

## Leaders

# Anal human papillomavirus and anal cancer

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In his eloquent introduction to the article "Anal warts and anal coitus", Oriol<sup>1</sup> briefly reviewed the historical belief, dating back to the Roman empire, that anal warts were invariably the result of anal intercourse and that, among afflicted men in particular, the reaction to such a malady was not sympathy, but "ribaldry and disgust". Today it is questionable whether the prejudice that such a reaction typified has greatly changed, but the knowledge surrounding the aetiology and biology of anogenital warts and their causative agents, the human papillomaviruses (HPVs) has increased dramatically over the past 20–30 years. It is the aim of this review to summarise what is currently known about anal HPV infection and its possible sequelae.

### Human papillomaviruses

The papillomaviruses are a group of epitheliotropic viruses classified as a genus of the Papovaviridae that includes the polyomaviruses (including the JC and BK viruses) and vacuolating agents. The virions themselves are icosahedral, non-enveloped particles with a diameter of 45–55 nm and, in HPVs, the genome is a circular, 7.9 kb double-stranded DNA that is divided into six early—that is, non-structural, genes (E6, E7, E1, E2, E4, and E5) and two late, structural genes (L1 and L2) followed by a short non-coding region, termed the long control region (LCR), or upstream regulatory region (URR). All the genes are encoded from one of the genomic strands.

The lack of cell culture system for HPVs has, for many years, hampered detailed investigation into the serological relations between the various HPV types and so their classification has always relied on some form of DNA analysis. The current taxonomy is based on the degree of nucleotide sequence identity in the L1, E6, and E7 genes, with a 10% divergence from any known HPV sequences in these regions defining a new type. This method has identified over 80 genotypes (some of which await formal classification) but, more importantly, the phylogenetic trees derived from such data have shown that the genetic differences between the various types identified correspond quite accurately with their biological activity, particularly concerning their tissue tropisms (cutaneous *v* mucosal). However, this system has not been able to predict which genotypes represent a high and low oncogenic potential as apparently minor genetic differ-

ences can lead to significant alterations in phenotype.

### HPV and cervical cancer

The most extensively studied aspect of HPV infection has been in relation to the epithelium of the anogenital tract and in particular that of the uterine cervix. At least 30 HPV genotypes have so far been isolated from the female genital tract and HPV prevalence in the female population is estimated to range from 3–30% depending on the study population and the HPV detection method employed.<sup>2</sup> Highly sensitive polymerase chain reaction (PCR) techniques for the detection of HPV DNA have shown that up to 90% of cervical infections are subclinical or latent with ultimate clearance of all virological, cytological, and histological evidence of infection. In clinical infection, HPV genotyping techniques have enabled correlations between lesion type and HPV genotype, and this has shown HPV types 6 and 11, and less frequently types 42, 43, and 44, to be most commonly associated with non-neoplastic epithelial changes including koilocytosis, flat condyloma, and condyloma acuminatum. Cervical intraepithelial neoplasia (CIN) associated with these genotypes is, if present at all, usually of the low grade type. Conversely, high grade CIN and invasive squamous cell carcinoma (SCC) of the cervix are most frequently associated with HPV types 16 and 18 and, to a lesser extent, types 31 and 33. Other types more rarely encountered in high grade lesions include HPV35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. In some series, over 90% of cervical SCC harbour a high risk HPV, most commonly type 16 or 18, the genome of which is usually integrated into the host DNA, although episomal viral DNA may also be present. In conjunction with epidemiological data showing that HPV infection and cervical SCC share a number of risk factors (early age at first intercourse and sexual promiscuity), the association between high risk HPVs and cervical SCC is now firmly established. However, as only 5–22% of high grade CIN typically progress to invasive carcinoma in untreated patients,<sup>3 4</sup> persistent HPV infection is thought to be a necessary factor but insufficient on its own to promote malignant invasion. The presumed cofactors in this process and their mode of action are currently poorly defined but it is hoped that HPV detection may assist in predicting the invasive potential of high grade CIN.

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### HPV infection and anal cancer

The establishment of a role for HPV infection in the development of cervical SCC raised the possibility of its involvement in other anogenital cancers. Anal infection was a particularly pertinent comparison because of similarities in the histological appearance of the cervix and anal canal. Similar to the cervix, the anal canal has a squamocolumnar junction or transformation zone, both deriving from the same cloacal membrane in the fetus.<sup>5</sup> It is this region that, in the cervix at least, appears to be particularly susceptible to HPV infection and the concomitant risk of intraepithelial neoplasia and carcinoma.

