Open access • Journal Article • DOI:10.1080/0284186X.2020.1730437

Incidence and malignant transformation of glottic precursor lesions in Denmark

Nina J. B. Sannino, Camilla Slot Mehlum, Ågot Møller Grøntved, Thomas Kjærgaard ...+3 more authors

Institutions: University of Copenhagen, Aarhus University Hospital

Published on: 25 Feb 2020 - Acta Oncologica (Taylor & Francis)

Topics: Malignant transformation

Incidence and malignant transformation of glottic precursor lesions in Denmark

Sannino, Nina J.B.; Mehlum, Camilla S.; Grøntved, Ågot M.; Kjaergaard, Thomas; Kiss, Katalin; Godballe, Christian; Tvedskov, Jesper F.

Published in:
Acta Oncologica

DOI:
10.1080/0284186X.2020.1730437

Publication date:
2020

Document version:
Accepted manuscript

Citation for pulished version (APA):
Sannino, N. J. B., Mehlum, C. S., Grøntved, Å. M., Kjaergaard, T., Kiss, K., Godballe, C., & Tvedskov, J. F. (2020). Incidence and malignant transformation of glottic precursor lesions in Denmark. *Acta Oncologica*, *59*(5), 596-602. https://doi.org/10.1080/0284186X.2020.1730437

Incidence and malignant transformation of glottic precursor lesions in Denmark

Running title: Glottic precursor lesions in Denmark

Nina J. B. Sannino, MD[1]; Camilla S. Mehlum, MD[2]; Ågot M. Grøntved, MD[2]; Thomas Kjaergaard[3], MD, PhD; Katalin Kiss, MD[4]; Christian Godballe[2], MD, PhD; Jesper F. Tvedskov, MD, PhD[1].

Key words: larynx; vocal cords; premalignant; dysplasia, precancerous lesions

1: Rigshospitalet, Department of Otorhinolaryngology, Head and Neck Surgery and Audiology, Copenhagen, Denmark

2: Research Unit for Department of ORL - Head & Neck Surgery and Audiology, Odense University Hospital, Odense, Denmark; University of Southern Denmark, Odense, Denmark

3: Aarhus University Hospital, Department of Otorhinolaryngology, Head and Neck Surgery, Denmark

4: Rigshospitalet, Department of Pathology, Copenhagen, Denmark

NS and CSM should be considered joint first author

Financial disclosure: No financial disclosures

Conflict of interest: No conflicts of interest

Corresponding author:
Nina Josefine Buhl Sannino, Department of Otorhinolaryngology, Head and Neck Surgery and Audiology, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen, Denmark. + 45 30 20 00 94. Josefinesannino@gmail.com

Abstract

Objectives: Glottic precursor lesion (GPL) is a well-known premalignant condition, but the existing knowledge of incidence and malignant potential is based on subpopulation studies. In this first nationwide study we report data from all verified cases of GPL in Denmark during a 10-year period with focus on incidence and malignant transformation of GPL.

Methods: Patients were identified by a search for GPL in the time period from 01.01.2000 to 31.12.2009 using the Danish Pathology Data Base, Patobank, which is a nationwide source of all cyto- and histopathological data obtained in Denmark. Data were validated and supplemented by medical chart review.

Results: A 10-year national cohort of 965 patients (median age 60 years, male-female ratio 2:1) with histologically verified GPL was analyzed. The overall malignant transformation rate was 18.3 % (mild dysplasia 7.7%, moderate dysplasia 19.8%, severe dysplasia 28.5%, and carcinoma in situ 40.3%) with a median progression time of 29 months. Eighty-eight percent of patients were active or former smokers. A significantly larger proportion of male patients (24.1 %) experienced malignant transformation compared to females (6.6%) ($p<0.001$).

Conclusion: This nationwide population-based study of GPL patients confirmed a stable incidence of GPL in Denmark from January 2000 to December 2009 and a considerable malignant potential, correlated to the grading of GPL according to the World Health Organization classification of laryngeal precursor lesions from 2005, WHOC2005. The recent update, WHOC2017, of low-grade versus high-grade lesions may thus contain less nuanced prognostic information than WHOC2005.

Key words: Head and neck cancer; Larynx; Precancerous lesions, Vocal Cords, Dysplasia

Level of evidence: 2b retrospective cohort study

Introduction

A glottic precursor lesion (GPL) is defined as specific architectural and cytological changes in the mucosal epithelium considered precancerous[1]. GPL is the most common and the most challenging subgroup of laryngeal precursor lesions due to the opposing demands for both radical treatment

and quality of voice. The World Health Organization classification from 2005 (WHOC2005)[2] categorizes GPL into mild, moderate or severe dysplasia, and carcinoma in situ (CIS) and has been widely accepted in the Western world[3]. Transformation rates to malignancy varies according to the grade of GPL and are reported from 0-12% for mild dysplasia[4,5,6,7,8,9] and up to 63% for carcinoma in situ (CIS)[4,10,11]. The existing knowledge of incidence and malignant potential of GPL is based on studies of selected smaller subpopulations[12] and the grading, classification and treatment of GPL remains a matter of debate[13,14,15].

