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Clinical and Epidemiological Studies of Wegener´s Granulomatosis

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Abstract

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Wegener´s granulomatosis (WG) is an unusual, serious, systemic vasculitis with specific clinical findings. The studies in this thesis aim at broadening our understanding of the aetiology and outcome of WG.

Patients with WG were identified in the In-patient Register 1975-2001. During this time the incidence increased three-fold, and neither ANCA-related increased awareness, nor diagnostic drift, seem to fully explain this trend, but it is still unclear if a true rise in incidence exists.

Anti- neutrophil cytoplasmic antibodies (ANCA) have been presented as highly specific for vasculitis. In a series of consecutive cANCA/PR3-ANCA positive patients, we investigated the positive predictive value for ANCA, and the outcome of patients with a positive cANCA/PR3-ANCA but not vasculitis. These patients have a low future risk of developing vasculitis, possibly indicating that ANCA, in this setting, reflects neutrophil activating properties not specific to vasculitis.

By linkage of the WG-cohort, and randomly selected population controls, to the Multi-generation register, we identified all first-degree relatives and spouses of patients and controls, totally encompassing some 2,000 patients and 70,000 relatives. Familial aggregation of WG was the exception, with absolute risks of < 1 per 1000.However, relative risks in first-grade relatives amounted to 1.56 (95% CI 0.35-6.90) such that a moderate familial aggregation cannot be excluded.

In the WG-cohort, cancer occurrence and risk was compared to that of the general population. Patients with WG have an overall doubled risk of cancer, with particularly increased risks of bladder-cancer, haematopoietic cancers including lymphomas and squamous skin-cancer. In a case-control study nested within the WG-cohort, treatment with cyclophosphamide was compared among bladder-cancer patients and matched cancer-free controls. Absolute risk of bladder cancer as high as 10% some years after diagnosis were found, and this risk can partly be attributed to cyclophosphamide-treatment, with a dose-response relationship.

Keywords: Wegener´s granulomatosis, incidence, time-trends, ANCA, familial aggregation, cancer risks, bladder cancer, cyclophosphamide

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List of papers

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I **Increasing Incidence of Wegener´s granulomatosis in Sweden, 1975-2001.** Knight A, Ekbom A, Brandt.L, Askling J. *J Rheum 2006; Vol 33:10 October*

II **What is the significance in routine care of C-ANCA/PR3-ANCA in the absence of systemic vasculitis? A case-series** (submitted)

III **Risks and relative risks of Wegener´s granulomatosis among close relatives patients with the disease** (submitted)

IV **Cancer incidence in a population based cohort of patients with Wegener´s granulomatosis.** Knight A, Asking J, Ekbom A. *Int J Cancer: 100, 8-85 (2002)*

V **Urinary bladder cancer in Wegener´s granulomatosis, risks and relation to cyclophosphamide.** Knight A, Askling J, Granath F, Sparen P, Ekbom A. *Ann Rheum Dis 63:1307-1311 (2004).*

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Contents

Abbreviations

ACR	American College of Rheumatology
ANA	Anti-nuclear antibody
ANCA	Anti-neutrophil cytoplasmic antibody
CHCC	Chapel Hill Consensus conference
CD	Cluster of differentiation
CNS	Central nervous system
CP	Citrullinated protein
ELISA	Enzyme-linked immuno-sorbent assay
ESR	Erythrocyte sedimentation rate
HLA	Human leucocyte antigen
IBD	Inflammatory bowel disease
ICD	International classification of disease
IDDM	Insulin dependent diabetes mellitus
IFN	Interferon
IIF	Indirect immuno-fluorescence
IL	Interleukin
IVIG	Intravenous immuno-globulin
MPA	Microscopic poly-angiitis
MPO	Myeloperoxidase
MS	Multiple sclerosis
NHL	Non-Hodgkin lymphoma
NRN	National registration number
OR	Odds ratio
PAN	Polyarteritis Nodosa
PPV	Positive predictive value
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RR	Risk ratio
SIR	Standardised incidence ratio
SMR	Standardised mortality ratio
SLE	Systemic lupus erythematosus
TNF	Tumour necrosis factor
UC	Ulcerous colitis
WG	Wegener´s granulomatosis

Introduction

This thesis includes studies of Wegener´s granulomatosis, a rare, small and medium-sized vessel vasculitis of unknown origin. The overall aim of this work has been to increase our knowledge of the occurrence and outcome of Wegener´s granulomatosis. Access to nation-wide population-based registers has made it possible to conduct large studies of incidence, outcome and familial associations of this uncommon disease.

There has been a general belief that the incidence of Wegener´s granulomatosis is increasing and this notion has been supported by several international studies. To further elucidate this subject, we conducted a nation-wide population based register-study of the incidence of Wegener´s granulomatosis in Sweden 1975-2001 (Paper I).

Owing to the fact that a large fraction of patients with Wegener´s granulomatosis have a positive anti-neutrophil cytoplasmic antibody-test (ANCA-test), Wegener´s granulomatosis is included in the so-called ANCA-associated vasculitis (AAVs), which, according to the Chapel Hill International Consensus Conference, also include microscopic polyangiitis and Churg-Strauss syndrome. To further understand the role of ANCAs in disease, we have assessed the outcome of a group of ANCA positive patients who *did not* have vasculitis at the time of testing. We also calculated the positive predictive value of ANCA testing for Wegener´s granulomatosis in a clinical setting. (Paper II).

In many rheumatological and autoimmune disorders, like rheumatoid arthritis, systemic lupus erythematosus and pelvospondylitis, the genetic background has been studied and described. However, the possible inherited susceptibility to Wegener´s granulomatosis is unknown. In a large population-based register study of Wegener´s granulomatosis we have assessed the possible familial aggregation of Wegener´s granulomatosis (Paper III).

Reports suggest an increased risk of urinary bladder cancer in Wegener´s granulomatosis. We assessed the occurrence of bladder cancer in a population-based cohort of Wegener´s granulomatosis, and also made a general assessment of any cancer in the same cohort. In a case-control study, nested within the same cohort, we assessed the risk of bladder cancer in relation to exposure to cyclophosphamide (Papers IV and V).

Background

Wegener´s granulomatosis

Classification and diagnosis

Wegener´s granulomatosis was first described in 1931 by Klinger in one patient, as a variant of polyarteritis nodosa (1), but in 1936 the German pathologist Friedrich Wegener defined the disease as a distinct clinical and pathological entity (2, 3).

Wegener´s granulomatosis is a relapsing necrotizing, granulomatous vasculitis of unknown origin, which typically involves the upper and lower respiratory tract and the kidneys, but involvement of skin, eyes, CNS, genitourinary and gastrointestinal tract has been frequently described (4). A more limited form of the disease engages the upper airways only, although subclinical inflammation in other organs, for example in the kidneys, can be found according to some reports (5), and a majority of patients eventually develop generalised disease (6).

Nasal congestion with secretion is a common symptom at presentation, and upper airway involvement occurs in 95% of the cases during the course of the disease, often as the first symptom. On physical examination inflammation of the nasal mucosa with oedema and bleeding may be seen. Computed tomography reveals thickening of the sinus mucosa. Blood supplies to the nasal septum, as well as to other cartilage and bone in the area, can be disrupted, causing the nasal bridge to perforate or collapse and giving the patient a typical appearance with a “saddle nose”. Subglottic tracheal stenosis, a potentially life threatening complication, occurs in about 10-20% of patients (7).

The lungs are affected in about 85% of the patients. Radiographic findings include bilateral nodular pulmonary infiltrates, cavities and ground-glass

infiltrates caused by alveolar haemorrhage, although other findings such as pleural effusions and transient infiltrates are also common.

Kidney involvement in the form of glomerulinephritis eventually develops in about 80% of the patients, although only 20% have renal disease at presentation. The glomerulinephritis is asymptomatic but can rapidly progress to acute renal failure. The glomerulinephritis is described as "pauci-immune" being focal, segmental, crescentic and necrotizising, but with few or no immune-complex deposits. Uro-genital involvement, with granulomas and inflammation in urethra, prostate, testis and bladder, has also been described (8).

Joint and muscle involvement, ranging from arthralgias and myalgias to oligo-articular arthritis, develops in two thirds of the patients. Eye involvement occurs in about 50% of patients, including scleritis, episcleritis and retro-orbital mass. Vision can be threatened. Skin lesions include palpable purpura, nail-fold infarctions and leucocytoclastic vasculitis. Peripheral neuropathy, often in the form of mono-neuritis multiplex occurs in 20 % of patients (Table 1).

Table 1. *Manifestations of Wegener´s granulomatosis*

Symptom	% of patients	% of patients at presentation
Upper airways	92	75-90
Lungs	85	45-70
Kidneys	80	20
Joints	80	40
Eyes	52	16
Skin	46	15
Nerves	20	5

Most aspects of Wegener´s granulomatosis are similar at all ages, but subglottic stenosis and nasal deformity is more common in childhood and adolescence (9).

According to the 1990 American College of Rheumatology (ACR) criteria for the classification of Wegener´s granulomatosis (Table 2), the diagnosis is made on the combination of clinical features and findings and a biopsy confirming granulomatous inflammation (10).

The Chapel Hill Consensus Conference (CHCC) proposed pathological definitions to discriminate between vasculitic diseases (11) (Table 3).The finding of a positive PR3-ANCA, which is not included in either of these classification criteria, supports the diagnosis (12).

Table 2. *The 1990 American College of Rheumatology criteria for the classification of Wegener´s granulomatosis (10)*

Criterion	Definition
1.Nasal or oral inflammation	Development of painful or painless oral ulcers or bloody nasal discharge
2.Abnormal chest radiograph	Chest radiograph showing the presence of nodules, fixed infiltrates or cavities
3.Pathological urinary sediment	Micro-hematuria or red cell casts in urine sediment
4.Granulomatous inflammation on biopsy	Histological changes showing granulomatous inflammation within the wall of an artery or in the peri- or extra-vascular area

For purpose of classification the patient is said to have Wegener´s granulomatosis if at least two of these four criteria are present.

Table 3.*The Chapel Hill Consensus Conference criteria for Wegener´s granulomatosis (11).*

Name	Definition
Wegener´s granulomatosis	Granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small to medium sized vessels, eg capillaries, venules, arterioles and arteries. Necrotizising glomerulinephritis is common.

Biopsies are not always conclusive. Histological changes are often patchy and a large amount of tissue is needed for diagnostic purposes. Only about 20 % of biopsies from nose, ear and throat are positive, and in transbronchial biopsies only 7%, but open lung biopsy from radiographically abnormal parenchyma is positive in 91 % (13).

The major findings in lung biopsies are parenchymal necrosis, vasculitis and granulomatous inflammation, but there is great variation and a broad spectrum of disease manifestations in the lung, emphasizing the complicated differential diagnoses that must be considered (14). Findings at kidney biopsies are segmental, pauci-immune inflammation, but granulomas are rarely found (15). Biopsies from skin are seldom conclusive as the skin pathology of Wegener´s granulomatosis is not specific for the disease.

Chest radiographs should always be performed as up to one third of patients without any pulmonary signs or symptoms have an abnormal chest radiograph.

Mandatory laboratory tests include blood tests of renal function, complete blood count, erythrocyte sedimentation rate (ESR), urinanalysis to look for red-cell casts, and a blood test for ANCA.
Diagnosing Wegener´s granulomatosis can be a challenge and it is important to distinguish it from other diseases, as listed in Table 3, as treatments are vastly different.

Table 4. *Important differential diagnosis of Wegener´s granulomatosis*

Infections
Malignancies (especially lymphoproliferative disease)
Connective tissue disease (systemic lupus, rheumatoid arthritis)
Other Granulomatous diseases (sarcoidosis, Crohn´s disease)
Other causes of glomerulinephritis and vasculitis (MPA, post streptococcal nephritis)

Epidemiology

Wegener´s granulomatosis can occur at all ages, and has been reported from infancy to old age. Approximately 15% of patients are less than 19 years at diagnosis (16) but the disease is most common among upper-middle-aged individuals with a peak at 64-75 years (17, 18).
According to some studies, Wegener´s granulomatosis is slightly more common among men than among women (19, 20).

Being an uncommon disease, the incidence is difficult to assess. As a result of this, data on incidence and prevalence have been scarce. In previous studies, covering mainly the 1990´s, the number of cases has been small and incidence figures have varied between studies (18, 21, 22).

The prevalence of Wegener´s granulomatosis in the United States has been approximated at 3/100,000 persons in a study undertaken 1979-1988 (18) and in Northern Norway more recent studies have revealed a prevalence of 9.5/100,000 (22).

From the south of Sweden, a prevalence as high as 19.7/100,000 was reported in 2006 (Mohammad ACR abstract 06)

Although an increasing prevalence most likely reflects an increasing survival from Wegener´s granulomatosis as a result of more effective treatment and care, incidence data from Europe have also indicated an increasing incidence of Wegener´s granulomatosis during the 1980´s and 1990´s. A possible greater physician awareness of Wegener´s granulomatosis with the introduc-

tion of ANCAs in the late 1980´s has been postulated as an explanation for the increased incidences noted (23, 24).