The proposition that a sexually transmitted agent was implicated in anal carcinoma was first proposed by Cooper *et al*<sup>6</sup> in 1979 who reported transitional or squamous cell carcinomas of the anus in four anoreceptive homosexual men. This hypothesis was supported by the citation of earlier studies that showed an increased incidence of anal carcinoma in women with a history of cervical SCC, and another case of anal SCC in a homosexual man.<sup>5 7 8</sup> Subsequent reports of 11 further cases of anal SCC or carcinoma in situ (CIS) in homosexual men supported the role for a sexually transmitted agent in anal malignancy.<sup>9-11</sup> As eight of these 11 cases had a history of anal warts or condyloma, both of which were known to be associated with HPV infection, suspicion fell on the HPVs. In fact, HPVs had previously been implicated in anal carcinoma from a number of case reports demonstrating malignant change of anal condylomata to CIS in which papillomavirus particles were identified by electron microscopy,<sup>12 13</sup> and similar reports continue to appear today.<sup>14</sup> This rare but well documented phenomenon may result from either simultaneous infection with high and low risk HPV genotypes, each being independently responsible for a characteristic lesion type<sup>15</sup> or, very occasionally, progression to malignancy of a condyloma solely associated with the high risk HPV16.<sup>16</sup> In addition, anal, as opposed to genital, warts were already known to be significantly more common in homosexual than heterosexual men.<sup>17</sup>

At the time of these early reports, anal cancer was recognised as a rare disease accounting for 1-6% of all anorectal tumours, being approximately five times more common in women than in men. In women, the annual incidence ranged from 0.9-1.3 per 100 000 population, whereas in men the figure was 0.3-0.7 depending on the test population.<sup>18-20</sup> Ensuing epidemiological and case-control studies confirmed that anal cancer, especially SCC, was strongly associated with overt male homosexuality and its presumed correlates (for example, never married). Daling *et al*<sup>21</sup> found a 20-fold excess over expected values of anal cancer in males with the same sex partner and a history of syphilis, and that 25% of all male anal cancers were in men who had never married, which was significantly different for the rate of rectal and colon cancer in the same group (7.8%). Similarly, the relative risk (RR) in never married or overtly homosexual men ranged

from 2.2-8.6,<sup>22-25</sup> although other, more explicitly defined practices, such as ever having had sex with another man or anal intercourse, carried RRs of 50 and 33, respectively.<sup>25</sup>

Significantly, no increased risk for anal cancer was seen in unmarried females.<sup>21 22 24</sup> In fact, the only independent risk factors for anal cancer in women were smoking, the use of haemorrhoid preparations, and disturbed bowel habits for longer than one month, the latter two of which may have been related to the cancer itself.<sup>20</sup> Further to this report, two epidemiological studies found an elevated risk of anal cancer (invasive and in situ), in women with a history of CIN or invasive cervical carcinoma (RR 4-5.5), which was greater than that for secondary vulvar cancer.<sup>26 27</sup> Clinical studies confirmed the association between anal SCC or anal intraepithelial neoplasia (AIN) and a history of high grade CIN and cervical or vulvar malignancy.<sup>28-30</sup>

Surprisingly, anal intercourse in women was found not to be a risk factor for anal HPV infection,<sup>20 31 32</sup> although one study found a higher HPV prevalence in women who had practised anal intercourse compared with those who had not (80% *v* 43%), but this did not reach statistical significance.<sup>33</sup> These data suggested that slightly different mechanisms—possibly reflecting the frequency of anal intercourse—were implicated in the development of anal cancer in women and in homosexual men, and that, although overall the disease was more prevalent in women than in men, homosexual men represented a subset of patients in whom the incidence of anal cancer was raised. Epidemiological data bore this out; Wexner *et al*<sup>34</sup> found that while only 8% of anal cancers were accounted for by homosexual men in the period 1959-67, this rose to 72% during 1978-86. The RR for anal cancer in this group was two to three times higher from 1980-81 than in the previous six years, and rose in never married men from 6.7 in 1979-84 to 10.3 in 1985-89.<sup>24 35</sup>

HPV infection, as manifested by a history of genital warts, was significantly associated with the development of anal SCC not only in homosexual men (RR 12.6), but also in heterosexual men and women (RR 4.4-32.5), but the period between the diagnoses of warts and anal cancer was shorter in homosexuals.<sup>23 25</sup>

Other significant risk factors for anal cancer in all groups included anal fissure or fistula (RR 2.4-9.1), cigarette smoking (RR 1.9-9.4), and more than 12 episodes of haemorrhoids.<sup>23 25</sup>

A number of studies of anal HPV infection in homosexual men have also been able to investigate the role of HIV infection in the development of HPV induced lesions because of the relatively high prevalence of HIV infection in this population. HPV infection is known to run a more aggressive course, be more refractory to treatment, and recur more frequently in men and women infected with HIV, and that these effects are worse in patients with HIV related disease.<sup>36 37</sup> One recent report found an overall RR of 63.4 for anal cancer in patients with an

AIDS diagnosis (83.4 in homosexual and bisexual men, 37.7 in heterosexual men), which was inversely proportional to both the patients' age (in homosexual and bisexual men) and time from AIDS diagnosis.<sup>35</sup>

While the weight of evidence implicating anal intercourse in homosexual men as a risk for anal HPV infection and its associated lesions is now overwhelming, HPV transmission in heterosexual men with anal warts cannot be explained by such a mechanism. A number of studies of heterosexual males with either a history of or current anogenital warts attending genitourinary medicine clinics have demonstrated perianal and anal canal warts in 8–33% of cases.<sup>1 31 38–41</sup> When additional criteria for HPV diagnosis were included (colposcopic appearance, acetowhite lesions), one study found that this figure rose to 88%.<sup>31</sup> Anal sexual practices (receptive anal intercourse, anodigital insertion) were not correlated with the presence of anal warts in any of these studies and the precise mode of acquisition of anal HPV in this group remains obscure.