The uncertainty regarding the malignant potential has caused varying treatment strategies internationally and nationally[16] dependent on institutional or personal preferences[17] and thus probably put patients at risk of both under- or overtreatment[18,19]. Recently, the World Health Organization classification was revised (WHOC2017)[1], into a two-category grading of low and high grade lesions, in an effort to harmonize the various grading systems internationally used for laryngeal precursor lesions.

The aim of this study was to investigate the incidence, characteristics and malignant potential in a Danish national 10-year cohort.

Materials and methods

This national population-based retrospective cohort study of GPL is based on tissue samples registered in the Danish Pathology Database, Patobank, which contains information of all cyto- and histopathological assessments performed in Denmark. Patobank is a real time database with immediate data transfer from Danish departments of pathology. It is associated with the national civil registration number, assigned to all Danish residents, and is controlled by the Danish Data Protection Agency.

Data was retrieved by a computer-assisted search using the specific classification codes for Systematized Nomenclature of Medicine (SNOMED) for the locations vocal cords, glottis and laryngeal commissures (SNOMED T24400, T24410, T24420, T24440, T24470 and TX2980) and for precancerous lesions, according to WHOC2005, dysplasia not otherwise specified (NOS),

mild, moderate, severe dysplasia and carcinoma in situ (CIS) (SNOMED M74009, M74A09, M74B09, M74C09, M80102 and M80702/M807*2). The aim of this study was to investigate incidence and malignant transformation of GPL and therefore biopsies initially registered as carcinomas were not included.

A total of 2518 histopathological records from 1534 patients diagnosed from 1.1.2000 to 31.12.2009 were retrieved from Patobank and data were extracted regarding the gender and age of the patient, number and the anatomical location of the tissue samples, any tissue samples classified with cancer, tissue samples performed prior to the year 2000, and number and diagnosis of subsequent tissue samples after primary diagnosis. Patients were excluded in case of previous or simultaneous laryngeal or hypopharyngeal cancer, cancer transformation within 3 months of the initial GPL, GPL prior to 01.01.2000, GPL solely on the ventricular folds or incorrect registration of the location of the lesions when validated by the medical chart. Sixteen cases of GPL restricted to the false vocal cords were registered as SNOMED T24400 (vocal cords) in Patobank, but excluded when location was revealed by review of information from the database. All primary cases of histologically verified GPL in Denmark in the period were thereby included for analysis. For details see Figure 1. A total of 966 patients were included and subsequently divided in two groups according to geographical area respectively Eastern Denmark (n=421) and Western Denmark (n=545)) representing approximately 45 % and 55% of the Danish population respectively[20].

Initially, the medical charts were requested for all patients from Eastern Denmark of which 326 of 421 (77%) charts were accesible. Information from the medical charts regarding the extend and site of lesion as well as cancer transformation during follow-up were compared to the data retrieved from Patobank in order to validate the data from Patobank.

After validating the data from patients of Eastern Denmark, a sample of 93 patients from Western Denmark were randomly selected by national civil registration number and their medical charts requested for review in order to ensure validity also of the data from Western Denmark. Of these 93 medical charts 73 (78%) were accessible. One patient (1.4%) was excluded from the sample of

Western Denmark as the medical chart revealed biopsy of leukoplakia on the ventricular fold and not the vocal fold.

Supplementary sociodemographic and clinical data was collected retrospectively from the 398 received medical charts regarding alcohol consumption, tobacco history, occupation, gastroesophgeal reflux disease (GERD), and the extend of the surgical procedure. The follow up period was defined as the time from the initial diagnosis of GPL to the last update of data from Patobank on 2nd of October 2017. In case of death the follow up was closed at the date of death. The data were processed in SPSS (SPSS inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS inc SPSS) and analyzed in Rstudio (Version 1.0.136 – © 2009-2016 RStudio, Inc.) Summary statistics for demographic and clinical characteristics were determined. Odds ratios for malignant transformation were calculated using a 2 x 2 table for each subgroup of GPL with the odds for mild dysplasia as baseline. Confidence intervals were calculated using the Woolfs formula. For comparison of the malignant potential among the subgroups of GPL, logistic regression was performed with binary outcome (malignant transformation yes/ no) based on the information retrieved from Patobank. Gender was included as a variable. Statistical methods and results were conferred with statisticians at the Faculty of Social Science, University of Copenhagen.

Results

A cohort of 965 patients (median age 60 years, male-female ratio 2:1) with histologically verified GPL was analyzed. Patient characteristics are summarized in Table 1. Data was provided from all the 23 departments of Otorhinolaryngology in Denmark at the time, including four University Hospitals (33.9% of patients) and a few private clinics (1.8% of patients).

Data regarding tobacco use were available in 388 (97%) of the 398 reviewed medical charts and revealed that eighty-eight percent of the patients were active or former smokers. Calculation of pack-years was possible for 152 of those patients with a median 41 pack-years (mean 44, range 4-125).

The median follow up time was 144 months (mean 155.3, range 93-204). After the initial diagnosis of GPL, subsequent tissue sampling was performed in 59 % of the patients due to clinically suspected persistence/recurrence of GPL. The number of subsequent tissue samples correlated positively to the severity of the initial histopathological diagnosis (Table 2).

