INCIDENCE, SURVIVAL, DIAGNOSTIC DELAYS AND PROGNOSTIC FACTORS IN LARYNGEAL CANCER

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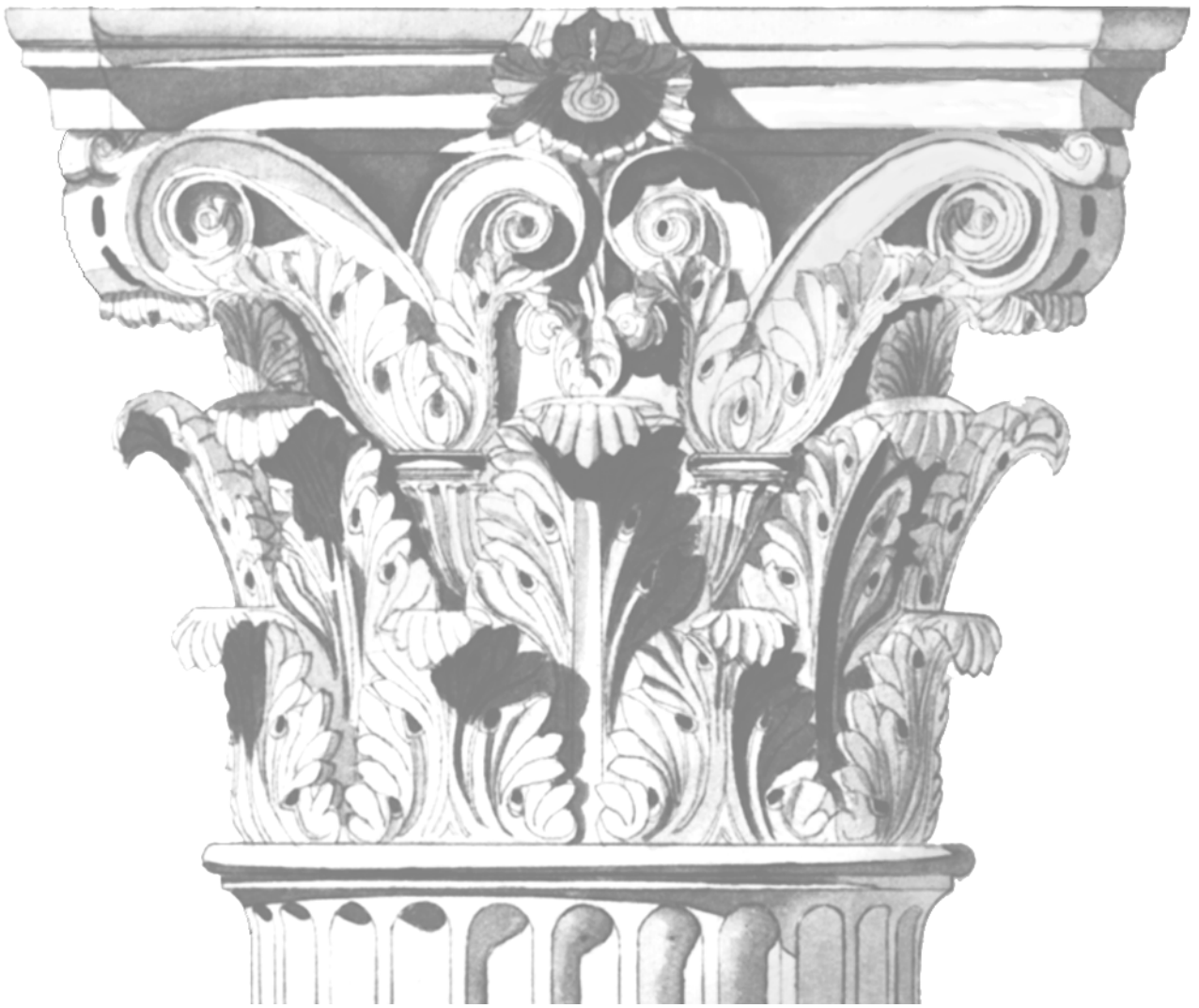

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Abstract

Incidence trends of laryngeal cancer in Finland were analyzed, especially in relation to survival, in a patient series of 5766 patients diagnosed in 1956–ndash1995 and identified from the Finnish Cancer Registry. The age-adjusted incidence rate decreased from 6.5 to 3.5 per 100 000 person-years in males and remained unchanged among females. Only minor improvement occurred in survival. In a hospital-based material from Northern Finland (353 patients with laryngeal squamocellular carcinoma, LSCC, diagnosed in 1976–ndash1995), the incidence among males decreased only for supraglottic cancer, diminishing the supraglottic to glottic incidence ratio from 1.4:1 to 0.5:1.

Evaluation of diagnostic delays and their impact on survival and risk of recurrence was undertaken in a sample of 66 LSCC patients. In only 38% of the patients was malignancy suspected at the initial visit to a physician; infection was the most common misdiagnosis (41%). Half of the first consultations resulted in referral, whereas 17% of the patients were neither referred nor controlled. The median patient delay was 2 months and median professional delay 3 months. The latter exceeded 12 months in 17% of the patients. The delays were not significantly related to any other clinical parameter, nor were they interrelated. Professional delay of 12 months or more resulted in increased relative hazard of death (HR = 4.74, p = 0.05), equalling the effect of advanced stage (stage IV).

One-third of the patients developed a recurrence. In univariate analysis, professional delay of 12 months or more increased the risk of local (p = 0.019) and neck (p = 0.019) recurrence. In a multivariate model, professional delay of 12 months or more indicated an adjusted relative hazard ratio (HR) of 4.6 for local recurrence (p = 0.02) and 9.5 for neck recurrence (p = 0.015).

Immunohistochemical factors p53, apoptosis, angiogenesis and proliferation were included in a multivariate model evaluating prognostic factors of LSCC in addition to clinical and sociodemographic factors. Advanced stage (stages III–ndashIV) (relative hazard ratio of death (HR) 8.9, p = 0.01), supraglottic site (HR 5.6, p = 0.02) and high apoptotic index (≥ 0.3) (HR 11.1, p = 0.05) were the best indicators of impaired prognosis.

Professional delay and enhanced apoptotic rate could be helpful in selecting LSCC patients for more aggressive primary treatment.

Keywords: apoptosis, diagnostic delay, incidence, laryngeal neoplasms, prognostic factor, survival

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The present study was carried out in the Department of Otolaryngology, Oulu University Hospital, during the years 1999-2002. This dissertation was completed during the spring 2003, while the author was working in the Department of Otolaryngology of Kanta-Häme Central Hospital in Hämeenlinna, Finland.

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Abbreviations

CI	confidence interval
ENT	ear, nose and throat
HPF	high-power field
HR	hazard ratio
Ki-67	nuclear antigen associated with cell proliferation
LSCC	laryngeal squamocellular carcinoma
MIB-1	antibody against Ki-67, used in Ki-67 staining
MVD	microvascular density, parameter to express the extent of angiogenesis
N, N0-3	N class (neck node status) in TNM classification
p53	tumour suppressor gene p53 protein
RSR	relative survival rate
SCC	squamocellular carcinoma
SD	standard deviation
SIR	standardised incidence ratio
T, T1-4	T class (extent of primary tumour) in TNM classification
TNM	classification of the clinical stage of the tumour
UADT	upper aerodigestive tract

List of original papers

This thesis is based on the following publications, which are referred to in the text by Roman numerals (I-IV), and on some previously unpublished data.

I Teppo H, Koivunen P, Sipilä S, Jokinen K, Hyrynkangas K, Läärä E, Pukkala E. Sovio U & Alho O-P (2001) Decreasing incidence and improved survival of laryngeal cancer in Finland. Acta Oncol 40:791-795.

II Teppo H, Koivunen P, Hyrynkangas K & Alho O-P (2003) Diagnostic delays in laryngeal carcinoma: professional diagnostic delay is a strong independent predictor of survival. Head Neck 25:389-394.

III Teppo H, Hyrynkangas K, Koivunen P, Jokinen K & Alho O-P (2003) Impact of patient and professional diagnostic delays on the risk of recurrence in laryngeal carcinoma. Submitted.

IV Teppo H, Soini Y, Melkko J, Koivunen P & Alho O-P (2003) Prognostic factors in laryngeal carcinoma: the role of apoptosis, p53, proliferation (Ki-67) and angiogenesis. APMIS 111:451-457.

Contents

1 Introduction

Cancer of the larynx practically always refers to laryngeal squamocellular carcinoma (LSCC). It is typically a disease of elderly smoking men. Even though LSCC is the most common head and neck malignancy, it comprises approximately only one percent of all new cancer cases in Finland. The trends in the incidence of LSCC in Finland have been exceptional among the western countries. In the first half of the twentieth century, supraglottis was the most common anatomical site of LSCC in Finland in contrast to the rest of the western world, where glottic cancer predominated. During the latter half of the century, a gradual switch from surpraglottic to glottic cancer has taken place in Finland parallel to a striking decrease in overall incidence among males. Meanwhile, many western countries have witnessed a relatively uniform increase in the incidence of LSCC. Subglottic cancer of the larynx is rare.

The overall prognosis of LSCC is relatively good compared to head and neck cancers in general. However, the treatment options available, especially those for advanced disease, are often detrimental, causing severe impairment of the quality of life. This warrants a search for more conservative, yet equally efficient treatment regimens. Also, methods for detecting these cancers at an earlier stage and for distinguishing the cancers with more aggressive behaviour and worse prognosis from the others are being sought since, despite the general improvement in cancer care during the past decades, the survival of LSCC has not improved significantly.

In spite of a multitude of published studies, our knowledge about the prognostic factors of LSCC has not markedly increased during the past decades. Even today, the disease stage at the time of diagnosis and the anatomical subsite of the tumour are the only solid and practical prognostic factors and tools in choosing the treatment protocol for LSCC. A wide variety of histopathological factors have been studied as potential indicators of poor prognosis and, consequently, aggressive treatment, but with conflicting results.

This study was conducted to explore the exceptional trends of the incidence of LSCC in Finland, especially in relation to survival trends. In addition, the present study aimed to increase our knowledge of the prognostic factors of LSCC and to find potential indicators of poor prognosis, which could be helpful in clinical decision-making in the future.

2 Review of the literature

2.1 Laryngeal cancer

2.1.1 Incidence

In the mid-twentieth century, Finland had the highest incidence of laryngeal cancer among the Nordic countries (1), and in contrast to the other western countries, supraglottic site of origin was more common than glottic, i.e., the vocal cords (2,3). Since the early 1970´s, the incidence of laryngeal cancer among males has decreased significantly in Finland: According to the Finnish Cancer Registry, the mean annual number of new laryngeal cancer cases among males decreased from 159 to 105 between 1968-1972 and 1993-1997 (4). In a study made in the Tampere University Hospital District, the age-adjusted incidence rate of laryngeal cancer among males decreased from 6.7 to 2.6 per 100 000 during the period 1962-1991 (5). Finland seems to be the only western country experiencing a decreasing trend in laryngeal cancer incidence in males (6,7). Increases in the incidence rates of laryngeal cancer among males have been reported from Sweden (8), Denmark (9), Canada (10), United States (11), United Kingdom (12), Switzerland (13) and Italy (14). The increase in the incidence has been especially alarming in Eastern and Southern Europe (15). This exceptional trend in Finland has generally been attributed to the decrease of smoking among Finnish males. Between 1960 and 1981, the proportion of adult daily male smokers dropped from 58% to 37% (16). The decrease in the incidence rate among Finnish males has been almost exclusively due to a decrease in the incidence of supraglottic cancer: according to Raitiola and Pukander, the ratio of glottic to supraglottic incidence increased from 0.5:1 to 1.9:1 among males between the years 1962 and 1991 (5). Since the beginning of the 1980´s, glottic cancer has also been more common in the epidemiological studies conducted in South-western Finland (17) and in the whole country (18). Thus, the subsite distribution of laryngeal cancer in Finland is nowadays in accordance with that prevalent in the other western countries.

Among females, the epidemiology of laryngeal cancer is completely different. Female cancer patients constitute approximately only 15% of the whole patient population (19).

In Finland, only 10-20 new female laryngeal cancers were diagnosed yearly in the 1990´s, equalling an age-adjusted incidence rate of 0.3 per 100 000, compared to over 100 patients yearly and an incidence rate of 2.6 in males in 1999 (4). In contrast to males, the incidence rate among females has not changed during the last few decades in Finland (5,18), with a consequent slight increase in the proportion of female patients. Among females, the increase in the incidence rate seen in the other countries has been even more pronounced, presumably due to the widely reported increase in female smoking in the western countries (7,10-12,20,21). Yet another dissimilarity between males and females is the subsite distribution of laryngeal cancer. Among females, the proportion of supraglottic cancer is clearly higher than that among males (12,22-24). In a study by Grénman *et al.* from South-western Finland, the male to female ratio was 48:1 in glottic and 4:1 in supraglottic cancer in 1981-1990 (17).

