site (2), thyroid (1), ovary (1), lymphoma (1), and pancreas (1).

5 Discussion

5.5 Paper I - The Prognosis in Patients with ANCA-GN

AAV is currently a chronic disease in which patients survival relies on immunosuppressive therapy. Quality longitudinal data is pivotal in monitoring long-term morbidity and mortality in this patient group. Although previous studies have indicated that patients with AAV live longer with the disease today, poorly standardised definitions, lack of hard end-points and a representative study cohort, have hampered the interpretation of the study results. Whether or not the changes in therapy over the last 5 to 6 decades have had a favourable impact on prognosis needs to be elucidated further in an unselected population. One of the aims of the NKBR is to evaluate the quality of medical care by studying the effect of diagnostics and therapy on temporal outcome.

In this biopsy-verified retrospective population-based cohort, we demonstrated an improved temporal prognosis in both patient and renal survival in patients with ANCA-GN. Short-term mortality was 61% higher in patients diagnosed between 1988 and 2002 compared to those between 2003 and 2012. Moreover, in elderly patients, there was a 2-fold increase in risk of death. This reflects both the advances in immunosuppressive treatment during the last few decades and the introduction of a rapid tool, ANCA serology, in the late 1980s. In addition, we postulate that increased awareness among clinicians has resulted in earlier detection and initiation of therapy, before irreversible organ damage occurs. This is demonstrated by our observation, that, after adjustment for baseline renal function, there is no significant change in renal prognosis between the different time periods. Therefore, baseline renal function might act as a marker of time to disease detection. A German single centre study by Holle *et al*. also demonstrated a trend towards earlier diagnosis; the interval between occurrences of

symptoms and diagnosis was reduced from 8 to 4 months during the last 4 decades (208).

Despite the progress in therapy, mortality still remains high. In our cohort, during the 1-year and long-term follow-up, the survival rate was estimated to be 16% and 37%, respectively. This trend is in agreement with reports from other observational studies (3, 182, 183, 188, 191-193, 208, 248). In our cohort, we observed a 2.8-fold higher mortality rate compared to the general population. The risk was substantially higher during the first year (SMR 10.8) after diagnosis, especially for older patients with impaired renal function. In subsequent years, the mortality ratio increased 1.7-fold compared to the general population. Our result correlates well with reports from clinical trials, which had an overall and subsequent mortality ratio of 2.6 and 1.3, respectively (3). This despite the fact that elderly patients and cases with lung haemorrhage were not enrolled in clinical trials. In addition, in a British case-control study of patients with WG, there was a 9-fold excess mortality during the first year after diagnosis compared to matched controls, while it was more comparable in subsequent years (198). Hence, it can be assumed that current national therapy regimens and follow-up protocols are up to the standard of international recommendations.

The most common causes of death within the first year after diagnosis are treatment and disease-related complications, which highlights the need to consider the risks and benefits of therapy in this patient group. During long-term follow-up, the most frequent cause of death in patients without ESRD was related to cardiovascular diseases, whereas patients who developed ESRD died due to infections. These observations underscore the negative impact of both the systemic vascular damage of AAV and the adverse effects of cumulative immunosuppression with CYC and corticosteroids.

The cumulative risk of ESRD at 1, 5, and 10 years was 16%, 25%, and 32%, respectively. Importantly, the cumulative 1-year risk of ESRD fell from 19% to 13% from 1988-2002 to 2003-2012, respectively. A partial explanation might be that patients are diagnosed at an earlier stage of disease course, with better baseline renal function (27 versus 37 ml/min per 1.73 m^2 in 1988-2002 and 2003-2012, respectively). The trend towards declining ESRD has also been described in other European cohorts (182, 208).

In our cohort, older age and low baseline eGFR were negative predictors of both death and ESRD. These patients, in particular, are susceptible to treatment-related toxicity and severe infections (3). Similar to these findings, Luqmani *et al.* (198) showed that during 5 years of follow-up, a 20% mortality rate was observed in 28 patients with renal GPA compared to no mortality in 22 patients with nonrenal GPA. Male patients had a higher risk of developing ESRD, but no significant sex difference was observed in mortality. In contrast, Holle and colleagues reported, in a historic single centre cohort of patients with WG, an almost 2-fold higher death ratio for men compared to the general population, while the risk was not significantly different for females (208). P-ANCA/MPO-ANCA positivity was a negative predictor of ESRD, which is associated with more advanced renal damage with irreversible scarring (53, 132, 188, 202). Interestingly, ANCA specificity had no predictive value on mortality in our cohort. This observation is supported by some studies (3, 53), but contradicted by others (249, 250). The reported discrepancy in the literature is probably due to the different composition of ANCA subtypes between the geographical areas, case inclusion from single or specialised centres that might result in selection bias, unadjusted mortality rates, or classification bias of ANCA specificity. Lastly, the percentage of patients with renal manifestations might vary between studies.

5.2. Paper II - A Histopathologic Classification of ANCA-GN

There are few established biomarkers in AAV, and repeated invasive biopsies are not desirable. Traditionally, biopsies were associated with inconsistent findings and had poor reliability and validity (251, 252). In 2010, a landmark study successfully predicted long-term ESRD by developing a histopathologic classification model (133). However, this model needs to be validated in other cohorts before it can be implemented in routine practice. A good prediction model, based on a baseline biopsy specimen, could aid the choice of therapy and diminish the need for additional biopsies. The NKBR contains one of the world's largest biobanks of kidney biopsies. Based on this registry, in the present validation study, we classified 250 biopsies according to the histopathologic classification model, as proposed by Berden and colleagues (133).

Our results show that this model is a moderate predictor of ESRD, particularly of the first year after baseline biopsy. Renal survival was best in the focal phenotype, with well-preserved renal function over time. The sclerotic class had the worst renal outcome, with almost 50% of cases progressing to ESRD within 5 years of follow-up, reflecting the severe irreversible scarring of the glomeruli. Patients in the sclerotic group are less likely to receive plasmapheresis, prednisone, and/or CYC (236), and have a poorer response to treatment (253). Nevertheless, our data shows that 50% of them regain renal function, and they should therefore receive induction therapy with immunosuppression. The mixed class had a slightly better prognosis than the crescentic class, with a cumulative 5-year renal survival of 75% and 69%, respectively. This is in contrast to reports by Berden *et al.* where 5-year survival was estimated to be 61% and 76% for these subgroups (133). A Japanese group has also observed a superior outcome in the crescentic category compared to the mixed class (241). However, the proportion of normal and sclerotic glomeruli was higher in the mixed than the crescentic class, which makes the discrepancy difficult to interpret. A likely explanation for the conflicting results in the literature between the mixed and

the crescentic class might be a difference in the percentage of normal glomeruli in the different classes between the studies, different study populations or therapy, average inter-rater reliability (242), or the lack of inclusion of tubulointerstitial changes (235). Nonetheless, most validation studies demonstrate similar eGFR and renal survival in patients with crescentic and mixed class biopsies (233, 234, 236-240, 242-244, 253). The mixed category might be too heterogeneous as a class to have a prognostic value. Thus, we suggest merging the mixed and crescentic classes to better utilise the classification model in clinical settings.

So far, four of the validation studies included more than 100 cases in the study cohort (133, 233, 234, 239). Merging the cumulative ESRD-free survival rate yielded a 1-year risk of 2%, 21%, 23%, and 57% for the phenotypes of focal, mixed, crescentic, and sclerotic, respectively. These estimates are equivalent to survival data observed in our study, which supports the predictive value of the histopathological model of renal outcome in patients with ANCA-GN.

In multivariate analysis, independent of baseline renal function, the sclerotic phenotype had a 9- and 3-fold higher risk of developing ESRD than the focal and mixed/crescentic classes, respectively. No difference in survival was observed between focal and mixed/crescentic classes, which highlights the negative impact of a low baseline eGFR. At the time of biopsy, renal function was significantly higher in patients with the focal phenotype compared to mixed and crescentic (54, 27, and 18 ml/min per 1.73 m^2; $p < 0.001$). A previous Norwegian report of patients with WG by Aasarød and colleagues has also described that baseline serum creatinine correlates well with the percentage of glomeruli with crescents and necrosis in the biopsy specimen (131). Other studies also showed a correlation between baseline renal function and the percentage of normal glomeruli (133, 233, 238).

The classification model assessment with ROC statistics showed that the histologic classes were of moderate quality for predicting renal outcome, with an estimated AUC of 0.72. In comparison, clinical parameters (age, gender, ANCA serology, and eGFR) yielded a higher AUC of 0.79, which strengthens the known prognostic value of age and baseline eGFR in long-term renal survival. Combining the two variables did not improve the model fit, with an AUC of 0.79. One should, however, interpret these results with caution, as the study sample is relatively small and other parameters, such as tubulointerstitial changes. The number of normal glomeruli and treatment were not incorporated in the models.

In their original paper, Berden et *al*. (133) stated that at least 10 glomeruli should be present in the biopsy specimen for adequate classification. However, in this paper, we have shown an equivalent prognostic value of the model in the excluded group of biopsies with 3-9 glomeruli. Ford (242) and Unlu (240) have also validated the model in their cohort of 120 and 141 biopsies with no minimum requirement for number of glomeruli required.

In conclusion, renal survival is best in patients with biopsies with the presence of the acute phenotypes of focal, mixed, and crescentic, and worst in sclerotic lesions. The ascending category order of focal, mixed, crescentic, and sclerotic class corresponded to the risk of ESRD at baseline, at 1- and at 5-year follow-up. However, assessing the performance of the histopathological classification model showed only an average predictive accuracy, and did not improve model fit compared to established clinical predictors. In the future, pathologists should perhaps report the biopsy findings with a breakdown into phenotypes of focal, sclerotic, or mixed/crescentic category with the percentage of normal glomeruli.

5.3. Paper III - The Risk of Cancer in Patients with ANCA-GN

Treatment-related malignancy due to heavy exposure to cyclophosphamide has previously been described in several studies. There is a well-known dose- and duration-dependent cancer risk associated with CYC, which is the first line therapy for AAV. In paper I, we showed that malignancy was a common cause of death in patients with ANCA-GN. We illustrated this further in paper III, where we investigated the occurrence of cancer in a nationwide cohort of patients with ANCA-GN. We demonstrated that during 3010 person years at risk, the occurrence of malignancy was 9% higher in this patient group compared to a matched general population, although a non-significant result (139, 155, 226).

Our finding is discrepant to previous studies on this topic (97, 101, 226-229, 232, 247); however, it is comparable with a German study of 445 patients with WG that showed an overall SIR of 0.8 (208). The discrepancy between our result and other studies probably reflects the fact that previous reports consisted of an older study population, which does not represent contemporary heterogeneous population receiving therapy. The landmark clinical trials on CYC-sparing regimens were published during the last 15 years (118-120, 158). In addition, several studies are limited by a relatively small study sample with short observation periods (101, 226, 227, 254). Another reason for the inconsistency might be that basal cell carcinoma of the skin is not registered in the NCR, and with squamous cell carcinomas, only one case per patient is included in the SIR calculations. Consequently, this methodological practice has probably underestimated the overall quantitative role of skin cancer in our study. However, most recent studies were from either tertiary centres (227, 229, 247) or included patients enrolled in clinical trials (228), limiting the generalizability of the results. Conversely, our study consists of an unselected nationwide population of patients with ANCA-GN, which limits the potential selection bias of the study cohort.

Pooling the SIR data from recent cohort studies on malignancy in AAV showed that the risk was 1.72-fold increased for all cancers. This finding is

comparable with the 1.74-fold risk increase shown in a recent meta-analysis of patients with AAV. However, this meta-analysis included a heterogeneous group of studies with a wide follow-up period ranging from 1966 to 2008 (255).