The mean annual national incidence of GPL was 95.6 (range 73 to 117 patients per year). According to the national institute of statistics, Statistics Denmark[21], the average population for adults (above or 18 years of age) was 4.216.714 inhabitants with a population growth of 0.26% from 2000-2009. This corresponds to a mean incidence of GPL for adults of 2.3/100,000 inhabitants a year (Figure 2). Three patients below 18 years of age were omitted from these calculations. The highest incidence of GPL of 7.7/100,000 a year was found for patients aged 50-69 years.

A total of 177 patients (18.3%) were diagnosed with glottic cancer during follow-up, with a median transformation time of 29 months (mean 41, range 3-172 months) from the initial diagnosis of GPL. Progression to cancer occurred within 120 months in 95.5% of cancer cases, within 60 months in 75% of cases and within 24 months for 46% of cases. The transformation rates and corresponding transformation times for subgroups of GPL are shown in Table 3 and Table 4. There was no substantial difference in cancer transformation or grade of GPL when seperating the data by geograhical area (data not shown elsewhere).

There was a considerable gender difference in the distribution of GPL, thus a significantly larger proportions of males were diagnosed with severe dysplasia or CIS and smaller proportions with mild dysplasia. For details, see Table 4. The overall malignant transformation rate for males was 24.1 % and for females 6.6 % with significant differences between all of the histopathological subgroups.

Details regarding the patient´s occupation, extend of the surgical procedure, alcohol intake, and GERD were unfortunately very inconsistently reported in the medical charts and therefore not analyzed further.

Discussion

To our knowledge this is the first report of a nationwide cohort of GPL. We confirmed a stable incidence of GPL in Denmark between 2000 and 2009 and a considerable malignant potential positively correlated to the severity of GPL, with an overall malignant transformation rate of 18.3% comparable to previous studies reporting transformation rates of 15 to 19%[16,9]. The incidence of GPL in Denmark was highest in patients aged 50-69 years (7.7/100,000 inhabitants per year). The rates of malignant transformation in our cohort were evenly proportional to the severity of GPL. Thus, we found that the histological classification according to WHOC2005[2] was an important prognostic factor as reported in other studies[12].

The recently updated WHOC2017[1] separates laryngeal precursor lesions into only two categories. The former categories of "hyperplasia" (no atypia or architectural disturbances), "mild dysplasia" (architectural disturbances and limited atypia in the lower third of the epithelium) and those of "moderate dysplasia" that only involve the lower half of the epithelium (WHOC2005 moderate dysplasia comprises architectural disturbances and limited atypia into the middle third of the epithelium), are fused into "low-grade dysplasia, LGD". The "high-grade dysplasia, HGD" comprises the former categories of carcinoma in situ, severe dysplasia, and those of moderate dysplasia that involves more than the lower half of the epithelial thickness. An unfortunate error in the latest edition of the reference book on WHOC20171 may cause confusion, as one table (Table 3.02, page 91) defines LGD as a spectrum of morphological changes restricted to the lower half of the epithelium, whereas another table (Table 3.03, page 92) restricts LGD only to the lower one-third. This error has recently been recognized by the authors of the reference book, and it was emphasized that LGD is correctly restricted to the lower epithelial half, as in Table 3.02 22,23,24 .

We found a considerable proportion of malignant transformation of mild dysplasia (7.7 %) in accordance with previous studies reporting malignant transformations in up to 12% of lesions with mild dysplasia [4,5,6,7,8,9,16,25,26] whereas moderate dysplasia carries a risk of malignant transformation of 4- 24 % [26], in our study 19.8%. Squamous hyperplasia has very limited or no malignant potential (0-

4.1%)[26,27]. We did not investigate this subgroup in our study, as it was not classified as dysplasia in WHOC 2005.

With recognition of the thorough work regarding the establishment of WHOC2017, we find that the results in our study encourages further discussion whether the term “Low grade” is justified and especially if it can be interpreted as “Low risk”.

Further, the options for stratified prognostication and intervention may be hampered with the implementation of WHOC2017 illustrated by the considerable differences in malignant potential for the four WHOC2005 categories of GPL reported here and in previous papers[12,16,28]. The WHOC2017 undoubtedly has important prognostic value and reflects significant difference in malignant potential of LGD and HGD. A cohort of 1444 patients from Slovenia was graded according to the amended Ljubljana classification on which the WHOC2017 is based, and malignant transformation was reported in 19/1204 (1.6%) of LGD over a period of 2–15 years as opposed to 30/240 (12.5%) of HGD over a period of 2–26 years ($p = 0.0001$) [1,27 29].

Based on the diagnostic criteria and corresponding malignant potential mentioned above, we do however find that the WHOC2017 pooling of squamous hyperplasia, mild dysplasia and partly moderate dysplasia into LGD raises some questions, as the malignant potential varies considerably within the group of LGD.

The inclusion of lesions (hyperplasia) with very low or no malignant potential will inevitably lower the overall rate of malignant transformation in the group of LGD. Thus, we are concerned that the risk of malignancy and need for treatment and follow-up may be underestimated, for a proportion of future patients diagnosed with LGD, who earlier would have been diagnosed “mild” or even “moderate” dysplasia.

In recognition of the thorough work behind the establishment of the WHOC2017, and its undeniable prognostic value, we suggest future research continue to strive to develop nuanced and applicable diagnostic criteria for laryngeal precursor lesions.

