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# The underestimated importance of acute infections by human papillomaviruses

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Approx. 2950 words in the main text, 40 references, 1 box and 3 figures.

**Key points (40 words or less)**

· Most HPV infections are acute and yet we know little about them compared to chronic infections.

· Acute HPV infections might affect fertility or cause long-term detrimental effects.

· Studying acute infections may help prevent and treat chronic infections and cancers, e.g. via immunotherapies.

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## Abstract

For human papillomaviruses (HPVs) and many other oncoviruses, there is a striking gap between our detailed understanding of chronic infections and our limited data on acute infections. We argue that studying the biology of HPV acute infections is necessary and timely. Disentangling early interactions will help explain why some infections become chronic or latent. A better description of immune effectors and pro-inflammatory pathways during the initial stages of infections can also lead to novel treatments, particularly immunotherapies. Furthermore, cervical cancer screening and vaccines impose novel iatrogenic pressures on HPVs, implying that monitoring viral epidemiological diversity and anticipating any evolutionary responses remain essential. Finally, given HPVs are ubiquitous worldwide, reports on the association between HPV acute infections and fertility deserve further investigations. Overall, the extent to which these 'benign' infections are virulent largely remains an open challenge.

136 words

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The most oncogenic viruses to humans are a group of Human papillomaviruses (HPVs). HPV-induced cancers typically occur after several years of HPVs infection (Figure 1). The importance of viral persistence in the natural history of the disease has driven most of the research to focus on chronic infections and to relatively neglect acute infections. Here, we first present recent progresses in the fight against HPVs before summarizing the current understanding about acute HPV 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 HPVs infections.

## State of the fight against HPVs

In 2012, chronic infections by HPVs were responsible for 30% of the 2.2 million new cancer cases attributable to infections [1]. As for other pathogens, we can use against infection-driven cancers the arsenal developed to fight 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 cervical and anal cancers, of a fraction of other anogenital cancers, and of a growing proportion of oropharyngeal cancers [1]. Contagion can be prevented by the use of safe and effective vaccines targeting the most oncogenic HPVs along with 'low risk' HPVs causing anogenital warts [2]. 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 [3].

Unfortunately, in spite of these successful programs, HPVs will continue to infect millions of people in the foreseeable future, thereby causing significant morbidity and mortality worldwide [4]. Indeed, vaccine coverage varies widely both within and between countries [2], as

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does access to screening programs. 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 on the rise. Finally, decades of successful fundamental research have focused on cervical cancer but we still know too little about the implication of HPVs in anal , oropharyngeal or even skin cancers which are all on the rise, albeit in different populations [1]. It therefore remains vital to unravel the biology and epidemiology of HPV infections.

# What we know about HPV acute infections

## A definition challenge

HPVs infections that are not chronic have been referred to as acute, non-persistent incident, resolving, transient, or clearing infections. Here, we follow Virgin *et al.* [5] and 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 [5]. In the case of HPVs the frontier between chronic and acute infections is 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 gamma-papillomaviruses 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 alpha-papillomaviruses infecting mucosa, a chronic infection can be achieved due to incomplete virus

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elimination after acute infection and the onset of immune evasion strategies. Alternatively, 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 [for more details on HPV chronic infections, see 6, 7].

## Most HPV infections are acute

We usually have better knowledge of the acute, clinically prominent phases of viral infections, rather than of the asymptomatic chronic ones [5]. Research on oncogenic HPVs stands out because its main goals are to unravel epidemiological links between chronic infection and disease, and to elucidate the immediate causes of virus-induced cell transformation. Yet, most novel HPV infections are transient and clear naturally, so that chronic infections represent a minority. For infections in the female genital tract, HPV16 is the most persistent type: after 12 months 40% of the infections have cleared or been treated because a (pre)neoplastic lesion was diagnosed (Figure 2). This proportion reaches 85% by 36 months [8]. For HPV6, which is a 'low risk' type, these numbers are 66% and 98% respectively.

