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Review

Why acute infections by human papillomaviruses matter

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Abstract: Most infections by human papillomaviruses (HPVs) are non-persistent. Yet, for HPVs, as for many other oncoviruses, there is a striking gap between our detailed understanding of chronic infections and our limited data on the early stages of infection. Here we argue that studying the biology of acute infections by HPVs is necessary and timely. Disentangling early interactions will help explain why certain infections become chronic or latent. A better description of immune effectors and pro-inflammatory pathways during the initial stages of infections has the potential to lead to novel treatments. Furthermore, cervical cancer screening and vaccines impose novel iatrogenic pressures on HPVs, implying that anticipating any evolutionary responses remain essential. Finally, hints at the associations between their acute infections and fertility deserve further investigation given their prevalence worldwide. Overall, understanding asymptomatic and benign infections may be instrumental in reducing HPV virulence.

Keywords: clearance; persistence; latency; fertility; virome; warts; cancer; evolution; immunotherapies; vaccination

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The most oncogenic viruses to humans are a group of Human papillomaviruses (HPVs) [1]. HPV-induced cancers typically occur after several years of infection (Figure 1). The importance of viral persistence in the natural history of these diseases has driven most of the research to focus on chronic infections and to relatively neglect acute infections. We first present recent progress in the fight against HPVs before summarizing the current understanding about acute HPVs infections. We then identify the main gaps in our knowledge about these infections, which, if filled, would have direct implications for preventing, controlling and treating infections by HPVs.

State of the fight against HPVs

Infection-driven cancers stand out because they can be fought using the arsenal developed against infectious diseases: identification of risk factors, prevention of transmission and early detection of infected individuals. Identification of risk factors has led to the recognition of a few, closely related 'high risk' HPVs as necessary etiologic agents of several cancers [1]. Contagion can be prevented by the use of safe and effective vaccines targeting the most oncogenic HPVs along with certain 'low risk' HPVs causing anogenital warts [2,3]. Finally, screening programs for early detection of (pre)neoplastic lesions caused by HPVs infections have been globally successful at decreasing the burden of cervical cancer [4].