Patients who were diagnosed with dysplasia NOS (n=114, 11.8%), were included for assessment of incidence. Histological grades of GPL may be difficult to distinguish by both the WHOC2005

and WHOC2017[1] with substantial intra- and interobserver variability[22,30]. Intra- and interobserver variability is suspected to be uniformly distributed across the different grades of GPL, but in our study the NOS diagnose was predominantly, though not exclusively, caused by difficulties in separating mild from moderate dysplasia. The heterogeneity of the subgroup made it unsuitable for detailed analysis.

In 2012 a national guideline for management of GPL was proposed by The Danish Glottic Study Group, an established collaboration of Danish health care professionals, as the DANGLOT protocol[31]. Surgical endoscopic cordectomy type 1-3, according to the European Laryngological Society´nomenclature[32] was proposed as the primary choice of treatment for suspected GPL to ensure complete removal of GPL and avoid progression to cancer. Up to this point the watchful waiting approach after initial simple biopsy was not uncommon neither in Denmark nor in other countries[17,33]. We found that 59% of patients had subsequent tissue samples performed after the initial diagnosis due to clinically suspicion of recurrent GPL or malignant transformation, which could suggest an incomplete surgical removal of the initial lesion. The available information from Patobank and the medical charts did however not reveal details enough to disprove or confirm this thesis and neither do we know whether patients continued smoking after the initial diagnosis.

Our data showed no linear correlation between time to cancer transformation and initial severity of GPL. However, a difference in mean and median, though not significant, was found as shown in Table 4. It is noticeable that 25.4 % of those patients who developed cancer did so after more than 60 months, as the recommended follow up period in Denmark is five years from the latest recurrence of GPL. According to literature the mean time to cancer transformation varies from 29 to 173 months for mild dysplasia, 22-56 months for moderate dysplasia, 25-132 months for severe dysplasia and from 10-192 months for CIS[12,28].

The overall age distribution and tobacco history of our cohort did not differ considerably from results reported in other studies[7,34] nor did the proportion of active smokers[16,35], whereas the proportion of females (33%) was higher than in most previous studies in which male-female ratios of 3:1 more often are reported[28,35]. Furthermore, the proportion of active smokers was larger for women than for men. This correlates to the fact that in Denmark the proportion of smokers has not

decreased so much among women as among men or among women in neighboring countries in the recent decades[36]. Somehow contradictory to this, we found that almost half (48%) of the females in our cohort had only mild dysplasia compared to 33% of the males (p=0.003), whereas a cumulative of 18% of the females and 30% of the males had severe dysplasia (p<0.001) or CIS (p<0.001) initially. Males were also significantly more prone to develop cancer than females in all subgroups as well as overall (24.1% and 6.6% respectively) as shown in Table 4. This surprisingly large difference in malignant transformation between the sexes has only rarely been reported, but was suggested by Rohde et al[16] who reported an overall transformation rate of 15% among 101 patients (18 mild dysplasia, 16 moderate dysplasia, 35 severe dysplasia and 32 CIS); fifteen of 82 males (18.3%) developed cancer, but none of the 19 included women. Several factors may contribute to the more frequent finding of the severe subtypes of GPL and the more frequent malignant transformation among males. A likely explanation may be a larger tobacco consumption among male smokers[37], but the more frequent male malignant transformation in all subgroups of GPL probably cannot be explained solely by a larger tobacco consumption. A possible causality is the larger alcohol consumption by males than females[38] as alcohol is known to have synergistic carcinogenic effect when combined with tobacco[1]. Furthermore, aspects like gender differences in patient compliance to follow-up, treatment delay[36] and ability to stop smoking after initial diagnosis may be considered, but is outside the scope of our study.

The gender difference in malignant transformation ought further investigation but might suggest for a longer and closer follow up for males and perhaps even more encouragement and support for smoking cessation.

Glottic cancer is the most common form of laryngeal cancer[39]. The annual number of cases diagnosed with glottic cancer in Denmark in the period 2000-2009 was approximately 139 and the corresponding incidence 2.6 per 100,000 persons[40]. With only around 95 annual new cases of GPL in Denmark, of which approximately 17 to 18 per year transforms into cancer, it seems as if only a limited fraction of all new patients with glottic cancer in those years have had biopsy proven GPL prior to the cancer diagnosis. However, since we excluded patients for whom the initial biopsy revealed cancer, further analysis of that cohort is outside the scope of our study and we cannot

comment on possible patients delay or the extent of benign (non-GPL) biopsies prior to the cancer diagnosis.

Limitations and strengths

Our data was based on histological assessments by several danish pathologists during 10 years. One therefore might expect a large inconsistency in data. However this did not seem to be the case as there was no substantial difference in data when seperated by geograhical area (data not shown elsewhere). The study was however limited by the fact that some medical charts were unaccessible for review and the requested data on smoking status after primary diagnosis, details concerning tobacco and alcohol consumption, GERD and information regarding the extend of the surgical procedure performed were largely unavailable.

To our knowledge, this study is one of the largest published cohort studies of GPL and the first nationwide study conducted in the field. The Patobank database ultimately proved valid and is to be considered a highly reliable source of information.