Smoking is widely accepted to be the major causative agent of laryngeal cancer. Thus, explanations for the changing laryngeal cancer trends are often sought from changes in smoking habits. In Finland, there is no controversy between the smoking and laryngeal cancer trends. In the 1920´s, smoking among Finnish males was estimated to be more common than anywhere else in the world (25), and in 1950, 76% of adult males and 13% of adult females were daily smokers (26). Since then, a 2.6-fold decrease in males and a 1.5-fold increase in females have occurred in these figures: in 2001, 29% of adult males and 20% of adult females smoked daily (27). This trend is clearly reflected in the abovementioned incidence rates of laryngeal cancer. The incidence trends in lung cancer, another well established tobacco-related malignancy, have been similar to those in laryngeal cancer in Finland: since the early 1970´s, the incidence rate has been steeply decreasing among males and slightly increasing among females (1,4). Since the reduced incidence of male laryngeal cancer has been largely attributable to a decrease in supraglottic disease, this subtype is speculated to be even more intimately related to the patients´ smoking history (28-30). In some other countries, laryngeal cancer incidence is on the rise despite a decreasing incidence of lung cancer and decreasing – or steady – tobacco smoking, suggesting other possible significant causes of laryngeal cancer, such as alcohol consumption (15,31). In Finland, the consumption of alcohol per capita has been rising continuously in the whole country and among both sexes during the past 30-40 years (32,33), making changes in drinking patterns a less plausible explanation for the trends of laryngeal cancer in Finland. A small minority of laryngeal cancers develop without a history of smoking or alcohol use; in a study by Agudelo *et al.*, 3% of 933 laryngeal cancer patients belonged to this group (34).

2.1.2 Survival

Since more than 95% of primary laryngeal malignancies are squamocellular carcinomas, and the other rare histological types have entirely different epidemiological and clinical characteristics (19), the rest of this thesis will concentrate specifically on laryngeal squamocellular carcinoma (LSCC), unless otherwise specified.

The prognosis of LSCC is relatively good, the second best among head and neck cancers following lip cancer (35). In Finland, the 5-year relative survival rate (RSR, ratio of

observed to expected rates) was 62% for males and 58% for females in 1985-1994 (36). The overall survival rate has increased only slightly since the 1960´s, and among patients with advanced cancer, survival has not improved at all (36,37). In Sweden, the 5-year RSR among males fluctuated between 62% and 72% in 1961-1987, and no increase in long-term survival was detected (8). Decreasing survival trends during the last few decades have been reported from Switzerland (13) and Canada (10), steady survival rates from Great Britain and Australia (20) and improving survival from Italy (14).

The prognosis of LSCC varies considerably between different patient groups and different tumour types (19,38-40). The current data on the effects of clinical, sociodemographic and histopathological factors on survival are presented in detail in Chapter 2.2.

2.1.3 Diagnostic delays

Commonly, patient delay refers to the interval between the onset of symptoms due to the disease and the first subsequent visit to a health professional, while professional delay refers to the interval between this initial consultation and the diagnosis of LSCC (41-43). The sum of these intervals is the total, or overall, diagnostic delay, which has also been referred to as the "duration of symptoms" (44,45). The reported delays vary to some extent between authors, partly because of the heterogeneity of the study populations; in many studies evaluating diagnostic delays, LSCC patients are analysed in conjunction with other head and neck or upper aerodigestive tract (UADT) cancer patients. Among their patients with LSCC, Pera *et al.* observed a median overall delay of 12 months (44), whereas in a Finnish study by Raitiola and Pukander, the median delay was 4 months (45). Habermann *et al.* differentiated between the patient and professional delays in LSCC and reported median durations of 2.5 months and 1 month for patient and professional delays, respectively (42). Among head and neck cancer patients, other than cancers of the lip and oral cavity, the patient delay noted by Amir *et al.* was as short as 3 weeks (43). In a study by Allison *et al.*, including all UADT cancer patients, the median patient delay was 2 months (range 0-18 months) and the median professional delay 1 month (range 0-12 months) (41). Finally, Koscielny *et al.* found patient delay to be twice as long in LSCC as in other head and neck cancers (46).

The reasons for patient delay are not well understood. According to Hackett (47), delay in seeking medical help among cancer patients appears to be a conscious and deliberate choice rather than a failure to comprehend the situation and its consequences, suggesting denial as a potential cause for the long patient delay. Patients with higher educational or social status sought medical attention significantly sooner than those less privileged. On the other hand, Guggenheimer *et al.* found no correlation between patient delay and educational level or any other sociodemographic characteristic among patients with oral or oropharyngeal cancer (48). Professional diagnostic delay is considerably easier to assess accurately, and its reasons are considerably easier to analyse, at least in LSCC. Preliminary diagnosis of a laryngeal neoplasm requires visualisation of the larynx, and this is considered difficult by primary health care physicians (49-52). In a study by Hoare *et al.*, general practitioners reached a sensitivity of 46% and a specificity of 24% in diagnosing cancer among patients with laryngeal symptoms (50). According to Klein, it was custo-

mary among general practitioners not to do mirror laryngoscopy when conducting a physical examination (51), largely because more than 70% of practising primary physicians were unable to perform it (52). Even for ENT specialists, visualisation of the larynx is not always easy: Barker and Dort found 52% of the mirror laryngoscopies performed by an ENT specialist to be successful (53), and in a study by Robinson and Weir, two ENT specialists agreed in 75% of cases on whether the finding was normal or pathological (54). In addition to the difficulties in visualising the larynx, the most common initial symptom of LSCC patients, hoarseness of voice (39,45), is quite a common complaint, especially among smokers (49). Of the patient characteristics, the presence of a comorbidity increased and older age and higher education reduced the risk of long professional delay among UADT cancer patients according to Allison *et al.* (55).

The significance of diagnostic delay in head and neck cancer is under debate. Since clinical stage at the time of diagnosis is by far the most important prognostic factor in LSCC (19,38-40), long diagnostic delay could be thought to worsen survival by resulting in more advanced stage at presentation. However, this has not been the case. In studies by Pera *et al*. (44), Allison *et al*. (41) and Raitiola and Pukander (45), no correlation between the delays and the prognosis of LSCC could be detected, although in the latter, a longer overall delay was associated with advanced stage. In the abovementioned study by Allison *et al*., a significant correlation between a professional delay of 1 month or more and late-stage disease was achieved when LSCC patients were pooled together with other UADT cancer patients (41). In oral and oropharyngeal cancer, diagnostic delay did not influence the clinical stage according to Guggenheimer *et al*. (48) and Kowalski *et al*. (56). In a study by Vernham and Growther, too, only 28% of head and neck cancer patients with advanced stage disease had had symptoms for more than 3 months, which further indicates that diagnostic delay and clinical stage are not significantly correlated (57). All these studies suffered from the methodological limitation of assessing the diagnostic delays from hospital records or by interviewing the patients personally or via a questionnaire after the diagnosis of malignancy had been set. Even though diagnostic delay in cancer is virtually impossible to study prospectively, a more sound methodology was used by Koivunen *et al*. (58), who determined the onset of symptoms from primary health care charts, where the clinical data concerning the initial consultation had been recorded prior to the cancer diagnosis. They found a significant correlation between longer patient delay and poor survival in patients with pharyngeal cancer. Unrelated to the diagnostic delays discussed above, another type of delay has been shown to have prognostic significance in head and neck cancer: delay in the initiation of oncologic treatment after the diagnosis clearly impaired the prognosis, especially in advanced cancer, according to Kowalski and Carvalho (59). Finally, diagnostic delay in LSCC is apparently becoming an important issue for other than clinical reasons, at least in the United States, where 83% of the malpractice lawsuits concerning LSCC are filed because of alleged delay in diagnosis (60)!

How could a shorter diagnostic delay and thus an earlier diagnosis in LSCC be achieved? Olofsson suggests a screening program for groups at high risk for LSCC (61). According to O´Hara and Bradley, screening of asymptomatic high-risk patients could lead to an improvement in survival in UADT cancers in general, but in LSCC, hoarseness is relatively a common first symptom and usually emerges when the disease is still at an early stage, thus reducing the need for undirected screening (62). Klein emphasises the importance of awareness of primary health care physicians and the call for better diagnos-

tic skills, either by learning to perform mirror laryngoscopy or by using fibre optics or rigid endoscopes to visualise the larynx (51,52).

2.1.4 Recurrences and second primary cancers

Disease recurrences and second primary malignancies are common in LSCC and in UADT cancers in general, presumably due to continuous exposure to common causative factors, smoking and excessive alcohol consumption (63-65). According to Franco *et al.*, patients diagnosed with UADT cancer experience a 10-fold risk of additional cancer related to tobacco and/or alcohol than the general population (65). The issue of recurrences and second primary malignancies in UADT cancers is complicated because of the diverse terminology and the difficulties in determining the temporal associations between individual tumours. There are no practical means to define whether a newly found squamocellular carcinoma in the head and neck region after a diagnosed and treated UADT index cancer is, in fact, a recurrence or a delayed metastasis of the index tumour or a second primary disease. However, some consensus has been reached: in the case of new squamocellular carcinoma in the larynx after the treatment of index LSCC, the tumour is usually considered a local recurrence if it occurs within 3 years after the treatment of the index tumour, whereas later occurrences are considered new primary LSCCs (66,67). Kuriakose *et al.* studied yet another problematic situation of simultaneously presenting head and neck and lung cancers and used the following criteria (68): The lesions are called synchronous tumours if they are both malignant by histological criteria and are anatomically distinct and separate. Among these synchronous primary tumours, the lesions are called simultaneous primary tumours rather than metastatic disease if the tumours are histopathologically different and/or the lung tumour is present without cervical adenopathy. Obviously, however, all this nomenclature – whether a local new disease is a recurrence or a new primary tumour, and whether a distant new disease is a delayed distant metastasis (or recurrence) or a new primary tumour – is based on negotiated agreements and is thus more or less artificial.

The incidence of recurrent LSCC is well documented. In a study by Brenner *et al.*, 20% of LSCC patients experienced a recurrence during a median follow-up time of 5 years (63). The majority (77%) were local recurrences, whereas neck and distant recurrences were clearly less common (12% and 11%, respectively). Spector *et al.* reported similar incidences of neck and distant recurrences in a study following 2550 patients with laryngeal and hypopharyngeal SCC for 5 years (69). In a study by Thomas *et al.*, 24 of 106 early (T1) glottic carcinomas developed a local recurrence, but in 10 cases the recurrence appeared more than 3 years after the treatment for the index tumour, and one of the recurrences appeared in the contralateral vocal cord, thus indicating a true local recurrence in only 13 (12%) of the patients (66). In advanced (T3-4) LSCC, an overall recurrence rate of 38% has been reported; two-thirds of these were local failures. Surgery as primary treatment yielded better results in terms of disease control compared to radiation (70). Parastomal recurrence after laryngectomy was studied by Ferlito *et al.* in a review encompassing nearly 17 000 patients with LSCC. They reported the overall incidence of parastomal recurrence to be 5.1%; 80% of the parastomal recurrences took place within 1

year and 94% within 2 years after laryngectomy. The prognosis of these patients was dismal (71). In a series by Kowalski *et al.* (72), parastomal recurrence was rare, but the most lethal complication of LSCC.

The major risk factors for recurrence of LSCC are advanced T stage and supraglottic disease for local and neck recurrence and positive neck nodes for distant recurrence (72,73). Continuation of smoking after the treatment of primary LSCC has also been a significant predictor of recurrence. According to Moore, 40% of those who continued to smoke developed a recurrence or a second primary UADT cancer compared to 6% of those who managed to stop smoking (74). On the other hand, McGuirt and Ray (67) could not confirm this finding.

In head and neck cancers in general, the incidence of recurrent disease is very similar to that in LSCC. Leemans *et al.* reported a local recurrence rate of 12% during a follow-up period of at least 5 years (75); 90 % of the local, regional (neck) and distant recurrences occurred within 2 years after the primary treatment. In a study by Boysen *et al.*, 33% of the patients with SCC of the head and neck developed a recurrence (76).

As mentioned earlier, second primary malignancies, especially UADT cancers, are also common after treatment of LSCC. In Denmark, among patients with early (T1) LSCC, 34% of patients with supraglottic and 23% of those with glottic disease developed a second primary cancer, the most common sites being the head and neck region and the lung. In the glottic group, second primary cancers were a more common cause of death than the original LSCC (77). In a study by McGuirt and Ray, 5% of the LSCC patients at risk of acquiring a new primary LSCC (i.e., those who had retained their larynx and lived for more than 3 years after the primary diagnosis) developed a second local LSCC (67). In a Swiss survey (64), LSCC patients had a significantly increased risk of second primary cancer (standardised incidence ratio (SIR) 3.0), and the most common sites for these second cancers were the oral cavity, pharynx, oesophagus and lung. Kuriakose *et al.* noted a 5% incidence of simultaneous lung cancer among patients with mucosal head and neck cancer (68).

Accordingly, the risk of developing LSCC after treatment of other UADT cancers also increases. Patients with oral cancer, which is closely related to smoking and alcohol consumption, similarly to LSCC, have been reported to show a 4- to 7-fold increase in the incidence of respiratory cancer (78). In a Finnish study, lip cancer patients experienced a 2-fold risk (SIR 2.0) of LSCC (79). In another study, 1% of patients with oral SCC developed LSCC during a median follow-up of 3 years (80).

2.1.5 Treatment

The treatment protocol for cancer of the larynx is based on the clinical stage of the disease at the time of diagnosis and the anatomical subsite of the tumour, although some variation exists between countries and head and neck centres (19). In Oulu University Hospital (in Northern Finland), the treatment is administered according to the generally accepted guidelines described here in a considerably simplified form.

