Time to change perspectives on HPV in oropharyngeal cancer. A systematic review of HPV prevalence per oropharyngeal sub-site the last 3 years

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ABSTRACT

Objectives: Human papillomavirus (HPV) as a risk factor in oropharyngeal squamous cell carcinoma (OPSCC) is well established. However, accumulating data imply that the OPSCC concept is too unspecific with regard to HPV prevalence and clinical importance. To further study the role of HPV in OPSCC by sub-site, a systematic review and meta-analysis was performed.

Material and method: PubMed was searched and all studies reporting HPV data (p16/HPV DNA/RNA) in both “lymphoepithelial associated” (i.e. tonsillar and base of tongue cancer; TSCC and BOTSCC respectively) and “non-lymphoepithelial” (“other” OPSCC) OPSCC were included. Pooled odds ratios by HPV detection method were analysed using a random effects model.

Results: In total, 58 unique patient cohorts were identified. Total HPV prevalence in TSCC/BOTSCC was 56%, 95%CI: 55–57% (59%, 95%CI: 58–60% for TSCC only) as compared to 19%, 95%CI: 17–20%, in “other” OPSCC. Significant association of HPV to TSCC/BOTSCC vs. “other” OPSCC was observed no matter HPV detection method used, but statistical homogeneity was only observed when studies using algorithm based HPV detection were pooled.

Conclusion: HPV prevalence differs markedly between OPSCC sub-sites and while the role of HPV in TSCC/BOTSCC is strong, the role in “other” OPSCC is more uncertain and needs further evaluation.

1. Introduction

Already in 1983 Syrjänen and colleagues published the first data suggesting that human papillomavirus (HPV) could be associated to a sub-group of head and neck squamous cell carcinoma (HNSCC) [1]. Since then, the field of HPV, especially HPV 16, in HNSCC has emerged considerably. Subsequently, in 2009, due to a large body of evidence the International Agency of Research of Cancer (IARC) declared that “there is a strong epidemiological evidence for the casual role of HPV16 in the aetiology of cancer of the oropharynx and tonsil” [2]. Today, research on HPV and HNSCC in general has shifted and focuses on HPV in oropharyngeal squamous cell carcinoma (OPSCC). Moreover, recent accumulating data imply that HPV in the oropharynx context may still be too broad and un-specific and that it is biologically and clinically necessary to narrow down the concept of oropharynx to specific sub-sites, more specifically to tonsillar and base of tongue squamous cell carcinoma (TSCC and BOTSCC) [3–6].

The oropharynx is namely a histological heterogeneous sub-site within the head and neck region that consists not only of the palatine tonsils and the base of tongue (including the lingual tonsils), but also the soft palate, the tonsillar pillars and the uvula. The histology of the palate, the pillars and the uvula is built up by a stratified squamous epithelium without a keratin layer, similar to what is observed in the oral cavity, whereas the histology of the tonsils and the tongue base is distinctly different. The tongue base and the tonsillar mucosa invaginates and forms “crypts” lined with reticulated epithelium, in which the basal lamina is discontinuous and the histological border between the epithelium and the underlying lymphoid stroma is indistinct (“lymphoepithelial tissue”) [7,8]. These crypts are normally not observed at the other sites of the oropharynx (or in e.g. oral cavity). There is now evidence demonstrating that HPV positive carcinomas develop within the histological characteristic crypts in the oropharynx, while HPV negative carcinomas emerge mainly from the surface epithelium [7,8]. Due to this morphological difference in tissue tropism and absence or...
presence of crypts, we speculate that HPV should be evaluated per sub-site in oropharynx. Here, a systematic review is presented of literature published 2013–2016 regarding HPV prevalence per cancer sub-site in the oropharynx, and we argue that sub-site within oropharynx matters.

2. Material and methods

2.1. Search strategy and data extraction

PubMed was searched for all studies published from 2013-01-01 to 2016-10-31 using the search terms (HPV OR Papillomaviridae[MeSH]) AND (oropharyngeal OR oropharynx OR tonsil OR tonsillar OR “base of tongue” OR “soft palate”) AND (cancer OR carcinoma) AND (2016[DP] OR 2015[DP] OR 2014[DP] OR 2013[DP]). The PRISMA statement was consulted to perform the search [9]. In total 1266 articles were identified and ultimately 64 met the inclusion criteria of which 58 unique cohorts were identified and for details see the flow chart in Fig. 1. More specifically, 965 articles remained initially for further analysis after filtering out 230 as review articles, 30 not written in English, and 41 without an abstract. Abstracts from these 965 articles were then reviewed by two researchers (AN and LH) and those reporting HPV data were then further reviewed by examining the “material and method” and the “result” section in the articles. Articles reporting HPV data by a molecular tissue specific method (PCR, ISH or p16 immunohistochemistry) in HPV related “lymphoepithelial” oropharyngeal sub-sites (i.e. tonsillar and base of tongue) and in HPV un-related “non-lymphoepithelial” oropharyngeal sub-sites (i.e. walls of oropharynx, uvula and soft palate) in an un-selected cohort (retrospective/prospective, randomized/non-randomized) were included (Fig. 1). For each study, only the cohort of OPSCC patients was considered and the numbers of patients with HPV positive and negative tumours per sub-site were calculated or extracted, together with the HPV detection method. A consensus was reached for each article. The main reason for exclusion was that the sub-sites of oropharynx were not specified (Fig. 1).