The borderline increase in overall SIR in our study was attributed to a high incidence of NMSC, which is associated with a significantly increased malignancy rate, compared to the general population. After exclusion of all cases with NMSC, the SIR decreased from 1.09 to 0.96. This finding confirms the results from studies by Heijl (228), Rahmattulla (229), and van Daalen (247), who showed that the overall risk of cancer was solely attributed to NMSC. However, in our pooled analysis of patients with non-NMSC, there was a 1.21-fold significant risk increase compared to the general population. NMSC occurs frequently in patients receiving immunosuppressive therapy and is responsible for over 90% of all skin cancers in patients, who have undergone transplants (230). NMSC is particularly associated with exposure to the immunosuppressive drug, azathioprine (101, 228, 229, 231, 256). The increased rate of NMSC was also observed in our study and vigilant dermatologic screening is advised in this patient group as part of routine follow-up. Novel biologic treatment with rituximab might have a protective effect in the development of NMSC, but this observation needs to be elucidated further (247).

Although there were many cases of haematologic malignancies in our cohort, none were acute myeloid leukaemia, which is strongly associated with CYC (226-228). Thirty percent of patients with myelodysplastic syndromes do, however, develop acute myeloid leukaemia (257). However, to our knowledge, no study has shown an association between AAV and chronic lymphocytic leukaemia. Although, there was a high occurrence of haematologic cancers in our cohort, which are known to occur with long latency (258), the majority of malignancies in our cohort occurred 1-5 years

after diagnosis. Interestingly, there was no increased risk of lymphomas or bladder cancer in our cohort. These malignancies are traditionally associated with patients with ANCA-GN and CYC (101, 138, 221, 222, 232). In contrast, previous studies showed a 2.4- to 33- fold increased risk of developing bladder cancer and a 1.1- to 11- fold higher risk of developing lymphomas (97, 101, 226, 228, 232). Nevertheless, recent groups have shown that the cancer risk was only observed in NMSC (229, 247). Our findings confirm the findings from these studies that contemporary treatment regimens are relatively safe in regards to urothelial cancers.

In subgroup analysis, we showed that there was no increased risk for cancer in patients with positive C-ANCA/PR3-ANCA compared to the general population. This is in contrast to the increased malignancy risk in patients with GPA/PR3-ANCA, described by Rahmattulla *et al.* (229). The C-ANCA/PR3-ANCA positive subgroup of patients is susceptible to frequent relapses and requires higher cumulative doses of CYC compared to patients with positive P-ANCA (161, 229). Furthermore, some studies suggest that they also have a more favourable overall survival rate compared to the latter ANCA subgroup (249, 250), which might increase the temporal risk of developing cancer. However, survival statistics demonstrated that there was no difference in mortality between the ANCA groups in our cohort ($p = 0.57$). Another explanation for our finding could be that the rate of relapses and exposure to CYC could have been more comparable between the two groups.

In conclusion, the result of this study shows that current treatment regimens with CYC in patients with ANCA-GN are not associated with an increased overall malignancy risk. We observed, however, an increased number of cases of NMSC and haematologic malignancies, which emphasises the judicious practice of immunosuppressive therapy in this patient group. Larger study samples with longer follow-up are warranted to

further investigate the long-term risk of cancer. Meanwhile, frequent follow-ups and preventive measures against heavy sun exposure are advised.

5.4. Methodological considerations and limitations

The patient sample

Selection bias

A selection bias emerges from the lack of comparability between the groups being studied. The renal biopsies in the registry are referred from all regions of Norway, and therefore are representative of ANCA-GN in Norway. However, this limits the external validity of the study since both disease phenotype and outcome are known to vary across other regions of the world. Furthermore, only patients with renal disease were included in our studies, with scarce information of other organ manifestations, which in turn reduces the possibility of generalising the results to patients, who do not have renal disease. Cases with renal failure where a biopsy was contraindicated are not reported. This might lead to an underrepresentation of patient subgroups, possibly, with poorer outcome. Furthermore, patients had to fill out a consent form before the data could be sent to the NKBR. Information on patients who did not fill out the consent form is unknown due to Norwegian privacy legislation. However, all biopsies have been examined and reported to the NKBR by the pathology departments (*p-skjema*) independent of clinical parameters (*k-skjema*). Hence, biopsies with missing consent forms could be identified and obtained when possible.

Nevertheless, this is a national population-based quality registry that reduces the risk for selection bias to a minimum. In comparison, most studies on ANCA-GN are clinical trial-based or from specialised referral centres. Majority of patients with AAV develop renal manifestations (90%) and a biopsy is considered as the gold standard in the diagnosis of AAV. Thus, it is probable that a renal biopsy-based cohort includes the majority of patients with AAV (259).

Sample size

Due to the rare nature of the disease, the sample size is relatively small, which might hinder adequate validity of the results. This particularly

affects long-term follow up where it becomes increasingly difficult to detect a statistically significant difference due to fewer cases reaching an end point. Nevertheless, the overall result in all three papers is consistent with previous studies of this patient population, which underscores the reliability of our findings.

Referral bias

The NKBR has national coverage and gets reports from all hospitals with a nephrology unit (25 hospitals). This reduces any chance of referral bias to a minimum.

Case definition, case capture, and case ascertainment

An epidemiologic study of a rare disease, such as ANCA-GN, can pose certain challenges that need to be addressed. First, the classification of vasculitis has been evolving over the last few decades, and distinction between similar diseases based on phenotypical characteristics might not always be straightforward. Second, a large population is necessary to include a representative amount of cases within a reasonable time frame. Finally, hospital-based case ascertainment is appropriate since most patients with this condition are being treated in secondary care institutions. In our study, we differentiated our cases from other diseases with serologic and histologic markers, which are distinct for ANCA-GN. However, this excludes patients, who are ANCA negative (10% of AAV) and patients without a biopsy. Nevertheless, the inclusion of patients from a population-based registry ensures adequate and representative case capture. The registry is based on information from secondary care units where most of these patients are treated in Norway.

Data collection

Information bias

All papers had a retrospective design; nevertheless, baseline data were registered at the time of the biopsy in NKBR with a standardised form (*"k-skjema" and "p-skjema"*). However, misclassification or measurement errors cannot be ruled out when obtaining clinical, biochemical, or immunologic data. Follow-up data, such as regarding ESRD, malignancy, and death were acquired from national quality registries and patients with missing or unknown data were excluded from the study cohort.

Confounder

A confounder is a third variable that is associated with the exposure and is blurring the effect of exposure on the outcome that is being studied (260), In study I and III, the cohort was compared to an age- and gender-matched general population, which controls for any confounding effect of these demographic variables. Furthermore, multivariate analyses were calculated in all three studies where adjustments were made for variables other than the studied predictor. Nevertheless, information about comorbidities, such as other renal diseases, for example, renal complications of diabetes mellitus, lupus nephritis, anti-glomerular basement membrane disease, or exposure to environmental toxins, such as smoking, are not available in the NKBR, and therefore are not adjusted for in our calculations. In addition, no adjustments for treatment, temporal improvement in healthcare, or time from symptoms to biopsy, are directly adjusted for in the regression models. However, in paper I, by stratifying the cohort into time period and baseline renal function, we showed a longitudinal improvement in survival.

Other epidemiologic fallacies, which we should be aware of, are lead-time bias and length-biased sampling. Lead-time bias occurs when patient survival is prolonged after implementation of a screening program. Moreover, patients are diagnosed earlier and live longer with their diagnosis, resulting in length-biased sampling. These limitations are unlikely in our study due to the rapid course of ANCA-GN, with weeks to months from debut of symptoms to

renal failure. Any potential lead-time cannot explain several years of improved long-term survival. Introduction of ANCA serology could have detected patients with localised or indolent disease, thereby showing a spurious improvement in overall outcome. Nevertheless, several studies, including ours, have also shown an improved survival in patients presenting with poor renal function (188, 191).

Paper I

To determine the availability of ANCA serology and practice of therapy, an email survey was sent out to all renal units reporting to the NKBR in Norway. Despite being standardised with a high response rate, recall bias is an inherent weakness of questionnaires. Nevertheless, information gathered with the survey was not used in any analyses, but had a more descriptive purpose since these data were not obtained in the NKBR.

Paper II

Although our study found that the histopathologic model was an independent predictor in multivariate analysis, it was only of average quality in predicting ESRD. However, we cannot exclude the prognostic importance of other histologic features, such as tubular atrophy or interstitial fibrosis, since these parameters were not included in the standard model. Also, the treatment the patients were exposed to prior to biopsy, at baseline and under follow-up, is missing in the registry and could not be corrected for in our analyses. Nonetheless, our study does provide information on prognosis by histologic class in the context of current therapy of ANCA-GN. To investigate this further, a large international validation study is currently being performed.

There might also be a problem with lack of inter- and intra-rater reliability, since only one pathologist reviewed and classified the biopsies. However, all histopathologic data in the NKBR has been examined and reported by a local pathologist (*"p-skjema"*), and re-examined by the

nephropathologist at the NKBR at Haukeland University Hospital. Also, in a previous study on renal histopathology in systemic vasculitis, good inter- and intra-observer agreement was found among four experienced nephropathologists when reviewing quantitative data (percentage of normal, crescentic, and sclerotic glomeruli) (261). A recent validation study by Ford *et al.* (242) showed good levels of agreement for classifying the sclerotic class among three pathologists, but only moderate agreement for classifying the remaining classes in the histopathologic classification model. Nonetheless, a non-differential misclassification tends to obscure the observed difference between the groups studied. Despite this, our finding shows that there is a significant difference in prognosis based on the histopathologic classes, after adjusting for potential confounders.

Paper III

The NCR obtains both clinical and pathological data from all health regions in Norway. However, the fact that basal cell carcinoma of the skin is not compulsorily reported in the NCR is a limitation. This underrepresentation is adjusted for in the paper by calculating the risk of cancer in patients with non-NMSC.

Statistics

Paper I

SMR was calculated in mortality analyses to compare our cohort with a matched general population. The expected number of deaths were retrieved and calculated from Statistics Norway. Meanwhile, the observed number of deaths was obtained from the Norwegian Population Registry. Despite that the retrieval of these two estimates comes from different sources, which might give a spurious mortality ratio, the death certificate filled out by the physician is the basis for the data in both registries. A potential reporting bias will affect the estimates evenly and not alter the mortality ratio.

A limitation of SMR statistics is that they have to be interpreted with caution when the sample size is small and there are very few deaths to compare between the groups studied. Another limitation is that the SMR cannot be used to compare different geographical areas, since the population mortality profile might vary between areas. Nevertheless, the SMR calculated in our study was based on national registries and reflects the Norwegian population. Also, a high prevalence of the disease itself in the general population could result in an underestimation of the true relative risk with a spurious low SMR (262).

Paper II

The ROC curve statistics are useful for measuring the accuracy of a diagnostic test. The AUC is the combined measure of how well the test predicts true end-points, in this case ESRD. An advantage with this method is that ROC plots are independent of prevalence of the disease. However, their value in clinical decision-making can be argued (263). Other studies have used continuous outcomes instead of dichotomous outcomes, and thereby also use alternative performance measures (multivariate linear regression models).

Paper III

Both the expected and the observed number of cancers in the SIR estimate are collected and reported in the same manner in the NCR. This ensures that there is no biased cumulative estimate of malignancies in either group, which could give a false SIR estimate. In accordance with the statistical practice of the NCR, only the primary occurring cancers were included in SIR analysis. This might lead to underrepresentation when comparing the results to other countries. However, the Netherlands Cancer Registry also applies this statistical methodology (264).