In a study from Norway, the annual incidence of Wegener´s granulomatosis increased from about 5 per million in the mid-eighties to 12 per million in the mid-nineties, representing the highest presented incidence rate so far. (22).These figures are similar to those recently presented from Finland with a five-fold increase in incidence between 1981-2000 (Takala et al Scand J Rheum 2006; 35 Supplement 121 abstract)

Table 5. *Incidence of Wegener´s granulomatosis in Europe 1980-2001 (per100 000)*

Country	Period	Population	Patients	Incidence	Reference
UK	1980-89	1 300 000	18	0.15-0.6	Andrews(23)
UK	1988-93	500 000	21	0.85	Car-ruthers(24)
Sweden	1975-86	200 000	19	1.6	Tidman (25)
Norway	1984-98	460 000	55	0.5-1.2	Koldings-nes(22)
UK+ Spain	1988-98	600 000	48+11	1.0 and 0.5	Watts(21)
Germany	1998-99	4 900 000	33+28	0.95	Reinhold-Keller(26)
Spain	1988-2001	200 000	12	0.3	Gonzalez-Gay(17)
UK	1988-97	430 000	40	1.0	Watts(27)
Germany	1998-2002	2 780 000	120	0.6-1.2	Reinhold-Keller (28)
Finland	1981-2000	5 000 000	487	0.2-1.0	Takala (abs)

Table 6. *Prevalence of Wegener´s granulomatosis in Europe (per 100.000)*

Country	Year	Population	Patients	Prevalence	Reference
N Germany	1999	449.498	27	6.0	Reinhold-Keller(26)
S Germany	1999	426.485	21	5.0	Reinhold-Keller (26)
Norway	1998	464.000	55	12.0	Koldingsnes (22)
UK	2000	429.000	46	10.9	Watts (27)
France	2002	1 093.515	24	2.4	Mahr (29)
Sweden	2003	287.479	45	15.7	Mohammad (abs)

Further studies from Great Britain, Spain and Norway have reported a north south gradient, indicating that Wegener´s granulomatosis may be more common in Northern Europe (20), possibly indicative of different exposures to triggering events such as infections. In a recent study from the southern hemisphere (30), the prevalence of Wegener´s granulomatosis in New Zealand was as high as that reported from Northern Norway (22). The population studied was 91% New Zealand European i.e. of Caucasian origin, and only 9 % Maori, Pacific Islanders, Chinese, Indians or others, reflecting the population of NZ. Further studies in the Southern hemisphere will be able to determine if the north-south gradient in the Northern hemisphere is reciprocated (30).

A seasonal variation in the onset of disease has been found in studies from Sweden (25) and from the United States (31), both indicating that the incidence of Wegener´s granulomatosis may peak during winter and spring months (December-May). Other studies have not been able to confirm this (18).

The disease predominantly affects patients of Caucasian origin (32), on the other hand studies from non-Caucasian populations are sparse. In a French urban multi-ethnic population consisting of 1.1 million adults, 28% being of non-European ancestry, the overall prevalence of vasculitides in the European population was twice that of the population of non-European ancestry. Of the total number of Wegener´s granulomatosis patients (n=21) only 3 (14%) were of non-European ancestry. Such a skewed distribution was not observed for other vasculitides (29).

Aetiology and pathogenesis

The aetiology of Wegener´s granulomatosis is unknown. Involvement of the upper airways and lungs with granulomatous inflammation suggests that Wegener´s granulomatosis may be initiated by an aberrant cell-mediated immune response to an exogenous antigen entering through the respiratory tract (33). But the fact that Wegener´s granulomatosis is associated with the occurrence of anti-neutrophil cytoplasmatic antibodies (ANCAs) suggests that humoral immunity also may play a role in pathogenesis (34).

Possible environmental factors triggering disease have been extensively studied. The seasonal variations reported, with a winter-spring peak in disease onset, could be supportive of an infectious trigger. It is well known that infections can be related to vasculitis, for example hepatitis B associated PAN, cryoglobulinemic vasculitis with hepatitis C, HIV and meningococcal septicaemia (35).

Silica exposure has been related to vasculitis (36) and in a study from Great Britain, silica exposure was significantly associated with primary systemic vasculitis (37).

Along with silica, inhaled fumes, particulates and pesticides have been associated with Wegener´s granulomatosis (38). Conflicting information on farming and the risk of vasculitis has been presented: in a study from the National Institute for Health, no such association was found (38) but in a study from Great Britain in 2003, farming was a significant risk factor for Wegener´s granulomatosis (37).

Reports of an association between allergy, especially between rhinitis, skin, drug and insect allergies, and Wegener´s granulomatosis have also been presented (37, 39).

Various drugs, including propylthiouracil, have also been associated with the initiation of AAVs, including cases of Wegener´s granulomatosis (40-42).

Is Wegener´s granulomatosis an autoimmune disease? The strong association of Wegener´s granulomatosis with the appearance of anti-neutrophil auto antibodies and the identification of the corresponding antigen proteinase-3 is indirect evidence of auto-immunity, as is the favourable response to immunosuppressive therapy.

But not all Wegener´s granulomatosis patients are ANCA-positive. Reports on correlation between ANCA and disease activity are diverging (12, 43-45), and direct evidence of auto-immunity is lacking. Antiglomerular basement membrane disease constitutes the classical example of the transfer of pathogenetic auto-antibodies in experimental animals. No such induction of disease has been shown with transfer of ANCAs. Trans-placental transfer of antibodies with evidence of disease in the foetus has not been reported. Non-pathogenic transfer of atypical ANCA to three patients receiving IVIG has been documented (46), so the modified Koch´s postulate for defining auto-immunity has not been completely fulfilled.(Witebsky´s modification of Koch´s postulate: An auto-antibody or a cell-mediated immune response must be recognized; the corresponding antigen must be identified; an analogous auto-immune response must be induced in experimental animals and the immunized animal must develop similar disease (47).

In summary, no specific environmental antigen(s), whether infectious or other, has yet been identified for Wegener´s granulomatosis. There is strong evidence that the disease is immunologic in origin. It is possible that the immunological events are triggered by one or several endo-or exogenous antigen(s).

A. Granulomatous inflammation

Granulomas can be defined as a focal, chronic, mononuclear tissue reaction to poorly degraded ingested antigens, first described by Virchow in the context of tuberculosis. Granulomas are the normal host defence reaction to infection with various intracellular pathogens such as mycobacteria (48), but it also occurs in hypersensitivity reactions to foreign antigens, as in sarcoidosis (49).
The common factor is the presence of an antigen. The granulomas´ function can be described as an attempt to contain the inflammatory focus within the granuloma, thereby minimizing damage to surrounding tissue. Both functional T cells and mature macrophages are necessary for the formation of an efficient granuloma; CD4+T cells and neutrophils are recruited, monocytes are differentiated to mature macrophages or epithelioid giant cells and form a tight granuloma surrounded by a rim of T cells. B cells are also present in the periphery of the granulomas, sometimes forming up to 20% of the cellular component (50, 51).Cytokines and chemokines play a vital role in coordinating granuloma formation and current investigations have suggested IFNγ, IL-12 and TNF as key actors in the formation and maintenance of the granulomas (48).Findings suggest that patients with Wegener´s granulomatosis have an immuno-regulatory defect that leads to excessive production of Th1 cytokines, mainly TNF and IFN-χ, by activated CD4+ positive cells in response to antigens such as infections or auto antigens. It may be that this excessive cytokine production initiates and maintains the granulomatous inflammatory vascular lesions characteristic of Wegener´s granulomatosis (33).

B. Anti-neutrophil cytoplasmic antibodies

PR3-ANCA is the predominant auto-antibody found in approximately 90% of patients with generalised Wegener´s granulomatosis.
PR3 is a 29kDa protein localized in the azurophilic granula of neutrophils and monocytes (52).

The majority of the PR3 found extracellularly is in its mature, active form. The resting neutrophil expresses PR3 on its membrane surface and this is increased by neutrophil activation in patients with acute inflammatory conditions, such as sepsis (53). PR3 has anti-microbial properties and an enzymatic activity that can cause both tissue damage and degradation of intracellular materials.

PR3 has several functions: it enhances neutrophil migration (54), endothelial cell binding and activation in vitro (55), cleavage of TNFα to a biologically more active form (56) and degradation of C1-inhibitor (57).The func-

tion of PR3 is in turn regulated by the serine proteinase inhibitor α-1-antitrypsin (58).

Several in-vitro observations suggest possible mechanisms for the contribution of ANCAs to the pathogenesis of Wegener´s granulomatosis. ANCA activated neutrophils can adhere to and kill endothelial cells in vitro (59) and also induce the release of pro-inflammatory cytokines such as IL-1 and IL-8 (60, 61).

Table 7. *Experimental evidence of the pathogenetic role of ANCA*

Effects of ANCA on neutrophils
1. Priming and apoptosis of neutrophils results in cell membrane expression of target antigens, making them accessible for ANCA.
2. Degranulation and oxidative burst of normal neutrophils primed with TNFα by binding simultaneously to Fcγ-RII receptor and to the corresponding antigen expressed on the cell surface
3. Activation of neutrophils by induction of the production of a leucotriene, a chemo-attractant for neutrophils
4. Induction of expression of IL-1β and IL-8 in neutrophils
Effects of ANCA on monocytes
5. Activation of monocytes by production of monocyte chemo-attractant-1
Effect of ANCA on endothelial cells
6. Induction of expression of adhesion molecules and enhancing the adhesion of neutrophils and mononuclear cells to the endothelium
Effect of ANCA on proteinase-3
7. Prevention of inactivation of PR3 by the natural inhibitor α 1-antitrypsin

No animal model has been able to prove that ANCAs mediate vascular injury, although experimental models with PR3 knockout-mice pre-treated with TNFα to activate the inflammatory response, and then treated with PR3-ANCA containing sera, showed larger inflammatory sites compared to mice treated only with TNFα. These experiments were interpreted as evidence of a potential for ANCAs to exacerbate inflammation initiated by primary stimulus (33).

However, several clinical observations argue against ANCA having a primary role in the pathogenesis of Wegener´s granulomatosis. Some patients with active Wegener´s granulomatosis do not exhibit a positive ANCA (62), the level of antibodies do not correlate well with disease activity (63) and Wegener patients in remission can still continue to have high antibody titres (45).

In summary, although the aetiology and pathogenesis of Wegener´s granulomatosis remains to be elucidated, current evidence supports the hypothesis that the initial event is an exposure to environmental antigen(s) that induce excess macrophage IL-12 response, leading to an unbalanced T cell response, mainly Th1, characterized by an overproduction of IFNγ and TNFα. This is followed by the establishment of a granulomatous inflammation as described above. The final event is inflammation-induced tissue breakdown and the release of intracellular material such as proteinase-3, which causes further immune response, in turn amplifying the primary inflammatory lesion.

The inflammation in the blood vessels leads to tissue damage and clinical symptoms in two ways: narrowing the vessel lumen leading to organ ischemia, and thinning of the vessel wall leading to aneurysms and haemorrhage.

However, the possible antigen(s) initiating this process, as well as the reason why the inflammation is not adequately controlled by the immunoregulatory system, remains unknown.

ANCA-testing

Though it originally was thought to be a response to arbo-virus infection, anti-neutrophil cytoplasmic antibody (ANCA), first described in 1982, emerged as a new diagnostic tool in the work-up of vasculitic disease in the 1980s (64). Neutrophil auto-antibodies have been assumed to exist since the indirect immunoflourescense technique (IIF) in the 1960s became a popular screening method for detecting auto-antibodies to tissue structures and cells. Two different ANCA patterns can be seen with IIF of ethanol fixed neutrophils: a cytoplasmic pattern, cANCA and the artifactual pattern pANCA. (65, 66). The major antigen for pANCA is myelo-peroxidase, a lysosomal enzyme found in neutrophils and the antigen for cANCA is proteinase-3. The cytoplasmic ANCA pattern (cANCA) has predominantly been associated with Wegener´s granulomatosis (67) and the peri-nuclear pattern (pANCA) with microscopic polyangiitis and other vasculitides.

One disadvantage of the IIF test for ANCA is that it is not an antigen-specific assay. It is therefore recommended that the target antigen reactivity of any ANCA detected by IIF should be verified. The use of a standardized

ELISA (enzyme-linked immuno-sorbent assay) for the detection of PR-3 and myelo-peroxidase adds specificity to testing. According to the International Consensus Statement on testing and reporting of Anti-neutrophil Cytoplasmic antibodies (68), ANCA is best demonstrated using a combination of IIF and ELISA that detects ANCA specific for proteinase-3 (or myeloperoxidase).

In a meta-analysis of the literature on IIF ANCA (cANCA)-testing for Wegener´s granulomatosis the sensitivities of cANCA for overall Wegener´s granulomatosis ranged from 34 to 92%, and the specificities from 88 to 100% (69). Disease activity was also assessed when presented in the studied literature (four articles). For active disease the pooled sensitivity was 91% and the pooled specificity 99%, for inactive disease the corresponding percentages were 63% and 99% respectively. The conclusion was that although c-ANCA results may serve clinicians in diagnosing Wegener´s granulomatosis, these studies imply that the ANCA- result must be viewed in the context of the patients' clinical picture and the prevalence of Wegener´s granulomatosis in the clinical setting at hand.

Likewise, in an evaluation of commercial immuno-assays for anti-neutrophil cytoplasmic antibodies directed against proteinase-3 (ELISA), there was great variation in test performance (70). In patients with a clinical and histologically proven diagnosis of Wegener´s granulomatosis, the sensitivities, ranged from 22 to 70%, and the specificities ranged from 93 to 100%.

The authors' conclusion is that IIF remains the "Gold standard" in ANCA-testing for Wegener´s granulomatosis. On the other hand, a group from Australia has argued for the technical advantages of the ELISA over IIF, as IIF is a subjective test that relies on the experience of the interpreter, and that there are difficulties associated with the distinction between perinuclear immunoflourescense and antinuclear antibodies (71).

One problem of commercial immuno-assays is the varying sensitivity caused by problems in choosing the optimal cut-off level. By assessing the optimal cut-off for the individual test kit, as done in a study by Holle et al, sensitivities of the PR3-ANCA increased to between 53 and 86%. For all tests analysed, changes in cut-off values were able to improve their overall performance (72).

By combining IIF with ELISA, as suggested by The International Consensus Group on ANCA-testing, the value of antigen detection can be increased. In a study from 1998, the combined testing increased the sensitivity for Wegener´s granulomatosis from 64 to 73% (62) .

