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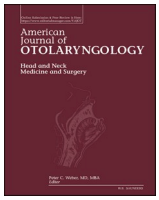

Prognostic role of histological depth of invasion in T1-2 oropharyngeal squamous cell carcinoma

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ABSTRACT

Background: Depth of invasion (DOI) is a well-established prognostic factor in oral cavity squamous cell carcinoma (OSCC). Incorporating DOI into the OSCC staging system has substantially refined T-classification and now informs key management decisions in patients with early-stage (T1–T2) disease. In contrast, the prognostic significance of DOI in oropharyngeal squamous cell carcinoma (OPSCC) remains relatively unclear. The aim of this study was to evaluate the prognostic value of DOI in T1–2 OPSCC.

Materials and methods: A population-based cohort representative of 1033 head and neck squamous cell carcinoma patients was used to identify T1–2 OPSCC patients treated with a curative intent and a tumor sample available for DOI measurement.

Results: In this retrospective cohort study of 74 patients, of whom 63.8% ($n = 44$) were p16-positive, 71.6% ($n = 53$) had a DOI $\geq$ 5 mm. While high DOI correlated with heavy alcohol consumption, it did not predict survival outcomes (5-year disease-specific survival HR 1.06; 95% CI 0.94–1.20; $p = 0.315$) or locoregional metastasis (HR 1.54; 95% CI 0.51–4.63; $p = 0.444$) in T1–2 tumors of the oropharynx. Instead, within the p16-negative cohort, increased DOI ($\geq$ 5 mm) was associated with an observable tendency toward poorer survival.

Conclusion: DOI was not a prognostic factor in p16-positive T1–2 OPSCC. Nevertheless, DOI may hold prognostic relevance for p16-negative disease. The patterns of local invasion and locoregional spread in OPSCC may reflect distinct biological mechanisms compared to those in other head and neck subsites.

1. Introduction

Within head and neck squamous cell carcinomas (HNSCCs), depth of invasion (DOI) is considered one of the most important prognostic factors for tumors originating from the oral cavity. DOI has been established as a significant predictor of diminished survival and an increased risk of cervical lymph node metastasis. Consequently, these findings carry profound implications for therapeutic stratification and prognostic accuracy [1–9]. The clinical significance of DOI is highlighted by its incorporation into the 8th edition of the American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC) staging system for oral cavity squamous cell carcinoma (OCSCC) [10–12]. In contrast to the oral cavity, the role of DOI in the adjacent oropharyngeal region remains relatively unexplored in literature.

While some studies on DOI have included small subsets of patients with oropharyngeal squamous cell carcinoma (OPSCC) [13–15], few have specifically focused on evaluating DOI in OPSCC, that has one of the most rapidly increasing incidences of any cancer in high-income countries [16] One contributing factor may be the inherent difficulty in determining true DOI in larger T3–T4 tumors, which are typically treated with radiotherapy (RT) or chemoradiotherapy (CRT), thereby complicating accurate histological evaluation. Furthermore, a concern of applying DOI in the oropharynx is the unique histological composition of the regional mucosa. Unlike in oral cavity, the oropharyngeal

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lymphoepithelium possesses a fragmented and discontinuous basement membrane that potentially facilitates more rapid invasion compared to a continuous histological barrier.

In 2020, Matos et al. reported that DOI as applied in the 8th edition of the AJCC/UICC staging system did not improve prognostic discrimination in OPSCC patients [17]. However, they proposed that this limitation could potentially be addressed through adjustments such as lowering the cutoffs DOI classification. Furthermore, the relationship between DOI and key clinical characteristics such as human papillomavirus (HPV) status, T-classification, and metastatic potential in OPSCC remains unclear. In this study, we investigate the role of DOI as a prognostic factor for survival outcomes in T1–2 OPSCC in a representative population-based HNSCC cohort [18–22].

