

From THE INSTITUTE OF ODONTOLOGY
Karolinska Institutet, Stockholm, Sweden

**ORAL MUCOSAL LESIONS, TOBACCO
USE AND THE LONG-TERM OUTCOME
IN A SWEDISH POPULATION**

Ann Roosaar



**Karolinska
Institutet**

Stockholm 2006

All previously published papers were reproduced with permission from the publisher.

Cover pictures: *Nicotiana glauca* from Linnean herbarium, the Swedish Museum of Natural History and *Usnea Filipendula* (a Lichen), photo by Sten Roosaar

Published and printed by



www.reproprint.se

Gårdsvägen 4 169 70 Solna

© Ann Roosaar, 2006

ISBN 91-7140-973-4

To my mother and in memory of my father

ABSTRACT

The overall objectives of this thesis were to study the natural history for Oral Lichen (OLL), Oral Leukoplakia (OL) and Snus Induced Lesions (SIL) and to correlate cancer and mortality among users and non-users of Swedish moist snuff (snus).

The population sub-sample comprised a historic cohort of 20,333 individuals, established in 1973-74. At the time for follow-up, data on 20,212 were still available. The cohort was followed for 27-29 years. Using the National Registration Number, a unique identifier for each individual in Sweden, the dataset was linked to the nationwide registers of the Total Population, Migration, Cancer and Deaths- all of which had been in operation for at least 15 years prior to cohort accrual.

For the nearly 30 year follow-up, in the first three papers, a sub-section of the cohort was selected, based on district of residence in 1973-74. This restriction was introduced in order to facilitate the subsequent field-work, when the cohort members were to be re-examined. Those who were still alive and residing in the area were offered a re-examination and 289 (68%) of the 422 invited individuals accepted. The register-based follow-up with data from The Cancer Register revealed oral cancer in one individual in the OLL cohort and three individuals each in the OL and SIL cohorts. There was no statistically significant increase for oral cancer in the three sub-cohorts. The clinical re-examination disclosed that around 40% of the lesions recorded in 1973-74 were no longer clinically detectable. For the OLL cohort this could be explained by the putative relapsing nature of the lesion and that there were no differentiations made between Oral Lichen Planus and Oral Lichenoid Reactions where a trigger factor might be present. For the OL cohort there was a strong association with smoking cessation and the disappearance of the lesion. Snus use was strongly associated with the appearance of SIL. In all cases where the individual reported cessation of using snus the lesion was no longer clinically discernible.

In the fourth study the subjects comprised all males from the original cohort (9,976). Tobacco habits (snus use and/or smoking) were used as exposure and cancer and mortality as outcome. The data set was linked to the health and population-based registers. Cancer had been notified to the cancer register for 1,575 of the subjects (only initial notification of cancer detected after day of entry into the study was included); 3,630 had died during the follow-up time. There was a statistically significant higher cancer incidence for those who were or had been daily smokers than for those who had never smoked (IRR 1.26, 95% CI 1.13-1.40). However, no such difference emerged with respect to snus habits (IRR 1.00). With respect to oropharyngeal cancer, there was a statistically significantly higher incidence among those who were or had been snus users compared to those who had never used snus, based on 11 exposed cases (IRR 3.1, 95% CI 1.5-6.6). For mortality ever daily smoking was strongly associated with death within the categories all cause mortality, cancer death, circulatory death and respiratory

death. Among ever daily snus users there was a 10% increase for all cause mortality compared to never snus users.

The following conclusions may be drawn from these results: There is no support for an increased incidence of oral cancer at the lesion sites. Both appearance and location of OLL change over time. There is a strong association between OL and smoking. The presence of SIL may be considered a marker for the use of snus. Use of snus seems to have a negative effect on health. It is associated with some increased risk for oropharyngeal cancer and overall mortality.

LIST OF PUBLICATIONS

- I. Roosaar A, Yin L, Sandborgh-Englund G, Nyrén O, Axéll T.
On the natural course of oral lichen lesions in a Swedish population-based sample.
Journal of Oral Pathology and Medicine 2006;35:257-61.
© Blackwell Munksgaard

- II. Roosaar A, Yin L, Johansson ALV, Sandborgh-Englund G, Nyrén O, Axéll T.
A long-term follow-up study on the natural course of oral leukoplakia in a Swedish population-based sample.
Journal of Oral Pathology and Medicine In press.
© Blackwell Munksgaard

- III. Roosaar A, Johansson ALV, Sandborgh-Englund G, Nyrén O, Axéll T.
A long-term follow-up study on the natural course of snus-induced lesions among Swedish snus users.
International Journal of Cancer 2006;119:392-7
© Wiley-Liss, Inc

- IV. Roosaar A, Johansson ALV, Sandborgh-Englund G, Axéll T, Nyrén O.
Cancer and mortality among users and non-users of snus.
In manuscript.

CONTENTS

1	Introduction	1
2	Background.....	2
2.1	Oral Lichen	2
2.1.1	Clinical appearance.....	3
2.1.2	OLP and malignancies	3
2.2	Oral leukoplakia	3
2.2.1	Clinical appearance.....	4
2.2.2	OL and malignancies	4
2.3	Snus - induced lesion	4
2.3.1	Clinical appearance.....	5
2.3.2	SIL and malignancies.....	5
2.4	Snus and smoking in Sweden	5
2.5	Oral and oropharyngeal cancer	5
3	Aims	7
4	Subjects and Methods.....	8
4.1	Subjects	8
4.1.1	Register-based follow-up (papers I–IV)	8
4.1.2	Samples	8
4.1.3	Examination procedures (papers I, II and III)	10
4.1.4	Criteria used for OLL (Paper I):	11
4.1.5	Criteria for OL (paper II):	11
4.1.6	Criteria for SIL (paper III):	12
4.2	Statistical methods	12
5	Summary of results.....	14
5.1	Register-based follow-up (Paper I-III)	14
5.2	Register-based follow-up (Paper IV)	16
5.3	Re-examination of selected lesions	17
6	General discussion.....	20
6.1	Methodological considerations.....	20
6.1.1	Study design and population.....	20
6.1.2	Diagnostic criteria and variables	22
6.1.3	Bias and confounders.....	23
6.1.4	Inter and intra-observer variation	24
6.2	Findings	25
6.2.1	OLL.....	25
6.2.2	OL	25
6.2.3	SIL.....	26
6.2.4	Snus use.....	26
6.3	Conclusions.....	26
7	Svensk sammanfattning (Swedish summary).....	27
7.1	Bakgrund.....	27
7.2	Material och metod	27
7.3	Delarbete I.....	28
7.4	Delarbete II.....	28
7.5	Delarbete III.....	28
7.6	Delarbete IV.....	28

8	Acknowledgements.....	30
9	References.....	32
10	Appendix 1.....	36

LIST OF ABBREVIATIONS

CI	Confidence Interval
EpC	Centre of Epidemiology (at the Swedish National Board of Health and Welfare)
GVHD	Graft-Versus-Host-Disease
HR	Hazard Ratio
ICD	International Classification of Diseases
IRR	Incidence Rate Ratio
NRN	National Registration Number
OL	Oral Leukoplakia
OLL	Oral Lichen
OLP	Oral Lichen Planus
OLR	Oral Lichenoid Reaction
RTB	Total Population Register
SCB	The Central Bureau of Statistic, Statistics Sweden
SIL	Snus Induced Lesion
SIR	Standardized Incidence Ratio
SMR	Standardized Mortality Ratio
SoS	The Swedish National Board of Health and Welfare
WHO	World Health Organization

1 INTRODUCTION

Our knowledge of the natural history of oral mucosal lesions is limited and incomplete, especially in non-patient populations. Although population-based studies have been undertaken to establish the prevalence of an array of oral lesions, there have been very few follow-up studies to determine their natural course (Gupta, PC 1980). In general, follow-up data on oral lichen and oral leukoplakia originate from selected populations. Subjects are sourced primarily from specialist clinics, frequently with sub-selection of subjects with malignant transformation as the main outcome (Silverman, S, Jr. 1984, Andersson, G 1991, Silverman, S, Jr. 1991, Schepman, KP 1998, van der Meij, EH 2003).

The oral use of smokeless tobacco is common in e.g. rural India and in parts of the United States and is there associated with an increased risk of oropharyngeal cancer (Pindborg, JJ 1975, Gupta, PC 1980, Winn, DM 1981). In Sweden the most popular form of smokeless tobacco is Scandinavian moist snuff, (“snus”). Whether this form of smokeless tobacco may also be associated with increased risk of oropharyngeal cancer has been investigated in a number of studies (Wynder, EL 1957, Blomqvist, G 1991, Lewin, F 1998, Schildt, EB 1998, Rosenquist, K 2005). Most of these studies have limitations, and their results and ability to capture clinically important associations have to some extent been debated.

Habitual use of snus is also associated with the occurrence of typical lesions in the oral cavity, so-called snus-induced lesions (Axell, T 1976, Andersson, G 1991). There are few follow-up studies on the natural course of these lesions (Andersson, G 1991).

2 BACKGROUND

Follow-up studies are viable in Sweden thanks to almost unique registers available. Every resident in Sweden receives a National Registration Number (NRN) at birth or on immigration. The NRN was first introduced in Sweden in 1947. As the NRN is used consistently in computerized population and health registers, it is possible to combine different registers and to follow non-patient populations over time.

Population statistics was started in Sweden as early as 1749, a quite unique phenomenon. The Central Bureau of Statistics (SCB) was established in 1858, and in 1968 a register over the total population (RTB) was introduced (Statistics Sweden 2005).

The Centre for Epidemiology (EpC) at the Swedish National Board of Health and Welfare (Socialstyrelsen, SoS) is responsible for several national registers, e.g. the Swedish Cancer Register and the Cause of Death Register (SoS/EpC 2001, SoS/EpC 2003).

The Swedish Cancer Register was founded in 1958 and covers the whole population of Sweden. It is mandatory for all health care providers to notify newly detected cancer cases. The information recorded in the Register includes patient data (NRN, sex, age, place of residence), medical data (site of tumour by ICD-code, histological type, basis of diagnosis, date of diagnosis, the notifying hospital/department, notifying pathology/cytology department), and follow-up data (date of death, cause of death, date of migration). The register has been shown to be over 98% complete (Mattsson, B 1984, Mattsson, B 1985).

Swedish statistics on causes of death are among the oldest worldwide and can be traced back to 1749, when a nationwide notification system was first introduced. Since 1911, statistics over causes of death in the Swedish population, recorded in the Cause of Death Register, have been published annually.

Three oral mucosal lesions were selected for study in the present thesis: oral lichen (OLL), oral leukoplakia (OL) and the snus-induced lesion (SIL). OLL and OL meet the WHO criteria for precancerous conditions/lesions (Kramer, IR 1978). SIL occurs in habitual users of snus, a common habit among Swedish men. The lesion is found in the oral mucosa at the site where the quid of moist tobacco is placed.