Most acute HPV infections are clinically asymptomatic or with limited cytopathic effect. Therefore, 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. 9, 10]. 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. This lack in data with a better sampling density explains why we know so little about the kinetics of these infections that last on average 8 to 18 months [8]. In fact, the little we know could be challenged by studies with higher resolution. For instance, results on unvaccinated girls aged 15-16

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years in Tanzania followed every three months found that the median time from reported sexual debut to first HPV infection was 5 months, while infection duration was 6 months [11]. Incidentally, 46.1% of the 76 girls who reported first sex during follow-up were positive for HPV DNA before the reported date of first sex, suggesting a role for non-sexual transmission or for transmission through non-penetrative sex, or alternatively an inappropriate reporting.

## The immunology of clearance

Why the majority of anogenital infections by HPVs are cleared and do not progress to cancer remains largely unresolved (Box 1). We know that HPV infections concur with a local anti-inflammatory environment, and that the efficacy of the adaptive immune response for resolving an ongoing infection and preventing future re-infections is limited (for a recent review on HPVs and immunology see e.g. [12]). 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 [13], 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 [14]. However our mechanistic understanding is still lagging behind. In the next section, we show that understanding acute infections would have tangible public health implications.

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# The challenges of HPV acute infections

## Genital warts

Infections causing cutaneous or genital warts are the paradigm of acute infections by HPVs. Warts (i.e. tumours) involve modified tissue structure, cellular damage and intense virion production [6]. However, several aspects of the warts biology deserve a better understanding. This is illustrated by 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 [15]. From a purely economical perspective, the total health care costs linked to treating genital warts should not be overlooked since it can exceed that of treating HPV-induced cancers [16], despite the obvious differences in severity and indirect impact of both diseases.

## Immunotherapies

Perhaps the most important practical application derived from understanding the mechanisms leading to HPV clearance would be 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 [17]. 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) [18]. The iatrogenic exposure to viral oncoproteins triggers a cellular immune response against the infected cells that the natural

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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 19]. Searching for effective adjuvants to use as stimulants of the innate immune response will progress faster with greater details into how HPVs create immunosuppressed environments and what roles effectors such as natural killer T cells or natural killer cells perform. Since vaccination will not be widespread for many years, development of treatments for acute infections and pre-cancerous lesions should remain a priority.

## Fertility

The Zika epidemic has reminded us of the risk viral infections have on pregnancies [20]. 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, 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 [21]. In terms of mechanisms, it was demonstrated using a murine model that viral infection of the cervix during pregnancy reduces barrier integrity thus leading to bacterial infections of the uterus, which are known to have adverse effects on fetuses [22]. In addition, viral DNA and even viral proteins can be detected in a significant number of placentae in pregnancies with complications, although the significance of such findings remains unknown [23].

In men, anogenital asymptomatic infections by mucosal and cutaneous HPVs are very common [10] and some are associated with penile cancer [1]. But the more stunning findings sug-

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gest that HPV DNA can also be found in human semen [24] and although a fraction of this viral DNA could originate from desquamating cells, data suggest the presence of HPVs DNA directly associated to sperm cells [25]. Evidence supporting a correlation between infection by HPVs in men and infertility is accumulating, although the exact mechanisms remain largely unknown. These could be linked to increased sperm disfunctionality or to an increase in the anti-sperm immune activity [for a review, see 26].

Finally, it appears that *in utero* transmission of HPVs is extremely rare [27]. There is, however, a sound body of evidence describing the transmission of HPVs during vaginal birth, as illustrated by the case of respiratory recurrent papillomatosis caused by HPV6 or HPV11 [15].

Overall, the presence of mucosal HPVs in the placenta and semen suggests non-classical tropisms, and viral life cycle in these cellular environments needs to be elucidated. Clinical studies investigating acute anogenital infections in pregnant women (along the various stages of pregnancy) and 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.

## Virus evolution and vaccination

Effective vaccines implemented with a high coverage rate exert a major selective pressure on pathogens, as reported for Hepatitis B virus, *Bordetella pertussis* or *Streptococcus pneumoniae* [28]. The risk of an evolutionary response from the pathogen's side relies on its genetic diversity. Importantly, this does not necessarily require *de novo* mutations if the pathogen population is already diverse to begin with. In the case of HPVs, the existing diversity is immense, with

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hundreds of HPV viral types, which themselves harbor significant genetic variation [29].