#### Detection of HPV infection

When considering the role of HPV in anal dysplasia and carcinoma, direct comparisons between studies have been hampered by differences in study design, the populations tested, and the method of HPV detection. Before the application of molecular biological techniques for the detection of HPV DNA, the laboratory diagnosis of papillomavirus infection relied solely on cytology and, more particularly, histology for the identification of characteristic virus induced lesions; techniques that continue to be successfully employed in cervical screening programmes. Methods of detection of HPV have included immunohistochemical staining for virus capsid antigen and Southern transfer hybridisation (STH), filter hybridisation, in situ hybridisation (ISH), hybrid capture, and polymerase chain reaction assay (PCR) for the detection of viral DNA.

Cytological and histological criteria used in the diagnosis of anal HPV infection and its associated lesions, have been defined by comparison with analogous lesions seen in cervical pathology. Cytologically, the major diagnostic markers for cervical HPV infection are koilocytosis, acanthosis, nuclear hyperchromasia, nuclear enlargement, dyskeratosis, and bi- or multinucleation.<sup>2</sup> Consequently, it is these features that have been primarily sought in anal Pap smears,<sup>42</sup> although virtually all the studies employing anal cytology have suggested that koilocytosis is a poor indicator of HPV infection; in contrast, the presence of koilocytes in cervical smears is considered pathognomic for HPV infection.<sup>28 41 43–45</sup> The reasons for this disparity are unknown, but indicate that cytological detection of anal HPV infection should rely on a range of diagnostic markers until a clearer picture emerges of the differences between anal and cervical pathology.

Histological classification of anal lesions, which remains the diagnostic gold standard, again looks to cervical histology for a taxonomic framework: CIN is graded either 1–3

with CIN 3 representing carcinoma in situ, or, in an attempt to resolve the highly subjective nature of such a schema, simply as high and low grade CIN. The term anal intraepithelial neoplasia (AIN), was introduced as an appropriate comparison with CIN and its diagnostic criteria are essentially the same as for CIN.<sup>46</sup> However, the invasive potential of AIN 3 is poorly defined.

Immunohistochemical staining of viral capsid antigen has been only infrequently employed in the context of anal HPV infection. The sensitivity is relatively poor<sup>20</sup> and, because it relies on the production of large quantities of viral capsid antigen (most often in the terminally differentiated epithelium), it will not detect genotypes, including HPV16, that in persistent infection at least do not express structural genes. As a group specific test, genotyping is not possible with this technique whereas DNA based methods do permit this.

The choice of HPV DNA detection method has depended on the pace of technological development, the type of specimen available, and the information required. ISH is uniquely able to localise HPV DNA and its physical state within tissue and thus directly to correlate its presence (or absence) with specific lesion types. Its main drawback is insensitivity, a positive signal requiring 20–50 copies of HPV DNA per cell for isotopically labelled probes, or 350 copies per cell for non-isotopically labelled probe DNA.<sup>47–49</sup>

STH, for many years the benchmark of HPV DNA detection, is considerably more sensitive than ISH, being capable of detecting 0.1 copy of HPV DNA per cell, and it is able to give some indication of the physical state of the viral genome (integrated or episomal, oligomeric, etc).<sup>50 51</sup>

Filter ISH, in which exfoliated cells are immobilised and lysed on a solid support before hybridisation with a DNA probe, yields only a qualitative result and is technically less demanding than STH; however, it is also relatively insensitive and prone to high background readings. It is also liable to sampling errors and gives no indication of the physical state of the viral DNA.<sup>47</sup>

More recently, PCR has been widely adopted in HPV DNA detection owing to its superior sensitivity (one HPV genome per 10<sup>5</sup> cells) and its ability to detect a broad spectrum of HPVs by the use of "consensus" primers derived from highly conserved regions of the HPV genome.<sup>52–54</sup> Post-PCR hybridisation with type-specific probes is still required for typing purposes. PCR does, however, suffer from the risk of false positive results because of carry over of amplified DNA to other specimens and reagents used in the test, and rigorous laboratory protocols are necessary to minimise this risk.

From the foregoing descriptions it is clear that there may be three strands to the investigation of anal epithelial abnormality: clinical, histological or cytological examinations, and the recent adjunct of HPV DNA detection. In spite of the aforementioned difficulties in comparing directly the methods used

in different studies, some comparative studies have been carried out with the aim of identifying AIN by means other than histology alone, and to elucidate the role of HPV in the development of these lesions.

