Diagnosis of genital infection caused by human papillomavirus using in situ hybridisation: The importance of the size of the biopsy specimen

F Gómez, M T Corcuera, E Muñoz, M Roldan, M J Alonso, M Abad, J I Paz, A López-Bravo

Abstract

Aim—To determine the size of a cervical biopsy specimen with human papillomavirus (HPV) infection required to enable in situ hybridisation to be carried out with a guarantee of a reliable result.

Methods—In situ hybridisation was carried out in 142 cervical uterine biopsy specimens classified histologically as low grade and high grade squamous intraepithelial lesions. Epithelial length at the level of the basal membrane was measured by image analysis. The specimens were divided into 10 groups based on epithelial length.

Results—Of the biopsy specimens, 61·2% were HPV positive. In specimens with an epithelial length below 5 mm 31·9% were HPV positive; in those between 5 and 9 mm in length 67·5% were HPV positive; and in those greater than 9 mm in length 81·8% were positive for HPV. For low grade squamous intraepithelial lesions (n = 90), 65·4% of specimens with an epithelial length greater than 5 mm were HPV positive. For high grade squamous intraepithelial lesions (n = 52), 86·8% of specimens with an epithelial length greater than 5 mm were HPV positive.

Conclusions—For a diagnosis of HPV infection using in situ hybridisation, the minimum length of epithelium in a cervical biopsy specimen should be 5 mm. For high grade squamous intraepithelial lesions, specimens over 5 mm in length are suitable. For low grade squamous intraepithelial lesions, to minimise the number of false negative results, the ideal minimum length is 10 mm.

(J Clin Pathol 1995;48:57–58)

Keywords: Human papillomavirus, in situ hybridisation, image analysis.

Several studies have shown that infection by human papillomavirus (HPV) is a common occurrence. It is clear that HPV infection is an essential event in the development of squamous intraepithelial lesions of the cervix. This association is based on the findings that squamous intraepithelial lesions usually contain HPV DNA and that normal cervical epithelium transfected by HPV DNA in vivo and in vitro will develop histological changes characteristic of squamous intraepithelial lesions.1,3

HPV infection has been diagnosed using colposcopy, cytology, histopathology, and electron microscopy. Immunohistochemical techniques have been used for antigen detection,4,6 while molecular hybridisation techniques (Southern blot hybridisation, dot blot, and in situ hybridisation) and gene amplification techniques (polymerase chain reaction (PCR), in situ PCR) have been used for the detection of specific HPV gene sequences.7,8

At present, one of the most commonly used techniques is in situ hybridisation with non-isotopic probes. This technique has many advantages: the location of positive cells can be correlated with the histopathological findings; it is simple and easy to use; it permits screening and typing of the HPV present in the lesion; and, as it can be carried out not only on fresh specimens but also on fixed paraffin wax embedded specimens, it can be used to carry out retrospective studies.

Most of the procedures involved in this technique (for example, fixation and enzymatic digestion) as well as the conditions that determine the specificity of hybridisation (hybridisation temperature and the concentrations of formamide and saline in the hybridisation buffer) have been standardised. However, the size of the biopsy specimen required for the procedure to be carried out with a guarantee of a reliable result has not been defined as yet.

Methods

A retrospective study of all lesions diagnosed as squamous intraepithelial lesions in our department between 1990 and 1992 was carried out. We studied 142 cervical uterine biopsy specimens histopathologically consistent with HPV infection and diagnosed as low grade squamous intraepithelial lesions (n = 90), equivalent to cervical intraepithelial neoplasia grade I (CIN I), and high grade squamous intraepithelial lesions (n = 52), equivalent to CIN grades II and III, in accordance with previously described criteria.8

An IMCO 10 image analyser (Kontron Bildanalyse) was used for image analysis, incorporating the Microm Image Processing program (MIP, Microm, Spain). Images were captured in real colour by a Hitachi KP-C503 TV camera linked to a Nikon microscope with a ×10 lens, and digitalised in a matrix measuring 5122 pixels. The length of epithelium in each biopsy specimen was then measured at the basal layer of epithelium.

