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Aspects of the etiology and survival of lower gastrointestinal cancers

Caroline Nordenvall

Karolinska Institutet

200 Years 1810 – 2010

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ABSTRACT

In the first two papers, the Inpatient Register was used to identify patients hospitalized with (i) condylomata acuminata, or with (ii) benign anal lesions. In both papers, the cancer incidence in the cohort was compared to the general population generating standardized incidence ratios (SIRs). Cancer cases occurring during the (i) first year, or (ii) first three years of follow-up and accrued person-years were excluded.

(i) Between 1965 and 1999, 10,971 patients were hospitalized with condylomata acuminata. During a median follow-up of 13 years, 473 cancer cases occurred, and the corresponding SIR for all cancers was 1.5. Although based on few cases, the risks of all anogenital cancers, except cervical cancer, were significantly elevated. The pattern of relative risks for different types of cancers resembled the patterns described for patients with a suppressed immune defense.

(ii) Between 1965 and 2002, 45,186 patients were hospitalized with benign inflammatory anal lesions (anal fissure, fistula, and perianal abscess), and 79,808 patients were hospitalized with hemorrhoids. Patients with inflammatory anal lesions had a 3-fold increased risk of anal cancer that persisted over time. There was no persistent risk elevation in patients hospitalized with hemorrhoids.

The third and fourth papers were based on the Construction Workers Cohort consisting of 336,381 males. Detailed information on tobacco use, smoking and Swedish moist snuff (snus), was collected at cohort entry in 1971-1992. Never-users of any tobacco served as reference group.

(iii) The third paper studied tobacco use and the incidence of colorectal and anal cancer. After up to 37 years of follow-up, pure smoking was marginally associated with colon cancer risk, modestly associated with rectal cancer risk, and there was a substantially increased risk of anal cancer. Snus use was not significantly associated with neither colorectal, nor anal cancer risk. However, the point estimates of colon cancer risk were similar in snus users and smokers.

(iiii) The fourth paper studied the impact of tobacco use on cancer survival, with a specific interest in colorectal cancer. There were 40,230 incident cancer cases in the cohort. Both smoking and snus use were associated with an increased risk of death, even though the estimates tended to be slightly higher for smokers. Smokers had a borderline 25% increased risk of rectal cancer-specific death, whereas there was no excess risk of colon cancer death. Snus use was not significantly associated with colorectal cancer death. Using data on comorbidity, a stratified analysis revealed no substantial differences between those with and without comorbid conditions. This suggests that an excess fatality among tobacco users might be a biological effect, possibly exerted by nicotine.

LIST OF PUBLICATIONS

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LIST OF ABBREVIATIONS

5-FU	Fluoropyramideine-based chemotherapy
APC	Adenomatous polyposis coli gene
APR	Abdominalperianal resection
AR	Anterior resection
BMI	Body mass index
CA	Condylomata acuminata
CI	Confidence interval
CIN	Chromosomal instability
CRC	Colorectal cancer
CT	Computer tomography
FAP	Familial adenomatous polyposis syndrome
GI	Gastrointestinal
HNPCC	Hereditary non-polyposis colorectal cancer
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HR	Hazard ratio
HRT	Hormone replacement therapy
IARC	International Agency on Cancer Research
ICD	International classification of diseases
MRI	Magnetic resonance imaging
MSI	Microsatellite instability
nAchRs	Nicotine acethylcholine receptors
NNK	4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone
NRN	National registration number
OR	Odds ratio
PET	Positron emission tomography
Rb	Retinoblastoma tumor suppressor protein
RR	Relative risk
RT	Radiotherapy
SIR	Standardized incidence ratio
STD	Sexually transmitted disease
TME	Total mesorectal excision
TNM	Tumor-node-metastasis

1 INTRODUCTION

The common theme in this thesis is cancer epidemiology. The concept of epidemiology stems from when infectious diseases constituted the bulk of health burden on society, and a previous definition was "the study of epidemics" [1]. A more recent definition is "a field of study concerned with methods for elucidating the causes of disease and for evaluating health services and treatments" [1]. The word 'epidemiology' has a Greek origin, *epi* (on), *demos* (people), and *logos* (study), and has been defined as "studies on people".

The thesis consists of four epidemiologic studies. As the thesis covers a broad range of subjects, the aim with the Background section is to equip the reader with a basic understanding of each of these. The discussion is divided into two sections; Methodological discussion and General discussion. In the Methodological discussion several epidemiological concepts will be described and their application in the four studies will be discussed.

The first two papers investigate the risk of cancer in two potential risk groups; patients hospitalized with condylomata acuminata, and patients hospitalized with benign inflammatory anal lesions. Both condylomata acuminata and benign anal lesions are common in the general population, and the current understanding of the cancer risks in these populations is scarce.

In Sweden, colorectal cancer is the second most common type of cancer in both genders. During the latest decades, smoking has become less common, whereas the use of Swedish moist snuff (snus) has increased. The last two papers study the association between the use of smoking and snus, and colorectal cancer incidence and survival. The association between smoking and colorectal cancer is under debate, and the literature is inconsistent. Sweden has the highest prevalence of snus users, and no study has previously investigated the association of Swedish snus and colorectal cancer incidence or survival.

2 BACKGROUND

Infectious diseases play a role in the development of cancer. The first section will describe human papillomavirus (HPV). HPV has been associated with several types of cancer including anal cancer. The most well-known HPV-associated cancer is cervical cancer, and cervical cancer will serve as a model in the description of HPV. This will be followed by a description of condylomata acuminata (CA), genital warts caused by HPV.

The second section will cover anal cancer. Even though anal cancer is associated with HPV, this section will be more focused on the role of benign anal lesions in this disease. The second study in this thesis is based on a cohort of patients with benign anal lesions. The aim with this introduction is to describe what the cohort consists of: (i) benign inflammatory lesions (anal fissure, anal fistula, and perianal abscess), (ii) hemorrhoids, and how these are treated.

In Sweden, one of the most common cancers in both genders is colorectal cancer (CRC). The third section will cover this topic. CRC is a complex disease with different carcinogenic pathways. The risk factors for CRC involve lifestyle and hereditary factors. Both the third and the fourth study focus on CRC and its association with tobacco. Smoking, as well as Swedish moist snuff (snus), and the association with cancer risk and survival will be discussed.

2.1 HPV AND CONDYLOMATA ACUMINATA

2.1.1 HPV

2.1.1.1 Epidemiology

HPV and cervical cancer was primarily described by zur Hausen who was awarded the Nobel Prize in 2008 [2]. Human papilloma viruses are non-enveloped, double stranded DNA viruses, and infect different parts of the body. The cervical infection is thoroughly studied and will serve as a model in this summary. HPV is the most common sexually transmitted infection. Among all sexually active adults, more than half will get a HPV-infection during their life-time, and at least 80% of all women have acquired the infection by the age of 50 [3]. In the United States it is estimated that 10% of the population have an active infection and 4% have an infection that has caused cytological abnormalities [4].

Each HPV type should be viewed upon as a separate infection, but due to a similar route of transmission, concomitant infections are common. There are more than 100 types of HPV [5]. Among the mucosal HPVs, the most common carcinogenic types are HPV 16 and 18, and the most common non-oncogenic types are HPV 6 and 11. Vaccines against these types were recently developed [3]. In Sweden the vaccines Cervarix and Gardasil are used, the former against HPV 16 and 18, and the latter against HPV 6, 11, 16 and 18. HPV 16 is the most common type in cervical cancer, accounting for about 50% [5], and more than 90% of HPV-positive tonsillar cancers [6]. HPV has also been associated with anogenital cancer [7-9], esophageal tumors [10-11], and with squamous cell carcinoma of the skin in immunocompromised patients [12].

In the Western world, the peak of cervical HPV infection is seen in the early twenties, and the prevalence decreases with age. Table 1 describes how the HPV-prevalence varies with age [13]. The number of sexual partners, race (more common among African Americans), poverty and level of education are also positively associated with HPV-prevalence. The median duration for the average HPV infection in young women is 5.6 months [14].

Table 1. Prevalence of HPV among American women 14-59 years old in 2003-2004 [13].

	HPV prevalence (%)	
Age	All (1921)	Sexually active (1477)
14-19	24.5	39.6
20-24	44.8	49.3
25-29	27.4	27.8
30-39	27.5	27.3
40-49	25.2	23.9
50-59	19.6	20.2

The decreasing prevalence with age could be due to clearing of the infection by the immune system, or a latent viral infection with undetectable levels. However, in some populations the prevalence has been shown to peak again or never substantially fall [15].

2.1.1.2 Pathophysiology

Trauma causes the HPV-infected surface to shed. The virus is probably transmitted to the recipient via small tears in the epithelium occurring during sexual intercourse or other sources of micro trauma. HPV has also been shown to be transmitted via anal intercourse, and between two women having sex [16].

The genome encodes for Early (E1, E2, E4-7) and Late open reading frames (L1, L2). Differences in L1 define different subtypes of HPV. The viral oncogenes E6 and E7 alter the host-cell metabolism to favor neoplastic development. High risk HPVs insert themselves into the host DNA, and their E6 and E7 can produce oncoproteins that regulate cell growth [17]. Apoptosis is the natural death for normal cells, and is induced in response to cellular stress. E6 binds to p53, a transcription factor that stimulates the expression of genes involved in cell cycle arrest and apoptosis [18]. The degradation of p53 results in blocking of apoptosis and accumulation of genetic abnormalities. E7 interacts with several factors that regulate cell growth. The most important effect is the interaction with the retinoblastoma tumor suppressor protein family (Rb). E7's binding to Rb results in a progression in the cell cycle from G1 to S-phase [19]. L1 and L2 encode for viral capsid proteins.

The virus targets for the stratified squamous epithelium and the metaplastic cells at the squamocolumnar junction of the cervix. It may also infect the glandular epithelium of the endocervix and induce glandular neoplasms.

HPV have several mechanisms to evade the host's immune response. HPV may induce a local immunodeficiency by reducing the number of intraepithelial cells involved in

the immune defense. Since the infection is epidermal, it is isolated from the blood and humoral immunity. The immune system usually clears the infection via interleukins that recruit the cell-mediated immune response and via interferons that slow the viral replication.

The natural history of HPV-infections is still uncertain. Cervical HPV infections tend to be detectable up to one year. HPV 16 infections persist longer. After two years of follow-up almost all HPV infections are either non-detectable, or has lead to precancer [20]. An increased risk of HPV-associated cancers are seen in transplant patients and Human Immunodeficiency Virus (HIV) infected patients following immunosuppression [21]. The recurrence of HPV-infections following immunosuppression has been interpreted as an outcome of a latent infection [22].

A persistent HPV-infection is crucial for development of precancer. Sometimes precancers appear to arise from oncogenic HPV infections without preceding microscopic lesions or an early stage [20]. The longer the infection persists, the greater the risk of cancer developing. The median age of onset of cervical cancer is 48 years [23]. The most advanced type of intraepithelial neoplasia might be diagnosed before the age of 25. However, cancer in this age group is rare, stressing the impact of time in cancer development [20].

In the discussion of time and cancer development, two concepts, "induction period" and "latent period", need to be defined (figure 1). "Induction period" is defined as the period from the causal action until the disease is initiated. For example, the time elapsed from when a person gets infected with HPV to the initiation of cancer development is called the "induction period". The "latent period" is the time elapsed from the initiation of a disease until it is discovered; hence the time from when the cancer development is initiated until the cancer is diagnosed.

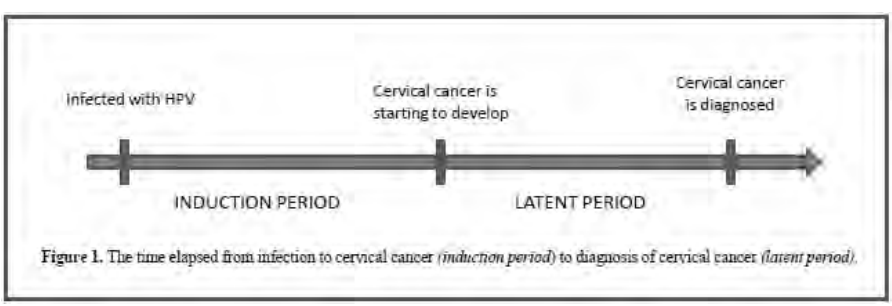

Figure 1. The time elapsed from infection to cervical cancer *(induction period)* to diagnosis of cervical cancer *(latent period)*.

In the past, number of sexual partners, age at first intercourse, previous sexual transmitted diseases, and smoking were the most frequently reported risk factors for cervical cancer. The knowledge of the causal relation between HPV and cervical cancer has resulted in a re-evaluation. Many of these risk factors simply reflect the probability of HPV infection [24].

All cervical cancers are caused by HPV [24]. This can be illustrated with the causal pie model (figure 2). Each pie is "a sufficient cause", and each component in the pie needs to occur before the event occurs. If one of the components appears in each *sufficient*

cause, then it is called a *"necessary" component cause* [25]. HPV infection is a *necessary cause component* in the development of cervical cancer, but it is not a *sufficient cause* of cervical cancer [24]. In addition to HPV, other factors are needed for the development of cervical cancer. Smoking, at least five years use of oral contraceptives, and five or more pregnancies are considered as probable risk factors for cervical cancer in HPV-positive women [24].

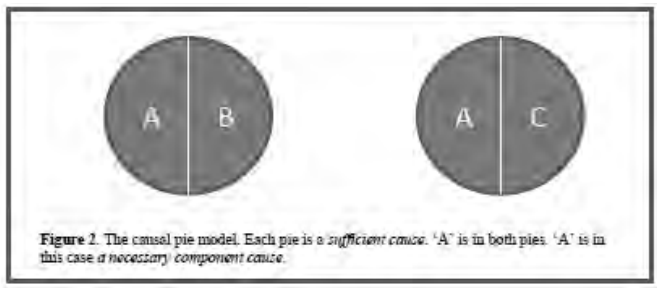

Figure 2. The causal pie model. Each pie is a *sufficient cause*. 'A' is in both pies. 'A' is in this case *a necessary component cause*.

2.1.2 **Condylomata acuminata**

2.1.2.1 Epidemiology

Condylomata acuminata (CA) are genital warts caused by low-risk HPVs. They are mainly transmitted by sexual contact and millions of people world-wide are affected by CA [14]. In the general population, about 1% has had CA [26]. In the United States, the prevalence in patients at STD (sexually transmitted disease) clinics has been estimated to be 13% in men and 9% in women [4]. A dose-response relationship with an increasing number of sexual partners has been reported [26]. A history of other STDs has been associated with an increased risk of 91%, and smoking with an increased risk of 11% [26]. CA grow during pregnancy, and a possible explanation could be altered hormone levels and changed immune competence [27].

HPV 6 and 11 are non-oncogenic, and 90% of all CA are positive for either of these viruses [28]. About 70% of those who have sexual intercourse with an infected partner develop genital warts [29]. The incubation period varies between 3 weeks to 8 months, with a mean of 2-3 months [29]. The reported prevalence of subclinical infection varies between 2/5 to more than 2/3 [14, 29].

2.1.2.2 Diagnosis

CA may be flat, pedunculated, dome- or cauliflower-shaped. CA occur anywhere in the squamous epithelium of the lower genital tract and cervix, and multiple sites are seen in 50% [14]. The vulva is the most common site, but perianal lesions are also common [14]. The color varies from skin colored, to red, pink, purple, white and brown.

Usually clinical inspection is enough for diagnosis [29]. Sometimes mild acetic acid is used to detect the lesions, but the specificity is low. Biopsy is recommended in case of therapy resistance, atypical appearance or pigmentation. HPV typing is not recommended. Differential diagnoses are condylomata latum (syphilis), benign skin lesions (seborrhoeic keratosis, nevi, microglandular hyperplasia, hymenal remnants,

penile papules), viral lesions (molluscum contagiosum and herpetic lesions), neoplastic lesions (verrucous carcinoma, bowenoid papulosis, malignant melanoma, Buschke-Lowenstein tumor) and inflammatory lesions (lichen nitidus).

2.1.2.3 Treatment

Many warts regress over time, and non-scarring treatments are recommended. Since none of the available treatments is superior, and recurrences are common, it has been suggested that type of treatment should be guided by patient preferences [14].

Imiquimod is an immune modifier that induces the local immune response. Imiquimod is available as an ointment in Sweden (Aldara). Imiquimod is only used for external HPV infections. In half of the cases the warts are cleared within 16 weeks [14, 29]. Other local treatments used are podophyllin resin or podophyllotoxin (purified podophyllin), where the latter has been shown to be more effective [29]. They are antimitotic agents that destroy warts by stopping cell division, inducing local necrosis.

Surgery is used for lesions causing obstruction, and the recurrence rate after 1 year is 19-29% [14]. Cryotherapy involves application of nitrous oxide or liquid nitrogen (-196) to induce necrosis in the genital warts. It is an effective treatment with 79-88% clearance rate, but recurrences are seen in 25-39% despite multiple treatments [29]. Electrosurgery (thermal coagulation or electrocautery) is used to destroy HPV lesions, and is considered second-lined treatment [29].

2.1.2.4 Cancer

A cohort study from Sweden included 3,620 patients with CA, with a mean follow-up of 7.8 years has studied the risk of different types of cancer in patients with CA [30]. Based on 22 incident male cancer cases, diagnosed at the time of CA or during follow-up, the relative risk (RR) of cancer was 1.6 compared to the general population [30]. For anogenital cancers, an increased risk of 160% was seen in men [30]. The study was limited by its size, and there was only one case of genital cancer in women [30].

A Danish register-based cohort study of women with CA revealed an increased risk of cancer, standardized incidence ratio (SIR) 1.7 based on 160 cases [31]. CA was associated with increased risks of vulvar, cervical and anal cancer. There was no case of vaginal cancer in the cohort [31]. For non-anogenital cancers, significantly elevated risks were seen for lung and bladder cancer [31]. Increased point estimates were also reported for head and neck, gastrointestinal tract, and kidney cancer [31].

2.2 ANAL CANCER AND BENIGN ANAL LESIONS

This section will start with a description of the anal canal followed by an introduction to anal cancer. In paper II, benign anal lesions are subdivided into inflammatory anal lesions (anal fissure, fistula, and perianal abscess), and non-inflammatory anal lesions (hemorrhoids). These benign anal lesions will be described one by one.

2.2.1 Anatomy and physiology of the anal canal

There are several definitions of the anal canal and also some uncertainties with respect to nomenclature [32-33]. The anal canal is variable in length but generally around 4 cm long [34]. Surgically it is often defined as the part of the bowel that starts at the level of the puborectal sling and ends at the anal verge or at the intersphincteric grove. Around circumference of the anal canal the anal sphincters are found (figure 3). The internal sphincter is continuous with the circular smooth muscle in the whole gastrointestinal tract (GI). It is innervated by the pelvic sympathetic nerves, the lower lumbar ganglia, and the preaortic/inferor mesenteric plexus – all parts of the autonomic nervous system [35]. The parasympathetic fibers arise from the sacral plexus. The internal sphincter has an important role in fecal continence by maintaining tonus and contributing to the resting pressure in the anal canal. In contrast to the internal sphincter, the striated muscle of the external sphincter is under voluntary control. The external sphincter is innervated by the right and left internal pudendal nerves, and the forth branch of the sacral plexus [35]. The levator ani muscles are important for maintaining the anatomic relationship between the anus and rectum during defecation [35].

The epithelium of the anal canal can be divided into three zones. The epithelium in the infra-dentate zone (1) is non-keratinized stratified squamous epithelium. It reaches from the anal verge (which is the level where the keratinized squamous epithelium with skin appendages ends) to the pectinate line (dentate line). Above the pectinate line is a short transitional zone, often referred to as the anal transitional zone (2) consisting of multilayered non-keratinized epithelium with various cell types and shapes. In this area endocrine cells and melanocytes are also present. The uppermost supradentate zone (3) consists of columnar epithelium, and is continuous with the rectal epithelium [36]. The anal valves are the mucosal folds that form the pectinate line, created by the fusion of the endoderm of the embryonic hindgut, and the ectoderm of the anal pit. The epithelium above the pectinate line receives autonomic innervations and lacks somatic sensory innervations. The epithelium below the pectinate line is innerved by the peripheral nervous system and diseases in this area may cause pain.

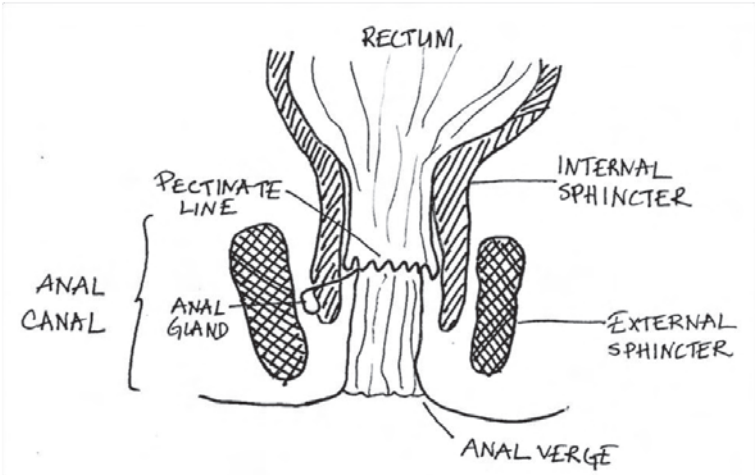

Figure 3. The anatomy of the anal canal.

There are 4-8 anal glands situated in the transitional zone, in the internal sphincter or intersphincteric space. They secrete mucus that lubricate and protect the sensitive epithelium in this area. The ducts discharge in the pectinate line. When an occluded gland is infected by gut microorganisms, a perianal abscess may arise [35, 37-38].

The anal cushions are located in the submucosa partly above and partly below the pectinate line. They are specialized vascular structures of fibro-connective tissue supplied by the three terminal branches of the rectal arteries. They are anatomically situated at 3, 7, and 11 o'clock (with 12 being upwards and the patient in the lithotomy position), and are believed to play an important role for fecal continence [35].

Lymphatic drainage of the anal canal below the pectinate line is mainly to the inguinal lymph nodes [38]. Many tumors of the anal canal metastasize to these lymph nodes.

2.2.2 **Anal cancer**

2.2.2.1 Epidemiology

Anal cancer is rare, but the incidence is rising [39]. In 2007 there were 41 male cases and 114 female cases diagnosed in Sweden [40]. About 80% of all anal cancer cases are squamous cell carcinoma, and 125 cases were diagnosed in 2007 [40]. Less than 10% are adenocarcinoma, and are treated as rectal cancer. Other rare tumors are melanoma, sarcoma and lymphoma. Anal cancer is more common among women, and most patients are over 50 years of age [40].

