On the pathogenesis and clinical outcome of ANCA-associated vasculitis

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Chapter V

Incidence of malignancies in patients diagnosed with ANCA-associated vasculitis between 1991 and 2013

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Abstract

Objective

To investigate the incidence of malignancies during longitudinal follow-up of patients with antineutrophil cytoplasmic antibody-associated vasculitis (AAV), and to examine the effect of immunosuppressive therapy on malignancy risk in these patients.

Methods

The study population consisted of patients with histopathologically confirmed AAV, diagnosed between 1991 and 2013 at a large university hospital. The mean duration of followup was 10 years. Malignancy incidence was assessed using the Dutch National Pathology Database. Incidence rates from the Netherlands Cancer Registry were used to compare malignancy incidence in the AAV cohort to that in the general Dutch population.

Results

Thirty-six of 138 patients with AAV developed a total of 85 malignancies during a mean followup of 9.7 years. The sex-, age-, and calendar year-adjusted malignancy risk was 2.21-fold higher (95% confidence interval [95% CI] 1.64-2.92) than that in the general population. Non-melanoma skin cancers occurred most frequently (standardized incidence ratio 4.23 [95% CI 2.76-6.19]). The incidence rates of other malignancies were not significantly increased. Malignancy risk was associated with the duration of cyclophosphamide (CYC) therapy and, interestingly, was not increased in patients who had received CYC for <1 year.

Conclusion

Patients with AAV have a higher risk of malignancy than the general population, but this risk is accounted for solely by non-melanoma skin cancers. Over the years, the risk of other malignancies – specifically bladder and hematologic malignancies – has decreased in patients with AAV. This finding reflects ongoing efforts to reduce CYC exposure by developing new treatment regimens.

Introduction

The antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs), including granulomatosis with polyangiitis (Wegener's) (GPA) and microscopic polyangiitis (MPA), are small-to-medium vessel vasculitides that affect multiple organs and are life-threatening when untreated[1]. The availability of immunosuppressive therapy has dramatically improved prognosis in patients with AAV. Before the introduction of immunosuppressive therapy for this disorder, the mortality rate within 1 year after diagnosis was 80%. Currently, the remission rate is 90%[2-4]. Moreover, the life expectancy of patients with AAV who survive 5 years after diagnosis approaches that of the general population[4, 5]. As a result, attention has shifted to the long-term complications experienced by patients with AAV. Malignancies have been shown to be the second most common cause of death >1 year after diagnosis[4]. In previous studies, the incidence of malignancies among patients with AAV has been shown to be increased compared to the general population[6-12]. This was particularly the case for bladder cancer, malignant lymphomas, leukemia, and non-melanoma skin cancers (NMSCs).

The results of earlier studies of malignancy risk in AAV must be interpreted in light of a number of factors. First, in previous studies, except for one published in 1992[6], followup was limited to ~5 years[7, 9, 10, 13]. Second, in some studies information on malignancy incidence was based on patient or physician questionnaires, which may have introduced reporting bias. Most importantly, the observation period in most studies dated from the 1960s to the 1990s[6-8, 13]. In recent years, immunosuppressive therapy regimens have changed, based on efforts to reduce exposure to steroids and cyclophosphamide (CYC)[14-16]. Therefore, previous data on malignancy incidence mostly reflect risks associated with therapy regimens that are no longer used.

In the current study, we investigated the incidence of malignancies in 138 patients with AAV treated with current immunosuppressive therapy regimens, who were followed up for a mean of 10 years. Specifically, we examined the effect of duration of CYC exposure on the risk of malignancy in these patients. A nationwide histology database was used to assess the incidence of malignancy in this cohort.

Patients and methods

Study population and data collection

This study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki.

