REVIEW

HPV infections and tonsillar carcinoma

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Since human papillomavirus (HPV) was first linked to laryngeal/oral carcinomas in 1983, several studies have confirmed its causal role in a subgroup of upper aerodigestive tract tumours. Of the non-genital cancers, tonsillar carcinomas (TCs) have the strongest association with HPV. By the end of 2002, 432 TCs had been analysed for HPV DNA. Overall detection rate was 51%, with HPV-16 being the most prevalent (84%). The original proposal that HPV-33 would be the most frequent HPV in TCs has not been confirmed, being present in only 4.6% of cases. HPV copy numbers are similar to those found in genital carcinomas (10–300 copies/cell), although HPV is mainly episomal in TC. The importance of this observation is unclear, although a role for subepithelial proliferative lymphatic tissue has been speculated. Patients with HPV-16 positive tumours have better overall and disease specific survival than HPV negative patients. They are also younger and the association with conventional risk factors— smoking and drinking—is less significant than in HPV negative patients. Thus, recent data suggest a distinct pattern for HPV-16 positive TCs.

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W aldeyer's ring consists of submucosal and subepithelial lymphatic tissues localised in the region of the pharynx. The distinct structures of Waldeyer's ring comprise the tubal, pharyngeal, palatine, and lingual tonsils (fig 1). The pharyngeal tonsils, also know as adenoids, consist of a single pyramidal mass of lymphatic tissue, located at the posterior superior nasopharynx. The surface is folded with no true crypts.[1] Palatine tonsils, frequently referred to as the tonsils, are bilateral structures situated in the tonsillar beds. Palatine tonsils consist of 10–30 crypts, lined by the surface epithelium. The lingual tonsils are an aggregation of lymphatic tissue located in the lamina propria of the root of the tongue. There is only one crypt for each nodule in the lingual tonsils.[1]

Pharyngeal, palatine, and lingual tonsils form part of the secondary immune system. They are exposed to ingested or inspired antigens that pass through the epithelial layers. The immunological structure is divided into four compartments: reticular crypt epithelium, extrafollicular area, mantel zone of the lymphoid follicle, and the germinal centre of the lymphoid follicles. The epithelium overlying the lymphatic tissues in the tonsil crypts is of the squamous cell type. As usual, antigens are presented to T helper cells,

thereby inducing a B cell response in the germinal centre, which results in antibody production. Secretory IgA is the main antibody produced in the tonsils.

Several microbial organisms can infect the tonsils, the best known agents being Epstein-Barr virus, adenoviruses, influenza A and B viruses, herpes simplex virus, respiratory syncytial virus, and parainfluenza virus.[2] During the past 10 years, increasing evidence has suggested that human papillomaviruses (HPVs) can also infect the tonsillar epithelium.[3][4] Similar to other mucosal sites, HPV infections have been associated with malignant transformation in this anatomical region.[3–9] However, there is much confusion in the literature regarding HPV infections in head and neck cancers. Head and neck cancer includes cancer of the lip, the oral cavity, the nose and sinuses (sinonasal cancer), the nasopharynx, the oropharynx, the hypopharynx, the larynx, the oesophagus, and the salivary glands, in addition to the soft tissues of the neck and ear (fig 1). Thus, the detection rates of HPV reported in head and neck cancer do not provide us with a detailed view on the association with HPV in the distinct entities, unless their detailed anatomical locations are given.

> "There is much confusion in the literature regarding human papillomavirus infections in head and neck cancers"

When studying the literature, even non-epithelial tumours, such as lymphomas and sarcomas, are often included among head and neck cancers in these reports. Consequently, assessment of the real detection rates of HPV DNA in tonsillar carcinomas is laborious, necessitating the scrutiny of the tables of all individual studies reporting on both head and neck cancers and cancers of the upper aerodigestive tract. In this review, these reports will be summarised by grouping the lesions according to their accurate anatomical location (whenever possible), to give the reader a more organised view on these complex data.