Capture-ANCA is an alternative ELISA method for PR3-ANCA, where a monoclonal antibody is used to capture proteinase-3, the result being a higher sensitivity combined with high specificity (73, 74).

What must be remembered is that the published tests on specificity and sensitivity have been performed in highly selected patient populations with confirmed disease to determine the tests´ operating characteristics. In a clinical situation, and when tests are applied to unselected populations, the tests´ discriminating capacity is less convincing. cANCA, as well as PR-3 ANCA has been found in a number of other, non-vasculitic conditions such as infections, inflammatory bowel disease, systemic lupus erythematosus and other rheumatic diseases and in otherwise healthy elderly people etc (62, 63, 69, 75).

To assess the likelihood that a patient with a positive test actually has the disease, the positive predictive value (PPV), which takes into account the relative prevalence of the disease in question, is a clinically more useful tool than the specificity and sensitivity (Fig 1).

Figure 1.

$$PPV = \frac{a}{a + b}$$

a = individuals tested positive who have the disease
b = individuals tested positive who do not have the disease

Wegener´s granulomatosis is highly prevalent in patients presenting the triad of upper respiratory, lung and renal disease. For such patients the PPV for ANCA is as high as 98-99% in some studies (43). However, since glomerulinephritis is present only in a minority of patients at presentation and both infectious disease and malignancy are more common than Wegener´s granulomatosis and since ANCA can be positive in a number of non-vasculitic conditions, the patients' potential for having another disease is higher. Because the prevalence of Wegener´s granulomatosis in these patients is low, the PPV for ANCA-test in this setting may be considerably lower. Among patients without a previous diagnosis of Wegener´s granulomatosis the sensitivity of ANCA may be as low as 34% (69).

Some authors have suggested test-ordering guidelines to improve test performance by specifying and thereby reducing the population subjected to testing (76). However, such guidelines are, to our knowledge, not widely used in clinical practice (Table 8).

Table 8. Clinical indications for ANCA testing as suggested by Mandl et al (76).

1. Glomerulinephritis
2. Pulmonary haemorrhage
3. Cutaneous vasculitis with systemic features; myalgia, arthralgia, arthritis
4. Multiple lung nodules
5. Chronic destructive disease of the upper airways
6. Long-standing sinusitis or otitis
7. Subglottic tracheal stenosis
8. Mono-neuritis multiplex or other peripheral neuropathy
9. Retro-orbital mass

Even when these guidelines were applied, the authors found a PPV of only 62% for ELISA-ANCA. However, the number of false positive tests decreased by 27%. No cases of vasculitis were missed.

However imperfect the ANCA-tests may be, they have become an important diagnostic aid in the work-up of vasculitic disease and are widely used in clinical practice. Our knowledge of what a false-positive ANCA, as described in a vast number of non-vasculitic conditions (63), actually means pathogenetically and for future outcome is limited. These "false-positive" ANCAs pose a problem in clinical practice and ANCA cannot serve as a basis for immunosuppressive treatment (77). Non-vasculitic conditions, most importantly infections associated with ANCA, must be ruled out in patients in whom vasculitis is suspected.

Treatment and prognosis

Untreated Wegener´s granulomatosis has a poor prognosis with a rapidly fatal course; mean survival has been reported to be 5 months and the mortality rate 82% within the first year of disease (19). The cause of death is usu-

ally acute renal failure or severe pulmonary engagement with haemoptysis (19). Advances in recognition as well as in treatment have considerably improved the outcome of the disease from being almost uniformly fatal to a chronic inflammatory disease characterized by remissions and relapses.

In the mid-sixties, glucocorticoids were reported to prolong survival to just over one year (78). In 1973, Fauci and Wolff proposed a treatment consisting of oral cyclophosphamide and high-dose steroids (prednisone 1 mg/kg daily tapered and discontinued after six to nine months and cyclophosphamide 2 mg/kg daily continued for 1 year past remission) (4), which increased survival rates to about 90 % the first year of disease (15). Even though remission can be obtained in 80 to 100% of patients, relapse of disease occurs in 50 %, usually after discontinuation of immunosuppressive therapy (79). Current treatment therapy still involves the use of high-dose corticosteroids and a cytotoxic agent, the first line choice usually being cyclophosphamide. The chronic and relapsing features of the disease are of prognostic concern due to the risk of tissue and organ damage with each relapse of disease (80) as well as the side effects caused by repeated immunosuppressive treatment (15) (Table 9).

Table 9. Complications of prednisolone-cyclophosphamide treatment for Wegener´s granulomatosis. Data from Hoffman GS et al Ann Intern Med 1992 (15).

Complication	% of patients
Sterility	> 50
Major infection	46
Cystitis	43
Cataracts	21
Fractures	11
Bladder cancer	3
Avascular bone necrosis	3
Lymhoma	2
Myelodysplasia	2

Various strategies have been tried to reduce cyclophosphamide toxicity. Intermittent intravenous cyclophosphamide as used in lupus-nephritis may be less toxic than daily oral doses but studies of Wegener´s granulomatosis have found higher rates of relapse with intermittent high dose intravenous regimens (81-83).

Using other agents to induce remission has also been tried. In milder cases of limited Wegener´s granulomatosis methotrexate as well as azathioprin has proved capable of inducing remission (84).

Changing to other agents after obtaining remission with cyclophosphamide is another way to reduce cyclophosphamide toxicity. Methotrexate has proven effective in maintaining remission induced by cyclophosphamide, the rate of relapse and the rate of serious toxicity being similar to those seen with cyclophosphamide maintenance therapy (85, 86).

Methotrexate can, however, pose a problem as it is contra-indicated in patients with renal insufficiency or severe pulmonary impairment, conditions which often are present in patients with Wegener´s granulomatosis.

An alternative may be azathioprin, as presented in a study in which patients, after remission was induced by cyclophosphamide, were randomized to treatment with cyclophosphamide or with azathioprin as maintenance therapy. Relapse rates were similar in both groups (87).

Mycophenolate mofetil has been tried as maintenance therapy with good tolerability and relapse rates around 45% (88, 89).

Other agents used are reported only in case reports and small series and include cyclopsporine, leflunomide and intravenous immunoglobulins, but these drugs should be considered only when standard regimens are contra-indicated or have proven ineffective, due to concerns about their efficacy and toxicity.

In the 1980s, trimethoprim-sulfamethoxazole (TMS) was reported to be of benefit to Wegener-patients with upper respiratory disease, although whether the positive effects were due to the antimicrobial actions or to an immunosuppressive effect remains unclear. One study has compared TMS or placebo as maintenance therapy after remission and concluded that although the recurrence of nasal and upper-airway disease was lower in the TMS treated group, there was no reduction of the relapse rate of major organ disease (90). TMS can be considered for treatment of limited discrete upper airway disease under close monitoring for the occurrence of major organ involvement but should never be used to treat glomerulonephritis or other major organ disease. However, TMS plays an important role in the prevention of Pneumocystis carinii pnemonia, which occurs in about 10% of patients receiving induction therapy with cyclophosphamide and glucocorticoids, and which may have a mortality rate of substantial 35%. Therefore, patients on induction therapy, who are not allergic to sulpha drugs, should receive prophylaxis with TMS (13).

New therapies, including the biologic agents, offer the promise of more targeted therapy and fewer side-effects. So far there are published trials on the safety and efficacy of etanercept and infliximab in Wegener´s granulomatosis. In the WGET-trial, where etanercept was combined with cyclophosphamide, a high rate of adverse events, particularly an increased occurrence of solid cancers, and an inability of etanercept to achieve durable remission,

was found. TNF-α blockers need to be further evaluated before justifying a possible role in the therapy arsenal of Wegener´s granulomatosis (91, 92).

The evidence for the role of ANCAs in the amplification of the inflammatory signals in vitro has led to attempts to specifically inhibit these antibodies. Rituximab, a chimeric monoclonal antibody directed against CD20+cells leads to B-cell depletion. Preliminary results in patients with Wegener´s granulomatosis have led to B-cell depletion and inhibition of ANCA-production and also remission induction (93, 94) .In these reports, rituximab has been well tolerated, induced remission in almost 100% of patients and has been successful also when used in the case of relapse (95, 96).However, in a pilot study of rituximab in refractory Wegener´s granulomatosis, ANCA titres failed to drop in spite of B-cell depletion, and granulomatous manifestations of the disease were not improved (97).

The possible efficacy of rituximab in Wegener´s granulomatosis and other ANCA-associated vasculitides thus needs to be further studied.

Hereditary susceptibility to Wegener´s granulomatosis

In inflammatory rheumatic auto-immune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, as well as in other auto-immune diseases like Hashimoto thyroiditis and pernicious anaemia, a hereditary predisposition is well established.

A genetic predisposition to Wegener´s granulomatosis is supported by several findings, each of which will be discussed in detail below:

1. Case reports of familial clustering of the disease
2. Varying prevalence in different ethnic groups
3. Expression of the primary antigen for ANCA being genetically determined
4. The finding of a risk allele for ANCA-positive Wegener´s granulomatosis
5. Genetic variations of CTLA-4 with enhanced T-cell activation
6. Cytokine gene polymorphisms
7. α1-anti-trypsine deficiency
8. HLA typing studies

1. Familial clustering

Shared environmental exposures, shared genes or an interaction between genes and environment may explain family clusters of disease.

Several case-reports of clustering within families, mainly limited to first-degree relatives, have been published (Table 8). Stoney et al reported Wegener´s granulomatosis in two brothers sharing the same HLA-haplotype, two further siblings being unaffected (98). Wegener´s granulomatosis in two young sisters, both parents and sisters healthy, was reported in 1986 (99). Wegener´s granulomatosis in mother and daughter, no similarity in HLA-haplotype found in a report from 1992 (100) and Wegener´s granulomatosis and Churg-Strauss syndrome in two first-degree relatives (101). On the other hand, twins discordant for Wegener´s granulomatosis has also been reported (102).

Infrequently, Wegener´s granulomatosis has been reported in distant family members; one case report presented a pedigree with three relatives with Wegener´s granulomatosis and a distant ancestor with fatal pulmonary disease of unknown cause (103).

It is important to realise that case reports lack denominator controls, and that they may well represent random incidents.

Studies of auto-immune diseases, especially on IDDM and Grave´s disease, as well as case reports, verify that clustering of auto-immune diseases within one individual exists. In 40% of Type I Diabetes mellitus families there is also some kind of auto-immune thyroid disease (104). Patients with Wegener´s granulomatosis have been reported with rheumatoid arthritis (105), Grave´s disease, Hashimoto´s thyroiditis, and relapsing polychondritis as well as with insulin-dependent-diabetes mellitus (IDDM) (103), arguing that there may be a genetic susceptibility to these diseases and that the same genes may be involved in different diseases.

Table 10. Reported familial cases of Wegener´s granulomatosis

Number of affected members	Relationship	Reference
3	2^{nd} and 4^{th} degree relatives	Nowack et al.J Am Soc Nephrol 1999 (103)
2	Siblings	Knudsen et al.Scand J Rheum 1988(106)
2	Siblings	Stoney et al. J Laryngol Otol 1991(98)
2	Siblings	Muniain et al. ARD 1986(99)
2	Siblings	Hay et al.Br J Rheum 1991(107)

2. Expression of proteinase-3

PR-3 is the main target antigen for anti-neutrophil auto-antibodies present in Wegener´s granulomatosis. Schreiber et al (108) studied a German cohort of Wegener´s granulomatosis patients and found that they demonstrated a significantly higher percentage of membrane PR3-positive neutrophils than healthy controls and patients with other inflammatory diseases. In twin studies they found that the percentage of membrane PR3-positive neutrophils was significantly correlated in monozygotic twin pairs. No correlation was found in dizygotic twins. The authors´ conclusion from these studies is that a high percentage of mPR3-expressing neutrophils are a risk factor for ANCA-associated vasculitis and that mPR3- expression is genetically controlled.

3. The PTPN22 620W allele as a risk factor for Wegener´s granulomatosis

Analyses of families with multiple auto-immune disorders have revealed a functional polymorphism, 620W, in the intra-cellular tyrosine phosphatase gene PTPN22 as a predisposing factor for type I diabetes, seropositive rheumatoid arthritis, systemic lupus erythematosus and Hashimoto thyroiditis. In these disorders the appearance of the PTPN22 protein seems to herald the development of auto-antibodies (109).Recently it has also been demonstrated that this PTPN22 620W allele frequency was significantly increased in ANCA-positive patients with Wegener´s granulomatosis compared to healthy controls. This association was particularly striking in patients with generalized Wegener´s granulomatosis (110). The impact that this described association of the PTPN22 620W risk allele to ANCA-positive Wegener´s granulomatosis, may have on a population, is yet unclear.

4. Wegener´s granulomatosis and T-cell activation

Patients with Wegener´s granulomatosis have an expanded T-cell population. The cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) mediates a negative regulation of cellular as well as humoral immune response. Animals deficient in CTLA-4 show a lethal phenotype with multiple organ inflammation including marked vasculitis. In a Swedish study a strong association between genetic variations of CTLA-4 and Wegener´s granulomatosis was found, correlating with an enhanced T-cell activity (111, 112).

5. Pro-inflammatory and anti-inflammatory cytokine genes

Cytokines such as TNF-α, TNF-β, IL-1α, IL-1β and IFNγ have important pro-inflammatory properties and have been demonstrated to be critical in granuloma formation. Other cytokines, such as IL-1Ra, IL-4, and IL-10, have an anti-inflammatory capacity and balance the immune response. Many genes encoding cytokines contain polymorphisms. In patients with Wegener´s granulomatosis, the IL-10G has been shown to be overrepresented (112). No

associations have been found with IL-4 and Wegener´s granulomatosis, or with TNF-α or IL-1β according to Swedish studies (111).