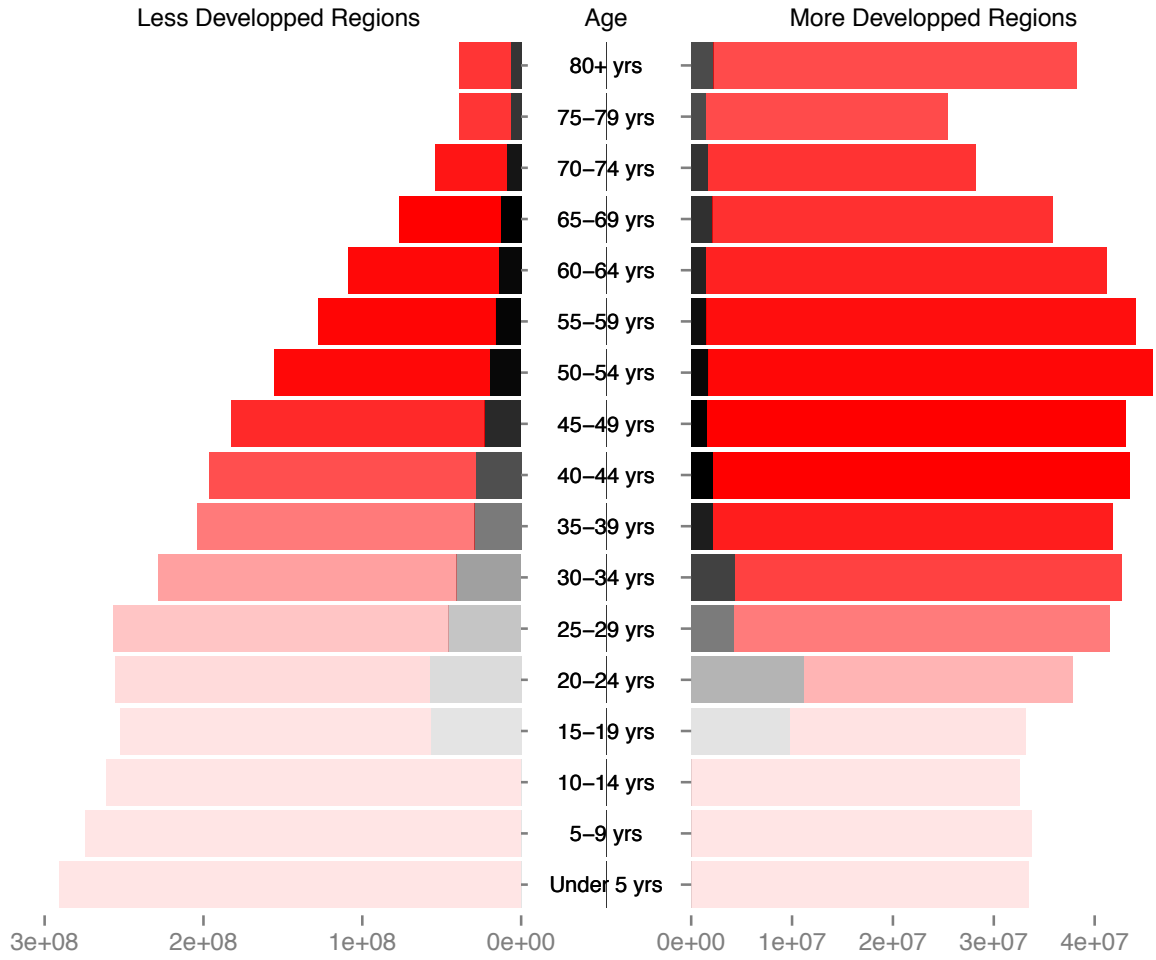

Figure 1. Number of women with asymptomatic cervical HPV infections (black bars) as a function of age class (red bars) and of country development status. Bar color intensity reflects the prevalence of cervical cancer in the corresponding age class (and hence the focus of current research). Data should be read as follows, using the 30-34 years of age as an example: in less developed regions, among the 228 million women in the age class, 40 million (17,8%) display a normal cytology but are actually infected by HPVs, and 30 thousand (0,013%) suffer from cervical cancer, while in more developed regions among the 42 million women in the age class, 4.3 million (10,2%) display a normal cytology but are actually infected by HPVs, and 6 thousand (0,015%) suffer from cervical cancer. Data correspond to HPV prevalence in women with normal cytology, as estimated in the HPV information center (http://hpvcentre.net/, [5]). Overall HPV prevalence values will be actually larger, as they will include women with abnormal cytology. No data were available for HPV prevalence in women below 15 years of age. Demographic data correspond to the UN projections for 2015 (note the shift in the logarithmic scale for the left and the right sides).

Unfortunately, HPVs will continue to infect millions of people in the foreseeable future, thereby causing significant morbidity and mortality worldwide [6]. Vaccine coverage varies widely both within and between countries [2], as does access to screening programs [7]. Beyond socio-economical factors, certain forms of cancer are more difficult to detect than others. This is the case for glandular forms of cervical cancer compared to the more common squamous carcinoma and recent trends show that the incidence and mortality of cervical adenocarcinoma are increasing [8]. Furthermore, decades of successful fundamental research have focused on cervical cancer, yet we still know too little about the implication of HPVs in anal [9], oropharyngeal [10] or even skin cancers [11], which are all on

the rise, albeit in different populations [1]. Finally, from an economical perspective, the total health care cost linked to treating genital warts should not be overlooked since it can exceed that of treating HPV-induced cancers [12], despite the obvious differences in severity and indirect impact of both diseases. It, therefore, remains vital to better understand the biology and epidemiology of HPV infections.

HPV acute infections

A definition challenge

HPV infections that are not chronic have been referred to as 'acute', 'non-persistent', 'incident', 'resolving', 'transient', or 'clearing' infections. Here, following Virgin *et al.* [13], we define acute infections as a non-equilibrium process that results either in infection clearance, host death or chronic infection.