2.1 ORAL LICHEN

OLL is a comprehensive term for lesions showing similar clinical and histopathological reaction patterns in the oral mucosa, but where the aetiology is still unclear. Its prevalence in a Swedish general population is 1.9%-2.4% and occurs predominantly in women (Axell, T 1976, Salonen, L 1990). OLL includes Oral Lichen Planus (OLP) and Oral Lichenoid Reactions (OLR). OLP is considered to be a systemic disease whereas OLR has an obvious trigger factor present (e.g., dental materials, drugs, graft-versus-host disease) (Scully, C 1998) However, differential diagnosis between OLP and OLR

may be difficult or impossible, especially on the basis of a single examination of the patient.

2.1.1 Clinical appearance

OLP is a chronic inflammatory condition, presenting at any site of the oral mucosa and is characterized by remissions and recurrences (Thorn, JJ 1988). OLP appears in various forms, often bilaterally. An early characterisation of the diverse forms of OLP was done by Andreasen (Andreasen, JO 1968). OLP may be separated into white and red lesions. The white variety of OLP can be characterised as papular, reticular or plaque-like, whereas the red variety may be divided into atrophic/erythematous, erosive/ulcerative, or bullous forms.

The two conditions OLP and OLR have similar clinical and histopathological features (Mattsson, T 1992, McCartan, BE 1997, Scully, C 1998). OLRs associated with a reaction to dental materials should meet the criterion of direct contact between the lesion and the dental material. This lesion often presents unilaterally. For OLRs due to drugs the differential diagnostic pathway is to change the medication and follow the lesion over time. This method, however, is often not recommended as it may jeopardise the patient's health. OLR in patients with graft-versus-host disease (GVHD) is another condition exhibiting clinical and histopathological features similar to OLP. If screening prior to the allogenic stem cell transplantation recorded healthy oral mucosa, then the probable diagnosis would be OLR (GVHD).

2.1.2 OLP and malignancies

According to WHO criteria (Kramer, IR 1978), OLP is considered to be a precancerous condition, but there are contradictory reports on the incidence of malignant transformation (Krutchkoff, DJ 1978, Holmstrup, P 1992). In previous studies where OLL has been shown to be associated with an increased risk for cancer development, the subjects have comprised possible high-risk patients, selected after referral to specialist clinics (Holmstrup, P 1988, Silverman, S, Jr. 1991, van der Meij, EH 2003, Gandolfo, S 2004, Rodstrom, PO 2004). A recently published retrospective study from UK on 690 patients showed a low risk for malignant transformation during the follow-up time (malignant transformation rate 1.9%) (Ingafou, M 2006). There are also recent studies reporting an increased risk for oral squamous cell carcinomas in patients with OLR (GVHD) (Curtis, RE 2005, Demarosi, F 2005).

2.2 ORAL LEUKOPLAKIA

The prevalence for OL in a Swedish population is 1.9%-3.6%, with a clear predominance among men 6% (Axell, T 1976, Salonen, L 1990). The definition of Oral Leukoplakia (OL) has been the subject of much discussion over the years (Axell, T, Holmstrup P, Kramer IRH, Pindborg JJ, Shear M 1984, van der Waal, I 2002). The major problem associated with the diagnosis is that it can be established only after

excluding all other possible conditions and/or trigger factors, *i.e.* it is an exclusion diagnosis. This fact influences on the determination of prevalence as there is no possible way to establish a positive OL diagnosis on the basis of a single examination, even with aid of a biopsy. No definite markers are present for OL and a white patch in the oral mucosa may be induced by many factors (e.g. OLL, trauma, Candida).

Tobacco use, especially smoking tobacco has a special place in the history of OL. The use of smoked tobacco has been used as an inclusion criterion for OL, and not for exclusion. Follow-up studies show that most oral white patches in tobacco smokers disappear on cessation of smoking, but some lesions persist and in other cases lesions disappear despite continued smoking (Silverman, S, Jr. 1984). This implies that tobacco use most certainly plays a role in some, but not all OL lesions.

2.2.1 Clinical appearance

The most commonly used definition of OL is “A predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion” (Kramer, IR 1978, Axell, T, Holmstrup P, Kramer IRH, Pindborg JJ, Shear M 1984). OL may present with different clinical characteristics, some of them considered to be more precancerous than others. The most common clinical distinction is made between the homogeneous and non-homogeneous types.

2.2.2 OL and malignancies

Non-homogeneous OL are associated with a higher risk of malignant transformation than homogeneous OL and idiopathic OL with a higher risk than tobacco-associated OL (Silverman, S, Jr. 1984, Gupta, PC 1989, Tischendorf, L 1990). Lesion site may also be important. OL located in the floor of the mouth is considered to be a high risk lesion (Kramer, IR 1978, Shell H, SA 1987). However, more recent reports have suggested that only size of lesion and presence of dysplasia may have a bearing on potential malignancy (van der Waal, I 2002). In order to better categorize lesions at risk a recording system for OL has been suggested, equivalent to the TNM-system for malignant tumours (Schepman, KP 1995, van der Waal, I 2002).

2.3 SNUS - INDUCED LESION

The prevalence of SIL in a Swedish male population has been reported to be 14.5%-15.9% (Axell, T 1976, Salonen, L 1990). Among habitual users of snus a typical lesion develops in the oral mucosa corresponding to the site at which the quid of tobacco is habitually placed (Axell, T 1976). A clinical and histological follow-up study has shown that the lesion is reversible (Andersson, G 1991).

2.3.1 Clinical appearance

The lesion is characterised by slight or heavy wrinkling of the mucosa, with or without whitish-yellowish to brown discolorations and with or without obvious thickening (Axell, T 1976).

2.3.2 SIL and malignancies

The question of whether lesions caused by smokeless tobacco should be considered premalignant has been debated. In a study from the US (Winn, DM 1981) evidence was presented for an increased risk for oral cancer at sites in the oral cavity exposed to dry snuff. No studies have proved malignant transformation of a SIL.

2.4 SNUS AND SMOKING IN SWEDEN

Data from the European regional office of WHO show that Sweden has one of the lowest proportions of daily smokers in Europe. (www.euro.who.int/informationources) This could possibly to some extent be ascribed to the use of snus.

Snus is a type of moist smokeless tobacco used intraorally. The product was developed from dry nasal snuff in the 19th century. The largest recorded consumption was 7,000 tons in 1919. Thereafter, consumption declined only to gain new popularity in the late 1960's. In 1973 portion-packed snus was introduced and this product has become very popular, especially among ex-smokers. In Sweden today (2004), the total annual consumption of snus is 6,800 tons. Over a million Swedes are snus users and 800,000 are daily users. From being an exclusively male habit its use has increased rapidly also among women. During the last 10 years snus use among women has almost tripled ranging from 54,000 users in 1993 to 191,000 in 2004 (data from www.swedishmatch.com).

Swedish snus is produced from air-dried non-fermented tobacco. The main ingredients are ground tobacco, water, salt (ordinary cooking salt) and sodium carbonate. Additional ingredients are aromatic compounds and humectants.

2.5 ORAL AND OROPHARYNGEAL CANCER

Oropharyngeal cancer accounts for approximately 4.5% of total cancers worldwide. In the year 2000 an estimated 450,000 newly diagnosed cases of oropharyngeal cancer occurred worldwide (Parkin, MD 2001).

In a global perspective, the incidence of oropharyngeal cancer varies considerably, e.g. it is the most common malignancy among men in India. In Europe the most recent report (2004) showed approximately 98,000 newly diagnosed cases of oropharyngeal

cancer, accounting for 3.4% of total cancers (Boyle, P 2005). In Sweden approximately 3% of all new cancer cases diagnosed are oropharyngeal (SoS/EpC 2003).

Epidemiological research on oral and pharyngeal cancer is complicated by the many anatomic sub-sites: lip, tongue, salivary glands, floor of the mouth, other parts of the mouth, oropharynx, nasopharynx, hypopharynx and pharynx (ICD7 codes 140-148). In this thesis, the following terminology has been adopted: “oral cancer” to denote cancer of the tongue (ICD7 141), floor of the mouth (ICD7 143) and other parts of the mouth (ICD7 144) and “oropharyngeal cancer” to denote ICD7 140-148.

3 AIMS

The general aims of this thesis, consisting of four papers, were to investigate the natural history of three possibly premalignant oral mucosal lesions/conditions, and to study cancer and mortality in a male cohort with users and non-users of snus.

SPECIFIC AIMS

To study the natural course of oral lichen lesions among men and women in a non-patient cohort follow-up for 27-29 years with population and health register data and with a re-examination after 19-22 years (Paper I)

To study the natural course of oral leukoplakia among men and women in a non-patient cohort follow-up for 27-29 years with population and health register data and with a re-examination after 19-22 years (Paper II)

To study the natural course of snus-induced lesions among men in a non-patient cohort follow-up for 27-29 years with population and health register data and with a re-examination after 19-22 years (Paper III)

To study cancer and mortality among users and non-users of snus followed up for 27-29 years with use of population and health register data (Paper IV)

4 SUBJECTS AND METHODS

4.1 SUBJECTS

During 1973-74 a population-based prevalence study of oral mucosal lesions was carried out in Uppsala County in central Sweden. At the time, a total of 30,118 individuals aged 15 or more resided in the area. All were offered clinical examination and 20,333 (10,036 men and 10,297 women) accepted (Axell, T 1976). For the follow-up studies presented in this thesis data were available for 20,212 individuals (Figure 1). Of the remaining 121 subjects of the earlier study, information was unavailable for the following reasons: in 84 cases the NRN was incorrect and 14 had changed NRN during follow-up. In eight cases the data were illegible and 15 individuals had inadvertently been examined twice in the original study (the second examination was excluded).

4.1.1 Register-based follow-up (papers I–IV)

After confirmation of correct identification by the Total Population Register (RTB), the dataset was linked to the national registers (the Swedish Cancer Register and the Cause of Death Register). Information on diagnosis based on ICD-codes (ICD7, ICD8, ICD9 and ICD10), date of diagnosis, death or emigration was retrieved and used in statistical analysis.

4.1.2 Samples

This thesis consists of three papers (paper I, II, III) based on subsets of the original study cohort from 1973-74 and one paper (paper IV) based on the whole male population from 1973-74 (Figure 1).

Selection of the subset for papers I-III was based on district of residence in 1973-74. The selection comprised Bålsta municipality, the town of Enköping and 15 surrounding parishes in total 16,144 individuals.

In 1993, samples originally diagnosed with OLL, OL or SIL were drawn from cohort members of the three subsets who had not died or moved out of the selected areas, for a face-to face interview and clinical re-examination. Because of limited resources, only a sample of the individuals was offered re-examination. The sampling procedures were different in Enköping, Bålsta and the surrounding parishes. In Enköping and the 15 rural parishes the selection was restricted to a few zip code areas chosen to preserve the socio-demographic distribution of the source population. In these zip code areas all individuals were offered re-examination. In Bålsta the entire study cohort was selected and offered re-examination. As the selection of subjects was unrelated to individual characteristics, and thus to outcome probability, these selection procedures were deemed equivalent to random sampling, but simplified the practical arrangements for the re-examinations. If a participant failed to attend the first appointment, at least one new appointment was offered.