ORAL AND PHARYNGEAL CARCINOMAS

Oral and pharyngeal carcinomas are classified topographically according to their site of origin into the following categories: lip, floor of the mouth, tongue, buccal mucosa, retromolar trigone, hard palate, base of the tongue, tonsillar area (including the anterior and posterior

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Abbreviations: CI, confidence interval; HPV, human papillomavirus; ICD, international classification of diseases; ORF, open reading frame; SCC, squamous cell carcinoma
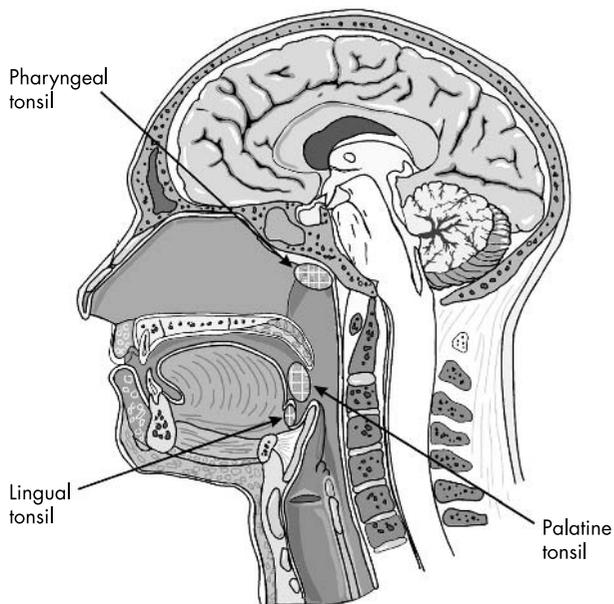
Figure 1 The localisation of the tonsils.

tonsillar pillars and the tonsillar fossa), soft palate, and pharyngeal folds.[10]

CANCER OF THE UPPER AERODIGESTIVE TRACT

The current literature uses this term to denote cancers of the oral cavity, pharynx (nasopharynx, oropharynx, and hypopharynx), larynx, and oesophagus.[11] Often, this term also includes the trachea and bronchus. The interesting recent evidence linking HPV infections to tracheal and bronchial carcinomas was discussed in a recent review in this journal.[12] In addition, the role of HPV in sinonasal carcinomas and those of the oesophagus has recently been reviewed in this journal.[13 14]

TONSILLAR CARCINOMA

According to the old disease classification, cancer of the palatine tonsils was the most common tumour of the oropharynx. However, the incidence of tonsillar cancer has declined significantly in many countries because of the adoption of the new international classification of disease (ICD) since 1993. In the previous classification, palatine tonsillar carcinomas were included in category 146 (malignant neoplasms of the oropharynx),[15] whereas the new ICD code is C09.[16] The discrepancy in the incidence and prevalence figures is because, in addition to lesions of the tonsillae and fossa tonsillaris, the former 146 code included lesions of the arcus pharyngealis and glossopalate, valleculae, and epiglottidis. Code C09 of the ICD system includes lesions of the tonsillar fossa (C09.0), tonsillar pillar (C09.1), unspecified palatine tonsil, and overlapping lesions of the tonsil (C09.8), but excludes lingual tonsil (C02.4) and pharyngeal tonsil (C11.1).

The most common malignant tumour of the palatine tonsils is lymphoma, followed by squamous cell carcinoma (SCC). In some cancer registries, lymphomas are included in the incidence and prevalence figures of tonsillar cancer, whereas in others they are not, adding further confusion to the global cancer statistics. SCC of the palatine tonsils represents approximately 15–23% of all intraoral and oral pharyngeal squamous cell carcinomas in the USA.[17 18] A recent epidemiological survey indicated a fourfold increase of tonsillar SCC among white women in Connecticut between 1945 and 1994, although it remained relatively constant in white men.[17] During 1973–1995, the incidence rates/1 million person years were considerably higher in blacks (31.6; 95% confidence interval (CI), 29.0 to 34.4 in men; 9.6; 95% CI, 8.3 to 10.9 in women) than in whites. No similar increase occurred for oral SCCs at non-tonsillar sites.[17]

In Finland, the age standardised incidence rate of cancer of the palatine tonsils doubled between 1956 and 2000 in both men and in women (fig 2). During 1996–2000, the incidence rates/1 million person years were 1.8 and 6.2 for women and men, respectively. The peak incidence for women is at the age of 45–69 years, and that for men at 45–59 years.[19] Interestingly, no increase in the incidence rate of tonsillar SCCs is detected in the neighbouring country of Sweden.[20]