2.2. Statistical analysis

Differences in HPV positive and negative patient numbers were calculated by using Fisher’s exact test (two-tailed) and Chi2-test (two-tailed) when appropriate. A p-value ≤0.05 was considered as significant. The metan command in Stata 11 (StataCorp, College Station, TX) was used to pool odds ratios (OR) with 95% confidence intervals (CI) across studies using the Der Simonian and Laird random-effects methods.

Fig. 1. Flow diagram of study population identification and selection.
| Author, Year       | Country | Oropharyngeal sub-site | HPV+ tumours | HPV- tumours | HPV prevalence | HPV detection | p-value¹ (TSSC vs. "other" OPSCC) | p-value¹ (TSSC only vs. "other" OPSCC) |
|--------------------|---------|------------------------|--------------|-------------|----------------|--------------|----------------------------------|----------------------------------|
| Bahl et al., 2014  | India   | Base of tongue         | 14           | 61          | 19% (18–20%)   | PCR          | NS                               | NS                               |
|                    |         | Tonsil                 | 10           | 15          | 40% (36–44%)   |              |                                  |                                  |
|                    |         | Soft palate            | 0            | 5           | 0% (0–0%)      |              |                                  |                                  |
| Bhosale et al., 2016 | India | Base of tongue         | 0            | 23          | 0% (0–0%)      | p16 IHC      | NS                               | NS                               |
|                    |         | Tonsil                 | 3            | 18          | 14% (11–18%)   |              |                                  |                                  |
|                    |         | Soft palate            | 0            | 5           | 0% (0–0%)      |              |                                  |                                  |
|                    |         | Posterior wall         | 1            | 4           | 20% (4–36%)    |              |                                  |                                  |
| Broglie et al., 2013 | Switzerland | Base of tongue | 22           | 28          | 44% (42–46%)   | p16 IHC      | NS                               | NS                               |
|                    |         | Tonsil                 | 31           | 37          | 46% (44–48%)   |              |                                  |                                  |
|                    |         | Post wall/ soft palate | 1            | 5           | 17% (4–29%)    |              |                                  |                                  |
| Broglie et al., 2015 | Switzerland | Base of tongue | 3            | 3           | 50% (34–66%)   | p16 IHC      | NS                               | NS                               |
|                    |         | Tonsil                 | 36           | 13          | 73% (72–75%)   |              |                                  |                                  |
|                    |         | Soft palate            | 0            | 5           | 0% (0–0%)      |              |                                  |                                  |
|                    |         | Posterior wall         | 1            | 4           | 20% (4–36%)    |              |                                  |                                  |
| Busso et al., 2014  | Italy   | Base of tongue         | 22           | 28          | 44% (42–46%)   | p16 IHC      | NS                               | NS                               |
|                    |         | Tonsil                 | 31           | 37          | 46% (44–48%)   |              |                                  |                                  |
|                    |         | Post wall/ soft palate | 1            | 5           | 17% (4–29%)    |              |                                  |                                  |
| Cerezo et al., 2014 | Spain  | Base of tongue         | 10           | 30          | 25% (23–27%)   | p16 IHC      | NS                               | NS                               |
|                    |         | Tonsil                 | 11           | 27          | 29% (27–31%)   |              |                                  |                                  |
|                    |         | Soft plate             | 5            | 8           | 38% (31–46%)   |              |                                  |                                  |
|                    |         | Pharyngeal wall        | 1            | 1           | 50% (1–99%)    |              |                                  |                                  |
| Dahlstrom et al., 2015 | United States of America | Base of tongue | 139          | 16          | 90% (89–90%)   | p16 IHC and ISH with/without PCR | 0.04                           | 0.04                           |
|                    |         | Tonsil                 | 172          | 22          | 89% (88–90%)   |              |                                  |                                  |
|                    |         | Other                  | 4            | 3           | 57% (43–71%)   |              |                                  |                                  |
| Davis et al., 2014  | United States of America | Base of tongue | 5            | 4           | 56% (45–66%)   | p16 IHC      | 0.003                           | 0.002                           |