While vaccines against HPVs are extremely effective and safe, they may be leaving the door open for an evolutionary response from the targeted viral populations [30]. Even when strictly adhering to the vaccination protocol, clinical trials revealed that some vaccinated women may still be infected by the HPVs targeted by the vaccine. For instance, in the Per-Protocol Efficacy Population, the median number of infections by at least one of four targeted viruses HPV6/11/16/18 and lasting more than six months was 3.6 for 1,000 person-years at risk for the nonavalent vaccine and 5.0 for the quadrivalent vaccine (data from Table S5 in [31]). In an ideal context of a high vaccine coverage achieved and millions of people vaccinated, this could still represent thousands of infections of vaccine-targeted HPVs occurring in vaccinated individuals. These infections will eventually be cleared and not result in malignant disease, so that the vaccine is efficacious. Nevertheless, such acute infections in immune individuals may last long enough to allow HPV variants (either pre-existing or appeared *de novo*) to be differentially transmitted, thus paving the way for viral adaptation to this special environment. 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.

The HPVs vaccines can also generate certain off-target activity and offer protection against closely related viruses non targeted by the vaccine formulation. But 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]. To evaluate the risk of viral evolution, we need genomic data of intra-individual and inter-individual viral populations collected through time. Since HPVs are double stranded DNA viruses replicated by the host polymerases, its mutation rate can be expected to be low, but we know little about polymerase fidelity in so-

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matic cells in general and during the course of a viral infection in particular. 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 [32]. Further, in the case of HPVs displaying acute, chronic and latent stages of the infection, the evolutionary dynamics may strongly depend on the varying viral transmission patterns, as described for Human herpes virus 3 (Varicella zoster virus) [33]. For HPVs, it would be particularly worth focusing on the acute infection since this is where the viral life cyle is most productive.

## 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. Regarding chronification, cells in the hair follicle seem to act as a reservoir for cutaneous HPVs, while in stratified mucosal epithelia the viral infection can become chronic in the basal cell layer.

Regarding the risk for malignisation, most HPVs-associated squamous cervical carcinomas may arise from a discrete population of cuboidal epithelial cells located in the transformation zone between the endo- and ectocervix [34]. The absence of such transformation zone in the vagina, vulva or penis could explain the much higher burden of HPVs infections, the higher incidence and the younger age at diagnosis for cervical cancers compared with other anatomical locations[1]. Additionally, cervical squamous and glandular cancers are associated with different HPVs and even with different very closely related viral variants, possibly also modulated by the host genetic background [35].

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This complex interactions between viral genotype, host genotype, cellular phenotype and environment highlight the importance and the need of better understanding the within-host ecology of the virus.

## 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' [36]. Certain oncoviruses are believed to leave behind such damage in their host cells, which can lead to cancer several years later [37]. For instance, acute infections can induce modifications in the cellular (epi)genome, creating the stage for pre-cancerous lesions [37]. Although this is 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 line [38].

This possible long-term impact of HPVs concerns anogenital but also infections at cutaneous sites. Indeed, virtually all humans in their life become infected by very diverse cutaneous HPVs, chiefly Beta- and Gamma-papillomaviruses, which can act as cofactors for the risk of developing non-melanoma skin cancers in certain human populations [39]. Because infections by HPVs are both very prevalent and generally benign, and because HPVs interact extensively with the immune system, they could represent an ideal model for studying 'under the radar' viral infections and their potential side effects on immune functioning.

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# Conclusions

Acute HPVs infections are the rule rather than the exception. Vaccine developments have put HPVs infections into the public spotlight and generated controversy among non-specialists, probably because it remains difficult to accept that potentially fatal viruses may be so prevalent and yet most often benign. A better understanding of HPV acute infections because of the enormous fundamental and public health implications they have (Figure 3).

In addition to the promise of identifying early markers of infection chronicity, understanding viral-immunity interactions can help design new treatments to boost natural immunity against novel infections. Such therapies may reduce the chances of an acute infection to chronify and to reach (pre)cancer stages. Detailed studies with long follow-up and short time intervals are further needed to precisely assess the role acute HPV infections could have on fertility or on the long-term 'immune scars' these infections may leave behind.

As for most infectious diseases, it is important to remain humble and to accept that eradication is unlikely. HPVs are so diverse that vaccines will 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 shifting from an eradication to control perspective.