Clinical features

Clinical symptoms and signs of tonsillar cancer include pain (especially in the ear), difficulties in swallowing, tonsillar asymmetry, palpable firmness or visible lesions in the tonsil, neck mass, and unexplained weight loss.[21] In the early stages the disease presents no symptoms. The overall five year survival rate is related to the stage of the disease, survival for stage I being close to 90%, whereas patients with stage IV disease have a survival rate of less than 20%.[22 23] Thus, the poor prognosis of the patients relates to the late detection of the disease. Surgery and/or radiotherapy are recommended for early disease (stages I/II) and a combination of radiotherapy and composite resection for stages III and IV.[22 23]

Risk factors

The most frequently reported risk factors for oral and pharyngeal carcinomas are smoking and alcohol.[24 25] These risk factors have not been specifically investigated in cancer of the palatine tonsils. Usually, patients with tonsillar cancer report a strong history of tobacco smoke and alcohol exposure. However, similar to oral cancer, increasing numbers of young individuals and elderly women without exposure to these chemical carcinogens have recently been reported to develop tonsillar SCC.[17 26] Because the incidence of tonsillar SCC has increased, whereas the frequency of smokers has declined in the same period, additional risk factors probably exist for this malignancy.[17 26 27] Indeed, studies during the past decade suggest that a substantial proportion of tonsillar SCCs may be associated with
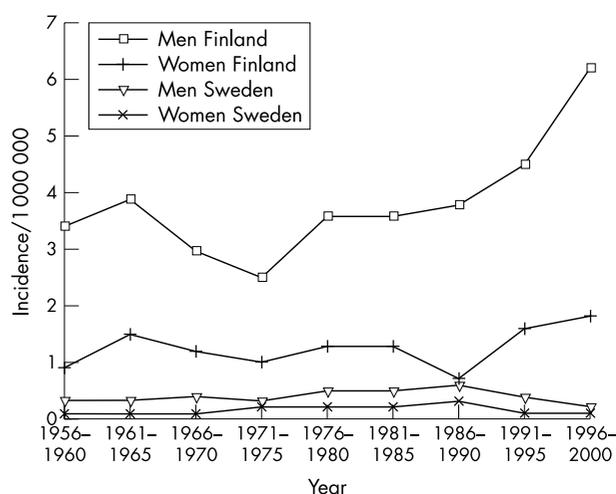

Figure 2 Incidence of tonsil cancer in Finland and Sweden 1956–2000.

oncogenic HPV infections.[28] In the subsequent discussion, the word tonsils is used to refer to the palatine tonsils only, unless otherwise indicated.

Tonsillar epithelium

Tonsillar epithelium is of mesodermic and ectodermic origin, with the crypts populated by lymphocytes. The surface of both the palatine and lingual tonsils is covered by stratified squamous epithelium and a similar type of epithelium also lines the crypts. In pharyngeal tonsils, however, the epithelium is of the ciliated, pseudostratified columnar type, similar to the lining of the respiratory passages.[1] In several studies discussed in detail below, it has been speculated that HPV associated tonsillar SCC originates from the epithelium of the crypts, whereas non-HPV related SCCs emerge from the tonsillar surface epithelium.[17 27] There are a few studies where in situ hybridisation has been used for HPV DNA detection in these lesions, but no attention has been paid to strict anatomical location of the infected epithelium or origin of the cancer.[5 29 30] Tyramide signal amplified in situ hybridisation is a very sensitive method that can detect even one single copy of HPV DNA. Using this method, HPV-16 DNA was detected in normal cryptal epithelium in a tonsillitis sample (fig 3). A carcinoma sample was also studied with the same method and nearly all the carcinoma cells labelled strongly with the HPV-16 DNA probe, indicating the presence of the episomal form of the virus.

EVIDENCE FOR HPV INVOLVEMENT IN TONSILLAR CARCINOMA
Epidemiological studies

Evidence suggesting an aetiological role for HPV in tonsillar carcinomas is derived from different approaches, including epidemiological and molecular studies.