2.2.2.2 Risk factors

Risk factors for anal cancer are HPV infection, immunosuppression, smoking, and anoreceptive intercourse [41]. A risk group is men who are not exclusively heterosexual [41]. The prevalence of HPV in anal precancers is over 90% [42]. The corresponding

number for anal cancer is 78-93% [42-43]. HPV 16 is the most common type, with a prevalence of 66-78%, followed by HPV 18 [42-43].

Smoking has been associated with anal cancer in case-control studies. Reported estimates for current smokers range from odds ratios (ORs) of 1.9 to 7.7 among women, and 2.7 to 14.6 among men [43-46]. The underlying biological explanation of the association between smoking and anal cancer is not fully understood. Phillips et al found higher levels of DNA adducts in anal epithelium among smokers compared to non-smokers. They concluded that components of tobacco smoke inflict genotoxic damage [47]. Nicotine has been proposed to be a promoter for malignant transformation in cells that are HPV infected [48]. Other theories are inhibition of apoptosis and immunosuppression as consequences of smoking [49-52].

Benign anal lesions have been associated with anal cancer in case-control studies [46, 53-54]. In a matched case-control study, infection, inflammation, or itching around the anus was associated with an OR of 1.6 (95%CI 0.9-2.9) after adjustments [46]. The same study revealed a risk increase associated with a history of hemorrhoids. There was a dose-response in the severity of the hemorrhoids and the risk increase [46]. In another case-control study, a history of anal fissure or fistula was associated with an OR of 2.4 [53]. In addition, more than 12 episodes of hemorrhoids were significantly associated with a risk increase of 160% [53]. In a case-control study by Frisch et al., a history of hemorrhoids was associated with an increased risk of anal cancer in men, but not in women [54]. Similarly, itching around the anus was associated with a 4-fold risk of anal cancer in men, but not in women [54]. The association between benign anal lesions and anal cancer has not been confirmed in cohort studies [55-56]. A cohort study by Frisch and colleagues revealed elevated risks for benign anal lesions during the first years of follow-up, but the risk elevations diminished with time [55]. They concluded that their results did not support a causal role of benign anal lesions in anal cancer [55].

2.2.2.3 Pathogenesis

The most common sign of anal cancer is a perianal mass, rectal bleeding and pain [39, 57]. The symptoms are often misinterpreted as benign anal lesions, which is the most common diagnosis made prior to cancer diagnosis [58]. Other symptoms are pruritus, anal discharge, and altered bowel habit. Advanced tumors that engage the anal sphincters can cause fecal incontinence. About 20% are asymptomatic at the time of diagnosis [59].

The staging for anal cancers is described using the TNM (tumor-node-metastasis) system (table 2). The classification Tx, Nx, and Mx is used when data on the variable is missing.

Table 2. TNM staging system for anal cancer [60].

TNM STAGING SYSTEM	
Primary tumor (T)	
Tis	Carcinoma in situ
T1	<2cm
T2	2-5cm
T3	>5cm
T4	Growing into the surrounding tissues or organs, such as the urethra, the vagina or the bladder
Regional lymph nodes (N)	
N0	No regional lymph node metastasis
N1	Metastases in perirectal lymph node (s)
N2	Metastases in unilateral iliac and / or inguinal lymphnode (s)
N3	Metastases in perirectal and inguinal lymph nodes and / or bilateral internal iliac and /or inguinal lymphnodes
Distant metastases (M)	
M0	No distant metastasis
M1	Distant metastases

2.2.2.4 Diagnostic procedure

Clinical examination often reveals an ulcerated discoid lesion at the anal verge which is hard to touch. Due to pain, general anesthesia is often required for thorough examination. Diagnosis is based on biopsy. Previously squamous-cell carcinoma of the anus was sub-grouped because of differences in histology. However, since the biology and prognosis are similar [59], this grouping has been abandoned with some rare exceptions [32]. Depending on its relation to the pectinate line, the squamous-cell carcinoma may be either keratinizing and non-keratinizing. On the other hand, anal adenocarcinoma behaves differently and should be treated as rectal cancer. In this thesis, anal cancer will refer to anal squamous-cell carcinoma.

Patients with anal cancer should undergo radiographic examinations with the aim of defining local tumor growth, presence of diseased nodes and distant metastases. The gold standard for local staging is endo-anal ultrasound [39]. The accuracy of endo-anal ultrasound in predicting nodal status is 80-85% [39]. In addition, positron emission tomography (PET) can be used to improve sensitivity in identification of nodal disease [41]. PET is also used in recurrent disease [39]. Magnetic resonance imagining (MRI) can be used to add staging information. When inguinal nodes are palpable, fine-needle aspiration is commonly used to detect or rule out malignant nodal spread.

2.2.2.5 Treatment

Until the mid-1980s, primary abdominalperineal resection (APR) was used. APR involves removal of the anus, rectum, and draining lymph nodes and all patients receive a permanent colostomy. Combined chemotherapy and radiotherapy was introduced as a treatment for anal cancer in the mid-1970s [61]. Today combined radio-chemotherapy is the gold standard for treatment of anal cancer, but APR is still used for patients with persistent or recurrent disease [62]. Primary surgery, in the form of local excision, can also be used to treat small cancers at the anal verge when further treatment is not required.

It has been shown that chemotherapy, in addition to radiotherapy, is superior to radiotherapy alone without being more toxic [63]. Internationally, Mitomycin and 5-fluoropyramideine-based chemotherapy (5-FU) are the chemotherapy agents most commonly used [41], but in Sweden Platinum-based chemotherapy is often used.

2.2.2.6 Prognosis

The overall survival rate is around 60% [35, 64]. For combined therapy, the 5-year disease free survival is around 50-60%, and recurrences are seen in 25-33% [65-66]. The colostomy-free rate in a recent study of patients treated with combined therapy was 80-90% [65].

Stage I and II lesions have a 5-year survival rate of 87-93% [64]. The similar number in patients with stage III disease is around 50% [64, 67]. Females may have a better prognosis than men [68-69], but these results are not consistent [64, 70]. Persistent disease after radio-chemotherapy is seen in 20% [66]. Surgery for recurrences can result in > 50% 5-year survival rates [62].

Smoking has been associated with higher recurrence rate and worse prognosis [71]. The effect of radiochemotherapy on anal cancer may be negatively influenced by smoking [72]. Smoking leads to tissue hypoxia [73-74]. Both the inhibition of apoptosis by nicotine [50], and the tissue hypoxia may affect the treatment efficiency.

2.2.3 **Anal fissure**

2.2.3.1 Epidemiology and risk factors

An anal fissure is a longitudinal or elliptical tear in the distal anal canal most commonly located in the posterior midline. It extends from below the pectinate line to the anal verge. It is usually seen in younger and middle aged adults. There is no great difference in incidence between the sexes. It is the most common cause of rectal bleeding in infants [75].

Anal fissure is more common among those who eat highly processed food, and less likely among those who eat a fiber-rich diet [75]. Anal fissure has been associated with constipation traumatizing the anal canal, as well as diarrhea [75]. In cases of multiple or lateral fissures, one should consider the possibility of an underlying pathology such as Crohn's disease, ulcerative colitis, tuberculosis, HIV infection and syphilis [75]. Further investigation with biopsies and/or cultures should be considered.

2.2.3.2 Pathogenesis

The etiology of anal fissures is unclear. The fissures are usually found in the posterior midline. However, 10% of women, and 1% of men, have a fissure in the anterior midline. Blood perfusion is less in the posterior midline than the rest of the anal canal, and ischemia is a suggested etiology [35, 75-76]. Increased anal pressure, due to abnormalities of the internal sphincter function, may cause chronic anal fissures. When a fissure is present for more than six weeks, it is classified as a chronic anal fissure [35].

2.2.3.3 Diagnostic procedure

The most common symptom is severe anal pain on defection that may last for some minutes to some hours. A small rectal bleed separated from the stool is seen in 75-100% [75]. Often, a dramatic increase in the resting tonus of the anal sphincter is present. History often reveals constipation, and sometimes diarrhea, whereas mucus discharge and pruritus are less common [75].

The history and presence of a sentinel pile (skin tag) support the diagnosis of anal fissure. Retraction of the perianal skin is often enough to reveal the fissure. If conservative treatment fails, further examination with rigid sigmoidoscopy is recommended to exclude other diagnoses or underlying diseases such as Crohn's disease, ulcerative colitis, syphilis, herpes, carcinoma, and lymphoma.

The triad of chronic anal fissure includes a sentinel pile, an indurated ulcer and a hypertrophied anal papilla situated at the proximal extent of the fissure [75].

2.2.3.4 Treatment

The majority of all acute fissures, and up to 40% of all chronic fissures, heal spontaneously [75-76]. Primary treatment involves treating constipation if present. Local anesthetic ointment is often used, but probably ineffective. Local application of steroids may increase healing, but most acute fissures heal anyhow [75].

Local administration of glyceryl trinitrate, a nitric oxide donor, leads to relaxation of the internal sphincter. More than 2/3 of all chronic fissures heal with this treatment [75-76]. Topical application of calcium-channel antagonists also reduces resting anal pressure, with similar results as for glyceryl trinitrate [77].

Purified botulinum toxin may be injected to the internal sphincter muscle to temporarily relax the muscle, and lower resting anal sphincter pressure [76]. In a Cochrane review, botulinum toxin was not better than glyceryl trinitrate [77]. Manual anal dilatation has recurrence rates varying from 2 to 57% [75]. In addition, about 40% get flatus incontinence or soiling, and up to 16% have fecal incontinence [75].

In case of chronic anal fissures that do not respond to conservative treatment, open and closed partial lateral internal sphincterotomy remains an option [78]. The procedure involves division of the lower half of the internal sphincter at 3 or 9 o'clock and may lead to healing of the fissure. Sphincterotomy is, however, associated with a risk of disturbances in continence [76].

2.2.3.5 Prognosis

Most acute fissures and up to 40% of chronic fissures heal spontaneously [75]. A 6-year follow-up of chronic anal fissures treated with lateral sphincterotomy showed a recurrence rate of 8% [75].

2.2.4 **Perianal abscess and fistula**

2.2.4.1 Epidemiology and risk factors

Perianal abscesses are two to three times more common among men than women [79]. Predisposing conditions for both abscesses and fistulas are ulcerative colitis, Crohn's disease, immunosuppression, infection, and anal fissure. However, most patients have no risk factors [79]. In patients with Crohn´s disease of both the small and large bowel, about 20% develop fistulas [80]. Similarly, 10% of patients with only large bowel disease develop fistulas [80].

2.2.4.2 Pathogenesis

Perianal abscesses may precede a fistula. Perianal abscesses form when the duct of the anal gland is occluded and becomes infected with *Streptococcus faecalis* and *Bacteroides*. If an abscess drains through the perianal skin a fistula is created. The fistula may also be iatrogenic secondary to inappropriate drainage of the abscess [35, 81].

2.2.4.3 Diagnostic procedure

The clinical presentation of an anal abscess depends on where the infected gland is situated, and by which route that the abscess chooses to empty its pus. Abscesses are often classified as intersphincteric, perianal or ischiorectal [81]. If the abscess remains within the intersphincteric space, the clinical presentation is acute anal pain and tenderness, and a pea-sized lump may be found. A perianal abscess is generally clinically obvious for the doctor as well as the patient. Ischiorectal abscesses are uncommon. They may occur if the infection travels through the external sphincter. The patient presents with fever, a longer history of pain, symptoms from both buttocks, and difficulties in sitting.

A patient with a fistula has a chronically discharging cavity in the perianal skin often in combination with pruritus and perianal discomfort. Anal fistulas may be classified in various ways. An internationally well-known classification was introduced by Parks where the fistulas were classified as intersphincteric, transsphincteric, supra-sphincteric, or extrasphincteric [82]. For most fistulas, examination under general anesthetic gives adequate information of the fistula tract. Goodsall's rule defines the extent and route of the fistula [83]. If a line is drawn from 3 to 9 o'clock, fistulas posterior of this line track circumferentially to the posterior midline, whereas fistulas anterior of this line track radially to the pectinate line. Recently, MRI and endo-anal ultrasound have been introduced for defining anal fistulas [84].

2.2.4.4 Treatment

An abscess is treated by drainage under general anesthetic. If there is a systemic infection, antibiotic treatment with broad-spectrum cephalosporins and metronidazole may be needed. If there is a history of GI symptoms, a full GI examination may be indicated. Most abscesses resolve after drainage [85], and inexperienced physicians are not recommended to search for a fistula if the perianal abscess has an uncomplicated healing [81]. The probing during the examination itself may cause a fistula.

Treatment of an established fistula is demanding because surgery always carries a risk of damage to the sphincter-apparatus resulting in continence disturbances. Low fistulas can usually be laid open to heal. An alternative is to place a seton, and then re-assess after 2-3 months. Various surgical techniques (e.g. advancement flap, fistula plug) have been described for the treatment of more complex or "high" fistulas [37, 85].

2.2.5 **Hemorrhoids**

2.2.5.1 Epidemiology and risk factors

The prevalence of hemorrhoids in the population is unknown. Reported number varies between 4-36% of the population [86], whereas a hospital-based proctoscopy study revealed a prevalence of 86%, where most were asymptomatic [86]. The precise etiology of hemorrhoids is unclear, but suggested risk factors are low fiber intake, prolonged straining at stool, and pregnancy [86].

2.2.5.2 Pathogenesis

Anal cushions have been suggested to be the precursors of hemorrhoids. The classical positions of the hemorrhoids are at 3, 7 and 11 o'clock, the same as for anal cushions. Prolapsing internal hemorrhoids are lined by an anesthetized covering, and the neck arises above the pectinate line. External hemorrhoids arise below the pectinate line.

Several theories exist regarding the pathogenesis of hemorrhoids. One includes degeneration of the collagenous fibers in the submucosa resulting in enlargement and distal displacement of the anal cushions [86]. Another theory includes increased local pressure, venous dilatation in the anal cushions, and engorgement of the valve-less venous system [86]. This is a possible explanation for the increased prevalence of hemorrhoids in pregnancy [86], but a hormonal effect on the laxity of the connective tissue may also play a role [35].

2.2.5.3 Diagnostic procedure

The most common symptom is rectal bleeding, followed by pain, mucosal discharge and pruritus. Rectal bleeding is usually intermittent, separated from the stool [35].

Proctoscopy is sufficient to diagnose and classify the hemorrhoids according to the Goligher classification [87]. Bleeding hemorrhoids that do not prolapse (normal external appearance) is classified as grade I. Grade II refers to hemorrhoids that prolapse, but reduce spontaneously. Grade III and IV refer to hemorrhoids that remain prolapsed, but the former can be manually replaced [86]. The different grades are not always related to the severity of symptoms [35, 88].

2.2.5.4 Treatment

Most conservatively treated hemorrhoids heal spontaneously [89]. The first treatment step is dietary advice to avoid constipation, and advice concerning toilet habits to avoid straining. Usually bulking agents and local topical treatment is prescribed. Medical treatment (for example local administration of local anesthetics and steroids) aims to reduce symptoms [86]. However, as symptoms may mimick those of colo-recto-anal malignancies, GI-investigations should always be considered [35].

Outpatient treatments are considered the primary option for grade I and II hemorrhoids, and include rubber band ligation, injection sclerotherapy, cryotherapy, and photocoagulation. Rubber band ligation is performed through direct visualization of the hemorrhoidical pedicle, resulting in ischemic necrosis of the hemorrhoid. It has been reported that rubber band ligation is the outpatient treatment procedure with the highest success rate varying between 69-94% [86].

When "officed-based" therapies fail or are insufficient surgical options can be considered. Hemorrhoidectomy has been shown to be the most effective treatment and several different techniques exist [86]. In Sweden, the technique described by Milligan-Morgan is commonly used [90]. Complications, such as pain, are more common than for outpatient procedures. More severe side effects include urinary retention, bleeding, sepsis, incontinence and anal stenosis [85-86].

Stapled anopexy has been suggested as the optimal treatment for prolapsed hemorrhoids (grade III and IV) [91]. Even though stapled anopexy has been associated with less postoperative pain, a higher rate of prolapse and need for reintervention than for patients treated with conventional hemorrhoidectomy has been reported [92]. Described complications include pain, fecal urgency, rectal obstruction and perforation, as well as pelvic sepsis [89, 93].

The newest treatment is Doppler-guided hemorrhoidal artery ligation leading to hemorrhoidal shrinkage mostly used for grade II and III. The success rate reported is 95%, and few complications have been reported so far [86].

2.3 COLORECTAL CANCER

2.3.1 Anatomy and physiology

The colon is about 1.5 meters long and extends from the ileocecal valve to the rectum. The start of the rectum is seen by the merging of the taenia coli of the sigmoid colon to the outer muscular tube of the rectum. The transverse and sigmoid colons have mesenteries making them mobile, whereas the ascending and descending colons are immobilized. The lower third of the rectum lies below the peritoneal floor of the pelvis. The epithelia consist of columnar epithelium mixed with mucus-secreting goblet cells.

The superior and inferior mesenteric arteries supply the colon. The two arteries anastomose, allowing collateral supply. The superior rectal artery is a continuation of the inferior mesenteric artery, whereas the middle and inferior rectal arteries are branches from the internal iliac arteries.

The venous drainage is via the portal vein. The lymphatic drainage of the colon is via the epicolic and paracolic nodes close to the colon, and to nodes at the origin of the mesenteric vessels. The lymphatic drainage of the rectum drains to the superior rectal and inferior mesenteric nodes [38, 80].

The large bowel absorbs sodium and water, especially in the right-sided colon, whereas the left-sided colon and rectum serve as a fecal storage [94]. Mucus is secreted as a

lubricant. The normal defecation-frequency ranges from three times per day to roughly every third day [80].

2.3.2 Epidemiology

CRC is the second most common type of cancer in both sexes in Sweden, the most common being breast cancer in women and prostate cancer in men [40]. CRC is the second most frequent cause of cancer-related death in Sweden with 2,615 deaths in 2007 [95]. CRC is the third most common cancer in both sexes in the United States [96]. In Europe, 412,900 CRC cases were diagnosed, and 207,400 CRC deaths were registered in 2006 [97].

Figure 4 shows the anatomical distribution of CRC. About 41.5% of all colorectal cancer are situated in the right side of colon, 28.7% in the left side of colon (including the splenic flexure), and 29.8% in the rectum [94].

The incidence of CRC differs in different parts of the world. High incidence rates are seen in Western Europe, North America, Australia, and Japan, and low incidence rates are seen in Africa, Asia and parts of South America [98]. The incidence among immigrants becomes similar to the original population [99]. This indicates that CRC is a disease sensitive to environmental and dietary changes [100].

The most well-known hereditary syndromes are Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC). FAP accounts for less than 1% of all CRCs, and HNPCC for 1-6% [101]. The genetic component of CRC has been estimated to 10%, and the remaining 90% may be caused by environmental and lifestyle factors [102].

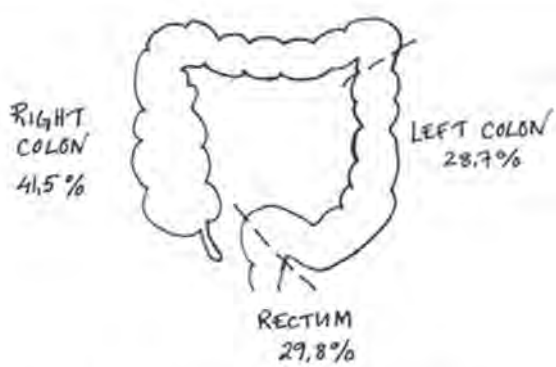

Figure 4. The distribution of colorectal cancer by subsite.

2.3.3 **Pathogenesis**

CRC can arise through two distinct mutational pathways; chromosomal instability (CIN) or microsatellite instability (MSI). CIN is more common than MSI, and present in 65-70% of all CRCs [103]. MSI is present in 15% of all CRCs [103]. The pathways are not yet fully understood.

2.3.3.1 Chromosomal instability

In order to transform the normal colonic epithelium to adenoma, and eventually cancer, an accumulation of mutations needs to occur. These mutations activate oncogenes or inactivate tumor suppressor genes, leading to genetic and epigenetic alterations [104-106].

The CIN pathway was described in 1990 [105]. The pathway is activated by a mutation or loss of the Adenomatous polyposis coli (*APC*) gene [107]. Inherited mutations in the *APC* gene are related to the Familial Adenomatous Polyposis syndrome (FAP) [108-109]. The CIN pathway is not only relevant for FAP, but also for 85% of all sporadic CRCs.

The oncogene *K-ras* is involved in the cellular processes of proliferation, differentiation, and apoptosis [110]. A mutation of the *K-ras* is seen in around 40-50% of all colorectal carcinomas, and has been suggested to be involved in environmental mechanisms of colorectal carcinogenesis (figure 5) [105, 111]. *K-ras* mutations are early events in the adenoma-carcinoma pathway [103]. *K-ras* mutations are more common in large than small adenomas suggesting a growth promoting role [112]. Even though *K-ras* mutations have been associated with worse prognosis [113], it has not yet been defined as independent prognostic factors for CRC [103]. Nevertheless *K-ras* mutation status is an established predictive marker for treatment with epidermal growth factor receptor inhibitors. Almost none of those with *K-ras* mutations respond to this treatment [103].

2.3.3.2 Microsatellite instability

Carcinogenic development may also be seen in the microsatellite instability pathway, where MSI is the consequence of a deficient mismatch DNA repair system (figure 5). The characteristic of MSI is increased, or decreased, tandem repeats of simple DNA sequences known as microsatellites. These sequences are easily mutated during replication and cannot be repaired due to a deficient DNA repair system.

MSI is the trademark of the Hereditary Non-Polyposis Colorectal Cancer syndrome (HNPCC). MSI is also present in sporadic tumors. The sporadic tumors are believed to arise via the "serrated polyp-neoplasia pathway" [114]. Almost all serrated adenomas have a mutation in *BRAF* [110]. *BRAF* is an oncogene involved in the cellular proliferation, differentiation, and apoptosis [110]. Through a two step process of dysregulated apoptosis, the serrated polyp is transformed to cancer [110]. MSI is more common in right-sided than left-sided colon cancer [115-117].

Figure 5 shows a proposed pathway of the colorectal carcinogenesis based on theories by Rowan et al [118].