Excluded cases

Paper II

To limit potential selection bias of the study cohort with ≥ 10 glomeruli (250 patients), we compared it with the group with 3-9 glomeruli (108 patients), and excluded cases with < 3 glomeruli (87 patients). A Cox regression model was utilised to compare the hazard ratios of ESRD between the groups when adjusting for age, gender, ANCA subtype, and baseline eGFR. In comparison of the group with ≥ 10 glomeruli versus the group with 3-9 glomeruli, we also adjusted for histologic classification. There was no significant difference between the groups in this multivariate analysis on ESRD. We can therefore conclude that the risk of selection bias between the studied and the excluded group is benign.

Paper III

Thirty-four patients had known cancer prior to renal biopsy and were therefore excluded from the study cohort. There might be an association with malignancy prior to AAV development, however, reports have yielded inconsistent results (225). Rahmattulla and colleagues have also showed that the risk of malignancy is comparable when comparing patients with and without a history of cancer prior to the AAV diagnosis (229). A recent group also found no support for the hypothesis that cancer might trigger AAV (264).

6 Conclusions

Paper I - The prognosis in patients with ANCA-GN

- Both patient and renal survival has improved significantly in patients with ANCA-GN from 1988 through 2012.
- Improved immunosuppressive therapy, shorter diagnostic delay due to ANCA serology, and more awareness among clinicians are important causal mechanisms of this trend.
- Mortality is still 3-fold higher compared to the general population with current treatment practices, particularly during the first year after diagnosis.
- Most common causes of deaths are related to disease and adverse effects of therapy.
- Independent negative predictors for short-term mortality were baseline eGFR < 15 ml/min per 1.73 m^2, older age (> 60 years), and serum albumin < 30 g/L.
- Independent risk factors for long-term ESRD were male sex and positive P-ANCA serology.

Paper II - Estimating the predictive value of a histopathologic classification model in patients with ANCA-GN

- The histopathologic classification model purposed by Berden *et al.* (133) significantly predicts renal survival.
- The sclerotic class had a significantly worse renal outcome compared to the focal and mixed/crescentic classes.
- There was no significant difference between the mixed and crescentic class on outcome. We therefore suggest that merging these two classes might simplify the future classification scheme.

- ROC statistics demonstrated that this histopathologic classification model is of moderate quality in predicting ESRD and not superior to clinical parameters.
- We have also validated the classification model in cases with 3-9 glomeruli in the biopsy specimen.

Paper III – Incidence of malignancy in ANCA-GN patients

- The risk of developing malignancy was not significantly increased in a Norwegian cohort of patients with ANCA-GN compared to the general population. This finding reflects current immunosuppressive therapy, with more restrictive use of CYC compared to previous times.
- There was a higher occurrence of NMSC and haematologic cancers in site-specific malignancies. The risk of bladder cancer was not increased.
- NMSC was the most prevalent cancer form and a major contributor to the observed cancer prevalence.

7 Future perspectives

- There is an increased risk of death in patients with ANCA-GN compared to the general population and particularly associated with toxic immunosuppressive drugs. Future prospective studies are pivotal to optimise treatment protocols, more specifically in older patients with severe organ damage. Tailoring treatment and prudent clinical monitoring are necessary to further reduce the disease burden in this patient group.
- We have validated the histopathologic classification model suggested by a consortium of international pathologists. However, our study was not able to detect any significant distinction between the mixed and the crescentic classes. Moreover, neither tubulointerstitial parameters nor percentage of normal glomeruli were included in our calculations as possible predictors of ESRD. Lastly, due to lack of treatment data in the NKBR, no adjustment was made with this regard. To address this further, a large and international prospective validation study is warranted.
- We did not observe a significantly increased risk of cancer in our cohort of patients with ANCA-GN. Nevertheless, there was still a 3-fold higher occurrence of NMSC and haematologic malignancies compared to the general population. Moreover, some cancer forms can develop after a long latent period. Larger studies with longer follow-up are therefore necessary to gather sufficient capabilities to detect late-occurring cancer cases. In addition, the effect of novel treatment protocols on the malignancy risk needs to be investigated in detail. Meanwhile, continued efforts in the search for safer and alternative therapies for ANCA-GN are warranted.

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Original Article

Improved prognosis in Norwegian patients with glomerulonephritis associated with anti-neutrophil cytoplasmic antibodies

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ABSTRACT

Background. Glomerulonephritis associated with anti-neutrophil cytoplasmic antibodies (ANCA) is associated with increased mortality and a high risk of end-stage renal disease (ESRD). Here, we investigated whether the prognosis has improved over the last 25 years.

Methods. Patients were identified in the Norwegian Kidney Biopsy Registry. We included all patients with pauci-immune crescentic glomerulonephritis and a positive ANCA test from 1988 to 2012. Deaths and ESRD in the cohort were identified through record linkage with the Norwegian Population Registry (deaths) and the Norwegian Renal Registry (ESRD). Outcomes of patients diagnosed in 1988–2002 were compared with outcomes of patients diagnosed in 2003–12.

Results. A cohort of 455 patients with ANCA-associated glomerulonephritis was identified. The mean follow-up was 6.0 years (range, 0.0–23.4). During the study period, 165 (36%) patients died and 124 (27%) progressed to ESRD. Compared with patients diagnosed in 1988–2002, those diagnosed in 2003–12 had higher mean initial estimated glomerular filtration rates (37 versus 27 mL/min/1.73 m^2) and lower risk of ESRD (1-year risk: 13 versus 19%; 10-year risk: 26 versus 37%). The composite endpoint, ESRD or death within 0–1 year after diagnosis, was reduced from 34 to 25%. In patients over 60 years old, 1-year mortality fell from 33 to 20%.

Conclusions. In Norwegian patients with ANCA-associated glomerulonephritis, prognosis was significantly better in 2003–12 compared with 1988–2002. This improvement was probably partly due to a shorter diagnostic delay, and better therapeutic management in older patients.

Keywords: ANCA-associated glomerulonephritis, diagnostic delay, end-stage renal disease, mortality, prognosis

INTRODUCTION

Anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (AAGN) is the most frequent form of the rapidly progressive glomerulonephritis syndrome. AAGN is an important cause of end-stage renal disease (ESRD) [1, 2]. Prior to the introduction of modern therapy, the prognosis of AAGN was very poor, and most patients died within 1–2 years after disease onset [3]. Cyclophosphamide (CYC) combined with corticosteroids (CS) was introduced as a treatment for AAGN more than 4 decades ago. This advent significantly improved prognosis, but morbidity and mortality remained high for several reasons [4–9]. First, treatment was often initiated after severe irreversible organ damage had occurred. Second, some patients had CYC-intolerance or resistance and, until recently, there were no effective

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alternative drugs for treatment. Third, both CYC and CS are associated with severe toxicity, which can be fatal. Fourth, AAGN often recurred after long periods of remission and, unless recurrent episodes were treated promptly, they could cause severe organ dysfunction, including ESRD [10–12]. Currently, rituximab has been proven as effective, but not superior, to CYC for inducing remission in patients with ANCA-associated vasculitis [13–15]. However, in Norway, CYC remains the first-line treatment for inducing remission in most patients with AAGN.

Despite the lack of new, safe pharmacological substances that are more effective than CYC, a number of measures have been taken that could improve the prognosis of patients with AAGN. The diagnostic delay has probably been reduced by an increased clinical awareness about AAGN and the availability of an ANCA analysis that provides an answer within 1–2 days. The more recent introduction of rituximab has increased the therapeutic possibilities, particularly for patients with AAGN that are resistant or intolerant to CYC treatment. Also, optimization of CYC usage in maintenance treatments for AAGN may have reduced treatment-related morbidity and mortality. For example, CYC usage may be optimized by reducing doses for older patients and patients with advanced renal failure; by transitioning patients from oral to intravenous CYC; and by substituting CYC with other, presumably safer agents, like azathioprine (AZA) after achieved remission. Finally, the diagnostic delay, and thus, the risk of ESRD may have been reduced by the increased awareness that life-long surveillance is necessary to detect recurrence in patients with AAGN [16–19].

A few recent reports have shown an improved prognosis in patients with ANCA-associated vasculitis over time, but specific data are limited for patients with biopsy-proven AAGN [20–22]. In Norway, since 1988, all patients with kidney biopsies that showed AAGN have been registered in the Norwegian Kidney Biopsy Registry (NKBR). Here, we studied the prognosis in patients with AAGN and compared outcomes between patients diagnosed in 1988–2002 versus those diagnosed in 2003–12. We hypothesized that Norwegian patients with AAGN have experienced improved survival and reduced risk of ESRD over time.

MATERIALS AND METHODS

This study was approved by the Regional Committees for Medical and Health Research Ethics (REC south-east).

Registries used in the study

The NKBR was established in 1988. Clinical and histopathological data from patients that underwent a diagnostic kidney biopsy in Norway (current population, 5 million) were registered starting in 1988, and the completeness of registration was estimated to be ∼90%.

The Norwegian Renal Registry is located at Oslo University Hospital, Rikshospitalet, Norway. Starting in 1980, all patients that started maintenance dialysis or received a kidney transplant were registered in the Norwegian Renal Registry. The tax office currently administers the Norwegian Population Registry, and all deaths are registered on a weekly basis. Information regarding the causes of death is recorded on the compulsory Norwegian death certificate, and this information is registered in the Cause of Death Registry, administered by Statistics Norway. The unique, 11-digit, Norwegian social security number made linking the different registries simple and reliable.

Identification of the study population

All patients were included when they had a first time kidney biopsy that showed a crescentic pauci-immune pattern and a documented positive ANCA test.

Observation period, identification and definition of study endpoints

The total follow-up (TFU) period of this study started on the date of the diagnostic kidney biopsy and ended on the date of death or on 31 December 2012. The observation period was further stratified into short (SFU = 0–1 years) and long (LFU > 1 years) follow-up periods. In the TFU period, the primary endpoints were progression to ESRD [commencement of chronic renal replacement therapy (RRT)] and standardized mortality ratio (SMR). In the SFU period, the study endpoints were all-cause deaths, SMR and ESRD. In the LFU period, the primary endpoints were SMR and ESRD. ESRD and deaths in the cohort were identified by linking the records of the study cohort to the Norwegian Renal Registry (ESRD) and the Norwegian Population Registry (deaths). Causes of death were identified by linking the study cohort to the Norwegian Cause of Death Registry; all deaths were categorized by cause, which included active vasculitis, infection, cardiovascular disease, malignancies and other causes.

Identification and definition of predicting factors in the study

Predicting factors were identified in the NKBR, and comparison groups were defined as follows:

(i) Early versus late study cohorts: patients with diagnostic biopsies performed before 31 December 2002 (early) were compared with those with biopsies performed after 31 December 2002 (late).

(ii) Age groups at time of biopsy: patients were compared in age groups of <60 years; 60–74.9 years and ≥75 years.

(iii) Renal function groups: pre-biopsy serum creatinine levels were converted to the estimated glomerular filtration rate (eGFR; modified modification of diet in renal disease formula, Caucasians). Patients with mean eGFR ≥15 were compared with patients with eGFR <15 mL/min/1.73 m^2.

(iv) ANCA specificity groups: patients with cytoplasmic (C)-ANCA (immunofluorescence, IF) or anti-proteinase 3 (PR3) (enzyme-linked immunosorbent assay, ELISA) were compared with those with perinuclear (P)-ANCA (IF) or anti-myeloperoxidase (MPO) (ELISA).

(v) Proteinuria groups: groups comprised patients with proteinuria ≥3 and those with <3 g/24 h.

(vi) Blood pressure: groups comprised patients with blood pressures ≥ or <140/90 mmHg.

(vii) Serum albumin: groups comprised patients with serum albumin ≥ or <30 g/L.

