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Risk of Anal Cancer Following Benign Anal Disease and Anal Cancer Precursor Lesions: A Danish Nationwide Cohort Study

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ABSTRACT

Background: Human papillomavirus (HPV) is associated with the majority of anal high-grade intraepithelial neoplasia (AIN) and anal cancers. Little is known about the risk of anal cancer following a diagnosis of benign anal disease and AIN.

Methods: Using data from nationwide, population-based Danish registries, a cohort of 126,174 individuals with either non-neoplastic anal disease or AIN 1 to 3 during 1970 to 2016 was followed until first occasion of anal cancer. Information on HIV status was obtained from the Danish HIV Cohort Study. The absolute risk of anal cancer was estimated using the Aalen-Johansen estimator taking into account censoring at emigration and end of follow-up and competing risk at time of death. Standardized incidence ratios (SIR) for anal cancer among individuals with non-neoplastic anal disease, including inflammatory lesions, hemorrhoids, and polyps, were estimated in Poisson models. Sex-, age-, and calendar period-specific national population rates were estimated using the Danish National Pathology Registry.

Results: Anal cancer risk increased with increasing severity of lesions, reaching 4% 5 years after diagnosis of AIN3. Even among those with non-neoplastic anal lesions, particularly inflammatory lesions, anal cancer risk was significantly higher than expected from Danish national anal cancer rates (SIR = 2.8; 95% confidence intervals, 2.3–3.2). The absolute 5-year risk of anal cancer following AIN3 was considerably higher among HIV-positive (14.1%) than HIV-negative (3.2%) individuals.

Conclusions: Anal cancer risk increases with increasing severity of lesions and is especially high among HIV-positive individuals.

Impact: Vaccination against HPV is important in the prevention of both high-grade AIN and anal cancer.

Introduction

Anal cancer is a rare disease, but over the past decades increases in the incidence have been observed in several high-income countries, both among women and men (1). This increase was primarily confined to the main histologic subtype: anal squamous cell carcinomas (SCC); whereas the incidence of anal adenocarcinomas (AC), the second most common histologic subtype, decreased or remained stable in most populations (1). High-risk groups of anal cancer include men who have sex with men (MSM), HIV-positive individuals, chronically immunosuppressed patients, and organ transplant patients (2).

Anal cancer is biologically similar to cervical cancer. Both occur at the squamocolumnar junction epithelium around the transformation zone and both cancers are causally linked to human papillomavirus (HPV) infection (3). Studies have shown that more than 80% of anal SCCs are associated with HPV infection, predominantly the high-risk type HPV16 (4). Anal cancer is preceded by high-grade anal intraepithelial neoplasia (AIN) including AIN grade 2 and grade 3 (2). AIN1 is not considered a direct precursor of invasive anal cancer, but may precede the later development of AIN2 or AIN3 (5). Furthermore, the possible role of nonneoplastic (hereafter denoted benign) anal lesions such as anal inflammations, hemorrhoids, and polyps in the development of anal cancer have been debated and different mechanisms hypothesized (6, 7).

Because of the low prevalence of anal cancer, anal cancer screening is not recommended in the general population (3) and therefore little is generally known about the risk of anal cancer following benign and anal precursor lesions. A number of studies have examined progression of high-grade anal precursor lesions to anal cancer, but primarily in high-risk groups such as HIV-positive patients, immunosuppressed patients, and MSM (8–20). Furthermore, most of the previous studies are limited by small study populations and short follow-up time. The risk of anal cancer among individuals diagnosed with benign anal lesions have been investigated in case–control studies (21–23) and cohort studies (24, 25); however showing conflicting results.

Given the rarity of large population-based studies investigating progression from precursor anal lesions to anal cancer and the conflicting results regarding the risk of anal cancer following benign anal lesions, we attempted to evaluate the risk of anal cancer using data from a large nationwide Danish cohort with a relatively long follow-up period. Our primary objectives were (i) to evaluate the relationship between benign anal lesions and anal cancer, and (ii) to determine the risk of anal cancer following anal precursor lesions of different severity. For the first objective, we evaluated the relationship for benign lesions overall and in subgroups including inflammatory lesions, hemorrhoids, and polyps. For the second objective, we determined the risk of anal cancer overall and for those with AIN3 we also evaluated the risk in subgroups according to sex, age, and HIV status. Furthermore,

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we aimed to compare the risk of anal cancer after AIN3 found in our study with the results from previous studies. The risk of anal cancer was investigated for all anal cancers combined and for the 2 main histologic subgroups of anal cancer, anal SCC and AC, respectively.

Materials and Methods

Data sources

The study is based on information from the nationwide, population-based Danish Civil Registration System and the Danish National Pathology Registry (DNPR). Information on HIV status was obtained from the Danish HIV Cohort Study. The fact that a unique personal identification number (PIN) is assigned to all Danish citizens secures accurate linkage of information from different registries.