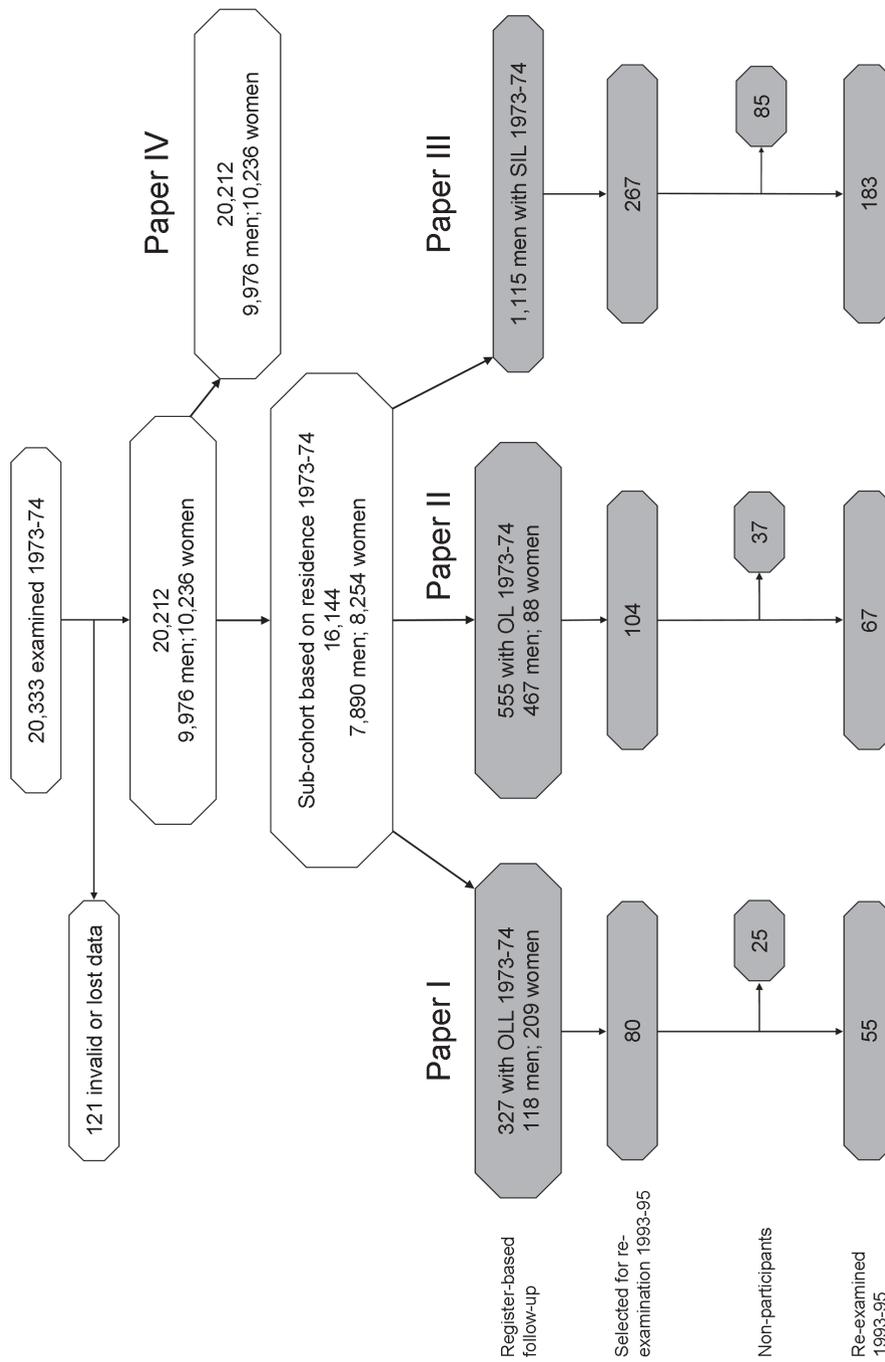


Figure 1. Flowchart describing the cohort and sub-cohorts.

In **Paper I**, all individuals registered with Oral Lichen Lesions (OLL) in 1973-74 in the selected follow-up area (327: 118 men and 209 women) were included in the register-based follow-up, and 55 of the 80 contacted individuals presented for re-examination in 1993-95. The lesions were registered either as no lesion, or presence of white and/or red forms of OLL.

In **Paper II**, all individuals registered with oral leukoplakia (OL) in 1973-74 in the selected follow-up area (555: 467 men and 88 women) were included in the register-based follow-up, and 67 of the 104 contacted presented for re-examination in 1993-95. The registrations used for analyses were no lesion or lesion.

In **Paper III**, all males registered with snus-induced lesions (SIL) in 1973-74 in the selected follow-up area (1,115 individuals) were included in the register-based follow-up and 183 of the 267 contacted presented for re-examination in 1993-95. A four-point scale (Axell, T 1976) was used for describing the SILs present at follow-up.

All male participants from the original examination in 1973-74 were included in the cohort used in **Paper IV** (9,976).

4.1.3 Examination procedures (papers I, II and III)

All individuals selected for re-examination in 1993-95 were contacted with a letter including information about the study and their prior participation in 1973-74. After two weeks they were contacted by phone by the investigator or the assisting dental nurse. They were offered an appointment at a clinic near their home or workplace for an interview and examination of the oral cavity. This was conducted in a standardized way using the same forms and criteria as in the initial survey in 1973-74 (Axell, T 1976). The questionnaire addressed tobacco habits (current and previous), type of tobacco (smoking or smokeless), years of habitual tobacco use and daily consumption (Appendix 1). Other topics addressed included alcohol consumption and medication and treatment of oral mucosal lesions during the follow-up interval. The clinical examination was conducted by one examiner (AR) who prior to the study start was calibrated with the examiner from the original survey in 1973-74. Calibration was made using photographs and inter- and intra-examiner agreement beyond chance was determined using Kappa statistics (Agresti, A 1990) The follow-up examiner had no knowledge of the subject's oral condition other than that the individual due to be examined and questioned had been diagnosed in 1973-74 with one or more of the three selected lesions (OLL, OL, SIL). So that the 1993-95 examiner would not be influenced by the prior findings, no data from the 1973-74 examination were accessible at the re-examination.

The examination was carried out in a dental chair with halogen light and dental mirrors. All lesions, persistent or new, were recorded using the same coding system as used at the baseline examination in 1973-74 (WHO 1973). Colour photographs were taken using Kodak Ektachrome professional 135/36 colour film from the same emulsion

batch and stored in a refrigerator. No biopsies were performed at re-examination. In cases where the clinical appearance was suspected for a malignancy the individual was referred to an oral and maxillofacial surgeon.

The clinical criteria used in this study were the same as at the base-line examination in 1973-74.

4.1.4 *Criteria used for OLL (Paper I):*

The OLL was classified as white (including papular, reticular and plaque type) or red (including erythematous/atrophic, erosive/ulcerative and bullous types) according to the following characteristics.

1. White, pinheaded-sized papules.
2. White, distinct striae forming patterns.
3. White plaque-like lesions with striae at the margins.
4. Red, erythematous areas with striae at the margins.
5. Atrophy of tongue papillae. The atrophic area has a whitish, dry surface.
6. Areas of erosion or ulceration with striae at the margins.
7. Vesicles or bullae in areas with lesions compatible with criterion 1,2,3,4 or 5.

The white structures cannot be rubbed off.

Papular type = 1+8 Reticular type = 2+8 Plaque type = 3+8
Atrophic type = 4+8 or 5+8 Erosive type = 6+8 Bullous type = 7+8

4.1.5 *Criteria for OL (paper II):*

A criterion common to all sub-classes was that the lesion must be a whitish patch of the oral mucosa that cannot be attributed to any other diagnosable lesion.

OLs were divided in two sub-classes:

1. OLs with demarcated margins or with indistinct boundaries blending into the adjacent normal mucosa (homogeneous).
2. OLs with one or more erythematous areas and/or with white, pinheaded-sized papules/nodules in parts of the lesion (non-homogeneous).

4.1.6 *Criteria for SIL (paper III):*

SIL is classified according to a four-point scale (Axell, T 1976)

1. A superficial lesion with a colour similar to the surrounding mucosa and with slight wrinkling. No obvious mucosal thickening.
2. A superficial, whitish or yellowish lesion with wrinkling. No obvious thickening.
3. A whitish-yellowish to brown, wrinkled lesion with intervening furrows of normal mucosal colours, obvious thickening.
4. A marked yellowish to brown and heavily wrinkled lesion with intervening deep reddened furrows and/or heavy thickening.

4.2 **STATISTICAL METHODS**

Descriptive statistics were used in tables describing the cohorts. (Paper I-IV)

In the analysis of the re-examined participants 1993-95, Fisher's exact test was used to test independence between lesions found at re-examination and exposures such as smoking (**Paper II**), snus use in hours per day and gram per day (**Paper III**). Age-at-entry and alcohol user status were also tested for independence of degree of lesion (**Paper II-III**). The missing value category was not included in the tests.

In the register-based follow-up for cancer analysis, we included only first cancers and disregarded malignancies detected incidentally at autopsy. After having confirmed the validity of the NRN through linkage with the registers for population, death and migration, person-time was calculated from the date of first examination until the date of death, occurrence of any cancer, emigration, or end of follow-up (January 31, 2002), whichever occurred first. The standardized incidence ratio (SIR), the ratio of the observed to the expected number of cancers, was used to estimate relative risk for oral cancer (WHO ICD7 codes 141: tongue; 143: floor of the mouth; 144: other parts of oral cavity) (**Paper I-III**) and any cancer (**Paper I and IV**) and some organ specific cancers (**Paper I**). The expected number of oral cancers was calculated by multiplying the observed person-time in gender-, 5 year age-, and calendar-year strata by cancer incidence rates in the corresponding strata, based on observed rates in the entire Swedish population. Confidence intervals (CI) of SIRs were calculated with the assumption that the observed number of events followed a Poisson distribution. Analogously, standardized mortality ratios (SMRs), were calculated for overall mortality (**Paper I and IV**) and some cause-specific deaths (**Paper I**). In the latter analyses, there was no censoring after cancer occurrence.

In **Paper IV** cancer diagnosis and cause of death were grouped in a number of categories. For cancer the groups were based on ICD7 codes: Any cancer (ICD7: 140-209); Smoke related cancers (based on (Levitz, JS 2004) (ICD7: 140-148, 150-151,157,161-162 180-181); and Oropharyngeal cancer (ICD7: 140-148). Mortality was grouped into four categories using ICD8, 9 and 10: All mortality; Cancer deaths (ICD8, ICD9: 140-209, ICD10:C00-D48); Cardiovascular deaths (ICD8, ICD9:390-458, ICD10:I00-I99); and Respiratory deaths (ICD8, ICD9: 460-519, ICD10: J00-J99).