Mortality analyses

We compared the mortality rate of patients with AAGN to that of the general population by calculating SMR. The SMR was defined as the ratio between the observed and expected number of deaths in the cohort. The expected number of deaths was calculated based on mortality data for the Norwegian population, stratified by age (20–24, 25–29 years, and so forth), time-period (1990–94, 1995–99 and so forth) and gender (Statistics Norway). Each participant had an expected risk of death that varied depending on age, gender, time-period and duration of follow-up. Thus, the expected number of deaths in the study cohort was the sum of all of these risks. A Poisson distribution for death incidence in the general population was assumed in calculating the 95% confidence interval (95% CI).

First, SMR was calculated for the TFU, SFU and LFU periods. Second, the LFU was stratified into not-RRT and RRT periods, and the SMR was calculated separately for these periods. Finally, the above calculations were repeated after the TFU was stratified into early (1988–2002) and late (2003–12) study cohorts.

Availability of ANCA test and treatment protocols for 1988–2012

A questionnaire was sent to hospitals that treated patients with AAGN to determine the availability and response time of the ANCA test. The questionnaire also asked whether the institution practiced substitution of CYC with AZA in maintenance treatments for AAGN, and when they started this practice.

Statistical methods

Continuous variables are expressed as the mean ± SD and categorical variables are expressed as the number (%). The χ^2-test was used to compare categorical variables, and the independent sample *t*-test was used to compare continuous variables. A P-value ≤0.05 was considered significant. Kaplan–Meier statistics were used to compare risks of ESRD and death. The log-rank test was used to test statistical significance. Cox regression statistics were used to analyse unadjusted and adjusted hazard ratios (HRs) for ESRD and/or death. Two adjusted HRs were calculated: the first was adjusted for age, gender and ANCA specificity; the second was adjusted for the same factors plus the eGFR.

RESULTS

A cohort of 455 patients with AAGN was identified. The TFU duration was 2720 patient-years, with a mean follow-up of 6.0 years (range, 0.0–23.4). The mean age was 59.6 years (SD 17), and 55% of patients were males. Number of patients with proteinuria ≥3 g/24 h was 94 (21%) and with hypertension 262 (58%). Other baseline characteristics are shown in Table 1. The outcomes of patients in the total study cohort are shown in Figure 1A. During the TFU period, 124/455 (27%) patients progressed to ESRD, and of those, 55/124 (44%) later died. Among the patients who did not progress to ESRD, 110/331 (33%) died. Thus, during the TFU, 165/455 (36%) deaths occurred. The cumulative risk of ESRD was 16% at 1 year, 25% at 5 years, 32% at 10 years and 42% at 15 years after the AAGN diagnosis.

Table 1. Baseline characteristics of patients diagnosed with AAGN in the early (1988–2002) and late (2002–12) study periods

	All (n = 455)	1988–2002 (n = 218)	2003–12 (n = 237)	P-value
Mean age, years (SD)	59.6 (17)	57.6 (19)	61.5 (18)	0.02
Age ≥75 years	94 (21)	31 (14)	63 (27)	0.001
Male gender	248 (55)	137 (63)	111 (47)	<0.001
C-ANCA	258 (57)	142 (65)	116 (49)	<0.001
P-ANCA	197 (43)	76 (35)	121 (51)	<0.001
Mean eGFR, mL/min/1.73 m^2 (SD)	32 (29)	27 (26)	37 (31)	<0.001
eGFR <15 mL/min/1.73 m^2	153 (34)	95 (44)	58 (25)	<0.001
S-albumin, g/L (SD)	31 (7)	31 (6)	32 (7)	0.03
Proteinuria, g/24 h (SD)	1.8 (1.9)	2.1 (2.2)	1.6 (1.5)	0.01
Systolic BP, mmHg (SD)	143 (22)	144 (21)	142 (22)	0.33
Diastolic BP, mmHg (SD)	81 (11)	82 (11)	80 (12)	0.05

ANCA, anti-neutrophil cytoplasmic antibodies; C-ANCA, cytoplasmic/proteinase3 ANCA; P-ANCA, perinuclear/myeloperoxidase ANCA; eGFR, estimated glomerular filtration rate; S-albumin, serum albumin; BP, blood pressure.
Values represent the number (%), unless otherwise indicated.

In the SFU period (<1 years after diagnosis), 69/455 patients (15%) progressed to ESRD, and 75/455 (16%) patients died (Figure 1B). Thirteen patients (3%) died after commencement of RRT. Thus, at the end of the SFU period, 324/455 (71%) patients without ESRD were alive, and 56/455 (12%) patients were alive, but were RRT-dependent.

At start of the LFU period (>1 year), 324 patients were alive without RRT (Figure 1C). Progression to ESRD occurred in 55/324 (17%) patients. The cumulative risk of ESRD was 13% at 5 years, 22% at 10 years and 37% at 15 years. During the LFU period, 90 patients died; of these, 48 patients had not progressed to ESRD, and 42 patients were established in RRT before death. At the end of the LFU period, 221 patients were alive without ESRD and 69 patients were alive with RRT.

Among the 165 deaths during the TFU, the causes of death were active vasculitis (n = 31; 19%), infection (n = 43; 26%), cardiovascular disease (n = 58; 35%), malignancies (n = 15; 9%) and other causes (n = 18; 11%). The causes of death in the different observation periods and in patients with or without RRT are shown in Figure 1A–C.

In the TFU period, the SMR was 2.8 (95% CI: 2.4–3.3). In the SFU period, the SMR was 10.8 (95% CI: 8.6–13.5), and in the LFU period it was 1.7 (95% CI: 1.4–2.1). In the not-RRT part of the LFU period, the SMR was 1.1 (95% CI: 0.8–1.5), and in the RRT part, the SMR was 4.3 (95% CI: 3.2–5.8).

Risk factors for ESRD in the TFU period

Several significant, independent risk factors for ESRD were identified for the TFU period (Table 2), including an initial

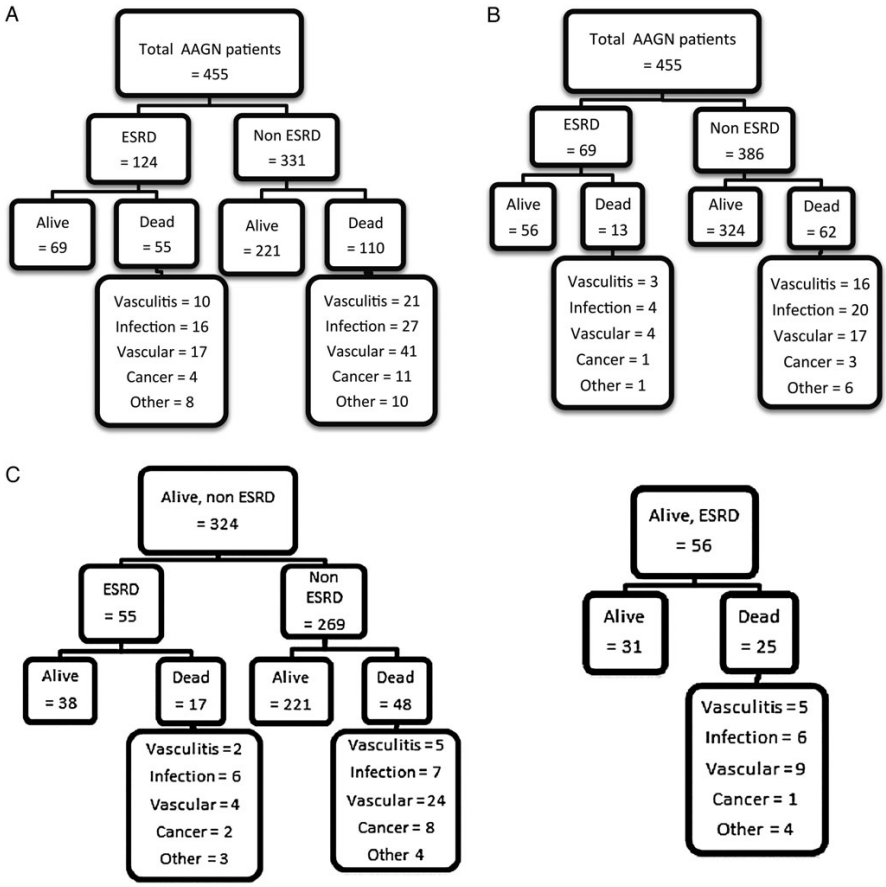

FIGURE 1: Flow chart shows the outcomes of 455 patients with AAGN in the study cohort. (**A**) All patients in the TFU period. (**B**) Patients included in the short follow-up period. (**C**) Patients included in the long follow-up period. AAGN, anti-neutrophil cytoplasmic antibody-associated glomerulonephritis; ESRD, end-stage renal disease.

eGFR <15 mL/min/1.73 m^2 (adjusted HR = 5.1), male gender (adjusted HR = 2.1), P/MPO-ANCA (adjusted HR = 1.8) and age bracket 60–74.9 years (adjusted HR = 0.7). Furthermore, other risk factors for ESRD identified for the TFU period were proteinuria ≥3.0 g/24 h (adjusted HR = 1.7, 95% CI: 1.1–2.4, P = 0.001) and blood pressure ≥140/90 mmHg (adjusted HR = 1.9, 95% CI: 1.2–2.9, P = 0.003), but not serum albumin <30 g/L (adjusted HR = 0.9, 95% CI: 0.6–1.3, P = 0.54).

Risk factors for death in the SFU period

Also shown in Table 2, several significant, independent risk factors for 1-year mortality were identified for the SFU period. These included an initial eGFR <15 mL/min/1.73 m^2 (adjusted HR = 2.2), age bracket of 60–74.9 years (adjusted HR = 4.0) and age ≥75 years (adjusted HR = 8.4). Moreover, an increased 1-year mortality rate was significantly associated with serum albumin <30 g/L (adjusted HR, 95% CI: 1.1.1–3.1, P = 0.01). Proteinuria ≥3.0 g/24 h and blood pressure ≥140/90 mmHg were not significantly associated with increased 1-year mortality.

Comparison of early (1988–2002) and late (2003–12) study cohorts

Compared with the early study cohort, the late study cohort had significantly higher initial renal function, measured as the mean eGFR (37 versus 27 mL/min/1.73 m^2), and significantly fewer patients with eGFRs <15 mL/min/1.73 m^2 (25 versus 45%) (Table 3). As shown in Table 1, there were some important differences in the baseline characteristics of these cohorts. The late study cohort had a higher mean age (62 versus 58 years), a larger fraction of patients ≥75 years (27 versus 14%), a larger fraction of female patients (53 versus 37%) and a higher frequency of type P/MPO-ANCA (51 versus 35%) than the early study cohort.