|                    |         | Tonsil                 | 12           | 3           | 80% (75–85%)   |              |                                  |                                  |
|                    |         | Soft palate            | 0            | 6           | 0% (0–0%)      |              |                                  |                                  |
| Doná et al., 2015  | Italy   | Base of tongue         | 26           | 34          | 43% (42–45%)   | PCR          | 0.002                           | 0.003                           |
|                    |         | Tonsil                 | 30           | 34          | 47% (45–48%)   |              |                                  |                                  |
|                    |         | Other oropharynx       | 1            | 15          | 6% (3–9%)      |              |                                  |                                  |
| Evans et al., 2013 | United Kingdom | Base of tongue and vallecula | 15           | 20          | 43% (40–46%)   | p16 IHC and ISH with/without PCR | 0.001                         | 0.0003                         |
|                    |         | Tonsil                 | 54           | 39          | 58% (57–59%)   |              |                                  |                                  |
|                    |         | Other oropharynx       | 0            | 10          | 0% (0–0%)      |              |                                  |                                  |
| Fahkry et al., 2014 | United States of America | Base of tongue | 52           | 36          | 59% (58–60%)   | p16 IHC      | 0.02                           | 0.002                           |
|                    |         | Tonsil                 | 39           | 19          | 67% (66–69%)   |              |                                  |                                  |
|                    |         | Soft palate            | 0            | 3           | 0% (0–0%)      |              |                                  |                                  |
|                    |         | Oropharynx NOS         | 14           | 9           | 61% (57–65%)   |              |                                  |                                  |
|                    |         | Fauical arch           | 0            | 1           | 0% (0–0%)      |              |                                  |                                  |
|                    |         | Pharyngeal oropharynx  | 0            | 8           | 0% (0–0%)      |              |                                  |                                  |
| Faust et al., 2016  | Sweden  | Base of tongue         | 15           | 12          | 56% (52–59%)   | PCR          | 0.001                           | < 0.001                         |
|                    |         | Tonsil                 | 75           | 28          | 73% (72–74%)   |              |                                  |                                  |
|                    |         | Oropharynx NOS         | 2            | 9           | 18% (11–25%)   |              |                                  |                                  |
| Fonmarti et al., 2015 | Not specified | Anterior/lateral oropharynx (tonsil, base of tongue and glossotonsillar sulcus) | 20           | 31          | 39% (37–41%)   | p16 IHC and PCR | < 0.001                         | –                               |
|                    |         | Other oropharyngeal sites | 0            | 20          | 0% (0–0%)      |              |                                  |                                  |
| Fujimaki et al., 2013 | Japan  | Lateral                | 27           | 23          | 54% (52–56%)   | p16 IHC and ISH | NS                             | 0.05                           |
|                    |         | Anterior               | 4            | 7           | 36% (28–43%)   |              |                                  |                                  |
|                    |         | Posterior              | 0            | 3           | 0% (0–0%)      |              |                                  |                                  |
|                    |         | Superior               | 0            | 2           | 0% (0–0%)      |              |                                  |                                  |
| Grisar et al., 2016 | Belgium | Tongue base            | 6            | 36          | 14% (13–16%)   | p16 IHC      | NS                             | 0.05                           |
|                    |         | Tonsil                 | 8            | 7           | 53% (47–60%)   |              |                                  |                                  |
|                    |         | Soft palate            | 1            | 4           | 20% (4–36%)    |              |                                  |                                  |
|                    |         | Oropharynx NOS         | 8            | 26          | 24% (21–26%)   |              |                                  |                                  |
| Habbous et al., 2013 | Canada | Base of tongue         | 159          | 50          | 76% (76–76%)   | p16 IHC      | < 0.0001                        | < 0.0001                        |
|                    |         | Tonsil                 | 308          | 83          | 79% (79–79%)   |              |                                  |                                  |

