Reviewer's report

**Title:** Human Papillomavirus-associated oropharyngeal cancer: an observational study of diagnosis, prevalence and prognosis in a UK population

**Version:** 1  **Date:** 21 February 2013

**Reviewer:** Richard Shaw

**Reviewer's report:**

Overall this paper is of very high quality and contains important data which should be published.

It is important that all the data from this series is published (perhaps as a large supplementary table) to allow subsequent use in metanalyses. Similarly it is important to clarify all details of lab techniques, specifications, PCR conditions, perhaps again as supplementary data - so that other groups can readily replicate the very high standards adopted by these authors.

All of the comments below are relatively minor, with the exception of some of the statements made in the abstract, which at first glance may not all be particularly reflective of the data:

P3L2 - text gives impression that infection might improve prognosis in existing tumour (what they mean is that HPV mediated cases have better prognosis than non HPV mediated cases?)

(Similarly P19L8 “dramatic improvements”… no patient’s prognosis has been improved here – a what is meant that the two groups of patients have distinct clinical features and distinct prognosis)

(Similarly P3 L21 “HPV positivity alters the behavior of OPC” is a similar minor grammatical error)

P7L3 – as around 50% of patients were not included in the data, it might be worth considering potential sources of systematic bias in discussion. Two possibilities spring to mind- firstly that patients having non-surgical treatment will have much less tissue available therefore less likely to meet DNA quality requirements, secondly that patients who are HPV+ve tend to have smaller primary tumours than HPV-ve in many series – perhaps again less likely to have good DNA.

How have HPV+ve OPSCC patients with occult primary tumours been dealt with?

P7L9 “local recurrence” seems to have been used as a term to include local, regional and locoregional recurrence – although this is clear in this paper, this may limit its usefulness in subsequent metanalysis and should be avoided.

P7L6 METHODS – there is no detailed account given about how clinical outcome
data has been recorded, other than by database. How is a patient who dies in a
peripheral hospital flagged at the database, how is the cause of death accurately
determined? Other UK series use the ONS which has evident limitations but this
group have a more reliable data source?

P11L15- although the use of a DNA quality threshold is laudable, and doubtless
an advance on the great majority of the literature, what is the rationale for using
this amplicon, how many cycles of PCR, what PCR conditions etc. What was the
effect of small variations in PCR machine, conditions and cycles of the overall
informativity of the series?
Is this test valid?

P12L5 – P16IHC does not assess HPV prevalence, so this statement needs a
grammatical adjustment

P12L17- “if all cases included” this is then presumably misleading data as the
negative cases by DNA are false negatives. This data can be presented but this
needs to be highlighted.

This misinterpretation seems to be carried through to results and even abstract
P3L17- “as a single marker, P16 was least affected by DNA quality and
correlated best with prognosis”. This seems the single most important correction
to consider in this paper - as the message of this is likely to be taken from the
published abstract without context / critical details that the authors present in the
main text.
This statement seems to be incorrect at 4 levels and should be rethought,
especially in the abstract:
1. how should a protein expression reasonably be expected to be affected by
DNA quality?
Is there any precedent for measuring degradation of a protein against a rather
robust macromolecule such as DNA? – there is an assumption made here not
explored in the detail.
2. although it is possible, it is evidently inappropriate, to compare P16 against
DNA tests in samples that have proven inadequate DNA. The authors have
moved this field on a little by establishing a minimum standard of DNA quality
(although this in itself is not validated) and should restrict this analysis to that
cohort with adequate tissue.
3. There were no significant differences between the prognostic ability as all the
C.I.s overlapped
4. We know that other cases have p16 overexpression in absence of HPV
aetiology (although the numbers are likely small) which seems to be ignored
here.
P13L16 .. had a mean difference in age of..
P15L3 & P18L6. Further there are many clinical trials being designed around the
potentially erroneous concept that smoking/HPV constitutes an entirely distinct
prognostic entity. This data leans on survival estimates derived from evidently sub-optimal HPV “diagnoses” - this is a key point for discussion and possibly abstract as the clinical implications are important.

P16L14 – It seems just as likely that the differences between HPV prevalence between the cited papers and this series might be due to dates of the samples analysed? (i.e. earlier samples will mean less HPV +ves, rather than a difference between the regions)

DISCUSSION – several papers point to RNA based tests being the gold standard – whether ISH or PCR – whilst discussing the relative merits of tests or combinations of tests, this might be worth a line or two in discussion?

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I have published a peer reviewed research paper with one of the authors in the past (MR)
I am co-applicant with one of the authors on a clinical trial grant (ME)