It was recently shown that patients with HPV associated anogenital cancer had a 4.3 fold higher risk of tonsillar SCC (95% CI, 2.7% to 6.7%).[26] The relative risk was significantly higher for tonsillar SCC than for other oral SCCs, supporting a causal role of HPV in the development of tonsillar SCC. The highest relative risk for tonsillar SCC was found in patients with anal SCC, which is common among male homosexuals (2.6%; 95% CI, 1.8% to 3.8%). However, the results of this study need to be interpreted with caution, because of the limited number of patients (only three human immuno-deficiency virus infected men with anal and tonsillar
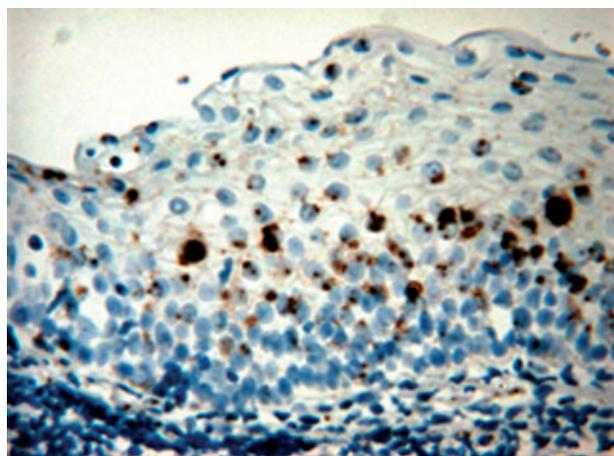

Figure 3 Tyramide amplified in situ hybridisation to detect human papillomavirus 16 DNA in the normal crypt epithelium of a tonsil during an episode of tonsillitis. Original magnification, ×250.

carcinoma). HPV unrelated cancers did not increase the risk of tonsillar carcinoma (0.8; 95% CI, 1.8% to 3.8%).[26]

Such an increased risk of tonsillar cancer was not associated with cervical HPV lesions in another study in Sweden.[27] Different rates of tonsillectomy might partly explain the discrepancy between these two studies. More importantly, however, it was shown that husbands of patients with HPV associated cervical cancer had an increased risk of tonsillar cancer (standardised incidence rate, > 2.00), which also suggests that HPV might be involved in tonsillar carcinogenesis. Such an association is further supported by the fact that HPV associated tonsillar carcinomas have been detected in transplant recipients, who have an increased risk of HPV infections.[31 32]

Detection of HPV DNA

Brandsma and Abramson were the first to report the presence of HPV-16 DNA in two of seven tonsillar SCCs using Southern blot hybridisation in 1989.[33] Since that preliminary report, large numbers of studies have reported on the detection of HPV DNA in tonsillar SCCs (summarised in table 1). However, practically no data are available on the detection of HPV DNA in the tonsillar tissues of Waldeyer's ring other than the palatine tonsils.

One year after the original report, Ishibashi and co-workers described an additional tonsillar SCC infected with an episomal form of HPV-16 DNA.[34] The same HPV type was also detected in two lymph node metastases, suggesting a direct role for HPV infection in the development of SCC. The two largest series of tonsillar carcinomas analysed for HPV so far have been reported by Mellin and co-workers (2002).[3 4 6] They analysed a total of 84 tonsillar carcinomas, 39 of which contained HPV DNA.[3 4 6]

> "It was shown that husbands of patients with human papillomavirus associated cervical cancer had an increased risk of tonsillar cancer"

At the time of writing (February 2003), the world literature comprises a total of 432 tonsillar SCCs analysed for the presence of HPV DNA by a variety of detection techniques (table 1). HPV DNA has been detected in 51% (221 of 432) of these lesions. Most of these studies are not focused solely on tonsillar SCCs, but on head and neck tumours in general. Thus, the cases of tonsillar SCCs listed in table 1 have been collected from the studies reporting oral, aerodigestive tract, or head and neck cancers.

The most prevalent HPV type detected in tonsillar SCCs seems to be HPV-16, being identified in 84% of the 216 HPV DNA positive tumours. In addition, HPV-16 was present as double infections (16/18 and 16/33) in 3% and 1.4% of the HPV DNA positive samples, respectively. HPV-33 has been found in only 4.6% of HPV positive tonsillar carcinomas. Interestingly, DNA from the low risk HPV types 6/11 has also been detected in 3% of HPV positive tonsillar carcinomas. In addition to the HPV types listed above (and some unidentified HPV types), the following HPV types have been detected in occasional tonsillar carcinomas: HPV types 5, 12, 31, 35, and 59 (table 1).