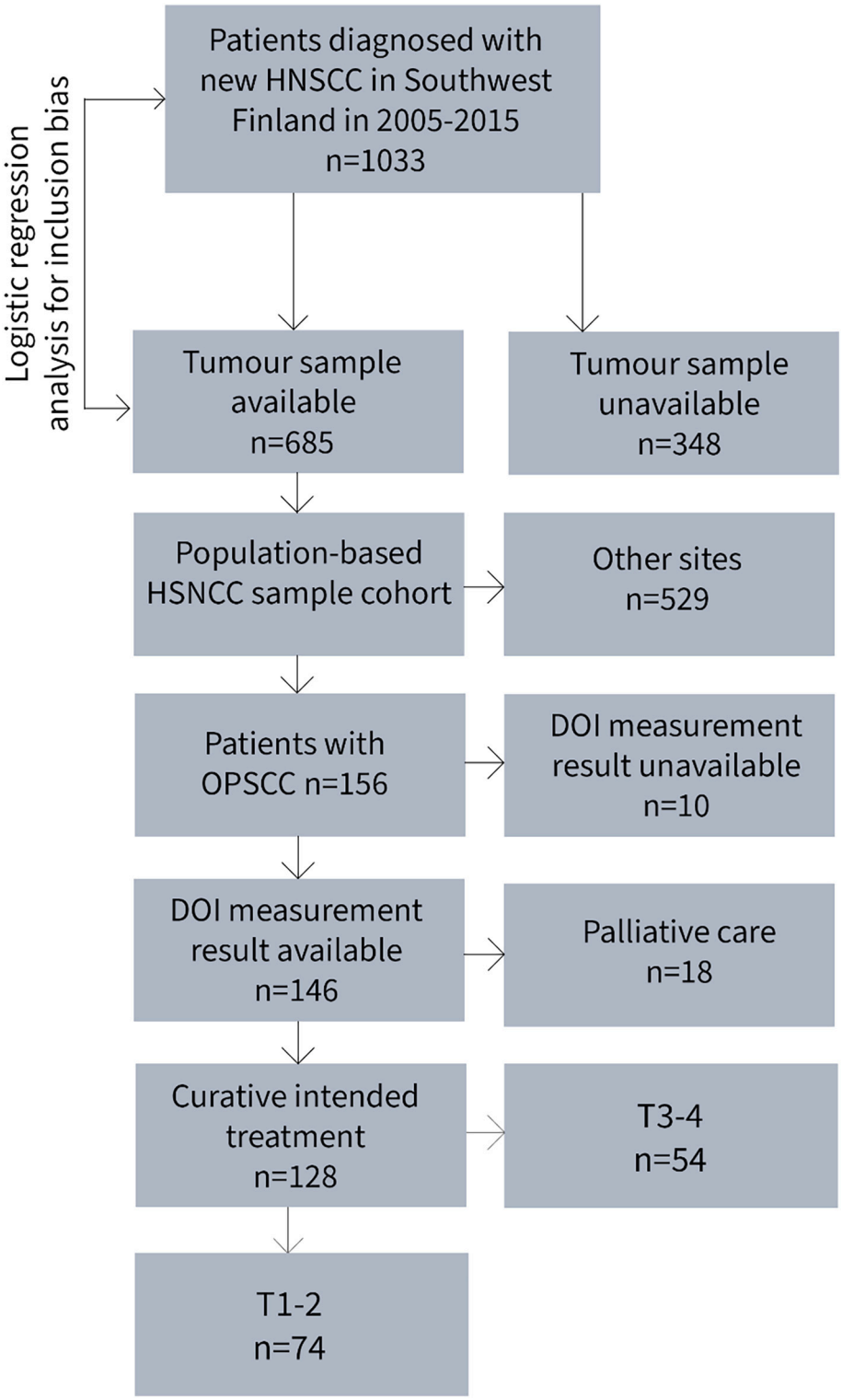

Fig. 1. Study population. All T1–2 oropharyngeal squamous cell carcinoma (OPSCC) patients of the population-based head and neck squamous cell carcinoma (HNSCC) sample cohort who had a depth of invasion (DOI) measurement result available and who were treated with curative intent were included in the study.