We enrolled patients with histopathologically confirmed AAV diagnosed at Leiden University Medical Center between 1991 and 2013. Patients with coexisting autoimmune diseases were excluded. Definitions provided in the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides[17] were used. Of the 138 patients enrolled, 134 (97%) had undergone renal biopsy in which AAV was confirmed histopathologically (i.e., crescentic and/or necrotizing glomerulonephritis with few or no immune deposits detected by immunofluorescence and/or electron microscopy). In the 4 patients who did not undergo renal biopsy, the biopsies showing AAV originated from the lung (3 patients), the ear/nose/throat (ENT) (3 patients), and the skin (2 patients). All 4 of these patients had a clinical diagnosis of GPA, and all exhibited ANCA positivity. Twenty of the 138 patients in the study were negative for ANCA or had an unknown ANCA serotype; AAV in these patients was confirmed histologically by renal biopsy findings as defined above. Seven of these patients also had a second biopsy from another organ showing AAV (skin [4 patients], lung [2 patients], and ENT region [1 patient]).

Primary malignancies were identified via the Dutch National Pathology Database, a nationwide histopathology and cytopathology network and registry in The Netherlands. The database includes data from all pathology laboratories[18]. The cohort was linked to the Central Population Registry to obtain information on death and emigration. The observation time started on the date of AAV diagnosis and ended on the date of death, the date of last followup, or on May 1, 2013, whichever occurred first. We retrieved medical records to collect information on sex, date of birth, clinical diagnosis (GPA/MPA), ANCA serotype, renal function at diagnosis, renal transplantation, renal transplantation date (when applicable), and use of immunosuppressive medication (type and duration).

Standardized incidence ratio calculation

Malignancy occurrence in our cohort was compared to that in the general population by determining standardized incidence ratios (SIRs), calculated as the observed number of malignancies divided by the expected number of malignancies. To obtain the most accurate value, SIRs were calculated with matching for sex, age (5-year age groups), and calendar-year period (1-year time periods). The observed number of malignancies was the total number of malignancies that occurred in the cohort. If a patient developed multiple NMSCs, only the first NMSC was taken into account in the analyses. The expected number of malignancies was defined as the person-years at risk multiplied by the national cancer incidence rate data provided by the Netherlands Cancer Registry. Subgroup analyses were performed for the following variables: sex, age at study entry (dichotomous according to the median value), renal transplantation, clinical diagnosis, ANCA serotype, followup duration, and history of malignancy before AAV diagnosis. Moreover, we considered that the landmark European Vasculitis

Society (EUVAS) trial Cyclophosphamide versus Azathioprine as Remission Maintenance Therapy for ANCA-Associated Vasculitis (CYCAZAREM), published in 2003[19], led to a drastic reduction in CYC exposure in patients with AAV. Consequently, we performed separate subgroup analyses of patients diagnosed before 2003 and patients diagnosed in or after 2003, to investigate whether this change in immunosuppressive therapy regimen had an effect on malignancy risk.

Statistical analysis

Student's *t*-test and the chi-square test were used to assess the significance of differences in baseline characteristics of patients who did and those who did not develop malignancies. Survival rates were analyzed by log rank test. Malignancy-free survival time was calculated with the Kaplan-Meier method; the observation time started at AAV diagnosis and ended at the date of the first malignancy diagnosis, death, or May 1, 2013, whichever occurred first. SIRs and 95% confidence intervals (95% CIs) were calculated with an exact Poisson regression analysis, which allowed incorporation of patients with multiple malignancies, assuming a Poisson distribution of the observed number of cases[20-22]. For all subgroup analyses, exact Poisson regression models were performed to calculate the relative risk. *P* values less than 0.05 were considered significant.

Results

Patient characteristics

A total of 138 patients with histopathologically confirmed AAV were included in the study. Of the 117 patients (85%) with available clinical diagnoses, 79 (68%) had a diagnosis of GPA and 38 (32%) had a diagnosis of MPA; none had a diagnosis of eosinophilic granulomatosis with polyangiitis (Churg-Strauss). Of the 128 patients (93%) with available data on ANCA serotype, 55 (43%) were positive for proteinase 3 (PR3)-ANCA; 47 (37%) for myeloperoxidase (MPO)-ANCA, and 5 (4%) for both PR3-ANCA and MPO-ANCA; 11 patients (9%) were positive for ANCA but with no available data on whether they were PR3-ANCA or MPO-ANCA positive, and 10 patients (8%) were negative for ANCA. The mean $\pm$ SD age at AAV diagnosis was 59.3 $\pm$ 14.8 years, and did not differ significantly between patients who did and those who did not develop malignancy during followup. Fourteen patients had a history of malignancy occurrence prior to the AAV diagnosis. No malignancy diagnosed prior to the AAV diagnosis metastasized during the followup period. Additional demographic and clinical data are summarized in Table 1.