In comparing the two cohorts, we found that, over time, the 1-year cumulative risk of ESRD decreased from 19 to 13%,

Table 2. Multiple Cox regression analyses for ESRD and 1-year mortality in patients with AAGN

Factor	*N*	Events	Unadjusted HR	Adjusted HR[a]	Adjusted HR[b]
			HR for ESRD, TFU period		
eGFR ≥15	302	53	1.0	1.0	
eGFR <15	153	71	4.07 (2.8–5.8), P < 0.001	5.06 (3.5–7.4), P < 0.001	
Female	207	44	1.0	1.0	1.0
Male	248	80	1.58 (1.1–2.3), P = 0.015	1.79 (1.2–2.6), P = 0.003	2.09 (1.4–3.1), P < 0.001
Age <60 years	189	64	1.0	1.0	1.0
Age 60–74.9 years	172	40	0.89 (0.6–1.3), P = 0.562	0.86 (0.6–1.3), P = 0.464	0.66 (0.4–1.0), P = 0.046
Age ≥75 years	94	20	1.09 (0.7–1.8), P = 0.736	1.05 (0.6–1.8), P = 0.861	0.76 (0.5–1.3), P = 0.316
C-ANCA	258	64	1.0	1.0	1.0
P-ANCA	197	60	1.56 (1.1–2.2), P = 0.015	1.78 (1.2–2.6), P = 0.003	1.78 (1.2–2.6), P = 0.003
			HR for death, short follow-up period		
eGFR ≥15	302	32	1.0	1.0	
eGFR <15	153	43	2.97 (1.9–4.7), P < 0.001	2.19 (1.4–3.5), P = 0.001	
Female	207	32	1.0	1.0	1.0
Male	248	43	1.16 (0.7–1.8), P = 0.536	1.33 (0.8–2.3), P = 0.241	1.44 (0.9–2.3), P = 0.134
Age <60 years	189	8	1.0	1.0	1.0
Age 60–74.9 years	172	33	4.85 (2.2–10.5), P < 0.001	5.01 (2.3–10.9), P < 0.001	4.02 (1.8–8.1), P < 0.001
Age ≥75 years	94	34	10.15 (4.7–21.9), P < 0.001	10.51 (4.8–23.1), P < 0.001	8.35 (3.8–18.6), P < 0.001
C-ANCA	258	41	1.0	1.0	1.0
P-ANCA	197	34	1.10 (0.7–1.7), P = 0.678	0.85 (0.5–1.4), P = 0.506	0.90 (0.6–1.4), P = 0.657

N, number of patients; HR, hazard ratio; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; ANCA, anti-neutrophil cytoplasmic antibodies; C-ANCA, cytoplasmic/proteinase3 ANCA; P-ANCA, perinuclear/myeloperoxidase ANCA.
[a]Adjusted for age, gender and ANCA; P-values compare to the top factor in each sub-group.
[b]Adjusted for age, gender, ANCA, and eGFR; P-values compare to the top factor in each sub-group.

Table 3. Baseline eGFR, stratified by age and study period

Age group	Mean eGFR (SD)			P-value	Patients with eGFR <15 (%)			P-value
	1988–2012	1988–2002	2003–2012	Early versus late	1988–2012	1988–2002	2003–2012	Early versus late
All	32 (29)	27 (26)	37 (31)	<0.001	34%	44%	25%	<0.001
<60 years	42 (34)	35 (30)	52 (37)	0.001	25%	34%	13%	0.001
60–74.9 years	27 (23)	21 (19)	32 (26)	0.002	36%	48%	25%	0.002
≥75 years	23 (22)	17 (17)	26 (24)	0.037	47%	65%	38%	0.016

eGFR, estimated glomerular filtration rates; SD, standard deviation.
Early study period: 1988–2002; late study period: 2003–12.

and the 10-year risk decreased from 37 to 26% (Figure 2). In the Cox regression model, the risk of ESRD changed with different adjustments. For the early study period, the HR adjusted for age, gender and ANCA was 1.6. However, after adding an adjustment for the initial eGFR, the HR decreased to 1.2, and the difference between cohorts was not significant (Table 4).

In comparing the two cohorts, we found that the 1-year mortality rate decreased over time from 18 to 15% and it decreased from 33 to 20% in those ≥60 years old (Figure 3). In multivariate analyses, the unadjusted HR for 1-year mortality (1.3) was not significantly different in the early compared with the late study period (Table 4). When this was adjusted for age, gender and ANCA (HR = 1.9), it was significantly higher than that of the late study period. Then, when it was adjusted also for the initial eGFR (HR = 1.6), it was only borderline significant compared with the late study period. In a sub-group analysis of patients ≥60 years old, the HR for 1-year mortality was 2.4 after adjusting for age, gender and ANCA, and 2.0 after also adjusting for the initial eGFR (Table 4).

In comparing the early and late study groups, we found that, over time, the risk of the composite endpoint of ESRD or death <1 year after the AAGN diagnosis (Figure 4) was reduced from 34 to 25% (all patients), from 21 to 13% (patients aged <60 years), from 38 to 25% (patients aged 60–74.9 years) and from 65 to 38% (patients aged ≥75 years). In the Cox regression analysis, the HR for ESRD or death <1 year after diagnosis was 1.8 after adjusting for age, gender and ANCA, but it decreased to 1.3 (not significantly different from the late study group) after also adjusting for the initial eGFR (Table 4).

During the SFU period, the difference in SMR between study groups was only borderline significant. The SMR was 14.8 (10.9–20.2) in the early cohort and 8.1 (5.8–11.3) in the late cohort. Otherwise, we found no significant differences in SMR between the early and late study cohorts (data not shown).

Availability of ANCA test and treatment protocols in 1988–2012

Most hospitals that treated patients with AAGN responded to our questionnaire. All reported good availability of the ANCA analysis, and the response time was one to two working days at most. Historically, all hospitals used CYC to treat AAGN, but in the last few years, some have used rituximab for induction and maintenance treatments. All respondents also practiced the substitution of CYC with AZA for maintenance treatments; this change in practice was introduced between 2003 and 2008 in most hospitals.

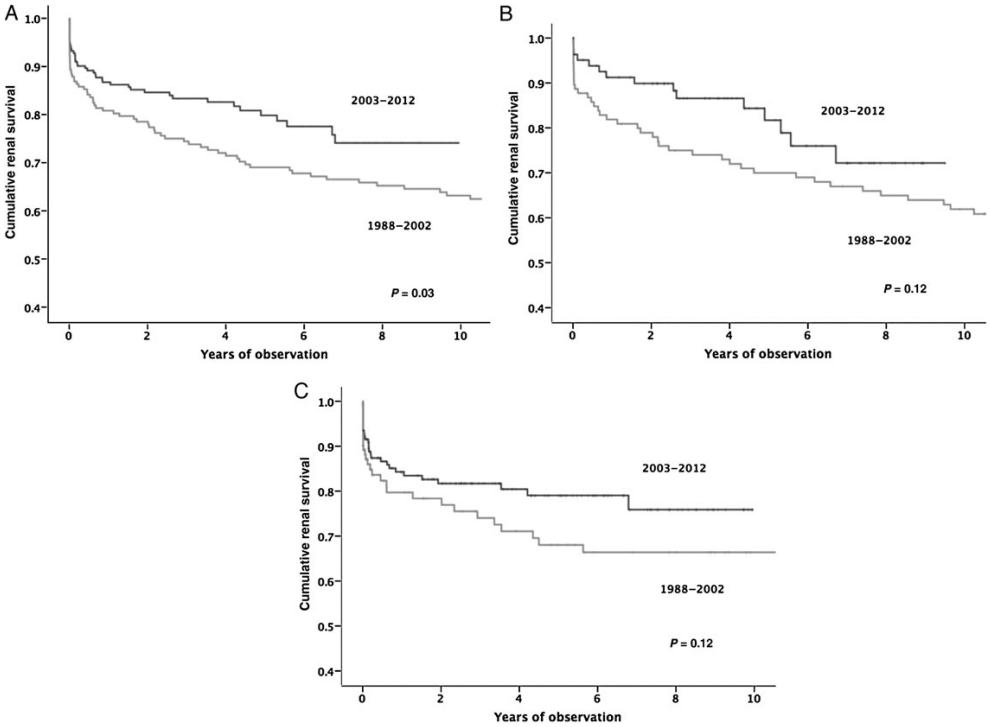

FIGURE 2: Kaplan–Meier plots show renal survival in 455 patients with AAGN stratified by early (1988–2002) and late (2003–12) study periods. (**A**) All patients. (**B**) Patients aged <60 years. (**C**) Patients aged ≥60 years. AAGN, anti-neutrophil cytoplasmic antibodies associated glomerulonephritis; ESRD, end-stage renal disease.

Table 4. Multiple Cox regression analyses compare the risk of ESRD, the 1-year mortality and the 1-year risk of ESRD or death for patients with AAGN in 1988–2002 versus 2003–12

	N	Events	Unadjusted HR	Adjusted HR[a]	Adjusted HR[b]
			HR ESRD 1988–2002 versus 2003–12		
2003–12	237	44	1.0	1.0	1.0
1988–2002	218	80	1.54 (1.0–2.3), P = 0.029	1.57 (1.1–2.3), P = 0.026	1.15 (0.8–1.7), P = 0.515
			HR 1-year mortality 1988–2002 versus 2003–12		
2003–12	237	35	1.0	1.0	1.0
1988–2002	218	40	1.31 (0.8–2.1), P = 0.243	1.87 (1.2–3.0), P = 0.011	1.61 (1.0–2.6), P = 0.056
			HR 1-year mortality patients age ≥60 years 1988–2002 versus 2003–12		
2003–12	154	30	1.0	1.0	1.0
1988–2002	112	37	1.99 (1.2–3.2), P = 0.005	2.35 (1.4–3.9), P = 0.001	2.01 (1.2–3.4) P = 0.009
			HR 1-year ESRD/death 1988–2002 versus 2003–12		
2003–12	237	58	1.0	1.0	1.0
1988–2002	218	73	1.49(1.1–2.1), P = 0.02	1.80(1.3–2.6), P < 0.001	1.34(0.9–1.9), P = 0.12

N, number of patients; HR, hazard ratio; ESRD, end-stage renal disease.
[a]Adjusted for age, gender and ANCA; P-values are compared with the 2003–12 study group.
[b]Adjusted for age, gender, ANCA and eGFR; P-values are compared with the 2003–12 study group.

DISCUSSION

The main finding in this study was that the prognosis of patients with AAGN in Norway improved significantly from the early (1988–2002) to late (2003–12) study periods. A similar trend has been described in several other studies in patients with ANCA-associated vasculitis. However, to our knowledge, this was the first, nationwide, unselected, population-based cohort with AAGN confirmed by kidney biopsies [10, 20–22].

A major complication of AAGN is progression to ESRD [23, 24]. We found that this risk decreased substantially over time; for the 1-year follow-up, it decreased from 19 to 13%, and for the 10-year follow-up, it decreased from 37 to 26%.

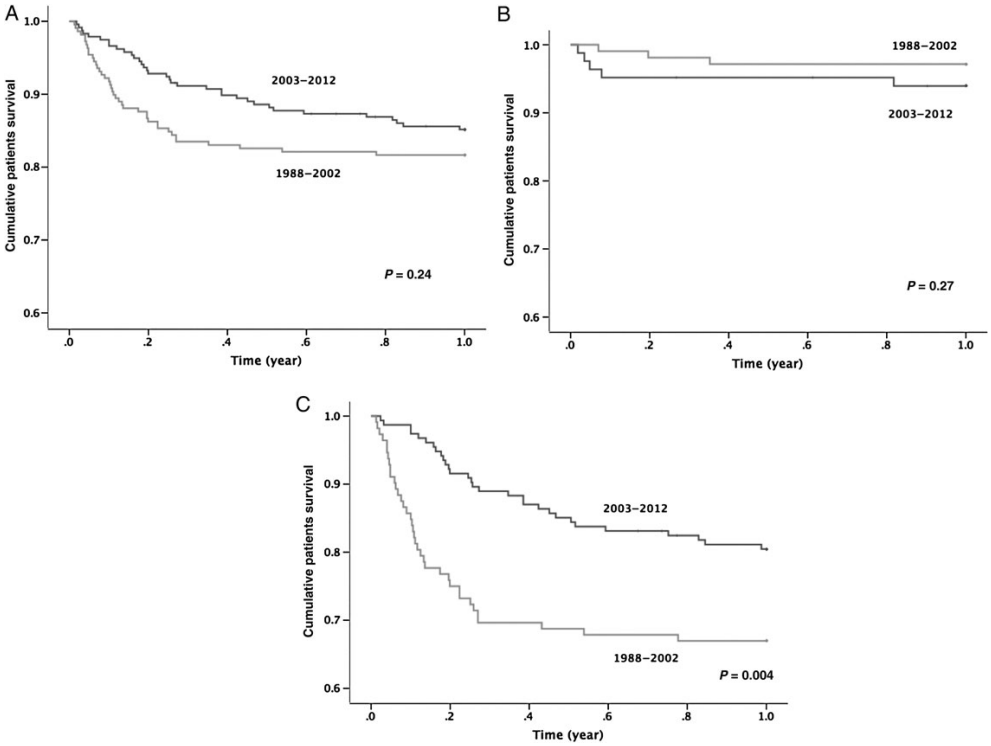

FIGURE 3: Kaplan–Meier plots show 1-year survival in 455 patients with AAGN stratified by study period (1988–2002 versus 2003–12). (**A**) All patients. (**B**) Patients aged <60 years. (**C**) Patients aged ≥60 years. AAGN, anti-neutrophil cytoplasmic antibodies associated glomerulonephritis; ESRD, end-stage renal disease.