Non-proportionality of hazards, i.e. interaction between age (underlying timescale) and covariates was investigated. Only participants with complete information on both outcome and all covariates were included in the models. Stata version 9 (StataCorp 2005) was used for the statistical analyses.

5 SUMMARY OF RESULTS

5.1 REGISTER-BASED FOLLOW-UP (PAPER I-III)

The register-based follow-up described in papers I-III comprised a total of 1,926 individuals. Both SIL and OL were registered in 45 subjects, 17 had SIL and OLL, 8 had OL and OLL and in one man all three diagnoses had been registered in 1973-74. Table 1 shows the characteristics of the four study cohorts used in papers I-IV. The OLL and OL cohorts include both men and women while the SIL cohort and individuals in Paper IV comprise men only. The age distributions were similar in the OLL and OL cohorts: 49% and 50% respectively were aged 45 to 64 years. The cohort representing the 1,115 individuals with SIL had the same age distribution as the total male population.

The majority of the OLL cohort (Paper I) had never smoked (74%), whereas only 11% in the OL cohort (Paper II) had never smoked.

Among the 555 individuals with OLL one case of oral cancer was detected in the Cancer Register. Data from the same register revealed three cases of oral cancer in the OL cohort, and three in the SIL cohort. It is noteworthy that one of the cases is represented in both the OL and the SIL. The cases are presented in Table 2.

With respect to all cause-mortality, 146/327 (45%) of the individuals in the OLL cohort had died during the follow-up time. The corresponding value for OL was 285/555 (51%). For those with SIL, 438/1,115 (39%) had died during the 27-29 years of follow-up.

The numbers of any (first) cancers showed variations similar to the all cause mortalities between the cohorts. The highest number of cancer cases was detected in the OL cohort 121/545 (22%), followed by 65/319 (20%) in the OLL cohort and 160/1,105 (14%) in the SIL cohort.

Table 1. *The four study cohorts stratified according to cancer (any and oral) overall mortality and other background factors*

		Paper I	Paper II	Paper III	Paper IV*	
		OLL	OL	SIL	Never snus user	Ever snus user
		n=327 (%)	n=555 (%)	n=1115 (%)	n=8416 (%)	n=1559
Age at entry	15-24	20 (6)	12 (2)	190 (17)	1360 (16)	252 (16)
	25-34	38 (12)	63 (11)	286 (26)	1833 (22)	396 (25)
	35-44	48 (15)	111 (20)	143 (13)	1447 (17)	179 (11)
	45-54	77 (24)	137 (25)	114 (10)	1384 (16)	161 (10)
	55-64	83 (25)	143 (26)	172 (15)	1252 (15)	235 (15)
	65-74	41 (13)	57 (10)	129 (12)	782 (9)	198 (13)
	75-84	17 (5)	28 (5)	62 (6)	309 (4)	108 (7)
	85-	3 (1)	4 (1)	19 (2)	49 (1)	30 (2)
Residence 1973-74	Rural	53 (16)	99 (18)	250 (22)	2940 (35)	630 (40)
	Small municipality	48 (15)	80 (14)	202 (18)	1280 (15)	222 (14)
	Small town	226 (69)	376 (68)	663 (59)	4196 (50)	707 (45)
Smoking 1973-74	Never daily	242 (74)	59 (11)	515 (46)	3107 (37)	867 (56)
	Ever daily	85 (26)	496 (89)	599 (54)	5309 (63)	692 (44)
	Missing	0	0	1	0	0
Alcohol 1973-74	Never	131 (40)	126 (23)	155 (14)	1342 (16)	133 (9)
	Ever	196 (60)	429 (77)	960 (86)	7068 (84)	1426 (91)
	Missing				6	0
Oral cancer**	Yes	1 (0)	3 (1)	3 (0)	4 (0)	4 (0)
	No	326 (100)	552 (99)	1112 (100)	8412 (100)	1555 (100)
Any cancer	Yes	65 (20)	121 (22)	160 (14)	1337 (16)	237 (15)
	No	254 (80)	424 (78)	945 (86)	7079 (85)	1322 (85)
Excluded***		8	10	10	105	11
All cause mortality	Yes	146 (45)	285 (51)	438 (39)	2988 (36)	641 (41)
	No	181 (55)	270 (49)	677 (61)	5428 (64)	918 (59)

*The cohort in paper IV includes all males (n=9976) in the table, data on snus use were unavailable for one individual

**Oral cancer include ICD7-codes 141, 143, 144

***Excluded due to first cancer registered in the Cancer Register prior to entry in the study

Percentages do not add to 100% due to rounding

Table 2. Description of oral cancer cases (paper I-III)

Gender/Age at entry	Year diagnosed	Lesion(s) in 1973-74	Site of oral cancer	Smoking habit*	Snus use**	Alcohol use***
Woman / 58	2000	OLL	Tongue, unspecified	No	No	No/low
Woman / 40	1990	OL	Base of the tongue, left	Yes	No	Moderate
Woman / 69	1985	OL	Cheek, right	Yes	No	Moderate
Man / 63	1984	SIL/OL	Lateral border of the tongue, right	Yes	Yes	Moderate
Man / 64	1974	SIL	Tongue, unspecified	No	Yes	Moderate
Man / 80	1975	SIL	Maxilla, right	Yes	Yes	Moderate

*No: Never ever daily smoking; Yes: Ever daily smoking

**No: Never ever daily snus use; Yes: Ever daily snus use

***Low: no consumption or less than once a week, Moderate: 1-2 times a week, High :> 2 times a week

5.2 REGISTER-BASED FOLLOW-UP (PAPER IV)

The cohort consisted of all men examined in 1973-74 (n=9,976), of whom snus use, prior or current, had been reported by 1,559 (16%). The cohort is described in Table 1. A difference in smoking habit was observed: 44% of those who used or had ever used snus daily also reported being or having been daily smokers whereas for those who had never used snus daily the corresponding figure was 63%. There were no differences between the two groups with respect to age at entry, residence or alcohol use.

Oral cancer and any cancer incidences were similar in the two groups but there was a small increase in the risk for all-cause mortality for those who had ever used snus daily (41%, compared to 35% for those who had never used snus daily).

We included all cohort members in a model where adjustments were made for smoking and snus use. No excess incidence of all cancer emerged for prior or current snus users relative to those who had never used snus (IRR 1.00). However, when the outcome was restricted to oropharyngeal cancers there was a statistically significant 3.1-fold (95% CI 1.5-6.6) higher incidence among prior or current snus users relative to those who had never used snus. This finding was though based on only 11 exposed cases.

In order to eliminate a residual confounding effect from smoking, the effects of snus in a never smoker stratum were also analysed. With cancer of all kinds as the outcome, a non-statistically significant increase (IRR 1.11, 95% CI 0.91-1.37) was disclosed for those who had never smoked but were prior or current snus users compared with those who had never smoked or used snus. For oropharyngeal cancer in the same stratum there was a more than 2-fold risk elevation, but this result was based on only 5 exposed cases and did not attain statistical significance (IRR 2.3, 95% CI 0.7-8.3).

As expected, those who smoked daily or had previously smoked daily showed a statistically significant (IRR 1.26, 95% CI 1.13-1.40) increased incidence for cancer of all kinds relative to non-smokers. This excess was also seen for smoke-related cancers (IRR 2.2, 95% CI 1.8-2.7).

Similarly we also estimated relative risks for all cause mortality, cancer death, cardiovascular death and respiratory death. Smoking was significantly linked to increased risks for all these mortality outcomes. Prior or current snus use was associated with a moderate but statistically significant increased risk for all cause mortality (HR 1.10, 95% CI 1.01-1.21).

We also calculated SMR and there was a statistically significantly lower risk for all cause mortality among the cohort members (SMR 0.8, 95% CI 0.78-0.83).

5.3 RE-EXAMINATION OF SELECTED LESIONS

In total, 298 individuals were re-examined in 1993-95. Seven individuals had had more than one of the selected lesions at base-line 1973-74. Re-examination disclosed the presence of SIL and OL in five individuals and the combinations SIL and OLL, and OL together with OLL in one individual, each.

Figure 2 shows the ratio of individuals with lesions present at the time of re-examination (Paper I-III).

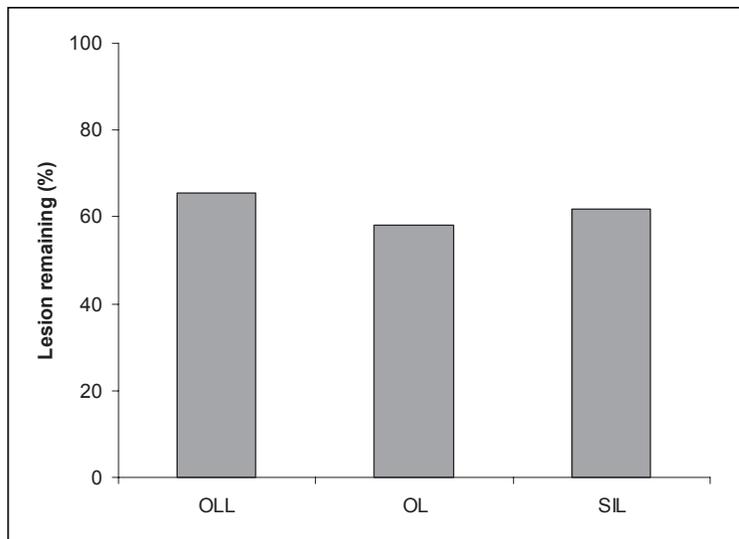


Figure 2. Percentage individuals with clinically observed lesions in the re-examined cohorts in 1993-95.

Among the 55 individuals in the OLL cohort, 19 (35%) had no lesions (clinically non-detectable) at re-examination. Of the 36 with remaining lesions, 22 (61%) were characterised as white OLL and 14 (39%) as red. The distribution between white and red lesions was comparable with the status at base-line: 36 (66%) and 19 (34%) for white and red respectively. Figure 3a illustrates one of the re-examined subjects showing OLL in the right cheek. This woman, aged 75 at the time of re-examination, had been diagnosed with a reticular OLL in 1973-74 (white OLL). She had never been a daily smoker. She showed a discrete persisting white OLL lesion at re-examination 20-years later.

Of the 67 individuals registered with OL in 1973-74, the lesion had disappeared in 28 (42%). There was a strong association between never/previous smokers in 1993-95 and the disappearance of OL.

Of the 23 never/previous smokers with no lesions, 78% reported that they had stopped smoking during the follow-up period. Figure 3b illustrates one of the re-examined individuals, a woman aged 42 at re-examination, a daily smoker for 32 years with an increase in consumption from 18 cigarettes/day in 1973-74 to 25 cigarettes /day in 1993-95. She was diagnosed with homogeneous OL in 1973-74 and showed a persisting, slightly more pronounced homogeneous lesion in 1993-95.

For those with SIL at baseline the lesion had disappeared in 38% of the individuals by the time of the re-examination. In the SIL cohort there was a strong correlation between the use of snus and the appearance of the lesion. In all cases where the individual had reported that he had stopped using snus (one year ago or more) there was no clinically detectable lesion. Figure 3c shows a man aged 57 at re-examination. He has been a daily snus user for 40 years using loose snus with the same consumption pattern over the years. The lesion was graded a three in 1973-74 and the persisting lesion graded two in 1993-95.