6. α1-antitrypsine-deficiency a risk factor for Wegener´s granulomatosis
The main inhibitor of PR3-ANCA is α1-antitrypsine. Studies of gene polymorphisms show that the frequency of the α1 AT deficiency allele PI*Z is increased in Wegener´s granulomatosis and strongly related not only to Wegener´s granulomatosis but also to its outcome (113, 114). Yet in a larger population study of PI*Z positive individuals, it was found that they did not suffer from any vasculitic symptoms. The incidence of the deficient α1- AT phenotype in ANCA-positive patients with Wegener´s granulomatosis is probably low, but its clinical relevance is emphasized by their poorer outcome (115).

7. HLA typing
No consistent relationship with human leukocyte antigen (HLA) alleles has been identified. A strong association with HLA-DR1 and the combined frequency of DR1-DQw1 and Wegener´s granulomatosis has been demonstrated in one population (116). In others, increases in HLA-B7, B8, B50 and DR9, DQw7, DR2, and DR4DQ7 have been described, in contrast, HLA-DR3 and HLA-DR13 and DR6 were under-represented.

8. Varying prevalence in different ethnic groups
80 to 97 % of patients with Wegener´s granulomatosis are of Caucasian origin and the disease is extremely rare in blacks (32). On the other hand studies in other ethnic environments are sparse. In a French urban multi- ethnic population, a significantly higher prevalence of small vessel vasculitides, including Wegener´s granulomatosis, was noted in subjects of European ancestry compared to non-Europeans (emanating from North Africa, Turkey and the French overseas territories), but the study only included a total of 21 patients with Wegener´s granulomatosis (29).
However, Wegener´s granulomatosis is also being increasingly recognised in India (117).

In summary, the genetics of Wegener´s granulomatosis are complex and involve multiple genetic factors. It seems likely that Wegener´s granulomatosis, like most autoimmune diseases, is polygenic, with no single gene being either necessary or sufficient for disease development.

Inflammatory disease and cancer

Cancer risks are known to be elevated in several rheumatic conditions and other chronic inflammatory diseases. For instance, there is an increased risk of lymphoma in patients with Sjögren´s syndrome (118) and rheumatoid arthritis (119), and for cancer of the intestine in patients with inflammatory bowel disease (IBD) (120). Also in systemic lupus erythematosus (121), sarcoidosis (122) and coeliac disease (123), increased occurrence of cancer has been reported. In the case of rheumatoid arthritis the increased lymphoma risk has been related to high inflammatory activity in the arthritic disease (124, 125), and in inflammatory bowel disease the increased risk for colorectal cancer is most likely to be a consequence of the inflammation (126, 127).

The potential mechanisms for cancer development in inflammatory disease include:

- Factors directly related to the inflammatory disease, i.e. extent or duration of inflammation
- Treatment used for the inflammatory disease; often immunosuppressive drugs with established effects on cell maturation and DNA replication
- Shared genetic predispositions for both the cancer and the inflammatory disease
- Shared environmental risk factors, for example viruses (Epstein-Barr virus) or tobacco smoking

Concerning Wegener´s granulomatosis, past studies have indicated an increased cancer risk, including a highly significant increased risk of urinary bladder cancer, risks that have been attributed to exposure to cyclophosphamide (128). In a recent study of etanercept and cyclophosphamide in Wegener´s granulomatosis, more solid tumours were observed in the combination therapy group. (92). This risk may, according to the authors, be attributed to the treatment, but the patients' age and duration of disease also play a role.

It is increasingly important to assess a possible association between cyclophosphamide, currently the mainstay induction treatment for Wegener´s granulomatosis and also the first-line treatment for a series of manifestations of more common rheumatological conditions such as rheumatoid arthritis and systemic lupus, and bladder cancer, as the prevalence of individuals with a history of cyclophosphamide exposure is likely to increase substantially in the future.

Cyclophosphamide

Cyclophosphamide is a commonly used drug in the treatment of malignant disease, for example as part of the CHOP-regimen used for malignant lymphomas. In the management of inflammatory rheumatic diseases, cyclophosphamide has proved to be highly effective and remains the standard treatment for severe extra-articular manifestations of rheumatoid arthritis, inflammatory alveolitis, and kidney- and CNS-manifestations of systemic lupus as well as for systemic vasculitides such as Wegener´s granulomatosis. Worldwide, cyclophosphamide was estimated to be used by 500,000 patients yearly in 1991 (129).

Cyclophosphamide, a cyclic phosphoramide, is an orally active form of the alkylating agent mustine (chlormethine). The fundamental pharmacological activities of cyclophosphamide are disruption of cell growth, mitotic activity, differentiation and function, mainly by cross-linking DNA strands. The capacity of cyclophosphamide to interfere with normal cell division in rapidly proliferating tissue provides the basis for its therapeutic effects as well as for many of its toxicities. Cyclophosphamide has to be metabolically activated before it can exert any of its therapeutic effects and the active metabolites are mainly generated in the liver. It is generally believed that the chlormethine metabolites are responsible for the therapeutic effects and acrolein for the toxic effects of the drug.

The most common general toxicity of cyclophosphamide includes bone-marrow depression, nausea and alopecia. The main organ specific toxicity is uro-toxicity, and to a much lesser extent pulmonary and cardiac toxicity. Sterile haemorrhagic cystitis is the main uro-toxicity, reported in up to 78% of patients with a mortality of 4% (130). In other studies the frequency of haemorrhagic cystitis ranges from 2 to 40% (131).

In a study by Cox, published in 1979, acrolein was identified as the causative agent for haemorrhagic cystitis (132), exerting a direct cytotoxic effect on the bladder epithelium. Attempts to decrease the uro-toxic properties of cyclophosphamide have led to the development of thiol compounds that inactivate cyclophosphamide in the bladder. Mesna (sodium-2-mercaptoethane-sulfonate) has been considered the most useful agent and has been shown to protect the bladder from developing haemorrhagic cystitis (132, 133). Mesna can be given orally or parenterally, it is oxidized to a stable, inactive disulphide and becomes active when excreted in the urine. Mesna has two ways of action: it binds to acrolein in the urine, hereby inactivating it, but it also has the capacity to inhibit the release of acrolein from 4-hydroxy-cyclophosphamide in the urine.

Bladder cancer has been reported as a side effect of cyclophosphamide treatment in both malignant disease, such as Non Hodgkin lymphoma (134,

135), and non-malignant conditions such as RA, systemic lupus erythematosus (136-138), and multiple sclerosis (139).

Bladder cancer

The incidence of urinary bladder cancer varies around the world, and shows a gender difference. In Egypt, bladder cancer accounts for a third of all malignancies, in contrast to in Asia where bladder cancer is very rare. In Sweden, cancer of the bladder accounts for about 7 % of all male cancers and 2, 5% of all female cancers (140). The clinical course and prognosis varies and is dependent on the stage of the cancer at diagnosis. The staging system is based on findings at palpation, biopsies from transurethral resection or cystoscopy and from radiological assessment and range from Tis (cancer in situ) to T4b (tumour overgrowth on abdominal wall or other intra-abdominal organs). The dominating histopathological type is urothelial cancer, which accounts for more than 80% of all bladder cancers in Sweden.

Like most malignancies, bladder cancer development is caused by the accumulation of various molecular changes. Through mutation or chromosomal aberration, the expression of specific oncogenes, tumour-suppressor genes, cell-cycle genes and DNA-repair genes is altered. Loss of heterozygosity of chromosome 9p and 9q has been shown to be important in the event of transformation of normal urothelium to papillary transitional cell carcinoma, while p53 is crucial in the development of carcinoma in situ (141).

Prognosis is generally good for cancer in stage Tis-T1 ,but relapse is common (71%) (142). However, in metastasized cancer or in cancer with muscular overgrowth (T2-4) the mortality is substantial within 2-5 years from diagnosis.

Treatment strategies depend on the histopathological type of cancer and staging. Tis-T1 cancers are usually locally resected. Laser-coagulation is reported to give fewer relapses and intra-vesical chemo- and immuno-therapy including intra-vesical BCG-therapy, which is the treatment of choice for cancer in situ, can be used both for prophylaxis after resection and as primary treatment. In more advanced cancers resection of the tumour or radical cystectomy is preferred.

Various risk factors and carcinogens are known to contribute to the generation of bladder cancer. A major risk factor is the compound b-napthylamine, an important carcinogen found in cigarette smoke, and smokers have a four-fold increased risk of bladder cancer (143). A higher incidence of bladder cancer is also found among workers in the rubber industry, who are heavily exposed to b-napthylamine and other aromatic amines such

as benzadine and phenacetine. Also workers exposed to coal exhausts exhibit increased incidence of urothelial bladder cancer (144).

In 1911, Ferguson reported a link between bilharziasis and squamous cell cancer of the bladder (145). Because of the papillomatous way of growth in bladder cancer, papilloma-viruses have been proposed as a possible aetiological agent (146).

Reports on possible hereditary susceptibility to bladder cancer are infrequent. Ryk et al have reported genetic polymorphism leading to altered repair that may influence the occurrence of p53 mutations in bladder cancer(147). There is also a study of familiar occurrence of bladder cancer in Sweden, reporting a relatively high sibling-to-offspring risk as well as possible gender specific effects in bladder cancer that may reflect an X-linked susceptibility gene(148).

Wegener´s granulomatosis and risk of bladder cancer

As stated above, several studies have indicated that patients with Wegener´s granulomatosis are at an increased risk of cancer of the urinary bladder(15, 128).The risk estimates have been as high as 33-fold and the increased risks have been attributed to the exposure to cyclophosphamide, but these studies rest on fewer than a dozen cases of bladder-cancer observed in two limited series (n= 145 resp. n= 158) of highly selected referral patients all treated with cyclophosphamide.

Studies of patients who have received cyclophosphamide as treatment for malignant conditions have indicated an association between exposure to cyclophosphamide and risk of bladder cancer (135), but have not indicated a dose-response relationship and the risk was not related to previous haemorrhagic cystitis. In an early study by Fairchild a nine-fold increase in the incidence of bladder cancer was noted in patients with a cancer diagnosis treated with cyclophosphamide compared to patients treated with other anti-cancer agents (149). There are also several reports on bladder-cancer occurrence in patients treated with cyclophosphamide for non-malignant disease, such as RA and systemic lupus erythematosus (136, 150).Consistent in the reports of bladder cancer following cyclophosphamide-therapy is the long delay between treatment and cancer development.

The histological type of bladder cancer usually associated with cyclophosphamide therapy is transitional cell cancer (uro-epithelial cancer). Squamous cell carcinoma (149),adenocarcinoma (151) and leiomyosarcoma (152), have also been described in the context of cyclophosphamide exposure. In an

analysis of 12 cases of cyclophosphamide-associated bladder cancer, the authors reported highly aggressive tumours, with high grade in staging at diagnosis and a potential of invasiveness and ability to metastasize (153).

The average dose of cyclophosphamide, given for non-malignant as well as malignant disease, in bladder-cancer cases reported in various studies has been 152 g (Table 11), and the lag time between treatment and cancer diagnosis on average 7.2 years, but cyclophosphamide-doses as small as 4 g have been implicated as causing bladder cancer (153),(136, 151, 154-158). A clinically relevant dose to compare these doses with is low-dose treatment (1-2 mg/kg) for one year, i.e., a 25-50 g cumulative cyclophosphamide dose.

Levine et al (131) suggest that patients who have received cumulative doses above 50g of cyclophosphamide, undergone high-dose intravenous therapy or had episodes of haemorrhagic cystitis should be subjected to routine screening for bladder-cancer including yearly urine cytology. If malignant cells are present, a more thorough investigation including urography, cystoscopy and biopsy of abnormal areas of the bladder should be performed and Fuchs has suggested the same screening routine for patients who have been on cyclophosphamide for more than two years (155).

Table 11. Published studies of bladder cancer risk in individuals treated with cyclophosphamide.

Diagnosis	Patients (n)	Median cumulative dose CY among cases (range)	Bladder cancers(n)	Relative risk	Cumulative risk (follow-up)	Author (reference)
NHL	6,171	37g (9-146)	9	4.5	not stated	Travis et al (1995) (134)
NHL	471	104g (27-148)	7	6.8	3.5% (8 yrs) 11% (12 yrs)	J Pedersen et al (1988) (135)
SLE	1,585	not stated	5	1.6	not stated	Mellemkjaer et al(1997) (159)
SLE/RA	43/11	46/56g (2-152)	1/1	not stated	not stated	Plotz et al (1979) (136)
RA	119	63g (18-108)	6		5%(11 yrs)	Baker et al (1987) (160) *
RA	119	120g (mean dose)	9	22	not stated	Radis et al (1995) (161) *
MS	70	61g(38-93)	5		5,7% (not stated)	de Ridder et al (1997) (139)
WG	145	144g (19-251)	7	31	5% (10yrs)	Talar-Williams (1996) (128) **
WG	158	not stated	4	33	not stated	Hoffman et al (1992) (15) **
WG	111	101(5-531)	3		2,7% (20 yrs)	Stillwell et al (1988) (162)

Abbreviations used in table: CY=Cyclophosphamide, NHL=non-Hodgkin's lymphoma, RA=rheumatoid arthritis, SLE=systemic lupus erythematosus, WG=Wegener's granulomatosis, g=grams, * and ** indicate studies with overlapping or identical study populations

Aims

Overall aim:

- To broaden our understanding of the aetiology and outcome of Wegener´s granulomatosis.

Specific aims of the thesis are:

- To assess the incidence of Wegener´s granulomatosis in Sweden and to relate possible time-trends to the introduction of ANCA testing in clinical practice.

- To assess the disease spectrum in patients with a positive ANCA - test but without Wegener´s granulomatosis and to assess their risk of later developing the disease.

- To assess occurrence of Wegener´s granulomatosis in first-degree relatives of patients with Wegener´s granulomatosis.

- To assess the general cancer incidence in patients with Wegener´s granulomatosis.