Establishment of chronic infections is a life history trait shared by very divergent viruses. For large viruses, such as herpesviruses, the infection often starts with an acute phase, but viral gene expression changes towards a different profile and the virus enters a latent state with limited or even no genome replication and no cellular damage, until a reactivation is triggered. In small viruses such as Torque-Teno viruses, the initial infection goes unnoticed (the acute infection stage has actually never been documented) and the viral genomes remain in the host and replicate chronically at very low levels [13].

In the case of HPVs the frontier between chronic and acute infections is somehow blurry because we lack knowledge about the immune and viral dynamics during the early stages of the infection and because HPVs are very diverse. For instance, some beta- and gammapapillomaviruses chronically infect stratified epithelia very early in the host's life and replicate at very low levels without any apparent clinical or cellular damage. For others, including the oncogenic alphapapillomaviruses infecting mucosa, some symptomatic infections can resolve naturally but in a matter of years. To add complication, the viral gene expression program can be diverted towards a latency state directly upon infection, depending on the precise cell type or differentiation stage of the infected cell, as suggested by animal models [14–16]. Although within-host mathematical mathematical models are starting to investigate this question [17], epidemiological evidence is still lacking to assess the prevalence of these latent infections.

Most HPV infections are acute

The acute, clinically prominent phases of viral infections, are generally better understood than the asymptomatic chronic ones [13]. Research on oncogenic HPVs is an exception because of its focus on chronicity and on virus-induced cell transformation.

It is currently thought that most infections by alphapapillomaviruses do not become chronic [4]. For infections in the female genital tract, HPV16 is the most persistent type, but by 12 months 40% of the infections have already cleared or been treated because a (pre)neoplastic lesion was diagnosed (Figure 2). This proportion reaches 85% by 36 months [18]. For HPV6, which is rarely associated with cancer and often associated to genital warts, these numbers are 66% and 98% respectively. Note that the proportion of chronic infections directly depends on our ability to detect latent infection [14,16]. Also, for beta- and gammapapillomaviruses, chronic infections might be more prevalent but our knowledge is limited.

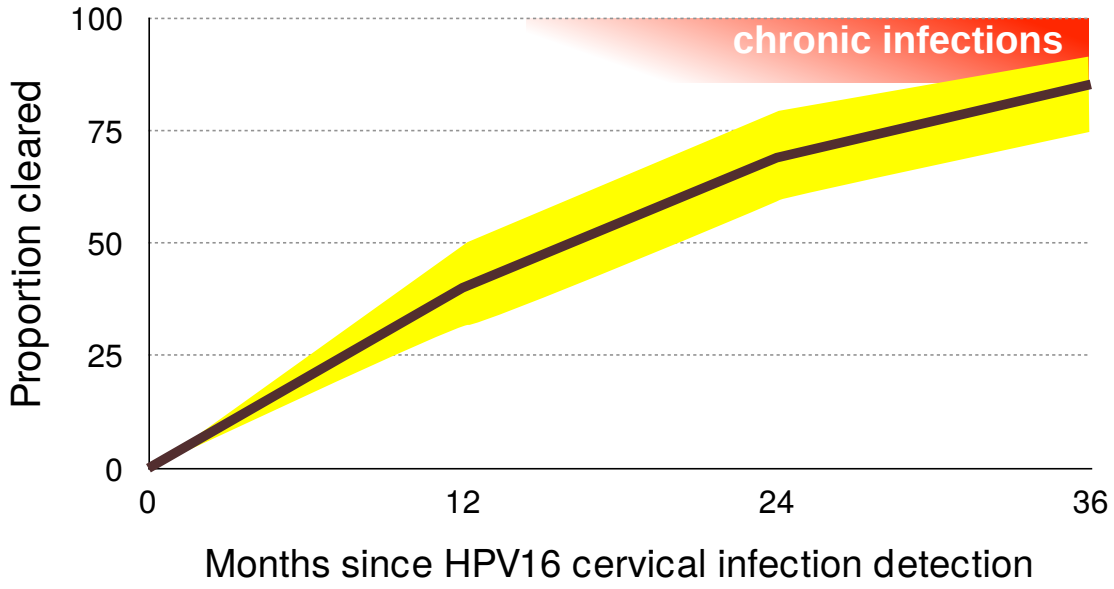

Figure 2. Proportion of HPV16 infections cleared 12, 24 and 36 months after the first positive visit. 95% confidence intervals are shown in yellow. Acute infections result in clearance or chronic infections, shown in red (note that chronic infections may clear as well). Data on clearance proportions originate from Table 4 in [18] and was obtained on 895 women aged 16 to 23 in the United States of America.