Table 1. Characteristics of the AAV patients included in the study

	Total sample	No malignancy occurrence	Malignancy occurrence	p-value
Mean age at diagnosis, years (SD)	59.3 (14.8)	58.9 (15.6)	60.7 (12.0)	0.533
Male, n (%)	87 (63)	62 (61)	25 (69)	0.355
Clinical diagnosis, n (%)				0.753
GPA	79 (57)	56 (55)	23 (64)	
MPA	38 (28)	28 (27)	10 (28)	
Unknown	21 (15)	18 (18)	3 (8)	
ANCA-serotype, n (%)				0.079
PR3-ANCA	55 (40)	35 (34)	20 (56)	
MPO-ANCA	47 (34)	39 (38)	8 (22)	
ANCA-negative	10 (7)	7 (7)	3 (8)	
PR3- and MPO-ANCA positive	5 (4)	5 (5)	0 (0)	
ANCA positive[1]	11 (8)	9 (9)	2 (6)	
Unknown	10 (7)	7 (7)	3 (8)	
Mean baseline serum creatinine, µl/l (SD)	337.0 (303.6)	332.5 (279.1)	348.0 (361.0)	0.813
Renal transplantation, n (%)	10 (7)	8 (8)	2 (6)	0.649
Previous history of malignancy, n (%)	14 (10)	11 (11)	3 (8)	0.675
Organ involvement, n (%)[2]				
Cutaneous	36 (31)	21 (25)	15 (46)	0.044
Eyes	35 (30)	21 (25)	14 (42)	0.075
Ear, nose, and throat	78 (67)	53 (63)	25 (76)	0.276
Chest	61 (52)	45 (54)	16 (49)	0.683
Cardiovascular	7 (6)	6 (7)	1 (3)	0.671
Gastrointestinal	16 (14)	13 (16)	3 (9)	0.551
Kidney	138 (100)	0 (0)	138 (100)	N/A
Nervous system	32 (27)	25 (30)	7 (21)	0.490
Deaths, n (%)	56 (41)	39 (38)	17 (47)	0.255[3]

The study population consisted of 138 patients with histologically confirmed antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis (AAV). Data on clinical diagnosis and ANCA serotype were available for 117 patients and 128 patients, respectively. Except where indicated otherwise, values are the number (%). GPA = granulomatosis with polyangiitis (Wegener's); MPA = microscopic polyangiitis.

[1] Patients who were ANCA positive, but with no information in the medical records regarding whether they were positive for proteinase 3 (PR3)–ANCA or myeloperoxidase (MPO)–ANCA.

[2] All 138 patients had renal involvement; data on involvement of other organs were available for 117 patients.

[3] P = 0.044 versus patients with no malignancy occurrence.

Observed malignancies

Thirty-six patients developed malignancies (total of 85 malignancies) during the followup period (mean 9.7 years, median 8.0 years; 1,339 person-years). Of these malignancies, 61 were NMSCs, and they occurred in a total of 22 patients. The NMSCs included 42 basal cell carcinomas and 19 squamous cell carcinomas. In addition, 3 colon carcinomas, 3 breast carcinomas, 3 prostate carcinomas, 2 lung carcinomas, 2 soft tissue sarcomas, 2 unknown primary malignancies, and a variety of malignancies that occurred only once were observed (Table 2). Malignancy-free survival at 2, 5, and 10 years of followup was 99%, 84%, and 80%, respectively (Figure 1).

