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**MAIN ARTICLE**

# Do patients with p16-positive oropharyngeal squamous cell carcinoma get more bone metastasis than p16-negative patients?

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## Abstract

*Background*: Oropharyngeal squamous cell carcinoma is thought to rarely metastasise to bone. This study hypothesised that in p16-positive disease there is a significant incidence of bony metastasis.

*Methods*: This was an ambispective cohort review. All patients with oropharyngeal squamous cell carcinoma diagnosed and treated at one centre were included.

*Results*: A total of 180 consecutive patients were identified over 5 years. Fifteen patients were excluded because of lack of p16 status, none of whom had bony metastasis. The final analysis included 165 patients: 48 (29.09 per cent) in the p16-negative group and 117 (70.91 per cent) in the p16-positive group. Ten patients (8.55 per cent) in the p16-positive group developed bony metastasis, compared with zero in the p16-negative group; this difference was statistically significant ($p = 0.036$).

*Conclusion*: Expression of p16 was associated with an increased incidence in bony metastasis in this cohort. This is the first study to explore this specific question.

**Key words:** Carcinoma; Squamous Cell; Oropharyngeal Neoplasms; Tonsillar Neoplasms; Papillomaviridae; Neoplasm Metastasis

## Introduction

It is a well-documented phenomenon that certain types of cancer have a propensity to metastasise to specific tissue and sites. This was first hypothesised by Stephan Paget in 1889, who, having reviewed a large set of women with breast malignancy, concluded that specific tumour 'seeds' are predisposed to grow in particular types of 'soil' (tissue).[1]

Head and neck squamous cell carcinoma (SCC) is traditionally considered to be a loco-regional disease, principally affecting the primary site and the cervical lymph nodes.[1–3] The most common site for distant metastasis is commonly understood to be the lungs; extra-thoracic distant metastases, for example metastases to bone, liver or brain, are vanishingly rare.[1] The focus for initial staging and for post-treatment follow up is therefore the head and neck, with the addition of thoracic imaging (usually only at initial staging unless specifically indicated).

In the last three decades, oropharyngeal SCC has been markedly increasing, and it has exhibited a change in behaviour and demographics.[4] Traditionally, this malignancy was highest in older patients with a large smoking and alcohol history; however, the biggest increase in recent years has been amongst younger patients without these risk factors.[4] The reason for this is now understood to be human papilloma virus (HPV), and a growing body of research has focused on defining and exploring this new and increasingly prevalent malignancy. Current opinion is that these cancers respond better to treatment compared to non-HPV-related SCC, and are generally thought to exhibit better outcomes in terms of disease-specific mortality.[4] Nevertheless, at the authors' institution, there has been a growing perception of an incidence of bony metastases in patients with HPV-related SCC following treatment, despite apparently successful loco-regional control.

P16 is a protein which has been shown to be expressed in HPV-related SCC. It is relatively simple to test and is a reliable surrogate for testing HPV itself.[5] Testing the presence of HPV itself is often not practical or cost effective in the clinical setting, and p16 testing has therefore become the primary method of defining these cancers as HPV-

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related, and has become a routine part of their initial investigation.

This project aimed to test the alternative hypothesis that p16 expression is associated with a higher incidence of bony metastasis in oropharyngeal SCC.

## Materials and methods

This was an ambispective cohort review. Data were collected as part of the patients' standard care in a prospective fashion, and were then collated retrospectively.

All patients with oropharyngeal SCC diagnosed and treated under the Aneurin Bevan University Health Board Head and Neck Multi Disciplinary Team were included.

Patients deemed to be receiving palliative care at the initial multidisciplinary team (MDT) meeting were excluded. Patients were identified from the local head and neck registry (in which all data on patients treated under the MDT are recorded prospectively). Patients diagnosed from January 2010 were included, as this was the time that tumour tissue began to be tested for p16 routinely in this centre. All patients were followed up in line with national guidelines and all included patients had at least one year of follow up. Malignancies other than SCC were excluded.

As part of their initial staging, all patients received a computed tomography (CT) scan of the neck and thorax, and an ultrasound of the neck. Many will have undergone magnetic resolution imaging of the neck, and a small number will have had a positron emission tomography with CT (PET-CT).

P16 status was confirmed by a consultant pathologist, and was defined as positive if there was global or full-thickness nuclear positivity or cytoplasmic positivity. P16 status was accepted from any biopsy site, unless evidence was found that the patient had two separate primary SCCs. In this case, evidence was needed that the result corresponded to the oropharyngeal SCC.