Future perspectives:

The recently introduced DANGLOT protocol[31] favoring cordectomy, and thus the intended removal of all neoplastic tissue in the initial procedure, is expected to minimize the risk of repetitive tissue samples and prevent partial biopsies that lead to a risk of underestimating severity. The treatment of GPL is now centralized to the five oncologic centers in Denmark to ensure a higher level of experience among those responsible for diagnosis and treatment and provide uniform management of Danish patients. Hopefully in time these initiatives will reduce the incidence of recurrent GPL and transformation to glottic cancer

Conclusion

This national population-based study of GPL patients confirmed a stable incidence of GPL in Denmark from January 2000 until December 2009 and a considerable malignant potential

positively correlated to the severity of GPL. With recognition of the thorough work regarding the establishment of the WHOC2017, the results provided by this study encourages further discussion and suggests that the new classification may contain less nuanced prognostic information than the WHOC2005.

References:

1. Gale N, Hille J, Jordan RC, Nadal A, Williams MD . Chapter 3: Tumours of the hypopharynx, larynx, trachea and parapharyngeal space. In: El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ, ed. *WHO Classification of Head and Neck Tumours*. Vol 9 4th ed. IARC Press: Lyon, France; 2017:91-93.
2. Gale N, Pilch BZ, Sidransky D, Westra WH, Califano J. Chapter 3 Hypoharynx, larynx and trachea. In: Barnes L, Eveson JW, Reichart P, Sidransky D, eds. *WHO Classification of Tumours. Pathology and Genetics of Head and Neck Tumours*. Vol 9 3rd ed. IARC Press: Lyon, France; 2005:140-143.
3. Gale N, Gnepp DR, Poljak M, et al. Laryngeal Squamous Intraepithelial Lesions: An Updated Review on Etiology, Classification, Molecular Changes, and Treatment. *Adv Anat Pathol*. 2016;23(2):84-91.
4. Sadri M, McMahon J, Parker A. Management of laryngeal dysplasia: a review. *Eur Arch Otorhinolaryngol*. 2006;263(9):843-852.
5. Plch J, Pár I, Navrátilová I, Bláhová M, Zavadil M. Long term follow-up study of laryngeal precancer. *Auris Nasus Larynx*. 1998;25(4):407-412.
6. Hojslet PE, Nielsen VM, Palvio D. Premalignant lesions of the larynx. A follow-up study. *Acta Otolaryngol*. 1989;107(1-2):150-155. http://www.ncbi.nlm.nih.gov/pubmed/2929313. Accessed February 2, 2017.
7. Blackwell KE, Calcaterra TC, Fu Y-S. Laryngeal Dysplasia: Epidemiology and Treatment Outcome. *Ann Otol Rhinol Laryngol*. 1995;104(8):596-602.
8. Sllamniku B, Bauer W, Painter C, Sessions D. The transformation of laryngeal keratosis into invasive carcinoma. *Am J Otolaryngol Neck Med Surg*. 1989;10(1):42-54.
9. Gallo A, de Vincentiis M, Della Rocca C, et al. Evolution of precancerous laryngeal lesions: a clinicopathologic study with long-term follow-up on 259 patients. *Head Neck*. 2001;23(1):42-47.
10. Hintz B, Kagan A, Nussbaum H, Rao A. A "'Watchful Waiting Policy'" for in situ carcinoma of the vocal cords. *Arch Otolaryngol*. 1981;107:746-751.
11. Stenersen TC, Hoel PS, Boysen M. Carcinoma in situ of the larynx. Results with different methods of treatment. *Acta Otolaryngol Suppl*. 1988;449:131-133.

12. Weller MD, Nankivell PC, McConkey C, Paleri V, Mehanna HM. The risk and interval to malignancy of patients with laryngeal dysplasia; a systematic review of case series and meta-analysis. *Clin Otolaryngol*. 2010;35(5):364-372.
13. Mannelli G, Cecconi L, Gallo O. Laryngeal preneoplastic lesions and cancer: challenging diagnosis. Qualitative literature review and meta-analysis. *Crit Rev Oncol Hematol*. 2016;106(2016):64-90.
14. Cosway B, Paleri V. Laryngeal dysplasia: an evidence-based flowchart to guide management and follow up. *J Laryngol Otol*. 2015;129(06):598-599.
15. Do Nascimento M, Coelho C, De V, et al. Correlation Between Clinical Diagnosis and Pathological Diagnosis in Laryngeal Lesions. *J Voice*. 2016;30(5):595-599.
16. Rohde M, Grøntved ÅM, Krogdahl A, Godballe C. Aggressive elimination of precancerous lesions of the vocal cords to avoid risk of cancer. *Dan Med J*. 2012;59(5):A4399.
17. Paleri V, Mackenzie K, Wight RG, et al. Management of laryngeal dysplasia in the United Kingdom: a web-based questionnaire survey of current practice. *Clin Otolaryngol*. 2009;34(4):385-389.
18. Fink DS, Sibley H, Kunduk M, et al. Subjective and objective voice outcomes after transoral laser microsurgery for early glottic cancer. *Laryngoscope*. 2016;126(2):405-407.
19. Sjögren E V. Transoral Laser Microsurgery in Early Glottic Lesions. *Curr Otorhinolaryngol Rep*. 2017;5(1):56-68.
20. Statistics Denmark: population figures based on area BEF1A07. https://www.statistikbanken.dk/bef1a07. Accessed April 25, 2019.
21. Statistics Denmark: population figures from the censuses HISB3. http://www.statistikbanken.dk/HISB3. Accessed April 25, 2019.
22. Mehlum CS, Larsen SR, Kiss K et al. Laryngeal Precursor Lesions : Interrater and Intrarater Reliability of Histopathological Assessment. *Laryngoscope*. 2018;128(10):2375-2379.
23. Mehlum CS, Groentved AM, Godballe C, et al. In response to Laryngeal precursor lesions: Interrater and intrarater reliability of histopathological assessment. *Laryngoscope*. 2019;129(3):E93.
24. M. Gale, Nina ; Hille Jos ; Jordan, Richard C ; Nadal A; W. Regarding Laryngeal Precursor Lesions : Interrater and Intrarater Reliability of Histopathological Assessment. *Laryngoscope*. 2019;Vol.129(3):pp.E91-E92.