This was an improvement of great clinical significance, because RRT is associated with reduced quality of life, increased mortality and high costs [25, 26]. The major mechanism of ESRD risk reduction over time seemed to be a shorter diagnostic delay. The mean eGFR at AAGN diagnosis increased significantly over time, from 27 to 37 mL/min/1.73 m^2, and the fraction of patients with eGFR <15 mL/min/1.73 m^2 decreased from 44 to 25%. In the Cox regression model, the unadjusted HR for ESRD in the early period (1.5) was significantly higher than that in the late period. Moreover, the HR was basically unchanged after adjusting for age, gender and ANCA sub-type. However, when we also adjusted for the initial eGFR, the HR dropped to 1.2. We interpreted this to indicate that the improvement in eGFR was a mechanistic factor, rather than a confounder.

A primary objective when treating patients with AAGN is to avoid fatal disease courses. In our study cohort, the mortality rate was significantly higher than expected for the Norwegian population (SMR 2.8). However, high mortality rates were only observed in the SFU period (SMR 10.8) and in patients dependent on RRT during the LFU period (SMR 4.3). In patients that survived the first year and did not develop ESRD, the mortality rate was equivalent to that of the general population, after adjusting for age and gender (SMR 1.1). The increased mortality rate observed in patients dependent on RRT was probably not specific to patients with AAGN. We previously showed similar data for patients with IgA nephropathy [27]. Nevertheless, it underscored the clinical importance of avoiding progression to ESRD in patients with AAGN.

The main concern regarding survival in patients with AAGN is the high 1-year mortality rate [10]. We did not observe significantly different short-term mortality rates in the early (18%) versus late (15%) study periods. However, among patients with AAGN aged ≥60 years, the 1-year mortality rate was significantly reduced from 33% (early cohort) to 20% (late cohort). Of interest, in this age group, the unadjusted HR for death at 0–1 year after AAGN diagnosis was 2.0 (P = 0.01), and this HR remained unchanged after adjusting for age, gender, ANCA type and eGFR. Thus, a shorter diagnostic delay did not appear to be a major explanatory factor for the improvement in short-term survival among older patients. We speculated that the improvement in short-term survival might be due to greater care taken in treating the older patients in the later compared with the earlier study period. For example, they may have received more frequent use of prophylactic measures, reduced doses of CYC and more rigorous surveillance, particularly during the induction phase of the treatment.

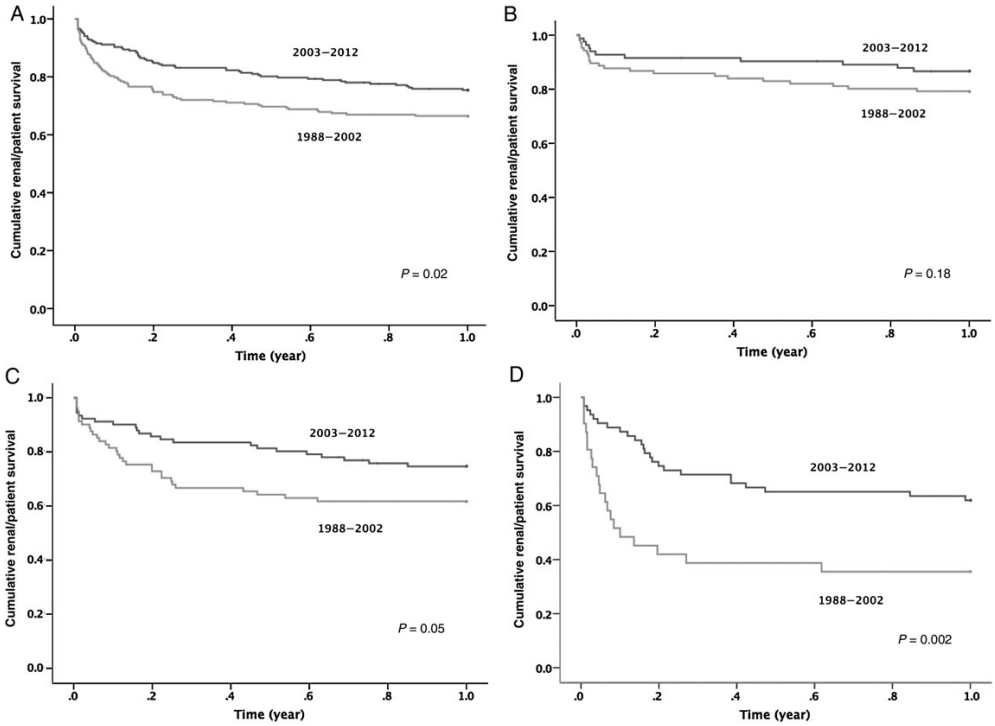

FIGURE 4: Kaplan–Meier plots show 1-year risk of ESRD or death in 455 patients with AAGN stratified by study period (1988–2002 versus 2003–12). (**A**) All patients. (**B**) Patients aged <60 years. (**C**) Patients aged 60–74.9 years. (**D**) Patients aged ≥75 years. AAGN, anti-neutrophil cytoplasmic antibodies associated glomerulonephritis; ESRD, end-stage renal disease.

The risk of the composite endpoint, ESRD or death, in the SFU period decreased from 34% (early cohort) to 25% (late cohort), which clearly indicated a clinically relevant improvement. In death-censored analyses, a reduced mortality rate could result in an increased risk of ESRD, because death and ESRD frequently occur concurrently, and they may compete as endpoints in the clinical setting of AAGN in older patients. Theoretically, a less toxic, but also less effective, treatment regimen could result in both decreasing the mortality rate and increasing the risk of ESRD. However, it was reassuring that, in our study cohort, the observed improvement in short-term survival-rate was not linked to an increased risk of ESRD; the 1-year risk of ESRD decreased from 18% (early cohort) to 15% (late cohort) among patients aged ≥60 years.

The prognosis of patients with AAGN was better in the LFU than in the SFU period. The mortality risk was, except in patients with RRT, similar to the age- and gender-adjusted general population. The annual risk of ESRD was 2–3% per year of follow-up. Because the death rate did not increase, we did not investigate the composite ESRD or death endpoint in the LFU period. In fact, the prognosis of AAGN in the LFU period was similar to our previous finding in patients with IgA nephropathy [27]. There were no significant differences in prognosis between the early as compared with late cohort in the LFU period.

The overall prognosis in our study cohort was quite similar to that of previous study cohorts from other countries. A substantial improvement in renal function at the time of AAGN diagnosis was also found in a recent study from the Netherlands. In that study, serum creatinine fell from ∼450 μmol/L (in 1979–2000) to 282 μmol/L (in 2001–09) [21]. Most studies reported that the long-term risk of ESRD was 20–40%, similar to our findings [9, 20, 28, 29]. Our finding that the 1-year overall mortality rate was 17% was consistent with previous reports from unselected study cohorts [21]. Finally, our SMR of 2.8 closely resembled the 2.7 SMR found in a study on patients recruited from four clinical European Vasculitis Study Group (EUVAS) trials [10].

This study showed that the prognosis of AAGN significantly improved from the 1988–2002 period to the 2003–12 period. However, even in the late study period, the 1-year mortality rate was 15%, the 1-year risk of ESRD or death was 25% and the 10-year cumulative risk of ESRD was 26%. Thus, there remains room for improvement. Further reduction in diagnostic delay may reduce the risk of ESRD. Optimization of treatment protocols, particularly for older patients, may reduce the short-term mortality from AAGN [28, 30]. More research is needed to find new solutions to these challenges.

The major strengths of this study were the large, nationwide, population-based, unselected nature of the study cohort

and the very long observation period with many clinically important (ESRD/death), reliably identified endpoints. This study also had some weaknesses. Because the NKBR had a purely renal focus, we could not categorize patients according to non-renal vasculitis activity. For example, we could not use the Birmingham vasculitis activity score. Furthermore, the histological data in the NKBR were, unfortunately, not sufficiently specific to apply the recently published histopathologic classification system for AAGN (focal/crescentic/mixed/sclerotic). Furthermore, we did not have detailed data on individual treatments; thus, we could not analyse the effects of different treatment protocols. Another limitation was that patients with AAGN without kidney-biopsy were not included in the study cohort, and such patients are not registered in the NKBR.

In summary, we have demonstrated that the prognosis of AAGN has improved significantly from 1988–2002 to 2003–12. Nevertheless, morbidity and mortality remain major concerns. More research is needed to identify strategies that can reduce diagnostic delay. Development of safer therapeutic strategies is also needed, specifically for older patients with AAGN.

CONFLICT OF INTEREST STATEMENT

The lead author, R.B., affirms that this manuscript is an honest, accurate, transparent account of the study reported; that no important aspects of the study have been omitted and that any discrepancies from the study as registered have been explained. The results presented in this article have not been published previously, except some of the data were presented in abstracts at the Nordic Nephrology meeting in Reykjavik, Iceland, 2013.

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Cancer in ANCA-Associated Glomerulonephritis: A Registry-based Cohort Study

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None declared.

Abstract

Background: Immunosuppressive therapy for antineutrophil cytoplasmic antibody-associated vasculitis has been associated with increased malignancy risk.

Objectives: To quantify the cancer risk associated with contemporary cyclophosphamide-sparing protocols.

Methods: Patients from the Norwegian Kidney Biopsy Registry between 1988 and 2012 who had biopsy-verified pauci-immune glomerulonephritis and positive antineutrophil cytoplasmic antibody (ANCA) serology were included. Standardised incidence ratios (SIRs) were calculated to compare the study cohort with the general population.

Results: The study cohort included 419 patients. During 3010 person-years, cancer developed in 41 patients (9.79%); the expected number of cancer cases was 37.5 (8.95%). The cohort had SIRs as follows: 1.09, all cancer types (95% CI, 0.81 to 1.49); 0.96, all types except non-melanoma skin cancer (95% CI, 0.69 to 1.34); 3.40, non-melanoma skin cancer (95% CI, 1.62 to 7.14); 3.52, hematologic cancer (95% CI, 1.32 to 9.37); 2.12, post-transplant cancer (95% CI, 1.01 to 4.44); and 1.53, during the 1-5-year follow-up after diagnosis (95% CI, 1.01 to 2.32).

Conclusions: Cancer risk did not increase significantly in this cohort with ANCA-associated glomerulonephritis. However, increased risk of non-melanoma skin cancer, post-transplant cancer, and hematologic cancer indicates an association between immunosuppression and malignancy.

Introduction

Historically, antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) was a fatal disease. The introduction of cyclophosphamide (CYC) treatment in the 1960s improved the prognosis and made long-term survival possible for patients with AAV [1, 2]. However, evidence soon emerged that the long-term survival of patients with AAV was associated with significant morbidity including a substantially increased cancer risk [3-7]. Immunosuppressive therapy using CYC was particularly associated with malignancy. Although the severe adverse effects have elicited a search for less toxic treatment regimens, CYC still remains the first-line drug [8].