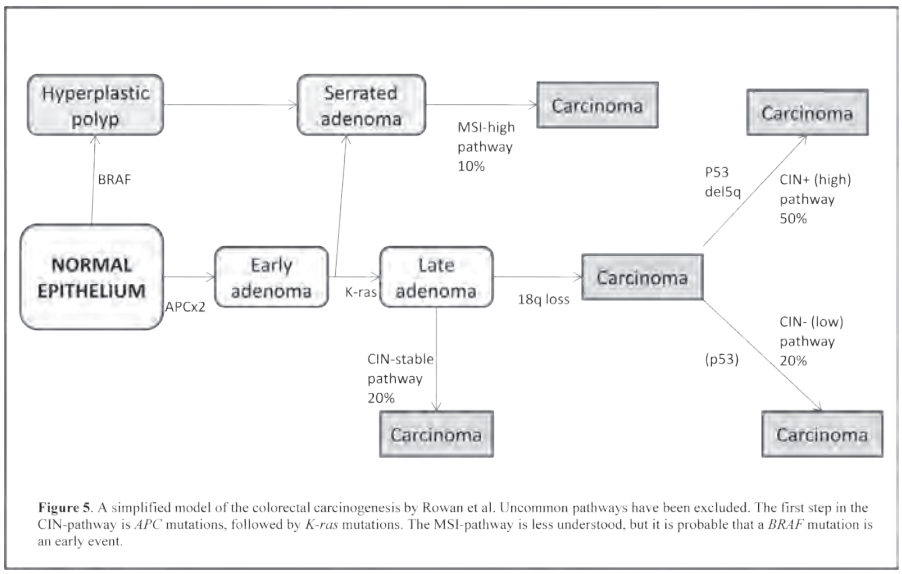

Figure 5. A simplified model of the colorectal carcinogenesis by Rowan et al. Uncommon pathways have been excluded. The first step in the CIN-pathway is *APC* mutations, followed by *K-ras* mutations. The MSI-pathway is less understood, but it is probable that a *BRAF* mutation is an early event.

2.3.4 **Environmental risk factors**

2.3.4.1 Smoking

Smoking is probably the best known environmental risk factor for cancer. No other known risk factor accounts for more cancer-related deaths than tobacco smoking [119]. Smoking has been estimated to be a contributory cause for about 25% of all male cancers, and about 4% of all female cancers [119]. In both sexes, approximately 16% of all cancers in developed countries are attributed to tobacco smoking [119]. The corresponding proportion in less developed countries is 10% [119]. Smoking is a well-established risk factor for lung cancer, but also for several types of GI-tract cancers. The positive association between smoking and anal cancer is well known [43-45]. In contrast to anal cancer, the role of smoking in the etiology of CRC is unclear and contradictive results have been reported. Adenomas are precursors of most colorectal tumors [120]. Smoking is an established risk factor for adenomas [121]. The inability to verify the association between CRC and smoking has been explained by a too short period of follow-up [122], and inclusion of adenomas in the control group [123-125]. Smoking has also been proposed to be an initiator of tumorigenesis, and a long duration of follow-up would be needed to observe an association [126]. From the 1950s to the 1980s no clear association between smoking and CRC was shown. In 1986, the International Agency for Research on Cancer (IARC) concluded that, based on existing evidence, tobacco smoking is not a risk factor for CRC [127]. During the latest decade accumulating evidence suggests that CRC is a tobacco-associated malignancy [128-137]. A reported earlier onset of CRC among smokers has initiated the discussion of smoking as a high-risk factor in screening programs [138]. Smoking has been associated with MSI status [117, 133, 139], methylation of CpG islands, and BRAF mutations [140].

Studies of head and neck, lung, bladder and cervical cancer have shown that many patients continue to smoke after cancer diagnosis. Smoking has been shown to decrease

cancer treatment efficiency [141-145], which has also been reported for CRC [146]. In addition, high consumption of alcohol and smoking have been associated with increased risk of anastomotic leakage in colorectal surgery [147].

2.3.4.2 Snus (Swedish moist snuff)

Nicotine is widely used among both men and women. Since smoking is a well-known risk factor for several malignancies as well as other diseases, many want to quit and replace tobacco smoking with snus use.

Snus contains tobacco specific nitrosamines [148]. American users of smokeless tobacco may be exposed to similar levels of 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK) as smokers [149]. However, the levels of NNK in Swedish snus are generally lower [150]. NNK and other nitrosamines have been shown to induce GI, lung and nasal tumors, and IARC has classified snus as a class I carcinogenic substance [151]. So far snus has been shown to accelerate the growth of gastric malignancies in mice [152]. Significantly increased risks have also been seen for pancreatic cancer [153-154], and for esophageal cancer [155]. American chewing tobacco has been associated with an increased risk of rectal cancer, but not colon cancer [156].

The carcinogenic substances in snus are not the only potential hazards. PH-values affect uptake of nicotine. A large part of the nicotine is in its unionized form at a pH of 6.5 or higher. In its unionized form, nicotine is able to cross biological membranes [151]. By changing the amount of free nicotine, the tobacco companies can make the snus more or less addictive. When snus is used continuously through the day, a snus user is exposed to similar nicotine levels as a smoker [157].

Nicotine is not carcinogenic in itself, but it is a substance with many biological effects that theoretically could promote cancer progression. Nicotine can stimulate nicotine acethylcholine receptors (nAchRs). This activation has been shown to promote angiogenesis, regulate the cell-cycle and apoptosis in several cell types [158-162]. Nicotine can activate Akt in human airway epithelial cells, stimulate the growth of lung cancer cells, suppress apoptosis [163], stimulate cell survival [164], and suppress growth inhibitory effects of *trans*-retinoic acid on lung cancer [165-166] . Oral administration of nicotine has been shown to promote human colon cancer growth and angiogenesis in a mice model [167]. Additionally, nicotine has also been shown to stimulate tumor invasion and metastasis in models of gastric cancer [168].

Snus users have previously been shown to have a slightly increased overall mortality [169-170]. Pure snus users from a Swedish cohort had an overall risk of death of any cause (hazard ratio (HR) 1.23 [95%CI 9-40%]) compared to never users of any tobacco [169]. The similar number for cancer mortality was HR 1.28 (95%CI 0.96-1.69) compared to never users of any tobacco [169].

2.3.4.3 Dietary factors

The importance of dietary factors has been debated. This section will include a summary of the available data. Contradictory results and opinions are common. In this

thesis, these factors are only of interest as possible confounders, and there will be no discussion concerning possible mechanisms.

Alcohol has been positively associated with CRC risk [137, 171], and a pooled analysis revealed a relative risk (RR) of 1.41 among those with high alcohol consumption [172].

A meta-analysis of coffee consumption and CRC risk reported no significant association [173]. However, in a Swedish case-control study coffee drinking was associated with a reduction in the risk of colon cancer, but not of rectal cancer [174]. In the same study, tea drinking was inversely associated with the risk of rectal cancer, but not of colon cancer [174]. A meta-analysis of green tea indicated that a high intake slightly decreased the risk of CRC [175]. However, in a sub-analysis restricted to cohort studies the significant effect disappeared [175]. An explanation might be methodological issues in case-control studies. Black tea was not associated with the risk of CRC [175].

Whereas the intake of red and processed meat was significantly associated with CRC risk, RR 1.21 and RR 1.19 respectively [137], the intake of animal fat and proteins was not associated with CRC risk [176]. Intake of fish and n-3 fatty acids has been inversely related to CRC risk in men [177]. This was not confirmed in a later cohort study [178].

Both calcium and vitamin D are proposed protective dietary factors of CRC. Milk intake was associated with a reduced risk of 20%, and the protective effect was more evident in left colon and rectal cancer [179]. A high intake of total calcium was associated with reduced risk of 22% [179]. A high intake of both calcium and vitamin D was associated with a reduced risk of 28% [179]. Similar results have been reported in Swedish studies [180-181] .

A diet rich in fruits and vegetables has been negatively associated with CRC risk [182-184]. However, the results are inconsistent [137, 185]. The European Prospective Investigation into Cancer and Nutrition study reported an inverse association between CRC risk and intake of fruits and vegetables in never and former smokers [184]. Surprisingly, the opposite was seen in current smokers [184]. The same cohort study reported that the highest intake of dietary fibers was associated with a significantly reduced risk of 42% [186]. Previously a pooled analysis of 13 prospective studies on the intake of dietary fiber revealed no association with CRC risk [187]. There are many difficulties in nutritional studies, and the intake of dietary fibers is likely to be confounded by lifestyle factors [188]. This is probably true for most dietary factors.

2.3.4.4 Non-dietary risk factors

Obesity has been associated with CRC. In a meta-analysis a higher body mass index (BMI) among men was significantly associated with a risk elevation of 24% for colon cancer [189]. This risk increase was more evident for left-sided colon cancer (28%), whereas the smallest risk increase was seen for rectal cancer [189]. The association between high BMI and CRC risk was smaller for women than men [189].

A meta-analysis revealed a 33% reduction in the risk of colon cancer in women who had recently used hormone replacement therapy (HRT) [190]. Rectal cancer was not

associated with HRT [190]. Two cohort studies reported inverse associations between HRT and both colon and rectal cancer [191-192]. Similar results were reported from a population-based case-control study where HRT use was associated with a 63% reduction in the risk of CRC [193]. Surprisingly, this protective effect was not seen in subjects who used aspirin and were more physically active [193]. Both physical activity [137, 194] and aspirin use [195] are associated with reduced risks of CRC.

2.3.4.5 Other diseases

Diabetes has been associated with an increased CRC incidence [196] and mortality [197]. An increased risk of CRC is also seen in patients with ulcerative colitis [198], and Crohn's disease [199]. In patients with Crohn's disease, the risk of CRC was significantly increased with 90% [199]. When CRC was subdivided, the site-specific risk of colon cancer in patients with Crohn's was elevated with 150% [199]. Cholecystectomy has been associated with a slightly increased risk of colon cancer, but not rectal cancer [200-201].

2.3.5 Genetic risk factors

Heredity plays an important role in CRC disease, and at least 10% of all CRCs are due to genetic factors [102]. The risk associated with having one first-degree relative (parent, sibling, child) with CRC compared to none is two-fold [202]. Similarly, the risk associated with having more than one first-degree relative with CRC is more than four-fold [202]. Figure 6 describes the distribution of sporadic and hereditary colorectal cancer cases in a Swedish county [203]. Even though the study was small [203], the findings were consistent with previous results. There was no case of FAP in this study, but the prevalence of FAP is known to be <1% [101].

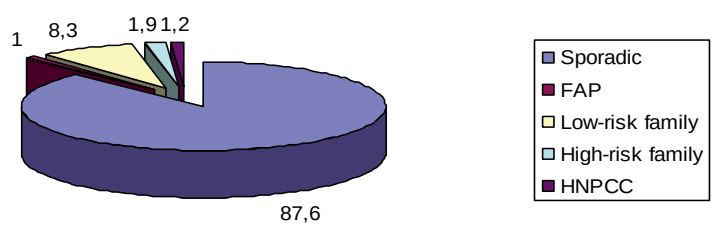

Figure 6. The distribution of different groups of colorectal cancer in Sweden. 87.6% are sporadic cancers, <1% FAP, 8.3% low-risk families, 1.9% high-risk families, and 1.2% HNPCC.

2.3.5.1 Familial Adenomatous Polyposis syndrome

Of the inherited CRC syndromes, FAP is the most well defined and understood type [204]. FAP is characterized by development of hundreds to thousands colorectal polyps and adenomas in late childhood and adolescence. Left untreated, these benign tumors will progress to cancer with an absolute risk of 100% before the age of 45 years [205]. It is an

autosomal dominant disease with a population frequency of 1 per 7,000-10,000 individuals, and FAP is responsible for about 1% of all CRCs [100, 205].

FAP is caused by a mutation in the *APC* gene [108-109]. More than 300 different mutations have been described. *APC* alterations are some of the first events in the CIN pathway.

Another form of the disease, Attenuated Familial Adenomatous Polyposis (AFAP), is associated with a smaller amount of adenomas, later clinical onset and a cumulative risk of CRC of 69% by the age of 80 [206].

2.3.5.2 Hereditary Non-Polyposis Colorectal Cancer syndrome

In 1966, Dr Henry Lynch described two families with an autosomal dominant predisposition of CRC at a young age, but with less polyps [207]. This disease is now known as HNPCC. The reported mean age at clinical presentation varies between 44 to 61 years [208-209]. HNPCC patients develop a modest number of polyps that rapidly progress to cancer. The cumulative risk of CRC has been estimated to 80% by the age of 75 [210]. HNPCC is the most common colorectal hereditary syndrome accounting for 1-6% of all CRC cases [101, 211-212]. HNPCC patients have an increased risk of several other malignancies.

Several clinical criteria for HNPCC have been proposed. The *Amsterdam II criteria* consist of three criteria. These are: (i) three or more relatives with HNPCC-associated cancers (colorectal, endometrial, small bowel, ureter, renal pelvis), one of whom is a first-degree relative of the other two, (ii) CRC involving at least two generations, (iii) one or more CRC diagnosed at age <50 years [101].

2.3.5.3 High-risk and low-risk families

In Sweden about 10-15% of all cases with a family history of CRC do not match the criteria for FAP and HNPCC, and 1.9% belong to so-called high-risk families [203]. High-risk families consist of three or more first-degree relatives affected with CRC in two generations. The responsible gene/genes are probably inherited in a dominant manner. The lifetime risk of CRC is probably similar to the risk for HNPCC families but with a later onset [213].

The remaining 8.3% belong to low-risk families [203]. These are families with two first-degree relatives that are affected with CRC. They have a higher risk of adenoma compared to high-risk families, and the cumulative risk of CRC has been estimated to 15% [213].

2.3.6 **Diagnostic procedure**

The symptoms of CRC vary and are non-specific such as intermittent rectal bleeding, blood mixed with mucus, altered bowel habit, iron deficiency anemia, weight loss, and lower abdominal pain of colic type [80]. Over 50% of patients have tenesmus [80]. Colonic tumors, in particular those of the cecum or sigmoid may be of considerable size and palpable through the abdominal wall. Since approximately 1/3 of CRC are rectal tumors [94], some tumors can be detected with a simple digital rectal examination.

Sigmoidoscopy and colonoscopy are used to visualize and biopsy the tumor. Sigmoidoscopy was introduced in the late 1960s, whereas complete colonoscopy was introduced a few years later [214]. Various radiographic examinations can be performed in CRC patients. Presently computer tomography (CT) is most commonly used to detect distant metastases and, for colonic tumors, local tumor growth. In rectal cancer, MRI of the pelvis gives valuable information [215]. Screening was introduced in the United States in 2001, and in the European Union in 2003 [216-217]. It has been recommended after the age of 50 for people with an average risk and is now being introduced in Stockholm [218]. Different methods, such as fecal occult blood testing, flexible sigmoidoscopy, and colonoscopy have been discussed. There is no consensus on which method to use [219]. The frequency of surveillance colonoscopy in patients with HNPCC is every third year, and is recommended to be initiated at the age of 20 to 25 [220]. Patients with ulcerative colitis and Crohn's colitis are recommended to initiate surveillance with colonoscopy 8 to 10 years after onset of inflammatory bowel disease [221]. As cancer risk is dependent on disease extension and duration, surveillance frequency varies.

CRC is classified according to the TNM system (table 3). The grouping of stages is based on the TNM classification (table 3).

Table 3. TNM classification for CRC cancer [60], and stage grouping.

TNM STAGING FOR COLORECTAL CANCER	
Primary tumor (T)	
Tx	Primary tumor cannot be assessed
Tis	Carcinoma in situ
T1	Cancer invades submucosa
T2	Cancer invades into muscularis propria
T3	Cancer invades through muscularis propria and into subserosa/adjacent non-peritonealized tissues
T4	Cancer perforates the visceral peritoneum or directly invades adjacent organs
Regional lymph nodes (N)	
Nx	The regional lymph nodes cannot be assessed
N0	No regional lymph nodes involved
N1	Metastases in 1-3 pericolic or perirectal lymph nodes
N2	Metastases in >3 pericolic or perirectal lymph nodes
N3	Metastases in lymph nodes along the course of major vessels
Distant metastasis (M)	
Mx	The presence of distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

STAGE GROUPING			
Stage	**T**	**N**	**M**
0	Tis	N0	M0
I	T1, T2	N0	M0
IIa	T3	N0	M0
IIb	T4	N0	M0
IIIa	T1, T2	N1	M0
IIIb	T3, T4	N1	M0
IIIc	Any T	N2	M0
IV	Any T	Any N	M1

2.3.7 **Treatment and prognosis**

The main treatment for colon cancer is surgery. The surgical principle is to remove the tumor-bearing segment of the bowel together with the regional lymph nodes *en bloc*. The surgical technique for colon cancer is under further development [222]. Presently, surgery is usually combined with post-operative chemotherapy in stage III colon cancers [223]. Radiotherapy is generally only used in palliative treatment. Survival after

colon cancer has improved. In 1999 the 5-year cumulative relative survival rate in Sweden was 57.2% [224].

Historically, treatment for rectal cancer was mainly surgery alone and the technique was blunt dissection of the rectum. In the 1980s total mesorectal excision (TME), where the tumor and local lymph nodes are removed *en bloc* using sharp dissection, was described as a treatment for rectal cancers [225]. It is now the gold standard in rectal cancer surgery. The introduction of TME has been reported to decrease local recurrence rates and improve survival [226-227].

About half of all rectal cancers in Sweden are treated with anterior resection (AR) [228]. In AR the entire or the upper part of the rectum is removed, and there is an anastomosis between the remaining rectum and the left colon. If the tumor is situated less than 6cm from the anal verge, abdominalperianal resection (APR) might be used. The entire rectum, anal canal and anus are removed, and a permanent colostomy is formed. Approximately a quarter of all rectal cancers in Sweden are treated with APR [228]. The corresponding number for patients treated with an extended Hartmann´s procedure is 15% [228]. Basically, rectum is resected as in an AR. However, in this procedure no anastomosis is constructed and the patient receives a colostomy. The anal canal or rectal remnant is left in situ.

Radiotherapy (RT) has been tried for rectal cancer in various settings since the 1920s. RT can be delivered pre- or postoperatively and can be fractionated either conventionally with 1.8-2 Gy per fraction or hypofractionated. Following randomized trials showing reduced local recurrence rates and possibly increased survival [229-230], short-course hypofractionated pre-operative RT has been established standard of care for many rectal cancer patients in Sweden. Since 1995 most hospitals in Sweden has used TME and pre-operative radiotherapy for rectal cancer [224]. Rectal cancer survival has improved, and in 1999 the 5-year cumulative relative survival rate in Sweden was 57.6% [224].

Overall, the prognosis for CRC has improved [231]. According to American data the 5-year relative survival has increased from 50.8% in 1975-77 to 65.3% in 1996-2004 [232]. In addition to stage and treatment, different factors that affect CRC survival have been described, such as the use of aspirin [233], vitamin D status [234], physical activity [235] and diabetes [197].

Other prognostic factors are CIN and MSI status (see section 2.3.3. for description). CIN-positive tumors are associated with a poorer prognosis (HR for death=1.45), and MSI-positive tumors with a better prognosis (HR=0.65) [103]. One study stratified survival on both MSI and CIN status [236]. They found that the survival benefit in CIN-negative patients was independent of MSI-status [236]. MSI is considered to be a prognostic marker. It has been recommended that CIN should be a prognostic marker as well [237]. It is rare that CRCs are both MSI and CIN positive, but about one third of all CRCs are both MSI and CIN negative [238].

3 AIMS

Study I: To investigate the risk of cancer in patients hospitalized with condylomata acuminata.

Study II: To investigate the risk of anal cancer in patients hospitalized with benign anal lesions, with a specific interest in benign inflammatory anal lesions.

Study III: To investigate the risk of colorectal and anal cancer associated with smoking and the use of snus.

Study IV: To estimate the effect of smoking and snus use on cancer survival, with special reference to colorectal cancer.

4 METHODS

4.1 SETTINGS

4.1.1 NRN

The national registration numbers (NRNs) are unique personal identifiers assigned to all residents in Sweden. The NRNs permits follow-up through linkages to nationwide and essentially complete registers of cancer, causes of death, the total population and migration.

4.1.2 The Swedish Cancer Register

The Swedish Cancer Register was started in 1958 by the Swedish National Board of Health and Welfare (Sw: Socialstyrelsen). According to law, physicians are obliged to report all malignant conditions to one of the 6 regional cancer registers. Each year, the results are summarized by the Swedish National Board of Health and Welfare. The register is coded according to the current revision of the International Classification of Diseases (ICD) and according to ICD-7.

The completeness of the register has been shown to be 96-98% complete [239-240], even though the completeness may vary by site [241]. The register does not include cases only documented on death certificates [242]. During 1971-1975, a code was introduced to distinguish incidental finding during autopsy. TNM Classification of malignant tumors has been reported in the register from 2004.

4.1.3 The Swedish Causes of Death Register

The Cause of Death Register started in 1952, and records data on all deaths among Swedish residents since 1961. It is held and maintained by the Swedish National Board of Health and Welfare, and is updated every year. The causes of death are classified according to ICD-7 through 1968, ICD-8 during 1969-1986, ICD-9 during 1987-1996, and ICD-10 thereafter. The overall completeness of the register is estimated to exceed 99% [243]. It holds information on the date of death, cause of death and contributing factors of all Swedish residents.

4.1.4 The Swedish Inpatient Register

The Inpatient Register (Sw: Slutenvårdsregistret) was established in 1964–1965 to document individual hospital discharges [244-245]. It is held by the Swedish National Board of Health and Welfare. The discharge diagnoses are coded according to ICD-7 through 1968, ICD-8 during 1969-1986, ICD-9 during 1987-1996, and ICD-10 thereafter. However, in some counties, the use of ICD-9 persisted until the end of 1997. The public medical service in Sweden is financially and geographically accessible to all residents. The number of patients treated in the private sector during the studied period is negligible. The hospitals delivering data to the register has increased steadily, from a 60% coverage of the Swedish population in 1969, to 85% in 1983, and 100% in 1987 [246]. Each Inpatient Register record corresponds to one in-hospital episode. The record contains (i) the patient's NRN, (ii) the date of hospital admission and discharge and (iii) up to 8 discharge diagnoses, including 1 main discharge diagnosis.

4.1.5 The Swedish Population and Migration Register

Statistics Sweden maintains the Swedish Population and Migration Register. The register contains information on current addresses of all Swedish residents since 1960, and migration information, within Sweden and across the borders, since 1968.