- To assess and characterize the risk of bladder cancer in patients with Wegener´s granulomatosis and relate this to exposure to cyclophosphamide.

Subjects and methods

Setting

The studies in this thesis were all conducted in Sweden. Sweden poses unique opportunities to perform epidemiological studies thanks to:

- the use of national registration numbers
- a long tradition of high quality census data
- the existence of nation-wide and population-based health-registers
- a public health care system with transparent referral systems
- an ethnically and socio-economically homogenous population
- a high public acceptance of registration

These factors, and their combination, act to increase the efficiency and validity of epidemiological studies.

Data-sources

The National Registration Number (NRN)

The NRN system was introduced in Sweden in 1947 as a unique ten-digit personal identifier (163, 164). It was assigned to all residents alive on January 1st 1947 and has been assigned to all residents born or immigrated thereafter.

The Inpatient Register

For administrative purposes, data on Swedish in-patient care has been registered in computerised form since 1964 in several counties. With an increasing number of counties enrolled the coverage became nationwide in 1987 (164). This registration is based on individual discharges rather than on individuals.

For each discharge, date of admission, date of discharge, main and up to five contributory discharge codes (According to the International Classification of Diseases, ICD 7-10 (165)) surgical procedures, department, hospital and the national registration number of the patient is recorded. By means of the NRN it is possible to obtain information on all in-patient care for a specific individual since 1964 (1987 nation-wide). The quality and content of the register is regularly validated, and completeness is estimated to be close to 100%.
The information in the In-patient-register has shown high diagnostic validity, but there are variations according to calendar period and type of diagnosis (Soc styrelsen pat reg). For instance, our studies of Wegener´s granulomatosis (see below) have found a diagnostic validity close to 90% of all episodes of in-patient care; the diagnostic validity of RA exceeds 90% (E.Baecklund, personal communication) and of Crohn´s disease and ulcerative colitis a validity of around 90 % is found (166, 167).

The Cancer Register

The Swedish Cancer Register was established in 1958 and contains data on cancer in the general population. Reporting is mandatory for both the treating clinician and for the pathologist, resulting in a completeness of 99% (168) .Apart from the ICD and the histopathology codes prevailing at the time of each cancer registration, all tumours are coded according to the ICD 7 by the cancer register to allow for longitudinal comparability. Within each cancer group, codes that distinguish different histological cancer types are used. Cancers only reported on death-certificates are not included. The cancer register is regularly linked to the cause of death register and it is thereby possible to obtain date and cause of death, if it has occurred, for each registered cancer diagnosis.

The Cause-of-death register

In Sweden, the numbers and causes of death have been registered since the 18th century in the parish registers. The cause of death register was set up in a computerised form in 1952 and includes the date of death, main and contributory cause of death (coded according to the ICD), and the NRN for all residents deceased during each year. The current completeness is estimated to exceed 99% (169).

The Register of Population and Population Changes

This register contains the official Swedish census data in computerised form since 1960. All residents alive in Sweden at the end of each year are included

with NRN, name, parish, community and county of domicile along with date of death for subjects deceased during the year. Since 1969 the register also contains information on dates of immigration and emigration.

The Multi-generation Register

In the early 1990s, Statistics Sweden created the Multi-generation Register by linking several different data-sources together. The resulting "generation register" provides information on vertical and horizontal first-degree relations (parents, siblings and children) for residents born in 1932 or later (index cases). To be included in the register, relatives have to be alive in 1960 or born thereafter. Adoptions and other non-biological relations are included and flagged. A new version of the register is created yearly when immigrated and new-born individuals (=new index cases) are added.
National registration numbers of both parents are identified for at least 83% of all index persons, and for over 90% of index persons alive in 1991 (170).

Paper I

In this study of the incidence of Wegener´s granulomatosis in Sweden between the years 1975 and 2001, the Swedish Inpatient Register was used to identify all patients discharged from hospital with a diagnosis of Wegener´s granulomatosis coded according to the ICD classification system (ICD-8 446.20, ICD-9 446 E, ICD-10 M31.3).

We hereby identified all 1,938 individuals registered in the In-patient Register between January 1, 1968 and December 31, 2001.

Since polyarteritis nodosa (PAN) was previously commonly used as a generic term for any systemic vasculitis, we similarly identified all individuals discharged with PAN as well as any other discharge code for defined (Goodpasture, Churg-Strauss, microscopic polyangiitis, midline granuloma) and undefined small or medium-vessel vasculitis during the same period (n= 5,306) .

To minimize the risk that patients with Wegener´s granulomatosis diagnosed before the start of our study period would be mistaken for incident cases by having the first hospitalisation for Wegener´s granulomatosis during our study period, we used a three-year wash-out period. However, testing for different wash-out periods (1-5 yrs) had little influence on the appearance of the incidence curve. As there was no specific ICD code for Wegener´s granulomatosis before the ICD 8 (1968) and since the coverage of the In-patient

Register reached 50% in the mid- seventies we chose to start the study period in 1975.

Taking into account that some individuals may initially have had a different vasculitis diagnosis code, we defined date of onset in such (5, 8 %) as the date of first discharge listing any vasculitic condition, but used the latest discharge to define the vasculitic diagnosis. From national statistics we collected information on the annual size-, and sex-and age-distribution of the Swedish population covered by the Inpatient Register. Incidences were standardized to the Swedish population as of 1990, and evaluated using Poisson regression.

Paper II

In this study of a series of patients positive for IIF-cANCA and ELISA-PR3-ANCA, we reviewed medical files and extracted information on reason for ANCA-testing, clinical symptoms, and final diagnosis.

The Dept of Clinical Immunology at Uppsala University Hospital serves a catchment area corresponding to Uppsala County and several smaller primary referral hospitals. The population in this area is approximately 2 million but ANCA testing is also performed at other laboratories. Between 1992 and 2002, 4,997 tests were performed in Uppsala.

The indirect immuno-fluorescence (IIF) was performed with human granulocyte substrate and the results given as positive or negative including staining pattern (Wieslab, Lund). The enzyme-linked-immuno-absorbent assay (ELISA) was performed with a commercial kit (Wielisa PR3-102X (171)). Capture ANCA was not performed. All the files of patients with a positive c-ANCA as well as a positive PR3-ANCA (n= 74) were reviewed and assessed for the underlying reason for testing, the medical condition at the time of testing, and for the possible development of any vasculitic disease (defined according to the ACR-criteria and, when applicable, also the CHCC-criteria), or any other new serious disease and death, during the study period, which ended in June 2006.

Paper III

This study is a population-based register study of the possible familial susceptibility to Wegener´s granulomatosis.

We identified a large cohort of patients (n= 2,288) with Wegener´s granulomatosis as main or contributory diagnosis, in the Swedish population-based Inpatient Register. These patients were then linked to the Register of Population and to the Multi-generation Register, thereby identifying all first-degree

relatives (parents, siblings, children and spouses) to the Wegener patients in our cohort. From the population register we also randomly selected ten controls for each Wegener´s granulomatosis patient, matched for age, sex, marital status and county of residence. Through a similar linkage with the Multigeneration register we also identified the first-degree relatives of these controls.

Through the NRNs the four cohorts i.e. the cohort of patients with Wegener´s granulomatosis , the cohort of their first-degree relatives, the control cohort, and the cohort of their relatives, were linked to the In-patient Register, the Causes of Death Register, and the Register of Population and Population changes, respectively.

By combining information from these sources we obtained information on vital status and discharges from in-patient care listing vasculitides for all the individuals.

Relative risks were assessed by comparing the occurrence of Wegener´s granulomatosis among the relatives of the patients with the disease to that among the first-degree relatives of the controls. For this comparison, we used COX proportional hazard regression adjusted for family structure.

To confirm the correctness of the ICD coded vasculitis diagnoses, we validated the medical files of all patients with Wegener´s granulomatosis who had a relative registered with vasculitis (n= 37), all these relatives registered with a vasculitis (n= 37) and, for the controls, all relatives registered with Wegener´s granulomatosis (n= 19).

Paper IV

In this study we assessed the overall cancer incidence in patients discharged from in-patient care with the diagnosis of Wegener´s granulomatosis in Sweden 1969 to 1995.

All individuals discharged with the diagnosis of Wegener´s granulomatosis (ICD 8 446.2, ICD 9 446E) as main or contributory diagnosis from 1969 through 1994 were identified (n = 1,176).

Using the NRN we linked the cohort to the Swedish Cancer Register, the Register of Causes of Death and the Register of Population and Population Changes. Through this linkage we obtained information on all registered cancers 1958-1995, all deaths from 1952-1995, all emigrations 1960-1995 as well as vital status at the end of the study period for each member of the study cohort. The start of the study period was set as the date of first discharge listing Wegener´s granulomatosis 1969-1994. The end of the study

period was defined as date of death, date of emigration or December 31, 1995, whichever occurred first.

As a measure of relative risk we calculated the standardized incidence ratios (SIR) of cancer (i.e. the ratio between the observed and the expected number of cancers). The expected numbers were estimated by multiplying sex-, -age and calendar-specific strata of person-years by the corresponding rates of cancer in the general population.

Paper V

The fifth study is a case-control study of Wegener´s granulomatosis and risk of bladder cancer in relation to exposure to cyclophosphamide, nested within the cohort used in Paper IV.

Using the same Wegener´s granulomatosis cohort as in Paper IV and by linking this to the Cancer Register we identified a total of 23 subjects diagnosed with bladder cancer between 1958 and 1995. Each bladder-cancer case was then matched with three controls from the same cohort using sex, year of birth and age at Wegener-discharge as matching variables. The controls had to be alive at the date of the bladder-cancer diagnosis of their cases.

All medical records of the cases and their controls were reviewed and data including date of symptoms of Wegener´s granulomatosis, extent of disease, accumulated cyclophosphamide dose in grams, duration of cyclophosphamide treatment in months, other treatments as well as information on smoking habits when possible, was abstracted.

All cases and controls were validated against the ACR-criteria for Wegener´s granulomatosis and patients for whom the diagnosis could not be confirmed were excluded. Likewise, all the bladder-cancer cases were validated, and the histological cancer diagnosis confirmed or refuted by a pathologist by re-assessment of the material obtained from the initial biopsy or operation. If bladder cancer could not be confirmed at re-evaluation, the case and its controls were excluded.

We estimated relative risks as odds ratios (ORs) and 95% confidence intervals (CI) for the association between cyclophosphamide and bladder cancer using conditional logistic regression analysis to account for the matched design.

A brief description of statistical methods used in this thesis

Standardised incidence ratios (SIR) and standardised mortality ratios (SMR)

Standardised incidence ratios and standardised mortality ratios both represent ways to measure the relative risk of an event through indirect standardisation (172).

SIRs and SMRs in this thesis are to be interpreted as the relative occurrence of an event in a cohort when compared with a hypothetical cohort of the general population of comparable composition. The expected numbers of events are calculated by first dividing the person-years of follow-up in the cohort under study according to standard demographic variables such as sex, age, and calendar period. Then these stratum specific person-years of follow-up are multiplied by the corresponding rates of the event in the general population. To obtain the total expected number of events in the cohort, the expected numbers of events in each such stratum, are summed. The SIR, or SMR, is calculated as the ratio of the observed number of events over the expected number.

Risk ratio

The "risk" is typically a measure of the chance of an event. In cohort studies, the risk of an event is expressed as the number of such events, divided by the number of all individuals at risk of this event at the beginning of the follow-up of the cohort. For instance, in a cohort of 100 patients all followed for one year, five developed Wegener's granulomatosis. The risk is 5/100, or 5%. In cohort studies, one cohort is typically compared to another, such that the risk in one cohort is divided by the risk in the other. The resulting ratio (the Risk Ratio), is a measure of relative risk.

Rate Ratio

In cohort studies, especially when individuals enter and exit the follow-up at different time points and are followed for varying periods of time, the "risk" cannot be calculated. Instead, the incidence, i.e., the number of events divided by the total observation time in the cohort is used. Similar to the risk ratio, the ratio of two incidences (the Rate Ratio) is used as an expression of relative risk.

Incidences can be compared to each other, and ratio ratios can be adjusted for other characteristics (sex, age, etc.) that may differ between the cohorts under comparison. *Poisson regression* is a statistical technique used to model the effect of one or several variables on another variable (the event) (173) when

this event is assumed to follow a Poisson distribution, and is thus a method of estimating the relative risk.

Cox' proportional hazards regression ("Cox regression") is an alternative to Poisson regression and the preferred method of estimating the relative risk in studies of survival, or "time to event". The underlying assumption is that the relative risk ("hazard ratio") is constant over time of follow-up, such that (for instance) "no matter how much or little treated patients die from time of diagnosis and onwards, untreated patients die twice as often".

Odds Ratio

The "odds" is an expression of chance, expressed as the number with a certain characteristic relative to 1 minus the number with this characteristic. For instance, of the patients in a waiting room, one has Wegener's granulomatosis. The odds is thus 1 to 9. In case-control studies, the preferred estimation of the relative risk is the odds ratio, which is calculated as the odds of exposure in the cases, divided by the odds of exposure among the controls.

Paper I

All patients with a discharge code for Wegener´s granulomatosis were identified in the In-patient register between 1968 and 2001. To avoid erroneously counting prevalent cases as incident such we used a three year wash-out period. Since there was no specific code for Wegener's granulomatosis in ICD7, and since the coverage of the Inpatient Register increased substantially during the 1970s, we arbitrarily chose to start the study period in 1975. From national statistics we collected information on the annual size, age-, and sex-distribution covered by the In-patient register. Incidences were standardized to the Swedish population as of 1990, and compared (e.g. trends) using Poisson regression.

Paper III

To assess the association between exposure (being a patient with Wegener's granulomatosis and not a control) and outcome (Wegener´s granulomatosis in a relative), i.e. the relative risk of also acquiring Wegener´s granulomatosis if a first degree relative has Wegener´s granulomatosis; we used Cox proportional hazard regression. The exposure (Wegener´s granulomatosis) is considered a family effect rather than a temporal effect, i.e. point of time of

Wegener´s granulomatosis in case /before or after/ its occurrence in the relative is regarded equal.