(continued on next page)
| Author, Year | Country | Sub-site | HPV+ | HPV- | HPV prevalence | HPV detection | p-value* (TSCC and BOTSCC vs. "other" OPSCC) | p-value* (TSCC only vs. "other" OPSCC) |
|-------------|---------|----------|------|------|---------------|--------------|------------------------------------------|----------------------------------|
| Hama et al., 2014 [28] | Japan | Anterior | 6 | 20 | 23% (20–26%) | PCR | < 0.0001 | < 0.0001 |
| &emsp; | &emsp; | Lateral | 73 | 44 | 62% (62–63%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Upper | 0 | 10 | 0% (0–0%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Posterior | 0 | 4 | 0% (0–0%) | &emsp; | &emsp; | &emsp; |
| Henneman et al., 2015 [29] | Netherlands | Base of tongue | 13 | 36 | 27% (25–28%) | PCR | 0.003 | 0.001 |
| &emsp; | &emsp; | Tonsil | 37 | 41 | 47% (46–49%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Other oropharynx | 1 | 18 | 5% (3–8%) | &emsp; | &emsp; | &emsp; |
| Hong et al., 2013 [30] | Australia | Base of tongue | 15 | 31 | 33% (31–35%) | PCR and p16 IHC | 0.001 | < 0.001 |
| &emsp; | &emsp; | Tonsil | 253 | 298 | 45% (45–45%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Other oropharynx | 9 | 41 | 19% (17–20%) | &emsp; | &emsp; | &emsp; |
| Hong et al., 2013 [31] | Australia | Base of tongue | 29 | 30 | 49% (47–51%) | PCR and p16 IHC | < 0.001 | < 0.001 |
| &emsp; | &emsp; | Tonsil | 181 | 222 | 45% (45–45%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Other oropharynx | 10 | 43 | 19% (17–20%) | &emsp; | &emsp; | &emsp; |
| Hong et al., 2014 [32] | Australia | Base of tongue | 11 | 18 | 38% (35–41%) | PCR and p16 IHC | 0.001 | < 0.001 |
| &emsp; | &emsp; | Tonsil | 99 | 84 | 54% (54–55%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Other oropharynx | 3 | 18 | 14% (11–18%) | &emsp; | &emsp; | &emsp; |
| Hong et al., 2013 [33] | United States of America | Base of tongue | 20 | 16 | 56% (53–58%) | RT-PCR | NS | 0.03 |
| &emsp; | &emsp; | Tonsil | 3 | 3 | 50% (34–66%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Soft palate/uvula | 10 | 10 | 50% (45–55%) | &emsp; | &emsp; | &emsp; |
| Iyer et al., 2015 [34] | United States of America | Base of tongue | 50 | 39 | 56% (55–57%) | p16 IHC | < 0.0001 | < 0.0001 |
| &emsp; | &emsp; | Tonsil | 48 | 18 | 73% (71–74%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Soft palate | 8 | 38 | 17% (16–19%) | &emsp; | &emsp; | &emsp; |
| Jiang et al., 2015 [35] | United States of America | Base of tongue | 12 | 3 | 80% (75–85%) | ISH | < 0.0001 | 0.0001 |
| &emsp; | &emsp; | Tonsil | 10 | 6 | 63% (57–68%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Soft palate | 0 | 10 | 0% (0–0%) | &emsp; | &emsp; | &emsp; |
| Kim et al., 2014 [36] | Not specified | Base of tongue | 5 | 12 | 29% (24–35%) | PCR | NS | NS |
| &emsp; | &emsp; | Tonsil | 15 | 32 | 32% (30–34%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Soft palate | 1 | 9 | 10% (4–16%) | &emsp; | &emsp; | &emsp; |
| Kim et al., 2015 [37] | South Korea | Base of tongue | 1 | 3 | 25% (4–46%) | p16 IHC | < 0.001 | < 0.001 |
| &emsp; | &emsp; | Tonsil | 79 | 25 | 76% (75–77%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Soft palate | 1 | 8 | 11% (4–18%) | &emsp; | &emsp; | &emsp; |
| Kwakami et al., 2013 [38] | Japan | Base of tongue | 4 | 9 | 31% (24–38%) | PCR | < 0.001 | 0.001 |
| &emsp; | &emsp; | Tonsil | 31 | 29 | 52% (50–53%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Other oropharynx | 5 | 26 | 16% (14–18%) | &emsp; | &emsp; | &emsp; |
| Kwon et al., 2016 [39] | New Zealand | Tonsil and tonguebase | 86 | 31 | 74% (73–74%) | p16 IHC | < 0.0001 | ~ |
| &emsp; | &emsp; | Other oropharynx | 0 | 14 | 0% (0–0%) | &emsp; | &emsp; | &emsp; |
| Lam et al., 2015 [40] | China | Base of tongue | 4 | 35 | 10% (9–12%) | PCR and E6*I mRNA | 0.01 | 0.003 |
| &emsp; | &emsp; | Tonsil | 36 | 88 | 29% (28–30%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Soft palate | 3 | 29 | 9% (8–11%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Other oropharyngeal walls | 0 | 12 | 0% (0–0%) | &emsp; | &emsp; | &emsp; |
| Lee et al., 2016 [41] | South Korea | Base of tongue | 15 | 4 | 79% (75–83%) | p16 IHC | < 0.0001 | < 0.0001 |
| &emsp; | &emsp; | Tonsil | 89 | 12 | 88% (87–89%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Soft palate | 0 | 4 | 0% (0–0%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Posterior wall | 0 | 2 | 0% (0–0%) | &emsp; | &emsp; | &emsp; |
| Van Limbergen et al., 2014 [70] | Belgium | Base of tongue | 16 | 67 | 19% (18–20%) | PCR and p16IHC | 0.002 | < 0.001 |
| &emsp; | &emsp; | Tonsil | 33 | 72 | 31% (31–32%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Soft palate | 0 | 11 | 0% (0–0%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Pharyngeal wall | 1 | 30 | 3% (2–4%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Unclear | 3 | 16 | 16% (12–20%) | &emsp; | &emsp; | &emsp; |
| Liu et al., 2015 [42] | Australia | Base of tongue | 7 | 13 | 35% (30–40%) | PCR and ISH | 0.002 | < 0.001 |
| &emsp; | &emsp; | Tonsil | 39 | 29 | 57% (56–59%) | &emsp; | &emsp; | &emsp; |
| &emsp; | &emsp; | Other oropharynx | 2 | 15 | 12% (8–15%) | &emsp; | &emsp; | &emsp; |