T1N0 tumours are treated with local radiotherapy or laser resection and T2N0 tumours with radiotherapy (in supraglottic cancer, the neck is also irradiated). Glottic T3N0

tumours are treated with local and bilateral neck radiotherapy, whereas patients with supraglottic T3N0 disease and all patients with T4N0 tumours undergo laryngectomy with local pre- or postoperative radiotherapy and bilateral neck irradiation. In T1-2 tumours with positive neck nodes (clinically or radiographically), the treatment consists of local radiotherapy and neck dissection on the affected side. Finally, T3-4 tumours with positive neck nodes are treated with laryngectomy, neck dissection on the affected side, and pre- or postoperative radiotherapy together with bilateral neck irradiation.

Nowadays, certain large primary tumours are treated with chemoradiotherapy, with or without subsequent surgery. However, this treatment option was not available during this study.

2.2 Prognostic factors of LSCC

2.2.1 Sociodemographic factors

There are so many interactions between age, gender, educational and social status, access to health care, smoking habits and alcohol consumption that the individual impacts of these factors are very difficult to determine (81).

The effect of patients´ age on the prognosis of LSCC has been studied in detail, but with conflicting results. Lefebvre *et al.* could not establish a significant difference between age groups in terms of cancer-related mortality (81). Similarly, in a multivariate model by Pera *et al.* (44), age did not have an independent impact on the prognosis, even though higher age significantly worsened survival in univariate analysis. On the other hand, Boffetta *et al.* (82) found advanced age to be an independent predictor of poor outcome in a multivariate analysis (hazard ratio of death (HR) 3.4 for patients between 60 and 69 years and 8.9 for patients older than 70 years), as did Kowalski *et al.* (72), and surveys from Finland (36) and Sweden (8), which used relative survival rate (RSR) as an indicator of survival, taking age-related increased mortality due to other causes into account. In summary, age seems to be an independent prognostic factor in LSCC.

Gender does not seem to be an important determinant of prognosis in LSCC. Even though Boffetta *et al.* found a significant difference in favour of women (82), studies by Robbins (83), Kowalski *et al.* (72), Dickman *et al.* (36), Stenbeck and Rosén (8) and Lefebvre *et al.* (81) failed to reveal a relationship between gender and survival in LSCC.

Socioeconomic status most likely has different implications in different studies, and it is probably the most difficult parameter to assess in the case of head and neck cancer patients. Unemployed, poorly educated and economically less privileged individuals are clearly over-represented among these patients (81). The matter is further complicated by the well-established correlations between this patient group and smoking, excessive alcohol consumption and a tendency to delay seeking medical help (47,81). Among the few studies including socioeconomic status in a multivariate model evaluating prognostic factors in head and neck cancers, Boffetta *et al.* (82) and Lefebvre *et al.* (81) found low socioeconomic status to be significantly related to poor survival, even though the latter

authors raised suspicion on this finding, suggesting other comorbidities linked with smoking and alcohol to be at least partly responsible.

The effect of smoking status on LSCC survival is also difficult to evaluate, since a non-smoking patient with laryngeal malignancy is a rarity (34). However, virtually all studies made on this topic have shown smoking to be a predictor of poor prognosis (34,44,74,81,82,84,85). However, different authors explain this phenomenon differently: Moore (74) reported a substantial increase in the risk of recurrence or second primary cancer among patients who continued to smoke after primary treatment, while Browman *et al.* (84) and Lefebvre *et al.* (81) reported that patients who continued to smoke during treatment of LSCC experienced worse survival because of the increased frequency of complications and the reduced efficacy of treatment. Agudelo *et al.* (34) found the impaired prognosis of smokers to be linked to their less favourable subsite distribution (larger proportion of supraglottic tumours), whereas Crosignani *et al.* (85) detected a dose-related relationship between heavy smoking and poor outcome.

Alcohol consumption has yielded results similar to those for smoking, and largely in the same studies. Excessive drinking impaired the prognosis of LSCC by being related to a higher proportion of supraglottic cancers (34) and by compromising the outcome of treatment (81). Some studies found no correlation between drinking patterns and survival (82,85).

2.2.2 Clinical factors

The extent, or clinical stage, of the disease at the time of diagnosis is by far the most significant determinant of prognosis in LSCC (19,36,39,44,72,81). As in most other malignant neoplasms, stage is commonly expressed in terms of the TNM classification (86), in which T class refers to the extent of the primary tumour, N class to the nodal status of the neck, and M class to the possible presence of distant metastases (Table 1).

T class, or the size and extent of the primary laryngeal lesion, carries a significant prognostic weight (44). Boffetta *et al.* observed 5-year survival rates of 65% and 50% for T1-2 and T3-4 tumours, respectively (82). In a study by Kowalski *et al.*, T2-3 tumours involved a hazard ratio of death (HR) of 1.6 and T4 tumours a HR of 3.3, compared to T1 lesions (72). According to Lefebvre *et al.*, the difference in survival is not significant between T1 and T2 or between T3 and T4, but is notable and significant between T1-2 and T3-4 tumours (81).

Lymph node involvement in the neck is certainly the most important individual clinical prognostic factor in LSCC (38,44,81,82). In head and neck cancers in general, the presence of at least one palpable node reduced the 5-year survival to half or less compared to N0 patients (81). In the same series, the number of nodes also turned out to be a significant determinant of prognosis: 5-year mortality increased from 65% to 84% when the number of neck nodes exceeded 3. Positive neck node status also clearly increases the risk of distant metastases in LSCC (73). The burden of neck lymph node metastases, measured by radiographically calculated tumour volume, has also shown some prognostic significance (87).

Table 1. TNM classification for clinical staging of laryngeal carcinomas used in this study (simplified) (International Union Against Cancer, 1987) (86).

T – Primary tumour	TX Primary tumour cannot be assessed
	T0 No evidence of primary tumour
	Tis Carcinoma in situ
	Supraglottis
	T1 Tumour limited to one subsite of supraglottis, normal vocal cord mobility
	T2 Tumour invades more than one subsite of supraglottis or glottis, normal vocal cord mobility
	T3 Tumour limited to larynx with vocal cord fixation and/or invades postcricoid area, pre-epiglottic tissues or deep base of tongue
	T4 Tumour invades through thyroid cartilage and/or extends into sof tissues of the neck, thyroid and/or oesophagus
	Glottis
	T1 Tumour limited to vocal cord(s), normal vocal cord mobility
	T1a Tumour limited to one vocal cord
	T1b Tumour involves both vocal cords
	T2 Tumour extends to supraglottis and/or subglottis, and/or impaired vocal cord mobility
	T3 Tumour limited to larynx with vocal cord fixation
	T4 Tumour invades through thyroid cartilage and/or extends to other tissues beyond the larynx
N – Regional lymph nodes	NX Regional lymph nodes cannot be assessed
	N0 No regional lymph nodes
	N1 Metastasis in a single ipsilateral lymph node, ≤3 cm
	N2a Metastasis in a single ipsilateral lymph node, >3 cm, ≤6 cm
	N2b Metastasis in multiple ipsilateral lymph nodes, ≤6 cm
	N2c Metastasis in bilateral or contralateral lymph nodes, ≤6 cm
	N3 Metastasis in a lymph node, > 6cm
M – Distant metastasis	MX Distant metastasis cannot be assessed
	M0 No distant metastasis
	M1 Distant metastasis

Clinical extent of the disease can be expressed as stage groups (I-IV) in line with the TNM classification (86). Stage I refers to T1N0M0 and stage II to T2N0M0, stage III includes T3N0M0 and T1-3N1M0, and finally, stage IV includes all T4 tumours, all N2-3 tumours and all M1 tumours. In an even shorter commonly used classification, stages I-II refer to early stage cancer and stages III-IV to late, or advanced, stage cancer. These classifications are simple to use, but unfortunately also oversimplify the issue. Nevertheless, some studies evaluating the prognostic effect of disease stage in LSCC have been made using this kind of staging. In Finnish Cancer Registry material from the years 1985-1994, the 5-year relative survival rates of LSCC in males were 75%, 27% and 13% for local, regional (confined to the neck) and distant cancers, respectively (36).

The anatomical subsite of the primary tumour is another undisputedly significant prognostic factor in LSCC. Among the two most common subsites, supraglottic cancer is associated with a markedly worse survival (35,44,72). In every T class, the 5-year survival rate is approximately 10% lower among patients with supraglottic disease compared to those with glottic cancer (39). Raitiola *et al.* (29) reported 5-year disease-specific survival rates of 81% and 70% and Silvestri *et al.* (23) 3-year adjusted survival rates of 83% and 46% for glottic and supraglottic cancers, respectively. This is due to at least two factors. Even minuscule anatomical changes in the vocal cords usually present as hoarseness

of voice, thus allowing early detection and a higher cure rate (40); in a Finnish study, the proportion of early stage lesions was significantly higher in glottic cancer (29). Secondly, lymphatic drainage is minimal in the vocal cords, delaying the metastatic spread of a glottic primary tumour (19). Jakobsen *et al.* found only 1% of patients with glottic LSCC to have primary lymph node metastases at diagnosis, compared to 29% in the supraglottic group (87). This higher incidence of neck involvement in supraglottic cancer has been repeatedly confirmed (23,29,38,73). Subglottic cancers, even though rare (under 1% of the total), also tend to metastasise and have poor prognosis (39).

The treatment protocol for LSCC is commonly based on the clinical stage and the anatomical site of the tumour, even though some variation exists between countries and head and neck centres (19,88). In general, it seems that surgery results in a superior outcome compared to radiation in advanced primary tumours. In supraglottic LSCC, the results of primary surgery and radiation were identical among patients with T1-3N0 tumours, but surgical treatment of T4N0 tumours was superior to radiation (5-year survival of 56% versus 14%) (19). Similarly, in glottic cancer, surgery and radiation yield comparable results in T1-2 tumours, whereas T4 lesions should be treated with radical surgery. In glottic T3 cancer, there is no consensus about primary treatment (40,89,90). A trend towards nonsurgical treatment and more conservative, function-preserving surgical techniques is gaining popularity, and its effects on the long-term survival of LSCC patients remain to be seen (91).

Some other clinical parameters that have been suggested as clinically useful prognostic factors in LSCC include the patient's performance status (81), blood haemoglobin level (3), endophytic or exophytic appearance of the tumour (81), ulceration of the neoplasm (44), extracapsular spread of neck node metastases (72) and tumour volume of the primary neoplasm (92).

2.2.3 Histopathological and immunohistochemical factors

Among the histopathological methods, only assessment of the degree of histological differentiation is in widespread clinical use in the diagnostic and therapeutic workout of LSCC (81). Histological differentiation of SCC is expressed as grades: Grade I means well differentiated SCC, whereas grade III refers to anaplastic, or undifferentiated, or poorly differentiated cancer (86). A four-step categorisation (grades I-IV) has also been used. In LSCC, the relationship between poorly differentiated or undifferentiated cancers and impaired survival is well established (44,93). Poorly differentiated cancer has a higher metastastic potential, but on the other hand, it also seems to be more responsive to radiation therapy (81). Numerous other histopathological and immunohistochemical methods for determining the prognosis in LSCC have been studied, but so far, none of them have been convincing enough to merit widespread use in clinical practice (94,95). Some of them will be presented in detail in the following chapters.

2.2.3.1 p53

Tumour suppressor protein p53 is involved in various critical cellular functions (96). Cellular stress, such as oncogene activation, DNA damage, hypoxia and loss of normal growth and survival signals, activate p53 (97) and lead to several responses, of which induction of cell cycle arrest and apoptotic cell death are the best understood (98). Loss of p53 strongly enhances tumour development in mice (97), and mutation or abnormal expression of p53 is the most common genetic abnormality in human cancer (99). Thus, p53 is believed to have a critical role in cancer development (100). The role of p53 in regulatory pathways of cell growth and its prognostic significance have been extensively studied, but the results in LSCC have been somewhat contradictory (94). This is at least partly due to methodologic reasons: most of the published studies have been made on small patient series using different immunohistochemical antibodies and methods. Also, both the detection of p53 mutation by DNA sequencing and the overexpression of p53 by immunohistochemistry have been used as methods to detect abnormal p53 status, even though these do not seem to correlate. In a study by Bradford *et al.*, over half of the tumours that overexpressed p53 did not have a mutation, and conversely, 40% of the samples with normal p53 expression did have a p53 mutation (101). There are also variations in the definition of overexpression (94).

In a study by Hegde *et al.*, 33% of patients with head and neck cancer had a mutated p53 protein, and this mutation correlated with an increased risk of recurrence and worse overall survival (102). Bradford *et al.* reached the same conclusion in a series consisting exclusively of LSCC patients (101).

Most of the studies evaluating the relationship between overexpression of p53 and survival in LSCC have revealed a significant correlation between overexpression and poor prognosis. In a study by Watling *et al.*, overexpression was observed in 62% of the samples, and it was clearly associated with histologic malignancy (103). Narayana *et al.* studied T1N0M0 glottic cancers: 82% of the tumours that recurred locally overexpressed p53 compared to only 29% of those with local control, indicating that p53 overexpression is a prognostic factor of early stage glottic cancer (104). According to Spafford *et al.*, increased expression of p53 was consistent with shorter survival in all stages (105). Lavertu *et al.* (106) and Jackel *et al.* (107) also confirmed the negative effect of p53 overexpression on survival in LSCC.