2. Materials and methods

2.1. Patients and samples

The established patient sample cohort was based on a previously collected population-based HNSCC cohort from the Southwest Finland tertiary referral area (Turku University Hospital), comprising all new HNSCC cases diagnosed between 2005 and 2015 (n = 1033) [19,20,23,24]. Of these, 685 (66.3%) had available primary tumor tissue and corresponding hematoxylin and eosin (H&E) stained HNSCC slides. The clinical data of these HNSCC patients were compared with a background population of all diagnosed HNSCC patients and cross-analyzed for possible inclusion bias [19,20,23,24]. Within this material, 156 patients had oropharyngeal SCC, and DOI could be assessed in 146 of the cases. Out of these 146 patients,128 were treated with curative intent. The final study cohort consisted of all curatively treated patients with T1–T2 oropharyngeal SCC and available DOI measurements ($n = 74$), as illustrated in Fig. 1. Supplement Table 1 compares the baseline characteristics of the study cohort with the T1–2 patients of the background population. Five- and three-year follow-up data were available for 79.7% and 95.9% of the patients, respectively.

Samples used for DOI measurements were H&E-stained and digitally scanned primary tumor samples (Pannoramic 1000 Digital Scanner, 3DHISTECH H-1141 Budapest, Öv u. 3., Hungary) that were retrieved from the Auria Biobank archives. Tissue samples were derived from resection specimens following diagnostic surgical interventions performed before CRT or RT. No post-treatment specimens were used. Immunohistochemistry (IHC) for p16 and in situ hybridization (ISH) for HPV were performed as previously described in detail [19,20]. Both immunohistochemical staining of p16 (Roche/Ventana clone E6H4) and HPV ISH were performed in a Ventana Bench-Mark XT staining automate (Ventana Medical Systems, Inc., Oro Valley, AZ, USA) in the laboratory of clinical pathology. Two independent authors analyzed the IHC staining and ISH score, and differences were conferred until consensus was reached. p16 immunostaining was graded positive if at least 70% of cells displayed strong nuclear and cytoplasmic staining intensity. Chromogenic ISH was performed using the ISH iView Blue Plus Detection Kit (760–097: Ventana Medical Systems Inc., Oro Valley, AZ, USA), the INFORM HPV III Family 16 Probe (800–4295: Roche, Basel, Switzerland), ISH protease 3 (780–4149: Roche, Basel, Switzerland), and Red Counterstain II (780–2218: Roche, Basel, Switzerland). The genomic cocktail contained dinitrophenol (DNP)-tagged probes targeting 12 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 ja 66). Visualization of the probe was achieved by biotin streptavidin-labelled alkaline phosphatase and 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) as the chromogenic substrate.

Overall survival (OS) was defined from end of treatment to end of follow-up or death from any cause. Disease-specific survival (DSS) was defined from end of treatment to end of follow-up or death from HNSCC. Cause of death was retrieved from the registry of the Finnish Digital and Population Data Services Agency. Disease-free survival (DFS) was defined as the time between the end of treatment and the first disease progression. Time from diagnostic resection to treatment averaged 3 weeks for surgical patients and 5 weeks for those receiving definitive RT or CRT.

2.2. Depth of invasion measurement

DOI was measured in line with the recommendations from the 8th edition of the AJCC for all samples [12]. The DOI measurement was defined as the plumbline from the basement membrane of the closest normal mucosa to the deepest point of tumor invasion [2,25]. All the slides' sections were examined carefully to identify the deepest part of tumor invasion. In ulcerated, exophytic or endophytic tumor, the epithelial baseline was reconstructed by drawing a horizontal reference line connecting the adjacent normal mucosal margins. In the absence of invasive front, estimated measurement was made till the deepest border of the specimen. All DOI measurements (by P.W. and M.K.) were carried out blinded to all patients' data, each case independently in millimeters, using digital pathology software (SlideViewer; 3DHISTECH H-1141 Budapest, Öv u. 3., Hungary). Discrepant scores were jointly reviewed, and an agreement value was recorded. For most statistical analyses, the DOI values were classified into two groups: low (< 5 mm) and high (≥ 5 mm). The 5 mm threshold was chosen since it corresponds to the AJCC/UICC 8th edition.