4.2 STUDY POPULATIONS

4.2.1 Study I

In study I, we selected all records in the Inpatient Register with a condylomata acuminata (CA) diagnosis (ICD7-039.20, ICD8-099.92, ICD9-078B, ICD10-A630) from January 1965 to December 1998. We defined CA as the main diagnosis if it was listed as the first discharge diagnosis for at least 1 hospitalization.

A total of 11,537 patients were identified as having ever been hospitalized with CA during the study period. After record linkages to the nationwide registers of Total Population, Cancer Register, Causes of Death and Migration, 566 records were excluded (25 with erroneous NRNs, 302 with a prevalent cancer, and 239 with other inconsistencies), and 10,971 patients remained for final analysis.

4.2.2 Study II

In study II, all records in the Inpatient Register with a diagnosis of benign anal lesion, including fissure (ICD7-574.00, ICD8-565.00, ICD9-565.A, ICD10 K60.1-K60.3), fistula (ICD7-574.10, ICD8-565.10, ICD9-565.B, ICD10 K60.3-K60.5), perianal abscess (ICD7-575.00, ICD8-566.00, ICD9-566, ICD10-K61.0), and hemorrhoid (ICD7-461.99, ICD8-455.99, ICD9-455, ICD10-I84) from January 1965 to December 2002 were initially selected.

We identified 135,276 individuals hospitalized for benign anal lesions during the studied period. After record linkages to the nationwide registers of Total Population, Cancer Register, Causes of Death and Migration, 10,282 records were excluded (316 with erroneous NRNs, 7,090 with prevalent cancer, and 2,876 with other inconsistencies), leaving 124,994 patients for the final analysis (figure 7). The cohort was broken down into two sub-cohorts, inflammatory lesions (fissures, fistulas, and perianal abscesses) and hemorrhoids. Patients who had been hospitalized for both were allocated to the inflammatory lesion sub-cohort.

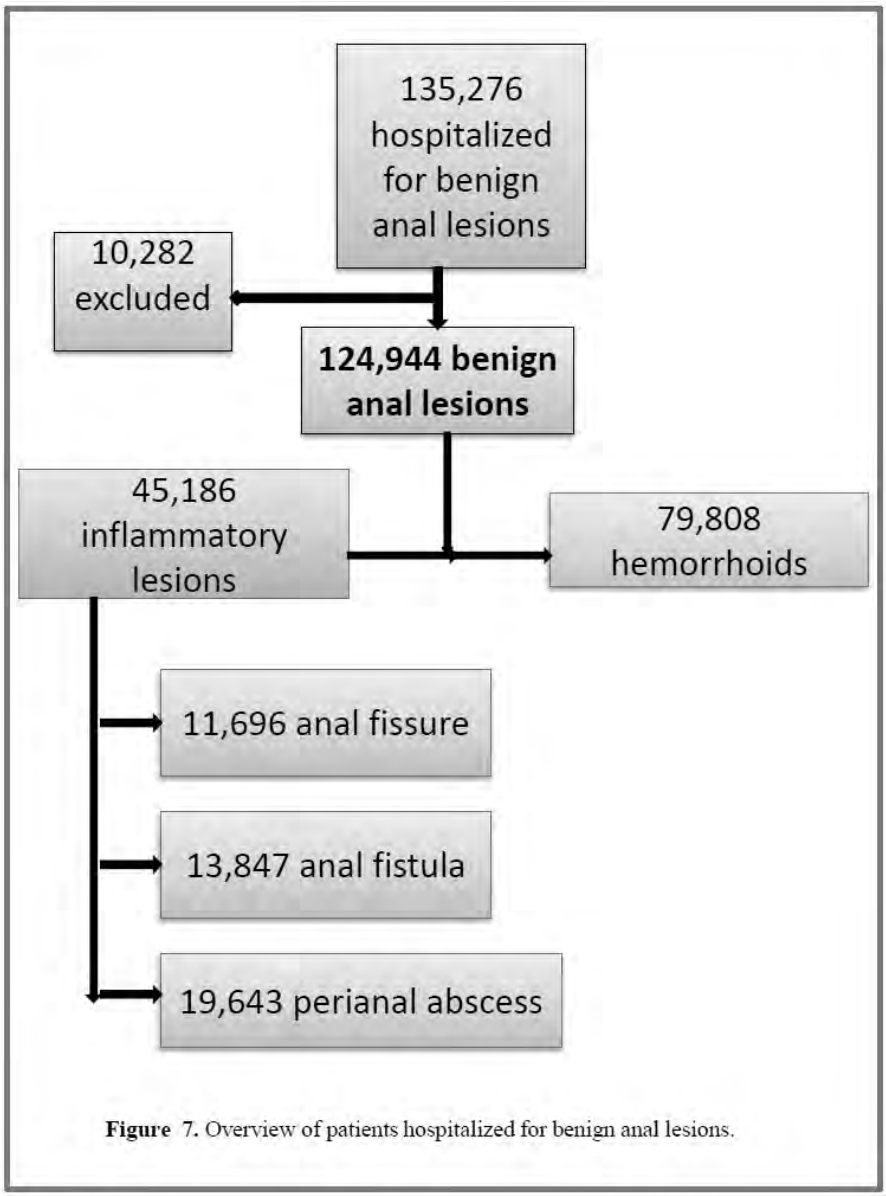

Figure 7. Overview of patients hospitalized for benign anal lesions.

4.2.3 **Study III and IV**

In 1969 to 1993, the Construction Workers Health Service, together with the trade unions and the Swedish Employers Association, offered free out-patient medical services to all employees in the building industry. The basic units were mobile or stationary clinics, usually staffed by a doctor and a few nurses. The aim was to provide preventive health check-ups to all construction workers, all of whom received a personal invitation. In 1971, data from these check-ups were registered in a computerized central register. Using personal invitations, and advertisements at most building sites, about 75% of the workers were registered in the Construction Workers

Cohort. The reason for non-participation was not registered. The mean number of visits was three, and more than 200,000 men had at least two visits.

Cohort members included in 1971-75 filled out a 200-item questionnaire with detailed questions about smoking and snus use. In connection with their personal visit at the clinic answers were double-checked by attending staff. During this period, non-smokers were instructed to simply skip the questions regarding smoking habits and move to the snus questions. Non-response was coded as non-use. However, non-response could be attributed not only to non-users but also to negligent smokers. The latter are more likely to also inadequately skip the snus question. Thus, the paradoxical consequence could be that never-users of any tobacco might contain more smokers than the non-smoking snus user category [155].

After a pause during 1976 through 1977, the collection of smoking and snus information was resumed in 1978 with a new form completed directly by the staff. However, at repeat visits after the primary visit using the new form, the registration of tobacco use may be incorrect. If the tobacco habits were unchanged, the questions on tobaccos habits were left unanswered. If so, the cohort member was incorrectly coded as non-user.

All data were compiled in a computerized central register. The number of visits for each cohort member ranged from 1 to 13. Repeat visits were variable in number and timing, and possibly linked to the probability of the studied outcome. In addition, since the validity of exposure data was uncertain at repeat visits after 1978, we only used the exposure information recorded at the first registered visit, which was defined as the entry into the cohort.

4.2.3.1 Study III

Figure 8 gives an overview of the cohort. The analyses were restricted to men (343,822), since less than 5% (17,458) of the participants were women. Additionally 7,441 men were excluded due to various reasons, including incorrect NRN (388), history of cancer (1,229), death (27), or emigration (2,765) before entry, or missing information regarding BMI (body mass index) (3,032), leaving 336,381 male workers for our final analyses.

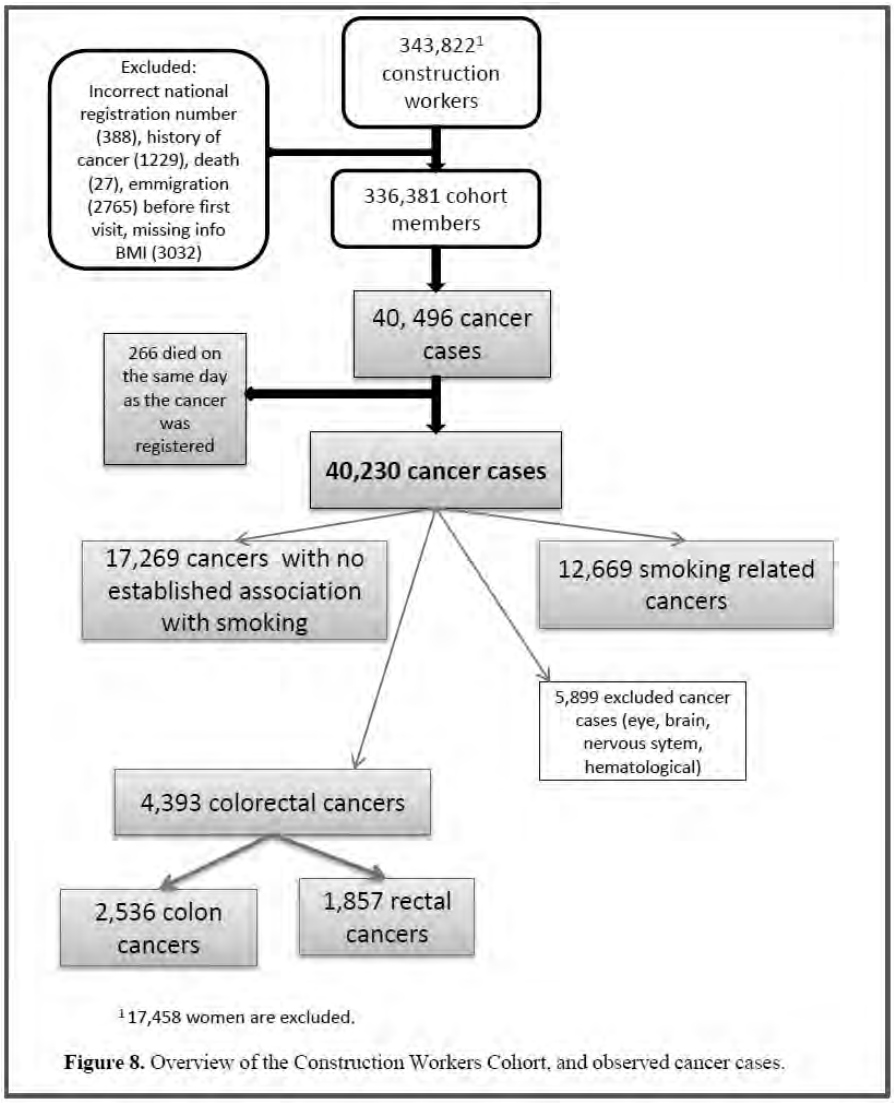

Figure 8. Overview of the Construction Workers Cohort, and observed cancer cases.

4.2.3.2 Study IV

Details of the assembly of cancer cohorts among the construction workers are shown in figure 8. Among 336,381 workers a total of 40,469 new first cancers were diagnosed. Of these cases, 266 died on the day of registration of cancer diagnosis, leaving 40,230 in the final cancer patient cohort.

Using information from the Cancer Register incident cancer cases were subdivided as follows: The *all cancers* category included all cases with ICD7 codes 140-209. This group was further divided into those with *smoking related cancers* (oral ICD7 140-48, esophageal ICD7-150, gastric ICD7-151, anal ICD7-154.1, hepatic ICD7 155-56, pancreatic ICD7-157, sinonasal ICD7-160, larynx ICD7-161, tracheal and lung ICD7-162, penile ICD7-179.0, renal and urinary tract ICD7 180-81), and *other cancers* with no established association with smoking (small intestine ICD7-152, peritoneum ICD7-

158, mediastinum ICD7-164, breast ICD7-170, male genital organs ICD7 177-78 and ICD7 179.1-179.9, skin ICD7 190-91, endocrine ICD7 194-95, bone ICD7-196, connective tissue ICD7-197). In this group some cancers were excluded (cancer of the eye ICD7-192, brain, and nervous system ICD7-193, UNS ICD7-199, hematological ICD7 200-9), including *colorectal cancer* (colon ICD7 153, rectal 154.0), which was analyzed separately due to its debatable association with smoking. A monography from 2004, published by IARC was used to define smoking related cancers [247], although penile cancer and anal cancer were also added.

4.2.3.3 Sensitivity analysis

Non-smokers entering the cohort in 1971-75 may have been erroneously classified as never-smokers while they, in fact, were ex-smokers or current smokers who skipped the smoking question inappropriately. Therefore sensitivity analyses, including only cohort members with visits after 1978, were performed in study III and IV.

In study IV, a sub-analysis was also done to investigate the importance of comorbidity. Data from the Swedish Inpatient Register on diagnoses registered up through the hospitalization when the cancer diagnosis was made was used. If the cancer was diagnosed at an outpatient clinic, only diagnoses registered before the date of cancer diagnosis were used. Patients were divided into three groups; (i) no recorded co-morbidity, (ii) any of chronic pulmonary disease, myocardial infarction or cerebrovascular disease, and (iii) other comorbidity (any discharge diagnosis other than chronic pulmonary disease, myocardial infarction or cerebrovascular disease).

Since data from the Inpatient Register could be accessed only until December 2004, cancer cases occurring after that were excluded in this sub-analysis. Counties started reporting to the Swedish Inpatient Register in different calendar years, and nationwide coverage of the Register was not attained until 1987. Only cohort members covered by the Inpatient Register for at least 5 years before cancer diagnosis were eligible for this sub-analysis.

4.3 STATISTICAL ANALYSES

SAS statistical software (release 9.1 and 9.2) and STATA 9 were used in the analyses.

4.3.1 **Study I and II**

In study I, individual person-time was calculated from the day of the first hospitalization with CA until the day of the first cancer (or cervical carcinoma in situ) diagnosis, death, emigration, or December 31, 1999, whichever came first.

In study II, individual person-time was calculated from the day of the first hospitalization until first cancer diagnosis, death, emigration, or December 31, 2002, whichever came first. Only anal squamous cell carcinomas were analyzed.

Person-time accrued and cancers observed during the first year of follow-up (study I), or first three years of follow-up (study II), were not counted in the main analyses. Cancer cases diagnosed during this period were assumed to be coincidental, and prone to selection and surveillance bias. Cancers found incidentally at autopsy were excluded from the analysis to avoid possible ascertainment bias relating to differential autopsy rates between the study cohort and the general population. The number of expected

events was calculated by multiplying the age-, sex- and calendar year-specific incidence rate (expected rate) in the general population by the person-time accrued in the cohort. In the calculation of expected rates, person-time at risk in the general population did not include person-time for prevalent cancer cases (individuals who were alive but who had been diagnosed with cancer). The number of observed events was divided by the number of expected events, producing a standardized incidence ratio (SIR) with 95% confidence intervals (CIs), assuming that the observed events followed a Poisson probability distribution [248]. Stratified analyses were performed by sex and follow-up duration. All p-values presented in this report are two-tailed, and the results were considered to be statistically significant at $p<0.05$.

In study I, SIRs were calculated for all cancers combined, as well as separately for each cancer type. In study II, SIR was calculated for anal cancer. In study II, stratified analyses by number of hospitalizations for inflammatory benign anal lesions or hemorrhoids were done. The analysis was stratified into patients with 1 and 2 hospitalizations. Person-years accrued before the second hospitalization was attributed to the 'only one hospitalization' stratum.

4.3.2 **Study III**

Each cohort member contributed person-time from the date of first registered visit until the date of any diagnosis of cancer, death, emigration or December 31, 2007, whichever came first. Since we only considered first cancers, all cohort members recorded with a previous cancer at time of entry were excluded. Only clinically diagnosed colorectal or anal cancers were counted, and those identified incidentally during autopsy were not included.

We computed the incidence of right- and left-sided colon cancer (ICD7 153.0-153.1 and 153.2-153.3 respectively), rectal (ICD7-154.0), and anal cancer (ICD7-154.1) by smoking and snus consumption categories, standardized to the distribution of person-time experienced by the entire construction workers cohort using 5-year age categories [249]. Cox proportional hazards regression models estimated hazard ratios (HRs) with corresponding 95% CIs as measures of relative risk, using attained age (in years) as the time scale. In addition to the inherent adjustment for age, all models were adjusted for body mass index (BMI, kg/m^2) at entry categorized into quartiles (<25, 25-29.9, ≥ 30). Cigarette smoking was categorized into 5 classes (0, 1-4, 5-14, 15-24, ≥ 25 cigarettes per day), and cigar smoking into 3 classes (0, 1-7, ≥ 8 cigars/day), whereas pipe smoking was divided into 4 classes (0, 1-29, 30-99, ≥ 100g/week). The categorization was initially the same as in the previously mentioned paper from this cohort [250], but the categorization of cigar smoking was reduced from 5 to 3 classes due to small numbers. The use of snus was only dichotomized (use versus non-use).

We grouped tobacco-using cohort members into pure smokers, pure snus users, and combined smokers and snus users. Never-users of any tobacco were used as the reference group in all analyses. The assumption of proportional hazards for tobacco use and covariates was examined by the method of Shoenfeld's partial residuals; there was no indication of violation of this assumption for any of the variables in the regression models [251].

At study entry the participants reported the duration of tobacco use. Cigarette smoking duration was categorized into 1-14, 15-24, and ≥25 years, while duration of snus use was divided into 1-9, 10-24, and ≥25 years. Since snus users were younger at study entry, we chose to use different categorizations for cigarette smokers and snus users. To better evaluate the importance of total duration of tobacco use on the risk of colorectal and anal cancer we estimated the total duration of cigarette smoking. This was done by adding follow-up time to self-reported duration of cigarette smoking at study entry, assuming a continued use throughout the follow-up period. The estimated total duration of cigarette smoking was categorized into 1-29, 30-39, 40-49, and ≥50 years. Similarly, we categorized estimated total duration of snus use into 1-24, 25-34, 35-44, and ≥45 years. The hazard ratios related to the estimated durations were calculated using time-dependent models.

To estimate a possible 'healthy workers effect', we computed the standardized incidence ratios (SIR) for CRC in the construction workers cohort. The number of expected events in the cohort was calculated by multiplying the age-, sex- and calendar year-specific incidence rate (expected rate) in the general population by the person-time accrued in corresponding strata in the cohort. The number of observed events was divided by the number of expected events, producing a SIR with 95% CIs, assuming that the observed events followed a Poisson probability distribution [248].

4.3.3 **Study IV**

Each cancer case contributed person-time from the date of cancer diagnosis to the date of death, emigration, or end of study, December 31, 2007, whichever came first. Overall mortality was defined as death due to any cause. For cancer-specific death, the cancer diagnosis recorded as the underlying cause of death had to be the same as the primary cancer diagnosis.

Using the information on tobacco habits obtained at the first visit at 'Bygghälsan', the cancer cases were classified as never or ever users of tobacco. Tobacco users were further subdivided into (i) never-smoking snus users, (ii) never snus-using smokers (cigarette, cigar, and/or pipe), and (iii) users of both snus and smoking tobacco (regardless whether the use was concurrent or in sequence).

Multivariate adjusted hazard ratios for overall and cancer-specific death, with 95% confidence intervals (CIs), were estimated with Cox proportional hazards regression models using time since cancer diagnosis as underlying time-scale. Never-users of all tobacco types were used as the reference group in these analyses. In all analyses, adjustments were made for body mass index (BMI, kg/m^2) at first visit at 'Bygghälsan', categorized into 3 groups (<25, 25-29.9, ≥30). In analyses of 'all cancer' and 'tobacco related cancer' additional adjustment was made for cancer site divided into 26 groups (ICD7 140-48; 150; 151; 152; 153-154.0; 154.1; 155-56; 157; 158; 160; 161; 162-63; 177; 178; 179; 180; 181; 190; 191; 193; 194; 195; 196; 197; 200-8; and the rest) to control for inherent differences in prognosis. Further, the proportional hazards assumption was examined using Shoenfeld's partial residuals [251]. Due to violation of this assumption the models were stratified according to age at cancer diagnosis categorized into 5 groups (<50 years, 50-59 years, 60-69 years 70-79 years and ≥80 years of age), period of diagnosis (1971-84, 1985-94, 1995-2007), and cancer group divided as indicated above.

Attribution of observed deaths to the cancer disease is sometimes problematic and misclassification could potentially be differential in regard to exposure status. To

estimate ‘net survival’, i.e., patients’ probabilities of surviving if their cancers were the only cause of death (survival corrected for the effect of other causes of death), we therefore calculated relative survival ratios [252] by different groups of tobacco users. The relative survival ratio is defined as the observed survival in the cohort regardless of cause of death, divided by the expected survival derived from the general Swedish male population divided into 1-year age and 1-year calendar period strata according to the Ederer II method [253]. Separate analyses were done for all cancers, CRC, smoking-related cancers, and other cancers.

Since we suspected that tobacco users and non-users might differ in their propensity to seek health care for early cancer symptoms, we tried to shed light on possible differences in their respective cancer stage distributions. Unfortunately, TNM classification data were not introduced in the Swedish cancer registration until 2004, so this analysis had to be restricted to 2004-2007.

All studies were approved by the Stockholm Regional Ethics Review Board.

5 RESULTS

5.1 STUDY I

Characteristics of the study cohort of 10,971 patients hospitalized for condylomata acuminata are summarized in table 4. The median follow-up was 13.2 years (15.1 years among men and 13.0 years among women). The median age at entry (the first hospitalization with CA) was 24 years, and CA was the main diagnosis in 74% of the participants. Following exclusion of the first year of follow-up, we ascertained altogether 473 incident invasive cancers, 106 in men and 367 in women.

Table 4. Characteristics of patients hospitalized with condylomata acuminata in Sweden in 1965-1998.

	MEN	WOMEN	TOTAL
Number of patients	1,685	9,286	10,971
Never main diagnosis[1]	382	2,518	2,900
Ever main diagnosis[2]	1,303	6,768	8,071
Median age at entry (years)	27	23	24
Median calendar year at entry	1982	1986	1985
Median years of follow-up	15.1	13.0	13.2
Total person-years at risk[3]	25,616	131,096	145,828
1-9 years of follow-up	12,750	73,067	85,817
10+ years of follow-up	11,206	48,805	60,011
Number of invasive cancers during follow-up[4]	106	367	473

[1]The main diagnosis was never CA. [2]The main diagnosis was CA at least once. [3]Person-years observed during the first year of follow-up were excluded. [4]Cancer cases occurring during the first year of follow-up were excluded.

The SIRs for overall and site-specific cancers among patients in the cohort are listed in table 5. Compared to the general population, the overall excess risk was somewhat higher in men (SIR 1.8) than in women (SIR 1.4). This excess risk of cancer at all sites was evident both after 1–9 years and after 10 or more years of follow-up (table 7). We found a more than 10-fold increased risk of all anogenital cancers except for invasive cervical cancer. However, the risk of cervical carcinoma in situ was almost doubled. The significant excess risk of anogenital cancers was still evident after 10 or more years of follow-up (table 7).