(continued on next page)
| Author, Year          | Country                | Sub-site                      | HPV+ tumours | HPV- tumours | HPV prevalence | HPV detection | p-value* (TSCC vs. “other” OPSCC) | p-value* (TSCC only vs. “other” OPSCC) |
|----------------------|------------------------|-------------------------------|--------------|--------------|----------------|---------------|--------------------------------|-------------------------------------|
| Ljokel et al., 2016  | Norway                 | Tonsil                        | 28           | 13           | 68% (66–71%)   | PCR           | < 0.0001**                  | < 0.0001**                          |
| Lybak et al., 2016   | Norway                 | Tonsil pillar                 | 1            | 12           | 8% (4–12%)     | PCR           | 0.04                           | 0.02                               |
| McIlwain et al., 2014| United States of America| Tonsil                         | 26           | 8            | 76% (74–79%)   | p16 IHC       | 0.03                           | –                                   |
| Mazul et al., 2016   | United States of America| Tonsil                         | 50           | 23           | 68% (67–70%)   | PCR           | NS                             | NS                                 |
| Melkane et al., 2014 | France                 | Lymphoid location             | 65           | 57           | 53% (52–54%)   | p16 IHC       | 0.03                           | –                                   |
| Melkane et al., 2014 | France                 | Lymphoid location (tonsillar and base of tongue) | 28            | 13           | 68% (66–71%)   | p16 IHC       | < 0.01                         | –                                   |
| Minamachi et al., 2013 | Japan                 | Lateral wall                  | 18           | 23           | 44% (42–46%)   | PCR           | NS                             | 0.04                               |
| Morbini et al., 2014 | Italy                  | Base of tongue                | 7            | 14           | 67% (51–82%)   | PCR           | NS                             | NS                                 |
| Naik et al., 2015    | United States of America| Tonsil                         | 70           | 6            | 60% (50–70%)   | mRNA ISH      | < 0.01                         | < 0.01                             |
| Nasman et al., 2013  | Sweden                 | Base of tongue                | 75           | 28           | 73% (72–74%)   | PCR           | < 0.0001**                   | < 0.0001**                         |
| Nichols et al., 2013 | United Kingdom         | Base of tongue                | 15           | 10           | 60% (56–64%)   | PCR           | < 0.01                         | 0.01                               |
| Nomura et al., 2014  | Japan                  | Lateral wall                  | 29           | 25           | 54% (52–56%)   | PCR and/or p16 IHC | 0.02                           | 0.05                               |
| Oguejiofor et al., 2013 | United Kingdom       | Base of tongue                | 32           | 27           | 54% (53–56%)   | p16 IHC       | NS                             | NS                                 |
| Ou et al., 2016      | New Zealand            | Base of tongue                | 15           | 5            | 75% (71–79%)   | p16 IHC and PCR | 0.02                           | 0.02                               |
| Quahius et al., 2015 | Germany                | Tonsillar                     | 59           | 76           | 44% (43–44%)   | PCR           | 0.03                           | 0.03                               |
| Rietbergen et al., 2013 | Netherlands          | Base of tongue                | 51           | 161          | 24% (24–24%)   | p16 IHC and PCR | < 0.0001**                   | < 0.0001**                         |