2.3. Statistical analysis

All statistical analyses were conducted using SPSS 30 software (SPSS, IBM, Armonk, NY, USA). Cox regression analysis was used to assess the survival effect of DOI. Alcohol use was defined as 10 doses or more a week and smoking as daily smoking at the time of diagnosis. Binomial logistic regression analysis was used to evaluate differences in the frequency of patients with high ($\geq$ 5 mm) and low ($<$ 5 mm) DOI in different patient groups. Survival curves were plotted using the Kaplan–Meier method and compared using the Cox regression analysis. Firth penalized likelihood approach was employed for p16-subgroup analyses. Hazard ratios (HRs) or odds ratios (ORs) with 95% confidence intervals (CI) and p values were reported. $P < 0.05$ were considered significant.

For analyses treating DOI as a continuous variable, Mann – Whitney U test was employed to study the difference in DOI between the 1) N0 and N+, 2) T1 and T2, and 3) p16+ and p16- groups. Additionally, median DOI values were reported.

2.4. Ethics

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Finnish National Supervisory Authority for Welfare and Health (V/39706/2019) and regional ethics committee of University of Turku (51/1803/2017 and 166/1801/2015) and the Auria Biobank Scientific Board (AB19–6863). Written informed consent was exempted as permission to use human tissues was granted by the Finnish National Authority for Medicolegal Affairs and the Auria Biobank.

3. Results

The clinicopathological characteristics of the patient cohort and the associations between these characteristics and high DOI are described in Table 1. The median follow-up time was 56 months (range 3–135 months, interquartile range 58–60). During the first 5 years after treatment, 17.6% (n = 13) of the patients experienced disease recurrence and 10.8% ($n = 8$) died of OPSCC. Similarly, 10.8% (n = 8) died of comorbidities. The median DOI result was 6.55 mm (range 1.0–25.0 mm). Out of all 74 patients, 28.4% (n = 21) had a DOI of <5 mm and 71.6% ($n = 53$) had a DOI of ≥5 mm. Representative samples of high and low DOI measurements are presented in Fig. 2A.

In the logistic regression analysis, presented in Table 1, heavy alcohol consumption, defined as 10 drinks or more a week, at the time of diagnosis was associated with lower DOI class ($<$ 5 mm). DOI differed significantly within the three different subsites of the oropharynx. The median DOI was 7.40 mm in tumors of the tonsils, 4.30 mm in tumors of the base of tongue, and 1.75 mm in tumors of the other sites of the oropharynx. Notably, the logistic regression analysis showed no association between nodal positivity and higher DOI class. The median DOI values in the N0 and N+ groups were 6.70 mm and 6.45 mm, respectively, with no statistically significant difference between the groups, as assessed by the Mann – Whitney U test ($p = 0.454$). Median DOI results for the T1 and T2 groups were 8.00 mm and 5.85 mm, respectively. The difference was not statistically significant, as determined by the Mann –

Table 1
Relationship between depth of invasion (DOI) and clinicopathological parameters. Alcohol use was defined as 10 drinks or more a week and smoking as daily smoking at the time of diagnosis. T-class and lymph node metastasis status were determined by pathologic staging.

	Total		DOI <5 mm		DOI ≥5 mm		Logistic regression	
	n	%	n	%	n	%	OR (95% CI)	*p* value
Age								
<60	36	48.6	8	22.2	28	77.8	1	–
≥60	38	51.4	13	34.2	25	65.8	0.55 (0.20–1.54)	**0.256**
Sex								
Female	22	29.7	3	13.6	19	86.4	1	–
Male	52	70.3	18	34.6	34	65.4	0.30 (0.08–1.15)	**0.078**
T-class								
T1	28	37.8	8	28.6	20	71.4	1	–
T2	46	62.2	13	28.3	33	71.7	1.02 (0.34–2.88)	**0.977**
Lymph node metastasis								
N0	20	27.0	7	35.0	13	65.0	1	–
N+	54	73.0	14	25.9	40	74.1	1.54 (0.51–4.63)	**0.444**
Smoking								
No	47	63.5	12	25.5	35	74.5	1	–
Yes	27	36.5	9	33.3	18	66.7	0.69 (0.24–1.93)	**0.475**
Alcohol use								
No	55	74.3	12	21.8	43	78.2	1	–
Yes	19	25.7	9	47.4	10	52.6	0.31 (0.10–0.94)	**0.038**
Site								
Tonsil	56	75.7	10	17.9	46	82.1	1	**–**
Base of tongue	14	18.9	8	57.1	6	42.9	0.16 (0.05–0.58)	**0.005**
Other	4	5.4	3	75.0	1	25.0	0.07 (0.01–0.77)	**0.030**
Treatment								
CRT	55	74.3	16	29.1	39	70.9		
RT	7	9.5	1	14.3	6	85.7	Not included	
Surgery only	2	2.7	1	50.0	1	50.0		
Combined[a]	10	13.5	3	30.0	7	70.0		
p16								
Positive	44	63.8	10	22.7	34	77.3	1	**–**
Negative	25	36.2	10	40.0	15	60.0	0.44 (0.15–1.28)	**0.133**
Unknown	5							