Table 5. Standardized incidence ratios (SIR) with 95% confidence intervals (CI) for cancer occurrence in patients hospitalized with condylomata acuminata.

	MEN		WOMEN		TOTAL	
Cancer type or site (ICD 7)	**N**	**SIR (95%CI)**	**N**	**SIR (95%CI)**	**N**	**SIR (95%CI)**
All sites (140-209)	106	1.8 (1.4-2.1)	367	1.4 (1.3-1.6)	473	1.5 (1.4-1.6)
Anogenital						
Vulva (176.0)	-	-	13	10.2 (5.4-17.4)	-	
Vagina (176.1)	-	-	4	12.0 (3.3-30.7)	-	
Cervix, invasive (171)	-	-	19	1.3 (0.8-2.0)	-	
Cervix, *in situ*	-	-	259	1.9 (1.7-2.1)	-	
Anal (154.1)	2	22.7 (2.8-82.0)	7	9.0 (3.6-18.6)	9	10.4 (4.8-19.8)
Penis (179.0)	5	21.9 (7.1-51.2)	-	-	-	-
Other						
Head and neck (140-148)	5	2.8 (0.9-6.6)	13	4.0 (2.1-6.8)	18	3.6 (2.1-5.7)
Esophageal[1] (150)	4	8.1 (2.2-20.7)	2	3.3 (0.4-11.9)	6	5.4 (2.0-11.8)
Stomach (151)	4	1.7 (0.5-4.4)	7	1.6 (0.6-3.3)	11	1.6 (0.8-2.9)
Colon (153)	6	1.5 (0.6-3.3)	11	0.8 (0.4-1.5)	17	1.0 (0.6-1.6)
Rectum (154.0)	4	1.5 (0.4-3.7)	2	0.3 (0.04-1.1)	6	0.7 (0.2-1.4)
Primary liver and bile duct (155)	2	1.9 (0.2-7.0)	6	1.6 (0.6-3.4)	8	1.6 (0.7-3.2)
Pancreas (157)	1	0.7 (0.0-4.0)	4	1.0 (0.3-2.5)	5	0.9 (0.3-2.1)
Lung (162-163)	10	1.8 (0.9-3.4)	30	2.8 (1.9-4.1)	40	2.5 (1.8-3.4)
Breast (170)	1	10.4 (0.3-58.2)	97	1.2 (1.0-1.4)	98	1.2 (1.0-1.5)
Corpus uteri (172)	-	-	12	1.0 (0.5-1.8)	-	-
Ovary (175)	-	-	12	0.9 (0.4-1.5)	-	-
Prostate (177)	12	0.9 (0.5-1.6)	-	-	-	-
Kidney (180)	5	2.5 (0.8-5.9)	5	1.2 (0.4-2.7)	10	1.6 (0.8-3.0)
Bladder (181.0)	7	1.8 (0.7-3.6)	8	2.0 (0.9-4.0)	15	1.9 (1.1-3.1)
Malignant melanoma of the skin (190)	7	2.2 (0.9-4.6)	16	1.0 (0.6-1.6)	23	1.2 (0.7-1.7)
Skin (nonmelanoma) (191)	7	3.0 (1.2-6.1)	15	3.1 (1.8-5.2)	22	3.1 (1.9-4.7)
Brain (193)	4	1.5 (0.4-3.8)	12	1.0 (0.5-1.7)	16	1.1 (0.6-1.8)
Thyroid (194)	0	-	5	0.9 (0.3-2.1)	5	0.8 (0.3-1.9)
Hodgkin lymphoma (201)	2	3.2 (0.4-11.8)	7	3.1 (1.2-6.3)	9	3.1 (1.4-6.0)
Non-Hodgkin lymphoma (200, 202)	4	1.6 (0.4-4.1)	19	2.9 (1.8-4.6)	23	2.6 (1.6-3.9)
Multiple myeloma (203)	2	2.2 (0.3-8.1)	2	1.0 (0.1-3.5)	4	1.4 (0.4-3.5)
Leukemia (204-207)	1	0.6 (0.0-3.1)	6	1.2 (0.5-2.7)	7	1.1 (0.4-2.2)

[1]Excluding adenocarcinoma. N=number of observed cancer cases in the cohort.

In addition to anogenital cancers, there was a more than 5-fold increased risk of cancer of the esophagus, and more than 3-fold increased risks of head and neck cancer, cancer of the skin (non-melanoma) and Hodgkin lymphoma (table 5). Elevated risks of 2-fold or more were also seen for cancer in the lung, bladder and non-Hodgkin lymphoma. Stratified analyses showed similar risk patterns among men and women, and within the 2 different periods of follow-up (table 5 and 6).

Table 6. Standardized incidence ratios (SIR) with 95% confidence intervals (CI) for cancer occurrence in patients hospitalized with condylomata acuminata, by duration of follow-up.

	1-9 YEARS		10+ YEARS	
Cancer type or site (ICD 7)	**N**	**SIR (95%CI)**	**N**	**SIR (95%CI)**
All sites (140-209)	213	1.4 (1.2-1.6)	260	1.6 (1.4-1.8)
Anogenital				
Vulva (176.0)	7	11.6 (4.7-23.9)	6	8.9 (3.3-19.4)
Vagina (176.1)	3	18.3 (3.8-53.5)	1	5.9 (0.2-32.8)
Cervix, invasive (171)	8	1.1 (0.5-2.1)	11	1.5 (0.7-2.6)
Cervix, *in situ*	191	2.0 (1.7-2.3)	68	1.6 (1.2-2.0)
Anal (154.1)	5	13.1 (4.3-30.6)	4	8.3 (2.3-21.2)
Penis (179.0)	5	45.9 (14.9-107)	0	-
Other				
Head and neck (140-148)	12	5.1 (2.6-8.9)	6	2.3 (0.8-4.9)
Esophageal[1] (150)	3	5.6 (1.2-16.3)	3	5.3 (1.1-15.5)
Stomach (151)	3	0.9 (0.2-2.6)	8	2.4 (1.1-4.8)
Lung (162-163)	15	2.0 (1.1-3.3)	25	2.9 (1.9-4.3)
Kidney (180)	4	1.3 (0.4-3.3)	6	1.9 (0.7-4.1)
Bladder (181.0)	7	1.8 (0.7-3.8)	8	1.9 (0.8-3.8)
Skin (nonmelanoma) (191)	9	2.7 (1.2-5.1)	13	3.4 (1.8-5.8)
Hodgkin lymphoma (201)	7	3.7 (1.5-7.7)	2	2.0 (0.2-7.2)
Non-Hodgkin lymphoma (200, 202)	13	3.1 (1.7-5.3)	10	2.1 (1.0-3.9)
Multiple myeloma (203)	2	1.4 (0.2-5.1)	2	1.3 (0.2-4.6)
Leukemia (204-207)	3	0.9 (0.2-2.6)	4	1.2 (0.3-3.1)

N=number of observed cancer cases in the cohort.

5.2 STUDY II

The final cohort consists of 124,994 patients. The hemorrhoids and inflammatory lesion sub-cohorts consisted of 79,808 and 45,186 patients, respectively. The latter was further divided into three non-overlapping sub-cohorts; that is, anal fissure (n=11,696), fistula (n=13,847), and perianal abscess (n=19,643), according to the diagnosis of their first hospitalization for benign anal lesions.

The cohort members were followed for a mean of 12.5 years, with more than 1.5 million person-years accumulated. Mean age at entry (the first hospitalization for a benign anal lesion) was close to 49 years (table 7). Mean duration of follow-up was similar in the inflammatory lesion and hemorrhoid sub-cohorts. However, members in the latter were, on average, hospitalized at an older age. There were no significant sex differences with regard to mean duration of follow-up or mean age at entry. Patients with inflammatory anal lesions were more likely to be hospitalized more than once than those with hemorrhoids (22.2 vs. 9.7%). Crohn's disease was more common in patients with inflammatory benign anal lesions (3.5%) than in patients with hemorrhoids (0.1%). In total, 53 anal cancer cases (squamous cell carcinoma) were observed during follow up (26 and 27 in the inflammatory lesion and hemorrhoid sub-cohorts, respectively). Mean age at anal cancer diagnosis was 62 years (table 7).

Table 7. Characteristics of patients hospitalized for benign anal lesions in Sweden in 1965-2002.

	BENIGN ANAL LESIONS		
	Inflammatory lesions[3]	**Hemorrhoids**	**Total**
All cohort members	45,186	79,808	124,994
Men	29,057	42,168	71,225
Women	16,129	37,640	53,769
Total number of person years	555,646	1,022,543	1,578,189
Men	350,194	537,228	887,442
Women	205,452	485,314	690,776
Mean duration of follow-up[1]	12.3	12.8	12.6
Men	12.0	12.7	12.5
Women	12.7	12.9	12.8
Mean age at entry[1]	42.2	52.6	48.8
Men	41.9	52.2	48.0
Women	42.7	53.1	49.9
Number of hospitalizations (%)			
1	77.7	90.3	
2+	22.2	9.7	
Presence of Crohn's disease (%)	3.5	0.1	1.3
Number of anal cancers	26	27	53
Age at diagnosis of anal cancer[1, 2]	59.5 (15.0)	65.0 (14.6)	62.3 (14.9)

[1]Years. [2]Mean (SD). [3]Anal fissures, perianal abscesses and fistulas.

SIRs by follow-up duration are shown in table 8. Among patients with inflammatory lesions, we observed six anal cancer cases compared with 0.25 expected, entailing an SIR of 24.0 (95% CI 8.8–52.3) in the first year of follow-up. Over eightfold excess risk was still noted 1–4 years after hospitalization which was mainly attributed to the highly significant excess risks in the second and third years of follow-up. The excess risks decreased somewhat, but were still significant, in the following years (SIR 5.4, 95% CI 2.0–11.7) for 5–9 years after hospitalization; SIR 2.6, 95% CI 1.0–5.7 for 10 or more years after hospitalization, respectively). The observed six cases of anal cancer after 10 years of follow-up were evenly distributed throughout the observation period (SIRs 2.2, 2.9, and 2.8 for 10–14, 15–19, and 20 or more years after hospitalization, respectively). After excluding the first three years of observation, SIR during years 3–37 was 3.3 (95% CI 1.8–5.7), based on 13 observed anal cancer cases. We observed a similarly significant excess risk for anal cancer in the first year of follow-up among hemorrhoid patients. However, relative risk dropped to approximately 3 in the second year of follow up, and was close to unity thereafter (SIR during years 3–37=1.3, 95% CI 0.7–2.1). Significant excess risks for CRCs (excluding anal cancer) were noted only in the first year of follow-up in both the inflammatory lesion and hemorrhoid cohorts. During years 3–37 there was an approximate 30% excess risk for lung cancer among patients with inflammatory lesions (SIR 1.3, 95%CI 1.1–1.4).

Table 8. Standardized incidence ratios (SIRs) with 95% confidence intervals (95%CI) for anal and colorectal cancer in patients hospitalized for benign inflammatory anal lesions and hemorrhoids, by duration of follow-up.

	INFLAMMATORY LESIONS[1]				HEMORRHOIDS			
Years	Anal cancer		Colorectal cancer		Anal cancer		Colorectal cancer	
	N	SIR (95%CI)	N	SIR (95%CI)	N	SIR (95%CI)	N	SIR (95%CI)
<1	6	24.0 (8.8-52.3)	71	3.9 (3.0-4.9)	11	14.9 (7.5-26.7)	200	3.6 (3.1-4.1)
1-4	8	8.3 (3.6-16.3)	88	1.3 (1.0-1.6)	2	0.7 (0.1-2.5)	228	1.1 (0.9-1.2)
1	4	16.3 (4.4-41.7)	21	1.2 (0.7-1.8)	2	2.8 (0.3-9.9)	68	1.3 (1.0-1.6)
2	3	12.3 (2.6-36.1)	22	1.3 (0.8-1.9)	0	-	55	1.0 (0.8-1.3)
3-4	1	2.1 (0.05-11.7)	45	1.4 (1.0-1.8)	0	-	105	1.0 (0.8-1.2)
5-9	6	5.4 (2.0-11.7)	97	1.3 (1.0-1.5)	6	1.8 (0.7-3.9)	243	1.0 (0.9-1.1)
10+	6	2.6 (1.0-5.7)	180	1.1 (0.9-1.3)	8	1.3 (0.6-2.5)	509	1.1 (1.0-1.1)
10-14	2	2.2 (0.3-7.9)	75	1.2 (0.9-1.5)	3	1.1 (0.2-3.3)	189	1.0 (0.8-1.1)
15-19	2	2.9 (0.4-10.6)	56	1.2 (0.9-1.5)	4	2.2 (0.6-5.6)	145	1.0 (0.9-1.2)
20+	2	2.8 (0.3-10.2)	49	0.9 (0.7-1.2)	1	0.6 (0.01-3.0)	175	1.2 (1.0-1.4)
3-37	13	3.3 (1.8-5.7)	322	1.2 (1.1-1.3)	14	1.3 (0.7-2.1)	857	1.0 (1.0-1.1)

[1]Anal fissures, perianal abscesses and fistulas. N= number of observed cancer cases.

Among patients with inflammatory lesions, stratified analyses (first three years excluded) showed no conspicuous variation in relative risks for anal or CRC by sex, age at index hospitalization, calendar period of index hospitalization, or number of hospitalizations for inflammatory anal lesions (table 9). Absence of any strong association with anal cancer or CRC was evident across different strata in patients with hemorrhoids (table 9).

Table 9. Standardized incidence ratios (SIRs) with 95% confidence intervals (95%CI) for anal and colorectal cancer in patients hospitalized for benign inflammatory anal lesions and hemorrhoids, by sex, age at entry, periods at entry, or number of hospitalizations[1].

	INFLAMMATORY LESIONS[2]				HEMORRHOIDS			
	Anal cancer		Colorectal cancer		Anal cancer		Colorectal cancer	
	N	SIR (95%CI)	N	SIR (95%CI)	N	SIR (95%CI)	N	SIR (95%CI)
Sex								
Men	4	2.2 (0.6-5.7)	223	1.2 (1.0-1.4)	6	1.5 (0.5-3.2)	489	1.0 (0.9-1.1)
Women	9	4.3 (2.0-8.2)	99	1.1 (0.9-1.4)	8	1.1 (0.5-2.3)	368	1.1 (1.0-1.2)
Age at entry								
<50 years	6	2.9 (1.1-6.3)	101	1.1 (0.9-1.3)	3	0.7 (0.2-2.1)	218	1.1 (0.9-1.2)
50+ years	7	3.8 (1.5-7.9)	221	1.2 (1.1-1.4)	11	1.6 (0.8-2.9)	639	1.0 (0.9-1.1)
Period at entry								
1965-1986	9	3.2 (1.5-6.1)	244	1.2 (1.0-1.3)	9	1.1 (0.5-2.1)	671	1.1 (1.0-1.2)
1987-2002	4	3.7 (1.0-9.4)	78	1.2 (0.9-1.4)	5	1.6 (0.5-3.8)	186	0.9 (0.8-1.1)
Number of hospitalizations								
1	10	3.3 (1.6-6.1)	241	1.1 (1.0-1.3)	14	1.4 (0.8-2.4)	774	1.0 (1.0-1.1)
2+	3	3.4 (0.7-9.9)	81	1.3 (1.1-1.6)	0	-	83	1.0 (0.8-1.2)

[1]Person-years and anal cancers occurring during the first three years of follow-up were excluded. [2]Anal fissures, perianal abscesses and fistulas. N= number of observed cancer cases.

In table 10 results for the three subtypes of inflammatory lesions are listed. After the first three years of follow-up, we observed five cases of anal cancer among fissure patients, corresponding to an approximate fourfold excess risk (95% CI 1.3–9.2). Excess risk was also noted for patients with perianal abscesses (n=6; SIR 5.3). It was less obvious for patients with anal fistulas (n=2; SIR 1.3). Stratified analyses by sex and follow-up duration showed similar results in most strata, except that for perianal abscess patients where excess risk was noted only among women (SIR 9.7 for women versus SIR 1.6 for men).

Table 10. Standardized incidence ratios (SIRs) with 95% confidence interval (95%CI) for anal cancer in patients with anal fissure, fistula, and perianal abscess [1].

	FISSURE		FISTULA		PERIANAL ABSCESS	
	N	**SIR (95%CI)**	**N**	**SIR (95%CI)**	**N**	**SIR (95%CI)**
Overall	5	3.9 (1.3-9.2)	2	1.3 (0.2-4.9)	6	5.3 (1.9-11.5)
Sex						
Men	2	4.7 (0.6-16.9)	1	1.3 (0.03-7.4)	1	1.6 (0.04-9.0)
Women	3	3.6 (0.7-10.4)	1	1.4 (0.04-7.6)	5	9.7 (3.1-22.6)
Follow-up duration[2]						
3-9	3	5.9 (1.2-17.3)	0	-	4	7.1 (1.9-18.3)
10+	2	2.6 (0.3-9.4)	2	2.1 (0.3-7.5)	2	3.5 (0.4-12.5)

[1]Person years and anal cancers occurring during the first three years of follow-up were excluded.
[2]Years. N= number of observed cancer cases.

For sensitivity analysis, 42,857 inflammatory anal lesion patients remained after excluding patients with a history of Crohn's disease, human immunodeficiency virus disease, CA, cervical cancer in situ, or organ transplantation. Overall SIR during years 3–37 was somewhat attenuated but still statistically significant (n=10; SIR 2.7, 95% CI 1.3–5.0), and the excess risk persisted after 10 years of follow up (n=5; SIR 2.3, 95% CI 0.7–5.3).

5.3 STUDY III

The cohort of 336,381 male workers was followed for up to 37 years (mean 24, quartile [Q] 1 19 years, median 25 years, and Q3 29 years) corresponding to 8,208,741 person-years under observation. The mean age at entry was 35 years (range 15-82 years, Q1 24 years, median 31 years, and Q3 44 years). Table 11 shows characteristics of the cohort members by age categories. Overall, 42% of the workers were ever smokers at time of entry, 12% were only snus users, 16% were combined smokers and snus users, and the rest never-users of tobacco. The prevalence of isolated snus use was higher among young workers at study inclusion.

A total of 2,552 cohort members were diagnosed with colon cancer during follow-up; 1,179 cancers were right-sided, 937 left-sided, and 436 had no registered sub-site (table 12). SIR for colon cancer in the total cohort, compared with the general Swedish male population of the same ages and during the same calendar periods, was 0.95 (95% CI 0.92-0.99). A small risk elevation by 8% for colon cancer was observed among pure smokers, just short of being statistically significant (table 12). The point estimate of the excess (8%) was of similar magnitude among snus users, but it was far from reaching statistical significance. Division of total colon cancer into right- and left-sided revealed a general tendency towards somewhat higher relative risks for the latter. However, although a 30% excess of left-sided colon cancer among combined users of smoking and smokeless tobacco attained statistical significance, the differences between right- and left-sided colon cancers were non-significant.

There were 1,863 cases of rectal cancer; SIR in the total cohort was 0.99 (95% CI 0.94-1.03). Among smokers the risk of rectal cancer was increased by a significant 16%, while the excess was only 5% (non-significant) among snus users. However, the difference between smokers and snus users was not statistically significant.

Among 53 cases with anal cancer, 30 cases were pure smokers with a statistically significantly elevated HR of 2.4. There were no indications of any association between isolated snus use and risk of anal cancer, but we only observed one exposed case.

Table 11. Characteristics of the Male Swedish Construction Workers Cohort

Age at entry (years)	Number of men	Year of entry (%)			No. of tobacco users (%)				Person-years of follow-up
		1971-75	1978-84	1985-93	Never users	Snus users[1]	Smokers[2]	Both[3]	
<30	155,082	39,956 (26)	57,523 (37)	57,603 (37)	55,134 (36)	30,448 (20)	45,677 (29)	23,823 (15)	3,865,880
30-34	41,364	16,838 (41)	15,993 (39)	8,533 (21)	10,659 (26)	3,339 (8)	19,070 (46)	8,296 (20)	1,107,251
35-39	32,682	13,524 (41)	12,487 (38)	6,671 (20)	8,530 (26)	1,862 (6)	16,556 (51)	5,734 (18)	856,630
40-44	26,797	12,336 (46)	8,839 (33)	5,622 (21)	7,178 (27)	1,195 (4)	14,193 (53)	4,231 (16)	679,857
45-49	23,162	12,508 (54)	6,730 (29)	3,924 (17)	6,116 (26)	892 (4)	12,774 (55)	3,380 (15)	561,634
50-54	22,112	13,650 (62)	5,991 (27)	2,471 (11)	5,579 (25)	909 (4)	12,289 (56)	3,335 (15)	494,739
55-59	19,650	12,489 (64)	5,513 (28)	1,648 (8)	4,840 (25)	1,154 (6)	10,789 (55)	2,867 (15)	385,006
60-64	13,544	9,368 (69)	3,372 (25)	804 (6)	3,401 (25)	983 (7)	7,234 (53)	1,926 (14)	228,539
≥65	1,988	1,779 (89)	159 (8)	50 (3)	522 (26)	150 (8)	1,056 (53)	260 (13)	29,206
Overall	336,381	132,448 (39)	116,607 (35)	87,326 (26)	101,959 (30)	40,932 (12)	139,638 (42)	53,852 (16)	8,208,741

[1]Pure snus users (never smokers). [2]Pure smokers (never snus users). [3]Combined smokers and snus users

Table 12. Hazard ratios of colorectal and anal cancer in different groups of tobacco users adjusted for age and BMI.

	All		Colon cancer		Right-sided colon cancer	
	N	**Person years**	**N**	**HR (95%CI)**	**N**	**HR (95%CI)**
Non-tobacco users	101,959	2,487,208	677	Ref.	324	Ref.
Pure smokers	139,638	3,443,126	1,282	1.08 (0.99-1.19)	602	1.07 (0.93-1.22)
Pure snus users	40,932	910,145	153	1.08 (0.91-1.29)	59	0.86 (0.65-1.13)
Both smokers and snus users	53,852	1,368,261	440	1.17 (1.04-1.32)	194	1.09 (0.91-1.30)
All	336,381	8,208,741	2,552		1,179	
	Left-sided colon cancer		**Rectal cancer**		**Anal cancer**	
	N	**HR (95%CI)**	**N**	**HR (95%CI)**	**N**	**HR (95%CI)**
Non-tobacco users	238	Ref.	467	Ref.	7	Ref.
Pure smokers	468	1.12 (0.95-1.30)	978	1.16 (1.04-1.30)	31	2.41 (1.06-5.48)
Pure snus users	60	1.28 (0.97-1.71)	99	1.05 (0.85-1.31)	1	0.61 (0.07-5.07)
Both smokers and snus users	171	1.30 (1.06-1.58)	319	1.21 (1.05-1.39)	14	3.48 (1.40-8.64)
All	937		1863		53	

[1]The numbers in the right and left colon column do not add up to the numbers in the colon column since all colon cancers were not defined as right or left.