The placebo arms of vaccine trials have been instrumental in increasing our understanding of the epidemiology of these infections by following thousands of young adults over time [e.g. 19–21]. The interest of these studies was to detect viral persistence of anogenital infections, and the typical interval between two visits was chosen around 6 months. In comparison, few studies have used denser sampling. In one of these [22], the sampling was extremely dense (twice per week) but this was at the expense of the duration of the follow-up (16 weeks) and of the accuracy of HPV detection (infections with low virion productivity might not be detected when using vaginal swabs). The results did show the importance of transient infections as well as the effect of the vaginal microbiota on HPV detection [23].

Two other studies sampled women every 3 months. One of these followed unvaccinated girls aged 15-16 years in Tanzania and found that the median time from reported sexual debut to first HPV infection was 5 months, while infection duration was 6 months [24]. Another study followed 150 adolescent women (aged 14 to 17) visiting primary care clinics for a median of 6 years [25]. Their main results was that 21% of HPV type-specific infections consisted of re-detection after apparent clearance (2 negative visits). However, women were infected by many HPV types, which could interfere with the detection of one of the types. More generally, the population studied exhibited high rates of sexually transmitted infections (71.2% for chlamydia and 49.3% for gonorrhea) and a mean of 10.6 sexual partners during the study. In spite of their limitations, these two studies suggest that the little we know about the duration and the actual clearance of acute infections could be challenged by studies with deeper resolution.

The immunology of clearance

Why some anogenital infections persist and progress to cancer remains largely unresolved (Box 1). We know that HPV infections concur with a local anti-inflammatory environment, and that although the adaptive immune response is very efficient at clearing the infection, its activation is variable and

sometimes insufficient to prevent future re-infections [20,26,27]. Recent research into the role of innate effectors (e.g. natural killer T cells, NKT) and Th-17 responses (e.g. $\delta\gamma$ T cells) in cancerous lesions has provided new insights for chronic infections [28], but the implications for acute infection clearance is not obvious.

Overall, we know that some cofactors such as HPV genotype, age of sexual debut, or coinfections, have an effect on clearance time [29]. However our mechanistic understanding is still lagging behind. In the next section, we show that understanding acute infections would have tangible public health implications.

Box 1: Open questions about HPV acute infections

- Do some innate immunity evasion mechanisms lead to delayed clearance? [28]
- How common is immunological tolerance to HPVs?
- What role, if any, do natural B-cell responses play in clearance?
- Are there type-specific differences in immunological clearance mechanisms? (i.e. are some mechanisms more effective against different HPV genotypes? or even across target cellular types, tissue structure or anatomical sites?)
- Are there long term effects after HPV infection clearance (immunological scarring)?
- How do HPV infections affect male and female fertility?
- How are do HPV infection kinetics affect transmission? [30]
- What is the role of the microbiota in HPV infection clearance, persistence and progression to cancer? [23,31]

Open challenges

Host cells and chronification

The mammalian skin is a complex environment with many different cell types, intricate tri-dimensional structure and strongly regulated cell differentiation programs. Not surprisingly, different epithelial cells are differentially targeted by different HPVs [32]. Cells in the hair follicle seem to act as a reservoir for chronic infection by cutaneous HPVs, while in stratified mucosal epithelia the viral infection can become chronic in the basal cell layer.

Many squamous cervical carcinomas associated to HPVs are now thought to arise from a discrete population of cuboidal epithelial cells located in the transformation zone between the endo- and ectocervix [33]. The absence of such transformation zones in the vagina, vulva or penis might explain why the cervix has a much higher burden of infection, higher incidence and younger age-at-diagnosis for cancers compared to other anatomical locations [32]. Cervical squamous and glandular cancers are associated with different HPVs and closely related viral variants, possibly also modulated by the host genetic background [34]. These complex interactions between viral genotype, host genotype, cellular phenotype and environment highlight the importance and the need to better understand the within-host ecology of the virus.