The Danish Civil Registration System (26) administers PIN and contains continuously updated information on dates of birth and death, addresses, and migration to and from Denmark. The DNPR (27) is a national registry containing information on pathology specimens from public and private pathology departments in Denmark since 1970. From 1997 all pathology departments were mandated to report to the databank through an online real-time data reporting system and therefore it is considered entirely complete from 1997 and onwards. The Danish HIV Cohort Study (28) is an open cohort with continuous enrolment including all HIV-infected individuals aged 16 years or older at time of HIV diagnosis seen in one of the 8 Danish HIV centers after December 31, 1994.

Identification of study population

We established a population-based study cohort comprising 128,478 individuals with at least one anal pathology specimen of less severity than anal cancer registered in the DNPR in the period from 1970 to 2016. Information about anal examinations was retrieved using the systematized nomenclature of medicine (SNOMED) topography codes T69000, T69010, T69015, T69110, T69120, T02507, and TY1701. Individuals residing in Greenland at time of first registration in DNPR ($n = 183$), and individuals with invalid PIN ($n = 280$) were excluded. For the remaining 128,015 individuals, information on all anal examinations, except for anal cancers, within the study period was extracted and subsequently categorized in 4 main groups according to severity as either benign (no dysplasia), AIN1, AIN2 [including "not otherwise specified" (NOS)], or AIN3 [including carcinoma *in situ* (CIS)] based on the SNOMED morphology codes (Supplementary Table S1). In case of more than one diagnosis registered at the same date, we counted only the most severe diagnosis. Among benign lesions, 3 main subgroups were identified including inflammatory lesions such as fistulas and pilonidal cysts, hemorrhoids, and polyps (Supplementary Table S1). To investigate the risk of anal cancer following registration with anal lesions of different severity we defined 4 cohorts. Each cohort included all individuals diagnosed with lesions at the given level of severity (benign, AIN1, AIN2, and AIN3, respectively), and who did not have more severe antecedent diagnoses. A person could be included in more than one cohort if progression to more severe lesions was experienced during the study period.

Outcome variables and follow-up

All eligible 128,015 individuals were followed until first occasion of anal cancer (all anal cancers combined and anal SCC and AC separately), emigration, death, or end of follow-up, whichever came first. The codes used to identify overall anal cancer, anal SCCs, and ACs are listed in Supplementary Table S1. Information on vital status, date of death, disappearance, and emigration was obtained from The Danish Civil Registration System. A total of 1,159 individuals did not provide any follow-up time; 1,088 were previously registered with anal cancer, and the remaining 71 died or emigrated on the date of entrance. Furthermore, as anal cancer rarely occurs before the age of 20, we started follow-up no earlier than 20 years of age and excluded those 682 individuals who were below 20 years of age at exit.

Hence, the final study base comprised 126,174 individuals who were eligible to be included in 1 or more of the 4 cohorts defined according to severity of lesion.

Statistical analysis

For each of the cohorts, the absolute risk of anal cancer following registration of the index diagnosis was estimated nonparametrically using the Aalen–Johansen estimator taking into account censoring at emigration and end of follow-up (December 31, 2016) and competing risk at time of death. Individuals were followed from the time of registration of the relevant lesion (benign, AIN1 AIN2, or AIN3) and applying delayed entry at 20 years of age. Median potential follow-up time in each of the 4 cohorts was estimated based on the reverse Kaplan–Meier method (29). Results considering the 2 main histologic subtypes SCC and AC, respectively, are provided whenever numbers are sufficiently large. In these analyses, only the first diagnosis was considered, treating other histologic subtypes as competing risks.

To evaluate whether the risk of anal cancer following a benign lesion is higher than expected from national population rates, standardized incidence ratios (SIR) with 95% confidence limits were estimated in multiplicative Poisson regression models with log transformed person-years times national population rates as offset. Sex-, age-, and calendar period-specific national population rates were estimated using the DNPR with age and calendar period in 5-year groups and person-years of risk estimated using mid-year population sizes as provided by Statistics Denmark (30). Stratified analyses by follow-up duration (<0.5, 0.5–1, 1–5, 5–10, 10+ years), sex (male, female), age (<50, 50+), period of diagnosis (<1997, 1997+), and HIV status at entrance (negative, positive) were performed. To exclude prevalent cases, results from analyses applying delayed entry at 6 months are provided.

Furthermore, to compare the absolute risk of anal cancer after AIN3 with that of other study populations, 5-year anal cancer risk estimates with 95% confidence intervals (CI) estimated using the Aalen Johansen estimator with delayed entry at 6 months after the AIN3 diagnosis were provided in subgroups according to sex, age, and HIV status at diagnosis. For those comparison studies where no 5-year anal cancer risk is provided in the paper, we calculated rough 5-year risk estimates as $1 - \exp(-5 \cdot rate)$; where *rate* is the estimated anal cancer incidence rate per year. The anal cancer incidence rate was extracted from the paper or alternatively estimated as the number of cases divided by the number of person-years of follow-up; using either reported value if provided or otherwise estimated as number of individuals times mean or median follow-up time.

Finally, as it differs whether studies include precursor lesions and anal cancers with topography codes T02507 (skin in anal region), a sensitivity analysis removing these was performed.