Table 13 elaborates on the associations by types of smoking tobacco and duration of cigarette smoking. Overall, there was a tendency for dose-response in the association with colon cancer, seemingly more evident for right-sided than for left-sided colon cancer. Even though significantly increased risks for rectal cancer were seen among cigarette and cigar smokers, this dose-response was less evident. The greatest risk increase for rectal cancer (71%) was seen among cigar users, based on 31 exposed cases. Smokers of cigarettes and pipes had substantially increased point estimates of relative risk for anal cancer, although based on few cases, and statistically non-significant for pipe smokers.

Table 13. Risk of colorectal and anal cancer in pure smokers in the Swedish Construction Workers Cohort, adjusted for age and BMI.

	All	Colon		Right colon		Left colon		Rectal cancer		Anal cancer	
		N	**HR (95%CI)**	**N**	**HR (95%CI)**	**N**	**HR (95%CI)**	**N**	**HR (95%CI)**	**N**	**HR (95%CI)**
Non-tobacco users	101,959	677	Ref.	324	Ref.	238	Ref.	467	Ref.	7	Ref.
Amount of tobacco											
≤15g/day	86,749	777	1.04 (0.94-1.16)	359	1.01 (0.87-1.17)	280	1.05 (0.89-1.25)	610	1.16 (1.03-1.31)	18	2.26 (0.94-5.42)
>15g/day	47,135	452	1.17 (1.04-1.32)	219	1.20 (1.01-1.43)	165	1.19 (0.98-1.46)	335	1.20 (1.05-1.38)	12	2.89 (1.14-7.36)
Missing	5,754	53		24		23		33		1	
Mixed smoking[1]	20,061	440	1.04 (0.89-1.21)	194	1.04 (0.89-1.21)	171	1.07 (0.84-1.36)	319	1.15 (0.96-1.37)	14	2.15 (0.58-8.00)
Cigarette[2]	98,183	690	1.10 (0.99-1.22)	331	1.12 (0.96-1.31)	238	1.08 (0.90-1.29)	539	1.18 (1.04-1.34)	18	2.57 (1.07-6.17)
1-4 cigs/day	24,171	101	0.98 (0.79-1.21)	42	0.87 (0.63-1.20)	34	0.96 (0.67-1.37)	90	1.22 (0.97-1.52)	2	1.79 (0.37-8.63)
5-14 cigs/day	28,045	226	1.00 (0.86-1.16)	117	1.08 (0.87-1.34)	66	0.83 (0.63-1.09)	184	1.12 (0.94-1.33)	5	1.94 (0.61-6.12)
15-24 cigs/day	36,910	293	1.22 (1.06-1.40)	139	1.23 (1.01-1.51)	116	1.37 (1.10-1.71)	216	1.22 (1.03-1.43)	8	2.95 (1.06-8.20)
≥25 cigs/day	6,138	58	1.22 (0.94-1.60)	28	1.28 (0.87-1.88)	17	0.99 (0.61-1.62)	41	1.21 (0.88-1.66)	3	6.35 (1.63-24.71)
Missing	2919	12		5		5		8		0	
Cigar[2]	1,938	24	0.89 (0.60-1.35)	12	0.94 (0.53-1.68)	10	1.01 (0.54-1.90)	31	1.71 (1.19-2.46)	0	-
1-7cigars/day	1,008	13	0.80 (0.46-1.38)	7	0.89 (0.42-1.88)	6	1.00 (0.44-2.25)	17	1.58 (0.97-2.56)	0	-
≥8 cigars/day	880	11	1.09 (0.60-1.97)	5	1.06 (0.44-2.56)	4	1.06 (0.39-2.85)	14	1.97 (1.15-3.35)	0	-
Missing	50	0		0		0		0		0	
Pipe[2]	16,988	258	1.07 (0.92-1.24)	106	0.91 (0.73-1.14)	112	1.27 (1.01-1.59)	188	1.14 (0.96-1.35)	7	2.95 (0.99-8.80)
1-29 g/week	3,851	40	0.76 (0.55-1.04)	14	0.55 (0.32-0.94)	23	1.20 (0.78-1.84)	35	0.98 (0.69-1.38)	2	3.95 (0.80-19.56)
30-99 g/week	12,134	206	1.16 (0.99-1.36)	86	1.01 (0.79-1.28)	85	1.31 (1.02-1.69)	145	1.20 (0.99-1.44)	5	2.86 (0.87-9.41)
≥100 g/week	990	10	0.87 (0.46-1.62)	5	0.91 (0.38-2.20)	3	0.71 (0.23-2.22)	8	1.00 (0.50-2.01)	0	-
Missing	13	2		1		1		0		0	
Smoking duration[3,4]	97,060	684		331		232		532		18	
1 to 14 years	49,527	166	1.06 (0.89-1.26)	75	1.01 (0.78-1.31)	55	1.10 (0.81-1.48)	149	1.30 (1.07-1.57)	5	2.60 (0.79-8.52)
15 to 24 years	27,562	210	1.12 (0.96-1.32)	103	1.19 (0.95-1.49)	68	1.03 (0.78-1.35)	153	1.08 (0.90-1.30)	5	2.29 (0.72-7.30)
≥25 years	19,971	308	1.12 (0.98-1.28)	153	1.16 (0.96-1.41)	109	1.07 (0.85-1.35)	230	1.18 (1.01-1.39)	8	2.84 (1.02-7.94)
Missing	1,123	6		0		6		7		0	
Estimated duration[3,5]	97,060	684		331		232		532		18	
1 to 29 years	17,086	114	0.98 (0.79-1.22)	49	0.85 (0.62-1.18)	39	1.17 (0.82-1.67)	89	1.15 (0.90-1.46)	2	1.33 (0.23-7.70)
30 to 39 years	31,067	162	1.02 (0.85-1.22)	80	1.13 (0.87-1.46)	53	0.94 (0.69-1.28)	147	1.17 (0.97-1.43)	5	2.98 (0.87-10.2)
40 to 49 years	32,050	217	1.14 (0.97-1.33)	101	1.19 (0.94-1.50)	73	1.01 (0.77-1.33)	181	1.23 (1.03-1.47)	9	4.58 (1.60-13.1)
≥50 years	16,857	191	1.22 (1.03-1.45)	101	1.27 (1.01-1.60)	67	1.18 (0.89-1.56)	115	1.14 (0.92-1.41)	2	1.26 (0.25-6.42)
Missing	1,123	6		0		6		7		0	

[1]Only pure smokers who combine the use of cigarettes, cigar and pipes. [2]Only pure smokers. [3]Cigarette smoking. [4]Smoking duration at inclusion. [5] Estimated duration = [Duration of tobacco use at inclusion]+[Time of follow-up]

Table 14 shows associations between colorectal/anal cancer risk and snus use by self-reported duration at study entry and by estimated total duration. Although the highest relative risk estimates were seen for left-sided colon cancers, only one of the estimates attained statistical significance, and no clear dose-response patterns emerged.

Table 14. Risk of colorectal and anal cancer in pure snus users in the Swedish Construction Workers Cohort, adjusted for age.

	ALL	Colon		Right colon		Left colon		Rectum		Anus	
		N	HR (95%CI)	N	HR (95%CI)	N	HR (95%CI)	N	HR (95%CI)	N	HR (95%CI)
Non-tobacco users	101,959	677	Ref.					467	Ref.	7	Ref.
Duration of snus[1]	40,600	153		59		60		97		1	
-1-10 years	26,124	39	1.33 (0.94-1.88)	16	1.09 (0.64-1.86)	11	1.55 (0.83-2.90)	15	0.71 (0.42-1.20)	0	-
-10-25 years	11,407	43	1.02 (0.74-1.38)	17	0.84 (0.51-1.37)	19	1.35 (0.85-2.17)	33	1.07 (0.75-1.53)	0	-
>25years	3,069	71	1.06 (0.83-1.36)	26	0.80 (0.53-1.19)	30	1.21 (0.82-1.78)	49	1.18 (0.88-1.60)	1	2.05 (0.23-18.1)
Missing	332	0		0		0		2		0	
Estimated duration of snus use [2]	40,600	153		59		60		97		1	
-0 to 20 years	10,555	27	1.15 (0.75-1.76)	13	1.07 (0.58-1.98)	5	1.09 (0.43-2.76)	12	0.81 (0.44-1.50)	0	-
-20 to 30 years	17,932	27	0.97 (0.65-1.43)	12	0.92 (0.51-1.66)	11	1.19 (0.64-2.21)	17	0.78 (0.47-1.27)	0	-
-30 to 40 years	8,388	33	1.01 (0.71-1.44)	9	0.63 (0.32-1.22)	20	1.66 (1.05-2.63)	27	1.10 (0.74-1.63)	0	-
> 40 years	3725	66	1.16 (0.89-1.50)	25	0.87 (0.57-1.31)	24	1.19 (0.77-1.82)	41	1.27 (0.92-1.77)	1	2.88 (0.31-26.9)
Missing	332	0		0		0		2		0	

[1] Duration at inclusion
[2] Estimated duration = [Duration of tobacco use at inclusion]+[Time of follow-up]

5.3.1 Sensitivity analysis

In the sensitivity analysis that only considered exposure data collected at the first visit after 1977, 20,740 workers were excluded (14,982 women, 334 with incorrect NRN, 4,032 with cancer/death/emigration before entry and 1,392 with missing information on BMI), leaving 279,897 for the final analysis. The relative risk of colon cancer was 1.08 (95% CI 0.96-1.21) among smokers, and 0.97 (95% CI 0.76-1.24) among snus users. The corresponding estimates for rectal cancer were 1.15 (95% CI 1.01-1.32) and 1.13 (95% CI 0.87-1.49), respectively. The relative risk of anal cancer among smokers was 2.4 (95% CI 0.97-5.82). The estimates in sub-analyses were similar to those presented for the entire cohort (data not shown).

To get an approximation of possible misclassification of tobacco use, we compared exposure data in workers who had registered visits both before and after 1977. Among 39,234 workers who were classified as never smokers at their first visit in 1971-75, 1,447 (3.7%) were coded as smokers at their first visit after 1977. Among 101,215 initially categorized as never-users of snus 4,224 (4.2%) were subsequently classified as snus users. With a mean interval of 7.5 years, 5,312 (10.7%) out of 49,817 workers reporting current smoking at their first visit in 1971-75 were classified as ex- smokers at the first visit after 1977.

5.4 STUDY IV

Table 15 presents some background characteristics of incident 40,230 cancer cases. There were 4,393 colorectal cancer cases, 12,669 cases with cancers which were a priori considered smoking related, and 17,269 cases with other cancers. There were no

obvious differences in the distribution of BMI or age at diagnosis between different groups. The mean age at diagnosis varied between 66 and 67 years.

Table 15. Characteristics of the total cohort of incident cancer cases identified among the construction workers (all cancer cases), broken down into three mutually exclusive sub-cohorts (colorectal cancer cases, smoking-related cancer cases, and other cancer cases).

	ALL CANCER CASES			COLORECTAL CANCER CASES		
	Number (%)	**All deaths**	**Cancer specific death[1]**	**Number**	**All deaths**	**Cancer specific death**
All	40,230	24,826	14,533	4,393	2,807	1,731
Age at diagnosis						
<50 years	3,815 (9.5)	1,579 (6.4)	1,369 (9.4)	299 (6.8)	141 (5.0)	125 (7.2)
50-59 years	6,969 (17.3)	3,762(15.2)	2,665 (18.3)	717 (16.3)	403 (14.4)	302 (17.5)
60-69 years	13,453 (33.4)	8,126 (32.7)	4,976 (34.2)	1,487 (33.8)	922 (32.8)	602 (30.7)
70-79 years	12,006 (29.8)	8,397 (33.8)	4,336 (29.8)	1,391 (31.7)	971 (34.6)	536 (30.7)
≥80 years	3,987 (9.9)	2,962 (11.9)	1,187 (8.2)	499 (11.4)	370 (13.2)	170 (9.8)
Mean age at diagnosis[5]	66 (19-100)			67 (22-96)		
Mean BMI[5]	25.1 (16-46)			25.5 (17-43)		
Period of diagnosis						
1971-1984	5,200 (12.9)	4,669 (18.8)	2,804 (19.3)	597 (13.6)	533 (19.0)	328 (19.0)
1985-1994	11,004 (27.4)	9,054 (36.5)	5,426 (37.3)	1,197 (27.2)	976 (34.8)	621 (35.9)
1995-2007	24,026 (59.7)	11,103 (44.7)	6,303 (43.3)	2,600 (59.2)	1,298 (46.2)	782 (45.2)
	SMOKING-RELATED CANCER CASES[2]			**OTHER CANCER CASES[3]**		
	Number	**All deaths**	**Cancer specific death**	**Number**	**All deaths**	**Cancer specific death[4]**
All	12,669	9,812	6,511	17,269	8,022	3,605
Age at diagnosis						
<50 years	984 (7.8)	583 (5.9)	434 (6.7)	1,430 (8.3)	282 (3.5)	170 (4.7)
50-59 years	2,627 (20.7)	1,859 (18.9)	1,299 (20.0)	2,352 (13.6)	688 (8.6)	411 (11.4)
60-69 years	4,429 (35.0)	3,503 (35.7)	2,334 (35.9)	5,787 (33.5)	2,381 (29.7)	1,216 (33.7)
70-79 years	3,615 (28.5)	3,007 (30.6)	1,932 (29.7)	5,624 (32.6)	3,289 (41.0)	1,398 (38.8)
≥80 years	1,014 (8.0)	860 (8.8)	512 (7.9)	2,076 (12.0)	1,382 (17.2)	410 (11.4)
Mean age at diagnosis[5]	66 (20-95)			67 (20-100)		
Mean BMI[5]	24.9 (17-45)			25.1 (16-46)		
Period of diagnosis						
1971-1984	2,157 (17.0)	2,016 (20.5)	1,247 (19.2)	1,533 (8.9)	1,293 (16.1)	631 (17.5)
1985-1994	3,870 (30.5)	3,354 (34.2)	2,291 (35.2)	4,071 (23.6)	3,173 (39.6)	1,502 (41.7)
1995-2007	6,642 (52.4)	4,442 (45.3)	2,973 (45.7)	11,665 (67.5)	3,556 (44.3)	1,472 (40.8)

[1]1,400 unspecified cancer cases were excluded from the cancer-specific analysis. [2]For definition, see text. [3]Other cancers where smoking is not an established risk factor, for definition see text. [4]65 unspecified cancer cases were excluded from the cancer-specific analysis. [5]Range is presented within parentheses.

The cumulative relative survival is presented for all cancer cases (figure 9), colorectal cancer cases (figure 10), smoking-related cancer cases (figure 11), and other cancer cases (figure 12) stratified on tobacco use.

The cumulative relative survival ratios among all cancer cases, by tobacco habit, are presented in figure 9. The curves diverged already within the first year after cancer diagnosis, probably partly due to differential risks of treatment-related deaths. The gap between them widened only marginally in the following years. Never-users of any tobacco fared best (cumulative relative survival ratio at 1, 5, 10 and 15 years were 0.86 [95% CI 0.85-0.87], 0.69 [0.68-0.70], 0.61 [0.59-0.63], and 0.56 [0.54-0.59], respectively) and pure smokers worst (corresponding ratios were 0.74 [0.73-0.74], 0.53 [0.52-0.54], 0.44 [0.43-0.45], and 0.38 [0.37-0.39]). With ratios of 0.82 [0.80-0.84], 0.64 [0.61-0.67], 0.55 [0.51-0.58], and 0.48 [0.43-0.53] for the same time points, the curve for pure snus users was below that for never-users of any tobacco, but closer to the latter than to that for pure smokers.

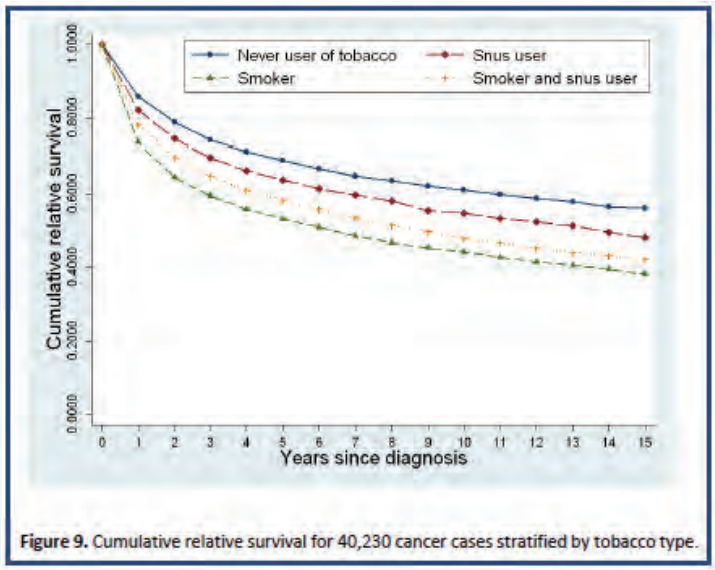

Figure 9. Cumulative relative survival for 40,230 cancer cases stratified by tobacco type.

In the CRC sub-cohort (figure 10), the curves did not separate significantly until after 3 years, but then a continuously widening gap between the point estimates became evident. In figure 10, the relative order between tobacco user categories was the same

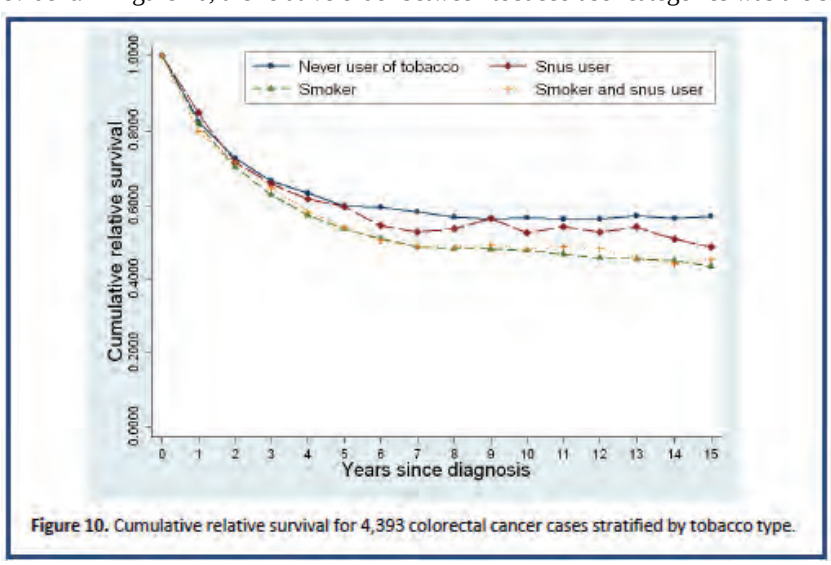

Figure 10. Cumulative relative survival for 4,393 colorectal cancer cases stratified by tobacco type.

as for total cancer, with never-users of any tobacco showing best relative survival, pure snus users second best, combined users third best, and pure smokers worst. While the curve for never-users of any tobacco leveled off after approximately 8 years, indicating cure from the colorectal cancer, the cumulative relative survival ratio continued to slowly fall among pure smokers and combined users, reflecting the sustained excess of all-cause mortality among smokers. Cumulative relative survival ratios at 5, 10 and 15 years were 0.60 (95% CI 0.56-0.63), 0.57 (0.52-0.62), and 0.57 (0.50-0.64) among never-users of any tobacco, and 0.53 (0.51-0.56), 0.48 (0.44-0.51), and 0.43 (0.39-0.48)

among pure smokers. The curve for pure snus users was below that for never-users of any tobacco, but not sufficiently stable to determine whether cure had been attained.

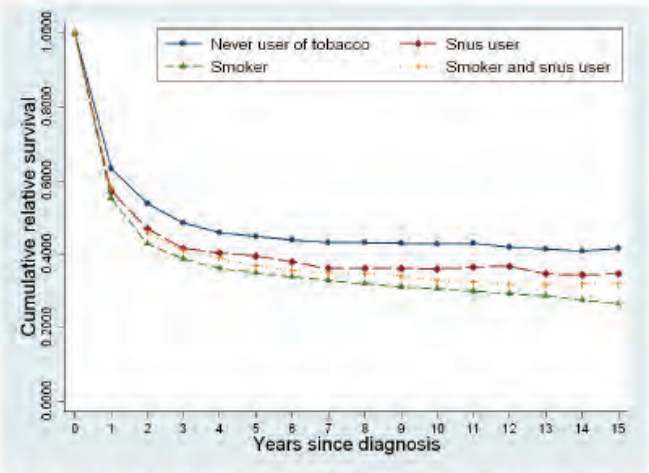

Figure 11. Cumulative relative survival among 12,669 smoking-related cancer cases stratified by tobacco type.

As shown in figure 11, cure of cancers considered to be smoking-related was evident after approximately 7 years among never-users of any tobacco. Albeit with a relative survival that was lower, cure seemed to be at hand also for pure snus users after about 7 years, but the curve was less stable towards the end. Cumulative relative survival ratios at 10 years were 0.43 (95% CI 0.39-0.46) among never-users of any tobacco, 0.36 (0.29-0.43) among pure snus users, and 0.31 (0.29-0.32) among pure smokers. For the latter, the ratio fell to 0.26 (0.25-0.28) at 15 years.

Figure 12 shows the cumulative relative survival ratios among workers with 'other cancers'. In this group the relative survival curves exhibited a continuous linear decline throughout the 15 years under observation. The gaps between the curves for never-users of any tobacco, snus users, and pure or combined smokers seemed to increase monotonically. Cumulative relative survival ratios at 5, 10 and 15 years were 0.85 (95% CI 0.83-0.87), 0.75 (0.72-0.77), and 0.66 (0.62-0.70) among never-users of any tobacco; 0.81 (0.77-0.85), 0.70 (0.63-0.76), and 0.59 (0.51-0.68) among pure snus users; and 0.78 (0.77-0.80), 0.63 (0.61-0.65), and 0.54 (0.51-0.57) among pure smokers.