An opposite conclusion was made by Hirvikoski *et al.*, who found overexpression of p53 (present in 68%) to be independently associated with favourable disease-free and overall survival in LSCC (108).

To make the issue even more confusing, many studies have failed to establish a significant correlation between p53 overexpression and prognosis in LSCC (109-116). In a study by Rowley *et al.* (109), p53 overexpression impaired survival in pharyngeal but not in laryngeal cancer, possibly explaining the striking differences in survival between these two cancers. According to Salam *et al.*, p53 overexpression did not correlate with subsite, clinical stage, histological grade, risk of recurrence, metastatic tendency or overall prognosis in LSCC (110).

2.2.3.2 Apoptosis

Apoptosis, or genetically regulated cell death, is an important self-repairing mechanism after cell trauma (117,118). Normal apoptosis involves deletion of damaged cells by collapse of the cell and fragmentation of its DNA followed by rapid phagocytosis by neighbouring cells (119). Apoptosis is regulated by a growing number of known anti-apoptotic and pro-apoptotic agents, of which p53 is the best known (120). Decreased or inhibited apoptosis resulting in uncontrolled accumulation of damaged or mutated cells is a feature of many malignancies (121): It is believed that the balance between cell survival and programmed cell death (apoptosis) is altered during multistage tumour progression (95). Enhanced apoptosis is also deliberately achieved in chemotherapy and radiation therapy of cancer (119). The rate of apoptosis seems to have prognostic significance in many tumours, including prostatic adenocarcinoma (122), cancer of the urinary bladder (123), small-cell carcinoma of the lung (124) and breast cancer (125).

In regard to LSCC, the majority of published papers addressing the prognostic impact of apoptotic rate, commonly measured as apoptotic index (number of apoptotic cells per field of vision in a microscopic examination), have reported similar results. Hirvikoski *et al.* (126) and Sikorska *et al.* (127) found an enhanced apoptotic index to be associated with poor survival, while Lera *et al.* (115) and Hotz *et al.* (116) reported a correlation between a low degree of apoptosis and better prognosis. On the other hand, in a Finnish study by Pulkkinen *et al.*, apoptosis and the markers associated with it did not seem to have predictive value in LSCC (111).

2.2.3.3 Cell proliferation

Cell proliferation has been shown to increase in almost all tumours (94), and cancer cells bear an indefinite proliferating capacity, avoiding all normal mechanisms of cell deletion in tissue homeostasis (95). The degree of cell proliferation is commonly measured by immunostaining the Ki-67 protein, also termed MIB-1, a nuclear antigen only present during cell division (94).

The degree of cell proliferation does not seem to have any convincing potential as a prognostic factor in LSCC (94). Lera *et al.* found a low degree of Ki-67 to be related to better local control (115), and Lavertu *et al.* reported a relationship between Ki-67 positivity and poor overall survival, though it was not marked enough to warrant treatment modifications (106). Usually, however, cell proliferation has proved to be insignificant as a predictor of outcome in LSCC (105,108,111,128).

2.2.3.4 Angiogenesis

The growth of solid neoplasms is always accompanied by neovascularisation, a critical factor in tumour development, growth, invasion and metastasis (129). This finding is believed to lead to the development of new, minimally toxic cancer therapies affecting the

various pro-angiogenic and anti-angiogenic cytokines that are being discovered at a rapid rate (130). The degree of neoangiogenesis can be measured by immunostaining microvessels with antibodies against CD-31 antigen or human factor VIII and evaluating the density of microvessels (MVD) in microscopy (102). However, the reliability of MVD as an indicator of neoangiogenesis is compromised due to marked heterogeneity in angiogenesis within tumours and differences in the methods used to measure it (94). Circulating angiogenic cytokines have also been used as tumour markers and candidates for prognostic significance (130). In prostatic (131) and breast cancer (132), high MVD has been related to metastatic disease and worse prognosis.

In head and neck cancer, the results on the predictive value of angiogenesis have been inconclusive. Increased MVD has been connected with a poor response to cancer treatment, an increased risk of recurrence and metastases and shorter disease-free and overall survival (133,134) or, in contrast, to a better response to radiation and better survival (135). Hegde *et al.* found no correlation at all between MVD and survival (102). Among circulating angiogenic markers, a high level of plasma endostatin and a low level of angiogenin (136) and elevated serum vascular endothelial growth factor (137) have been linked with impaired prognosis.

2.2.3.5 Other immunohistochemical factors

Numerous other immunohistochemical markers have been suggested and studied as prognostic factors in LSCC, with varying degrees of success. Among the factors involved in cell adhesion, cytoplasmic accumulation of α-catenin (138) and a low degree of staining against CD-44 receptor (139) have been related to aggressive LSCC and poor survival, whereas an enhanced amount of CD-44 (105) and strong staining for syndecan-1 (140) have been associated with improved survival. Related to p53 and apoptosis, the proteins p21 and p27 are negative regulators of the cell cycle (114). Jeannon *et al.* reported that increased p21 expression significantly impaired prognosis in LSCC (114), whereas Hirvikoski *et al.* found no such evidence, even though p21 status correlated with advanced stage and poor differentiation (141). In a study by Fan *et al.*, positive staining for p27 improved survival in LSCC (142). Proto-oncogene bcl-2, a negative regulator of apoptosis, has not shown any predictive potential in LSCC (105,111). Finally, Bentzen *et al.* studied the prognostic influence of the mitotic index and the mean nuclear volume in LSCC but found no correlations (143).

3 Aims of the present study

The aims of the present study were:

1. To study the changes in the incidence and survival of LSCC in Finland, with a special emphasis on Northern Finland.
2. To evaluate the patient delay after the onset of symptoms, the results of the following first medical consultation and the subsequent professional diagnostic delay leading to the diagnosis of LSCC.
3. To evaluate the impact of patient and professional diagnostic delays on survival and the risk of recurrence in LSCC.
4. To assess the relative prognostic significance of immunohistochemical markers p53 and apoptosis and the degree of cell proliferation and angiogenesis in LSCC.

4 Patients and methods

4.1 Incidence and survival of laryngeal cancer in Finland (I)

A total of 5766 patients were reported to the Finnish Cancer Registry as having cancer of the larynx between the years 1956 and 1995. The registry files are practically complete in terms of the cancer cases diagnosed in Finland, including more than 99% of solid cancers (37,144). This 40-year period was divided into four 10-year periods (1956-1965, 1966-1975, 1976-1985 and 1986-1995) to detect time-related trends. Age-adjusted incidence rates and 5-year relative survival rates (RSR) (145) were calculated by sex, age and clinical stage for each period. The expected survival rates for RSR analyses were obtained from the population life tables by age, sex and year of follow-up supplied by Statistics Finland. A comparison of incidence trends in males between the five university hospital districts in Finland was also made.

4.2 Incidence and survival of laryngeal cancer in Northern Finland (I)

Using the Finnish Cancer Registry material described above, a separate analysis of age-adjusted incidence rates and relative survival rates by sex and age was made for the patients who lived in the Oulu University Hospital District, or Northern Finland, which accounts for approximately 50% of Finland´s area and 14% of its population. This analysis was restricted to the latter two 10-year periods (1976-1985 and 1986-1995), because Oulu University Hospital was only founded in the early 1970´s. According to the Finnish Cancer Registry files, the number of laryngeal cancer patients from Northern Finland totalled 398 during that period.

Since the anatomical subsites of laryngeal tumours are not recorded in the cancer registry files, hospital-based data of the laryngeal cancer patients diagnosed and/or treated in Oulu University Hospital during the same period (1976-1995) were collected, including 353 patients, of whom 3 patients with subglottic cancer (0.8%) were excluded to simplify the analysis. Even though Oulu University Hospital is the only tertiary referral centre in Northern Finland, and all patients with cancer of the larynx are assumed to be

treated there, a drop-out rate of 11% was observed compared to the cancer registry data. Therefore, the hospital data were used only to characterise the patient series and to calculate incidence and survival rates in relation to anatomical subsite. Data were collected on age, sex, domicile, socioeconomic status, smoking, alcohol consumption, duration of symptoms at the time of diagnosis, histology and grade of the tumour, anatomical subsite and clinical stage according to the TNM classification. Relative survival rates were calculated in relation to anatomical subsite. Throughout the 20-year period, the data were uniformly collected and the patients diagnosed and/or treated by the same ENT surgeon.

Finally, age-adjusted incidence rates in Northern Finland by sex and anatomical subsite were calculated for three 7-year periods (1976-1982, 1983-1989 and 1990-1996) to illustrate the incidence trends in relation to anatomical subsite (data not previously published in the original publications).

4.3 Diagnostic delays and their impact on survival (II)

For the series II and III, all patients (n=99) who lived in the Oulu University Hospital District and were diagnosed with LSCC (ICD codes 1610-1619 and C32.0-9) between 1990 and 1995 were identified from the hospital's patient registers. Four patients were diagnosed incidentally in tertiary care, and 16 patients were diagnosed during follow-up for chronic laryngitis and were excluded. Furthermore, 13 patients were excluded because their primary care documents were deficient or not available, thus leaving 66 patients for the final analysis of the delays (Fig. 1). The patients excluded from the study did not differ from those diagnosed intentionally after referral from primary care with respect to age, sex, socioeconomic status, smoking or drinking habits or general health status. However, they had localised disease at diagnosis more often, since the majority of excluded patients were under surveillance because of chronic laryngitis, and among them, glottic cancer was over-represented. Accordingly, their prognosis was better (5-year disease-specific survival rates 93% and 74% in the excluded patients followed up because of chronic laryngitis and in the final study population, respectively).

Detailed data concerning the first visit and the previous onset of symptoms were recorded from the primary health centre. Data on the demographic and clinical variables were drawn from the tertiary centre. The cause and date of death data were obtained from Statistics Finland. Patient delay was defined as the interval between the perception of the first symptoms and the initial professional evaluation, which was the first visit after the onset of symptoms regardless of which symptom the patient gave as the main reason for the first visit. Professional delay was determined as the interval between the initial consultation and the date of histological verification of the diagnosis. Total delay was the sum of the patient and professional delays.

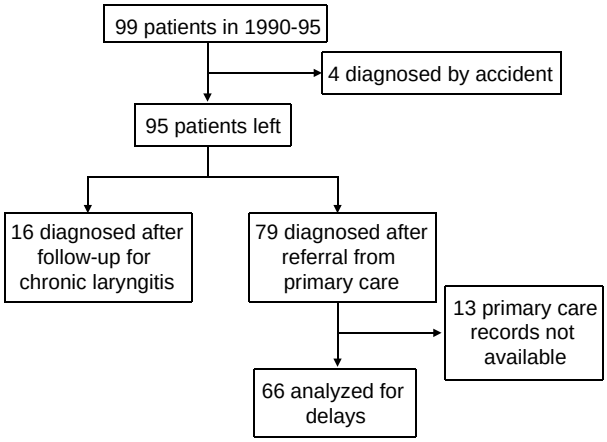

Fig. 1. Flow chart of the diagnostic pathways of patients with laryngeal squamocellular carcinoma in Northern Finland in 1990-1995.

Patient delay was divided into 2 categories (<3 months and ≥3 months) and professional delay into 3 categories (<6 months, 6-12 months and ≥12 months), and the impact of these delay categories on disease-specific survival was analysed. Finally, a multivariate analysis was performed to evaluate the independent significance of these delays on survival. The following prognostic factors were included in the model: clinical stage (stages I-III vs. stage IV), anatomical subsite, age (< vs. ≥65 years) and smoking status (smoking vs. no smoking).

4.4 Impact of diagnostic delays on the risk of recurrence (III)

In study III, the same patient population and data gathering methods were used as in study II.

In addition to the clinical variables recorded for series II, data were gathered on the primary treatment regimen for LSCC, the date and localisation of possible recurrence and the treatment administered for the recurrence. Recurrence of LSCC was defined as the appearance of histologically verifed SCC within a follow-up period of 5-10 years after primary treatment of LSCC either locally, in the neck or in some other distant location. SCC of the lung (n=4) was defined as a distant recurrence rather than a new primary malignancy. Patients with a residual tumour in the larynx after primary radiotherapy (radiotherapy failure, n=2) were included in the group of patients with a local recurrence. Throughout the 6-year study period, the patients were diagnosed and followed up by the same ENT surgeon, and the decisions concerning their treatment regimens were made by one interdisciplinary team.

The impact of patient and professional diagnostic delays on the risk of recurrence of LSCC was analysed, as were the associations between the different prognostic factors and the different types of recurrence.

4.5 Prognostic factors of LSCC (IV)

For study IV, LSCC patients diagnosed in the Oulu University Hospital (criteria, data collection and study region described above) between 1985 and 1996 were enrolled in the study, constituting a total of 196 new cases. On the basis of the availability of representative and sufficient histological material, 100 patients (52%) were included in the study. The distribution of the main patient and tumour characteristics (age, sex, smoking status, anatomical subsite and clinical stage) were identical between the final study population of 100 and the original population of 196 patients.

All histological analyses were made by two pathologists from formalin-fixed, paraffin-embedded pre-treatment biopsies. The pathologists were blinded to all clinical data, and in cases of disagreement, consensus was reached through discussion.