Paper IV

As a measure of relative risk we calculated standardizes incidence ratios (SIRs) of cancer, i.e. the ratio between the observed and the expected numbers of cancers. SMRs for cancer were calculated in a similar way, using expected death rates from the Swedish Register of Causes of Death. Patients who died or had their cancer registered at their first discharge with Wegener´s granulomatosis were not included in the analytical cohort.

Paper V

In the nested case-control study we estimated relative risks as odds ratios (ORs) for the association between cyclophosphamide and bladder cancer. In the assessment of the cumulative risk of bladder cancer in the entire cohort, we calculated the absolute risks using Kaplan-Meier curves.

As an assessment of the relative prevalence of bladder cancer in patients with Wegener´s granulomatosis already at the time of their first hospitalisation listing Wegener's granulomatosis, we calculated the ratio of the observed prevalence of bladder cancer in the cohort of Wegener patients (at cohort entry) and compared this to the expected prevalence in the general population (of the same sex-, age- and calendar period composition and taking fatal bladder cancers into account).

Results

Paper I

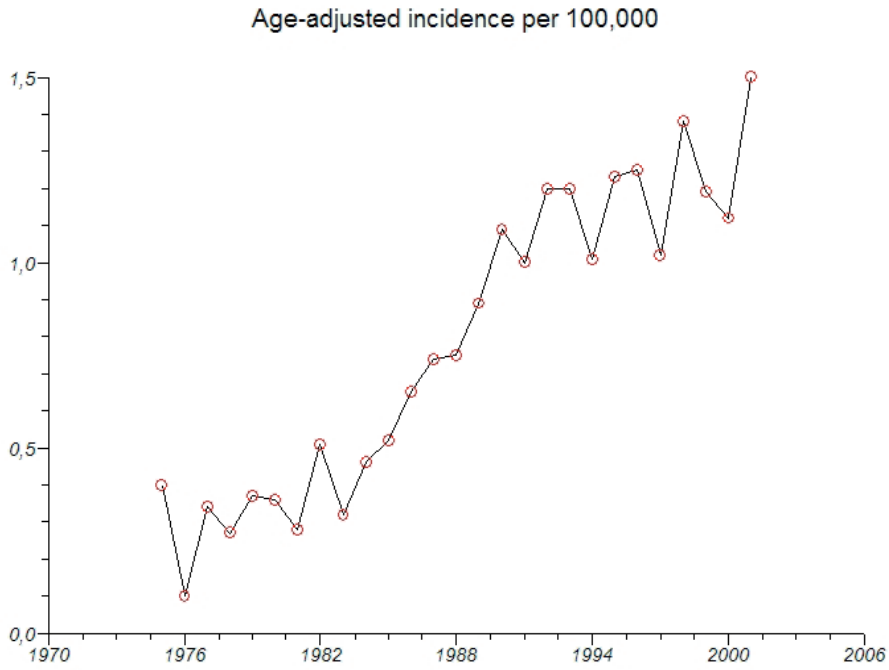
Between 1975 and 2001 a three-fold increase in the incidence of Wegener´s granulomatosis in Sweden was noted.

1,636 incident cases of Wegener´s granulomatosis were identified during the study period (1975-2001), representing 885 men and 751 women, with a mean age at diagnosis of 59.4 years and 62.1 years respectively. These individuals, discharged with Wegener´s granulomatosis, corresponded to a mean incidence of 0.78 (95% CI 0.74-0.82) per 100.000; 0.86 among men (95%CI 0.80-0.91) and 0.70 among women (95% CI 0.65-0.76). The overall incidence increased during the study period from 0.33 (95% CI 0.28-0.39) in 1975-1984 to 0.77 (95% CI 0.69-0.85) in 1985-1990 to 1.19 (95% CI 1.12-1.26) in 1991-2001.

Overall and within each of these time periods the annual increase was statistically significant (all p values <0.05). The time trend was similar among men and women and the age at first discharge remained constant during the study period, as did the proportion of patients whose first discharge with Wegener´s granulomatosis was preceded by at least one discharge listing another vasculitic disease.

Estimations of the prevalence of Wegener´s granulomatosis in Sweden based on the incidence data from our study, revealed a dramatic increase also in prevalence. The prevalence increased from 36/million in 1993 to 112/million in 2001.

Fig 1. Incidence of Wegener´s granulomatosis in Sweden 1975-2001.



As PAN may have been used as a general diagnostic code for all vasculitic diseases during the earlier period of our study, the incidence curve of PAN was calculated and combined with that for Wegener´s granulomatosis. The increased incidence of Wegener´s granulomatosis before the mid -1980s was mirrored by a declining incidence of PAN, resulting in a stable combined incidence. Between the mid-1980s and the late 1990s, the increase in incidence of Wegener´s granulomatosis was not clearly reciprocated by that of PAN and the combined incidence. After the late 1990s the incidence of PAN declined, resulting in a decline in the combined incidence of PAN and Wegener´s granulomatosis, despite a continued increase of the latter. A similar pattern was observed when the incidence of Wegener´s granulomatosis instead was contrasted with that of all other small-and medium-sized vessel vasculitis.

Paper II

In this case series of ANCA-positive patients, 18 of a total of 74 patients did not fulfil the ACR- or CHCC-criteria for systemic vasculitis at the time of testing, nor did they develop any vasculitic disease during the mean follow-up of 6.8 years. Of the 74 patients with a positive c-ANCA and a positive PR3-ANCA, 56 had some systemic vasculitis (46 patients had Wegener´s granulomatosis). The remaining 18 patients suffered from a heterogeneous group of inflammatory and infectious diseases and conditions (Table 12).

The values of the first positive ELISA test among the eighteen patients without a confirmed vasculitis varied from 10-140 U/ml (mean 36, median 15). Although these values were lower than those of the 56 patients with confirmed vasculitis at the time of testing, this difference did not reach statistical significance (Mann-Whitney p=0.11).

During the follow-up period from first positive c- and PR3-ANCA until June 2006 (range 3 years to 12 years, mean 6.8 years) none of the eighteen ANCA-positive patients without a confirmed diagnosis of vasculitis at the time of testing developed such a disease, nor any new signs or symptoms suggestive thereof, nor any other new serious condition. Four patients died, all above the age of 75 years, and from conditions unrelated to vasculitic disease.

The PPV (positive predictive value) of the combined testing for c-ANCA and PR3-ANCA for any vasculitis in this setting (i.e. in a non-selected cohort of patients with clinical symptoms warranting an ANCA-test according to their treating physician) was 76%. In the patients with a confirmed Wegener´s granulomatosis in this group, according to the ACR- and CHCC-criteria (n= 46), the PPV was 62%.

Table 12. Characteristics of the 18 patients with a positive c-ANCA and PR3-ANCA but no concurrent evidence of vasculitic disease

Age	Number of positive PR3-ANCAs (n)	Sex	Symptoms prompting ANCA-testing	Final diagnosis
38	10	F	Fever,malaise,SR	Ankylosing spondylitis
33	1	M	Renal failure,hematuria,anuria	Nephropathia epidemica
83	1	M	Fever,pulmonary infiltrates,renal failure	Sarcoidosis
70	1	M	Resp tract symptoms,purpura	Terminal liver failure
56	2	F	Upper resp tract symptoms,SR	Primary Sjögrens syndrome
66	6	F	Acute renal failure,hematuria	Nephropathia epidemica
31	8	M	Fever,exantema,arthralgia,malaise,SR	Rheumatic fever
84	1	F	Epistaxis,ear pain	External otitis
66	1	M	Fever,SR,Malaise,back-pain	Endocarditis,discitis
75	5	M	Exanthema,mononeuritis	Systemic sclerosis ,infection
32	5	M	Blocked nose	Observation for possible WG
22	1	M	Renal failure,proteinuria	Amyloidosis
35	1	F	Renal failure,proteinuria	Diabetes nephropathy
54	1	M	Ulcerative colitis	Ulcerative colitis
17	7	F	Ulcerative colitis,fever	U.C,urinary tract infection
66	1	M	Ulcerative colitis, renal failure, elevated liver enzymes	U.C, hepatit C,diabetes
84	1	F	Fever,cutaneous vasculitis	Erytema multiforme,infection
32	1	F	Artralgia,resp tract symptoms,fatigue	Chronic fatigue syndrome

Paper III

For 1,944 of the 2,288 Wegener´s granulomatosis patients identified in the register, we could identify 6,670 first degree relatives. Among these we found two relatives in the same family with Wegener´s granulomatosis (mother and son). Among the 68,994 relatives of the 19,655 controls (none of the controls were registered with Wegener´s granulomatosis) we identified 14 relatives with the disease (3 parents, 6 siblings, 4 children and one spouse).
The corresponding relative risks of Wegener´s granulomatosis among the first-degree relatives of patients with the disease were 1.56 (95% CI 0.35-6.90) for all biological relatives combined, 3.36 (95 % CI 0.35-32.0) for parents, 0.00 for siblings, 2.56 (95% CI 0.29-22.8) for offspring and 0.0 for spouses.

The tendency for increased relative risks of Wegener´s granulomatosis was apparent only in attained ages below 50 years, but numbers were small and confidence intervals wide.

Table 13. Number of cases with, and relative risks of, Wegener´s granulomatosis in 6,670 first degree relatives of 1,939 Swedish patient with Wegener´s granulomatosis, compared to68, 994 relatives of 19,606 population controls

Type of relative	Relatives of patients	Relatives of controls	RR(95 % CI)
All 1st degree	2/6,670	13/68,994	1.56 (0.35-6.90)
- Parents	1/1,586	3/15,422	3.36 (0.35-32.0)
- Siblings	0/1,274	6/13,303	0.0
- Children	1/3,810	4/40,269	2.56 (0.29-22.8)
Spouses	0/428	1/4,812	0.0

Paper IV

In this study the total cancer incidence in a population-based cohort of patients diagnosed with Wegener´s granulomatosis was estimated. A total of 110 cancers were registered during the 5,708 person-years follow-up, which corresponded to a SIR of 2.0 (95% CI 1.7-2.5). In 68 cases, cancer was registered as the underlying cause of death, resulting in a SMR (standardised mortality rate) for all cancers of 2.2 (95% CI 1.7-2.8).

There were 14 cases of bladder cancer (SIR 4.80, 95%CI 2.6-8.1). The risk was elevated for both men and women and tended to increase with time of follow-up, (p for trend = 0.12). Other cancers with significantly increased risks included squamous skin cancer (SIR 7.3, 95% CI 4.4-12, n = 18), malignant lymphomas (SIR 4.2, 95% CI 1.8-8.3, n = 8), leukaemia (SIR 5.7, 95%CI 2.3-12, n = 7) and cancer of the liver (SIR 3.8, 95% CI 1.2-8.8). The SIR estimate for lung cancer suggested an increased risk (SIR 2.0 95% CI 0.9-8.8), but the confidence interval was wide and included the null value.

Table 14. Number (n) and type (ICD 7) of registered cancers, standardized incidence ratios (SIR) and 95% confidence intervals (95% CI) of cancers in a population-based cohort of 1,065 patients with Wegener´s granulomatosis in Sweden 1969-95.

Site (ICD7)	n	SIR (95% CI)
All sites (140-200)	110	2.0 (1.7- 2.5)
Primary liver (155)	5	3.8 (1.2-8.8)
Lung (161-163)	8	2.0 (0.9-3.9)
Nose and middle ear (160)	1	14.1 (0.4-79)
NMSC (191)	18	7.3 (4.4-12)
Bladder (181)	14	4.8 (2.6-8.1)
All haematopoietic (200-209)	15	3.8 (2.1-6.3)
Malignant lymphoma (200-202)	8	4.2 (1.8-8.3)
Leukaemia	7	5.7 (2.3-12)

Paper V

In this nested case-control study within our Wegener´s granulomatosis-cohort, a dose response relationship between treatment with cyclophosphamide and risk of bladder cancer is suggested.

Of the 14 registered bladder cancers, two cases had to be excluded as they did not meet the ACR-criteria for Wegener´s granulomatosis and in one case the medical file could not be retrieved. Of the remaining eleven cases ten (91%) had been exposed to cyclophosphamide and 20 of the 25 controls (80%). The median cumulative dose of cyclophosphamide among the cases was 113 g compared to 25g among the controls (Wilcoxon p for difference = 0.01) (Fig 2).