While histological diagnosis remains the gold standard for AIN detection, significant interobserver variation in its identification, particularly in low grade AIN, is known to occur,<sup>55</sup> and it is therefore important that this bias is appreciated and controlled for during the course of a study. Anal cytology has been investigated by a number of groups as a less invasive alternative to biopsy, which may be repeated at regular follow up. Medley<sup>56</sup> first demonstrated the feasibility of using anal cytology as a screening tool for occult HPV infection in a study of 102 homosexual men with a history of anal condyloma, but no detailed comparisons were made in that report between cytological results and histological and virological findings.

Where comparisons have been made between the presence of clinical lesions and the detection of HPV and AIN by anal cytology, the latter's sensitivity has been highly variable. In one study of homosexual men, Surawicz *et al*<sup>57</sup> found that only 36% of 78 patients with colposcopically identified HPV associated anal lesions (wart, wart ring or flat white epithelium) had dysplasia by cytology. Two other studies investigating anal warts in homosexual men showed cytology to identify 42% and 70% of patients with warts when HPV and/or dysplasia were included as diagnostic criteria.<sup>43-58</sup> Similarly, Law *et al*<sup>44</sup> found cytological evidence of HPV/AIN in 62% of homosexual men with anal condyloma. In a smaller study of heterosexual women the same authors found the sensitivity of cytology in the identification of anal HPV infection to be 65% compared with clinical examination and the detection of viral DNA by dot blot.<sup>33</sup> However, abnormal cytology was seen in 83%, 90%, and 98% of patients attending genitourinary medicine clinics with perianal warts, acetowhite lesions, and anal canal warts, respectively,<sup>41</sup> while acetowhite lesions were found in 43% of a similar group of patients in the absence of clinical warts.<sup>31</sup> Given that the patients in all these studies could be classified as being at high risk for STDs, the differences in the detection rates of HPV/AIN by cytology is not readily explicable. Poor sensitivity may in part be explained by the difficulty in exfoliating representative atypical cells from a highly keratinised lesion such as an external wart,<sup>43</sup> and in fact seven of 11 false negatives by cytology were from external rather than anal canal lesions.<sup>44</sup> This would still not explain the low sensitivity seen by Surawicz *et al*<sup>57</sup> where cytology was performed on patients selected on the basis of their colposcopically visible HPV associated lesions. Conversely, the very high sensitivity seen by Sonnex *et al*<sup>41</sup> raises questions about the specificity of cytological testing in relation to the presence of clinical lesions, which was obviously not considered by the other studies, and this issue remains largely unaddressed.

The rate of cytological abnormality in patients without clinical lesions in the above studies ranged from 21–58% and it was presumed that at least some of these patients were either harbouring HPV genotypes that did not give rise to clinically overt lesions or had subclinical HPV infection. In support of this, Haye *et al*<sup>43</sup> reported that all six patients with abnormal cytology but with no evidence of anal warts had flat condyloma following biopsy and 26 of 34 (76%) comparable patients in the study of Law *et al*<sup>44</sup> had detectable HPV DNA. Taken together these data suggest that abnormal cytology is often not correlated with external anal warts, and that such lesions may be present only within the anal canal and therefore require thorough clinical examination.<sup>59</sup>

The physical appearance of lesions either clinically or anoscopically has been shown not to predict the histological findings: low and high grade AIN was found with similar frequency in a range of HPV associated lesions of the anal canal, and neither lesion type could be correlated with the presence of external anal condylomata.<sup>57-59</sup> In addition, high grade dysplasia (with concurrent HPV16 infection) may occasionally be present in the absence of any colposcopically visible lesion.<sup>60</sup> Scholefield *et al*<sup>61</sup> successfully identified 18 of 23 (78%) cases of histologically proven AIN by endoscopy in a group of patients attending genitourinary medicine clinics with anal HPV/condyloma, but this level of success has not been duplicated elsewhere and may reflect a high degree of patient selection. Biopsy of acetowhite lesions demonstrated HPV in 85–90% of cases.<sup>28-41</sup>

Where cytology and histology have been performed in parallel, the two techniques have not always been compared directly. Histology detected dysplasia in 92% of homosexual men including high grade AIN in 27%, compared with 36% and 7%, respectively for cytology, implying a poor sensitivity for the latter,<sup>57</sup> whereas a significant correlation was found between the AIN grade in nine anal biopsies and their corresponding smears although cytology tended to underestimate the grade of lesion. The cytological results on a further 36 normal biopsies were not reported.<sup>59</sup> Conversely, AIN was histologically confirmed in 27 of 32 (84%) biopsies from homosexual men with cytological evidence of AIN.<sup>62</sup>

Direct comparisons between cytology and histology have been investigated in only two studies. Scholefield *et al*<sup>28</sup> detected AIN by cytology in 10 of 29 (34.5%) biopsy proven AIN in women with preexisting CIN 3 including only five of 11 cases of AIN 3. No extra cases of AIN were suggested by the cytological findings. In the sole report that specifically set out to compare anal cytology and histology, de Ruyter *et al*,<sup>45</sup> in a study of homosexual men, demonstrated that cytological sensitivity and specificity depended crucially on the diagnostic criteria employed. If markers of both AIN (with or without HPV) and HPV alone were included as such criteria, then cytology showed a sensitivity of 87.5% (including the identification of eight of nine cases of AIN 3) but an unacceptable specificity of 16.3%. However, if

this definition was narrowed to exclude all cases of HPV alone, specificity rose to 72.5%, but the sensitivity dropped to only 33.9% (identifying just three of nine cases of AIN 3).