25. Blackwell KE, Fu Y, Calcaterra TC. Laryngeal dysplasia. A clinicopathologic study. *Cancer*. 1995;75(2):457-463.
26. Gale N, Michaels L, Luzar B, et al. Current review on squamous intraepithelial lesions of the larynx. *Histopathology*. 2009;54(6):639-656.
27. Gale N, Blagus R, El-Mofty SK, et al. Evaluation of a new grading system for laryngeal squamous intraepithelial lesions—a proposed unified classification. *Histopathology*. 2014;65(4):56-64.
28. Van Hulst AM, Kroon W, van der Linden ES, et al. Grade of dysplasia and malignant transformation in adults with premalignant laryngeal lesions. Eisele DW, ed. *Head Neck*. 2016;38 Suppl 1(S1):E2284-E2290.
29. Gale Nina, Poljak M, Zidar N. Update from the 4th Edition of the World Health Organization Classification of Head and Neck Tumours: What is New in the 2017 WHO Blue Book for Tumours of the Hypopharynx, Larynx, Trachea and Parapharyngeal Space. *Head Neck Pathol*. 2017;11(1):23-32.
30. Fleskens SA, Bergshoeff V, Voogd A et al. Interobserver variability of laryngeal mucosal premalignant lesions: A histopathological evaluation. *Mod Pathol*. 2011;24(7):892-898.
31. Waldemar T, Tvedskov J, Charabi B et al. Vejledning for håndtering af laryngeal intraepitelial neoplasi (LIN) og T1A- glottiscancer. *DAHANCA Guidel*. 2012;(september):1-26. https://www.dahanca.dk/page1.html#header1-u. Accessed February 4, 2018.
32. Remacle M, Eckel HE, Antonelli A, et al. Endoscopic cordectomy. a proposal for a classification by the Working Committee, European Laryngological Society. *Eur Arch Oto-Rhino-Laryngology*. 2000;257(4):227.
33. Ahn A, Wang L, Slaughter JC, Nguyen AM, Ossoff RH, Francis DO. Serial full-thickness excision of dysplastic vocal fold leukoplakia: Diagnostic or therapeutic? *Laryngoscope*. 2016;126(4):923-927.
34. Montgomery J, White A. A decade of laryngeal dysplasia in Paisley, Scotland. *Eur Arch Otorhinolaryngol*. 2012;269(3):947-951. doi:10.1007/s00405-011-1684-x.
35. Karatayli-Ozgursoy S, Pacheco-Lopez P, Hillel AT, et al. Laryngeal Dysplasia, Demographics, and Treatment. *JAMA Otolaryngol Neck Surg*. 2015;141(4):313.
36. Juel K. [Life expectancy and mortality in Denmark compared to Sweden. What is the effect of smoking and alcohol?]. *Ugeskr Laeger*. 2008;170(33):2423-2427.

37. Zuo J, Tao Z, Chen C, Hu Z. Characteristics of cigarette smoking without alcohol consumption and laryngeal cancer : overall and time-risk relation . A meta- analysis of observational studies. *Eur Arch Oto-Rhino-Laryngology*. 2017;274(3):1617-1631.
38. Schnohr P, Jensen JS, Scharling H, et al. Alkoholstatistik 2015 - Nationale data Sundhedsstyrelsen og Statens Serum Institut (Alcoholstatistics 2015, national data, Danish Health and Medicines Authority). 2015;1:17-22 Available at. https://www.sst.dk/da/udgivelser/2015/alkoholstatistik-2015. Accessed January 12, 2018.
39. Nahavandipour A, Jakobsen KK, Grønhøj C, Hebbelstrup Jensen D, Kim Schmidt Karnov K, Klitmøller Agander T, Specht L von BC. Incidence and survival of laryngeal cancer in Denmark: a nation-wide study from 1980 to 2014. *Acta Oncol*. 2019;58(7):977-982.
40. Lyhne NM, Johansen J, Kristensen CA, Andersen E, Primdahl H, Andersen LJ, Oksbjerg S OJ. Lyhne, N. M., et al. (2016). Incidence of and survival after glottic squamous cell carcinoma in Denmark from 1971 to 2011 - A report from the Danish Head and Neck Cancer Group. *Eur J Cancer*. 2016;May; 59:46-56.

Figure legends:

Figure 1: Patient selection flowchart (GPL: glottic precursor lesion).