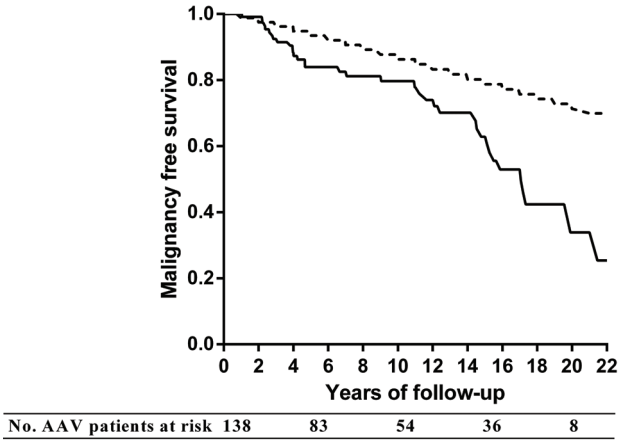

Figure 1. Years of malignancy-free survival in patients with antineutrophil cytoplasmic antibody-associated vasculitis (AAV) (solid line) compared to the age-and sex-matched general population (dashed line).

Comparison with the general population

The sex-, age-, and calendar year–adjusted malignancy risk among the AAV patients in this cohort was 2.21-fold higher than the risk in the general population (95% CI 1.64-2.92) (Table 2). This increased risk was attributable solely to an increased risk of developing NMSC (SIR 4.23 95% CI 2.76-6.19). We observed only 1 bladder carcinoma and 1 non-Hodgkin's lymphoma in the cohort. These incidences were not significantly increased compared to the general population, with SIRs of 1.43 (95% CI 0.04-7.96) and 1.37 (95% CI 0.03-7.63), respectively. The risk of developing any of the other reported malignancies was also not increased compared to that in the general population (Table 2).

Table 2. SIRs calculated for malignancies overall and per observed malignancy

Malignancy type	PY	N malignancies	SIR	95% CI	p-value
All malignancies	1339	85	2.21	1.64 – 2.92	<0.001
NMSC	1339	61	4.23	2.76 – 6.19	<0.001
Non-NMSC	1339	24	1.46	0.93 – 2.17	0.095
By malignancy					
Breast carcinoma	1339	3	2.04	0.42 – 5.96	0.367
Colon carcinoma	1339	3	1.85	0.38 – 5.41	0.444
Prostate carcinoma	795	3	0.99	0.20 – 2.89	1.000
Lung carcinoma	1339	2	0.75	0.23 – 3.30	0.993
Soft tissue sarcomas	1339	2	7.69	0.93 – 27.79	0.057
Unknown primary malignancy	1339	2	3.92	0.47 – 14.17	0.187
Brain carcinoma	1339	1	5.56	0.14 – 30.95	0.329
Parotis carcinoma	1339	1	33.33	0.84 – 185.72	0.059
Bladder carcinoma	1339	1	1.43	0.04 – 7.96	1.000
Uterus carcinomas	544	1	4.00	0.10 – 22.29	0.442
Non-Hodgkin lymphoma	1339	1	1.37	0.03 – 7.63	1.000
Renal cell carcinoma	1339	1	2.44	0.06 – 13.59	0.672
Chondrosarcoma	1339	1	28.57	0.72 – 159.19	0.068
Esophageal cancinoma	1339	1	2.70	0.07 – 15.06	0.602
Melanoma	1339	1	1.92	0.05 – 10.71	0.811

The analysis encompassed 1,339 person-years (except for the sex-specific malignancies prostate carcinoma [795 person-years] and uterus carcinoma [544 person-years]). Standardized incidence ratio (SIR) (ratio of observed to expected malignancies) was adjusted for sex, age (5-year age groups), and calendar time period (1-year time periods). 95% CI = 95% confidence interval; NMSC = non-melanoma skin cancer.