While all these studies are, as many of the authors accept, prone to errors of sampling and interpretation, the overall impression is that, although anal cytology might be a useful adjunct in the detection of some anal lesions, anoscopy and biopsy in particular are still essential in the diagnosis and grading of AIN.

#### HPV DNA detection

The introduction of testing for HPV DNA has added another dimension to the investigation of both AIN and CIN and it is hoped that an appreciation of the clinical significance of HPV in these lesions and tissues will be a useful prognostic indicator as well as enhancing the understanding of the pathogenesis of HPV infection. A working knowledge of the differences in the performance characteristics of these tests is important when trying to compare HPV prevalences in different studies. As mentioned previously, a number of approaches based on hybridisation with HPV specific probes have been adopted and some investigations have set out to compare their relative merits.

Before the introduction of PCR, the most sensitive test for the detection of HPV DNA was STH, although one report showed comparable sensitivity using a dot blot format following alkaline hydrolysis of anal carcinoma tissue.<sup>63</sup> ISH is known to be highly specific but its sensitivity relative to STH is only of the order of 50–83% when applied in clinical studies.<sup>51 63 64</sup> This is not surprising given its low theoretical sensitivity, although one group have claimed a sensitivity of only 1–2 genomes per cell for ISH using a biotin labelled DNA probe.<sup>65</sup>

Comparisons of dot blot and filter ISH with STH have shown that dot blot in particular provides a similar level of HPV detection to STH, with filter ISH being slightly less sensitive. When STH was taken as the gold standard, the sensitivity and specificity of dot blot ranged from 68–96% and 90–100%, respectively,<sup>51 62 66–68</sup> but when PCR was taken as the gold standard, some groups have found dot blot to be slightly more sensitive than STH.<sup>66 67</sup>

Where immunohistochemical staining has been attempted, positive results have most frequently been observed in the warty and condylomatous lesions associated with HPV6 and 11 rather than the more severe lesions associated with the high risk HPVs. In two small studies, virus capsid antigen was detected in seven of 10 and two of three warty or condylomatous tissues,<sup>69 70</sup> confirming an estimated 40–80% positivity rate seen by others in similar tissue.<sup>20 71</sup>

When anal carcinoma tissue has been studied, capsid antigen has been demonstrable in only 0–50% of cases and then only in areas of AIN 3 that were positive for HPV6 or 11 rather than invasive or HPV16 containing tissue.<sup>20 70–72</sup> The necessity for the presence of

capsid antigen and, by implication, mature virus particles, may make immunohistochemical staining inappropriate for the detection of high risk HPVs that give rise to severe dysplasia and carcinoma in which structural genes are not expressed and integration of the viral genome may have occurred.

The introduction of highly sensitive PCR assays into HPV DNA detection has overshadowed and, to some extent, superseded these older technologies. A number of laboratory based and clinical studies have demonstrated that the sensitivities of ISH, STH, hybrid capture, dot, blot, and filter ISH compared with PCR were 21–65%, 38–69%, 50%, 58%, and 58%, respectively, and although some groups still consider STH and PCR as complementary,<sup>57</sup> the majority of reports has shown that PCR has the highest HPV detection rate and that alternative techniques only rarely detect HPV DNA in PCR negative specimens.<sup>49 51 65–68 73 74</sup> In addition, up to 75% of those specimens untypeable by STH were typeable by PCR.<sup>67</sup>

The superior sensitivity of PCR over STH has been exploited by some groups to distinguish patients with high viral load (PCR and STH positive) and those with low viral load (PCR positive, STH negative), and then to identify their associated clinical correlates.<sup>32 62 75</sup>

HPV PCR protocols are not internationally standardised, consequently there exists the possibility of significant interlaboratory variation in assay performance depending upon the particular protocol employed. The most commonly used techniques employ a set of consensus primers derived from highly conserved regions of the HPV genome—usually L1 or E1—that are able to detect a broad spectrum of HPV genotypes.<sup>52 54 76</sup> Further testing by the selective use of type specific probes, type specific PCR, and either sequencing or restriction fragment length polymorphism analysis of the PCR product enable identification down to the genotype level.<sup>77–79</sup> The choice of PCR primers, probe, and conditions of PCR and hybridisation will all affect the final result. Noffsinger *et al*<sup>80</sup> found L1 consensus primers to be significantly less sensitive than E6 derived type specific primers for HPV16 and 18 in detecting HPV DNA from anal carcinoma tissue, most probably owing to the disruption of the L1 gene (but not the E6/7 genes) during integration of the viral genome, which is thought to be a prerequisite for malignant transformation. Also, type specific primers that generate short (~ 100 base pair) PCR products may be more sensitive than consensus primers in the detection of HPV16 and 18.<sup>81</sup> Balanced against the undoubted benefits of PCR are the risk of false positive results owing to adventitious contamination of reagents with previously amplified DNA, as well as false negative results owing to PCR inhibitors present in the specimen. The latter consideration is usually assessed by amplification of a portion of human DNA before HPV PCR.