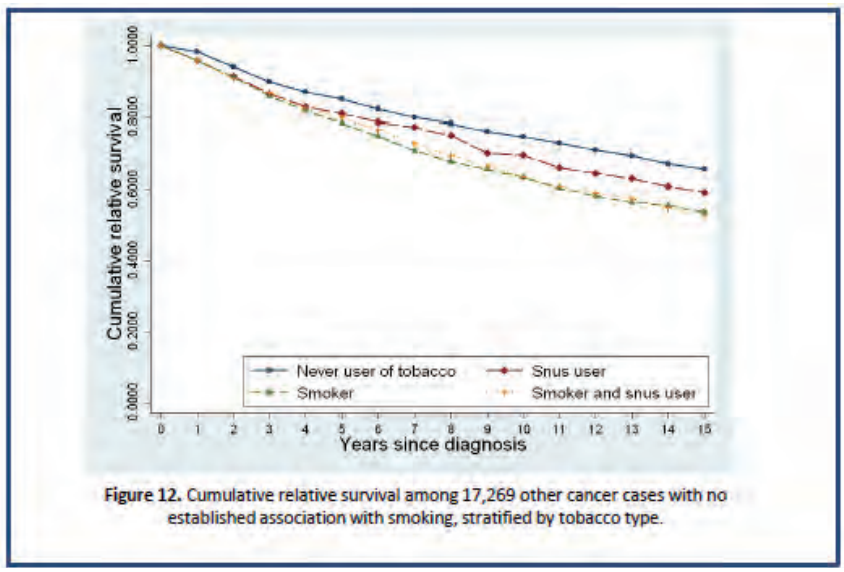

Figure 12. Cumulative relative survival among 17,269 other cancer cases with no established association with smoking, stratified by tobacco type.

Table 16 presents HRs for death from any cause and cancer-specific death by pattern of tobacco habits among workers with any cancer, and in sub-cohorts of workers with colorectal cancer, smoking-related cancer, and other cancer. Not surprisingly, smokers exhibited a significant excess risk of dying; the risk was 21% higher than among never-users of any tobacco. Interestingly, a statistically significant 13% excess was noted also among never-smoking snus users.

Table 16. Hazard ratios (HRs) of any death and cancer-specific death by pattern of tobacco use among all cancer cases, and after division of the cancer cases into three mutually exclusive sub-cohorts (colorectal cancer cases, smoking-related cancer cases, and other cancer cases). Adjustments in the multivariable Cox regression models were made for age at cancer diagnosis, period of diagnosis, and BMI.

	ALL CANCER CASES						COLORECTAL CANCER CASES					
	RELATIVE RISK (HR) OF DEATH[1]						RELATIVE RISK (HR) OF DEATH					
	OVERALL			CANCER-SPECIFIC[2]			OVERALL			CANCER-SPECIFIC		
	N	HR	95%CI	HR	N	95%CI	N	HR	95%CI	N	HR	95%CI
Never tobacco users	4,994	Ref		2,899	Ref		689	Ref		448	Ref	
Ever tobacco users	19,832	1.19	1.15-1.23	11,634	1.12	1.08-1.17	2,118	1.14	1.05-1.25	1,283	1.04	0.93-1.15
-only snus user	1,060	1.13	1.05-1.20	663	1.16	1.06-1.26	159	1.04	0.87-1.24	96	0.97	0.77-1.21
-only smoker	14,913	1.21	1.17-1.25	8,752	1.13	1.08-1.18	1,496	1.15	1.05-1.26	908	1.05	0.94-1.18
-combined user[5]	3,859	1.17	1.12-1.22	2,219	1.07	1.01-1.14	463	1.16	1.03-1.30	279	1.01	0.87-1.17
ALL	24,826			14,533			2,807			1,731		

	SMOKING-RELATED CANCER CASES						OTHER CANCER CASES[3]					
	RELATIVE RISK (HR) OF DEATH[1]						RELATIVE RISK (HR) OF DEATH[1]					
	OVERALL			CANCER-SPECIFIC			OVERALL			CANCER-SPECIFIC[4]		
	N	HR	95%CI	N	HR	95%CI	N	HR	95%CI	N	HR	95%CI
Never tobacco users	1,193	Ref		721	Ref		2,122	Ref		990	Ref	
Ever tobacco users	8,619	1.21	1.13-1.29	5,790	1.24	1.14-1.34	5,900	1.19	1.14-1.26	2,615	1.13	1.05-1.21
-only snus user	296	1.19	1.04-1.35	195	1.28	1.09-1.51	402	1.14	1.02-1.27	194	1.22	1.04-1.42
-only smoker	6,830	1.22	1.15-1.31	4,630	1.26	1.16-1.37	4,236	1.20	1.13-1.26	1,882	1.13	1.05-1.22
-combined user[5]	1,493	1.15	1.06-1.24	965	1.16	1.05-1.28	1,262	1.20	1.12-1.29	539	1.08	0.97-1.20
ALL	9,812			6,511			8,022			3,605		

[1]Further adjusted for cancer group to control for inherent differences in prognosis (please see text). [2]1,400 unspecified cancer cases were excluded from the cancer-specific analysis. [3]Other cancers where smoking is not an established risk factor, for definition see text. [4]65 unspecified cancer cases were excluded from the cancer-specific analysis. [5]Both snus user and smoker

Not only all-cause mortality, but also risk of cancer-specific death, was elevated, both among pure smokers and pure snus users, who showed statistically significant 13% and 16% excesses, respectively. The risk for cancer-specific death were substantially increased among tobacco users who were diagnosed with cancers that were considered

smoking-related – HR 1.26 (95% CI 1.16-1.37) among pure smokers and HR 1.28 (95% CI 1.09-1.51) among pure snus users – but also among those with cancers not perceived as smoking-related (HR 1.13 [95% CI 1.05-1.22] among pure smokers and HR 1.22 [95% CI 1.04-1.42] among pure snus users). On the other hand, among colorectal cancer patients, cancer-specific death did not appear to be importantly linked to tobacco use. Although with less precision due to fewer observed deaths, the HR point estimates for pure smokers and pure snus users were 1.05 (95% CI 0.94-1.18) and 0.97 (95% CI 0.77-1.21), respectively.

Table 17. Hazard ratios (HRs) of any death and cancer-specific death by pattern of tobacco use among cases with colon cancer and those with rectal cancer. Adjustments in the multivariable Cox regression models were made for age at cancer diagnosis, period of diagnosis and BMI.

	COLON CANCER CASES						RECTUM CANCER CASES					
	RELATIVE RISK (HR) OF DEATH						RELATIVE RISK (HR) OF DEATH					
	OVERALL			CANCER-SPECIFIC			OVERALL			CANCER-SPECIFIC		
	N	HR	95%CI	HR	N	95%CI	N	HR	95%CI	N	HR	95%CI
Non users of any tobacco	420	Ref		270	Ref		269	Ref		178	Ref	
Ever tobacco users	1,211	1.11	0.99-1.24	722	0.99	0.86-1.14	907	1.22	1.06-1.40	561	1.13	0.95-1.34
-pure snus users	99	1.13	0.90-1.41	64	1.09	0.83-1.43	60	0.93	0.70-1.23	32	0.80	0.55-1.17
-pure smokers	841	1.10	0.98-1.24	493	0.97	0.84-1.13	655	1.25	1.08-1.45	415	1.19	1.00-1.43
-combined users[1]	271	1.13	0.97-1.31	165	0.99	0.82-1.21	192	1.22	1.01-1.47	114	1.04	0.82-1.32
ALL	1,631			992			1,176			739		

[1]Both snus user and smoker

We further divided the CRC sub-cohort into cases with colon cancer (2,536) and those with rectal cancer (1,857) (table 17). HRs for cancer-specific death among tobacco users with colon cancer was close to unity (95% CI 0.86-1.14), and judged by the point estimates there were no indications of important excesses in any of the categories of tobacco use. HR for cancer-specific death was 1.13 (95% CI 0.95-1.34) among tobacco users with rectal cancer. While the point estimate was 0.80 (95% CI 0.55-1.17) among pure snus users, it was borderline significant 1.19 (95% CI 1.00-1.43) among pure smokers.

TNM stage data from 2004-07 in the 'all cancer' cohort revealed no important differences between never-users of any tobacco and pure snus users, whereas the T-stage distribution among smokers was shifted towards more advanced stages (table 18). Similarly, while the N- and M-stage distributions did not differ much between never-users of any tobacco and pure snus users, there was a tendency for more advanced stages among smokers. However, for N and M stage, about 50% of the data points were missing. Among CRC cases, there were no great differences between UICC stage group distributions for non-users of any tobacco and smokers (data not shown), whereas the number of snus users with such data (27) was too small for meaningful comparisons.

Table 18. TNM classification for all cancers diagnosed in 2004-2007.

	Non users of any tobacco	Pure snus users	Pure smokers	Combined user
T0	4 (0.2%)	2 (0.6%)	13 (0.4%)	2 (0.2%)
T1	749 (40.9%)	154 (43.5%)	1,092 (31.2%)	407 (35.8%)
T2	502 (27.4%)	82 (23.2%)	890 (25.7%)	306 (26.9%)
T3	336 (18.4%)	64 (18.1%)	769 (22.2%)	246 (21.6%)
T4	132 (7.2%)	32 (9.0%)	423 (12.2%)	108 (9.5%)
Ta	32 (1.8%)	9 (2.5%)	127 (3.4%)	32 (2.8%)
Tis	11 (0.6%)	1 (0.3%)	14 (0.4%)	2 (0.2%)
Tx	64 (3.5%)	10 (2.8%)	129 (3.7%)	34 (3.0%)
∑	1,830	354	3,457	1,137
Missing	561	118	967	363
N+	3 (0.2%)	1 (0.3%)	6 (0.2%)	2 (0.2%)
N0	470 (25.8%)	91 (25.9%)	975 (28.4%)	322 (28.5%)
N1	92 (5.1%)	20 (5.7%)	252 (7.3%)	86 (7.6%)
N2	70 (3.8%)	18 (5.1%)	244 (7.1%)	60 (5.3%)
N3	8 (0.4%)	5 (1.4%)	122 (3.6%)	23 (2.0%)
Nx	1,178 (64.7%)	217 (61.7%)	1,832 (53.4%)	637 (56.4%)
∑	1,821	352	3,431	1,130
Missing	570	120	993	370
M0	637 (36.1%)	132 (38.5%)	1,328 (39.5%)	447 (40.8%)
M1	151 (8.5%)	42 (12.2%)	580 (17.3%)	151 (13.8%)
Mx	979 (55.4%)	169 (49.3%)	1,452 (43.2%)	499 (45.5%)
∑	1,767	343	3,360	1,097
Missing	624	129	1,064	403

Using Cox regression models for all-cause and cancer-specific deaths, and stratification for co-morbidity status, we examined the importance of recorded comorbidity among 27,253 cancer cases (any site); (i) 4,374 with no recorded co-morbidity (ii) 5,941 with any of chronic pulmonary disease, myocardial infarction or cerebrovascular disease, and (iii) 16,938 with other co-morbidity (table 19). There were no important differences between the groups. In particular there were generally small differences in point estimates of HRs for cancer-specific death.

5.4.1 Sensitivity analysis

In the sensitivity analysis restricted to construction workers with at least one health check-up after January 1, 1978 and using only exposure information collected at the first visit after this date, we included 27,403 cancer cases. The numbers of deaths of any cause and cancer-specific deaths were 13,632 and 9,148, respectively. The HRs were similar to those in the original cohort; for example, in the 'all cancers' cohort, HR among ever tobacco users, relative to never-users, was 1.18 (95% CI 1.13-1.23) for death of any cause and 1.08 (95% CI 1.02-1.13) for cancer-specific death. The HR for cancer-specific death among pure snus users (HR 1.06; 95% CI 0.95-1.19) and pure smokers (HR 1.09; 95% CI 0.98-1.13) were essentially the same.

Table 19. Hazard ratios (HRs) of any death and cancer-specific death by pattern of tobacco use among all cancer cases stratified into three groups depending on the presence or absence of recorded comorbidity. Analyses were adjusted for age at cancer diagnosis, period of diagnosis, BMI, and cancer group.

	NO COMORBIDITY						
		RELATIVE RISK (HR) OF DEATH					
		OVERALL			CANCER-SPECIFIC[1]		
	All cancer cases	N	HR	95%CI	N	HR	95%CI
Non users of any tobacco	1,254	547	Ref		401	Ref	
Ever user of any tobacco	3,120	1,704	1.22	1.10-1.36	1,184	1.13	1.00-1.28
-only snus user	222	93	1.10	0.86-1.40	70	1.09	0.83-1.43
-only smoker	2,266	1,298	1.24	1.11-1.38	883	1.12	0.99-1.28
-combined user[2]	632	313	1.20	1.04-1.40	231	1.17	0.98-1.39
	CHRONIC PULMONARY/CEREBROVASCULAR DISEASE/MYOCARDIAL INFARCTION						
		RELATIVE RISK (HR) OF DEATH					
		OVERALL			CANCER-SPECIFIC[2]		
	All cancer cases	N	HR	95%CI	N	HR	95%CI
Non users of any tobacco	1,120	722	Ref		328	Ref	
Ever user of any tobacco	4,821	3,568	1.13	1.04-1.23	1,856	1.10	0.97-1.25
-only snus user	227	153	1.08	0.89-1.29	81	1.19	0.92-1.55
-only smoker	3,636	2,727	1.16	1.06-1.26	1,427	1.12	0.99-1.28
-combined user[2]	958	688	1.07	0.96-1.20	348	1.01	0.86-1.18
	OTHER COMORBIDITY						
		RELATIVE RISK (HR) OF DEATH					
		OVERALL			OVERALL[3]		
	All cancer cases	N	HR	95%CI	N	HR	95%CI
Non users of any tobacco	4,152	2,216	Ref		1,393	Ref	
Ever user of any tobacco	12,786	8,261	1.17	1.12-1.23	5,237	1.09	1.03-1.16
-only snus user	828	459	1.15	1.04-1.27	300	1.10	0.97-1.25
-only smoker	9,296	6,232	1.19	1.13-1.25	3,936	1.10	1.03-1.18
-combined user[2]	2,662	1,570	1.12	1.05-1.19	1,001	1.05	0.96-1.14

Unspecified cancer cases were excluded from the cancer-specific analysis, [1]105 cancer cases, [2]254 cancer cases, and [3]670 cancer cases.

6 METHODOLOGICAL DISCUSSION

The aim with this section is to describe common methodological issues in epidemiology. In section 7, a general discussion regarding the studies' results (I to IV) will follow.

6.1 VALIDITY IN ESTIMATION

Errors in estimation refer to systematic or random errors. Systematic errors are often called bias. The opposite of bias is validity. The opposite of random error is precision, and this will be conferred separately.

There are two types of validity, internal and external. The internal validity refers to whether the result is representative of the source population. In studies of causation, the internal validity is related to the accuracy of the estimate (excluding random error). The lack of internal validity could be due to selection bias, information bias, and confounding, and each topic will be discussed separately. Internal validity is a prerequisite for external validity.

External validity refers to the generalizability of the results to populations outside the source population. CA and benign anal lesions are usually treated at out-patient clinics. Study I and II are based on patients hospitalized for these diseases, and the results might not be generalizable to all patients with CA or benign anal lesions.

External validity is also an issue in study III and IV where the source population is based on male construction workers. Working people are generally healthier than the general population, known as the "healthy worker effect". If a working population is compared to the general population, the "healthy worker effect" might be a concern [254-255]. However, the estimates in our studies will not be affected since the comparison groups are also construction workers. However, the generalizability might be affected.

To approximate a possible healthy worker effect, we calculated the standardized incidence ratios for all cancers, SIR 1.00 (95% CI 0.99-1.01), for colon cancer, SIR 0.95 (95% CI 0.92-0.99), and for rectal cancer, SIR 0.99 (95% CI 0.4-1.03). The "healthy worker effect" does not seem to be of such magnitude that it affects generalizability. However, in study III and IV the results are not generalizable to women since they are not included in the studies.

6.2 BIAS

From an epidemiologist's point of view, bias is a systematic error that results in a false estimate of the association between exposure and outcome. There are different types of bias. Some of the most common ones will be discussed below.

6.2.1 Selection bias

Selection bias is a systematic error. It occurs when the association between exposure and disease differs for those who do, or do not, participate in the study. Selection bias

is most common in case-control studies. A selection bias is introduced if the selected cases or controls are not representative of the population generating the cases.

Selection bias may also occur in cohort studies, for example due to loss to follow-up. If the loss to follow-up is large-scaled or related to the exposure or outcome, a bias may be introduced. Due to the linkage to high quality nationwide registers, the follow-up in this thesis was virtually complete.

6.2.2 **Information bias**

Information bias is a systematic error. It occurs when the information collected about the study subjects is incorrect. Information bias may occur in both case-control and cohort studies. Different types of information bias are surveillance bias, recall bias, and interviewer bias. If the incorrect information is measured on the categorical scale, and the consequence is an erroneous categorization of the study subject, the information is misclassified. The misclassification is either differential, or non-differential. The misclassification is differential if it is dependent on the exposure or the outcome. The misclassification is non-differential if it is unrelated to the exposure or the outcome.

6.2.2.1 Differential misclassification

Differential misclassification can either exaggerate or underestimate the effect. One example of differential misclassification is recall bias. Recall bias may occur in retrospective studies when the exposure information is collected after the disease has occurred and relies on the study subject's memory. Recall bias might result in a differential misclassification since the memory of the exposure information may differ depending on the presence of disease. For example, if two smokers are asked about their smoking habits 20 years ago, a person with lung cancer may recall differently than a person without lung cancer. Recall bias is not an issue in our studies being either register-based, or based on exposure data collected before the outcome was known.

6.2.2.2 Non-differential misclassification

The bias generated by non-differential misclassification is more predictable than that produced by differential misclassification. Non-differential misclassification of a dichotomous variable bias the estimate toward the null. However, this may not be true for a variable with more than two levels, or if the misclassification depends on errors made in other variables [256].

In study III and IV, there were potential sources of non-differential misclassification in the exposure data (tobacco use). Firstly, the habits of tobacco use may have changed during follow-up. The group of never-users of tobacco may contain smokers if non-smokers started to smoke during follow-up. However, usually people start smoking at a young age, and smoking cessation is probably a bigger source of misclassification. We chose not to subdivide smokers into current or ex-smokers. Since smoking has become less common in Sweden during the last decades, a large portion of the smokers may be ex-smokers. This potential misclassification could lead to an overestimation of the exposure, and may explain our inability to confirm an association between smoking and CRC. However, we believe that the misclassification is non-differential since it is most

likely unrelated to outcome. Data from cohort members with more than one visit were used to approximate the misclassification. Among 49,817 pure smokers, 10.7% had quit smoking at the second visit. This data should be interpreted with caution since it is based on a subset of the cohort. It is possible that individuals who quit smoking paid more attention to their health, and were more likely to have a second visit. However, the dose-response tendency should not be attenuated since heavy smokers are less likely to quit [257-259]. In addition, anal cancer serves as a positive control. The increased risk of anal cancer associated with smoking contradicts a great underestimation of the result. In contrast to smoking, the use of snus often is sustained once the habit has been initiated [260]. The age of onset of snus use occurs during a longer period compared to smokers, and about one third of all smokers switch to a combination of cigarette and snus use [260]. Snus is used as a medium to quit smoking. Combined users are more likely to quit smoking than to quit using snus [260]. It is possible that some of the registered smokers in this cohort started to use snus during follow-up, which would affect the estimates for pure smokers, but not for pure snus users.

Secondly, the quality of the exposure data collected before 1977 was limited. To get an approximation of the possible non-differential misclassification, a comparison of data on tobacco use collected before and after 1977 was done for a subset of the cohort members with several visits. Of those registered as never-smokers before 1977, 3.7% were later registered as ever-smokers. Likewise, among never-users of snus before 1977, 4.2% were registered as users of snus after 1977. However, sensitivity analyses in both study III and IV showed similar results as the original analyses, and indicated that the result was robust.

6.2.3 **Surveillance bias**

Surveillance bias, also known as detection bias, occurs when the exposure leads to a closer surveillance. It may result in a greater probability of detection of outcomes among the exposed compared to the unexposed. Surveillance bias can either be viewed upon as information or selection bias. In a cohort study, surveillance bias may be regarded as information bias if exposed individuals undergo more frequent or more careful examinations than unexposed. In case-control studies, surveillance bias may be considered as selection bias if exposed cases are more likely to be identified/selected into the study. The former is the case in our studies.

In study I, for example, there was no significant excess risk of invasive cervical cancer, but a 2-fold excess risk of cervical carcinoma *in situ* was noted. Detection of carcinoma *in situ* depends strongly on the intensity of medical surveillance. The incidence of carcinoma *in situ* increased markedly after introduction of screening programs [261]. If women with CA undergo gynecological examination with a Pap smear more frequently than women in general a surveillance bias is introduced. Women who are not screened with Pap smears according to the recommendations have an increased risk of cervical cancer [262]. It is also conceivable that more liberal diagnostic criteria are applied among women with CA. Hence, both intensity of medical surveillance and variations in diagnostic criteria might account for the excess risk of cancer *in situ*. An increased detection and treatment of cancer *in situ* could explain the lack of association with

invasive cancer. To decrease possible surveillance bias, cases diagnosed during the first year were excluded.

Similarly, patients with benign anal lesions are more likely to undergo a rectal examination than the general population. Elevated rates of anal cancer were expected during the first years of follow-up. To diminish possible surveillance bias (and misclassification), cases diagnosed during the first three years of follow-up were excluded or reported separately.

Since the autopsy rate varies in different counties, and may vary in the general population by exposure status, all incident cancers diagnosed at autopsy were excluded in the thesis.

6.2.4 **Lead-time bias**

Lead-time bias may be of importance in survival studies where follow-up starts at diagnosis. Screening introduces lead-time bias. The time elapsed from the earlier detection of the cancer (due to screening), to the usual clinical detection is known as the *lead time* (figure 9). The consequence of screening is the appearance of an improved survival, even if the mortality is unchanged. This is known as lead-time bias.