Figure 2: Annual national incidence for adults (above or at 18 years of age) of glottic precursor lesions in Denmark 2000-2009 (per 100,000 inhabitants). Three patients below 18 years of age were omitted from these calculations.

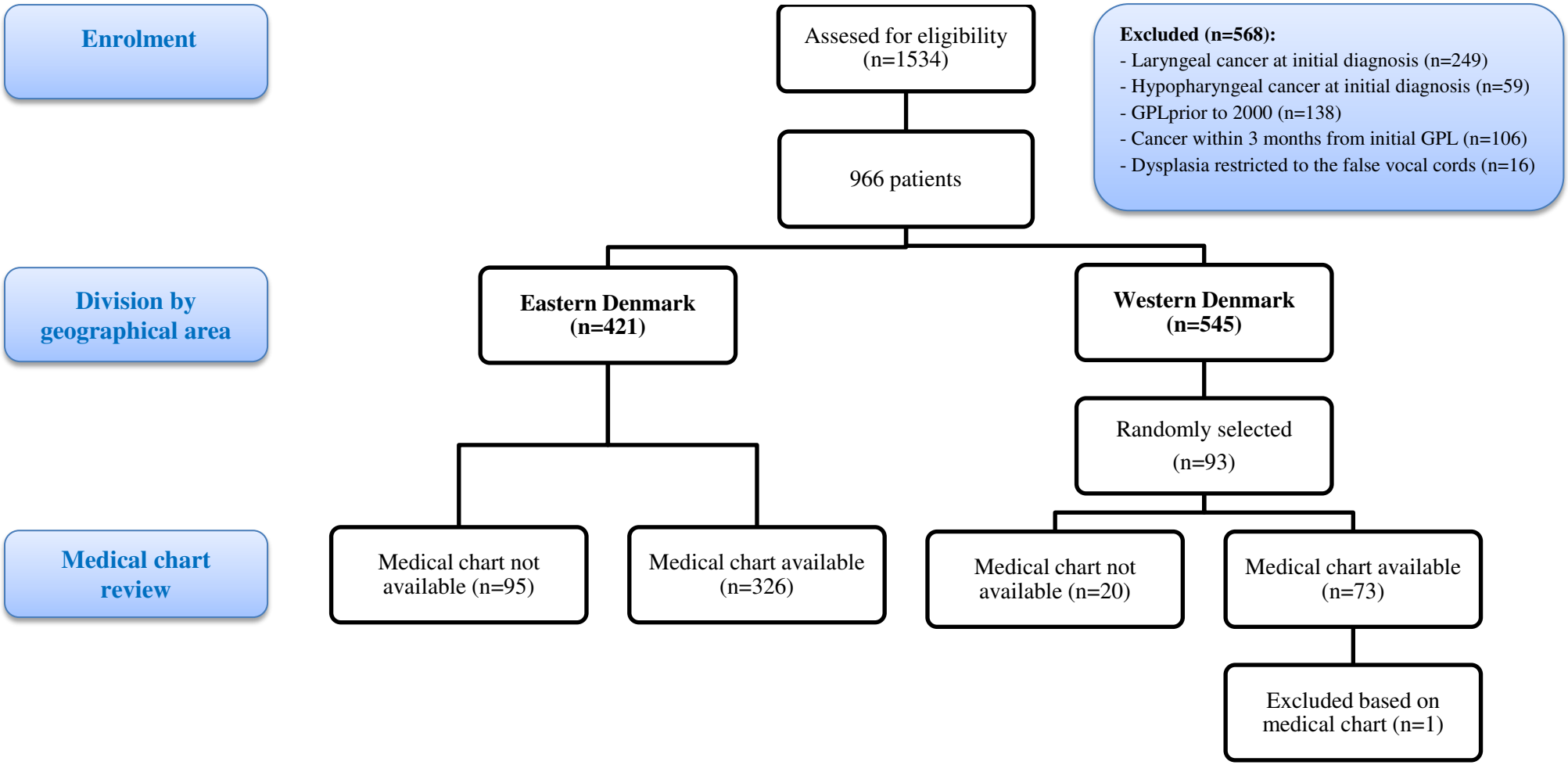
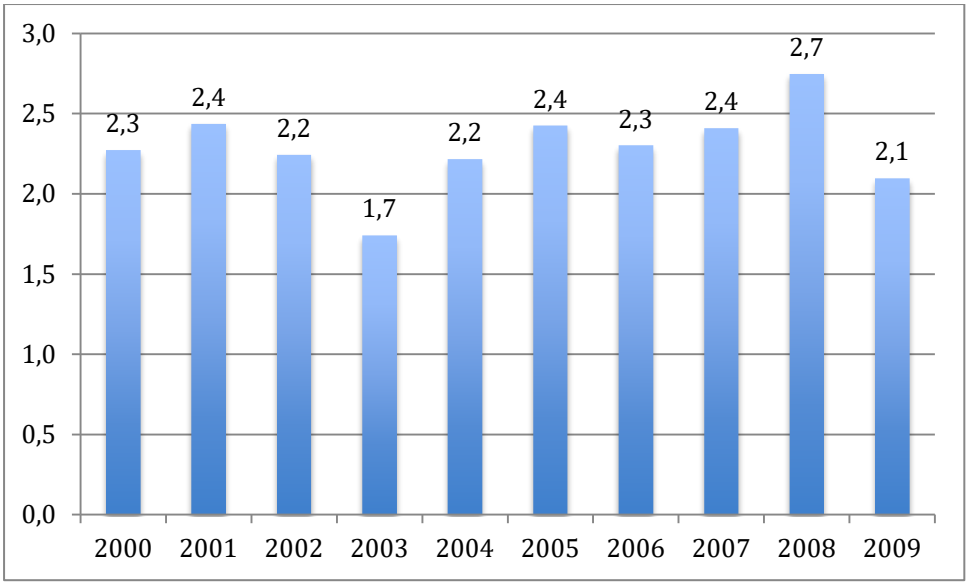