(continued on next page)
3. Results

3.1. Prevalence of HPV at different OPSCC sub-sites

In total, 64 articles were included in the analysis, with a total of 11710 patients in these studies. The number of patients varied between 30 and 1474 (mean 202 patients per study) (Table 1) [10–73]. The sub-sites tonsils and base of tongue dominated the oropharyngeal cancer sites (83%), whereas only a minority of the tumours were located in the soft palate and the oropharyngeal walls (17%). Total oropharyngeal HPV prevalence per study varied between 7% and 88% (Table 1).

Notably, HPV was more commonly found in “lymphoepithelial” tissues (TSCC and BOTSCC) as compared to “non-lymphoepithelial” tissues (other OPSCC) of the oropharynx (Table 1, Fig. 2A). Total HPV prevalence in TSCC/BOTSCC was 56%, 95% CI: 55–57% (59%, 95% CI: 58–60% for TSCC only) as compared to 19%, 95% CI: 17–20%, HPV prevalence in “other” OPSCC (Table 1).

Furthermore, since there is a risk of misclassification of large mobile tongue cancer into BOTSCC and vice versa, a sub-group analysis was performed comparing only TSCC and “other” OPSCC. The differences observed between “lymphoepithelial” and “non-lymphoepithelial” tissues were here even more pronounced (Table 1 and

Table 1 (continued)

| Author, Year   | Country   | Oropharyngeal sub-site | HPV+ tumours | HPV- tumours | HPV prevalence | HPV detection | p-value* (TSCC and BOTSCC vs. “other” OPSCC) | p-value* (TSCC only vs. “other” OPSCC) |
|---------------|-----------|------------------------|--------------|--------------|----------------|--------------|---------------------------------------------|----------------------------------------|
| Rietbergen et al., 2013 [61] | Netherlands | Base of tongue | 13 | 54 | 19% (18–21%) | P16 IHC and PCR | < 0.001 | < 0.0001 |
|              |           | Tonsil | 23 | 60 | 28% (27–29%) | PCR | - | - |
|              |           | Soft palate | 0 | 31 | 0% (0–0%) | PCR | - | - |
|              |           | Oropharynx NOS | 5 | 54 | 8% (6–9%) | PCR | - | - |
| Saito et al., 2015 [62] | Japan | Lateral wall | 45 | 48 | 48% (47–49%) | p16 IHC | 0.005 | 0.002 |
|              |           | Base of tongue | 12 | 29 | 29% (27–31%) | PCR | - | - |
|              |           | Superior wall | 1 | 10 | 9% (4–14%) | PCR | - | - |
|              |           | Posterior wall | 0 | 5 | 0% (0–0%) | PCR | - | - |
| Schache et al., 2013 [64] | United Kingdom | Base of tongue | 5 | 8 | 38% (31–46%) | qRT-PCR | NS | NS |
|              |           | Tonsil | 22 | 21 | 51% (49–53%) | PCR | - | - |
|              |           | Soft palate | 4 | 9 | 31% (24–38%) | PCR | - | - |
|              |           | Oropharynx NOS | 2 | 7 | 22% (13–31%) | PCR | - | - |
| Schache et al., 2016 [65] | United Kingdom | Base of tongue | 179 | 183 | 49% (49–50%) | p16 IHC and PCR | < 0.0001 < 0.0001 | 0.0001 |
|              |           | Tonsil | 528 | 326 | 62% (62–62%) | PCR or ISH | - | - |
|              |           | Soft palate/uvula | 8 | 80 | 9% (8–10%) | PCR | - | - |
|              |           | Oropharynx NOS | 49 | 121 | 9% (28–29%) | PCR | - | - |
| Schouten et al., 2016 [66] | Not stated | Base of tongue | 12 | 7 | 63% (58–68%) | p16 IHC | NS | NS |
|              |           | Tonsil | 12 | 6 | 67% (62–72%) | PCR | - | - |
|              |           | Oropharynx NOS | 3 | 4 | 43% (29–57%) | PCR | - | - |
| Steinhaus et al., 2014 [67] | United States of America | Base of tongue | 149 | 64 | 70% (70–70%) | PCR | < 0.0001 | < 0.0001 |
| Saraiya et al., 2015 [63] | China | Tonsil | 201 | 49 | 80% (80–81%) | PCR | - | - |
| Goodman et al., 2015 [25] | Germany | Other oropharynx | 46 | 48 | 49% (48–50%) | PCR | - | - |
| Strojan et al., 2015 [68] | Slovenia | Base of tongue | 4 | 16 | 20% (16–24%) | E6/E7 mRNA | NS | 0.05 |
|              |           | Tonsil | 12 | 28 | 30% (28–32%) | ISH | - | - |
|              |           | Other oropharynx | 4 | 35 | 10% (9–12%) | ISH | - | - |
| Tural et al., 2013 [69] | Turkey | Base of tongue | 12 | 15 | 44% (41–48%) | PCR | NS | NS |
|              |           | Tonsil | 26 | 19 | 58% (56–60%) | PCR | - | - |
|              |           | Other | 4 | 5 | 44% (34–55%) | PCR | - | - |
| Wang et al., 2016 [72] | China | Base of tongue | 6 | 68 | 8% (7–9%) | PCR | NS | < 0.0001 |
|              |           | Tonsil | 7 | 3 | 70% (61–79%) | PCR | - | - |
|              |           | Soft palate | 3 | 47 | 6% (5–7%) | PCR | - | - |
|              |           | Oropharynx NOS | 6 | 48 | 11% (10–12%) | PCR | - | - |
| Ward et al., 2014 [73] | United Kingdom | Base of tongue | 40 | 28 | 59% (57–60%) | p16 IHC and ISH | < 0.0001 | < 0.0001 |
|              |           | Tonsil | 99 | 57 | 63% (63–64%) | ISH | - | - |
|              |           | Other oropharynx | 10 | 36 | 22% (20–23%) | ISH | - | - |
| Wagner et al., 2015 [71] | Germany | Tonsil | 20 | 12 | 63% (60–65%) | P16 IHC | - | < 0.0001 |
|              |           | Other than tonsil | 12 | 84 | 13% (12–13%) | PCR | - | - |

* p-value calculated by chi-2 test (tonsil and tongue base vs other oropharynx and soft palate; or tonsil vs other oropharynx and soft palate) after patient numbers had been extracted from article.

** p-value calculated by chi-2 test (tonsil and tongue base, overlapping tonsil vs tonsil pillars other oropharynx and soft palate) after patient numbers been extracted from article.