A related issue that continues to be debated is the role of latency in HPVs chronic infections. We know that some acute HPV infections originate from viral reactivation rather than from a new infection [14,16]. However, we still largely ignore how common this phenomenon could be. While certain herpesviruses are the paradigm of latency with their particular viral gene expression patterns, this does not seem to be the case for papillomavirus latent infections. Most of the evidence on latency in papillomaviruses originates from animal models, and latency is often defined as persistent infection in the absence of lesion [35,36]. Understood like this, latency might originate from the incomplete elimination of the virus during an acute infection. However, it has also been suggested that some infections could directly become latent, without going through an acute phase [16]. In addition to molecular virology investigations into latency mechanisms, studies that combine a detailed initial follow-up (to demonstrate clearance), a long follow-up (to demonstrate chronic infection) and

sequencing (to demonstrate that the virus causing the acute infection persisted) will provide us with a definitive answer regarding latency. HPVs could prove to be a particularly promising model to better understand why some infections may become chronic in general [13].

Immunotherapies

Since vaccination will not be widespread for many years [37], the development of treatments remains urgent. Understanding the mechanisms of HPV clearance could lead to novel applications. The most promising are the development of immunotherapies, which consist in treating a disease by stimulating or suppressing the immune system.

Currently, the bulk of clinical and animal models research into HPV immunotherapies is, understandably, focused on cervical cancer treatment [38]. One of the most successful examples to date is that of the therapeutic vaccine VGX-3100, a plasmid containing synthetic versions of *E6* and *E7* genes of HPV16 and HPV18, which showed efficiency in a controlled trial at improving the regression of high-grade lesions (CIN2/3) [39]. The iatrogenic exposure to viral oncoproteins triggers a strong cellular immune response against the infected cells that the natural infection is not able to initiate. An alternative approach is to reverse the anti-inflammatory microenvironment that the virus creates during infections, in order to alert the immune system and boost its functioning [reviewed in 40].

Practically, immunotherapies cannot be envisaged to treat all acute infections given their prevalence. However, knowledge on the acute stage of the infections could be transferable to fight the chronic stage. Furthermore, there could be more focused applications, for instance in the case of respiratory recurrent papillomatosis, a rare chronic condition that imposes a recurrent burden to the patients to control the benign clinical presentations of the disease, which can progress fatally to the lungs [41].

Fertility

The Zika epidemic has reminded us of the risk viral infections have on pregnancies [42]. Anogenital infections by HPVs deserve to be studied in this context because rare deleterious effects could translate into an important burden, given their high prevalence. There is a well-established link between cervical disease and pregnancy complications [43], but we are only beginning to understand the connection between clinically asymptomatic HPVs infections and complications such as pre-eclampsia, fetal growth restriction or pre-term delivery [44]. Asymptomatic cervical viral infections can also reduce barrier integrity [45] and viral DNA can be detected in the placentae [46].

In men, anogenital asymptomatic infections by mucosal and cutaneous HPVs are very common [21] and some are associated with penile cancer [1]. Surprisingly, HPV DNA can also be found in human semen [47] and although this viral DNA could originate from desquamating cells, data suggest the presence of HPVs DNA directly associated to sperm cells [48]. Evidence supporting a correlation between infection by HPVs in men and infertility is still controversial [49] and the exact mechanisms remain largely unknown [50].

Finally, *in utero* transmission of HPVs is rare [51] but there is a sound body of evidence describing transmission of HPVs during vaginal birth, as illustrated by the case of infantile respiratory recurrent papillomatosis caused by HPV6 or HPV11 [41].

Overall, the presence of mucosal HPVs in placenta and semen suggests non-classical tropisms, and viral life cycles in these particular cellular environments need to be elucidated. Clinical studies investigating acute anogenital infections in pregnant women (along the various stages of pregnancy) as well as in their partners are required to unravel how HPVs (independently of their oncogenic potential) may be either directly or indirectly increasing the risk of infertility, spontaneous abortions, pre-term labor, preeclampsia or other complications.