The expression of p53 was determined using immunohistochemical staining with monoclonal mouse anti-human p53 antibody (D-07®, Dako A/S, Glostrup, Denmark) at a dilution of 1:50 with a three-stage immunoperoxidase method. The percentage of p53-positive cells was estimated from one representative high-power field (HPF, objective x 40, diameter of the field 400 μm) and divided into four categories (0%, 1-10%, 11-25% and >25%). Negative p53 status was used as a synonym for 0%, whereas tumour blocks with any number of cells staining for p53 were taken to represent positive p53 status. Two stainings were considered inadequate.

In order to determine the level of cell proliferation in the tumour blocks, immunohistochemical staining was performed using diluted monoclonal mouse anti-Ki-67 following trypsin digestion and heat-induced antigen recovery (Zymed Inc., San Francisco, CA, USA). A representative HPF was selected, and the percentage of Ki-67-positive cells in the HPF was estimated using four categories (0%, 1-10%, 11-25% and >25%). Ninety-three stainings were considered adequate to allow an analysis of proliferation.

Microvascular density (MVD) representing neoangiogenesis in tumour tissue was determined from sections immunohistochemically stained with endothelial cell-specific monoclonal mouse anti-human CD-31 antibody (Dako A/S, Glostrup, Denmark) following antigen retrieval by heating in 10 mmol/l citrate buffer. The number of CD-31-positive microvessels in five different HPFs within a representative section of the tumour was calculated, and the mean value was used as an estimate of MVD. In 81 cases, there was enough tissue in the tumour block and the staining was successful enough to allow analysis.

To detect apoptotic cells, *in situ* labelling of the 3´-ends of the DNA fragments generated by apoptosis-associated endonucleases (TUNEL) was performed using the ApopTag® *in situ* detection kit (Oncor, Gaithersburg, MD, USA). Apoptotic index (percentage of apoptotic cells in a given area) was estimated by counting the apoptotic cells and bodies in a HPF and dividing this figure by the number of tumour cells in the same HPF. For each tumour sample, 10 HPFs were analysed, and the mean of the indexes was the final result. Analysis of apoptosis was performed last, and during the study, some tumour blocks were exhausted, leaving only 63 samples for the analysis of apoptosis.

In addition to the abovementioned four immunohistochemical factors, the following known or suggested prognostic factors of LSCC were included in the analysis of their relative significance in the survival of LSCC: sex, age, smoking status, anatomical site of

the tumour and clinical stage. The statistical interrelationships of these factors were also evaluated.

4.6 Statistical methods

4.6.1 Series I

Study I was descriptive in nature, and no tests for statistical significance were made except for the difference in the relative survival rates (RSR) between the anatomical subsites in the hospital-based material from Northern Finland, for which 95% confidence intervals (CI) were calculated in the Finnish Cancer Registry using the Relative Survival Analysis program (145). Using the same software, RSRs were calculated for the nationwide registry material. The incidence rates were adjusted for age to the world standard population.

4.6.2 Series II and III

The differences in categorical data were analyzed by using the chi-square test. For continuous data, the groups were compared using the Kruskal-Wallis test. Disease-specific survivals (series II) and disease-free periods (series III) were determined using the Kaplan-Meier method and compared with Breslow´s test (series II) or the log rank test (series III). Multivariate analyses were undertaken using the stepwise Cox regression model with disease-specific survival (series II) or the disease-free period (series III) following diagnosis as the outcome measure. Spearman's coefficient was used to test for possible correlations between the patient and professional delays.

4.6.3 Series IV

In the case of the variables interpreted primarily categorically (p53, cell proliferation), the categories were compared for survival using the Kaplan-Meier method and Breslow´s test. Cut-off points with the best effect on separating the categories in terms of survival in univariate analysis were selected for dichotomisation. In the case of the prognostic factors assessed as continuous variables (apoptotic index, MVD), the data were divided into quartiles, and similar dichotomisation was carried out with the Kaplan-Meier method. Sociodemographic and clinical prognostic factors were primarily recorded as dichotomised data (male vs. female, < vs. ≥65 years, smoking vs. no smoking, glottic vs. supraglottic tumour), with the exception of clinical stage, which was analyzed first categorically (stages I-IV) and then as dichotomised into early (stages I-II) and late stage (stages III-IV) disease.

The prognostic significance of all factors studied was evaluated as disease-specific survival following the diagnosis with univariate analysis (Kaplan-Meier method, Breslow´s test) using both original categorisation and dichotomisation. In the multivariate analysis using Cox regression model with disease-specific survival as the outcome measure, all four immunohistochemical factors were included in addition to the following, previously known prognostic factors: clinical stage, tumour site and smoking status. The relationships between the different dichotomised prognostic factors studied were estimated with the chi-square test using simple cross-tabulation.

5 Results

5.1 Incidence and survival of laryngeal cancer in Finland (I)

From 1956-1965 till 1986-1995, the age-adjusted incidence rate of laryngeal cancer in males in Finland decreased from 6.5 to 3.5 per 100 000 person-years. In females, the rate remained unchanged (Table 2). The decrease took place after the early 1970´s and was constant in all stages (local, regional and distant) and relatively more pronounced among patients younger than 65 years. Age-adjusted incidence was considerably higher among patients older than 65 years, with an approximately 10-fold difference. The decreasing trend in males was similar in all the five university hospital districts in Finland (Fig. 2).

The changes in survival were small. Among males, the 5-year RSR between 1956-1965 and 1986-1995 increased from 57% to 62%, while among females, it decreased from 61% to 58% (Table 3). Among patients older than 65 years, a noticeable improvement in RSR was noted (from 38% to 57%). No significant differences between the different stages were observed in the survival trends.

Table 2. Trends in the age-adjusted incidence rate of laryngeal cancer in Finland in 1956-1995 and in Northern Finland in 1976-1995 by sex, age and stage. Data derived from the Finnish Cancer Registry.*

		Finland				Northern Finland	
		1956-65 (n=1453)	1966-75 (n=1652)	1976-85 (n=1420)	1986-95 (n=1241)	1976-85 (n=206)	1986-95 (n=192)
Males (n=5307)		6.5	6.4	4.7	3.5	5.2	3.8
Age:	<65 years	4.9	4.5	2.8	1.9	3.4	1.9
	≥ 65 years	27.9	31.4	28.8	24.6	29.6	30.1
Stage:	local	4.4	4.0	3.1	2.2	3.4	2.4
	regional	1.1	0.70	0.52	0.29	0.91	0.46
	distant	0.40	0.51	0.34	0.21	0.29	0.12
	unknown	0.60	1.2	0.74	0.80	0.60	0.82
Females (n=459)		0.33	0.35	0.33	0.30	0.36	0.42
Age:	<65 years	0.02	0.03	0.24	0.19	0.22	0.29
	≥ 65 years	1.7	1.4	1.5	1.7	2.3	2.1
Stage:	local	0.24	0.22	0.22	0.18	0.26	0.25
	regional	0.05	0.02	0.05	0.05	0.09	0.08
	distant	0.02	0.04	0.02	0.02	-	0.03
	unknown	0.02	0.07	0.04	0.05	0.01	0.06

*Incidence rate per 100 000 person-years adjusted for age to the world standard population.

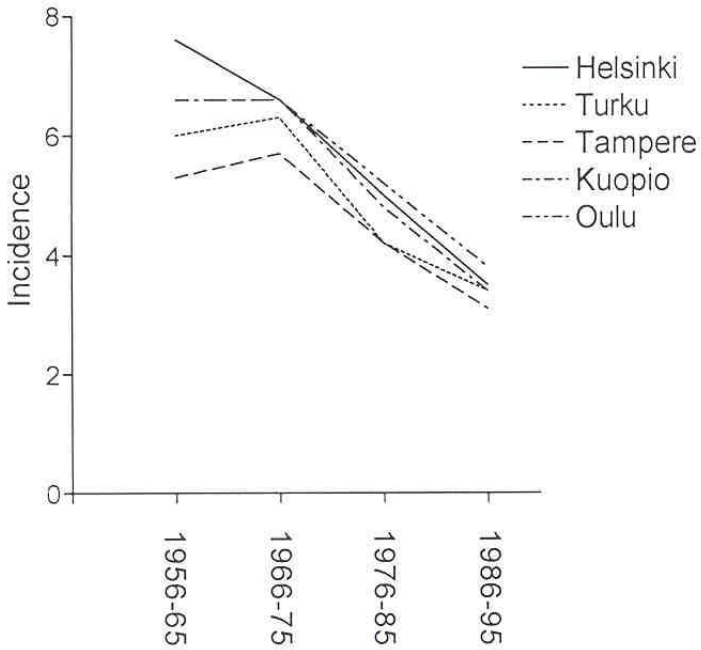

Fig. 2. Age-adjusted incidence rate (per 100 000) of laryngeal cancer in men in 1956-1995 in five University Hospital regions in Finland. Data derived from the Finnish Cancer Registry.

Table 3. Trends in the five-year relative survival rate (RSR, %) of laryngeal cancer in Finland in 1956-1995 and in Northern Finland in 1976-1995 by sex, age and stage. Data derived from the Finnish Cancer Registry.

		Finland					Northern Finland	
		Overall RSR (n)*	1956-65 RSR (n)*	1966-75 RSR (n)*	1976-85 RSR (n)*	1986-95 RSR (n)*	1976-85 RSR (n)*	1986-95 RSR (n)*
All patients		59 (5766)	57 (1453)	59 (1652)	61 (1420)	62 (1241)	61 (206)	67 (192)
Males		59 (5307)	57 (1358)	58 (1544)	60 (1295)	62 (1110)	60 (189)	66 (169)
Females		63 (459)	61 (95)	67 (108)	64 (125)	58 (131)	74 (17)	69 (23)
Age:	<65 years	63 (3523)	61 (1092)	64 (1094)	65 (761)	65 (576)	70 (122)	69 (85)
	≥65 years	50 (22443)	38 (361)	46 (558)	55 (659)	57 (665)	45 (84)	64 (107)
Stage:	local	70 (3740)	68 (995)	68 (1025)	72 (946)	75 (774)		
	regional	36 (690)	34 (241)	43 (178)	38 (157)	29 (114)		
	distant	19 (518)	11 (92)	29 (195)	13 (123)	14 (108)		
	unknown	55 (818)	50 (125)	55 (254)	55 (194)	59 (245)		

*Total number of patients at the beginning of the follow-up.

5.2 Incidence and survival of laryngeal cancer in Northern Finland (I and previously unpublished data)

Based on the Finnish Cancer Registry material, the incidence rates were slightly higher in Northern Finland than in the whole country between 1976 and 1995 among both sexes and in all stages (Table 2). During the last period (1986-1995), the relative survival rates were somewhat more favourable in Northern Finland among both sexes and age groups (overall 5-year RSR 67% vs. 62%) (Table 3).

The patient and clinical characteristics of the hospital-based material from Northern Finland in 1976-1995 are presented in Table 4. The patients' mean age was 64 years, male smokers made up the majority, and glottic cancer was slightly more common than supraglottic cancer. Over 90% of the tumours were invasive LSCCs (others were carcinoma *in situ* 9, small-cell carcinoma 2, fibrosarcoma 1, rhabdomyosarcoma 1, transitiocellular carcinoma 1, and undefined 16). Clinical stage was generally more advanced among the patients with supraglottic disease.

Table 4. Patient characteristics and five-year relative survival rates of laryngeal cancer in Northern Finland in 1976-1995 by anatomical subsite[1].

	Supraglottic (n=170)	Glottic (n=180)
Mean age	63	64
Male sex	145 (85%)	168 (93%)
Smoking	151 (98%)	163 (95%)
Heavy alcohol consumption[2]	12 (15%)	17 (15%)
Low socioeconomic status[3]	90 (65%)	96 (64%)
Urban domicile	75 (45%)	102 (58%)
Mean duration of symptoms (months)	6	7
Histology		
Squamous cell carcinoma	162 (99%)	158 (93%)
Other[4]	2	12
Grade		
Well differentiated	33 (28%)	58 (46%)
Moderately well differentiated	56 (47%)	52 (42%)
Poorly differentiated	22 (18%)	7 (6%)
Indeterminate	9 (8%)	8 (6%)
Stage		
I	35 (21%)	94 (53%)
II	29 (17%)	30 (17%)
III	67 (40%)	43 (24%)
IV	36 (21%)	8 (5%)
not staged	2	1
5-year relative survival rate (95% confidence interval)	64% (55-73)	80% (71-88)

[1]3 patients (0.8%) with subglottic cancer were excluded to simplify the table. [2]Daily or heavy drinking or alcoholism. [3]Based on profession and employment status according to WHO. [4]Including carcinoma in situ. Percentages calculated among the subpopulations of patients with the data available.

In males, the age-adjusted incidence rate of supraglottic disease decreased clearly from 1976-1985 to 1986-1995 (from 2.6 to 1.2 per 100 000), whereas an increase was observed for glottic cancer (from 1.9 to 2.3). This resulted in a substantial change in the subsite distribution of the incidence rates: the supraglottic to glottic ratio diminished from 1.4:1 to 0.5:1. In females, the incidence rates remained unchanged for both main anatomical subsites (0.3 and 0.1 per 100 000 in supraglottic and glottic cancer, respectively). In consequence, the proportion of women among the patients with supraglottic disease increased from 11% to 20%. The incidence trends of laryngeal cancer in Northern Finland between the years 1976 and 1996 are presented graphically in three 7-year periods in Fig. 3 (previously unpublished data).