The study design stipulated that no biopsies would be taken by the examiner. In case of doubtful diagnosis or suspected malignancy the individuals were referred to an oral-maxillofacial surgeon. Only two of the 289 subjects who were re-examined were referred: in both cases the clinical appearance of the lesions suggested malignancy. One lesion was diagnosed clinically as OLL and the other as OL. Histopathology confirmed the clinical diagnosis. Neither case proved to be malignant.

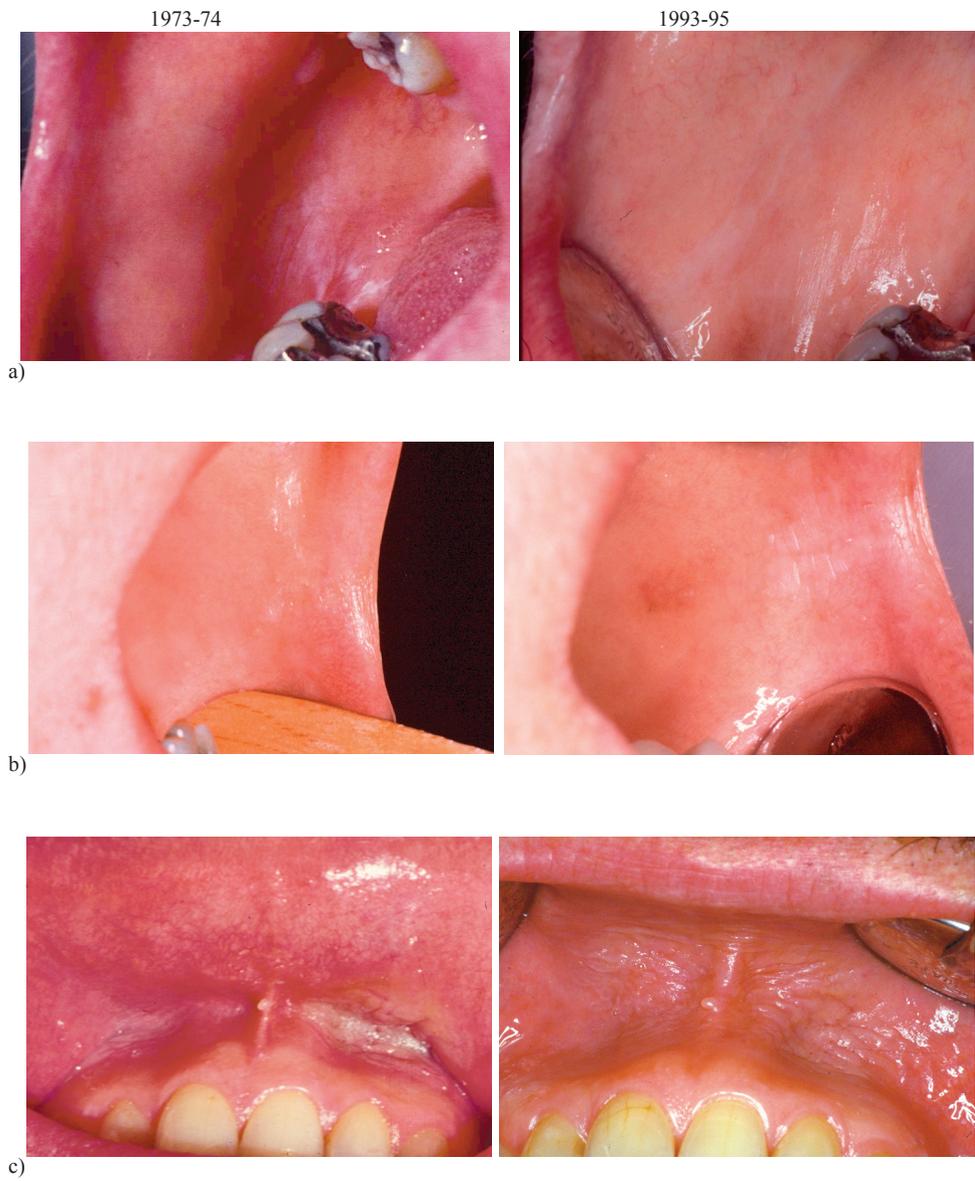


Figure 3. In the left column are clinical photos of lesions in 1973-74, and to the right clinical photos from the same individual in 1993-95: a) OLL, b) OL and c) SIL

6 GENERAL DISCUSSION

To establish the true natural history of oral mucosal lesions in a non-patient population is a major challenge, well beyond the scope of the present thesis. The diagnostic criteria for OLL and OL are under continuous review and to date there is no gold standard. Thus at this point the challenge will probably remain just a challenge. Nevertheless, within the limitations of the present study we have tried to shed some light on the natural course of these possibly premalignant lesions. Our results highlight a number of unanswered questions and unresolved issues. There is however, an indication that in a normal population there is a very low risk for development of serious disease associated with the diagnosis of OL, OLL or SIL.

For several reasons longitudinal studies of oral mucosal lesions in non-patient cohorts are rare. Since the incidence of oral cancer, the outcome of primary concern, is relatively low, large cohorts and long follow-up are required. Few countries have the fortunate combination of well-functioning population administration, with unique personal identifiers assigned to every resident at birth or on immigration, and nationwide, complete and high-quality population and health registers that consistently use these personal identifiers. Therefore, in most countries, follow-up requires investigators to keep track of all cohort members and to actively contact them for verification of the outcome. This makes such studies time consuming and very expensive.

In Sweden, however, the introduction of the 9-10-digit National Registration Number (NRN) in the mid 1900's and the establishment of computerized and complete population and health registers, including the registers of Cancer and Causes of Death, that all use the NRNs as identifiers, have made it possible to attain essentially complete follow-up at comparably low cost by using well established techniques for linking records. This study includes an almost 30 year register-based follow-up with a clinical re-examination in a sub-sample after 19-21 years. To perform a second clinical re-examination at the end of the follow-up would have been valuable but unrealistic due to limited financial resources.

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Study design and population

This population-based follow-up was made possible thanks to the far-sighted planning of the prevalence study undertaken in 1973-74 (Axell, T 1976). The present follow-up of a non-patient cohort with clinically confirmed oral health status at base-line is therefore unique.

The 20,333 individuals examined in 1973-74 represented 68% of the total adult population (15 years of age or more at the year of examination) of the area.

Of 60 different kinds of oral mucosal lesion detected in 1973-74, three entities, OLL, OL and SIL were selected for follow-up in the present study. In 1973-74 biopsies were obtained from lesions where there was doubt as to the clinical diagnosis and for validation purposes. Colour photographs were taken of all lesions where the diagnosis was doubtful and of all lesions to be biopsied.

The register-based 27-29 year follow-up was made possible because of the availability of data from essentially complete population and health registers (nationwide registers of the Total Population, Migration, Cancer and Deaths- all in operation for at least 15 years prior to cohort accrual).

For the nearly 30 year follow-up we selected a sub-section of the cohort based on district of residence in 1973-74. This restriction was introduced in order to facilitate the subsequent field-work, when the cohort members were to be re-examined. Individuals who were alive and still residing in the selected areas were offered a clinical re-examination. All the clinical examinations were conducted during the years 1993-94 by the one examiner (AR), following calibration with the 1973-74 examiner (TA).

With a participation of only 68% of the total population (30,118) the representativity of the cohort must be addressed. Efforts to minimize selection bias in the original cross-sectional study, by a random selection procedure among the non-participants including examinations at homes for elderly, hospital wards etc (Axell, T 1976, Axell, T 1987) probably increased the external validity of the follow-up part. However, a “healthy participant effect” was noticeable when disease incidence and mortality rates in our cohort were compared to the corresponding rates in the matching general population. Unless linked to the exposures under study, this expression of a generally healthier lifestyle among participants does not automatically confound our relative risk estimates, but when the general population serves as a reference (as in the standardized incidence ratio – SIR), risk elevations among exposed cohort members tend to be underestimated. Internal comparisons within the cohort are less likely to be affected. Moreover, we cannot exclude the possibility that the factors linked to participation are effect modifiers of the studied associations. Since these factors are undefined and thus not measured, we are unable to confirm or refute the existence of such effect modification.

The long (nearly 30-year) follow-up for cancer and mortality was close to complete thanks to the NRN and the population and health registers used. The Cancer Register is more than 98% complete, i.e. essentially the occurrence of all cancers is recorded (Mattsson 1984, Mattsson 1985). All deaths are recorded in the Cause of Death Register (Mattsson, B 1984, Mattsson, B 1985, Johansson, LA 2000).

The clinical re-examination of OLL, OL and SIL in 1993-94 resulted in a low number of participants 289 (68%) of the 422 invited individuals. This was primarily due to lack of interest and/or time to participate, personal integrity, people moving out from the area, and secondly the limited financial and personnel resources to undertake such a

time-consuming study. Although the remaining participants did not differ markedly from the original sample with regard to recorded background factors, we cannot confidently exclude the possibility that important selection bias was introduced through the losses to follow-up. Moreover, the reduction in the number of subjects investigated resulted in low power in the analyses. This represents a weakness in the clinical part of the study.

6.1.2 Diagnostic criteria and variables

Criteria and classifications of diseases typically evolve over time as the understanding of aetiologies and underlying mechanisms improves. The definitions of the lesions studied were based on state-of-the-art knowledge in 1973-74. Accordingly, the differentiation of OLL types into OLP and OLR was not known at the time of cohort accrual. Studies revealing trigger factors, such as dental materials (Lind, PO 1986, Blomgren, J 1996, Scully, C 1998, Thornhill, MH 2003, Issa, Y 2004), medications (McCartan, BE 1997, Scully, C 1998) and allogenic bone marrow (stem cell) transplants leading to GVHD (Schubert, MM 1984, Mattsson, T 1992) have resulted in suggested somewhat modified diagnostic criteria for OLL. We did not have access to the information needed to re-classify our OLL cases. The question of whether a clinical diagnosis of OLL should be verified by histopathology has been raised, but the poor correlation between clinical and histopathological features of the lesion would seem to limit the value of such verification (van der Meij, EH 2003).

Over the years, diagnostic procedures leading to a true OL diagnosis have been the subject of some debate and are still under discussion. OL is seen as an exclusion diagnosis, as a number of differential diagnoses need to be ruled out before a final diagnosis of OL can be confirmed (e.g. frictional keratosis, chronic candidosis, OLL, and tobacco-induced oral mucosal changes). There are also suggestions that a definitive OL diagnosis can be made only when other potential factors have been excluded and the final diagnosis includes both clinical and histopathological features (van der Waal, I 2002). The recommended diagnostic procedure precludes a correct diagnosis of OL on the basis of a single examination.