Malignancy risk by clinical diagnosis and ANCA subtype

We performed prespecified subgroup analyses with stratification by sex, age, followup duration, clinical diagnosis, ANCA subtype, history of malignancy before AAV diagnosis, diagnosis and treatment initiation before or after publication of the CYCAZAREM trial (2003), and renal transplantation (Table 3). The risk of malignancy became increased compared to the general population after the duration of AAV exceeded 5 years (Table 3).

Table 3. SIRs for malignancy in the AAV patients stratified into various subgroups

	N patients	N malignancies	SIR (95% CI)	SIR p-value	RR (95% CI)	RR p-value
Gender						
Male	87	61	2.33 (1.64 – 3.21)	<0.001		
Female	51	24	1.93 (1.03 – 3.31)	0.041	0.83 (0.40 – 1.60)	0.680
Age						
≥ 61 years	68	44	2.20 (1.47 – 3.18)	0.001		
< 61 years	70	41	2.22 (1.39 – 3.36)	<0.001	0.99 (0.55 – 1.82)	1.000
Clinical diagnosis						
MPA	38	23	1.89 (0.98 – 3.30)	0.058		
GPA	79	54	2.53 (1.76 – 3.52)	<0.001	1.34 (0.68 – 2.83)	0.478
ANCA-serotype						
MPO-ANCA	47	16	1.87 (0.93 – 3.34)	0.076		
PR3-ANCA	55	53	2.72 (1.80 – 3.93)	<0.001	1.46 (0.70 – 3.24)	0.371
Renal transplantation						
No	128	78	2.17 (1.59 – 2.91)	<0.001		
Yes	10	7	3.33 (1.08 – 7.78)	0.037	1.53 (0.48 – 3.85)	0.495
Follow-up						
0 – 5	55	6	1.60 (0.52 – 3.73)	0.414		
5 - 10 years	29	19	2.91 (1.45 – 5.21)	0.004	1.82 (0.58 – 6.67)	0.385
>10 years	54	60	2.17 (1.50 – 3.03)	<0.001	1.36 (0.53 – 4.44)	0.702
Year of diagnosis						
<2003	83	73	2.43 (1.76 – 3.26)	<0.001		
≥2003	55	12	1.34 (0.49 – 2.92)	0.586	1.81 (0.77 – 5.20)	0.220
Previous history of malignancy						
No	124	80	2.24 (1.64 – 2.98)	<0.001		
Yes	14	5	1.97 (0.54 – 5.05)	0.297	1.13 (0.41 – 4.34)	1.000

Standardized incidence ratio (SIR) (ratio of observed to expected malignancies) depicts malignancy risk compared to the general population. Relative risk (RR) depicts malignancy risk compared to the referent group. None of the RR values were statistically significant. 95% CI = 95% confidence interval (see Table 1 for other definitions).

Analysis of the clinical diagnosis and ANCA serotype subgroups showed a significantly increased malignancy risk in patients with GPA and/or with PR3-ANCA compared to the general population, but not in patients with MPA and/or MPO-ANCA. However, within the AAV cohort, the malignancy risk was not significantly different between those who had GPA and/or were positive for PR3-ANCA and those who had MPA and/or were positive for MPO-ANCA (Table 3).

Malignancy risk in patients with a history of malignancy before AAV diagnosis

Fourteen of the 138 patients had a history of malignancy before AAV diagnosis and 124 did not. The total number of malignancies that developed in these 2 groups during followup were 5 and 80, respectively. SIRs were similar between patients with and patients without a history of malignancy before the AAV diagnosis (Table 3).

Malignancy risk before and after publication of the CYCAZAREM trial in 2003

Eighty-three of the 138 AAV patients were diagnosed before 2003, and 55 in or after 2003 (Table 3). The mean ± SD duration of CYC therapy was 14.5 ± 17.4 months and 5.1 ± 4.1 months in patients diagnosed before 2003 and patients diagnosed in or after 2003, respectively ($P < 0.001$). The 83 patients diagnosed before 2003 developed a total of 73 malignancies, which resulted in a 2.43-fold increase in malignancy risk compared to the general population (95% CI 1.76-3.26). The 55 patients diagnosed in or after 2003 developed a total of 12 malignancies; the malignancy risk in these patients was not significantly increased compared to the general population (SIR 1.34 [95% CI 0.49-2.92]).