Odds ratios (HR), 95% confidence intervals (95% CI), and statistical significance were calculated using binomial logistic regression analysis.
[a] Surgery and chemoradiotherapy (CRT) or surgery and radiotherapy (RT).

Whitney U test ($p = 0.251$).

Consequently, we studied the effect of DOI on survival in OPSCC. As demonstrated by Fig. 3B–D, DOI class had no significant effect on OS, DSS or DFS. Emerging evidence suggests that utilizing DOI as a continuous variable may provide superior risk stratification compared to categorical modeling [9]. Thus, the effect of DOI on survival was additionally evaluated by treating DOI as a continuous variable in a Cox regression analysis. The survival analysis, presented in Table 2, demonstrated that DOI, assessed as a continuous covariate, did not have prognostic significance for 5-year survival.

P16 immunohistochemistry was utilized as a surrogate marker for human papillomavirus (HPV) status. Of the 69 patients with available p16 data, 44 (63.8%) were p16-positive and 25 (36.2%) were p16-negative. Among the p16-positive subgroup, HPV ISH results were available for 43 patients, with 38 (88.4%) demonstrating HPV ISH positivity. Whereas, within the p16-negative subgroup, 23 patients were evaluated via ISH; only one (4.3%) was HPV ISH-positive, while the remaining 22 (95.7%) were negative. These findings demonstrate a high degree of concordance between p16 immunohistochemistry and HPV ISH within our cohort. To maintain analytical clarity, we utilized p16 status as the primary indicator for HPV status in the following DOI analyses.

The median DOI for p16-positive and -negative patients were 7.25 mm and 5.60 mm, respectively. No statistical significance was determined using the Mann – Whitney *U* test ($p = 0.111$). In terms of overall survival, DOI did not appear to be a prognostic factor in either p16-positive or p16-negative patients (Fig. 3A–B). However, among the p16-negative subgroup ($n = 25$), patients with a high DOI (≥5 mm) demonstrated an apparent trend toward inferior survival, though this difference did not reach statistical significance, likely reflecting the limited sample size. Subsite-specific analyses for tonsillar and base-of-tongue tumors are provided in Supplementary Fig. 1.

4. Discussion

In this study, we evaluated DOI as a prognostic factor in T1–2 OPSCC in a population-based HNSCC cohort. Our findings indicate that, unlike in oral cavity SCC, DOI did not predict poor survival in T1–2 tumors of the oropharynx when both p16-positive and p16-negative patients were included. Instead, low DOI was associated with heavy alcohol consumption, a known prognostic risk factor in HNSCC [23]. Moreover, high DOI was not associated with the presence of cervical lymph node metastases. However, in the p16-negative subgroup, a DOI of ≥5 mm was linked to an evident trend toward poorer DSS and DFS.

Our results are consistent with Matos et al. who applied DOI as incorporated in the 8th edition of the AJCC/UICC staging system in a dataset of 60 OPSCC patients who received surgery as primary treatment. The integration of DOI, as reported in their study, compromised the discriminatory capacity of the pT classification. Moreover, patients who were upstaged based on DOI exhibited a paradoxically better prognosis compared to those who remained within the same pT stage group [17]. Alkureishi et al. suggested based on a sample of 38 OPSCC patients that a smaller cutoff (2 mm) would be more appropriate for tumors of the oropharynx [15]. However, results from our continuous variable analysis did not support the hypothesis that DOI would confer a prognostic benefit in T1–2 OPSCC. Given that DOI varied significantly across the three oropharyngeal subsites, we presume that subsite-specific calculations would likely be necessary to establish optimal thresholds. Additionally, HPV status should ideally be accounted for when determining DOI cutoffs.