Results

Characteristics of study cohorts

Within the study period, 126,174 individuals were registered with at least one noncancerous anal lesion and were at risk for incident anal cancer after the age of 20. The majority (99.4%) only served as exposed to 1 of the 4 exposure cohorts, whereas the remaining 814 were

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Table 1. Characteristics of the 4 study cohorts.

	Benign ($n = 115,770$) *n* (%)	AIN1 ($n = 8,941$) *n* (%)	AIN2[a] ($n = 799$) *n* (%)	AIN3 ($n = 1,491$) *n* (%)
Characteristics at diagnosis				
Sex				
Male	55,161 (48%)	4,009 (45%)	338 (42%)	378 (25%)
Female	60,609 (52%)	4,932 (55%)	461 (58%)	1,113 (75%)
Age				
<50 years	69,401 (60%)	7,106 (79%)	325 (41%)	642 (43%)
≥50 years	46,369 (40%)	1,835 (21%)	474 (59%)	849 (57%)
Median age (P25–P75)	45 (34–58)	35 (27–47)	55 (41–69)	52 (43–65)
Calendar year				
<1997	35,805 (31%)	2,630 (29%)	173 (22%)	324 (22%)
≥1997	79,965 (69%)	6,311 (71%)	626 (78%)	1,167 (78%)
HIV				
No	115,363 (99.6%)	8,736 (97.7%)	757 (94.7%)	1,433 (96.1%)
Yes	407 (0.4%)	205 (2.3%)	42 (5.3%)	58 (3.9%)
Follow-up				
Median years of follow up (P25–P75)	13.3 (6.3–21.5)	13.1 (5.9–21.8)	11.1 (6.1–18.5)	11.6 (5.3–18.8)
Number of deaths	22,928 (20%)	1,093 (29%)	229 (29%)	307 (21%)
Number of incident anal cancers	245 (0.2%)	62 (0.7%)	49 (6.1%)	276 (18%)

[a]Including precursor lesions not otherwise specified, which constitutes approximately one third.

registered with progressions during follow-up and thereby included in more than 1 of the 4 cohorts defined according to severity of lesion. Most of these [$n = 509$ (63%)] were initially registered with a benign diagnosis, which was later followed by a diagnosis of AIN1. The cohort of individuals with benign registrations consisted of 115,770 individuals. A diagnosis of AIN1 without prior lesions of higher severity was experienced by 8,941, AIN2 by 799, and AIN3 by 1,491 individuals. In the benign cohort, almost half were men (48%), whereas in the cohort with AIN3, the majority were women (75%). The median age at diagnosis among those with high-grade lesions (AIN2: median age 55 years; AIN3: 52 years) was higher than among those presenting with benign lesions (median age 45 years). The AIN1 cohort was the youngest with a median age of 35 years at diagnosis. The proportion of HIV-positive individuals was generally low, but it was markedly higher among individuals with AIN lesions compared with those with benign lesions. The median potential follow-up time for the 4 cohorts ranged between 11 and 13 years (**Table 1**).

The cohort of 115,770 individuals with a benign registration was further divided into a subcohort of 25,797 individuals with inflammatory diseases (22%), 41,239 with hemorrhoids (36%), and 31,982 (28%) with polyps. Most of the remaining 16,752 individuals were registered with normal tissue without further specification such as fibrosis or dermatitis. Among 3,192 (3%) individuals registered with more than 1 of the 3 subtypes, 1,809 with inflammatory lesions were placed in that group, whereas the remaining 1,383 were classified as hemorrhoids. Contrary to the group with polyps where only one third were men (33%), those with inflammatory lesions were dominated by men (67%), and they were younger at diagnosis (median age 37) than those with hemorrhoids (median age 48) or polyps (median age 46; Supplementary Table S2).

Anal cancer risk following benign anal disease

During follow-up we identified 245 anal cancer cases among the 115,770 individuals in the benign cohort; 161 (66%) were SCCs and 57 (23%) were ACs. Among the remaining 27 anal cancer cases, 18 were registered as other histologic types, whereas 9 were carcinoma NOS. The absolute risk of anal cancer after registration of a benign anal diagnosis showed a steep increase immediately after the diagnosis, and the same pattern was seen for the risk of SCCs and ACs (**Fig. 1A**). The risk of SCCs continued to increase, whereas the risk of ACs seemed to level off. Of the 245 cases observed within the total follow-up period, 89 cases appeared within the first 6 months. The pattern of an excess risk just after the diagnosis was confirmed in the SIR analysis, where we observed almost 60 times more anal cancer cases than expected from national population incidence rates [SIR 59.3 (95% CI, 47.8–72.5)] and with an overall SIR for the total follow-up period of 4.2 (95% CI, 3.7–4.8; **Table 2**). The risk remained significantly elevated for up to 10 or more years of follow-up. After excluding the first 6 months, the increased risk of anal cancer was still highly significant (SIR 2.8; 95% CI, 2.3–3.2), and this applied to both histological subtypes: SCC (SIR 2.8; 95% CI, 2.3–3.3) and AC (SIR 2.8; 95% CI, 1.9–3.9). The results remained unchanged when restricting to the period after 1997 when the DNPR is considered to be complete (SIR 3.1; 95% CI, 2.5–3.8) for all anal cancers 0.5+ years of follow-up) and existed in both genders and according to age in groups below and above age 50 years. The elevated risk of anal cancer was however most pronounced in men (men: SIR 3.8; 95% CI, 3.1–4.7; women: SIR 2.0, 95% CI, 1.6–2.6) and was extremely high among those diagnosed with HIV before the benign anal diagnosis (SIR 172; 95% CI, 95–283; **Table 2**).