Malignancy risk in patients with renal transplants

Ten patients (7%) underwent renal transplantation during followup. The mean ± SD time from AAV diagnosis to renal transplantation was 6.7 ± 4.8 years. Two of the 10 patients with renal transplants developed a total of 7 malignancies, during a mean ± SD followup of 12.8 ± 4.8 years. These malignancies included 3 squamous cell carcinomas, 2 basal cell carcinomas, 1 prostate carcinoma, and 1 parotid carcinoma. Malignancy risk compared to the general population was significantly increased among patients with AAV who had undergone renal transplantation (SIR 3.33 [95% CI 1.08-7.78]) as well as those who had not undergone transplantation (SIR 2.17 [95% CI 1.59-2.91]). We did not observe a significant effect of transplantation on malignancy risk in these subgroups ($P = 0.495$); however, since only 10 patients in the cohort had undergone transplantation, this subanalysis was presumably not sufficiently powered to detect a significant difference.

Effects of immunosuppressive therapy on malignancy risk

Data on immunosuppressive therapy were available for 117 patients (85%) (Table 4). All 117 patients received prednisolone, 110 (94%) received CYC, 62 (53%) received azathioprine (AZA), and 24 (21%) received mycophenolate mofetil (MMF) at some time during their disease. Moreover, 4 patients (3%) received methotrexate, and 5 patients (4%) received rituximab. The mean ± SD duration of CYC therapy was 17.6 ± 17.9 months and 8.0 ± 11.8 months, respectively, in patients who did and those who did not develop a malignancy during followup ($P = 0.001$) (Table 4).

Table 4. Immunosuppressive therapy in the AAV patients

	Total sample (n=117)	No malignancy occurence (n=84)	Malignancy occurence (n=33)	p-value
Ever treatment, n (%)				
Prednisolone	117 (100)	84 (100)	33 (100)	N/A
Cyclophosphamide	110 (94)	80 (95)	30 (91)	0.374
Azathioprine	62 (53)	43 (51)	19 (58)	0.533
Mycophenolate mofetil	24 (21)	20 (24)	4 (12)	0.159
Mean duration, months (SD)				
Prednisolone	37.7 (42.3)	29.7 (39.2)	58.1 (43.7)	0.001
Cyclophosphamide	10.7 (14.4)	8.0 (11.8)	17.6 (17.9)	0.001
Azathioprine	18.5 (31.4)	14.2 (28.3)	29.5 (36.5)	0.017
Mycophenolate mofetil	7.6 (21.1)	7.5 (16.7)	7.8 (29.9)	0.943

Some of the patients with antineutrophil cytoplasmic antibody–associated vasculitis (AAV) received consecutive treatments with cyclophosphamide and/or azathioprine and/or mycophenolate mofetil (47 patients received cyclophosphamide and azathioprine, 8 patients received cyclophosphamide and mycophenolate mofetil, 1 patient received azathioprine and mycophenolate mofetil, and 13 patients received cyclophosphamide, azathioprine, and mycophenolate mofetil).

NA = not applicable (analyses on prednisolone were not possible as all patients were treated with this immunosuppressant).

The duration of CYC exposure was directly associated with malignancy risk (Table 5). The malignancy risk in patients with AAV who had received CYC for <1 year was not significantly different from that in the general population. Patients treated with CYC for 12–24 months and those treated for >24 months showed increased malignancy risks, with SIRs of 3.83 (95% CI 1.98–6.70) and 4.67 (95% CI 2.55–7.83), respectively.

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The risk of malignancy in patients treated with CYC and corticosteroids was not different from that in patients treated with AZA or MMF maintenance therapy following CYC treatment (SIR 2.62 [95% CI 1.53–4.19] and SIR 2.02 [95% CI 1.29–3.01], respectively). The average duration of CYC treatment was 14 months in patients treated with CYC and corticosteroids and 10 months in patients who received other immunosuppressive agents for maintenance therapy after CYC treatment ($P = 0.234$).