Physical state of HPV in tonsillar carcinomas

The physical state of HPV in tonsillar carcinomas has not been analysed systematically and, in fact, such data are available in a few studies only. In 1991, Bercovich and co-workers reported both integrated and episomal forms of HPV-6a in tonsillar SCCs.[35] HPV-6 is included among the low risk mucosal HPV types that are rarely found in malignant tumours.[28 42] However, HPV-6 or HPV-11 DNA has occasionally been found in vulvar carcinomas, cervical carcinomas,
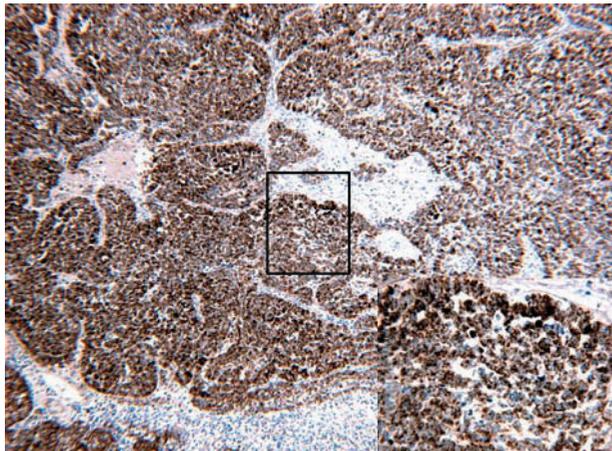
Figure 4 Tyramide amplified in situ hybridisation in the detection of human papillomavirus 16 DNA in a tonsillar squamous cell carcinoma. Original magnification, ×40. The insert shows a high power view (original magnification, ×250) of the area marked with a rectangle.

laryngeal papillomas on malignant transformation, and in most cases of Buschke-Löwenstein tumours (giant condylomas).[28][42] Subsequently, the same authors reported that in this particular tonsillar SCC, HPV-6a was integrated into chromosome 10q24.[53] At the HPV-6a integration site, there were also breakpoints affecting protooncogenes Hox11 and Lyt10, and genes related to cell division.[53] There is a fragile site in the same region and, interestingly, integration of HPV-18 into the chromosome at 10q24 has also been reported.[53]

More recently, Mellin and colleagues provided evidence that all HPVs detected in 11 of the 22 tonsillar carcinoma samples analysed were in the episomal form.[3] The physical state was analysed by a very sensitive restriction enzyme digestion, ligation, and inverse PCR method. Previously, Snijders et al (1992) had described two HPV-16 positive tonsillar carcinomas, where HPV-16 was episomal. They also found two HPV-33 positive carcinomas, where HPV was either integrated or in both the episomal and integrated form.[54][55] It is currently unknown why HPV is mostly in the episomal form in tonsillar carcinomas. One possible explanation could be genetic alterations of the long control region of extrachromosomal HPV, leading to dysregulation of the viral oncogenes. The sequence analyses of episomal HPV-16 in cervical cancers has revealed sequence variation in YY1 binding sites, leading to increased activity of the viral oncogene promoter.[56][57] Similarly, Mellin et al (2002) reported episomal but deleted HPV-16 in three tonsillar carcinomas.[3] In all three cases, HPV-16 was disrupted at the end of the E1 open reading frame (ORF), and E2 was missing. In two carcinomas, the deletion of HPV-16 continued into the long control region, whereas in the third case deletion continued into the E6 ORF.[3] The biological implications of the deletions remained obscure. As described below, it seems that the pathogenesis of HPV induced tonsillar carcinoma is different to that of cervical carcinoma, where HPV is mostly in the integrated form.