Countries from which the patient material and data were collected.

Patients reported in Stainau et al. presented.
In addition, a separate analysis including only studies reporting HPV prevalence data divided by tonsillar, base of tongue, soft palate/uvulae and oropharynx was performed. As depicted in Table 2, HPV prevalence was highest in TSCC, followed by BOTSCC, and lower at the other sites (Table 2).

3.2. HPV is significantly more prevalently found in TSCC and BOTSCC compared to other OPSCC sites

The odds ratio of having HPV in TSCC and BOTSCC as compared to “other” OPSCC was calculated and studies were grouped by HPV detection method, i.e. either HPV DNA PCR alone, or p16 IHC alone, or a HPV DNA based algorithm, i.e. combining HPV DNA and p16 overexpression. The odds having HPV in TSCC and BOTSCC as compared to “other” OPSCC was significantly higher, no matter which detection method that was used as depicted in Fig. 3 (PCR: OR 4.60 95% CI 2.95–7.16, p < 0.001; p16 IHC: OR 4.26 95% CI 2.41–7.53, p < 0.001; algorithm: OR 5.19 95% CI 4.24–6.34, p < 0.001). Notably, no statistical heterogeneity (Chi² = 8.84 (d.f. = 15) p = 0.885; Estimate of between-study variance Tau-squared=0.00) was observed when applying the algorithm using the presence of HPV in combination with p16 overexpression as defining positive HPV status (Fig. 3C). In contrast, when using either HPV DNA PCR positivity or p16 alone, gave significant statistical heterogenic results (PCR: Chi² = 36.09 (d.f. = 16) p = 0.003; Estimate of between-study variance Tau-squared=0.39 and

Fig. 2. Heat map of HPV prevalence by oropharyngeal cancer sub-site. (A) Prevalence of HPV, defined by each included study, stratified by tonsillar (TSCC) and base of tongue (BOTSCC) squamous cell carcinomas vs. “other” (i.e. walls of oropharynx, uvulae and soft palate) oropharyngeal squamous cell carcinomas (OPSCC). (B) Prevalence of HPV, defined by each included study, stratified by TSCC only vs. “other” OPSCC.
Table 2
HPV prevalence by oropharyngeal sub-site (data extracted only from studies reporting HPV data separated by tonsils, tongue base, soft palate/uvula and oropharyngeal walls).

| Oropharyngeal sub-site | HPV+ tumours | HPV- tumours | HPV prevalence (95% CI) |
|------------------------|--------------|--------------|-------------------------|
| Tonsil*                | 1577         | 1238         | 56% (54–58%)            |
| Base of tongue*        | 590          | 881          | 40% (38–43%)            |
| Soft palate            | 59           | 429          | 12% (9–15%)             |
| Posterior wall*        | 122          | 537          | 19% (16–22%)            |

* This table only presents data from studies that have divided by oropharyngeal sub-sites: base of tongue, tonsil, soft palate and posterior wall. Following studies where included: 11, 14–16, 21, 24, 26, 28, 33, 37, 40–41, 43–45, 47, 50, 53, 55, 57, 60–62, 64–65, 70, 72.

4. Discussion

In this systematic review, HPV prevalence was significantly higher in “lymphoepithelial” sites of the oropharynx, i.e. tonsil and base of tongue, as compared to “non-lymphoepithelial” sites of the oropharynx, i.e. soft palate and oropharyngeal, irrespectively of HPV detection method.

Numerous previous studies have focused on differences in HPV prevalence between different head and neck cancer sites and different geographic areas [6,74], but few have addressed the relevance of sub-sites within oropharynx. As there has been a focus on OPSCC in contrast to HNSCC in general, many studies have unfortunately not specified these oropharyngeal sub-sites and very few studies have verified the sub-sites by histopathology. Recently however, Garnaes et al. [4] subdivided TSCC into specified TSCC (“lymphoepithelial”) and non-specified TSCC (“non-lymphoepithelial”) by histomorphology. This study reported that HPV prevalence was higher and increased over time in specified TSCC, while the prevalence of HPV was lower and stable over time in non-specified TSCC. Notably, the authors also observed a significant discordant HPV DNA and p16 IHC positivity in non-specified TSCC as compared to specified TSCC. Likewise, Marklund et al. have also presented similar results with discordant p16 status and HPV DNA positivity by PCR in oropharyngeal sub-sites outside the tonsils and the tongue base [5]. Analogous data have also been conveyed in oral carcinomas [75]. Moreover, in a recent meta-analysis of HPV prevalence in different head and neck sites, 24.2% (18.7–30.2) of the oral carcinomas were reported to harbour HPV DNA [6]. Comparable prevalence data were here described for “other” OPSCC (19%, 95% CI: 17–20%), which – together with the overlapping histomorphology – may suggest that “other” OPSCC are more comparable with oral carcinomas than TSCC/BOTSCC. Hence, we argue that not only geographic region and detection method should be considered when reporting HPV prevalence, but also oropharyngeal sub-site.

