University of BRISTOL

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headandneck 5000

Using saliva to identify human papillomavirus driven oropharyngeal cancers

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Scientific Outline

Summary

Most cancers of the head and neck are squamous cell cancer. Tobacco and alcohol use are risk factors. Oral infection with human papillomavirus (HPV), especially HPV type 16, is a risk factor for oropharyngeal cancer (OPC). The proportion of OPCs caused by HPV infection varies and is estimated to be around 30% in Europe (though around 70% of OPC in head and neck 5000 are HPV seropositive). The ability of salivary measures to identify HPV driven OPC requires clarification.

We have already measured HPV serology measurements at the German Cancer Research Center (DKFZ) in Heidelberg. Assays included the E6 and E7 proteins of all high-risk HPV available at the DKFZ laboratory, i.e. HPV 16, 18, 31, 33, 35, 45, 52 and 58. For HPV 16 and 18 this also included E1 and E2. We are currently measuring molecular markers including Human Papilloma Virus (HPV) DNA and RNA and cellular protein p16ink4a on up to 1000 formalin fixed tissue blocks selected on the basis of their HPV serology and location.

We will measure the HPV DNA positivity of 19 HR or probable high-risk (pHR) HPV types (HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a and b, 70, 73, and 82) and two low-risk (LR) HPV types (HPV6 and 11), using a type-specific multiplex genotyping (TS-MPG) assay, which combines multiplex PCR and bead-based Luminex technology.

Keywords: Human papillomavirus, lifestyle behaviour, survival

Proposed work

We will have 250 participants with oropharyngeal cancer with serological assays and tissue measure. So we estimate that around 200 (80%) will have a usable saliva sample and based on a seroprevalence of ~70% then 140 will be seropositive and 60 will be seronegative.

All participants were asked to provide a saliva sample at recruitment. The participant was asked to rinse his/her mouth with water several times prior to collection. The participants then allowed the saliva to flow in the mouth and emptied saliva by spitting into the sterile empty screw-top container. Where possible at least 1ml of saliva was collected. No fixative was added. The sample was then transported at ambient temperature by first class post. The samples were divided into 7x1ml tubes and stored at −80°C.

We will transfer up to 200x1ml saliva samples to Massimo Tommasino's laboratory at the International Agency for Research on Cancer (IARC). They will be blind to the HPV status of the samples. We will send an initial pilot of 50 samples (25 HPV positive and 25 HPV negative tumours) in participants with more than one stored tube of saliva.

Saliva will be processed using a similar process to that described for gargles (1,2). With gargles DNA is extracted from 15 ml of gargles mixed 1/1 with preservcyt. The DNA is resuspended in 100 microliters of water/TE and 10 microliters used for our assay. HPV DNA positivity will be determined by using a type-specific multiplex genotyping (TS-MPG) assay, which combines multiplex PCR and bead-based Luminex technology (Luminex Corporation, Austin, TX). This assay detects 19 HR or probable high-risk (pHR) HPV types (HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a and b, 70, 73, and 82) and two low-risk (LR) HPV types (HPV6 and 11), as well as cellular beta-globin gene, which is used to control for DNA quality. After PCR amplification, 10 µl of each reaction mixture will be analyzed by multiplex HPV genotyping (MPG) using Luminex technology (Luminex Corporation, Austin, TX) as described previously [3, 4].

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