> "It is currently unknown why human papillomavirus is mostly in the episomal form in tonsillar carcinomas"

We recently described an HPV-33 positive cell line established from a vaginal mild dysplasia lesion, where HPV was mostly detected episomally at early passages.[58] However, at passages 18–20 onward, only the integrated form was detected.[58] After identifying the exact integration site of HPV-33 at chromosome 5p14, we were able to trace the

Table 1 Detection of HPV DNA in tonsillar carcinomas

Technique	No of positive cases/total	HPV types								First author (year)
		6/11	16	18	16/18	16/33	31	33	X	
SB	2/7	1	1							Brandsma (1989)[33]
SB	1/1		1*							Ishibashi (1990)[34]
ISH	6/28		6							Niedobitek (1990)[29]
SB	1/	1								Bercovitch (1991)[35]
ISH	5/				5					Arndt (1992)[30]
PCR	10/10		4			1		3	2	Snijders (1992)[5]
PCR	12/14		7			1		2	2	Snijders (1994)[36]
PCR	2/3									Brachman (1992)[37]
PCR, SB, Virapap	3/6		3							Watanabe (1993)[38]
PCR, SB	1/4		1							Ogura (1993)[39]
PCR	2/4		2							Lewensohn-Fuchs (1994)[40]
PCR	3/7								ND	Brandwein (1994)[41]
PCR, SB	9/15		8						1	Paz (1997)[9]
PCR, SB	1/1		1							Turazza (1997)[42]
PCR, SB	11/21		8					1	2	Andl (1998)[43]
PCR, SB	19/44	3	40							Schwarz (1998)[44]
PCR	14/22		11					1	2	Wilczyski (1998)[45]
PCR	2/2	1*			1*					Badaracco (2000)[46]
PCR	6/13						6†			Lopez-Lizarraga (2000)[47]
SB	1/1		1							Ishiji (2000)[48]
PCR	2/4	1			1					Badaracco (2000)[49]
PCR	32/52									Gillison** (2000)[7]
PCR	26/60		26			1				Mellin (2000)[6]
PCR	12/22		11					1		Mellin (2002)[3]
PCR	14/24		12‡					1	1	Klussman (2001)[50]
PCR, SB	24/52		21¶						3§	Strome (2002)[51] Ringström (2002)[52]
Total (%)	221/432 (51)	7 (3)	186 (84)	7 (3)		3 (1.4)	6 (2.8)	9 (4.6)	13 (6)	

*Triple infection with HPV types 6, 16, and 18; †one infection with several HPV types 6, 18, 31, and 35; ‡double infections with HPV types 5/16, ADX1/16; §HPV-12, HPV-59; ¶one unidentified and HPV-59; **includes also tumours of the lingual tonsils.
HPV, human papillomavirus; ISH, in situ hybridisation; PCR, polymerase chain reaction; SB, Southern blotting.

Table 2 Detection of HPV in normal tonsillar mucosa or benign lesions

Type of lesion	Technique	No of cases	6/11	16	18	31	33	First author (date)
Tonsillitis	SB	0/20						Brandsma (1989)[33]
Chronic inflammatory disease	ISH	0/30						Niedobitek (1990)[29]
Tonsillitis	PCR	0/7						Snijders (1992)[5]
Normal posterior tonsillar pilar	PCR, SB	1/3	1					Smith (1993)[60]
Chronic tonsillitis	PCR, SB	4/28		4				Watanabe (1993)[38]
Adenoid hyperplasia and chronic tonsillitis	PCR, SB	0/8, 5/38		5				Fukushima (1994)[61]
Condyloma	SB	1/1	1					Tominaga (1996)[62]
Normal mucosa	SB	3/3	3					Tominaga (1996)[62]
Chronic tonsillitis	PCR	0/14						Klussmann (2001)[50]
Tonsillar hyperplasia	PCR, SB	3/48		3				Strome (2002)[51]

In total, 17/200 (8.5%) samples were HPV positive.
HPV, human papillomavirus; ISH, in situ hybridisation; PCR, polymerase chain reaction; SB, Southern blotting.

presence of the integrated form of HPV-33 at early passages and even in the original biopsy. However, the copy numbers of the integrated form of HPV-33 were very low.[58] In line with these results, we also found that most premalignant HPV-16 positive lesions (cervical intraepithelial neoplasia I–III) contained the integrated form of HPV-16, mixed with episomal forms, by using a new real time polymerase chain reaction method.[59] Our studies with genital lesions suggest that integration is an early event in HPV induced carcinogenesis.