Table 5. SIRs calculated according to duration of cyclophosphamide therapy

Cyclophosphamide therapy duration	N patients	N malignancies	SIR (95% CI)	SIR p-value	RR (95% CI)	RR p-value
0 – 6 months	65	15	1.52 (0.78 – 2.64)	0.212		
6 – 12 months	21	14	1.82 (0.79 – 3.59)	0.156	1.20 (0.43 – 3.19)	0.847
12 – 24 months	16	30	3.83 (1.98 – 6.70)	<0.001	2.53 (1.04 – 6.17)	0.040
> 24 months	15	18	4.67 (2.55 – 7.83)	<0.001	3.08 (1.31 – 7.32)	0.008

Standardized incidence ratio (SIR) (ratio of observed to expected malignancies) depicts malignancy risk compared to the general population. Relative risk (RR) depicts malignancy risk compared to the referent group. 95% CI = 95% confidence interval.

Discussion

This is the first published study to investigate malignancy incidence in relation to current therapy regimens in a cohort of patients with AAV followed up for a long period (mean 10 years). During this period, the risk of malignancy was 2.21-fold higher in patients with AAV compared to the general population. This increased risk was attributable solely to the occurrence of NMSC. There was no increased risk for all other reported malignancies compared to the general population. In particular, there was no increased risk of bladder cancer, leukemia, or malignant lymphomas, in contrast to data presented in previous reports[6–11,13]. In our cohort, risk of malignancy was directly associated with the duration of CYC therapy. Interestingly, malignancy risk was not increased among patients who had been exposed to CYC for <1 year.

Previous studies on malignancy risk in AAV showed 2.4-33-fold increases in the risk of developing bladder cancer, 3.2-5.9-fold increases in the risk of developing leukemia, and 1.1-11-fold increases in the risk of developing malignant lymphomas[12]. Followup periods in those studies were ~5 years[7,9–11] or were

not reported[8, 13], except in the case of one study conducted from 1967 to 1990 with a median followup of 8 years[6]. As noted, patients in our AAV cohort had no increased risk of developing any of these malignancies after a mean followup period of 10 years. Only 1 patient in our cohort, who received CYC for 4 years, developed bladder carcinoma (9 years after AAV was diagnosed). In addition, there was 1 non-Hodgkin's lymphoma (which occurred 13 years after the AAV diagnosis). This patient was treated with CYC for 4.5 years and AZA for 3.8 years.

The discrepancies between our findings and those of previous studies likely reflect the changes in AAV treatment regimens over the years. Most previous studies were conducted from the 1960s to the 1990s[6-8, 13]. Since that time, large, international therapeutic trials have been conducted[14-16, 19], which resulted in dramatic reductions in exposure to CYC. A previous 5-year followup study of patients initially recruited in those trials demonstrated no increased risk of bladder or hematologic malignancies compared to the general population[10]. Our study validates those results and, moreover, shows that even after 10 years of followup, the risk of developing these malignancies is not increased.

The followup period of our study (1991–2013) overlaps publication of the reports of the therapeutic trials conducted by the EUVAS[14-16, 19]. In the landmark EUVAS CYCAZAREM study published in 2003, it was concluded that early withdrawal of CYC and substitution of AZA at the time of remission did not increase relapse rates in patients with AAV, and that the duration of exposure to CYC therefore could be safely reduced by switching to maintenance therapy at the time of remission achievement[19]. In our cohort, the mean duration of CYC therapy was ~3 times longer in patients diagnosed before 2003 compared to patients diagnosed in or after 2003. Interestingly, we found that the malignancy risk compared to the general population is significantly increased among AAV patients diagnosed before 2003, but not among those diagnosed later. This indicates that the reduction in CYC exposure over time has resulted in a decrease in malignancy risk among patients with AAV.