High occurrence of cancer has been demonstrated in patients with AAV treated with cumulative CYC doses exceeding 36 g [9-11] or when the treatment duration lasted for more than a year [4, 12-14]. In the current clinical practice, cumulative CYC doses and treatment duration rarely exceed these limits [15-18], which questions the relevance of previous CYC-associated increased cancer risk observations in patients with AAV. There is a relative paucity of data regarding cancer risk in AAV patients treated with current therapy protocols, and to our knowledge, only 4 studies have published such data [5, 11, 13, 19]. In these studies, increased incidence of malignancy was observed. After excluding non-melanoma skin cancer (NMSC) from the analyses, the incidence of malignancy no longer significantly increased in any of these investigations. However, these 4 studies were relatively small, with a limited statistical power to detect small- to medium-range associations between cancer and AAV. Furthermore, most of these reports are single-center studies, which limits their generalizability.

Interestingly, in the most recent study, the standardised incidence ratio (SIR) of all cancer types treated with CYC was 4.61 (95% confidence interval (CI) 1.16 to 39.38) times higher than that with rituximab-based therapy. However, after excluding NMSC from their

analysis, the risk was only 1.30 (95% CI not reported) times higher with CYC compared with rituximab-based therapy [19].

To further investigate the association of cancer and AAV, we analysed data from the Norwegian Kidney Biopsy Registry (NKBR) and the Norwegian Cancer Registry (NCR). Additionally, we merged data from the present and the 4 recent studies on this topic and investigated the cancer risk in a total of 1532 patients with AAV diagnosed after 1988.

Materials and Methods

The study was approved by the Regional Committees for Medical and Health Research Ethics (REC South-East 2013/1083).

Study population and registries

The NKBR was established in 1988. We estimate that ~90% of all kidney biopsies are registered. The registry contains morphological, laboratory, and clinical data collected when the biopsy was performed. The NCR was established in 1953. Reporting to this registry is mandatory and based on reports from clinical and pathological departments and death certificates. A near complete registration (98-99%) of solid tumours, except for basal skin cell carcinomas, is documented [20]. Annual sex-specific incidence rates for cancer and cancer subtypes are available for age groups and time periods in 5-year intervals. These data allow for an accurate calculation of the expected cancer case numbers in the study cohort. The Norwegian Cause of Death Registry is part of Statistics Norway and based on the mandatory Norwegian death certificate. The Norwegian Renal Registry was established in 1980 and has registered all patients with end-stage renal disease (ESRD), defined by the commencement of maintenance dialysis or receiving kidney transplantation.

Data collection and definitions

We included patients registered in the NKBR and diagnosed from 1988 to 2012, with a pauci-immune necrotising glomerulonephritis and a positive ANCA serology. Patients with cancer prior to the AAV diagnosis were excluded. Baseline clinical data, including sex, age, ANCA specificity, and estimated glomerular filtration rate (eGFR) (determined by the Modification of Diet in Renal Disease equation) [21] were obtained from the NKBR. The primary study end-point, cancer incidence, was identified by linking the study cohort with the

NCR, using the unique 11-digit Norwegian personal number. Causes of deaths in the study cohort were identified through linkage with the Norwegian Cause of Death Registry and classified as vascular, malignant, infectious or active inflammation, and other causes. The observation period was from the kidney biopsy date to incident cancer, end of 2013, or death, whichever came first. Patients with ESRD and those receiving kidney transplants were identified through record linkage with the Norwegian Renal Registry. When we calculated the cancer risk in transplanted patients, the observation period started from the kidney transplantation date.

SIR calculation

We calculated the SIR as the ratio between the observed and expected cancer case numbers in the cohort. The expected cancer case number was calculated as follows: First, the number of person-years was calculated in the cohort, stratified by 5-year age groups and 1-year time periods. Second, this person-time was multiplied with the corresponding incidence rate in the general population to get the expected number of cases in each age group and 1-year period. The total number of expected cancer cases was then calculated as the sum of expected cases across age groups and time periods. The observed cancer case number was determined by record linkage of the study cohort and NCR using the 11-digit unique Norwegian personal number. A Poisson distribution of cancer incidence was assumed when 95% CIs were calculated. We calculated the SIR throughout the study period and stratified in accordance with disease duration periods (0-1, 1-5, 5-10, and >10 years after kidney biopsy), sex, ANCA specificity [cytoplasmic ANCA (C-ANCA) or perinuclear ANCA (P-ANCA)], time periods (1988-2002 and 2003-2012) and post-transplantation observation period.

Pooled analysis

A systematic PubMed search was conducted to identify previous studies reporting the cancer risk in patients with AAV diagnosed after 1988. The search was restricted to papers published in English language. Studies who do not report SIR data were excluded. The SIRs were calculated as the sum of the observed cancer cases divided by that of the expected cancer cases in all studies.

Treatment

Information regarding cumulative CYC doses administered to the patients is not available in the NKBR. In a previous Norwegian study including patients with Wegener's granulomatosis diagnosed between 1988 and 1998, the majority of patients received intravenous CYC with a median cumulative dose of 17 g. In patients receiving oral CYC, the median cumulative dose was 48 g [22]. Most centres since approximately 2003 have substituted CYC with azathioprine for maintenance treatment, substantially lowering the exposure to CYC [23]. In some patients, rituximab has been used for induction and maintenance treatment. Some patients have also received plasma exchange treatments [24].

Statistical analyses

Continuous variables were expressed as medians with 25th and 75th percentiles and categorical variables as numbers (%). Comparisons of continuous and categorical variables in the baseline characteristics were calculated using the Mann-Whitney *U*-test and the X^2 or Fisher's exact test, respectively. A two-tailed p-value of ≤ 0.05 and 95% CI was considered statistically significant. All statistical analyses were performed using the SPSS software, V.23 and STATA software, V.14.

Results

Baseline characteristics

Between 1988 and 2012, 454 patients diagnosed with AAV and glomerulonephritis were identified. Of these, 35 were excluded as a result of cancer diagnosis prior to the observation period. Thus, 419 patients were included in the study cohort. As shown in Table 1, the median age in the cohort was 62 years [interquartile range (IQR), 48 to 72 years], and 229 (55%) were men. The median eGFR at the time of kidney biopsy was 23 mL/min/1.73 m^2 (IQR, 11 to 46). A positive C-ANCA was found in 237 (57%) and P-ANCA in 183 (43%) patients.

The median length of follow-up was 5.7 years (IQR, 2.8 to 11.3). The mean length of follow-up was 7.2 years (SD, 5.8), and the total number of person-years of observation was 3010. A total of 148 (35%) patients died during the observation period. Causes of deaths are registered for 138 patients, of which 69 (50%) died from infectious disease or active inflammation/vasculitis, 36 (26%) cardiovascular disease, 12 (9%) malignancy, and 21 (15%) from other causes. Kidney transplantation was performed in 60 (14%) patients.

Observed cancer cases

During follow-up, 46 cancer cases were reported in 41 (9.5%) patients. The first occurring cancer cases were as follows: NMSC (7 cases), lung (7), prostate (5), hematologic (4), urinary bladder and ureter (3), rectum (3), colon (2), uterus (2), central nerve system (2), unknown primary site (2), thyroid (1), ovary (1), lymphoma (1), and pancreas (1). Five patients had 2 distinct cancer diagnoses; the second occurring cancer cases were as follows: lung (1), NMSC (1), prostate (1), hematologic (1), and stomach (1). Seven patients who received transplants were diagnosed with cancer; the first occurring cancer cases were as follows: lung (4), prostate (1), lymphoma (1), and NMSC (1). Two of the 5 patients with 2

distinct cancers underwent transplant, with the cases of secondary cancer involving the prostate and lungs.

Comparison of patients with and without cancer

The comparison of the patients with and without cancer during follow-up is shown in Table 1. The patients with cancer were significantly older [65 years versus 61 years (p=0.04)] at the time of AAV diagnosis, and a significantly higher percentage were men (71% versus 53% (p=0.03). There were no significant differences in eGFR and ANCA specificity between those with cancer and those without cancer during follow-up. A higher percentage of patients with cancer died during follow-up [25 (61%) versus 123 (33%) (p<0.001)].

Comparison with the general population

As shown in Table 2A, the SIR of overall malignancy was 1.09 (95% CI 0.81 to 1.49). The SIR of all cancer types, except NMSC, was 0.96 (95% CI 0.69 to 1.34). In the 1-5-year period after AAV diagnosis, the cancer risk in the study cohort significantly increased compared to that in the general population (SIR, 1.53; 95% CI 1.01 to 2.32). The SIR did not increase in the first year or >5 years of follow-up after AAV diagnosis. In gender-, ANCA- and time period specificity-stratified analyses, SIR of cancer was not significantly increased for males of 1.27 (95% CI 0.88 to 1.83), in the C-ANCA positive group of 1.17 (95% CI 0.78 to 1.74) and in the 1988-2002 time period of 1.14 (95% CI 0.78 to 1.68). Compared to the general population, the transplanted patients in the study cohort had a significantly increased malignancy risk (SIR, 2.12; 95% CI 1.01 to 4.44), whereas the non-transplanted patients had no increased risk (SIR, 0.99; 95% CI 0.71 to 1.39).

The SIR calculation for the most common site-specific cancer cases showed significantly increased risks of NMSC (SIR, 3.40; 95% CI 1.62 to 7.14) and hematologic malignancies

(SIR, 3.52; 95% CI 1.32 to 9.37). The SIR did not significantly increase in any other site-specific cancer type (Table 2B).

Pooled analysis of the 5 cohort studies

The separate and merged SIRs of all cancer types in the present and the 4 previously reported studies are shown in Table 3 and Figure 1. This analysis included a total of 1532 patients, 8801 patient-years of observation, and 236 cancer cases. The merged SIR of all cancer types was 1.72 (95% CI 1.51 to 1.95) and that of all cancer types, except NMSC, was 1.21 (95% CI 1.01 to 1.45).

Discussion

In the present study, there was no statistically significant increase in the cancer incidence in the patients with AAV and glomerulonephritis compared to the age- and sex-matched general population (SIR, 1.09; 95% CI 0.89 to 1.49). Excluding the study by Holle *et al.* which reported an SIR of 0.82 (95% CI 0.45 to 1.38) [25], most previous studies, including those investigating patients with AAV diagnosed after 1988, have reported a significantly increased cancer risk [3-5, 9, 11, 13, 14, 19]. A couple of methodological discrepancies might partially explain the contrasting findings between the present and the majority of previous studies. First, basal skin cell carcinomas are not registered in the NCR and were thus excluded in our analysis. Second, among patients with diagnoses of several cancers, we only included the primary cancer when calculating the SIRs. In contrast, other studies have included subsequent cancer cases, particularly NMSC, in their SIR estimates.

The studies investigating cancer incidences in patients with AAV diagnosed after 1988 reflect contemporary treatment regimens, in which NMSC accounts for the majority of the observed increased malignancy risk [5, 11, 13, 19]. In the present study, the SIR decreased from 1.09 to 0.96 (95% CI 0.69 to 1.34) when NMSC cases were excluded. Further, in the pooled analysis (Table 3), the SIR decreased from 1.72 (95% CI 1.51 to 1.95) to 1.21 (95% CI 1.01 to 1.45) after excluding NMSC. Moreover, part of the residual cancer risk after excluding NMSC can be attributed to post-transplant malignancies, with the SIR post-transplant of 2.12 (95% CI 1.01 to 4.44) in the present study and 4.31 (95% CI 1.17 to 11.04) in that by van Daalen *et al.* [19].