Vaccine escape

Effective vaccines with high coverage rates exert major selective pressures on pathogens [52]. The risk of an evolutionary response from the pathogen relies on its genetic diversity, and does not necessarily require *de novo* mutations if it is already diverse. Extant diversity in papillomaviruses is immense, with hundreds of viral lineages, which themselves harbor significant genetic variation [53].

While vaccines against HPVs are effective and safe, they may be leaving the door open for an evolutionary response [54]. This can occur if some vaccinated individuals are infected by HPV vaccine types (due to infection before vaccination, immunosuppression or failure to mount a sufficiently strong immune response). In the clinical trial for the nonavalent vaccine, the median number of infections by one of the targeted viruses was 3.6 for 1,000 person-years at risk (data from Table S5 in [55]). If millions are vaccinated, this represents thousands of infections that would be eventually cleared and not result in malignant disease. Nevertheless, such acute infections may last long enough to allow specific viral lineages (either pre-existing or *de novo* generated viral variants) to be differentially transmitted, thus paving the way for viral adaptation to this special environment. HPV vaccines can also generate off-target activity and offer protection against closely related viruses not targeted by the vaccine formulation [56,57]. Yet viral diversity remains so large that in the few regions where vaccination has reduced the prevalence of vaccine-targeted types, most other HPVs continue to circulate [2,58]. Overall, vaccination against HPVs will undoubtedly have a strong and highly desirable impact in disease prevention, but it will create novel host environments to which the viruses may adapt.

Evaluating the potential for HPV evolution, requires genomic data of intra- and inter-individual viral populations collected through time. Since HPVs are double-stranded DNA viruses replicated by host polymerases, mutation rates are expected to be low. Nevertheless, we know little about polymerase fidelity in somatic cells, both in general and during a viral infections. Investigation of long follow-up data sets with short sampling intervals are lacking, especially since evolutionary rates may exhibit periods of rapid increase followed by long periods of stasis, as demonstrated for Influenza A virus [59]. Further, in the case of HPVs, displaying acute, chronic and latent stages of the infection, the evolutionary dynamics may strongly depend on the presence of a latent phase and on the epidemiological transmission patterns, as described for Varicella-zoster virus [60]. For HPVs, focusing on the acute infection could therefore yield novel insights since this is where the viral life cycle is most productive.

Scars that matter long after clearance?

Even when viral clearance does occur, recent work shows that acute infections can impair the immune system causing chronic inflammation or 'immunological scaring' [61]. Certain oncoviruses are believed to leave behind damage in their host cells, which can lead to cancer several years later [62]. For instance, acute infections can induce modifications in the cellular (epi)genome, creating the stage for pre-cancerous lesions [62,63]. Although an old hypothesis, the 'hit-and-run' effects of acute infections are poorly understood for bacterial or viral infections, and exploring how HPVs may cause this kind of damage remains an important research direction [64].

Quite speculatively, such a long-term impact could concern anogenital but also infections at cutaneous sites. Indeed, virtually all humans become infected by very diverse cutaneous HPVs, chiefly beta- and gammapapillomaviruses, which can act as cofactors for the risk of developing non-melanoma skin cancers in certain human populations [11]. Since HPVs generally cause very prevalent, benign infections, and because HPVs interact extensively with the immune system, they could be an ideal model for studying 'under-the-radar' viral infections and their potential side-effects on immune functioning.

Conclusions

Better understanding acute infections by HPVs is urgent because of their enormous fundamental and public health implications (Figure 3). As with most infectious diseases, it is important to remain humble and to accept that eradication is unlikely. HPVs are so diverse that vaccines will likely never remove them from our virome. In fact, this might not be desirable since HPVs might be occupying a niche, which, if vacant, could be filled by more virulent pathogens. This is why the development of vaccines should not stop us from improving our characterization of HPVs and to shift from an eradication to control perspective.