TABLE I
COMPARISON OF INCLUDED VERSUS EXCLUDED PATIENTS

| Variable | Included patients | Excluded patients | *p* |
|---|---|---|---|
| Number | 165 | 15 | |
| Average age (years) | 59.34 | 60.13 | 0.77 |
| Males (%) | 76.97 | 86.67 | 0.52 |
| Tumour (T) stage (% of cases) | | | 0.62 |
| – $T_1$ | 14.55 | 20 | |
| – $T_2$ | 23.64 | 13.33 | |
| – $T_3$ | 17.58 | 6.67 | |
| – $T_4$ | 44.24 | 60 | |
| Node (N) stage (% of cases) | | | 0.72 |
| – $N_0$ | 23.64 | 26.67 | |
| – $N_1$ | 7.27 | 6.67 | |
| – $N_2$ | 63.64 | 46.67 | |
| – $N_3$ | 5.45 | 20 | |

Data were obtained from the electronic and hard-copy patient records. Data collected included: patient-specific variables such as age (at diagnosis) and gender; disease-specific variables such as staging at diagnosis, date of diagnosis and p16 status; treatment given; and the finding of bone metastasis during the follow-up period, how and when it was confirmed, and any symptoms prior to confirmation.

Data were collated in Microsoft Excel 2010 and processed in Statistical Product and Service Solutions software, version 22 (SPSS; IBM, Armonk, New York, USA). Statistical significance was taken at the 5 per cent level; *p*-values were calculated using Fisher's exact test, chi-square test or student's *t*-test.

### *Ethics*

In line with the institution's research ethics policy and the National Health Service Health Research Authority guidelines, research ethics committee approval was not required for this project. This was because of the retrospective methodology, with no changes to any patient's care, and all authors were a part of the team that cared for these patients.

All data were recorded as part of the care of these patients and were collated by the authors in an anonymous form.

## Results

A total of 180 patients fulfilled the inclusion criteria between 1st January 2010 and 31st December 2015. Data were correct as of 1st January 2017, giving at least one year of follow up. Fifteen patients (8.33 per cent) were excluded because a record of p16 status could not be identified. The final analysis therefore included 165 patients, with 48 (29.09 per cent) in the p16-negative group and 117 (70.91 per cent) in the p16-positive group.

A comparison of the included and excluded patients is shown in Table I. This demonstrates no significant differences in age, gender or staging between these groups. No patients in the excluded group were found to have bony metastasis. No patients with distant metastasis at diagnosis were included, as all were treated palliatively.

A comparison of the p16-positive and p16-negative groups is shown in Table II. Ten patients (8.55 per cent) in the p16-positive group developed bony metastasis, compared with zero in the p16-negative group. A Fisher's exact test (two-tailed) comparing these groups showed this difference to be statistically significant ($p = 0.0356$). A statistically significant difference was also seen between the groups in terms of tumour (T) stage, with the p16-negative group presenting on average with a more advanced primary tumour ($p = 0.001$). Length of follow up is taken from the date of diagnosis to 1st January 2017 or date of death. There was a longer average length of follow up in the p16-positive group ($p = 0.022$).

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TABLE II
COMPARISON OF P16-POSITIVE AND P16-NEGATIVE GROUPS

| Variable | P16-positive group | P16-negative group | *p* |
|---|---|---|---|
| Number | 117 | 48 | |
| Bony metastasis (*n* (%)) | 10 (8.6) | 0 (0) | 0.036 |
| Average age (years) | 58.4 | 61.5 | 0.08 |
| Males : females (*n* (%)) | 89 (76.1) : 28 (23.9) | 39 (81.3) : 9 (18.8) | 0.54 |
| Mean tumour stage | 2.74 | 3.35 | |
| Tumour (T) stage (*n* (%)) | | | 0.001 |
| – $T_1$ | 20 (17.1) | 4 (8.3) | |
| – $T_2$ | 34 (29.1) | 5 (10.4) | |
| – $T_3$ | 20 (17.1) | 9 (18.8) | |
| – $T_4$ | 43 (36.8) | 30 (62.5) | |
| Node (N) stage (*n* (%)) | | | 0.11 |
| – $N_0$ | 25 (21.4) | 15 (31.3) | |
| – $N_1$ | 8 (6.8) | 4 (8.3) | |
| – $N_2$ | 78 (66.7) | 27 (56.3) | |
| – $N_3$ | 7 (6.0) | 2 (4.2) | |
| Treatment (*n* (%)) | | | 0.019 |
| – Primary surgery | 36 (30.8) | 11 (22.9) | |
| – Post-operative radiotherapy | 7 (6.0) | 4 (8.3) | |
| – Post-operative chemoradiotherapy | 16 (13.7) | 5 (10.4) | |
| – Radiotherapy | 15 (12.8) | 16 (33.3) | |
| – Chemoradiotherapy | 48 (41.0) | 17 (35.4) | |
| – Chemotherapy & chemoradiotherapy | 18 (15.4) | 4 (8.3) | |
| Deaths (*n* (%)) | 17 (14.5) | 26 (54.2) | 0.0001 |
| Mean time from diagnosis to death (days) | 575 | 466 | 0.37 |
| Mean length of follow up (days) | 1116 | 843 | 0.0219 |