Studies by others have shown that HPV status defined by only p16 IHC or PCR alone in OPSCC may be too unspecific, and that if the methods are combined in an algorithm there is a high concordance with presence of active HPV infection [61]. Although the odds ratios, reported in this study, of having HPV in TSCC and/or BOTSCC as compared to “other” OPSCC was higher independent of method used, there was a significant heterogeneity between studies using p16 or PCR alone. In contrast, statistical heterogeneity was not observed when uniting studies using an algorithm combining HPV DNA and p16 overexpression, which suggests that using only a PCR or p16 based HPV detection method is too unspecific and may detect false HPV positive samples in non-tonsillar non-base of tongue OPSCC.

Notably, HPV prevalence per oropharyngeal sub-site is not only of academic concern, it is in fact of clinical importance. In a recently published Danish study, patients with specified TSCC and BOTSCC had a better clinical outcome if their tumours were both HPV DNA and p16 positive as compared to being only p16 positive, while an analogous difference in clinical outcome was not observed in patients with non-specified TSCC [3]. Similar results were reported by Ljokjel et al.[44] In that study, patients with HPV positive TSCC and BOTSCC were reported to have a better clinical outcome, but no differences in clinical outcome were observed between patients with HPV positive and negative “other” OPSCC. Likewise, a study by Marklund et al.[5] showed that HPV infection was not correlated to patient outcome if the patients had a non-tonsillar, non-base of tongue OPSCC.

Currently, it is discussed whether oncological treatment can be tapered in patients with HPV positive OPSCC, and randomized controlled studies have shown a beneficial survival in patients with HPV positive OPSCC. However, since patients with TSCC and BOTSCC dominate the OPSCC patient group, there is a risk that patients with TSCC and BOTSCC in published survival studies supersede patients with “other OPSCC”. This could lead to that patients with “other OPSCC” could disfavour from the introduction of tapered treatment, as well as that de-escalated therapy could be offered to patients with HPV positive “other” OPSCC, where survival benefit is doubtful. Notably, according to the newest 8th AJCC staging system, all oropharyngeal malignancies should be staged depending on their p16 status [76]. In light of data presented and discussed here, this approach could potentially be problematic. Subsequently, sub-specific survival analysis studies in oropharyngeal are highly warranted.

There are recognisable limitations in this study. First of all, since OPSCC still is a relatively rare disease, there is a risk that same patients are included in different studies/cohorts. To reduce this risk, we have restricted our analysis to patient cohorts included in reports published during the three last years, still allowing for the inclusion of more than 11,000 patients. We also focused on the patient cohort description in the material and method sections, but there could still be a risk for non-described overlapping patients between studies. Secondly, there is also a possibility of misclassification of tumours within the oropharyngeal region. This is especially evident in the distinction between large mobile tongue carcinomas and BOTSCC, in which only the latter is HPV associated. Relatedly, sub-coding of TSCC is infrequently presented. As stated in the introduction section, the histology and, most likely, the HPV prevalence differs between specified TSCC (ICD-10 C09.0) and e.g. carcinomas of the tonsillar pillars (ICD-10 C09.1). Furthermore, few studies have sub-classified OPSCC by histo-morphology [4]. Nevertheless, misclassification of sub-sites would most likely only dilute the HPV prevalence numbers and thus reduce the HPV differences between TSCC/BOTSCC and “other” OPSCC. Lastly, it has been documented that HPV prevalence differs between geographic regions [6] and studies included in this report are obtained from different geographical regions with different risk factors. Nonetheless, since the difference in HPV prevalence between sub-sites is studied here, and not absolute numbers, the impact of patient nationality should be minor.

To conclude, combining HPV DNA and p16 overexpression is safer for defining HPV positivity compared to using HPV DNA or p16 alone, and with this algorithm HPV was significantly more prevalent in TSCC/BOTSCC as compared to “other OPSCC sites”. The clinical role of HPV in “other” OPSCC must be further evaluated before initiation of de-escalation trials in these patients.

Conflict of interest statement

None declared.
**Fig. 3.** Forrest plot with odds ratios (OR) of having HPV in tonsillar and base of tongue squamous cell carcinomas (TSCC and BOTSCC respectively) vs. “other” (i.e. walls of oropharynx, uvulae and soft palate) oropharyngeal squamous cell carcinoma (OPSCC) presented by molecular detection method. (A) OR (95% CI) of having HPV, defined by presence of HPV DNA by PCR, in TSCC/BOTSCC vs. “other” OPSCC. (B) OR (95% CI) of having HPV, defined by overexpression of p16 immunohistochemistry (IHC), in TSCC/BOTSCC vs. “other” OPSCC. (C) OR (95% CI) of having HPV, defined by an algorithm combining presence of HPV DNA and overexpression of p16 IHC, in TSCC/BOTSCC vs. “other” OPSCC.
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