Viral load in tonsillar carcinoma
Mellin and co-workers recently showed that there is a wide variation in copy numbers of HPV DNA in tonsillar carcinomas.[3] Most tonsillar carcinomas contained between 10 and a few hundred copies for each copy of β actin DNA.[3] Notably, six patients with tumours containing > 190 copies/β actin showed a significantly better clinical outcome, as measured by recurrence free three year survival after diagnosis ($p = 0.026$), and better overall survival rates ($p = 0.039$), than did the five patients with tumours containing < 190 HPV copies/β actin.[3] They also showed that overall survival was better for patients with HPV DNA positive tumours than for those with HPV negative cancers. Similar results have also been shown by others.[50]

Expression of viral oncogenes in tonsillar carcinoma
It should be emphasised that the detection of viral DNA per se does not confirm that the virus has a causal association with malignant transformation. However, based on the still limited material, it now seems that HPV-16 E6 and E7 are actively transcribed in most of the tonsillar carcinomas that have been analysed.[8][45][54][55] Snijders and co-workers (1992) showed that independent of the physical state of the virus, all tumours expressed E7 encoding HPV-33 E6*I mRNA. They also suggested that the transcription of HPV-16 E6/E7 mRNA in tonsillar carcinomas is not necessarily dependent on viral DNA integration.[54][55]

HPV DETECTION IN NORMAL TONSILS OR TONSILLITIS
Normal tonsillar tissues have been assessed in only a few studies. By the end of the year 2002, only 200 normal tonsillar samples and/or biopsy samples from tonsillitis were analysed for the presence of HPV DNA. In total, 8.5% (17 of 200; table 2) of the samples contained HPV DNA, either type 16 (12 samples) or 6/11 (five samples). As in other anatomical sites, the positivity rate of HPV is expected to increase when more samples of normal tissues are analysed.

CONCLUSIONS
The HPV detection rate of 51% is among the highest in any extragenital human malignancy.[28] In fact, it is more than twice as high as that established in the current literature for other upper aerodigestive tract carcinomas, such as sinonasal, bronchial, and oesophageal carcinomas.[11–13]

In several other respects, tonsillar carcinomas have special features that mark them out among the known and emerging HPV lesions. First, tonsillar carcinomas are mostly infected with HPV type 16; second, the virus is predominately in episomal form; and third the virus is transcribed. How the virus can remain in carcinoma tissues as episomes with relatively high copy numbers is not yet known.

''The human papillomavirus detection rate of 51% is among the highest in any extragenital human malignancy''

Take home messages

- Tonsillar carcinoma is the most prevalent oropharyngeal carcinoma
- Cigarette smoking and alcohol are the primary risk factors traditionally associated with the development of this malignancy
- The association of human papilloavirus (HPV) infection with tonsillar carcinoma has been suggested by the following observations: HPV DNA has been detected in around 50% of tonsillar carcinomas; HPV-16 is the predominant virus type in tonsillar carcinomas; HPV-16 is present in episomal form in most tonsillar carcinomas; HPV is transcribed in tonsillar carcinomas and HPV DNA is detectable in carcinoma cells by in situ hybridisation; patients with anogenital cancer might have an increased risk for tonsillar carcinoma; HPV DNA has also been detected in lymph node metastases
- Patients with HPV-16 positive tumours seem to have a better survival than HPV negative patients, thus presenting a distinct group among patients with tonsillar carcinoma
- The new international disease classification has led to a decreased incidence of palatine tonsillar carcinomas, because the posterior part of the tongue is no longer included in the C09 code

The mode of entry of HPV into tonsillar tissue is not known. Tonsillar crypt epithelium is known to capture and process antigens, which might facilitate viral access to the basal cells. The possibility of persistence of the virus in the crypt epithelium and even in lymphoid tissue cannot be totally excluded. If this is true, tonsillar tissue could represent a reservoir of HPV in the upper aerodigestive tract. This view is partly supported by the fact that when oral samples are collected by oral rinse, the detection rate of HPV is much higher that that with swabs. Finally, the persistence of HPV in tonsillar tissue might be of importance in the immune response to HPV.

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