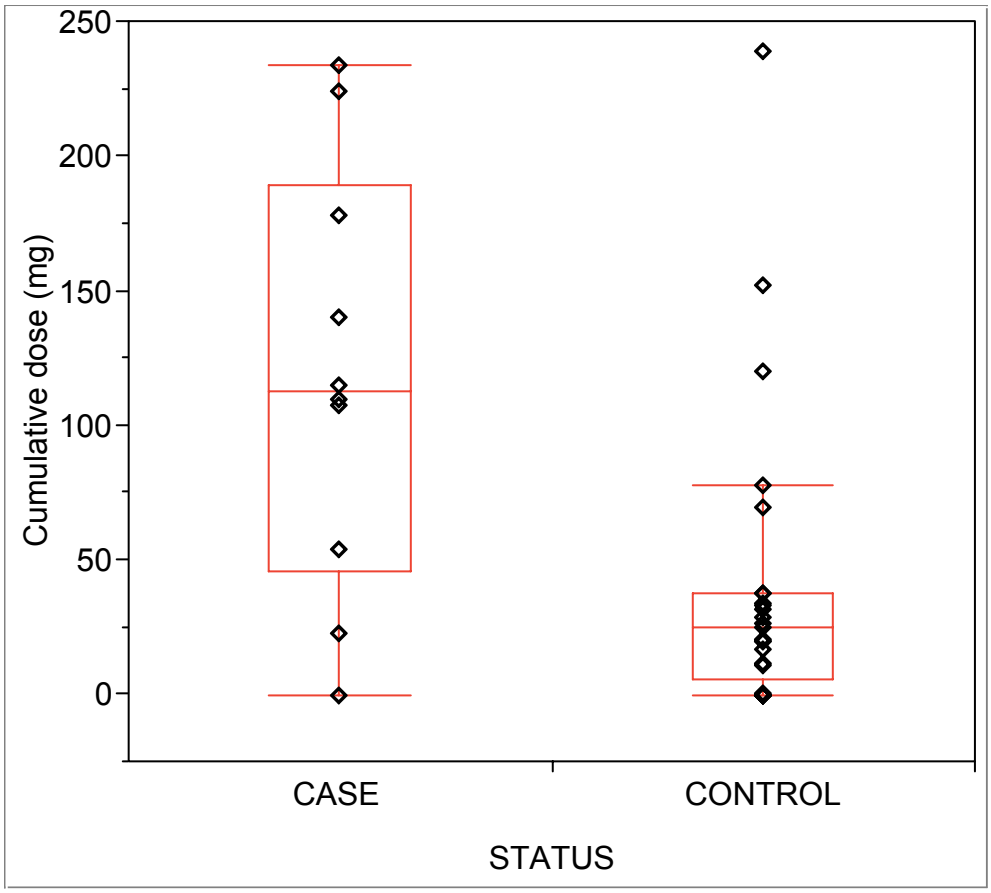


Fig 2. Distribution of the cumulative dose of cyclophosphamide among the eleven cases and their 25 controls. (Wilcoxon p for difference = 0.01).

The median duration of treatment was 86 months (7 yrs and 2 months) among the cases and 13 months among the controls. Each 10g increment in cumulative cyclophosphamide dose was associated with a doubled risk of bladder cancer (OR 2.0, 95%CI 0.8-4.9). Similarly, a cumulative dose above (versus below) the median dose among the controls (25g) was associated with a five-fold increase in the risk for bladder cancer (OR 5.2, 95% CI 0.8-36). There was also a tendency towards increased bladder cancer risk with longer duration of treatment, and exposure to cyclophosphamide for longer than 13 months (median among the controls) was associated with a near eight-fold increased risk (OR 7.7, 95% CI 0.9-69).

The cumulative risk for bladder cancer was 2 % (95% CI 0.1-3.9) after ten years of follow-up but increased to10% (95% CI 2.7-17) after 16 years

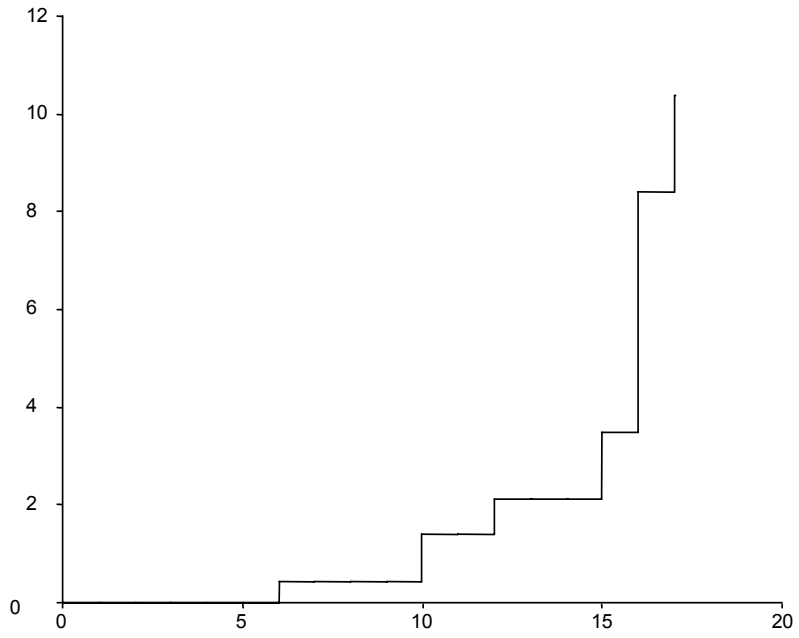


Fig 3. Cumulative incidence (%) of bladder cancer by time since first discharge for Wegener´s granulomatosis (yrs)

The latency from stopping cyclophosphamide treatment to the development of bladder cancer varied from 0 to14 years, with a median latency of 2 years and 7 months.

One of the controls developed urinary bladder cancer after the end of the study. This patient had received a cumulative dose of 70 g of cyclophosphamide, and the treatment duration was 4 years.

Eight of the 1,065 patients with Wegener´s granulomatosis had developed bladder cancer *before* being diagnosed with Wegener´s granulomatosis. The median time-interval from bladder-cancer diagnosis to Wegener´s granulomatosis was 1.5 years (2 months to 9 years). Compared to the expected prevalence of bladder cancer in Sweden (adjusted for sex and age), this corresponds to a relative risk of 2.1(95% CI 0.6-3.6).

Discussion

Incidence of Wegener´s granulomatosis

The finding in this study of a significant three-fold increase in the incidence of Wegener´s granulomatosis in Sweden between 1975and 2001 is in keeping with several other European studies (22, 24, 27, 174), (Takala, abstract Scand J Rheumatol 2006; S:121) and also in a study from New Zealand (30) and India (117), all of which have suggested increasing incidences of Wegener´s granulomatosis. Some studies have also found a north-south gradient indicating that Wegener´s granulomatosis is more common in northern Europe than in the south (20, 21).

In contrast to these studies, an investigation conducted in Germany between 1998 and 2002 (28) reported stable incidences of primary systemic vasculitides including Wegener´s granulomatosis , the incidence levels being comparable with the high incidences noted in Northern Norway (22).

No previous study, however, has encompassed such a large number of patients (n = 1,638) or covered such a long period of time (26 years) as the one presented in this thesis.

Estimations of the prevalence of Wegener´s granulomatosis in Sweden based on the incidence data from our study revealed a dramatic increase in prevalence as well. Our figures are very similar to those reported from smaller studies in Norway, with 49/ million in 1993 and 95 /million in 1998 (22) and from the UK, were a point prevalence of 63/million in 1997 has been reported (27). The noted increase in prevalence most likely reflects the improved survival of these patients and is also an estimation of the disease burden in the population. These patients are likely to be heavy users of medical facilities due to the seriousness of the disease, the risk of relapses and the requirement for long-time monitoring.

If the noted increases in incidences reflect a true rise in incidence or are merely the effect of increased physician awareness, new diagnostic tools or a diagnostic “drift” in favour of Wegener´s granulomatosis remains to be clari-

fied. In our study, we have approached these possible explanations of increased incidence.

ANCA was gradually introduced in Sweden from 1987 and onwards, and has been widely used from 1992. This can be mirrored by the increased incidence of Wegener´s granulomatosis during this time period. However, incidence already rose prior to ANCA-introduction and has continued to increase.

Of course, even increased physician awareness of the disease following the articles of Fauci et al on Wegener´s granulomatosis published in 1983 and the discovery of ANCA in 1982 and the subsequent literature on the subject may have influenced diagnostic precision.

New ICD codes for the classification of disease may also influence incidence rates. In ICD 7 (-1968) there was no specific code for Wegener´s granulomatosis and vasculitides were generally diagnosed as PAN. The possibility of a diagnostic drift from PAN to Wegener´s granulomatosis can be illustrated by the findings that the incidence of PAN decreased slightly during the early 1980s, while the combined incidence of Wegener´s granulomatosis and PAN remained stable.

Throughout the study period we used the same case definition (hospitalisation) to define Wegener´s granulomatosis. Despite possible differences in case ascertainment between our study and previous epidemiological studies, our results are remarkably consistent with respect to sex, mean age at onset, distribution and the magnitude of observed incidence and prevalence during the 1990s. Our method of case identification may have failed to include patients never hospitalized, even primarily for other causes than Wegener´s granulomatosis, but if this was the case the true incidence would be even higher than noted. In a study from the most southern part of Sweden in 2002, where the sources of case identification were medical files from hospitals in the area, ANCA databases from laboratories as well as records from pathology departments, an incidence of Wegener´s granulomatosis of 1.6 /100.000 and a prevalence of 19.7/100.000 was found (Mohammad, abstract ACR 2006), findings that are consistent with those presented in our study, for the same time period.

A method of finding Wegener´s granulomatosis patients solely treated in an out-patient setting is to search for ANCA positive individuals in laboratory registers. However, in our survey of 74 ANCA-positive patients, none of the 56 Wegener´s granulomatosis-patients had been treated on out-patient basis only (Paper II).

The classification of vasculitic diseases is complicated by the existence of at least two major classification schemes, the ACR-criteria and the CHCC-criteria. We have chosen to use the ACR-criteria as they are widely used, recognise that histological material is not available from all patients and they

are validated with a specificity of 92% and a sensitivity of 88% for Wegener´s granulomatosis (175, 10).

However, other studies may have used different criteria which make comparisons of incidence from different studies and different geographical areas difficult. Recently, a methodology for classification of the ANCA-associated vasculitides and polyarteritis nodosa, using a step-wise approach incorporating the ACR- and CHCC, as well as the Lanham criteria (for the diagnosis of Churg- Strauss vasculitis) has been put forward, the aim being to permit comparisons of the epidemiology between different geographical areas (176).

Correct and consistent classification of disease is vital in studies of incidence and prevalence as well as when comparing studies. What is just as important, however, is an adequate size of the studied cohort, especially when studying such a rare disease as Wegener´s granulomatosis, and this requirement is fulfilled by large population-based studies such as ours.

Although our study indicates that increased disease awareness due to ANCA testing or diagnostic drift from PAN to Wegener´s granulomatosis are not likely to be the *only* explanation of the dramatic time trends observed, a stabilisation of incidences during the coming years would argue for an impact of these factors on disease incidence. On the other hand, continuing increases or apparent differences in different geographical areas will indicate that environmental, and possibly genetic, factors contribute to the changing incidence of Wegener´s granulomatosis.

The significance of ANCA in the absence of systemic vasculitis

This population-based study indicates that patients who test positive for c-ANCA and PR3-ANCA, but do not fulfil the criteria for systemic vasculitis at the time of testing display a wide array of medical conditions but have a low risk of developing vasculitis.

In Wegener´s granulomatosis overall 65-90% of patients ever develop ANCA (73), but whether this antibody precedes clinical disease is not known. Accumulating evidence, although some based on in-vitro experiments, support the hypothesis that ANCA is involved in the pathophysiology of Wegener´s granulomatosis (73, 74).

In rheumatoid arthritis anti-CCP has been shown to precede clinical symptoms with several years (177). In SLE 88% of patients have at least one autoantibody present up to 9 years (mean 3.3 years) before diagnosis (178).

The finding in our study that none of the c-ANCA and PR3-ANCA-positive individuals developed systemic vasculitis during a mean follow-up of 6.8 years may suggest that ANCA reflects neutrophil activating properties not specific to vasculitic disease. ANCAs have been described in rheumatoid arthritis and then been associated with long-standing disease (179), and disease activity and inflammation (180).

The pro-inflammatory properties of ANCA may enhance or even maintain the inflammatory process, possibly accounting for the greater relapse-rate suggested in patients with a persisting ANCA (181-183). On the other hand, some studies have shown that a rising ANCA does not correlate well with disease relapse (34, 45). A practical clinical implication of this study may be that a positive ANCA *in itself* does not warrant extensive and prolonged monitoring, especially not in the low value range.

The PPV for vasculitic disease overall, including such diseases as PAN and microscopic polyangiitis, was in our study 76%. The PPV of 62% for Wegener´s granulomatosis is in keeping with several other studies (63, 184) and underlines the necessity to handle the ANCA results in the context of the patient's clinical picture, disease activity and the prevalence of Wegener´s granulomatosis in the clinical setting in which the patient is seen.

Our study was designed as a routine care assessment, as such being a clinical counterpart to studies of the ANCA-test performance in highly selected and clinically typical patients with diagnosed vasculitis from referral centres (43). A limitation is our retrospective assessment of diagnosis and follow-up. We used the ACR-criteria to define the vasculitis, but 67% of the patients had had their diagnosis confirmed by biopsy. Applying the newly proposed algorithm for diagnosis of vasculitis did not change the outcome (176). The follow-up was performed using the patients' medical charts or by direct contact with their treating physician, minimizing the risk of serious disease passing undetected.

Familial association of Wegener´s granulomatosis

In this large population based assessment of risks of Wegener´s granulomatosis in first degree relatives of patients with Wegener´s granulomatosis, we found that the *absolute* risk of Wegener´s granulomatosis among the closest relatives with the disease amounts to less than one in a thousand, and we observed no clear pattern of extensive clustering in certain families. In terms of *relative* risks, our results essentially rule out pronounced increased familial risks as those suggested for eg. MS, IBD, psoriasis and SLE (185-190) but

are compatible with an increased familial occurrence of the same magnitude as reported for e.g. rheumatoid arthritis (191).

Our previous knowledge of familial aggregation of Wegener´s granulomatosis rests on case-reports only (98, 99, 103, 106, 107). Although several candidate genes or gene polymorphisms have been put forward, including α_1-anti-trypsine deficiency (113, 192-196), no single genetic marker has so far been tied to Wegener´s granulomatosis-susceptibility. Many of the genetic features found in Wegener´s granulomatosis are also found in other chronic inflammatory diseases, such as PTPN22 polymorphisms (110) and CTLA4 variations (111, 197). If these common "inflammation-genes" make up an important part of the genetic susceptibility for Wegener´s granulomatosis, only limited familial aggregation of Wegener´s granulomatosis can be expected, as found in our study.