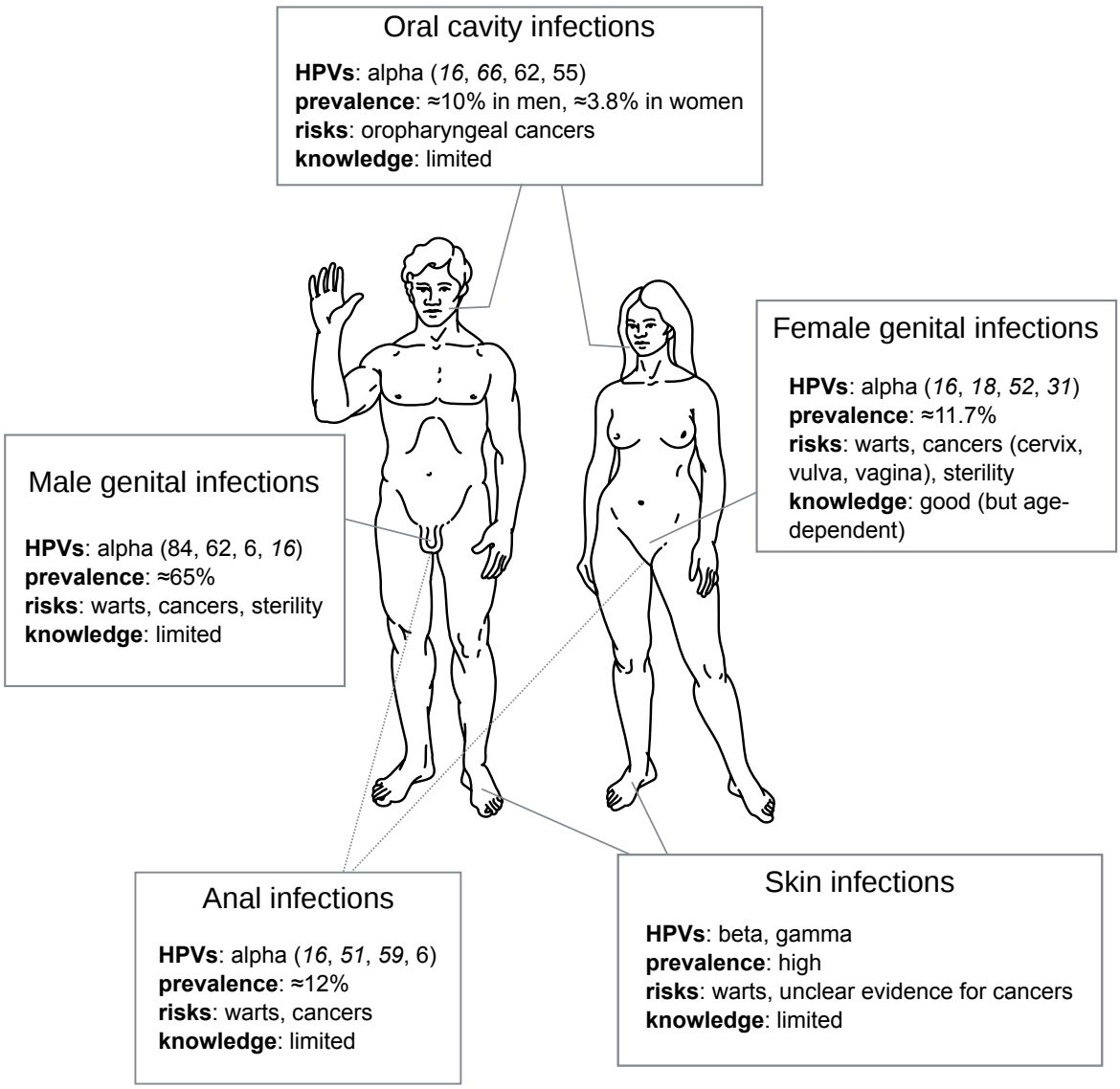

Figure 3. Challenges of HPV acute infections per anatomical location. For each location, HPV types are ordered per prevalence and high risk types are in italic.

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Abbreviations

The following abbreviations are used in this manuscript:

HPVs	Human Papillomaviruses
LR	Low Risk
HR	High Risk

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