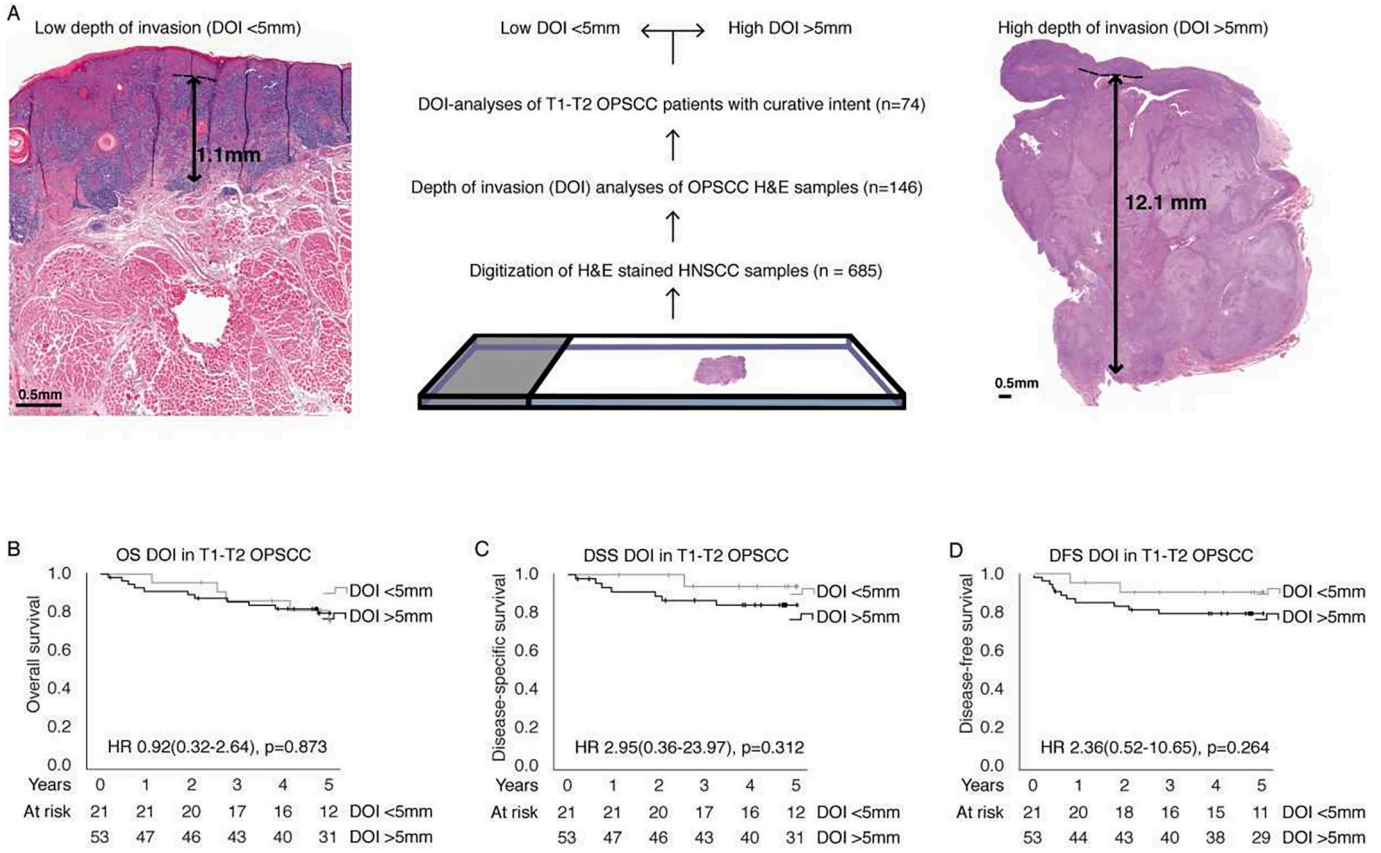

Fig. 2. A: Representative images and workflow of depth of invasion (DOI) measurement. The basement membrane is delineated in the image by a dashed line. B–D: Survival in relation to DOI in the T1–2 oropharyngeal squamous cell carcinoma (OPSCC) cohort ($n = 74$). Low DOI was defined as <5 mm and high DOI as ≥5 mm. Hazard ratios (HR), 95% confidence intervals (95% CI), and statistical significance were calculated using Cox regression analysis. Overall survival (OS); disease-specific survival (DSS); disease-free survival (DFS).

In contrast to results reported for other HNSCC subsites, our analysis did not demonstrate an association between DOI and lymph node metastases [9,26–29]. One possible explanation is the proximity of oropharyngeal tissues to the lymphatic system, particularly in the tonsillar region, which possesses a unique anatomical architecture. In 2004, Wenzel et al. suggested that DOI loses its prognostic value in the presence of lymph node metastases and especially when capsular rupture is present. This conclusion was based on a cohort of 66 patients diagnosed with either OCSCC or OPSCC [13]. In contrast to oral cavity cancers, where DOI guides elective neck dissection, our findings suggest it may not hold the same utility in OPSCC, specifically among p16-positive cases. Despite a high rate of nodal metastasis in our cohort, no significant association with DOI was observed, suggesting that DOI is unlikely to guide neck dissection decisions in this context.

The limitations of this study include retrospective nature and small sample size of the subgroup analyses. Furthermore, treatment modalities were not standardized across the cohort. However, all patients included in the study received curative intended cancer treatment according to national guidelines. We also acknowledge the limitations of using p16 as a surrogate