Figure 1: Patient selection flowchart. GPL: glottic precursor lesion.


3,0
2,5
2,0
1,5
1,0
0,5
0,0
2,3
2,4
2,2
1,7
2,2
2,4
2,3
2,4
2,7
2,1
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009

		Females	Males	Total
Gender (n, % of total)		318 (33%)	647(67%)	965
Age (years)	<40	29	42	71
	40<50	57	63	120
	50<60	102	178	280
	60<70	69	208	277
	70<80	46	124	170
	≥80	15	32	47
	Mean	57.0	60.7	59.4
	Median	57	61	60
Tobacco	History known	114	274	388
	Active	87 (76%)	156 (57%)	243
	Former	10 (9%)	86 (31%)	96
	Never	17 (15%)	32 (12%)	49
	Packyears (mean/med)	41.0/35	45.4/42	44.0/41

Table 1: Patient characteristics
Tobacco history was based on information available in 388 (97%) of the 398 reviewed patient medical charts. Packyears was registered for 156 of the 388 patients with known tobacco history (1 packyear is equivalent to smoking twenty cigarettes a day for one year).

Initial diagnosis		**Number of subsequent tissue samples after initial diagnosis**		
	n	0	1-2	>2
Dysplasia, NOS	114	51 (45%)	34 (30%)	29 (25%)
Mild dysplasia	365	214 (59%)	92 (25%)	59 (16%)
Moderate dysplasia	237	94 (40%)	74 (31%)	69 (29%)
Severe dysplasia	172	34 (20%)	68 (40%)	70 (41%)
Carcinoma in situ	77	9 (12%)	29 (38%)	39 (51%)
Total	965	402 (42%)	297 (31%)	266 (28%)

Table 2: Number of subsequently obtained tissue samples during follow-up based on the initial diagnosis of glottic precursor lesion.
NOS: dysplasia not otherwise specified.

		Malignant transformation					
Initial diagnosis	n	No	Yes	Odds	Odds ratio	CI 2.5%	CI 97.5%
Dysplasia, NOS	114	92	22 (19.3%)				
Mild dysplasia	365	337	28 (7.7%)	0.083	1		
Moderate dysplasia	237	190	47 (19.8%)	0.247	2.977	1.817	4.961
Severe dysplasia	172	123	49 (28.5%)	0.398	4.795	2.905	8.054
Carcinoma in situ	77	46	31 (40.3%)	0.674	8.111	4.479	14.830
Total	965	788	177 (18.3%)				

Table 3: Distribution of glottic precursor lesions at initial diagnosis graded according to World Health Organization Classification 2005 and Malignant Transformation.
NOS: dysplasia not otherwise specified is not included in the statistics but the data for overall transformation. Mild dysplasia is the baseline for comparison.

		Distribution of GPL			Malignant transformation				Months to malignant transformation	
Initial diagnosis	**Total (n=965)**	**Women (n= 318)***	**Men (n= 647)***	**p-value (Gender diff)**	**Total (n=177)#**	**Women (n=21)§**	**Men (n=156)§**	**p-value (Gender diff)**	**Median**	**Mean**
Dysplasia, NOS	114 (11.8%)	36 (11.3%)	78 (12.1%)	n.s	22 (19.3%)	3 (8.3%)	19 (24.4%)		36.3	41.7
Mild dysplasia	365 (37.8%)	152 (47.8%)	213 (32.9%)	<0.01	28 (7.7%)	3 (2.0%)	25(11.8%)	<0.001	34	48.5
Moderate dysplasia	237 (24.6%)	73 (23.0 %)	164 (25.3%)	<0.01	47 (19.8%)	4 (5.5%)	43(26.2%)	<0.001	34	43.3
Severe dysplasia	172 (17.8%)	44 (13.8%)	128 (19.8%)	<0.001	49 (28.5%)	8 (18.2%)	41 (32.0%)	<0.001	26	36.3
CIS	77 (8.0 %)	13 (4.0%)	64 (9.9%)	<0.001	31 (40.3%)	3 (23.1%)	28 (43.8%)	<0.001	16	38.0
Overall					177 (18.3%)	21 (6.6%)	156 (24.1%)		29	40.8

Table 4: Distribution of glottic precursor lesions and transformation to cancer of based on gender.
CIS: carcinoma in situ, NOS: dysplasia not otherwise specified. n.s. not significant
** percentages of subgroup of glottic precursor lesion among 318 women resp. 647 men*
 # percentages of malignant transformation in the specific histological subgroup
§ percentages of malignant transformation in the specific histological subgroup among women resp. men