Table III gives further details for the 10 patients with bony metastases. The median time from diagnosis (taken as the MDT date confirming diagnosis) to a scan confirming a bony metastasis was 491 days (range, 219–869 days). All of these patients had positive cervical nodes at initial staging. Four patients had biopsy confirmed bony metastasis; the remainder were diagnosed on radiological appearance. All patients with bony metastasis had vertebral involvement; other sites included the scapula, femur and pelvis. Thirty per cent had only bony metastasis; amongst the others, three patients had widely disseminated disease. Pulmonary, hepatic and mediastinal metastasis occurred equally in this group. Only one patient had local recurrence, in conjunction with an isolated thoracic vertebral body metastasis. Seven of the 10 patients were symptomatic with localised pain, prior to a scan showing bony metastasis. One patient had a pathological fracture that led to the diagnosis.

## Discussion

### *Outcomes and evaluation*

This ambispective cohort study, of five years' consecutive patients at one centre, showed a statistically significant higher incidence of bony metastasis in the p16-positive group. This is the first study to explore this specific question. It included consecutive patients and every effort was made to minimise exclusions. Furthermore, of the 8.33 per cent excluded, none have developed bony metastasis in the study period; therefore, if these could be included they would

TABLE III
PATIENTS WITH BONY METASTASIS

| Tumour–node (TN) stage | Time from diagnosis to metastasis (days)* | Position of metastases | Biopsy? | Symptoms prior to scan | Local recurrence? |
|---|---|---|---|---|---|
| $T_1N_{2b}$ | 219 | Thoracic vertebrae | Yes | Pain | No |
| $T_{4a}N_{2b}$ | 223 | Thoracic vertebrae | No | Pain + fracture | Yes |
| $T_3N_{2c}$ | 496 | Thoracic vertebrae | No | Pain | No |
| $T_{4a}N_3$ | 486 | Vertebrae, pleura, mediastinum | No | None | No |
| $T_4N_{2c}$ | 424 | Thoracic vertebrae, mediastinum, pulmonary, brain | No | None | No |
| $T_1N_{2a}$ | 710 | Vertebrae, scapula, femurs, pulmonary, liver | Yes | Pain | No |
| $T_{4a}N_1$ | 609 | Vertebrae, liver | Yes | Pain | No |
| $T_3N_{2b}$ | 544 | Lumbar vertebrae, pelvis | Yes | Pain | No |
| $T_3N_{2b}$ | 869 | Lumbar vertebrae, lung | No | Pain | No |
| $T_{4a}N_{2b}$ | 314 | Vertebrae | No | None | No |

*Median time = 491 days

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likely strengthen the conclusions and are not seen as a possible source of bias.

Other statistically significant differences were found between the two study groups. The p16-negative group had a higher proportion of $T_3$ and $T_4$ tumours than the p16-positive group, but there was no significant difference in cervical nodal involvement.

There were differences in the treatment the groups received. A larger proportion of patients in the p16-negative group were treated with primary radiotherapy; surgery was more commonly used in the p16-positive group. In both groups, chemoradiotherapy was the commonest primary treatment. It is also apparent that a higher proportion of patients received chemotherapy (in conjunction with any other treatment) in the p16-positive group. As chemotherapy is a systemic treatment (as opposed to local and regional in relation to surgery and radiotherapy), it might be expected to have an effect on the rate of distant metastasis. As a higher number of distant metastases was seen in the group receiving a higher level of systemic treatment, this was not felt to be a confounding factor.

There was a significant difference in average follow-up duration, with the p16-positive group having a significantly longer follow up. This is likely to be because of the increased survival in the p16-positive group. Full survival analysis has not been undertaken in this study, but a significantly higher proportion of the p16-negative group died during the study period, which is in keeping with larger studies.[4] Clearly, this difference may have had an effect on the amount of bony metastasis found in the p16-negative group.