It should be clear from the foregoing summary that the choice of HPV DNA detec-

tion method can have a significant effect on the final results; therefore, it is important that the appropriate technique is selected so that the aims of a particular investigation are satisfied.

#### **Anal HPV infection: prevalence and risk factors**

Investigations into anal HPV prevalence, risk factors, etc, have largely been cross-sectional and retrospective in nature and, in contrast to the situation in cervical HPV infection where a representative test population is readily available through the cervical screening programme, studies into anal HPV have tended to concentrate on homosexual men in whom such infection is very common as well as on patients with anal carcinoma. In the case of male homosexuals, it has, in many instances also been possible to investigate the effects of HIV mediated immune suppression on HPV infection as dual infection with both viruses is relatively common in this group.

The prevalence of anal carcinoma and cytological or histopathological markers of anal HPV infection have long been known to be increased in homosexual men with and without HIV infection<sup>6 9-11 71 82 83</sup> and may currently be on the increase, particularly in HIV positive patients.<sup>18</sup> HPV DNA was not sought in the majority of these early studies but many recent investigations have used some form of HPV DNA detection to examine the role of HPV in anal pathology in symptomatic and asymptomatic patients.

Prevalence rates vary considerably between studies depending on the criteria used for patient selection and which HPV DNA detection technique was used. In patients with anorectal symptoms, the HPV prevalence rate was found to be only 17% by dot blot,<sup>84</sup> whereas the corresponding figure for patients presenting with anal condyloma (using ISH) was 87%.<sup>85</sup> However, by PCR, 86% of HIV negative and 98% of HIV positive patients with internal anal lesions were positive for HPV DNA.<sup>57</sup>

In cases where patients have not been selected by anal symptoms (most frequently genitourinary and HIV clinic attendees), anal HPV prevalences have ranged from 8–40% by dot blot or STH, 29–78% by PCR, 33–82% by cytology, and 24–65% by dot blot, cytology or both in HIV negative patients. The values for HIV positive patients were 27–61% (dot blot), 87–92% (PCR), and 70% (dot blot/cytology).<sup>43 44 51 58 59 62 75 86-89</sup>

Anal cytological abnormality (including changes indicative of HPV infection) and AIN have been found in 14–46% of unselected homosexual men,<sup>41 43 44 51 56 59 62 87 88 90</sup> with one study recording a figure of 82%,<sup>58</sup> but these figures represent a range of patients with and without HIV infection and its attendant immunosuppressive effects. In addition, histologically confirmed AIN has been found in 29% and 43% of patients with anal condylomas although the majority of these, where stated, were AIN 1.<sup>85 91</sup>

Studies comparing cytology and HPV status in homosexual men have shown a strong, inde-

pendent association between HPV infection and abnormal cytological appearance, with odds ratios of 4.6 and 6.1 compared with patients with normal cytology.<sup>59 87</sup> Abnormal cytology (viral changes, dysplasia or both) has been correlated with both high and low risk HPV genotypes, but generally speaking, high grade AIN tended to have higher HPV prevalences, a greater proportion of which were accounted for by the high risk HPVs. Infection with multiple genotypes and a high viral load (with multiple or single genotypes) were also independently associated with abnormal cytology in HIV positive patients, but detection of HPV DNA by PCR alone (a low viral load) tended to weaken the association between cytological abnormality and HPV infection, presumably by detecting a greater number of latent HPV infections.<sup>51</sup>

HPV DNA can also be detected by non-amplification techniques in up to 40% of patients with normal anal cytology, implying a high rate of latent and subclinical infections, although the distribution of genotypes included a greater proportion of low risk HPVs compared with patients with cytological lesions.<sup>44 51 59 62 87 88</sup>

#### **The role of HIV**

The relatively high prevalence of HIV infection in the male homosexual population has enabled its role in anal pathology to be investigated. Virtually all studies to date have identified an increased rate of anal epithelial abnormality and HPV infection in HIV positive individuals, although these differences have not always reached statistical significance.<sup>51 57 58 62 84 86 88 89</sup> Only one study has been able to demonstrate any difference in the distribution of genotypes between HIV positive and negative individuals, with the high risk HPV16 and 18 being more common in HIV positive patients.<sup>57</sup>