Apart from increased malignancy risk induced through immunosuppressive therapy regimens, it has been suggested that an increased risk of malignancy may arise from chronic stimulation of the immune system due to vasculitis[23]. Moreover, more severe disease with greater disease activity is generally associated with longer duration of immunosuppressive therapy; thus, it is difficult to separate disease-related and therapy-related effects on malignancy risk. Our finding of a high incidence of malignancies only after 5 years of followup (i.e., after longer exposure to immunosuppressive therapy) suggests that this therapy may have an important effect on the development of malignancies, because intrinsic disease-associated malignancies would be more likely to occur within the first 5 years. Moreover, we found that the malignancy risk in patients with AAV decreased after reduction of CYC exposure, which clearly indicates a dose-dependent effect.

Subgroup analyses demonstrated a significantly increased malignancy risk among patients with GPA and/or PR3-ANCA positivity, but not among patients with MPA and/or MPO-ANCA positivity. Possible explanations for this could be that patients with GPA and/or PR3-ANCA positivity had superior survival or a higher risk of relapses and need for more immunosuppressive treatment compared to patients with MPA and/or MPO-ANCA positivity. Further analyses demonstrated that survival was not different between patients with GPA and/or PR3-ANCA positivity and patients with MPA and/or MPO-ANCA positivity (data not shown). However, the duration of CYC treatment was significantly longer among patients with PR3-ANCA positivity than among those with MPO-ANCA positivity (mean 14 months and 8 months, respectively). This finding further supports the notion that immunosuppressive treatment exposure increases malignancy risk in patients with AAV.

Currently, immunosuppressive therapy for AAV seems to be complicated by the occurrence of NMSC. It has been suggested that AZA might accelerate NMSC development by sensitizing the skin cell genome to ultraviolet A radiation[24]. Previous studies have shown an association between AZA exposure and the occurrence of NMSC in patients with transplants, myasthenia, autoimmune inflammatory rheumatic diseases, and inflammatory bowel disease[25-29]. Our data imply that there is also an association between AZA and NMSC in patients with AAV (Table 4). In contrast, we found no difference in the occurrence of NMSC between patients treated with CYC alone and those treated with AZA following CYC therapy, despite similar durations of CYC exposure. However, our analysis may not have been sufficiently powered to detect a significant difference. Although mortality associated with NMSCs is low, their impact should not be underestimated because they can cause significant morbidity. Our results support the notion that patients with AAV who receive immunosuppressive therapy should undergo regular skin cancer screening and be advised to protect themselves against ultraviolet radiation exposure[30].

One limitation of this study is the fact that the clinical data were collected retrospectively. However, for most patients these data were accessible and recorded punctually. Moreover, the analysis was confined to a Dutch patient population, which should be taken into account in comparing our results with those from the previous multicenter European study[10]. Finally, the limited number of patients included in this study could have resulted in insufficient power to detect significant differences, particularly in the subgroup analyses. One of the strengths of the study is the long followup period. This is the first study on malignancy risk in patients with AAV with a mean followup duration as long as 10 years. Moreover, the diagnosis of AAV was confirmed histologically in all of our patients. Finally, no malignancy in our patient cohort could have been missed, due to the accurate data reporting through the Dutch National Pathology Database.

In conclusion, the results of this 10-year followup study indicate that patients with AAV have a higher risk of NMSC than the general population. There was no increased risk of bladder cancer, leukemia, or malignant lymphomas, as was previously reported in patients with AAV. With current treatment regimens, CYC treatment appears to be safe for up to 1 year without causing an increase in malignancy risk. Longer exposure to CYC increased the risk of malignancy occurrence. Our results demonstrate that with treatment regimens currently in use for AAV, the risk of developing bladder and hematologic malignancies is lower than with previous standard regimens, underscoring the success of international efforts to find less cytotoxic treatment regimens for the disease. However, other toxicities associated with current therapies, e.g., in terms of infertility and infection, remain a concern. Quite recently, rituximab was introduced as part of AAV treatment regimens, showing success and with the promise of further reducing malignancy risk in AAV patients[16, 31, 32]. Continued efforts aimed at developing additional safe and effective therapies for AAV are, nevertheless, still warranted.

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