Ideally, all patients included would have five years of follow up, with a larger p16-negative group. However, as p16 has only been routinely tested in oropharyngeal SCC cases in this centre since 2010, the study period has by necessity been limited to these years. Therefore, this study can only comment on differences within the early follow-up period.

In addition, this study was confined to one centre, and it is possible, although unlikely, that the local population differs in some way to other developed Western populations. Further studies should therefore expand on this investigation, to confirm that these findings can be globally applied.

Because of the relatively small (although significant) number of patients with bony metastasis, it was not possible to conduct a multivariate analysis to provide stronger evidence that p16 status is an independent risk factor for bony metastasis. Further corroboration with a larger sample is required.

### *Distant metastasis*

Here we consider distant metastasis in oropharyngeal SCC. Several papers have commented on the unusual behaviour of p16-positive SCC; however, no studies were identified which specifically explored the incidence of bony metastasis.

Huang *et al.* conducted a large cohort study (613 patients) investigating atypical clinical behaviour in oropharyngeal SCC; however, 48 per cent of their patients were excluded as they were not tested for p16.[6] These authors concluded that p16-positive disease is more likely to involve unusual sites. However, only 3 per cent of the p16-positive cohort in that study developed bony metastasis, and they found no difference overall in the rate of distant metastasis between p16-positive and p16-negative groups.[6]

Müller *et al.* described four cases of unusual p16-positive oropharyngeal SCC metastasis, of which three involved distant bony metastasis.[7] Metastasis involved the sternum and thoracic vertebrae in the first two cases respectively, and multiple bony sites in the third case (clavicle, humerus, ribs, sacrum and vertebrae).

Studies of oropharyngeal SCC that did not differentiate p16 status have shown a range of incidence for metastasis to bone. Kowalski *et al.* reviewed 2327 patients with oral and oropharyngeal SCC, of which 1.2 per cent had bony metastasis within a variable follow-up period, and p16 was not commented on.[3]

### *Clinical implications*

If the findings of this paper are proven to apply to other populations, then this has important clinical implications. Currently, clinical review and investigations, at initial staging and at post-treatment follow up, do not seek to identify bony metastasis. At the very least, clinicians should be aware that bony symptoms should trigger investigation, and clinical follow up should entail some screening questions.

Furthermore, consideration should be given to imaging the skeleton at diagnosis and in the follow-up period. What form this imaging should take is another point of contention, with some sources suggesting PET-CT.[2] However, this has implications for resources and funding, as serial examinations for this entire (and expanding) group would entail a significant work load.

All patients with bony metastasis in this series had positive cervical nodes at initial staging. This correlation between nodal staging and distant metastasis is similar to other studies, and may be a useful guide to the level of surveillance required.[1] This needs further corroboration, however, and it should be noted that one patient with bony metastasis in this study had only limited cervical node involvement (initial nodal (N) staging of $N_1$).

In this series, the average time to the identification of a bony metastasis was approximately 16 months, which is similar to that reported in other studies.[6,7]

Another unanswered question is the best treatment for these patients.[2] This study did not explore in depth the methods or response to treatment for bony metastasis. Half of the patients received radiotherapy for their bony metastasis (predominantly to help palliate pain), which resulted in a subjective improvement

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in symptoms for all patients. Three of these patients had follow-up CT scans, which revealed a partial response to radiotherapy.

With regard to prognosis, one patient died within weeks of diagnosis of an upper gastrointestinal haemorrhage, and the remaining patients subsequently developed further distant metastases and all died within a year. Haigentz *et al*. explored the evidence base for treating distant metastasis.[8] These authors commented that radiotherapy is the most frequently utilised therapy to treat and prevent pain and fractures, although surgery may be offered in selected patients. With the current trend of increasing p16-positive SCC, this area will quickly need further evidence.

- **Oropharyngeal squamous cell carcinoma (SCC) is thought to rarely metastasise to bone**
- **Growing evidence shows that p16-positive SCC behaves differently to p16-negative SCC**
- **This is the first study to investigate the risk of bony metastasis related to p16 expression**
- **There was a statistically significant increase in bony metastasis in p16-positive compared to p16-negative patients**

## Conclusion

This single-centre cohort study of five years' consecutive patients with oropharyngeal SCC showed that p16 expression is associated with an increase in incidence of bony metastasis within the early follow-up period. The realisation that p16-positive disease behaves in a different fashion to the traditional p16-negative disease may lead to differences in post-treatment surveillance practices between the groups. This is the first study to ask this specific question, and more studies are now required to establish if this outcome is consistent in a larger population.

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