For SIL, an important factor is that during the follow-up period there have been changes in the product causing the SILs. The tobacco-specific nitrosamine content of Swedish snus has decreased considerably (Osterdahl, BG 2004). Snus is now also available in pre-packaged, individual portions. The lesions associated with portion-packed snus are less pronounced than those associated with bulk (loose) snus (Andersson, G 1991).

6.1.3 Bias and confounders

In a study like this, a number of biases and confounders could potentially influence the results.

In the following section an attempt is made to list and examine biases and confounders.

Selection bias and the influence of the non-participants:

For the register-based follow-up the selection bias was limited to the original cohort that has been discussed previously. For the cohorts invited for re-examination, attendance was not 100% and the selection that led to invitation was based on individuals alive and living in the areas. This probably influenced the results in that only the healthy and younger persons responded to the invitation.

Misclassification of diagnoses has also been discussed earlier. The lesions selected for this follow-up are such that in most cases several visits are necessary to establish a definite diagnosis. As the initial classification among the subjects in this investigation was based on only one examination, some misclassification of the “exposure” (or initial lesion status) was probably inevitable. In general terms, misclassification of the exposure leads to underestimations of associations with the outcome. Hence, this misclassification could have led to a somewhat underestimated correlation between the occurrence of lesions in 1973-74 and the occurrence in 1993-95. However, the misclassification was kept to a minimum. A validation sub-study in 1973-74 (Axell, T 1976) revealed high rates of agreement between the clinical diagnosis made by the examiner and the final clinical/histopathological diagnosis: 97% for OLL, 93%, for OL, and 100% for SIL.

Misclassification of the outcome could to some extent have been random (or non-differential with regard to exposure status), but also differential (detection bias) if the evaluator in 1993-95 had been aware of the lesion status in 1973-74. This element of differential misclassification might have been somewhat amplified by the calibration efforts of the examiners in 1973-74 and 1993-95. On the other hand, this attention to measurement validity and reliability should have reduced overall misclassification. Moreover, the examiner at the re-examination in 1993-95 was only aware of the fact that the subject to be examined was invited on the basis of having one or more of the selected lesions, but the examiner had no information on the base-line data. Non-differential misclassification of the outcome would tend to attenuate the indices of association between lesion status in 1973-74 and 1993-95, with some modification depending on whether the misclassification led to over- or under-ascertainment (Rothman, K 1998) while differential misclassification in this case probably led to a slight overestimation of the correlation between 1973-74 and 1993-95. Overall, the net effect of the misclassifications is likely to be small. The new criteria that have been established during the follow-up time have not been taken in consideration as the base-line data were not prepared for that.

Although the register-based follow-up for cancer was essentially complete, detection bias could still exist if the exposure status determined the probability of detection of prevalent malignancies. Such a link between exposure status and probability of detection is conceivable if the lesions were noted by the subjects' regular dentists, and if the latter encouraged the subjects to keep these lesions under observation. Detection bias is, moreover conceivable if risk factors for the lesions (like tobacco use) led to higher general morbidity and increased probability of health care contacts. On the other hand, detection bias presupposes recruitment of cancer cases from a pool of indolent malignancies that would otherwise pass unnoticed. Since such indolent cancers are very rare in the oral cavity, and virtually all oral cancers develop into clinically manifest disease, detection bias, if any, with regard to the cancer outcomes is likely to be unimportant.

Information on exposure: The data presented in this thesis are based on one (for the register-based cohorts) and two (for the re-examined cohorts) questionnaire(s) addressing exposure to tobacco, alcohol and medications. This limits the analysis as there is no information on changes over time. Although it is conceivable that subjects with an impending cancer might have changed their habits, potentially leading to exposure misclassification that is differential in relation to the outcome, misclassification of any aetiologically relevant exposure in this prospective cohort study is most likely non-differential. If the misclassification is substantial, this will lead to some underestimation of associations between exposures and outcomes. Non-differential misclassification of confounding factors will lead to less efficient adjustments, and thus to some residual confounding.

A limitation of paper IV is the limited availability of information about potentially confounding factors. We could only control for the factors that had been measured (age, area of residence, tobacco habits and alcohol use) in our analyses. In recent decades, variables such as dietary habits, weight and socioeconomic factors have been shown to be important in the outcomes of oral cancer (La Vecchia, C 1997, Chainani-Wu, N 2002, De Stefani, E 2005, Kreimer, RA 2006).

6.1.4 Inter and intra-observer variation

Prior to the re-examination a calibration was performed between the two examiners (TA) and (AR). The agreement between the two examiners was considered good according to Kappa statistics. In a subsequent blinded evaluation of colour photos of OLL, OL, SIL, the inter-examiner agreement for OLL and OL, measured as Kappa value was 0.74 (95% CI 0.26-1.0). AR's intra-examiner agreement was 0.74 (95% CI 0.26-1.0). For SIL the inter-examiner agreement was substantial with a weighted Kappa value 0.81; 95% CI 0.63-0.98 and AR's intra-examiner agreement 0.85; 95% CI 0.69-1.00. These results are in conformity with inter and intra-observer variability on clinical assessment of OLL (van der Meij, EH 2002).

A definitive strength in this follow up is that any doubtful data presented in the data files could be checked against the original paper charts that are still available. To reduce errors when transferring the information from the charts filled out in 1993-95 the data input was made by one person (AR). The enormous progress in digital techniques since 1973-74 is another aspect that has to be taken in account. Modern computers have almost unlimited memory and the potential to process information in many different ways. Incorrect data (e.g. duplicates, missing/incorrect data) can be detected and excluded, leading to more accurate analyses and estimates.

Small samples in the re-examined cohorts limited statistical analysis to descriptive statistics. For the register-based follow-up the power to detect any statistically significant findings was limited to the whole male cohort described in paper IV.

6.2 FINDINGS

6.2.1 OLL

Our aim was to study the natural history of OLL with both a register-based and a clinical follow-up. The re-examination disclosed that OLL lesions changed both appearance and localisation from baseline in 1973-74. Among the re-examined individuals 35% showed no OLL in 1993-95, indicating a higher remission rate than has been reported earlier (Thorn, JJ 1988). One probable explanation is that we could not make any distinctions between true OLP and OLR; another explanation may be the putative relapsing nature of OLP. With respect to malignant transformation, the clinical re-examination did not reveal any cases. The long-term register-based follow-up showed no statistically significant elevated risk for oral cancer. The expected number of 0.4 and the finding of 1 oral cancer in our small cohort of only 327 individuals did not allow us to draw any conclusions. The findings do not indicate that individuals in a non-patient population who are diagnosed with OLL should be monitored more often than at their regular dental visits.

6.2.2 OL

The aims for OL were the same as for OLL. In 42% of the subjects the OL lesion was not clinically detectable at follow-up. In most such cases, a possible explanation was that the subjects had stopped smoking. Among never-smokers with disappearing OL a misdiagnosis must be considered (e.g. trauma, OL, Candida infection). The question of whether OL also has a putative relapsing nature warrants further investigation and has not been addressed in this thesis. In the register-based follow-up an increased, although not statistically significant, risk for oral cancer was seen among women with OL. Because of the small sample, chance findings cannot be ruled out. With respect to monitoring of individuals diagnosed with OL, the results of the study lead to the same conclusions as for OLL.

6.2.3 *SIL*

In the SIL cohort the aim was to follow the lesions diagnosed in 1973-74 over time. The re-examination revealed that after cessation of snus use the lesion was no longer clinically detectable. This is completely in accordance with previous findings (Andersson, G 1991). This study also supports earlier published data (Andersson, G 1991) disclosing less pronounced lesions among most subjects who had switched to portion-packed snus. We found no evidence of an increased risk for oral cancer at the site of the SIL.

6.2.4 *Snus use*

In Paper IV, inclusion of all men from the study cohort allowed greater precision in the analyses. One finding that does not conform to previous studies on snus is that among those who have ever used snus there seems to be an increased risk, albeit moderate, for oropharyngeal cancer. There is also a statistically increased risk for overall mortality among those who have ever used snus, but with no prior or current history of smoking.

6.3 **CONCLUSIONS**

This population-based follow-up study has shed some light on the natural history of the selected oral mucosal lesions (OLL, OL and SIL) and the possible health consequences of tobacco use. Taking into consideration the advantages and disadvantages in the design of the study, the following conclusions may be drawn with respect to the above lesions:

- There is no support for an increased incidence of oral cancer at the lesion sites.
- Both appearance and location of OLL change over time.
- There is a strong association between OL lesions and smoking.
- The appearance of SIL is strongly associated with snus use and within a year of cessation of the habit the lesion is no longer clinically discernible.
- Use of snus seems to have a negative effect on health. It is associated with some increased risk for oropharyngeal cancer and overall mortality. The product should not be regarded as a harmless alternative to other forms of tobacco.

7 SVENSK SAMMANFATTNING (SWEDISH SUMMARY)

7.1 BAKGRUND

Munslemhinneförändringar är vanligt förekommande i den allmänna populationen och prognos och utveckling över tid är frågeställningar som diskuterats. Resultaten från olika studier varierar avseende både risken för malignisering och huruvida förändringarna är reversibla. Majoriteten uppföljningsstudier är baserade på patientmaterial och populationsbaserade långtidsuppföljningar är mycket ovanliga.

Grunden till det aktuella projektet lades under perioden 1973-74 då en populationsbaserad studie av 20 333 individer bosatta i några kommuner i Uppsala län genomfördes. Studien resulterade i väldefinierade kohorter av individer med respektive utan olika munslemhinneförändringar, liksom av individer med och utan tobaksbruk vid tiden för munhåleundersökningen. Den nu utförda uppföljningen har gett oss en unik möjlighet till att studera en större oselektad population under en lång tid.

7.2 MATERIAL OCH METOD

Av de ursprungliga 20 333 individer har data kunnat säkerställas för 20 212 individer (för 121 individer har data eller journaler gått förlorade eller varit oläsbara).

I delarbete I-IV har data från ett antal register såsom Befolkningsregistret (kontroll av personnummer, datum för emigration), Cancerregistret (diagnos enligt ICD7-kod och datum för diagnos), samt Dödsorsaksregistret (dödsorsak enligt ICD-kod och dödsdatum) använts.

För delarbeten I-III, som behandlar uppföljning av munslemhinneförändringar, har en subpopulation valts ut. Av studietekniska skäl begränsades urvalet till individer som vid tillfället för undersökningen 1973-74 var bosatta i Bålsta, ett urval efter postnummer i Enköping samt i 15 församlingar i landsbygden runt Enköping och Bålsta. Kohorter som följdes upp bestod av individer som vid undersökningen 1973-74 hade registrerade lesioner av oral lichen (delarbete I), oral leukoplaki (delarbete II) eller snus-inducerad lesion (delarbete III).