The absolute risk of anal cancer was higher in the subgroup of individuals diagnosed with inflammatory lesions compared with those with hemorrhoids and polyps throughout the follow-up period (**Fig. 1B**). Compared with national population rates, the incidence of anal cancer overall was significantly increased in all 3 subgroups within the first 5 years after diagnosis. However, except for inflammatory lesions (SIR 3.1; 95% CI, 2.0–4.5; 5+ years), the risk of anal cancer was not significantly increased after 5 years (hemorrhoids: SIR 1.3; 95% CI, 0.9–1.8, 5+ years; polyps: SIR 1.2; 95% CI, 0.6–2.2, 5+ years; Supplementary Table S3). The elevated risk after inflammatory lesions was evident both for SCCs (SIR 3.8; 95% CI, 2.5–5.5, 0.5+ years) and ACs (SIR 6.3; 95% CI, 3.3–10.9, 0.5+years) also after excluding the first half year after diagnosis.

In a sensitivity analysis where cancer cases with topography code T02507 (skin in anal region) were excluded, we observed 230 cases with

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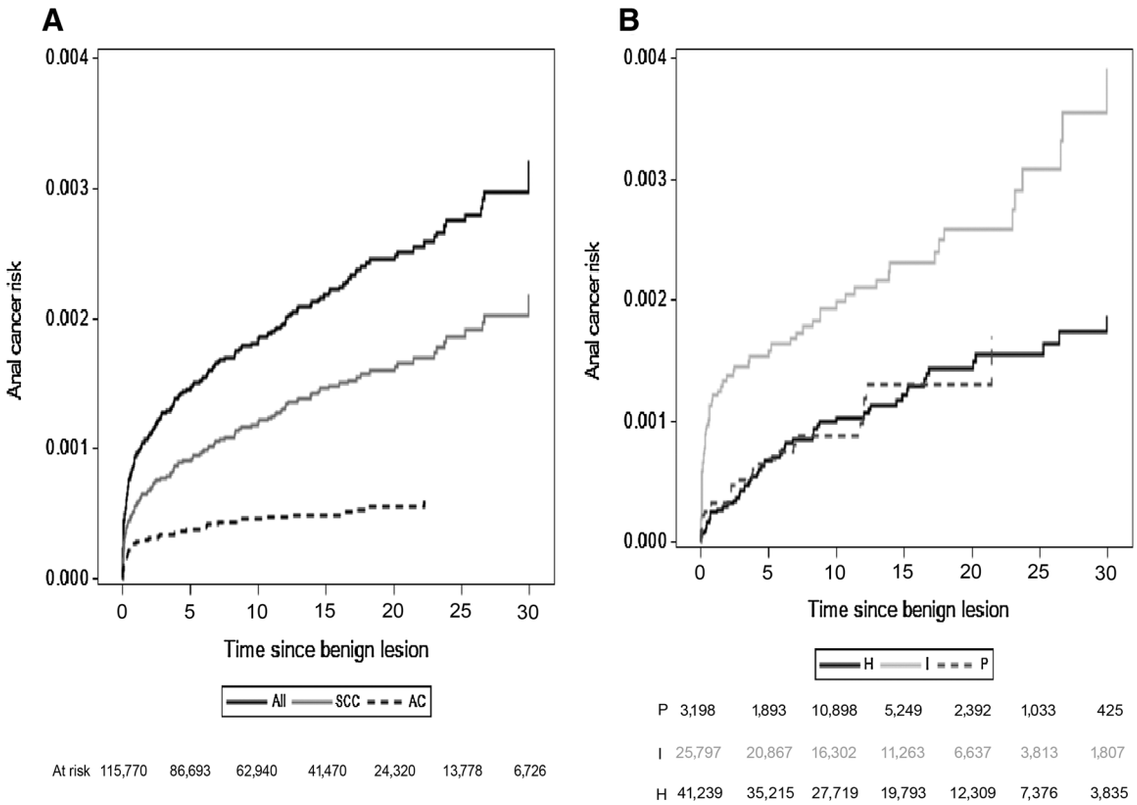

Figure 1.