In the organ-specific sub-analysis, the SIR of NMSC was 3.40 (95% CI 1.62 to 7.14), which is consistent with that of previous studies [5, 9, 11, 13, 14, 19]. An increased risk of NMSC is observed in immunocompromised patients and associated with both environmental factors as chronic human papillomavirus infection [26-29] and a direct effect of individual

immunosuppressant, e.g. azathioprine [19, 30]. A significantly increased hematologic cancer risk was also found (SIR, 3.52; 95% CI 1.32 to 9.37). Traditionally, CYC use was associated with a very high risk of acute myelogenous leukaemia (AML) [9, 14, 31]. Interestingly, no case of AML was observed in our cohort, and the increased haematologic malignancy risk was caused by 2 cases of myelodysplastic syndrome and 2 cases of chronic lymphocytic leukaemia. To what extent these cases are related to CYC-based therapy or immunosuppression specifically is unclear [32]. In comparison, Zycinska *et al.* found an increased AML risk in their study (SIR, 4.3; 95% CI 2.1 to 11.7). Notably, oral CYC was used in the majority of their patients, and 22% received >36 g of cumulative CYC dosage. In addition, a significantly increased urothelial cancer risk was also found in their study, in contrast to those of other recent groups (SIR, 3.4; 95% CI 1.6 to 5.2) [11].

Some previous groups have indicated that the cancer risk, perhaps caused by a higher tendency of relapses and thus higher cumulative immunosuppressive drug doses, is higher in C-ANCA than in P-ANCA positive patients [5, 13]. In contrast, we observed no significantly increased risk in these subgroups compared to the general population.

An important measure to reduce cancer risk in patients with AAV has been replacing CYC with azathioprine for maintenance treatment. This practice change occurred around 2003 concurrent with the publication of the CYCAZAREM study [23, 33]. In the present study cohort SIR of cancer was not significant increased, neither in the 1988-2002 nor in the 2003-2012 time periods. Of notice, in a study including a sub-group of the 1988-2002 cohort, cumulative doses of CYC were found fairly low, median 17 grams in intravenous CYC treated patients [34].

The findings by van Daalen *et al.* indicate that rituximab use is associated with a substantially lower risk of malignancy. However, this difference was primarily related to excess NMSC cases. The calculated SIR of cancer, except NMSC, was 1.14 (95% CI 0.49 to

2.25) with CYC and 0.88 (95% CI 0.11 to 3.19) with rituximab treatment. Thus, the SIR of cancer, except NMSC, was only 1.30-fold higher (CI not reported by van Daalen *et al*.) in the CYC group than in the rituximab group. Further, the SIR of cancer, except NMSC, was only marginally higher in the present CYC-treated study cohort than in the rituximab group in their study, estimated SIR of 0.96 (95% CI 0.69 to 1.34) and 0.88 (95% CI 0.11 to 3.19), respectively. Finally, no adjustment for post-transplantation malignancies has been performed when comparing CYC- and rituximab-treated patients [19]. In summary, whether replacing low-dose CYC regimens with rituximab would have a beneficial effect on non-NMSC malignancy risk remains uncertain.

NMSC occurrence is still substantially increased in patients with AAV. Although NMSC by no means should be considered as an inconsequential morbidity, deaths caused by these tumours are rare [35]. A number of measures can be taken to reduce the risk and morbidity related to NMSC in immunosuppressed patients; the most important measures include limiting sun exposure of the skin and vigilant monitoring with early NMSC detection and treatment when they appear. Interestingly, van Daalen *et al.* observed that rituximab use is associated with a lower NMSC risk than CYC use, which represents a new possible solution to such complication in patients with AAV [19]. However, the decision to replace CYC with rituximab as the first-line treatment in patients with AAV must also include considerations, such as efficacy, treatment-related complications, and cost-benefit.

The major strengths of the present study include its population-based approach and identification of patients with AAV from quality registries with histologic and serologic data. Information on expected and observed cancer cases was retrieved from the same registry, the NCR, which limits potential information biases in the SIR calculation. The NCR also has high accuracy and only few missing cases owing to mandatory reporting of cancer cases. Moreover, the pooled analysis strengthens the statistical power and increases the detection

rate of significant differences. Some weaknesses of our study must be admitted. Most importantly, we could not correlate our findings to the cumulative CYC doses administered. Treatment data were unavailable in the NKBR. However, we have shown from previous reports of this cohort that these patients have received therapy according to international recommendations [22-24]. Another weakness is the lack of information regarding extra-renal and relapsing disease. Relapsing disease in particular is associated with increased treatment length and cumulative doses of immunosuppressive drugs that might affect risk of cancer development. Also, owing to a lack of sufficient data, we could not calculate pooled SIRs for single-site cancers or for malignancy after excluding post-transplant cancers.

In summary, we have demonstrated that the risk of malignancy in patients with AAV and glomerulonephritis is not significantly increased. However, significantly increased NMSC, hematologic malignancy, and post-transplantation cancer risks were found. These findings indicate the presence of associations, although relatively weak, between immunosuppression and cancer development. However, recently published data suggest that substituting CYC with rituximab could eliminate the risk of developing NMSC. Our findings confirm that the long-term international efforts of developing CYC-minimising strategies had an important beneficial effect on cancer morbidity in patients with AAV.

Disclosure

None.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this article.

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Authors' Contributions

All authors contributed to the generation of hypotheses. T.Å.M. and B.M. performed statistical analyses, S.S.[1] and R.B. drafted the manuscript, and all of the authors contributed to revisions and approved the final version of the manuscript.

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Table Headings

Table 1. Baseline demographics of 419 Norwegian patients with AAV

Table 2A. Standardised incidence ratios for cancers in all sites in the study population

Table 2B. Standardised incidence ratios for the most common organ-specific cancers in the study population

Table 3. Studies on cancer incidence in patients with AAV

Legend to figure

Figure 1. Forest plot showing the risk of malignancy except for non-melanoma skin cancer in observational studies of patients with ANCA-associated vasculitis.

SIR, standardised incidence ratio; 95% CI, 95% confidence interval

Table 1.

Characteristic	Total	Non-Malignancy	Malignancy	P-value
Age (median, IQR)	62 (49-72)	61 (48-72)	65 (56-73)	0.04
Male sex (%)	229 (55%)	200 (53%)	29 (71%)	0.03
eGFR (median, IQR)	23 (11-46)	24 (11-47)	19 (9-39)	0.29
C-ANCA positivity	237 (57%)	213 (56%)	24 (59%)	0.89

AAV, ANCA-associated vasculitis; IQR, interquartile range; eGFR, estimated glomerular filtration rate, mL/min/1.73 m^2; C-ANCA, cytoplasmic ANCA

Table 2A.

Characteristic		Observed	Expected	SIR	95% CI
All		41	37.5	1.09	0.81 to 1.49
Non-NMSC		34	35.4	0.96	0.69 to 1.34
Sex					
	Male	29	22.9	1.27	0.88 to 1.83
	Female	12	14.6	0.82	0.47 to 1.44
Follow-up period					
	0-1 year	3	4.3	0.70	0.22 to 2.16
	1-5 years	22	14.4	1.53	1.01 to 2.32
	5-10 years	11	11.1	0.99	0.55 to 1.78
	>10 years	5	7.6	0.66	0.27 to 1.57
Transplantation					
	Yes	7	3.3	2.12	1.01 to 4.44
	No	34	34.2	0.99	0.71 to 1.39
ANCA serology					
	C-ANCA	24	20.6	1.17	0.78 to 1.74
	P-ANCA	17	16.9	1.01	0.63 to 1.62
Study period					
	1988-2002	26	22.7	1.14	0.78-1.68
	2003-2012	15	14.8	1.02	0.61-1.68

SIR, standardised incidence ratio; 95% CI, 95% confidence interval; NMSC, non-melanoma skin cancer; C-ANCA, cytoplasmic ANCA; P-ANCA, perinuclear ANCA

Table 2B.

Organs	Observed	Expected	SIR	95% CI
NMSC	7	2.1	3.40	1.62 to 7.14
Hematologic	4	1.1	3.52	1.32 to 9.37
Lung	7	4.0	1.73	0.83 to 3.63
Colon	2	1.2	1.73	0.43 to 6.93
Urothelium	3	2.0	1.48	0.47 to 4.59
Prostate	5	7.0	0.72	0.30 to 1.73
NHL	1	1.2	0.86	0.12 to 6.12

SIR, standardised incidence ratio; 95% CI, 95% confidence interval; NMSC, non-melanoma skin cancer; NHL, non-Hodgkin lymphoma

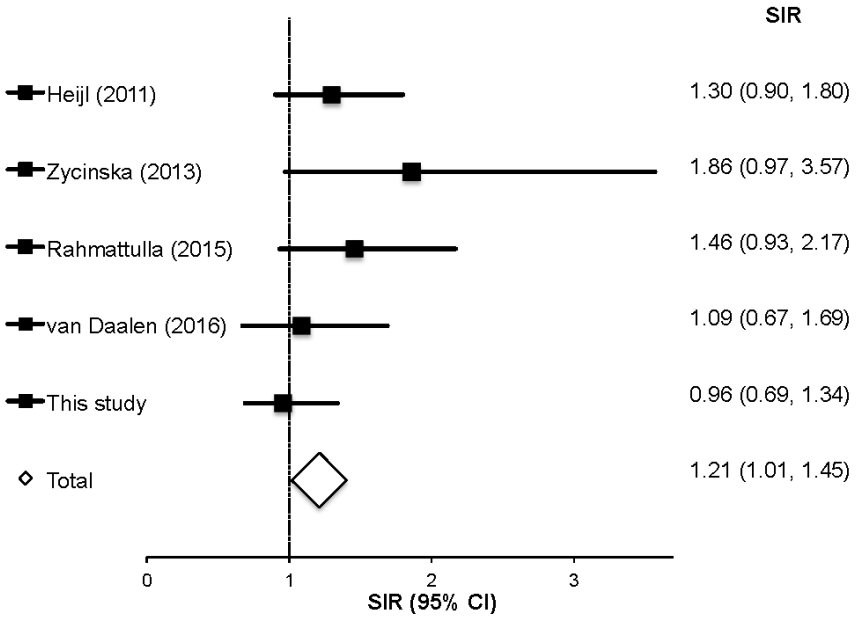
Table 3.

Characteristic	Heijl (2011)	Zycinska (2013)	Rahmattulla (2015)	van Daalen (2016)	This study	Total
Study period	1995-2007	1990-2008	1991-2013	2000-2014	1988-2012	
Number of patients	535	117	138	323	419	1532
Cumulative person-years	2650	NR	1339	1802	3010	8801
No. of observed cancers	50	15	85	45	41	236
No. of expected cancers	31.7	6[a]	38.5[a]	23.8	37.5	137.5
SIR (95% CI)	1.58 (1.17 to 2.08)	2.50 (1.20 to 2.90)	2.21 (1.64 to 2.92)	1.89 (1.38 to 2.53)	1.09 (0.81 to 1.49)	1.72 (1.51 to 1.95)
All non-NMSC sites						
No. of observed cancers	35	9[a]	24	20	34	122
No. of expected cancers	25.9	4.8[a]	16.4[a]	18.33	35.4	100.8
SIR (95% CI)	1.30 (0.90 to 1.80)	1.86[a] (0.97 to 3.57)	1.46 (0.93 to 2.17)	1.09 (0.67 to 1.69)	0.96 (0.69 to 1.34)	1.21 (1.01 to 1.45)

[a]Not reported; calculated by the authors of this study

SIR, standardised incidence ratio; 95% CI, 95% confidence interval; NR, not reported; NMSC, non-melanoma skin cancer

Figure 1


SIR
Heijl (2011)
1.30 (0.90, 1.80)
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1.09 (0.67, 1.69)
This study
0.96 (0.69, 1.34)
Total
1.21 (1.01, 1.45)
0
1
2
3
SIR (95% CI)