Den kliniska uppföljningen som ägde rum under perioden 1993-95 utfördes av en undersökare (AR) som kalibrerats med undersökaren från 1973-74 (TA). Undersökningsformulär och enkätfrågor som användes vid uppföljningen var identiska med dem som användes vid ursprungsundersökningen med tillägg för frågor om portionssnus då detta inte fanns 1973-74. Inför undersökningen kontaktades samtliga individer brevledes och tidsbokning skedde via telefon. Undersökningen utfördes på folktandvårdskliniker nära individernas hem/arbetsplats och tog ca 20-30 min. Samtliga kvarvarande lesioner fotograferades. Inför studien hade beslut tagits att remiss till käkkirurgisk klinik skulle skickas i de fall en lesion uppvisade misstanke om malignitet. Inga biopsier togs inom ramen för studien.

Delarbete IV är registerbaserad och populationen som studerats är samtliga män från den ursprungliga kohorten. Register som använts är samma som i arbete I-III.

7.3 DELARBETE I

Denna studie är en uppföljning av 327 (118 män, 209 kvinnor) individer med lichenoida lesioner. Femtiofem individer (18 män, 37 kvinnor) undersöktes kliniskt, och samtliga 327 följdes upp under 27-29 år via registersamkörningar för att fastställa incidensen av olika cancerformer samt mortalitet. Den kliniska uppföljningen visade att 1/3 av de lesioner som registrerades 1973-74 hade försvunnit vid uppföljningen 1993-95. De övriga kvarstod oförändrade eller hade ändrat karaktär vilket är överensstämmande med tidigare publicerade studier: Oral lichen/lichenoida förändringar kan variera i sitt kliniska utseende över tid.

Den registerbaserade uppföljningen visade inte på någon förhöjd incidens av vare sig av oral cancer eller av någon annan cancerform. Inte heller fanns någon ökad risk för mortalitet hos individerna med oral lichen jämfört med den övriga kohorten.

7.4 DELARBETE II

Kohorten består av de 555 (467 män, 88 kvinnor) individer som uppvisade oral leukoplaki 1973-74. En klinisk uppföljning av 67 individer (8 kvinnor och 59 män) har genomförts, samtliga individer har följts upp under 27-29 år via register avseende incidensen av oral cancer (ICD7 141, 143, 144). Kliniskt påvisades en stark koppling mellan rökning och kvarstående leukoplaki. Icke rökare/tidigare rökare var överrepresenterade bland individerna vars OL lesion hade försvunnit i jämförelse med dem som hade kvarstående lesioner (82 % mot 47 %). Samkörningen med cancerregistret gav 3 fall av oral cancer (2 kvinnor och 1 man). Detta var fler än förväntade 0,7 respektive 0,07 men på grund av en alltför låg precision kan inte någon säker konklusion dras om överrisken för oral cancer.

7.5 DELARBETE III

Snusinducerade lesioner hos 1115 män har följts upp under 27-29 år via registersamkörningar för att fastställa incidensen av oral cancer (ICD7 141, 143, 144). I en klinisk undersökning ingick 183 av dessa män. Den kliniska uppföljningen visar att lesionen i hög grad är reversibel när snus vanan upphör. Hos samtliga individer som slutat snusa (n=62) hade lesionen försvunnit.

Registeruppföljningen visade på en något förhöjd incidens av oral cancer bland de 1115 individerna. Lokalisationen för oral cancer var inte i något fall angiven till platsen för den tidigare observerade snusinducerade lesionen.

7.6 DELARBETE IV

Syftet med denna artikel är att studera cancerincidens och mortalitet hos snusande män. Av de 9 976 män som ingick i undersökningen 1973-74 hade 1 559 uppgett att de använde eller hade använt snus dagligen. Hypotesen som testats är om dagligt snus användande ökar risken för cancer respektive tidig död. Endast första registrerade cancer diagnosen ingår i analysen av cancerincidens och de har klassificerats i grupperna oropharyngeal cancer, rökingsrelaterad cancer samt cancer oavsett typ.

Mortalitet analyserades avseende total dödlighet, död i cancer, cirkulatoriska sjukdomar respektive respiratoriska sjukdomar. För de 9 976 män som har studerats har under uppföljningsperioden, som sträcker sig från 1973-74 till 31 januari 2002, 1 575 fall av cancer rapporterats till cancerregistret och 3 630 individer har avlidit enligt dödsorsaksregistret

Cox proportional regression har tillämpats i den statistiska analysmodellen. Efter vederbörlig justering för confounders inklusive rökning och alkoholanvändning fann vi att snusanvändare har en signifikant ökad risk för oropharyngeal cancer och även en ökad mortalitet.

8 ACKNOWLEDGEMENTS

This thesis would not have been possible without the generous support and assistance of many people. I would like to thank my family, supervisors, colleagues, friends and others who have shared this long journey with me.

In particular I am indebted to the following people for their valuable contributions:

My supervisors Tony Axéll, Olle Nyrén and Gunilla Sandborgh Englund, for all your incredible support and encouragement.

Tony, thank you for introducing me to the field of oral medicine and for your unfailing confidence in me. I am especially indebted to you for your foresight with respect to the original study in the 1970's, which enabled the present thesis to become a reality.

Olle, I am grateful to you for generously sharing your expertise in epidemiology, for making it comprehensible and for always keeping your door open. It has been a pleasure working with you.

Gunilla, thank you for encouraging me and never giving up, and being a fantastic figure-maker.

Anna Johansson – statistics can be fun! Thank you for your excellent pedagogic skill, your patience and all the help with organizing data. It has been great working with you. I will not forget to breathe.

Maja Johansson, for your invaluable help during the re-examination procedures. For accompanying me to all the clinics, no matter what the time or the weather. We had a lot of good laughs in the car.

Bo Elander, for your never-failing guidance with the original data, creating forms and programs and for being a constant good friend.

Thore Martinsson, former head of the Department of Oral Diagnostics of the Institute of Odontology, Björn Klinge, former head and Eva Hellsing, present head of the Institute of Odontology, for providing me with the opportunity to participate in the doctoral program.

Hanna Svensson and Li Yin, for good statistical assistance.

Mats Jontell, for all your support and steadfast friendship.

Torsten Mattsson, for taking the time whenever needed.

Anders Hjerne and your colleagues at EpC for introducing me to all your registers.

Joan Bevenius-Carrick for support and arranging all my words so the thesis would be comprehensible.

The staff of the public dental clinics in Bålsta, Enköping, Kallhäll, Sollentuna and St Eriks hospital for generously allowing me to use your facilities at no cost and for being so helpful and supportive.

Colleagues in oral medicine units around Sweden for invaluable support and encouragement.

Colleagues at the Institute of Odontology, Huddinge for all your encouragement.

Sten, my husband, for all your love, support, cooking and for putting up with me.

Essie and Mira for just being there and coming for walks whenever I needed it.

All of my family for their faith in me and in my work.

This project was supported financially by grants from The Swedish Cancer Society, The Swedish Tobacco Medical Research Council, The Swedish Medical Research Council, The Swedish Dental Society and travel grants from the Institute of Odontology and the Swedish Association of Hospital Dentists.

9 REFERENCES

- Agresti, A. *Categorical Data Analysis*: John Wiley & Sons, Inc. New York, 1990.
- Andersson, G. Snuff-induced changes associated with the use of loose and portion-bag-packed Swedish moist snuff. A clinical, histological and follow-up study. *Swed Dent J Suppl* 1991;75:1-89.
- Andreasen, JO. Oral lichen planus. 1. A clinical evaluation of 115 cases. *Oral Surg Oral Med Oral Pathol* 1968;25(1):31-42.
- Axell, T. A prevalence study of oral mucosal lesions in an adult Swedish population. *Odontol Revy* 1976;27(36):1-103.
- Axell, T. Occurrence of leukoplakia and some other oral white lesions among 20,333 adult Swedish people. *Community Dent Oral Epidemiol* 1987;15(1):46-51.
- Axell, T, Holmstrup P, Kramer IRH, Pindborg JJ, Shear M. International seminar on oral leukoplakia and associated lesions related to tobacco habits. *Community Dent Oral Epidemiol*. 1984;12:145-54.
- Axell, T, Mornstad, H, Sundstrom, B. The relation of the clinical picture to the histopathology of snuff dipper's lesions in a Swedish population. *J Oral Pathol* 1976;5(4):229-36.
- Blomgren, J, Axell, T, Sandahl, O, Jontell, M. Adverse reactions in the oral mucosa associated with anterior composite restorations. *J Oral Pathol Med* 1996;25(6):311-3.
- Blomqvist, G, Hirsch, JM, Alberius, P. Association between development of lower lip cancer and tobacco habits. *J Oral Maxillofac Surg* 1991;49(10):1044-7; discussion 1048-9.
- Boyle, P, Ferlay, J. Cancer incidence and mortality in Europe, 2004. *Ann Oncol* 2005;16(3):481-488.
- Chainani-Wu, N. Diet and Oral, Pharyngeal, and Esophageal Cancer. *Nutrition and Cancer* 2002;44(2):104-126.
- Curtis, RE, Metayer, C, Rizzo, JD, Socie, G, Sobocinski, KA, Flowers, ME, Travis, WD, Travis, LB, Horowitz, MM, Deeg, HJ. Impact of chronic GVHD therapy on the development of squamous-cell cancers after hematopoietic stem-cell transplantation: an international case-control study. *Blood* 2005;105(10):3802-11.
- De Stefani, E, Boffetta, P, Ronco, AL, Correa, P, Oreggia, F, Deneo-Pellegrini, H, Mendilaharsu, M, Leiva, J. Dietary Patterns and Risk of Cancer of the Oral Cavity and Pharynx in Uruguay. *Nutrition and Cancer* 2005;51(2):132-139.
- Demarosi, F, Lodi, G, Carrassi, A, Soligo, D, Sardella, A. Oral malignancies following HSCT: graft versus host disease and other risk factors. *Oral Oncol* 2005;41(9):865-77.
- Gandolfo, S, Richiardi, L, Carrozzo, M, Broccoletti, R, Carbone, M, Pagano, M, Vestita, C, Rosso, S, Merletti, F. Risk of oral squamous cell carcinoma in 402 patients with oral lichen planus: a follow-up study in an Italian population. *Oral Oncol* 2004;40(1):77-83.
- Gupta, PC, Bhonsle, RB, Murti, PR, Daftary, DK, Mehta, FS, Pindborg, JJ. An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. *Cancer* 1989;63(11):2247-52.

Gupta, PC, Mehta, FS, Daftary, DK, Pindborg, JJ, Bhonsle, RB, Jalnawalla, PN, Sinor, PN, Pitkar, VK, Murti, PR, Irani, RR, Shah, HT, Kadam, PM, Iyer, KS, Iyer, HM, Hegde, AK, Chandrashekar, GK, Shiroff, BC, Sahiar, BE, Mehta, MN. Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. *Community Dent Oral Epidemiol* 1980;8(6):283-333.

Holmstrup, P. The controversy of a premalignant potential of oral lichen planus is over. *Oral Surg Oral Med Oral Pathol* 1992;73(6):704-6.

Holmstrup, P, Thorn, JJ, Rindum, J, Pindborg, JJ. Malignant development of lichen planus-affected oral mucosa. *J Oral Pathol* 1988;17(5):219-25.