A, The absolute risk of overall anal cancer, anal SCC, and anal AC after a benign lesion. **B,** The absolute risk of overall anal cancer following subtypes of benign lesions, including inflammatory lesions (I), hemorrhoids (H), and polyps (P).

anal cancer corresponding to an overall SIR of 4.9 (95% CI, 4.3–5.6) and a similar SIR for anal SCC (SIR 4.9; 95% CI, 4.2–5.7). After excluding the first 6 months there were 144 cases with anal cancer and the relative risk of overall anal cancer (SIR 3.2 (95% CI, 2.7–3.8) as well as anal SCC (SIR 3.3; 95% CI, 2.7–4.0) was still highly significant. Thus, these analyses showed the same, or even a higher, excess risk pattern among those diagnosed with benign lesions when comparing with national population rates.

Anal cancer risk following anal precursor lesions

In total, 62 anal cancer cases were observed during follow-up in the AIN1 cohort, 49 in the AIN2 cohort, and 276 in the AIN3 cohort. The proportion of cases diagnosed within the first 6 months after diagnosis of the precursor lesion was however high and increased with increasing severity of the lesion (AIN1: 44%, AIN2: 55%, and AIN3: 68%). The estimated risk of anal cancer after excluding the first half year after diagnosis and separating CIS from the rest of AIN3s was increasing with increasing severity of lesion (**Fig. 2**). The 5-year risk of anal cancer following a diagnosis of AIN3 was 3.7%, but even after an AIN1 diagnosis the 5-year risk was 0.15%; approximately twice as much as the risk level following a benign diagnosis (**Table 3**). The absolute 5-year risk of anal cancer following AIN3 was slightly higher among men, increased with age, and was highly elevated among those diagnosed with HIV before the AIN3 lesion. Among the 1,190 individuals who were HIV-negative at the AIN3 diagnosis, 6 individuals were registered as HIV-positive after the AIN3 diagnosis and none of them developed anal cancer in the follow-up period. The same patterns were seen when restricting to SCC's only. It was not possible to study ACs in subgroups due to few observed cases.

Discussion

In this large nationwide cohort study, we report the subsequent anal cancer risk among all Danish citizens registered with an anal specimen in DNPR. The risk of anal cancer increased with increasing severity of lesion, but even among those with a benign lesion, the anal cancer risk was significantly higher than expected from gender, age, and calendar-specific Danish national anal cancer rates. The excess risk was however most pronounced among those with inflammatory lesions, and for those diagnosed with hemorrhoids or polyps we could only show a significantly increased risk up to 4 years after the initial diagnosis of the benign lesion. The overall excess risk pattern was similar looking at SCCs and ACs respectively as the outcome; however based on low numbers for ACs.

The risk of anal cancer among those with AIN3 reached a level of nearly 4% 5 years after diagnosis when excluding the first half year after the diagnosis, whereas the estimated 5-year risk of SCC was 3.4%. The absolute risk of anal cancer following AIN3 was considerably higher among HIV-positive than HIV-negative individuals excluding the first half year after the diagnosis, reaching a level of 14% 5 years after diagnosis among HIV-positive compared with only 3% among HIV-negative individuals.

Our finding of an increased risk associated with benign inflammatory anal lesions is consistent with findings from a Swedish cohort

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Table 2. SIRs for all anal cancers, SCC, and AC according to time since diagnosis, sex, age at diagnosis, calendar period at diagnosis, and HIV status before diagnosis among all 115,770 individuals with benign anal lesions.

	All anal cancers		SCCs		ACs	
	Obs	SIR (95% CI)	Obs	SIR (95% CI)	Obs	SIR (95% CI)
Time since diagnosis						
0–0.5 years	89	59.3 (47.8–72.5)	52	50.6 (38.0–65.6)	27	91.2 (61.0–130)
0.5–1 years	20	13.4 (8.3–20.1)	13	12.7 (7.0–20.9)	6	20.5 (8.1–41.5)
1–5 years	53	4.5 (3.4–5.9)	36	4.5 (3.2–6.1)	10	4.4 (2.2–7.7)
5–10 years	30	2.2 (1.5–3.1)	23	2.5 (1.6–3.6)	6	2.3 (0.9–4.7)
≥10 years	53	1.8 (1.3–2.3)	37	1.8 (1.3–2.4)	8	1.4 (0.6–2.6)
All period	245	4.2 (3.7–4.8)	161	4.0 (3.4–4.6)	57	5.1 (3.9–6.6)
≥0.5 years	156	2.8 (2.3–3.2)	109	2.8 (2.3–3.3)	30	2.8 (1.9–3.9)
Sex						
Men, ≥0.5 years	86	3.8 (3.1–4.7)	56	4.2 (3.2–5.4)	19	3.2 (1.9–4.8)
Women, ≥0.5 years	70	2.0 (1.6–2.6)	53	2.0 (1.5–2.6)	11	2.3 (1.2–3.9)
Age						
<50 years, ≥0.5 years	60	2.3 (1.8–3.0)	46	2.2 (1.6–2.9)	10	3.3 (1.6–5.7)
50+ years, ≥0.5 years	96	3.1 (2.5–3.8)	63	3.4 (2.6–4.3)	20	2.6 (1.6–3.9)
Calendar period						
<1997, ≥0.5 years	76	2.5 (1.9–3.1)	47	2.4 (1.8–3.1)	18	2.6 (1.5–3.9)
1997+, ≥0.5 years	80	3.1 (2.5–3.8)	62	3.2 (2.5–4.0)	12	3.2 (1.7–5.4)
HIV before diagnosis						
No, ≥0.5 years	143	2.5 (2.1–3.0)	98	2.5 (2.0–3.0)	30	2.8 (1.9–3.9)
Yes, ≥0.5 years	13	172 (94.6–283)	11	179 (93.2–307)	0	—