for HPV infection, as discussed in our previous work [19]. The main strength of this study is its unbiased, population-based patient and tissue sample collection [20,24]. To the best of our knowledge, this represents the largest cohort of patients with OPSCC in which the associations between DOI and overall survival, disease-specific outcomes, locoregional metastasis, and p16 expression have been systematically investigated.

As a conclusion, our findings do not support the implementation of DOI on T1–2 p16-positive OPSCC. Nonetheless, DOI may represent a meaningful prognostic indicator in T1–T2 p16-negative OPSCC. Larger studies are required to further elucidate this association and to determine whether the prognostic value of DOI varies across different oropharyngeal subsites. Consistent with our previous research [20,21,30], this study further underscores the importance of site specificity in prognostic studies of HNSCC. Finally, our observations support the hypothesis that local invasion and locoregional dissemination of especially p16-positive OPSCC follows a distinct biological pathway compared to other HNSCC subsites.

CRediT authorship contribution statement

Linda Nissi: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Amr Elseragy:** Writing – original draft, Visualization, Investigation. **Awais Wahab:** Writing – original draft, Visualization, Investigation. **Pia Wennerstrand:** Writing – review & editing, Investigation. **Maisa Knuutila:** Writing – original draft, Investigation. **Johannes Routila:** Writing – review & editing, Investigation, Data curation. **Teemu Huusko:** Writing – review & editing, Investigation, Data curation. **Heikki Irjala:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Tuula Salo:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition. **Sami Ventelä:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Financial support