Immune function in HIV infection, measured by CD4 count, CD4:CD8 ratio or clinically (whether the patient is symptomatic with an AIDS defining illness) has also been recorded in many of these reports, but its precise significance in relation to anal pathology remains unclear. A high HPV viral load, HIV infection, and immune dysfunction have all been identified as risk factors for AIN,<sup>58 62 88 90</sup> although a low CD4 count was not an independent risk factor for anal epithelial abnormality after controlling for HPV status in a number of studies<sup>44 51 59 87</sup>; however, immune dysfunction alone has been correlated with HPV infection by others.<sup>84 86-89</sup>

Confusingly, Kiviat *et al*<sup>84</sup> found that in HIV positive patients, a depressed CD4 count was not associated with HPV infection compared against those with normal CD4 levels, whereas Palefsky *et al*<sup>88</sup> reported the opposite result, a positive association between a low CD4 count and abnormal anal cytology in patients with group IV disease as defined in the Centers for Disease Control and Prevention (CDC; Atlanta, Georgia, USA) classification.<sup>59</sup> However, HIV positive patients without immune dys-

function were still more likely to have HPV infection, a higher viral load, and AIN than HIV negative patients.<sup>62</sup>

Overall, these data have led to a consensus theory that the immunosuppressive effects of HIV infection, rather than a direct effect of HIV itself, lead to an enhanced expression of HPV infection, and this in turn leads to HPV induced epithelial abnormality.<sup>51 87 89 90</sup> This model clearly represents a simplified scheme as it not only omits the possible effects of various epidemiological and lifestyle factors, but also excludes the possibility of alternative mechanisms for the mode of action of HIV in anal pathology. These include HIV mediated immune modulation in the recognition of HPV induced neoplasia, non-specific potentiation of HPV infection by HIV, and direct interaction between gene products of HIV and HPV, and should therefore be regarded as a framework from which long term natural history studies should begin.<sup>62</sup>

### Anal cancer

In contrast to the cross sectional population based studies outlined above, many investigators have retrospectively examined tissue taken from patients with anal lesions, including carcinoma, for the presence of HPV DNA. Croxson *et al*<sup>10</sup> and Nash *et al*<sup>92</sup> first implicated a role for HPV in AIN in homosexual men while three other studies found an elevated risk for AIN and anal carcinoma in women with a history of intraepithelial neoplasia involving the cervix and vulva or vagina, and that 48.5–51% of anal biopsies from this group contained HPV16 DNA.<sup>28–30</sup> Given the histological similarities between CIN and AIN and the former's known malignant potential and association with HPV infection, HPV DNA was soon sought in anal carcinoma tissue.

The HPV prevalence rate in anal SCC is inevitably dependent to some degree on the performance characteristics of the HPV detection method. ISH using type specific probes has detected HPV DNA in 0–81% of in situ and invasive squamous cell carcinomas with the corresponding figures for STH and PCR being 23–60% and 46–100%, although because of the rarity of anal carcinoma, a number of these reports only examined a small number of tissues.<sup>15 28 48 49 63–65 70 72 74 80 93–100</sup> Viral capsid antigen has been detected by immunohistochemistry in none of 38 and five of eight cases of invasive and in situ carcinoma.<sup>20 71</sup>

Attempts to detect HPV DNA in tumours with a basaloid pattern of differentiation (cloacogenic carcinoma) have yielded conflicting results. Wolber *et al*<sup>94</sup> failed to detect HPV DNA in any of 14 such tumours while only one of 42 transitional cell carcinomas contained detectable HPV DNA,<sup>48</sup> but both these studies used ISH and may have underestimated the true HPV prevalence. Subsequent reports using both ISH and PCR have detected HPV DNA and RNA in up to 90% of basaloid tumours, strengthening the view that it actually represents a variant of squamous cell carcinoma rather than a distinct histopathological entity.<sup>15 72 74 101</sup> HPV16 is by far the most com-

monly encountered genotype, being present either singly or in association with other genotypes, in up to 93% of invasive squamous tumours and 69% of CIS with the equivalent figure for HPV18 being only 10%,<sup>15 30 48 49 63–65 70 72 74 80 93–97 100–102</sup> although HPV18 DNA has occasionally been found in anal adenocarcinoma.<sup>48 103</sup>

Although HPV6 and 11 are usually associated with low grade lesions, they have occasionally been identified in both CIS and invasive carcinoma (by PCR and ISH), apparently in the absence of any of the commonly encountered high risk genotypes<sup>49 74 80 94 96 100</sup>; one study, using ISH, detected HPV6 in eight of 23 (34.7%) HPV positive anal carcinomas.<sup>48</sup>

In carcinoma, HPV DNA is virtually always found integrated into the host chromosome, at least with types 16, 18, 31, and 33, but it is frequently coexistent with episomal DNA in the cell nucleus.<sup>64 65 74 98</sup> Scheurlen *et al*<sup>50</sup> also identified a rearranged 10.7 kb oligomeric episome containing duplications of E7 and E1 and parts of E6 and E2. This latter observation may represent an alternative mechanism to viral integration for the disruption to E2 function and the consequent derepression of E6 and E7 leading to uncontrolled cellular proliferation and HPV induced aneuploidy.<sup>50 104</sup>