study (6) in patients hospitalized for benign anal lesions. Similar to our results, they also reported no association with hemorrhoids. Other cohort studies found elevated risks of anal cancer among patients with benign anal lesions within the first year of diagnosis of the benign disease, but these studies were underpowered to detect long-term excess risks (7, 24). Furthermore, case–control studies from Denmark and Sweden (21) and the United States (22, 23) observed increased risks of anal cancer among patients with fissure and fistula and/or hemorrhoids.

There is a growing body of evidence that inflammation and chronic activation of the immune system can promote cancer development (31, 32), which supports our finding of an increased risk of anal cancer associated especially with benign inflammatory lesions. We did not observe the same elevated risk among benign, noninflammatory lesions (hemorrhoids and polyps). Different mechanisms and possible pathways linking inflammation to cancer initiation have been suggested and the interplay between inflammation and HPV infection in the development of cancer has been discussed (33). However, it is our belief that inflammatory lesions do not progress directly to cancer but may potentiate progression of HPV-related disease. Thus, benign inflammatory anal lesions may facilitate viral access to the epithelium; potentially act as a cofactor to promote HPV-related carcinogenesis or simply be a marker of infection with high-risk HPV causing inflammation. Furthermore, it has been suggested that a chronic inflammation requires accelerated cell turnover, which might lead to the acceleration of cell division and thereby increase mutation rates.

Progression rates or proportions provided in the literature regarding anal cancer risk following high-grade AIN are based on follow-up periods of substantially varying length, which makes direct comparison impossible. Instead, we attempted to use 5-year anal cancer risk as the comparison measure, and in cases where no 5-year estimates were provided, we calculated rough 5-year estimates based on the available information (**Table 4**). In our study, the estimated 5-year risk of anal cancer based on 10 cases among 47 HIV-positive individuals was 14%. This is in line with Fazendin and colleagues (18), whereas lower (13–16, 20) and substantially higher risk estimates (9, 11, 17), respectively, were extracted or derived from other studies among HIV-positive individuals with high-grade AIN. However, all studies, including ours, were based on small populations. This is reflected in the wide confidence interval connected to our point estimate, and is also applying to almost all of the point estimates from the other studies. Few studies on progression of high-grade AIN in populations with none or few HIV-negative individuals have been published so far, and most of them are single-institution clinical studies involving very small groups of patients (8, 10, 12). However, a recent large population-based cohort study among 2,074 patients with AIN3, based on data from the Surveillance, Epidemiology, and End Results registry, reported a 5-year risk of anal squamous cell carcinoma of almost 10% following a diagnosis of AIN3 (19). This is significantly higher than the risk level of 3%–4% in our population-based cohort study of 1,237 individuals diagnosed with AIN3 and alive without anal cancer 6 months after the AIN3 diagnosis. There may be several explanations for the difference between the anal cancer risk reported in our study and the study by Lee and colleagues (19). First, although we provided estimates considering cases appearing within the first 6 months as prevalent cases, Lee and colleagues only regarded cancers diagnosed within the first 2 months as synchronous. Second, as their study had no information about HIV status, it cannot be ruled out that their risk estimates are partly influenced by a higher proportion of their study population being HIV-positive. Finally, the study by Lee and colleagues did not take into account death as a competing risk, which most likely overestimated the risk.

The risk of anal cancer following high-grade anal precursor lesions reported in this study and other studies is generally much lower than that reported for progression of CIN3 to cervical cancer (34). One possible explanation may be that high-grade AIN regress more often than high-grade CIN (35, 36). Furthermore, even though anal HPV infection in the general population is relatively common, clearance of anal HPV is high whereas persistence of anal HPV is lower than that for cervical HPV (36).