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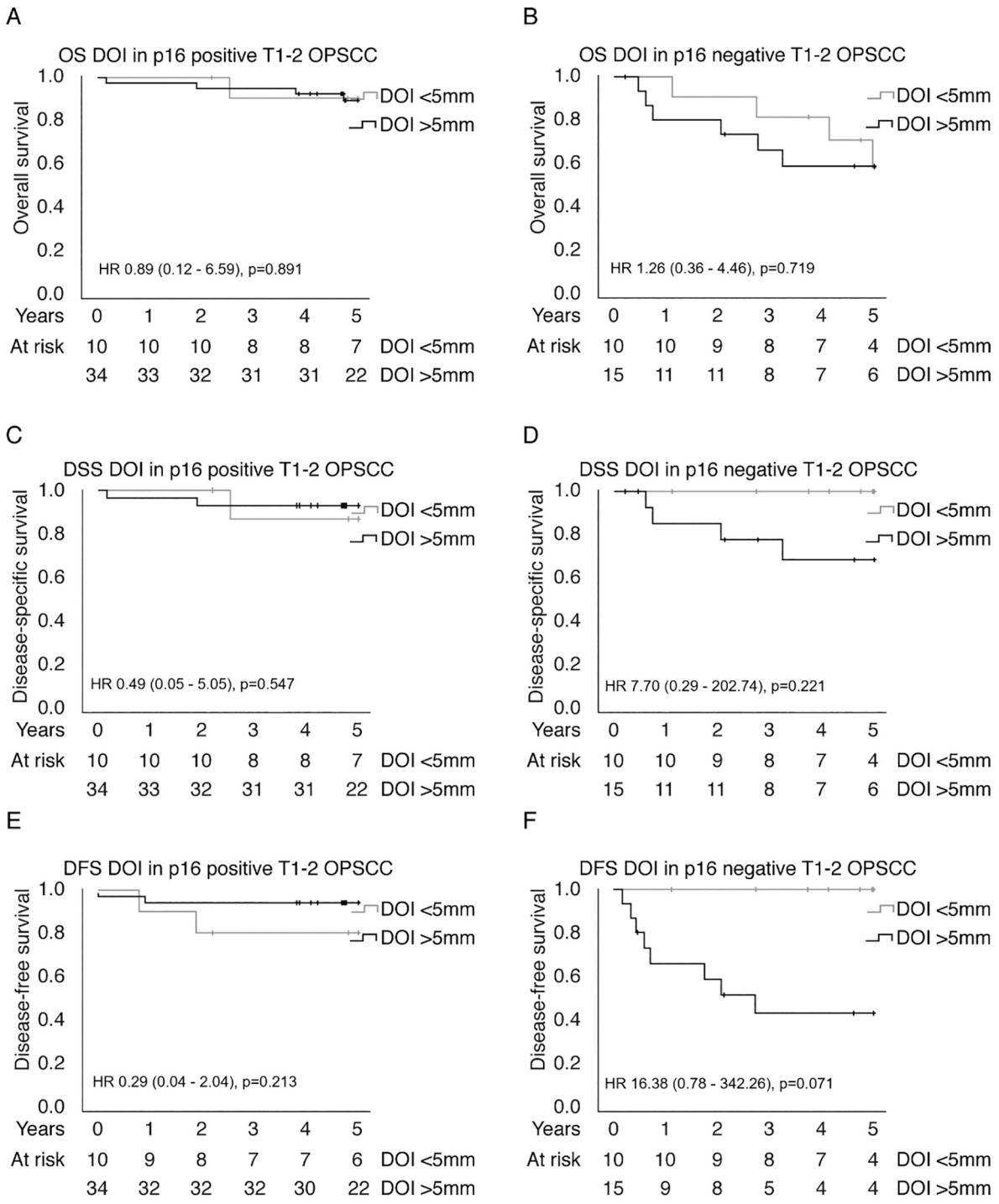

Fig. 3. Five-year overall survival (OS), disease-specific survival (DSS), and disease-free survival (DFS) in relation to depth of invasion (DOI) and p16 status of the primary tumor used as a surrogate marker for human papillomavirus (HPV). Low DOI was defined as <5 mm and high DOI as ≥5 mm. Results from Cox models with Firth penalized likelihood approach.

Table 2
Survival effects of DOI as a continuous variable. Hazard ratios (HR), 95% confidence intervals (CI), and *p* values were calculated using Cox regression analysis. OS (overall survival), DSS (disease-specific survival), DFS (disease-free survival).

	5-year OS		5-year DSS		5-year DFS	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
DOI/1 mm	1.01 (0.92–1.12)	0.787	1.06 (0.94–1.20)	0.315	1.04 (0.94–1.15)	0.444

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Declaration of competing interest

The authors declare that they have no conflicts of interests that are directly or indirectly related to the work submitted for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amjoto.2026.104869.

Data availability

De-identified aggregate data and analytic code available on reasonable request; individual-level data restricted.

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