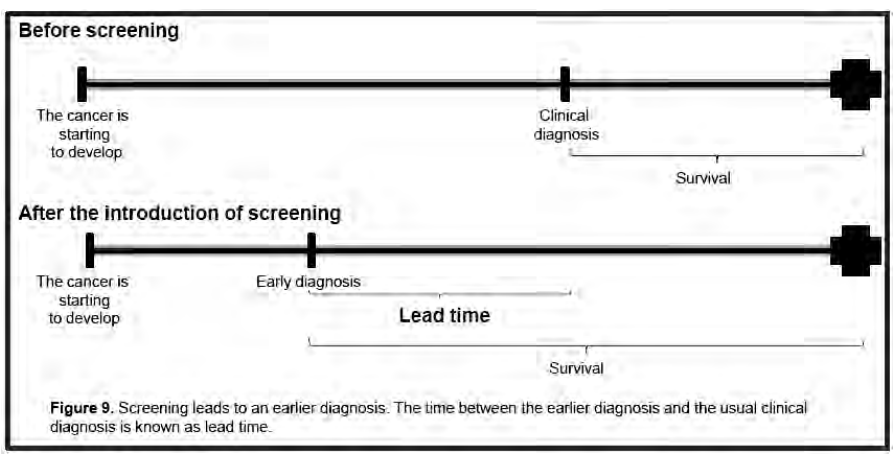

Figure 9. Screening leads to an earlier diagnosis. The time between the earlier diagnosis and the usual clinical diagnosis is known as lead time.

During follow-up in study IV, screening was introduced for several cancers, and diagnostic methods improved. So far, a screening program for CRC is under evaluation. Since screening methods, like colonoscopy and fecal occult blood testing, have been more frequently used, different time periods may not be comparable. It is also possible that the frequency of screening varies in different groups of tobacco users. Non-smokers are generally more health conscious, and probably more likely to participate in screening programs.

To reduce potential influence of bias and a possible confounding effect of time, we stratified for study period in the regression model (1971-84; 1985-94; 1995-2007). It would be impossible to take every type of cancer into account. Since one of the main interests in the paper was CRC, we chose to base our categorization on the

development of diagnostic methods and treatment of CRC. In 1985, CT scanning was available in most hospitals. In addition, the surgical technique of TME was introduced in Sweden in the 80's together with preoperative radiotherapy for rectal cancer treatment [224]. In 1995, TME was an established technique throughout Sweden [224]. Unfortunately, no further adjustments were possible.

6.3 CONFOUNDING

Confounding is a systematic error mainly seen in observational research. A confounder is both related to the exposure and the outcome (figure 10). It should not be an intermediate (represent a step) in the causal pathway. A predictor of disease is a possible confounder, but it is only a confounder when the presence of the predictor varies in different groups of exposure.

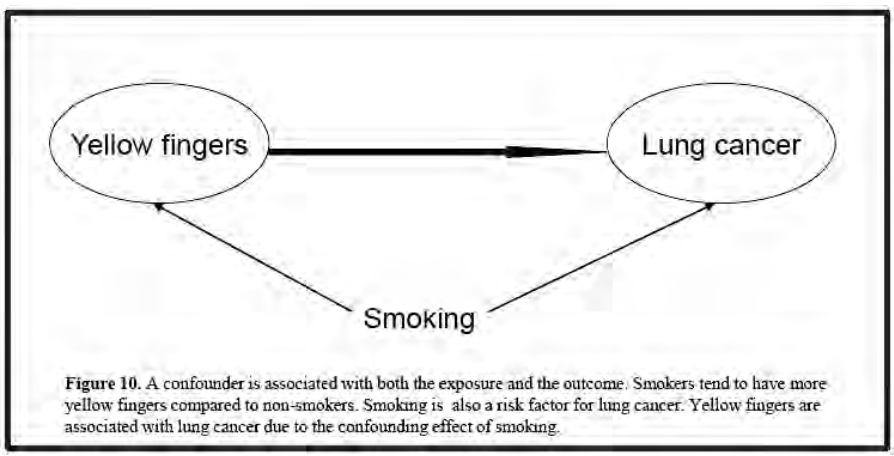

Figure 10. A confounder is associated with both the exposure and the outcome. Smokers tend to have more yellow fingers compared to non-smokers. Smoking is also a risk factor for lung cancer. Yellow fingers are associated with lung cancer due to the confounding effect of smoking.

In study I, for example sexual habits and possibly smoking are associated with CA, as well as anogenital cancers [26, 43-45]. We saw an overall increased risk for smoking-related cancers indicating an overrepresentation of smokers. Both sexual habits and smoking are potential confounders that we were unable to control for.

In study II, we estimated the confounding effect of smoking by investigating the incidence of lung cancer in the cohort. We found an increased incidence of 30%, and concluded that smoking cannot explain the observed threefold excess risk for anal cancer. Other potential confounders were organ transplantation, Crohn's disease, HPV and HIV infection. However, a sensitivity analysis, where subjects with condylomata acuminata, cervix cancer *in situ*, HIV, or organ transplantation were excluded, revealed similar results as the original analyses.

Restriction, randomization, and matching are the three most common ways to control for confounding. Restriction means that all study subjects have the same value for a variable that is a possible confounder. Restriction is used in study III and IV. We restricted the smoking analyses to never-snus users, and the snus analyses to never smokers. Only men were included in the analyses, and a confounding effect of gender

unlikely. It is also probable that the variation in lifestyle factors in the Construction Workers Cohort is less than in the general population.

In study III, BMI could be considered as a confounder. Smokers have a lower BMI [263], and a high BMI is positively associated with CRC [189]. We adjusted for BMI in the regression model. Another potential confounder in study III is physical activity. Physical activity reduces the risk of CRC [137, 194], and smokers perform less physical activity [263]. In addition, there seems to be an association between physical activity and CRC survival, which is of interest in study IV. It was recently shown that physical activity performed after the cancer was diagnosed was associated with a better CRC-specific survival [235]. There was no correlation between the physical activity before and after cancer diagnosis [235]. Furthermore, there was no significant association between physical activity performed before the cancer was diagnosed and the prognosis [235].

Another potential confounder is alcohol. Smokers tend to have a higher consumption of alcohol [264-265], and similar results have been reported for young snus users [266]. Alcohol is positively associated with the risk of CRC [137, 171-172]. Low socio-economic status is also associated with higher cancer mortality [267], but the association has been shown to be independent of smoking habits [267].

Other potential confounders in study III and IV that may affect both CRC incidence and survival are the use of aspirin [195, 233], vitamin D status [179, 234], and diabetes [197, 268]. Smoking is positively associated with type 2 diabetes [269], probably due to an effect of nicotine [270]. An increased risk of diabetes for snus users has not been convincingly demonstrated [271-273]. Diabetes and other diseases associated with smoking could result in a less aggressive treatment and a higher treatment-associated mortality.

Randomization is only used in experimental trials. Matching means that you match two groups (exposed or unexposed) on a potential confounder, resulting in an equal distribution of the confounder. Matching deals with confounding in cohort studies, but is rarely used due to inefficiency. Matching in case-control studies might introduce confounding where there was none from the beginning. It might also introduce selection bias. However, matching may make the study more efficient, and the introduced selection bias can be controlled for in the analysis.

If it is not possible to control for a confounder in the study design, it can be done during data analysis by stratification and use of regression models. For examples, in study III we controlled for age and BMI at entry in the regression model. Similarly, in study IV we controlled for age at diagnosis, period of diagnosis, and cancer type. We also stratified for comorbidity.

In paper IV, cancer stage is a possible confounder. We only had data on TNM for the last 3 years, and it was incomplete. For all cancers, it indicated a more advanced T-stage for smokers. This was not the case for snus users who had a similar TNM pattern as never-users of tobacco. For CRC, smokers had a similar stage pattern as never-users of tobacco. However, stage should only be treated as a confounder if smokers have a more advanced disease due to a delayed contact with health care. If an advanced

disease in smokers is due to a carcinogenic effect of tobacco, it is part of the causal pathway and should not be treated as a confounder.

Another possible confounder in study IV is comorbidity. Smoking is a risk factor for several diseases such as cardiovascular disease, diabetes and chronic pulmonary disease [269, 274-275]. The association with snus is less established [169, 271-273, 276-277]. To estimate the impact of comorbidity on cancer survival, we used data from the Inpatient Register. This method was imprecise since data from outpatient care was not available. In addition, the possible influence of residual confounding cannot be excluded. However, the results were surprisingly consistent and there were no large differences between the strata.

6.4 RANDOM ERROR

Random error is related to the precision of the estimation. It is the error that remains after the systematic errors have been addressed. Precision can be improved by increasing sample size. If the number of study participants is limited, various study designs can be used to increase efficiency and reduce random error.

Confidence intervals (CIs) or p-values are used to statistically describe precision. CIs are the inferred ranges around the point estimate. If the level of confidence is set to 95%, the point estimate would appear within the CI in 95% of the time. A wide CI suggests low precision, whereas a narrow CI suggests high precision. Point estimates with corresponding CIs that do not include 1.0 are usually referred to statistical significant.

P-values are used in a similar way as CIs. However, some find CIs superior to p-values since they describe the range. P-values are also used to test specific hypothesis, and are often used to confirm or refute statistical significance. The probability of rejecting a false test hypothesis is called the power of the study. An incorrect rejection of a hypothesis is called type I error. Type II error occurs when the test hypothesis is false, but not rejected. Type II errors arise when the effect is of small magnitude, the study is too small, or bias are present. In study III and IV, the inability to describe an evident association between snus and CRC risk and survival may be due to type II error.

This thesis is based on cohort studies. Even though the study bases are large, precision is still a problem when the outcome is rare. This is the case for several cancers in study I, and for anal cancer in study II. In study III and IV, the precision is low despite the common outcome, since the possible association is of a small magnitude.

7 GENERAL DISCUSSION

7.1 STUDY I

In this cohort, patients hospitalized for CA, we found a 50% increased risk of all cancers. When the cancers were subdivided, elevated risks were not only seen for anogenital cancer, but also for several other types of cancers. The elevated risks of anogenital cancers were expected, since anogenital cancers are associated with HPV [7-9, 42], and concomitant infections are common.

The increased risks of non-anogenital cancers are somewhat more puzzling. Head and neck, esophageal, stomach, lung, and bladder cancer are associated with smoking [247]. HPV infection is more common among current smokers [278]. Whether this is due to an increased acquisition or an increased risk of HPV infection is unknown. Smoking has been described as an immunosuppressant [279-280], and smoking has been shown to weaken the immune response against viral infections [281]. This could speak in favor of an increased susceptibility of HPV-infection in smokers.

Most HPV-infections are non-detectable after 2 years [20], but some develop a persistent infection. Patients with CA are usually treated at out-patient clinics, and not included in this study. It is likely that patients hospitalized for CA have a more severe disease than the rest of the patients with CA. In 1988, women aged over 40 years with recurrent CA were recommended to be investigated regarding the presence of immunosuppression [282].

The risk increase of cancer in our study is similar to the pattern of cancer risk in patients with immunosuppression. In a meta-analysis of cancer risks in HIV-infected and transplant patients, elevated risks were reported for cancer related to Epstein-Barr virus (EBV), Hepatitis B and C (HBV/HCV), *Helicobacter pylori*, and HPV [21]. Increased risks were also reported for some other malignancies such as kidney cancer and bladder cancer, the latter only in transplant patients. Since lifestyle-related factors probably are different between HIV-infected and transplant patients, the aim with the meta-analysis was to understand which cancers were 'truly' associated with immune deficiency. The final conclusion was that the pattern of cancer risk was similar in both groups. Mostly, the elevated risks were observed for cancers with a known, or assumed, infectious cause.

Table 20 shows the estimates for cancer risk in patients hospitalized with CA (data from our study), together with estimates for cancer risk in patients with HIV and transplant patients (data from a meta-analysis [21]). The resemblance of the patterns of cancer risk in patients hospitalized for CA and in patients with immunosuppression supports the theory that patients with persistent/severe CA have, at least partly, a weakened immune system. If this theory is true, it could be important for clinicians to be aware of. It is possible that these patients (as most patients) should be supported to quit smoking. Smoking is associated with several cancers and other smoking-related diseases. As previously mentioned, smoking might also weaken the immune response against viral infections [281], and if so, smoking cessation could affect the CA prognosis positively. This is an interesting field, and further studies are needed for clarification.

Table 20. SIR of different types of cancers in patients hospitalized for condylomata acuminata (our results) as well as in HIV and transplant patients (data from a meta-analysis [21]).

	CONDYLOMATA ACUMINATA SIR (95%CI)	HIV/AIDS SIR (95%CI)	TRANSPLANT SIR (95%CI)
CANCERS			
EBV-related			
Hodgkin´s lymphoma	3.1 (1.4-6.0)	11.0 (8.4-14.4)	3.9 (2.4-6.3)
Non-Hodgkin's lymphoma	2.6 (1.6-3.9)	76.7 (39.4-149)	8.1 (6.4-10.2)
HBV/HCV related			
Liver	1.6 (0.7-3.2)	5.2 (3.3-8.2)	2.1 (1.2-3.9)
***Helicobacter pylori* related**			
Stomach cancer	1.6 (0.8-2.9)	1.9 (1.5-2.4)	2.0 (1.5-2.8)
HPV-related			
Cervix uteri	1.3 (0.8-2.0)	5.8 (3.0-11.3)	2.1 (1.4-3.3)
Vulva	10.2 (5.4-17.4)	6.5 (4.1-10.2)	22.8 (15.8-32.7)
Vagina	12.0 (3.3-30.7)		
Penis	21.9 (7.1-51.2)	4.4 (2.8-7.1)	15.8 (5.8-34.4)
Anus	10.4 (4.8-19.8)	28.8 (21.6-38.3)	4.9 (1.4-17.3)
Head-neck	3.6 (2.1-5.7)	2.3 (1.7-3.3)	3.2 (2.4-4.4)
Possibly HPV-related			
Esophagus[1]	5.4 (2.0-11.8)	1.6 (1.2-2.2)	3.1 (1.9-5.0)
Non-melanoma skin	3.1 (1.9-4.7)	4.1 (1.1-16.6)	28.6 (9.4-87.2)
Other			
Lung	2.5 (1.8-3.4)	2.7 (1.9-3.9)	2.2 (1.9-2.6)
Kidney	1.6 (0.8-3.0)	1.5 (1.2-1.8)	6.8 (5.7-8.1)
Bladder	1.9 (1.1-3.1)	0.8 (0.4-1.3)	2.5 (1.8-3.3)

[1]For CA patients, only squamous cell carcinoma are included.

7.2 STUDY II

In this cohort, patients hospitalized for benign anal lesions, we found an increased risk of anal cancer in patients with inflammatory anal lesions but not in patients with hemorrhoids. The risk persisted after 10 years of follow-up, although based on few cases. Two previous cohort studies found no persisting association between benign anal lesions and anal cancer [55-56]. One of them was limited by size [55], and another did not subdivide the lesions into inflammatory anal lesions and hemorrhoids [56].

Benign anal lesions are commonly treated in out-patient clinics. It is likely that those in need of hospitalization have a more severe disease. In our cohort, 22% of those with inflammatory lesions were hospitalized more than once for this disease. In contrast to hemorrhoids, anal fissures and fistula are often infected, and infection is always present in perianal abscesses. Chronic inflammation is common in these lesions, and probably more frequent in patients who require hospitalization. Chronic inflammation has been said to cause cancer by favoring oncogene activation, genomic instability and consequently DNA damage, or by weakening tumor suppressor function [283].

HPV may explain the association between inflammatory anal lesions and anal cancer. HPV is present in almost 80% of all anal cancers [42]. So far HPV has not been shown to be present in all anal cancers, and HPV might not be a *sufficient cause* for anal cancer. In benign inflammatory anal lesions and hemorrhoids treated with open hemorrhoidectomy an injured area with a broken skin barrier is present. However, chronic inflammation is probably less common in the latter. The chronic inflammation in benign inflammatory anal lesions might facilitate the establishment of an HPV

infection. As discussed for the CA patients, smoking might weaken the immune response against viral infections [281]. Smoking has also been shown to have a negative effect on wound healing [284], and smoking cessation would probably improve healing of lesions. As for CA, these results are insufficient for any recommendations.

7.3 STUDY III

In this large cohort study with a follow-up of 37 years, we found no evident association between smoking and CRC. No significant association between snus and CRC was found, although type II error is a possibility. The association between anal cancer and smoking was confirmed.

There is an established association between smoking and colorectal adenoma [121, 126]. On the other hand, the relationship between smoking and CRC is more disputable. In a meta-analysis by Botteri et al, the reported risk increases in both genders were 7% in current smokers, and 17% in former smokers [134]. The corresponding numbers in males were 12% and 18% [134]. The mechanisms behind a possible causal relationship between tobacco use and colorectal cancer development remain speculative. Smokers have a longer colonic transit time than non-smokers [285]. A longer exposure of carcinogenic substances in the stool might affect the risk of cancer. Another suggestion is that a possible carcinogenic effect of cigarette smoke is mediated by the systemic circulation and not a direct effect [286]. Different mechanisms, such as MSI [117, 133], methylation of CpG islands and BRAF mutations have been proposed [140]. In a human colon cancer cell line, cigarette smoke extract induced the release of factors capable of promoting angiogenesis, and cell proliferation [287].

Previously, no association with colon cancer, and a small excess risk of rectal cancer, were reported from the same cohort as in the present study, during a mean follow-up of 18 years [250]. It has been proposed that the induction and latency period for colorectal cancer after tobacco smoke exposure might be as long as 40 years [122]. Therefore we undertook the present study with an addition of 16 years of follow-up and a bigger study population. However, the present study revealed no different results.

The inability to verify an association between smoking and CRC risk could be due to methodological considerations already discussed. However, an association between smoking and CRC risk in our study ought to have been indicated by a dose-response relationship. The only obvious dose-response relationship was for anal cancer and cigarette smoking. In addition, an increased risk of anal cancer was seen with duration of follow-up. There was a tendency to a dose-response relationship for the risk of rectal cancer and cigar smoking, and for left colon cancer and pipe smoking.

The association of smoking and colon cancer risk by subsite has previously been addressed in four case-control studies [115, 288-290]. To our knowledge, this is the first cohort study investigating this association, and we found no significant differences between right-sided and left-sided colon cancer. Although non-significant, there was a tendency to a dose-response relationship for smoking and right-sided colon cancer. On the other hand, the risk of left-sided colon cancer was more evident among snus users. However, imprecision makes the results hard to interpret.

Swedish moist snuff (snus) has been shown to accelerate the growth of gastric malignancies in mice [152], and significantly increased risks have been seen for pancreatic cancer [153-154], and esophageal cancer [155]. The association between snus and CRC has

not previously been investigated. The use of snus was not significantly associated with the risk of CRC or anal cancer. However, the pattern of the point estimates for colon cancer was similar to the pattern among smokers. The mechanistic link with smokeless tobacco, if any, remains to be delineated.

7.4 STUDY IV

In this study of 40,230 incident cancer cases in the Construction Workers Cohort, a history of tobacco-use at entry into the cohort was associated with a 19% excess hazard of all-cause death, and 12% excess of cancer-specific death compared to never-users of tobacco.

Interestingly the estimates for smokers and snus users were similar, even though the risks for the latter tended to be somewhat higher. Significantly increased hazards of cancer-specific death in pure smokers and pure snus users (13% and 16% respectively) are of note. This was confirmed in the analyses of cumulative relative survival. In all curves, smokers had the poorest survival, and the curves for snus users were constantly below never-users of tobacco. One exception was the relative survival of CRC where the curve for snus users was unstable and hard to interpret. The Cox proportional hazards regressions of cancer-specific death showed no obvious differences in the results for smoking-related cancers and other cancers. However, the highest estimates were seen for cancer-specific deaths after smoking-related cancers (HR 1.26 for smokers, and HR 1.28 for snus users).

Smoking has been shown to decrease cancer treatment efficiency for CRC [146] and other cancers associated with tobacco [141-145]. Present in both smoking tobacco and snus, nicotine has growth regulating effects that could affect cancer survival [159-161, 163, 165-167].

While there were associations between tobacco use and survival of both smoking-related and other cancers, the association with CRC survival was less apparent. Notwithstanding a possible type II error, tobacco use was not associated with colon cancer survival. There was a borderline significantly increased risk of rectal cancer death in smokers. This provides some support for the theory of colon and rectal cancer being two different diseases and should be treated as such [94]. Another explanation could be the treatment. Even though CRC surgery is major surgery, the procedure-related risks are higher in rectal cancer surgery. Thus, both less radical surgical treatment and higher postoperative mortality among smokers with rectal cancer may, at least in part, explain the increase in fatality among smokers with rectal cancer. However, this theory was not supported by the cumulative relative survival curves for CRC. The curves for tobacco strata did not clearly diverge until after three years.

The elevations of the risk estimates are of small magnitudes, and methodological limitations discussed in the previous section must be considered. To estimate whether tobacco use was associated with stage, we compared the recorded tumor stage for a small subset of incident cancer cases diagnosed in 2004 and later. Even though the data was sparse, and should be interpreted with caution, it indicated that smokers had a more advanced disease compared to non-smokers. Although based on even fewer cases, this did not seem to be the case for snus users. To estimate whether comorbidity could

explain the increased risk of death in smokers, data from the Inpatient Register was used. A limitation with this method is the lack of knowledge of patients who were only treated in the outpatient care. Stratification by comorbidity revealed no large differences in the hazards of death for different groups of tobacco use. This indicates that the observed association between tobacco use and cancer survival is not entirely due to confounding by comorbidity.

Cancer patients are interested in knowing how they may affect their prognosis. Our study has shown an increased risk of cancer-specific death in both smokers and snus users. The excess risk for tobacco users was apparent for both smoking-related cancers and other cancers. On the other hand, there was no similar association with colon cancer, and only an uncertain signal in regard to rectal cancer. Our results indicate a biological effect of tobacco on cancer survival, possibly excreted by nicotine. This is of interest for cancer patients as well as physicians. Further studies are needed to verify the association and clarify a possible mechanism behind it.

8 CONCLUSIONS

Patients hospitalized with condylomata acuminata had an increased risk of anogenital cancers, as well as other cancers. The pattern of cancer risk for patients hospitalized with condylomata acuminata was similar to the pattern for patients with immunosuppression from HIV-infection of by pharmacological means.

Patients hospitalized with benign inflammatory anal lesions had a 3-fold increased risk of anal cancer, and the risk persisted over time. There was no persistent risk elevation in patients hospitalized with hemorrhoids.

Smoking was marginally associated with colon cancer risk, modestly associated with rectal cancer risk, and substantially associated with anal cancer risk. Snus use was not significantly associated with either colorectal or anal cancer risk. However, the point estimates of colon cancer risk were similar in snus users and smokers.

A history of both smoking and/or snus use was associated with an increased risk of death in cancer patients. Smoking was not strongly associated with mortality from colon cancer, and only an uncertain signal in regard to rectal cancer. Snus use was not associated with colorectal cancer survival.

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