HPV DNA is most abundant in the superficial layers of the infected epithelium containing the non-keratinised, most differentiated tumour cells, in contrast to the concentration of viral DNA that is seen in the koilocytes of condylomatous tissue, but HPV16 DNA has been detectable throughout the less differentiated tumour mass in some cases.<sup>48</sup> The proportion of infected cells visible within a histological section is variable but often greater than 50% and the HPV genome copy number per cell ranges from 1–500.<sup>64 65 98</sup> Although the rate of HPV detection increases with increasing severity of lesion, there often remains a minority of HPV negative tumours,<sup>49 74 98 102</sup> suggesting the possibility of HPV positive and HPV negative tumours as histologically distinct entities.<sup>72</sup> However, the lack of association between the detection of HPV16 DNA in anal SCC and any clinical and pathological correlates also implies its generic involvement in this condition rather than representing a distinct subset of anal squamous carcinomas.<sup>98</sup>

Lymph node metastases from HPV16 positive anal tumours have been found to harbour HPV16<sup>64 104</sup> although others have not been able to reproduce this finding.<sup>49</sup> Worldwide, HPV16 has been found with variable frequency in anal cancers from at least six countries although the geographic variations observed suggested different global distributions for the various HPV genotypes.<sup>105</sup>

Taken as a whole, the circumstantial evidence linking HPV with precancerous lesions and the development of invasive carcinoma of the anus is highly persuasive, but there is currently a dearth of natural history studies of AIN to support this association. That AIN truly represents a precancerous lesion with malignant potential is suggested by the histological observation of AIN and CIS adjoining

frank tumour tissue and the detection of HPV DNA in the AIN/CIS from HPV positive, but not HPV negative, tumours.<sup>65 72 93 102 104</sup> Such evidence does not unequivocally prove that anal CIS necessarily progresses to invasive carcinoma, but the uncertainty regarding its malignant potential has meant that, on ethical grounds, longitudinal studies have used CIS as their follow up end point.<sup>75</sup> However, this uncertainty has meant that there is as yet no consensus on how best, or even whether, to treat anal CIS and its presumed precursors and clinical decisions must therefore be taken on a case by case basis.

Natural history studies of AIN in HIV positive and negative homosexual men have shown that the rate of progression to a high grade lesion ranges from 2.6–16% over a 9–48 month follow up period.<sup>75 106 107</sup> Although no progression to invasive carcinoma was observed in any of these cases, or in any of nine cases of histologically confirmed AIN 3 over a five year period,<sup>70 108</sup> isolated observations of apparent progression to carcinoma have been reported. Ogunbiyi *et al*<sup>30</sup> reported on the case of a female with a history of vulvar cancer and current AIN 3 that was found to be invasive 18 months later. Similarly, Scholefield *et al*<sup>28</sup> reported on another female with multifocal grade 3 lesions of the cervix, vulva, and anus, the last of which appeared invasive two months after presentation. The HPV status of both these patients was not recorded.

Cytological abnormality was found to regress to either normality or a lower grade lesion in only 10% of HIV positive men with group IV disease, whereas 40% progressed to a more severe grade over a 17 month period, but this was not correlated with CD4 count in this highly immunocompromised group.<sup>106</sup> Persistent HPV infection, particularly with the high risk types 16 and 18, is associated with progression to abnormal cytology, including AIN 2 and 3, in homosexual men, especially if the virus is present at high levels. In addition persistent infection is more common in symptomatic and asymptomatic HIV positive than negative men.<sup>75 89</sup> Immunosuppression, measured by a low CD4 count, was independently associated with the development of AIN 2/3 in HIV positive men, as was HIV infection per se although the mechanism by which this could occur is unknown.<sup>75</sup>

The relative rarity of anal SCC, compared with cervical SCC suggests that progression to invasion in anal carcinoma is not a common event, and the factors that bear on it, although showing much in common with cervical carcinoma, may differ. Alternatively, it must be borne in mind that AIN itself is a rarely encountered entity and, as noted above, vanishingly few natural history studies have been carried out to date. It is therefore possible that untreated AIN does progress to invasive SCC at a similar rate to comparable CIN lesions but is merely observed less frequently. The relatively common occurrence of high risk HPV DNA in anal SCC tissue would support this hypothesis.

## Conclusions

The anal and cervical transformation zones have a similar histological appearance and both are susceptible to HPV infection. Anal HPV infection is highly correlated with epithelial abnormality in a similar fashion to cervical infection and, although the potential of anal CIS to progress to invasive SCC is unknown, it is a rarely observed event. Conversely, high risk HPVs are frequently detected in anal SCC tissue. Homosexual men are at particular risk of anal infection, probably via anoreceptive intercourse, but this does not appear to be a risk factor for anal infection in women. HIV coinfection is associated with anal HPV, frequently leading to more severe disease, resultant in part from HIV induced immunosuppression. Guidelines on the clinical management of patients with high grade AIN require further natural history studies to assess its malignant potential.

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