Also, the results of this study rules out existence of any substantial family clustering of Wegener´s granulomatosis, which might have been indicative of specific "Wegener´s granulomatosis-genes". The few previous reports of clustering of Wegener´s granulomatosis within families are indirect support of our results, although case-reports cannot be used to quantify risks and the risk of reporting bias is obvious.

Apart from genetic susceptibility, environmental factors can also lead to clustering of disease. Some previous studies on environmental risk factors have suggested the possibility of silica exposure (36, 37, 198) as well as inhaled organic solvents and farming (37) as potential environmental hazards connected to the development of Wegener´s granulomatosis. The absence of marked familial clustering in our study would argue that whatever environmental factors that are involved, they seem to occur on an individual rather than household level.

The strengths of the study lies in its nation-wide population based setting allowing us to identify a large cohort of patients with Wegener´s granulomatosis, a large set of general population controls and an unbiased identification of relatives and spouses. All register-based outcomes were validated by scrutiny of the medical files before analyses. And although all our outcome was identified on the basis of hospitalisation, the overwhelming proportion of patients with Wegener´s granulomatosis in Sweden are treated on an inpatient basis at some time, for diagnostic or therapeutic reasons.

Moreover, the incidence of Wegener´s granulomatosis found among the relatives in this study is well in accordance not only with our results presented in Paper I, but also with incidences from other settings (20, 22, 28, 199), supporting that we by using hospitalisation as outcome identification, manage to include the majority of Wegener´s granulomatosis patients in Sweden.

Despite the very large, nation-wide cohort of Wegener´s granulomatosis patients accrued over many years, and although our data strongly argues against substantial familial aggregation as in MS, IBD, SLE or psoriasis, the statistical precision around the observed RR of 1,56, was limited.

We did not check the diagnostic correctness of *all* the 2,288 individuals registered with Wegener´s granulomatosis in the In-patient Register, but previous validations and a review of medical files within the current study has found a correctness of 85-90%. Possible misclassification of the diagnosis Wegener´s granulomatosis could potentially slightly underestimate the relative risk in this study, but even if the relative risk of 1,56 is increased by 10-15 %, this does not alter the clinical or the etiological conclusion.

In conclusion, our results suggest that the risk of Wegener´s granulomatosis among close relatives of patients with the disease is low, and do not support the existence of extensive clustering within some families. However, our results are compatible with some increase familial occurrence of the disease, and thereby also of a genetic susceptibility to Wegener´s granulomatosis.

Cancer incidence in Wegener´s granulomatosis

In this study we observed an overall doubled risk for any cancer in individuals diagnosed with Wegener´s granulomatosis. Increased risks were noted for bladder cancer, squamous skin cancer, malignant lymphoma and leukaemia. Our five-fold increased risk of bladder cancer suggests lower relative risks than those previously reported from Hoffman et al (15) who noted a 33-fold increase and Talar-Williams (128) who reported a 31-fold increased risk of bladder cancer. These studies were however considerably smaller, 158 and 145 patients respectively (4 and 7 cases of bladder cancer respectively), resulting in uncertain risk estimates. Also the patients were recruited from referral centres which may have led to the inclusion of more severely ill patients

In a Swedish study from Lund, a 5-fold risk of bladder cancer was noted in a cohort of patients with Wegener´s granulomatosis and microscopic polyangiitis, all with glomerulinephritis (200). These figures however, are not directly comparable to ours due to the inclusion also of MPA and the fact that all patients had kidney involvement, suggestive of serious generalised disease.

Similarly to the increased risk of bladder cancer, we estimated a four-fold increased risk of malignant lymphomas, which is also lower than the 11-fold increase reported from Hoffman et al (15). The relative risk of malignant

lymphoma is comparable to the risk noted for other rheumatic conditions, for instance in a Swedish study reporting a two-fold increased risk for lymphomas in patients with rheumatoid arthritis (124). Detailed studies of this RA-cohort has indicated an association between increased disease activity and lymphoma (125).

The increased risk of skin cancer is higher than previously reported but lower than in that found in organ transplant patients (201). This increased risk is unlikely to be caused only by exposure to immuno-suppressants as the increase is apparent already at the start of follow-up, indicative of detection bias. However, as the increased risk persists even after the first year, this persistent risk could be related to immunosuppressive treatment.

The finding of a doubled, or greater, risk of cancer in tissues frequently involved in Wegener´s granulomatosis, that is, nose and middle ear, lungs and kidneys, is noteworthy. This finding may indicate a carcinogenic effect of the disease process itself, as some previous case-reports have indicated (202). However, the numbers are small, the confidence intervals wide and include the null value and neither surveillance bias nor misclassification can be excluded, as these files could not be scrutinized for diagnostic correctness.

The strengths of this study includes the large number of subjects (n=1,065) selected in a population based and nation-wide setting, the independent gathering of exposure (Wegener´s granulomatosis) and outcome (incident cancer) and the long study period (26 years).

Diagnostic accuracy remains a concern, as the exposure was identified through discharge codes and not ACR-criteria for Wegener´s granulomatosis, but in the subset of validated medical files the diagnostic accuracy was close to 90%. In addition, inclusion resting on hospitalisation may have included more severe cases. In Sweden, however, most cases of Wegener´s granulomatosis irrespective of disease extent are at some time treated or diagnosed on an in-patient basis. In the subset of our cohort that we validated closely, 30% of the patients had limited disease, that is, not pulmonary or renal disease, and had still been seen as in-patients. Also, in our study of ANCA-positive individuals, all vasculitis cases had been treated as in-patients at some time.

In this the largest cohort-study of Wegener´s granulomatosis and cancer incidence so far, we observed a double risk of cancer overall, with a particularly high risk for cancer of the urinary bladder, and the haematopoietic system including lymphomas and of squamous skin cancer. The risk of cancer in this group is fully comparable to that reported from other rheumatic conditions, and highlights the necessity to establish the causes of cancer in this group, whether related to treatment, disease intensity or common aetiological factors.

Bladder cancer in Wegener´s granulomatosis and relation to cyclophosphamide

The results of this study suggest that cyclophosphamide in doses frequently encountered in clinical practice when treating patients with Wegener´s granulomatosis is associated with a dose-response related increase in the relative risk of bladder cancer.

Cyclophosphamide is a commonly used drug in the treatment of malignant conditions and there are studies of secondary malignancy, including bladder cancer, in patients with NHL (135, 203) and ovarian cancer (204) as well as other malignancies (149, 205). A dose-response relationship between cyclophosphamide treatment for NHL and bladder cancer has been reported with significantly increased risks with cumulative cyclophosphamide doses above 20 g. The risk with doses of 20-49 g was reported to be 6 times elevated, and at doses > 50 g the reported risk was 14.5-fold (134).

Cyclophosphamide is also the first-line treatment not only of severe Wegener´s granulomatosis but also for lung, kidney and central nervous system manifestations of systemic lupus, systemic manifestations of rheumatoid arthritis, autoimmune alveolitis and other vasculitides etc. As a result of these treatment regimens, and the fact that the prognosis of many of these conditions has improved dramatically over the years, the prevalence of subjects ever exposed to cyclophosphamide in doses similar to those of the present studies, is increasing.

Also in these “benign” conditions treated with cyclophosphamide an increased occurrence of bladder cancer has repeatedly been reported (136-138). However, not many studies have assessed the risk of bladder cancer after exposure to cyclophosphamide and our current knowledge has rested on a few cases within two small cohorts of Wegener´s granulomatosis patients (15, 128).In the study by Talar-Williams the seven cases of bladder cancer cases observed in a cohort of 145 Wegener´s granulomatosis patients corresponded to a 31-fold relative risk and a cumulative risk of 5% at 10 years and 10% at 15 years. In the Talar-Williams study, all patients had been treated with cyclophosphamide, in the Hoffman study, 90% of the patients were treated with cyclophosphamide. Six of the seven cancer cases had received cumulative cyclophosphamide doses above 100 grams and treatment duration exceeding 2.7 years.

In our cohort, the relative risk was five-fold at cumulative doses above 25 g and the absolute risk was 2% after 10 years, rising to 10% after 16 years, to be compared to a *life-time* risk of bladder cancer in Sweden of 2 %. Our study also indicates that there are no “safe” doses of cyclophosphamide.

The mechanism of bladder-cancer development after cyclophosphamide treatment is not fully understood. Haemorrhagic cystitis is a well known side-effect of cyclophosphamide and the metabolite acrolein is responsible for the toxic effect on the bladder cancer epithelium causing cystitis and bladder fibrosis (132), but whether haemorrhagic cystitis also predisposes to bladder cancer is a debated matter (135). In our study none of the bladder-cancer cases had been subjected to cystoscopy because of hematuria before the diagnosis of bladder cancer.

Another metabolite of cyclophosphamide is phosphoramide mustard. In a study by Khan et al, the mutation spectrum of the p53 tumour-suppressor gene, with a clustering at exon 6, and a majority of mutations being G:C-A:T, is linked to phopsphoramide mustard and differs distinctly from mutations caused by tobacco or schistosomiasis (206). The main mutation occurring after acrolein was G:C-T:A and in a minority G:C-A:T. Concluding, this study suggests that phosphoramde mustard is the key mutagen in iatrogenic bladder cancer.

Mesna is not likely to be able to block phosphoramide-mustard mutagenicity as it cannot enter into cells; its action against cyclophosphamide toxicity on bladder epithelium is by binding acrolein in the bladder, resulting in an inactive product that is eliminated in the urine. Chemoprotective agents which bind nitrogen mustard, such as amifostine, might be considered as bladder cancer protectants (207, 208).

Even if recent reports have suggested shorter induction regimens for cyclophosphamide in Wegener´s granulomatosis (87) and even if alternative treatments are being sought (91, 93, 209, 210) cumulative doses above 25 g will remain a common finding as the relapse of Wegener´s granulomatosis still poses an unsolved problem, and cyclophosphamide still remains the mainstay treatment for severe systemic Wegener´s granulomatosis.

Our study also points to the possibility of increased risks for bladder cancer even before the diagnosis of Wegener´s granulomatosis, suggesting common aetiologies or risk factors. A relationship between Wegener´s granulomatosis and cancer has also previously been demonstrated in a study by Tatsis et al (211) who found an increased risk of renal carcinoma in Wegener´s granulomatosis patients. In our study of cancer incidence in a large cohort of Wegener´s granulomatosis patients, we found a doubled risk for renal cell carcinoma, but we have not in detail studied the time relation between the diseases or the use of cyclophosphamide in these cases.

Smoking could be a common risk factor for Wegener´s granulomatosis and cancer, but we are unaware of any studies linking Wegener´s granulomatosis to smoking. On the contrary, one study by Hayworth et al showed no correlation between smoking and pulmonary haemorrhage in Wegener´s granulomatosis (212).In our case-control study smoking did not emerge as a

strong risk factor, but information on smoking habits was scarce and the results should be interpreted with caution.

However, screening for bladder cancer in patients subjected to high doses or long durations of cyclophosphamide treatment should be recommended, although cystoscopy and voided urine cytology (VUC) are considered the golden standard in bladder-cancer detection, cystoscopy is an invasive method and cytology is limited by its low sensitivity. New tumour biomarker tests can prove to be convenient and reliable in bladder-cancer detection (213, 214).

The strength of this nested case-control study lies in the population-based, nationwide setting of the source cohort. The identification of bladder cancer was independent of the exposure and the diagnosis of Wegener´s granulomatosis, and most likely to be complete thanks to the almost 100% coverage of the Cancer Register in Sweden. However, the power of the study was limited and several risk estimates did not reach beyond border significance. Surveillance bias cannot be excluded, but in this study only one patient had a cystoscopy performed long before the bladder cancer diagnosis (revealing chronic cystitis) and among the diagnosed bladder cancer cases 5/14 were invasive at the time of diagnosis, and six of the bladder-cancer cases died with bladder cancer noted on the death certificate, suggesting that early detection of cancer was not the case.

In conclusion, patients with Wegener´s granulomatosis run a markedly increased risk of bladder cancer, and this risk may be partly attributed to cyclophosphamide, but other factors operating even before the diagnosis of Wegener´s granulomatosis may be of importance. To avoid an accumulating number of cyclophosphamide-related bladder-cancer cases in this population, increased clinical attention should be directed towards this group of patients. Cyclophosphamide doses and treatment durations should be kept as low as possible, other treatments should be considered and regular urine cytology tests or cystoscopic evaluations should be liberally performed. Agents' protective of the mutagenic capacity of cyclophosphamide and its metabolites should be investigated. Furthermore, other risk factors for bladder cancer, such as smoking, should be avoided.

Conclusions

Based on the findings in Papers I-V, the following conclusions are made:

The incidence of Wegener´s granulomatosis in Sweden increased three-fold between 1975 and 2001.Neither ANCA-related increased awareness nor diagnostic drift from other vasculitic diseases fully explain this dramatic increase over time, but it still remains an open question whether the observed time trends reflect a true increase in incidence.

In clinical practice, the significance of c-ANCA/PR3-ANCA in patients who test positive for both these tests but do no present sufficient evidence for a vasculitis diagnosis appears limited. Patients, who are positive for c-ANCA /PR3-ANCA but do not present sufficient evidence for a systemic vasculitis diagnosis at the time of testing, appear unlikely to develop vasculitis in the future.

In absolute terms, the risk for Wegener´s granulomatosis among close relatives of patients with the disease is low. However, a moderately increased risk among close relatives cannot be excluded. Although a genetic predisposition to Wegener´s granulomatosis may exist, the clinical occurrence of the disease is likely to be strongly dependent on exogenous triggers.

Patients with Wegener´s granulomatosis have an overall doubled risk of developing cancer and there is a particularly increased risk of cancer of the urinary bladder, cancers of the haematopoietic system including lymphomas and squamous skin cancer.

The markedly increased risk of developing bladder-cancer in patients with Wegener´s granulomatosis can at least in part be attributed to the use of cyclophosphamide, with a dose-response relationship.

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