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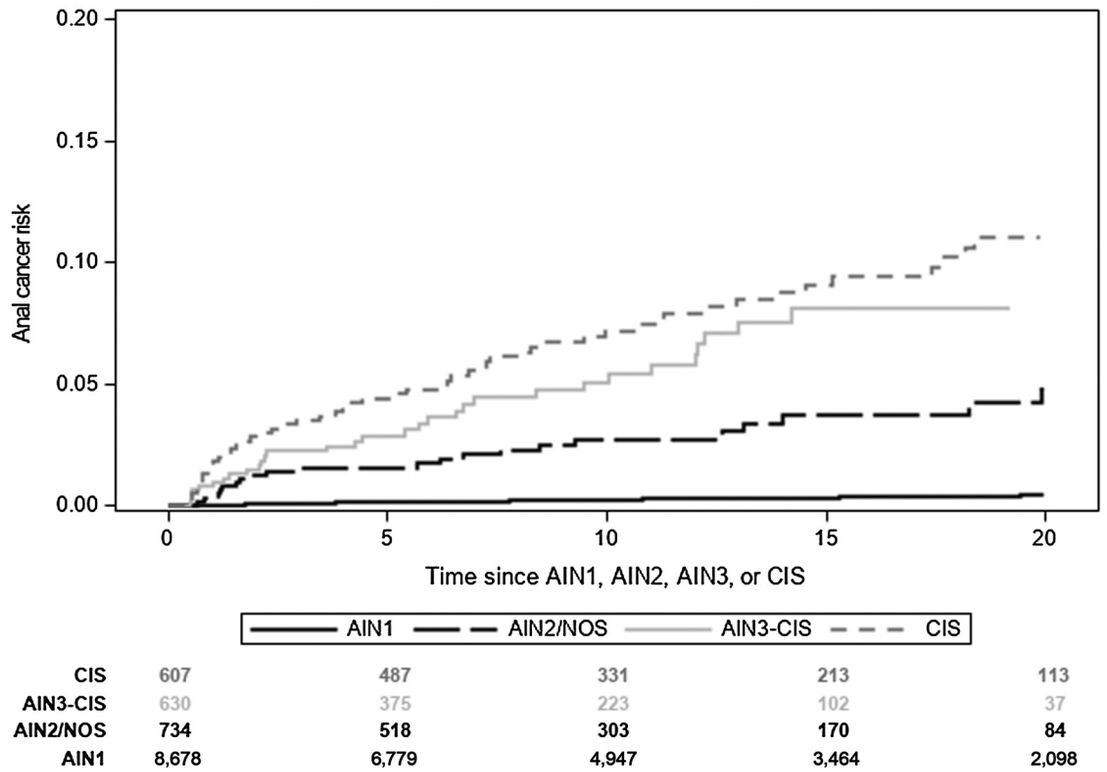

Figure 2.
The absolute risk of overall anal cancer according to time since diagnosis of AIN1, AIN2, AIN3 (excl. CIS), and CIS, respectively (excluding the first half year of diagnosis).

Following the hypothesis that persistent infection with HPV is required for anal cancer development this may partially explain why anal cancer incidence is much lower than cervical cancer incidence (36). Finally, it may reflect differences in the microenvironment between the cervix and anus including hormones, microbiomes, etc.

To our knowledge, this is the first study investigating the risk of anal cancer in a nationwide cohort of all individuals in the general population, who for some reason had an anal specimen taken. Furthermore, it is by far one of the largest studies with the longest follow-up time investigating the risk of anal cancer in individuals diagnosed with anal precursor lesions. Potential limitations relate to the registry-based nature of the study making it unknown to what extend estimates of anal cancer risk based on our referred cohort are proper estimates of natural history progression risk. As no anal cancer

Table 3. Estimated 5-year risk of anal cancer and anal SCC following benign anal disease, AIN1, AIN2, and AIN3 overall and AIN3 by sex, age, year of diagnosis, and HIV status excluding the first 6 months after diagnosis.

		All anal cancers		SCC anal cancers	
	Number in cohort	Number of anal cancers	Five-year risk (%) (95% CI)	Number of anal SCCs	Five-year risk (%) (95% CI)
Benign	112,305	156	0.07 (0.05–0.09)	109	0.05 (0.03–0.06)
AIN1	8,678	35	0.15 (0.07–0.24)	32	0.14 (0.06–0.22)
AIN2	735	22	1.57 (0.65–2.48)	18	1.13 (0.35–1.92)
AIN3	1,237	87	3.7 (2.6–4.7)	80	3.4 (2.3–4.4)
Sex					
Male	298	23	4.6 (2.2–7.1)	22	4.3 (1.9–6.7)
Female	939	64	3.3 (2.1–4.5)	58	3.1 (1.9–4.3)
Age					
<50 years	598	43	2.1 (0.9–3.3)	40	1.9 (0.8–3.1)
50–60 years	277	11	2.9 (0.8–5.0)	11	2.9 (0.8–5.0)
≥60 years	362	33	6.9 (4.2–9.6)	29	6.3 (3.7–8.9)
HIV status					
Negative	1,190	77	3.2 (2.2–4.3)	70	3.0 (2.0–4.0)
Positive	47	10	14.1 (3.6–24.7)	10	14.1 (3.6–24.7)

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Table 4. Overview of previous studies with estimates of 5-year anal cancer risk.