A commercial in situ hybridisation kit with a HPV specific biotinylated DNA probe (Kreatech Biotechnology, Amsterdam, The Netherlands) was used to detect HPV. For each specimen, three serial sections were processed: one was treated with the HPV specific probe; one was used as a positive control, which was treated with a biotinylated probe specific for human DNA (Biomeda Corporation, Foster, USA); the third section was a
Distribution of specimens based on epithelial length and cervical lesion. The total number of HPV positive biopsy specimens on in situ hybridization for each group is given.

<table>
<thead>
<tr>
<th>Epithelial length (mm)</th>
<th>LSIL (CIN I)</th>
<th>HSIL (CIN II + CIN III)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of specimens</td>
<td>HPV positive</td>
<td>No. of specimens</td>
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<tr>
<td></td>
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<tr>
<td>&lt;2</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2-3</td>
<td>4</td>
<td>2</td>
<td>3</td>
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<td>3-4</td>
<td>2</td>
<td>2</td>
<td>5</td>
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<tr>
<td>4-5</td>
<td>20</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5-6</td>
<td>9</td>
<td>4</td>
<td>5</td>
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<td>6-7</td>
<td>7</td>
<td>3</td>
<td>1</td>
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<td>7-8</td>
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<td>8-9</td>
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<td>3</td>
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<tr>
<td>9-10</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>&gt;10</td>
<td>31</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

LSIL = low grade squamous intraepithelial lesions; HSIL = high grade squamous intraepithelial lesions.

negative control and was treated with a biotinylated probe specific for the pBR322 plasmid (Kreatech Biotechnology).

Results
Based on epithelial length, as measured by image analysis, the 142 cervical biopsy specimens were divided into 10 groups ranging from less than 2 mm to more than 10 mm in length (table).

Of the specimens with epithelial length less than 5 mm, 31.9% were HPV positive; this figure was 67.5% for specimens between 5 and 9 mm in length; and was 81.8% for specimens over 9 mm in length. For the 90 low grade squamous intraepithelial lesions 24.6% of specimens with an epithelial length less than 5 mm were HPV positive; this figure was 61.5% for specimens between 5 and 10 mm long; and was 74.2% for those greater than 10 mm long. For the 52 high grade squamous intraepithelial lesions, 50% of specimens with an epithelial length less than 5 mm were HPV positive; this figure was 70.5% for specimens between 5 and 9 mm long; and was 100% for those greater than 9 mm long.

Discussion
The routine use of in situ hybridisation for the diagnosis of genital tract infection caused by HPV has revealed that, for positive results, there is a wide variation in the percentage of labelled cells detected. In some positive biopsy specimens most of the epithelial cells are labelled, whereas in others the positive reaction is caused by the presence of either a few labelled cells randomly distributed throughout the specimen or by one or two strongly positive cells. Several factors account for this variation in the number of positive cells, including the type of viral infection (productive or non-productive), the number of copies of the viral genome in each cell as well as its physical state (episomic or integrated), and the sensitivity of the technique (25–50 copies of HPV per cell). We believe that the size of the specimen is an important factor which should be taken into account in any attempt to reduce the number of false negative results.

Our findings indicate that the minimum size of the specimen should be 5 mm, as the percentage of positive results obtained using specimens less than 5 mm long (23%) indicates that these positive results are not reliable. The highest percentage of positive results was obtained with specimens over 9 mm long, and therefore we consider this to be the ideal minimum epithelial length. Furthermore, the finding that 100% of high grade squamous intraepithelial lesion specimens over 9 mm long were HPV positive supports this conclusion; of the specimens over 7 mm long, 93% were HPV positive indicating that its length is also suitable for in situ hybridisation. By contrast, in low grade squamous intraepithelial lesions biopsy specimens with minimum epithelial lengths over 5 mm, no significant changes were observed in the percentage of positive results, suggesting that, for this type of lesion, specimens over 5 mm long can be used for in situ hybridisation. To minimise the number of false negative results, the ideal minimum length is 10 mm. In specimens less than 5 mm long, the number of false negative results could be reduced by studying multiple sections of the same specimen, but this would increase the cost of diagnosing this infection using in situ hybridisation.

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doi: 10.1136/jcp.48.1.57

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