During the whole study period, the 5-year relative survival rates were 80% (95% CI 71-88%) for glottic and 64% (95% CI 55-73%) for supraglottic cancer (Table 4).

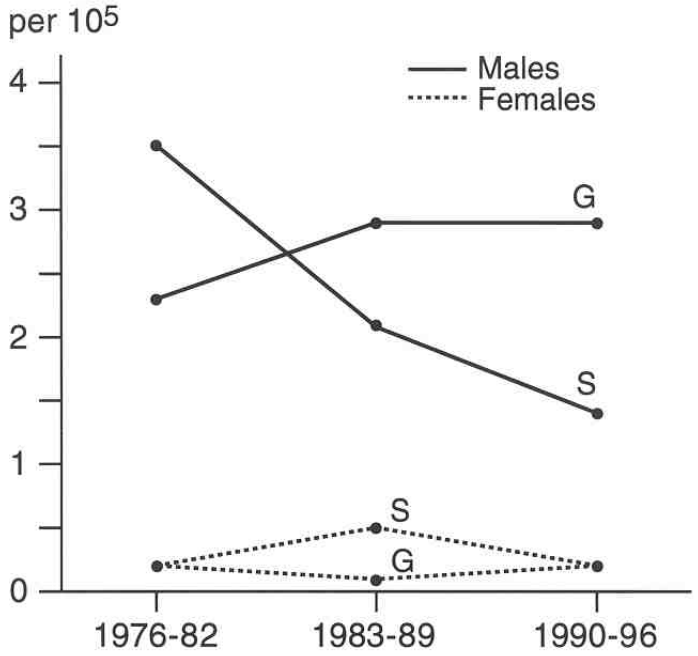

Fig. 3. Age-adjusted incidence rate (per 100 000) of laryngeal cancer in 1976-1996 in Northern Finland by anatomic subsite and sex. G= glottic cancer, S= supraglottic cancer.

5.3 First medical consultation (II) and diagnostic delays (II, III) in LSCC

In the hospital-based patient material drawn for series II and III from Northern Finland (n=66), the patients' mean age was 65 years, 92% were males, 92% reported previous or present smoking, 65% had glottic disease, and the stage distribution was as follows: stage I 45%, stage II 12%, stage III 32% and stage IV 11%. The overall disease-specific 5-year survival rate was 74% (95% CI 63-85%).

Fifty-one (77%) patients had hoarseness as their initial symptom, whereas other symptoms were less common (Table 5). Hoarseness was more common in patients with glottic than supraglottic carcinoma (88% vs. 57%, p=0.03). In only 38% of the patients was malignancy suspected at the first visit, and although the malignant nature of the lesion was correctly suspected more often in supraglottic than in glottic tumours, this difference was not statistically significant. The most common misdiagnosis was infection, which was suspected in 41% of the patients. Approximately half of the initial consultations resulted in a referral to a specialist, and 29% of the patients were not referred, but were asked to come back for clinical control; 17% of the patients were neither referred, nor controlled after the first medical visit.

The median patient delay was 2 months (mean 4 months, range 1 day to 5 years), and for 46% of the patients, the patient delay was 3 months or more (Table 5). No statistically significant differences were observed in patient delays between supraglottic and glottic carcinomas. Longer patient delay (≥3 months) was not significantly associated with stage IV disease at either tumour site (p=0.58 for glottis and 0.08 for supraglottis), nor was it associated with the clinical stage in general (p=0.72). The median durations (and ranges) of symptoms were as follows: hoarseness 2.0 months (0.2-61), pharyngalgia 3.1 months (0.5-6.1), dyspnoea 2.0 (1.0-3.0) and neck lump 0.2 months (0.1-1.0). Neck lump made patients seek medical advice sooner than the other initial symptoms (p=0.005). The duration of the main symptoms was similar for tumours at both sites. Patient delay of 3 months or longer was associated with lower socioeconomic status (p=0.009), but not with any other patient characteristics.

The median professional delay was 3 months (mean 7 months, range 6 days to 6 years) and similar for both tumour sites. Eleven patients (17%) had a professional delay of 12 months or more (Table 5). Longer professional delay was not significantly related to age, gender, socioeconomic status, smoking status, comorbidities or clinical stage of the disease (p=0.61 for professional delay of < or ≥12 months vs. TNM stages I-IV).

The median overall diagnostic delay was 6.0 months (range 1-79 months). There was no correlation between the patient and professional delays (Spearman's correlation coefficient, r_s=0.21, p=0.09).

Table 5. Details of the first visit in primary care and the diagnostic delays in 66 patients with laryngeal carcinoma.

	All (n=66)	Glottic (n=43)	Supraglottic (n=23)	p[3]
Initial symptom				0.03
hoarseness	51 (77%)	38 (88%)	13 (57%)	
pharyngalgia	9 (14%)	3 (7%)	6 (26%)	
neck lump	4 (6%)	1 (2%)	3 (13%)	
dyspnea	2 (3%)	1 (2%)	1 (4%)	
Suspected diagnosis				0.42
malignant tumour	25 (38%)	14 (33%)	11 (48%)	
benign tumour	5 (8%)	4 (9%)	1 (4%)	
infection	27 (41%)	19 (44%)	8 (35%)	
not defined	9 (14%)	6 (14%)	3 (13%)	
Result of the visit				0.44
referred	36 (55%)	22 (51%)	14 (61%)	
not referred	30 (45%)	21 (49%)	9 (39%)	
Patient delay[1]				0.11
<1 month	16 (24%)	7 (16%)	9 (39%)	
≥1 ,<3 months	20 (30%)	15 (35%)	5 (22%)	
≥3 months	30 (46%)	21 (49%)	9 (39%)	
median (range), months	2.0 (0-61)	2.2 (0-61)	1.5 (0-14)	
Professional delay[2]				0.85
<6 months	44 (67%)	28 (65%)	16 (70%)	
≥6, <12 months	11 (17%)	7 (16%)	4 (17%)	
≥12 months	11 (17%)	8 (19%)	3 (13%)	
median (range), months	3.0 (0-75)	3.1 (0-75)	2.9 (0-21)	
Overall delay				
median (range), months	6.0 (1-79)	6.7 (1-79)	5.2 (2-21)	

[1]From initial symptom to first visit to a physician. [2]From first visit to a physician to histological diagnosis. [3]Groups compared collectively using the chi-square test for categorical variables and Kruskal-Wallis test for continuous data.

5.4 Impact of diagnostic delays on survival in LSCC (II)

Patient delay of 3 months or more was not related to overall survival (Fig. 4) or to survival with glottic (p=0.28) or supraglottic (p=0.21) cancer. In contrast, the length of professional delay was significantly associated with survival: longer delay implicated worse prognosis (Fig. 5). Survival declined strongly after 6 months of professional delay in supraglottic carcinoma (5-year disease-specific survival rate dropped from 60% to 43%, p=0.12), but only after delay of 12 months or more in glottic carcinoma (from 91% to 55%, p=0.008). The marked and independent effect of professional delay on survival was confirmed in the multivariate analysis (Table 6), in which professional delay of 6 months or longer led to a relative hazard of death ratio (HR) of 1.88, and professional delay of 12 months or longer to a HR of 4.74 (p=0.05). The impact of professional delay of 12 months or more was equal to that of advanced disease stage (stage IV), the only other significant factor in the model. Patient delay was not related to survival in the multivariate model, either.

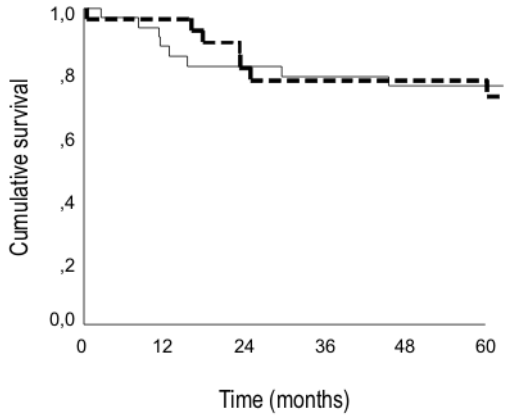

Fig. 4. Kaplan-Meier disease-specific survival curves for patients with laryngeal squamocellular carcinoma (n=66) divided by patient delay: <3 months (—) and ≥3 months (- - - -) (p=0.96, difference tested with Breslow´s test).

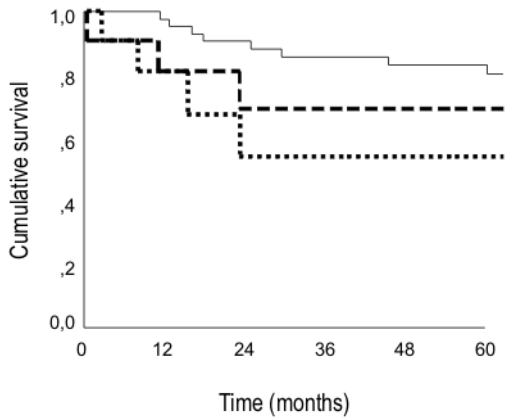

Fig. 5. Kaplan-Meier disease-specific survival curves for patients with laryngeal squamocellular carcinoma (n=66) divided by professional delay: <6 months (—), 6-12 months (- - - -), and ≥12 months (▪▪▪▪) (p=0.05, difference tested with Breslow´s test).

Table 6. Adjusted relative hazard of death for prognostic factors in laryngeal cancer in a multivariate analysis using the Cox regression model (n=66).

	Relative hazard of death	95% confidence interval	p
Patient delay[1]			0.41
<3 months	1.0		
≥3 months	1.73	0.48-6.25	
Professional delay[2]			0.05
<6 months	1.0		
≥6, <12 months	1.88	0.47-7.44	
≥12 months	4.74	1.30-17.3	
Stage			0.02
I-III (n=59)	1.0		
IV (n=7)	5.18	1.26-21.3	
Anatomic subsite			0.16
glottic	1.0		
supraglottic	2.33	0.73-7.48	
Age			0.78
<65 years	1.0		
≥65 years	1.17	0.39-3.47	
Smoking			0.55
no	1.0		
yes	1.92	0.23-15.9	

[1]From initial symptom to first visit to a physician. [2]From first visit to a physician to histological diagnosis.

5.5 Recurrences in LSCC (III)

In all of the 66 patients, the primary treatment (39% surgery, 61% radiation therapy) was administered with a curative intent. A third of the patients developed a recurrence within the follow-up period of 5-10 years (Table 7); 10 (15% of the total) were local, 7 (11%)

neck and 6 (9%) distant recurrences. All recurrences developed to male patients. The sites of distant metastases were lungs and bone. The median disease-free period before recurrence was 13 months (range 2-60 months). A majority (87%) of the recurrences took place within 2 years after the primary treatment. Local recurrences were treated mainly surgically, and a majority of these patients (6/10) underwent total laryngectomy. The options for neck or distant recurrences were chemotherapy, radiotherapy, neck dissection and palliation. Early stage disease and glottic site diminished the risk of recurrent disease (Table 7).

Table 7. Patient and tumour characteristics and treatment modalities for recurrences in 66 patients with laryngeal squamocellular carcinoma.

	No recurrence (n=43)	Local recurrence (n=10)	Neck recurrence (n=7)	Distant recurrence* (n=6)
Men	38 (88%)	10 (100%)	7 (100%)	6 (100%)
Mean age (range), years	65 (43-86)	65 (38-79)	66 (49-84)	65 (60-69)
Anatomic subsite				
glottic	31 (72%)	6 (60%)	3 (43%)	3 (50%)
supraglottic	12 (28%)	4 (40%)	4 (57%)	3 (50%)
Stage				
I	26 (60%)	2 (20%)	1 (14%)	1 (17%)
II	4 (9%)	3 (50%)	-	1 (17%)
III	11 (26%)	5 (50%)	3 (43%)	2 (33%)
IV	2 (5%)	-	3 (43%)	2 (33%)
Treatment for recurrence				
total LE	-	6 (60%)	-	-
partial LE	-	1 (10%)	-	-
local resection	-	1 (10%)	-	-
only neck dissection	-	-	3 (43%)	-
radiotherapy	-	-	1 (14%)	2 (33%)
chemotherapy	-	-	2 (29%)	-
no treatment	-	2 (20%)	1 (14%)	4 (66%)

*Lung n=4, bone n=2. LE=laryngectomy, partial LE=supraglottic laryngectomy or hemilaryngectomy.

5.6 Impact of diagnostic delays on the risk of recurrence in LSCC (III)

Patient delay did not affect the risk of local, neck or distant recurrence in the univariate analysis as a continuous variable (Table 8) or in the multivariate analysis dichotomised into <3 months or ≥3 months (Table 9).

Table 8. The associations between various prognostic factors and local, neck and distant recurrences in 66 patiens with laryngeal carcinoma (univariate analysis).