Study	Population size *N*	Number of anal cancer cases detected during follow-up *n* (%)	Median/mean follow-up time (range) *T*	Proportion of HIV-positive	Rate per 100 person years	5-year cumulative incidence
High HIV prevalence						
Sobhani (2004)	26 AIN3	6 (23)	35.1 months (±10 months)	84%	7.89[c]	32%[f]
Devaraj (2006)	28 AIN3	3[a] (11)	32 months (13–130)	100%	4.02[c]	18%[f]
Weis (2012)	124 (AIN2/3)	2[a] (2)	1.6 years[b]	100%	1.01[c]	5%[f]
Tong (2013)	119 (AIN2/3)	2[a] (2)	Mean 1.4 years (total 161.5 person-years)	74%	1.2[d]	6%[f]
Dalla Pria (2014)	90 AIN3	4 (4)	4.8 years[b]	100%	1.06[e]	3.2%[d]
Gautier (2016)	46 AIN3	1 (2)	35 months (27–43)	65%	0.75[c]	4%[f]
Tinmouth (2016)	35 AIN2/3	5 (14)	2.3 years (1.1–3.9)	100%	5.2[d]	23%[f]
Fazendin (2017)	31 AIN3	2[a] (6)	1,029 days (total mean follow-up)	100%	2.29[e]	11%[f]
Arens (2019)	592 AIN3	33 (6)	6 years	100%	0.92[c]	5.7%[d]
No/low HIV prevalence						
Marchesa (1997)	47 Bowen's disease	3 (6)	104 months (16–273)	No information	0.74[c]	4%[f]
Scholefield (2005)	35 AIN3	3[a] (9)	63 months (14–120)	0%	1.63[c]	8%[f]
Watson (2006)	45 AIN3	6[a] (13)	60 months (18–112)	7%	2.67[c]	13 %[f]
Lee (2018)	2,074 AIN3	171 (8)	4.0 years (1.8–6.7)	No information	2.06[c]	9.5%[d]

[a]SCCs.
[b]Calculated weighted average.
[c]Rate estimated by number of cases (n) divided by number of individuals in the population (N) times median/mean follow-up time in years (T) ($n/(N \cdot T)$).
[d]Provided in paper.
[e]Rate estimated by number of cases (n) divided by total number of follow-up years (T).
[f]Estimated as $1 - \exp(-5 \cdot rate)$.

screening exists in Denmark, our cohort primarily comprises individuals with lesions causing symptoms. Therefore, although the coverage of DNPR from 1997 and onwards is close to 100% (27), referral bias due to the fact that only those with symptoms are included may tend to overestimate the risk. Mathews and colleagues (37) compared estimates of AIN progression rates in a cytology inception cohort with a subset of patients referred for high-resolution anoscopy (HRA). Transition rates were generally overestimated in the HRA subcohort relative to the inception cohort. However, the rates only varied slightly for the transition from high-grade squamous intraepithelial lesions to invasive anal cancer [0.014 (95% CI 0.009–0.021) versus 0.011 (95% CI 0.007–0.017)], suggesting that the effect of referral bias on high-grade progression rates might be limited. Another potential limitation is that, as no active follow-up exists, neither the exact time of lesion development nor the exact onset of cancer is known. Consistent with international practice, Danish clinicians did not routinely screen for these lesions even in high-risk populations. However if identified, treatment options for AIN included, for example imiquimod, topical fluorouracil, and electrocautery. Unfortunately, we had no information about treatment or HPV status, and furthermore the study relies on the quality of the pathology diagnoses provided by several different pathologists.

Because of the low prevalence of anal cancer, routine screening is not offered in the general population but it has been proposed for high-risk groups (38). Treatment of high-grade AIN is especially challenged by poor to moderate response rates and recurrence of lesions is common (38, 39). A large prospective randomized trial, the Anal Cancer/HSIL Outcomes Research (ANCHOR) study, is currently in progress to determine whether treatment of high-grade AIN reduces the risk of anal cancer in HIV-positive men and women. One long-term approach to reduce the risk of anal cancer is HPV vaccination. The efficacy of vaccination depends on the timing of vaccination and the vaccine coverage in the target population; if only low levels of vaccination are achieved or vaccination primarily occurs after acquisition of anal HPV, then the vaccine may not have a great impact on anal cancer incidence. Furthermore, men are not being offered vaccination in most countries. Heterosexual men may benefit from some herd protection through vaccinated women, but MSM do not. Thus, in order to reduce the risk of anal cancer by HPV vaccination, high vaccine coverage, and vaccination of boys is essential (40).

In conclusion, we found that the risk of anal cancer increased with increasing severity of precursor lesions, reaching a level of around 4% 5 years after diagnosis of AIN3. However, even among those with benign lesions, particularly inflammatory lesions, the anal cancer risk was significantly higher than expected from Danish population anal cancer rates. Small differences in cancer risk following AIN3 were observed with regard to sex and age, whereas the absolute risk of anal cancer following AIN3 was considerably higher among HIV-positive than HIV-negative individuals. One approach to reduce anal cancer risk is HPV vaccination of girls and boys.

Disclosure of Potential Conflicts of Interest

J. Palefsky is a consultant/advisory board member for Vir Biotechnologies, Vaccitech, Virion Therapeutics, Merck, and Antiva Biosciences and reports receiving commercial research grants from Antiva Biosciences, Merck, and Cel-sci. S.K. Kjaer previously received a lecture and scientific advisory board fee from Merck, and a research grant through her institution from Merck. No potential conflicts of interest were disclosed by the other authors.

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