Ingafou, M, Leao, J, Porter, S, Scully, C. Oral lichen planus: a retrospective study of 690 British patients. *Oral Diseases* 2006;12(5):463-468.

Issa, Y, Brunton, PA, Glenny, AM, Duxbury, AJ. Healing of oral lichenoid lesions after replacing amalgam restorations: a systematic review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;98(5):553-65.

Johansson, LA, Westerling, R. Comparing Swedish hospital discharge records with death certificates: implications for mortality statistics. *Int J Epidemiol* 2000;29(3):495-502.

Kramer, IR, Lucas, RB, Pindborg, JJ, Sobin, LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. *Oral Surg Oral Med Oral Pathol* 1978;46(4):518-39.

Kreimer, RA, Randi, G, Herrero, R, Castellsagué, X, La Vecchia, C, Franceschi, S, For the IARC Multicenter Oral Cancer Study Group. Diet and body mass, and oral and oropharyngeal squamous cell carcinomas: Analysis from the IARC multinational case-control study. *International Journal of Cancer* 2006;118(9):2293-2297.

Krutchkoff, DJ, Cutler, L, Laskowski, S. Oral lichen planus: the evidence regarding potential malignant transformation. *J Oral Pathol* 1978;7(1):1-7.

La Vecchia, C, Tavani, A, Franceschi, S, Levi, F, Corrao, G, Negri, E. Epidemiology and prevention of oral cancer. *Oral Oncol* 1997;33(5):302-12.

Lewin, F, Norell, SE, Johansson, H, Gustavsson, P, Wennerberg, J, Björklund, A, Rutqvist, LE. Smoking tobacco, oral snuff, and alcohol in the etiology of squamous cell carcinoma of the head and neck: a population-based case-referent study in Sweden. *Cancer* 1998;82(7):1367-75.

Levitz, JS, Bradley, TP, Golden, AL. Overview of smoking and all cancers. *Med Clin North Am* 2004;88(6):1655-75, xiii.

Lind, PO, Hurlen, B, Lyberg, T, Aas, E. Amalgam-related oral lichenoid reaction. *Scand J Dent Res* 1986;94(5):448-51.

Mattsson, B, Rutqvist, LE, Wallgren, A. Undernotification of diagnosed cancer cases to the Stockholm Cancer Registry. *Int J Epidemiol* 1985;14(1):64-9.

Mattsson, B, Wallgren, A. Completeness of the Swedish Cancer Register. Non-notified cancer cases recorded on death certificates in 1978. *Acta Radiol Oncol* 1984;23(5):305-13.

Mattsson, T, Sundqvist, KG, Heimdahl, A, Dahllof, G, Ljungman, P, Ringden, O. A comparative immunological analysis of the oral mucosa in chronic graft-versus-host disease and oral lichen planus. *Arch Oral Biol* 1992;37(7):539-47.

- McCartan, BE, McCreary, CE. Oral lichenoid drug eruptions. *Oral Dis* 1997;3(2):58-63.
- Osterdahl, BG, Jansson, C, Paccou, A. Decreased levels of tobacco-specific N-nitrosamines in moist snuff on the Swedish market. *J Agric Food Chem* 2004;52(16):5085-8.
- Parkin, MD, Bray, F, Ferlay, J, Pisani, P. Estimating the world cancer burden: Globocan 2000. *International Journal of Cancer* 2001;94(2):153-156.
- Pindborg, JJ, Mehta, FS, Daftary, DK. Incidence of oral cancer among 30,000 villagers in india in a 7-year follow-up study of oral precancerous lesions. *Community Dent Oral Epidemiol* 1975;3(2):86-8.
- Rodstrom, PO, Jontell, M, Mattsson, U, Holmberg, E. Cancer and oral lichen planus in a Swedish population. *Oral Oncol* 2004;40(2):131-8.
- Rosenquist, K, Wennerberg, J, Schildt, EB, Bladstrom, A, Hansson, BG, Andersson, G. Use of Swedish moist snuff, smoking and alcohol consumption in the aetiology of oral and oropharyngeal squamous cell carcinoma. A population-based case-control study in southern Sweden. *Acta Otolaryngol* 2005;125(9):991-8.
- Rothman, K, Greenland, S. Modern epidemiology. 2nd edition: Lippincott Williams & Wilkins, Philadelphia; 1998.
- Salonen, L, Axell, T, Hellden, L. Occurrence of oral mucosal lesions, the influence of tobacco habits and an estimate of treatment time in an adult Swedish population. *J Oral Pathol Med* 1990;19(4):170-6.
- Schepman, KP, van der Meij, EH, Smeele, LE, van der Waal, I. Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. *Oral Oncol* 1998;34(4):270-5.
- Schepman, KP, van der Waal, I. A proposal for a classification and staging system for oral leukoplakia: a preliminary study. *Eur J Cancer B Oral Oncol* 1995;31B(6):396-8.
- Schildt, EB, Eriksson, M, Hardell, L, Magnuson, A. Oral snuff, smoking habits and alcohol consumption in relation to oral cancer in a Swedish case-control study. *Int J Cancer* 1998;77(3):341-6.
- Schubert, MM, Sullivan, KM, Morton, TH, Izutsu, KT, Peterson, DE, Flournoy, N, Truelove, EL, Sale, GE, Buckner, CD, Storb, R. Oral manifestations of chronic graft-v-host disease. *Arch Intern Med* 1984;144(8):1591-5.
- Scully, C, Beyli, M, Ferreiro, MC, Ficarra, G, Gill, Y, Griffiths, M, Holmstrup, P, Mutlu, S, Porter, S, Wray, D. Update on oral lichen planus: etiopathogenesis and management. *Crit Rev Oral Biol Med* 1998;9(1):86-122.
- Shell H, SA. Zur Lokalisationshäufigkeit von benignen und präkanzerösen Leukoplakien und von Karzinomen der Mundhöhle. *Z Hautkr* 1987;62:798-804.
- Silverman, S, Jr., Gorsky, M, Lozada, F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. *Cancer* 1984;53(3):563-8.
- Silverman, S, Jr., Gorsky, M, Lozada-Nur, F, Giannotti, K. A prospective study of findings and management in 214 patients with oral lichen planus. *Oral Surg Oral Med Oral Pathol* 1991;72(6):665-70.

SoS/EpC. The Cause of Death Register. In: The National Board of Health and Welfare; 2001. <http://www.sos.se/epc/english/dorseng.htm>

SoS/EpC. The Swedish Cancer Register. In: The National Board of Health and Welfare; 2003. <http://www.sos.se/epc/english/cancereng.htm>

StataCorp. Stata Statistical Software Release 9. 2005. <http://www.stata.com>

Statistics Sweden. In: Statistics Sweden; 2005. <http://www.scb.se>.

Thorn, JJ, Holmstrup, P, Rindum, J, Pindborg, JJ. Course of various clinical forms of oral lichen planus. A prospective follow-up study of 611 patients. *J Oral Pathol* 1988;17(5):213-8.

Thornhill, MH, Pemberton, MN, Simmons, RK, Theaker, ED. Amalgam-contact hypersensitivity lesions and oral lichen planus. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics* 2003;95(3):291-299.

Tischendorf, L, Giehler, U. [Studies on the quality of leukoplakias of the oral mucosa and lips]. *Dtsch Z Mund Kiefer Gesichtschir* 1990;14(4):301-5.

van der Meij, EH, Schepman, KP, Plonait, DR, Axell, T, van der Waal, I. Interobserver and intraobserver variability in the clinical assessment of oral lichen planus. *J Oral Pathol Med* 2002;31(2):95-8.

van der Meij, EH, Schepman, KP, van der Waal, I. The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;96(2):164-71.

van der Meij, EH, van der Waal, I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. *Journal of Oral Pathology and Medicine* 2003;32(9):507-512.

van der Waal, I, Axell, T. Oral leukoplakia: a proposal for uniform reporting. *Oral Oncol* 2002;38(6):521-6.

WHO. Application of the international classification of diseases to dentistry and stomatology. ICD-DA. Geneva; 1973.

Winn, DM, Blot, WJ, Shy, CM, Pickle, LW, Toledo, A, Fraumeni, JF, Jr. Snuff dipping and oral cancer among women in the southern United States. *N Engl J Med* 1981;304(13):745-9.

Wynder, EL, Hultberg, S, Jacobsson, F, Bross, IJ. Environmental factors in cancer of the upper alimentary tract; a Swedish study with special reference to Plummer-Vinson (Paterson-Kelly) syndrome. *Cancer* 1957;10(3):470-87.

10 APPENDIX 1

Questionnaire for tobacco habits.

Registration number _____

1. Do you use tobacco (smoking, snus use, chewing)?

- Never Occasionally Daily

2. Did you first begin to use tobacco during the last 3 months?

- Yes No

3. Approximately how much tobacco do you use?

- | | | | |
|--------------------------|----------------------------------|--------------|--------------|
| <input type="checkbox"/> | Cigarettes without filter | _____ no/day | Brand? _____ |
| <input type="checkbox"/> | Cigarettes with filter | _____ no/day | Brand? _____ |
| <input type="checkbox"/> | Cigarillos | _____ no/day | Brand? _____ |
| <input type="checkbox"/> | Cigars | _____ no/day | Brand? _____ |
| <input type="checkbox"/> | Pipe, one can (50g) lasts approx | _____ days | Brand? _____ |
| <input type="checkbox"/> | Snus in the mouth approx | _____ h/day | Brand? _____ |
| <input type="checkbox"/> | Snus (50g), lasts approx | _____ days | Brand? _____ |
| <input type="checkbox"/> | Snus portion bags | _____ no/day | Brand? _____ |
| <input type="checkbox"/> | Chewing tobacco, in the mouth | _____ h/day | Brand? _____ |

4. For how long have you

- | | | |
|--------------------------|----------------------------|-------------|
| <input type="checkbox"/> | Smoked? | _____ years |
| <input type="checkbox"/> | Used loose snus? | _____ years |
| <input type="checkbox"/> | Used snus in portion bags? | _____ years |
| <input type="checkbox"/> | Chewed tobacco? | _____ years |

5. Do you use a cigarette holder?

- Yes No

6. If you smoke, do you inhale?

- Yes No

7. If you use snus, do you always put the quid in the same place in the mouth?

- Yes No

8. Have you previously used tobacco daily?

- | | | |
|--------------------------|--------------------------------------|-------------|
| <input type="checkbox"/> | No | |
| <input type="checkbox"/> | Yes, I smoked for | _____ years |
| <input type="checkbox"/> | Yes, I used snus, (loose) for | _____ years |
| <input type="checkbox"/> | Yes, I used snus, (portion bags) for | _____ years |
| <input type="checkbox"/> | Yes, I chewed tobacco for | _____ years |

9. If you have completely stopped using tobacco, when? _____ years ago

Questionnaire for alcohol use

How often have you used alcohol the last 3 months?

	Never	Seldom	Once a week	A few times/week	Daily
Beer	<input type="checkbox"/>				
Wine	<input type="checkbox"/>				
Hard Liquor	<input type="checkbox"/>				