	No recurrence (n=43)		Local recurrence (n=10)		Neck recurrence (n=7)		Distant recurrence[1] (n=6)		p[2]
	n (%)	median (range)	n (%)	median (range)	n (%)	median (range)	n (%)	median (range)	
Patient delay (months)		2 (<1-60)		1 (<1-6)		1 (<1-6)		3 (<1-6)	0.56
Professional delay (months)		2 (<1-26)		10 (1-75)		10 (2-23)		2 (<1-16)	0.007
Age (years)		66 (43-86)		67 (37-79)		68 (48-84)		66 (60-69)	0.90
Male sex	38 (88)		10 (100)		7 (100)		6 (100)		0.41
Supraglottic tumour	12 (28)		4 (40)		4 (57)		3 (50)		0.37
Advanced stage (III-IV)	13 (30)		5 (50)		6 (86)		4 (67)		0.02
T-category 3-4[3]	14 (33)		5 (50)		4 (57)		4 (67)		0.26
N-category 1-3[4]	1(2)		0		4 (57)		2 (33)		<0.001
Poor differentiation[5]	4 (9)		1 (10)		2 (29)		2 (33)		0.25

[1]Lung n=4, bone n=2. [2]Groups compared collectively using the chi-square test for categorical variables and Kruskal-Wallis test for continuous data. [3]Vocal cord fixation or invasion beyond larynx by the primary tumour. [4]Positive neck node status at the time of diagnosis either clinically or in ultrasonography or CT-scan. [5]Histological grade 3-4.

Table 9. Adjusted relative hazard rate (HR) of local, neck and distant recurrence for prognostic factors of laryngeal carcinoma (multivariate analysis with Cox regression model) (n=66).

	Local recurrence		Neck recurrenc		Distant recurrence[1]	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Patient delay		0.49		0.68		0.59
<3 months	1.0		1.0		1.0	
≥3 months	0.62 (0.17-2.36)		1.46 (0.24-8.81)		1.61 (0.28-9.10)	
Professional delay		0.02		0.015		0.32
<12 months	1.0		1.0		1.0	
≥12 months	4.62 (1.25-17.1)		9.50 (1.55-58.3)		3.67 (0.28-48.7)	
Stage		0.29		0.04		0.11
I-II	1.0		1.0		1.0	
III-IV	2.10 (0.54-8.16)		13.8 (1.20-158.9)		6.55 (0.65-65.9)	
Age		0.45		0.83		
<65 years	1.0		1.0		NA[2]	
≥65 years	1.86 (0.37-9.44)		1.23 (0.20-7.56)			
Anatomic subsite		0.96		0.65		0.92
glottic	1.0		1.0		1.0	
supraglottic	1.04 (0.24-3.82)		1.48 (0.28-8.0)		0.92 (0.14-6.03)	

95% CI= 95% confidence interval. NA = not analysed. [1]Lung n=4, bone n=2. [2]All patients (n=6) with a distant recurrence were older than 65 years.

On the other hand, professional delay of 1 year or longer, compared with shorter delay, clearly increased the risk of local recurrence (p=0.019) (Fig. 6) and delayed neck metastasis (p=0.019) (Fig. 7), but did not affect the risk of distant metastasis (p=0.66). When divided by stage, this phenomenon was particularly conspicuous among patients with early stage (stages I-II) LSCC (n=38): professional delay of 6 months or more increased the risk of local recurrence from 7% (2/28) to 30% (3/10) (p=0.066). Professional delay of ≥1 year had an even more pronounced effect: compared with a professional delay of <1 year, the risk of local recurrence increased from 6% (2/31) to 43% (3/7) (p=0.01) and the risk of neck recurrence from 0% (0/31) to 14% (1/7) (p=0.03) in early stage disease. In late stage disease (stages III-IV, n=28), neither of the diagnostic delays had a significant effect on the risk of recurrence.

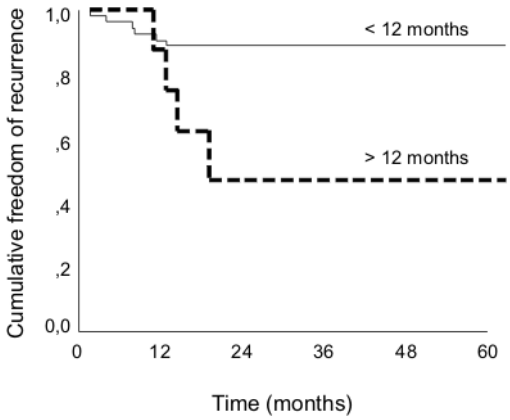

Fig. 6. Risk of local recurrence displayed as Kaplan-Meier curves for cumulative disease-free time among 66 patients with laryngeal squamocellular carcinoma divided by professional delay (<12 months and ≥12 months) (p=0.019, difference tested with the log rank test).

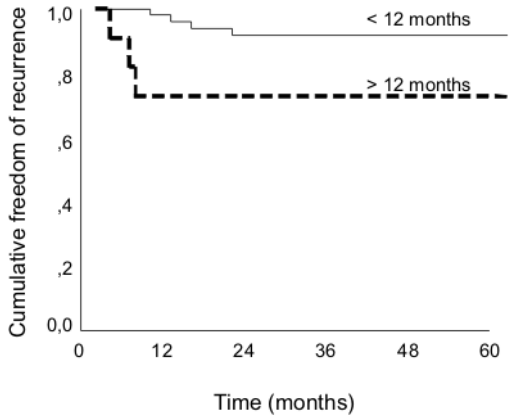

Fig. 7. Risk of neck recurrence displayed as Kaplan-Meier curves for cumulative disease-free time among 66 patients with laryngeal squamocellular carcinoma divided by professional delay (<12 months and ≥12 months) (p=0.019, difference tested with the log rank test).

In the univariate analysis where the delays were considered as continuous variables, the impact of professional delay on the risk of local and neck recurrence was further confirmed (p=0.007) (Table 8). Among the other variables analysed, disease spread to the neck at the time of diagnosis (N categories N1-3) (p<0.001) and advanced stage (stages III-IV) (p=0.02) increased the risk of subsequent neck and distant recurrence.

The independent nature of longer professional delay as a determinant of an increased recurrence risk was finally confirmed in the multivariate analysis (Table 9). Professional delay of 1 year or longer indicated an adjusted relative hazard ratio (HR) of 4.6 for local recurrence (p=0.02) and 9.5 for neck recurrence (p=0.015) compared with shorter delay. The only other factor significantly associated with the risk of recurrence in the multivariate model was late stage disease, which increased the HR for neck recurrence considerably, but did not significantly affect the risk of local or distant recurrence.

5.7 Relative roles of sociodemographic, clinical and immunohistochemical factors on survival in LSCC (IV)

The patient characteristics and tumour data of series IV are presented in Table 10. Among the continuous immunohistochemical factors, the mean apoptotic index was 0.97 (range 0-4) and the mean MVD (angiogenesis) 8.0/HPF (range 2.4-22/HPF). Forty-three percent of the p53 stainings were negative (category 0%). In Ki-67, the most common category was 1-10% (33% of the samples).

In the univariate analysis, the most powerful predictors of poor survival were advanced stage (p<0.001) and supraglottic disease (p=0.009) (Table 10). A trend towards impaired prognosis was detected for female sex (p=0.43), age of 65 years or more (p=0.19) and smoking (p=0.18), but the differences were not statistically significant. Among the four immunohistochemical factors studied, the original categorisation showed no significant associations with prognosis (Table 10). When dichotomised with the cut-off point that differentiated the data most effectively in terms of survival, a higher degree of apoptosis (apoptotic index ≥0.3) reached statistical significance as a predictor of worse survival (p=0.05).

Among the demographic and clinical prognostic factors, smoking status, clinical stage and anatomical site of the primary tumour seemed to be interrelated (Fig. 8). Smoking was associated with advanced stage (p=0.02) and supraglottic disease (p=0.08), and a strong correlation emerged between advanced stage and supraglottic disease (p<0.001). Female sex was clearly associated with supraglottic site: 73% of female patients had supraglottic tumours compared to 38% of males (p=0.007). Among the immunohistochemical factors, a correlation between negative expression of p53 and a high degree of proliferation (Ki-67 ≥25%) was observed (p=0.014). The immunohistochemical factors were not associated with any of the clinical or sociodemographic factors.

Table 10. The disease-specific 75th percentile survival times (months) for demographic, clinical and histopathological prognostic factors in a series of 100 patients with laryngeal squamocellular carcinoma (univariate analysis, Kaplan-Meier method).

	n	75th percentile survival time	p[1]
Sex			0.43
male	85	48	
female	15	29	
Age			0.19
<65 years	43	71	
≥65 years	57	29	
Smoking			0.18
no	11	>60[5]	
yes	83	31	
Anatomic subsite			0.009
glottic	57	>60[5]	
supraglottic	43	22	
Stage			<0.001
I	38	>60[5]	
II	18	91	
III	32	25	
IV	12	10	
p53 expression			0.28
0%	43	67	
1-10%	10	23	
11-25%	11	45	
>25%	34	25	
Proliferation[2]			0.48
0%	20	23	
1-10%	31	24	
11-25%	22	48	
>25%	20	>60[5]	
Apoptotic index[3]			0.18
<0.3	14	>60[5]	
≥0.3, <0.8	20	25	
≥0.8, <1.4	14	>60[5]	
≥1.4	15	48	
Angiogenesis[4]			0.33
<5.8	20	29	
≥5.8, <7.3	21	71	
≥7.3, <9.6	20	91	
≥9.6	20	15	

[1]Calculated with the Breslow´s test. [2]Ki-67 expression. [3]Mean of percentages of apoptotic cells in 10 high power fields. [4]Microvessel density (microvessels/high power fields). [5]The 5-year disease-specific survival rate was over 75%.

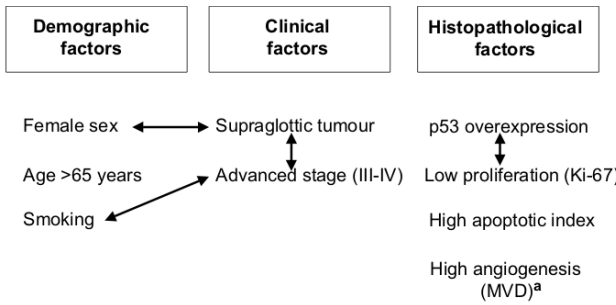

Fig. 8. Statistically significant interactions (arrows) (p<0.05, chi-square test) between different prognostic factors of laryngeal squamocellular carcinoma in a series of 100 patients ([a]=microvascular density). Supraglottic location, advanced stage and high apoptotic index proved to be statistically significant and independent predictors of poor outcome in multivariate analysis.

In the multivariate analysis, advanced stage (stages III-IV) (relative hazard ratio (HR) 8.9, p=0.01) and supraglottic site (HR 5.6, p=0.02) were again the best predictors of impaired survival (Table 11). High degree of apoptosis (apoptotic index ≥0.3) was also significantly associated with poor prognosis (HR 11.1, p=0.05). The other factors showing a trend towards impaired outcome were non-smoking status (HR 10.1, p=0.08) and low degree of proliferation (Ki-67 <25%) (HR 8.5, p=0.07). p53 (p=0.73) and angiogenesis (p=0.34) did not have a significant effect on survival.

Table 11. Adjusted relative hazard of death for dichotomized prognostic factors of laryngeal cancer in a multivariate analysis with Cox regression model (n=100).

	n	Relative hazard of death	95% confidence interval	p
Stage				0.01
early (I-II)	56	1.0		
late (III-IV)	44	8.9	1.67-47.0	
Anatomical subsite				0.02
glottic	57	1.0		
supraglottic	43	5.6	1.29-24.6	
Smoking				0.08
yes	83	1.0		
no	11	10.1	0.76-135	
Apoptotic index[1]				0.05
<0.3	14	1.0		
≥0.3	49	11.1	0.97-128	
Proliferation[2]				0.07
≥25%	20	1.0		
<25%	73	8.5	0.86-84.5	
p53 expression				0.73
-	43	1.0		
+	55	1.3	0.34-4.59	
Angiogenesis[3]				0.34
<9.6	61	1.0		
≥9.6	20	2.3	0.42-12.4	

[1]Mean of percentages of apoptotic cells in 10 high power fields. [2]Ki-67 expression. [3]Microvascular density (microvessels/high power field).

6 Discussion

6.1 Incidence and survival of laryngeal cancer

Previous studies and surveys have shown a decrease in the incidence rate since the 1970´s (4,5,18) and a shift from supraglottic to glottic sites (5,18,28) in laryngeal cancer among males in Finland. This study confirmed both of these findings as well as the steady state of the incidence rate among females. Moreover, the decreasing trend in men was found to be very similar in all of the five university hospital districts in Finland. However, in contrast to a study conducted in the Tampere region (5), an increasing trend in glottic cancer among males was observed in Northern Finland, even though the general decreasing trend in incidence and the shift from supraglottic to glottic cancers were essentially similar. The reason for this unexpected increase in glottic disease remains speculative: the observed significant decrease in the proportion of adult male smokers in Finland after the 1950´s (26,27) agrees well with the decrease of overall incidence and the incidence of supraglottic cancer in men but contradicts the increase of glottic cancer among males in Northern Finland, suggesting other possible causative factors. The increasing alcohol consumption in Finland (32,33) is one possible explanation, as is the possible increase in the incidence of glottic cancers caused by human papillomavirus (HPV), an infection with a debated role in laryngeal carcinogenesis (146-148). However, supraglottic cancer seems to be even more closely related to previous smoking than glottic cancer, as previously documented (28-30).

The decreasing incidence of laryngeal cancer among Finnish males during the last few decades has been widely termed as an exception among the western countries (6,7). Perhaps Finland was not so “western” in the 1950´s and early 1960´s, at least from the viewpoint of health behaviour. Smoking was extremely common among Finnish males in the 1950´s (26), and since then, an exceptionally marked decrease has taken place (27). Denmark serves as a good point of comparison: in 1960, the incidence of laryngeal cancer in men was less than 50% of that observed in Finland, whereas today, laryngeal cancer among men is nearly twice as common in Denmark as in Finland. The alarming increase in laryngeal cancer among Danish men has been preceded by a clearly increasing consumption of tobacco (31). Also, alcohol consumption per capita is 50% higher in Denmark compared to Finland (1), which further explains the difference seen today.

In this study, the relative survival of patients with laryngeal cancer did not improve significantly between 1956 and 1995. This is in accordance with the previous studies (8,10,13,20,36,37). This universally observed unexpected steady state of laryngeal cancer survival despite the undisputed progress in cancer diagnostics and cancer care, not to mention the significant improvements in cancer survival in general (36), has been largely attributed to a persistently high rate of recurrences and second primary cancers among UADT cancer patients (149). Part of the explanation may lie in the more conservative treatment strategy applied in the recent years, which has markedly reduced the proportion of patients treated with total laryngectomy (8,91).

The future will probably not bring any dramatic changes into the incidence rate of laryngeal cancer in Finland. The decrease in smoking among males has stopped, as has the increase of smoking among females (32), and according to predictions by the Nordic Cancer Registries (1), the marked decrease in the incidence among men is now over, and the incidence rate will remain largely unchanged until the 2020´s or even longer. However, due to aging of the population, the number of new laryngeal cancer cases will rise slightly during the next few decades. Also, because of the strongly increasing proportion of daily female smokers during the 1980´s and the relatively small difference in smoking patterns between the genders observed today (27), the proportion of female laryngeal cancer patients can be expected to increase for some time.

6.2 Diagnostic delays and their impact on survival and the risk of recurrence in LSCC

It is difficult to determine the duration of diagnostic delays in LSCC. The timing of the onset of the first symptoms is very unlikely to be accurate: most LSCC patients are lifelong smokers and suffer from chronic laryngitis, which produces symptoms similar to those of laryngeal cancer. Moreover, anamnesis of the symptoms after the diagnosis of malignancy is likely to be easily biased. The durations of diagnostic delays in LSCC vary considerably between different reports (41-45), suggesting different health care infrastructures and difficulties in assessing the precise onset of symptoms leading to the diagnosis of LSCC. The study populations are also heterogeneous: many reports have utilised patient data pooled from all head and neck or UADT cancers (41,43,46). In this study, the median patient delay was 2 months (mean 4 months) and the median professional delay 3 months (mean 7 months). The delays reported earlier tend to be markedly shorter (42,45). This could be at least partly explained by methodological differences. The abovementioned studies have the common methodological limitation of assessing the diagnostic delays from hospital records or by interviewing the patient personally or via a questionnaire after the malignant diagnosis had been established, whereas in this study, the data concerning the onset of symptoms were obtained from the primary health care records written before the true nature of the underlying condition had been discovered. Even though a prospective study design is virtually impossible in the evaluation of diagnostic delays in cancer, the method employed in this study minimises the possibility of methodological bias with respect to the timing of the onset of symptoms.

Even though delayed diagnosis could be suspected to lead to a more advanced disease stage at the time of diagnosis, and thus to poorer prognosis, this has not been verified in laryngeal cancer (41,44,45,57). However, a significant correlation between long professional diagnostic delay and impaired survival in LSCC was observed in this study: professional delay of 12 months or longer was an independent predictor of poor survival in a multivariate model and equalled the effect of advanced clinical stage. The difference in the conclusions between this study and previous reports can be at least partly explained by the methodological discrepancies discussed above. In this study, patient delay had no impact on survival in LSCC, and neither of the delays were significantly related to clinical stage at the time of diagnosis. Obviously, tumour growth rate varies considerably, partly explaining the lack of correlation between diagnostic delays and stage.

According to Shaw (49), head and neck cancer offers a possibility for earlier detection for two reasons. First, the head and neck region is readily accessible in a routine physical examination. Secondly, these tumours (at least in the case of glottic cancer) tend to remain localised for a long period and only to metastasise relatively late in the course of the disease. The accessibility of the larynx and the presumed ease of diagnosis can naturally be utilised only to shorten the professional delay, in which case the primary health care professional evaluating the patient during the first medical consultation is in a key position. However, long professional delay cannot always be attributed to the physician: patients may continue to deny the severity of their symptoms and fail to show up for control examinations or referral appointments, contributing elements of patient delay into professional delay. Since delay in seeking medical help seems to be a conscious decision, suggesting denial as the primary cause of patient delay (47), there are no useful means for shortening this type of diagnostic delay. On the other hand, one can influence the length of professional delay by improving the medical education of physicians. The visualisation of the larynx with indirect laryngoscopy should belong to the repertoire of every practising primary health care physician (51,52). In addition, the awareness of physicians about LSCC should be enhanced: hoarseness or other laryngeal symptoms should be suspected to be caused by a malignant process until proven otherwise, especially among smoking patients. The results of this study provide evidence in favour of this viewpoint. In only 38% of the cases was malignancy suspected during the first visit, and 17% of the patients were neither referred, nor asked to reappear for a clinical control visit should the symptoms continue unabated. Over half (6/11) of the patients with a professional delay of 12 months or longer belonged to the group of patients who were neither referred nor controlled after the first visit.

In addition to survival, there are other outcomes of interest in the treatment of LSCC: ability to use one's own voice and voice quality (150), absence of pain and other treatment-related symptoms (151,152), psychosocial adjustment (153), general post-treatment quality of life (154), and above all, disease-free period (risk of recurrence). The incidences of the different types of recurrence seen in this study (local recurrence 15%, neck recurrence 11% and distant recurrence 9%) are in accordance with previous reports, as is the disease-free time period after treatment (median 13 months, 87% occurred within 2 years) (63,69,75). The impact of diagnostic delays on the risk of recurrence has not been evaluated in LSCC before. In this study, lengthened professional delay increased significantly the risk of local and neck recurrence in early stage (stages I-II) LSCC. Since only 21% of the patients with early stage disease developed a recurrence, which, in most cases

of local failure, led to total laryngectomy, this finding is important. Longer delay among early stage LSCC patients could be used, in conjunction with supraglottic site, as an indicator of an increased risk of recurrence and, accordingly, a need for more aggressive primary treatment – early stage LSCC patients are today routinely treated conservatively with either local radiotherapy or local resection. At least, these patients would benefit from more meticulous follow-up after the primary treatment.

In summary, long professional delay proved to be an independent and powerful predictor of poor prognosis in LSCC. According to Greenman (94), for a prognostic factor to have significant practical value in clinical work, it should provide some of the following information influencing the management of an individual patient: its impact on the risk of local failure, risk of delayed neck metastasis in N0 neck, risk of distant recurrence or second primary malignancy and, finally, the possibility of radiosensitivity or radioresistance. According to the present results, lengthened professional delay fulfils this criterion in LSCC.

6.3 Relative roles of sociodemographic, clinical and immunohistochemical prognostic factors in LSCC

To the present day, clinical stage at the time of diagnosis (19,36,39,44,72,81) and the anatomical subsite of the tumour (23,29,35,39,44,72) remain the only solid and useful variables for determining the prognosis and for selecting the treatment protocol in LSCC. In this study, the essential role of these factors in the prognosis of LSCC was repeatedly confirmed. Among the histopathological or immunohistochemical parametres, histologic malignancy (grade) correlates with aggressiveness of the disease and survival (93) and is commonly used in clinical work. Numerous studies evaluating the impact of these factors on the prognosis of LSCC have been published, but with mutually conflicting results (94,95). Only overexpression or mutation of p53 protein (101-107) and enhanced apoptotic rate (115,116,126,127) have been consistently related to poor survival in LSCC. However, methodological variations and small materials compromise the generalisability of these results.

In this study, enhanced apoptosis emerged as a significant, independent determinant of poor prognosis in LSCC in a multivariate analysis, including clinical stage, anatomical subsite of the tumour and smoking status. Similar results have been reported earlier. These results suggest that increased apoptosis in pre-treatment biopsies could be used to identify patients with a need for more aggressive primary treatment. The mechanisms whereby increased apoptosis is linked to impaired survival are yet to be confirmed (126). Enhanced apoptosis may be an indicator of a higher tumour cell turnover rate and aggressiveness of the neoplasm. In addition, apoptotic cell death may promote the selection of tumour cells with a good ability to survive within the host. p53, proliferation (Ki-67) and angiogenesis failed to show significant associations with survival in LSCC.

The interrelationships between the different prognostic factors observed in this study have also been previously documented (female sex and supraglottic site (22-24); supraglottic site and advanced stage (23,29,38,73,87); smoking and both supraglottic site and advanced stage (34)). Opposite to practically all studies previously published on the topic

(34,44,74,81,82,84,85), non-smoking status appeared to be an independent predictor of worse prognosis in LSCC. This finding could be explained by the use of a multivariate model, which takes confounding factors into account. Smoking was clearly associated with advanced stage and supraglottic site, and when these variables were controlled, non-smokers seemed to have a more dismal prognosis and hence more aggressive disease. However, the importance of this result should not be exaggerated. The number of non-smokers in this study was small, and data on patients´ smoking habits are very susceptible to bias.

6.4 General discussion: representativeness of patient material and results

The patient data for this study were drawn from the Finnish Cancer Registry (series I) and the patient register of Oulu University Hospital (series I-IV). The files of the Finnish Cancer Registry are practically complete in terms of the cancer cases diagnosed in Finland, with a coverage of over 99% (37,144), due to the Finnish legislation which obliges all hospitals, pathological laboratories and practitioners to report to the Cancer Registry every cancer case that comes to their attention. Oulu University Hospital is the only tertiary referral centre in Northern Finland, and all laryngeal cancer cases from the area are supposed to be treated there. Moreover, the Finnish health care system is based on a comprehensive health insurance scheme providing equal access to medical and hospital services for everyone. Thus, the patient material from Oulu University Hospital can be considered representative, even though the patient population is relatively small (population approximately 700 000 persons). The dates and causes of death were obtained from the official causes of death register of Statistics Finland, which can also be considered an extremely reliable source of information.

The size of the patient sample in series I (n=5766 for the whole of Finland and 398 (Cancer Registry) or 353 (University Hospital) for Northern Finland) is abundant. In the series II and III (n=66) and in series IV (n=100), the number of patients included in the final analyses can be considered small.

In the series II and III, 33% of the original population of 99 patients were excluded from the analyses. Twenty (20% of the total) were excluded due to a diagnosis of LSCC either incidentally (4%) or after follow-up for chronic laryngitis (16%), both obstructing the evaluation of diagnostic delays, and their impact on the study end points. In the followed-up group, early stage disease and glottic site were over-represented, resulting in better survival. However, the diagnostic delays were not significantly related to clinical stage, and both stage and anatomical subsite were included in the multivariate analyses. Hence, this selection bias does not undermine the key conclusions of the studies II and III. The rest of the drop-outs in the patient series II and III (13%) were due to insufficient or missing primary health care charts, again suggesting a possibility of small selection bias. The missing cases, however, did not differ from the rest of the sample with regard to age, gender or clinical stage at the time of diagnosis.

In series IV, only 52% of the patients out of the original population of 196 were included in the study on the basis of the availability of sufficient and representative histologi-

cal material, suggesting that the analysed sample may be biased towards larger tumours, but because clinical stage (which includes tumour size or T class) was included in the multivariate analysis, this does not jeopardise the main conclusions of the study. Moreover, the percentage of tissue samples accepted into the study is in accordance with the studies previously published on the topic (108,126).

In terms of demographic and clinical characteristics (mean age, distribution of sex and clinical stage, anatomical subsite of the tumour and smoking status), the present patient data were similar to those published previously (5,28-30,44,63,69,72,75,82,105,108,126).

7 Conclusions

1. The incidence of laryngeal cancer has been decreasing among Finnish males since the 1970´s consistently throughout the country. Even though the decrease is – at least in Northern Finland – exclusively due to a shift from the more lethal supraglottic to the glottic subtype, no significant improvement in survival has taken place.
2. The median durations of diagnostic delays in LSCC were 2 months for patient delay and 3 months for professional delay. The proportion of patients with extremely long professional delay (≥ 12 months) was unexpectedly high. In only 38% of the patients was malignancy suspected at the first medical visit, infection being the most common misdiagnosis. Almost one-fifth of the patients were left without follow-up after the first medical consultation.
3. Lengthened professional delay (≥ 12 months) is an independent and powerful predictor of impaired survival in LSCC and of an increased risk of local or neck recurrence in the early stage (stages I-II) LSCC. Thus, longer professional delay could be used to identify the patients with a need for more aggressive primary treatment or more meticulous follow-up. Patient delay has no impact on survival or the risk of recurrence in LSCC.
4. Enhanced apoptotic rate is an independent determinant of poorer prognosis in LSCC and could be used as an additional indicator of a need for more aggressive primary treatment.

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