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# Box 1: Open questions about HPV acute infections

- Do some innate immunity evasion mechanisms lead to delayed clearance? [13]
- 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?
- What is the role of the microbiota in HPV infection clearance, persistence and progression to cancer? [40]

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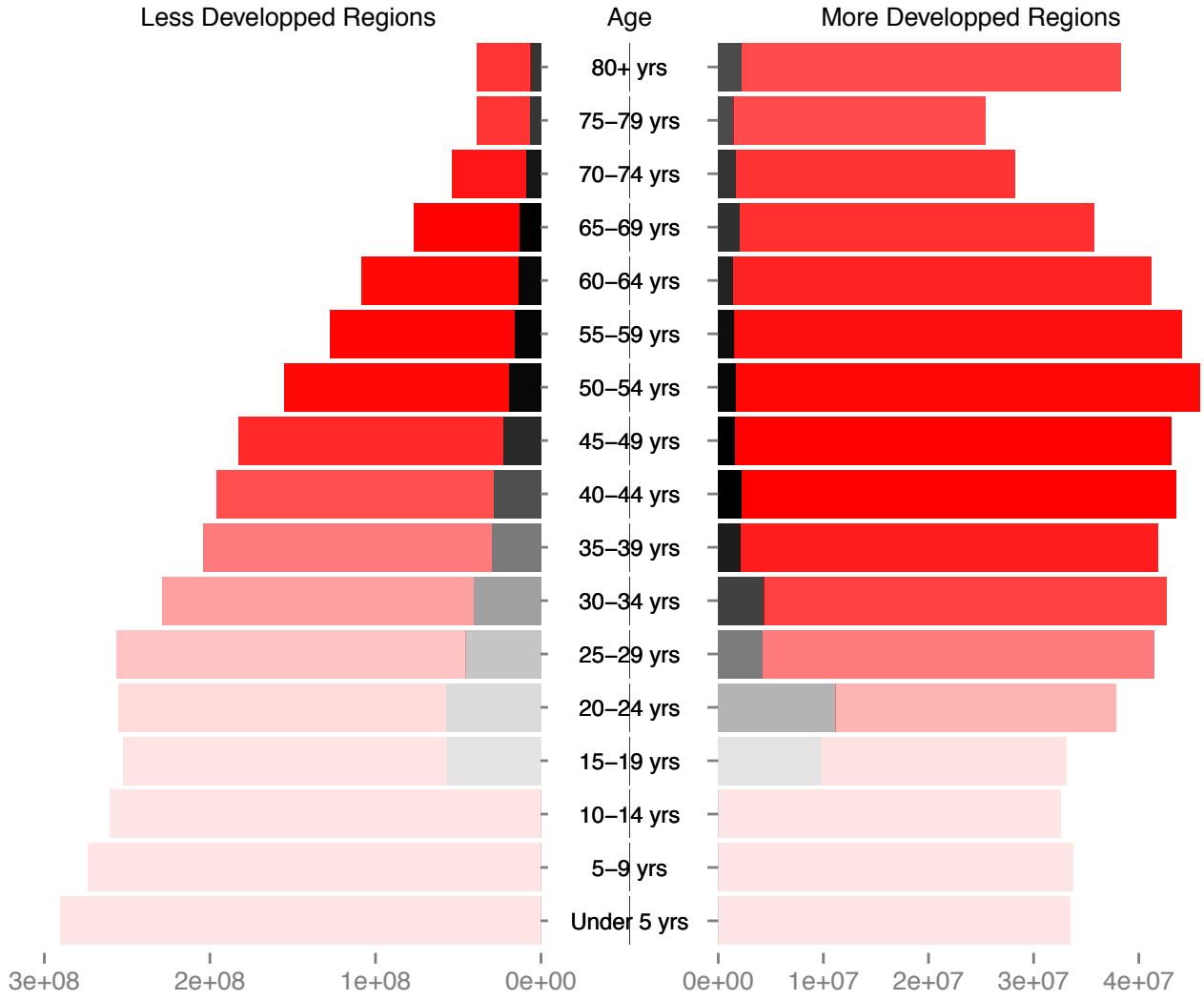


Figure 1: **Number of women with asymptomatic cervical HPV infections (black bars) as a function of age class (red bars) and of country development status.** Bar color intensity reflects the prevalence of cervical cancer in the corresponding age class (and hence the focus of current research). Data should 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/). 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).

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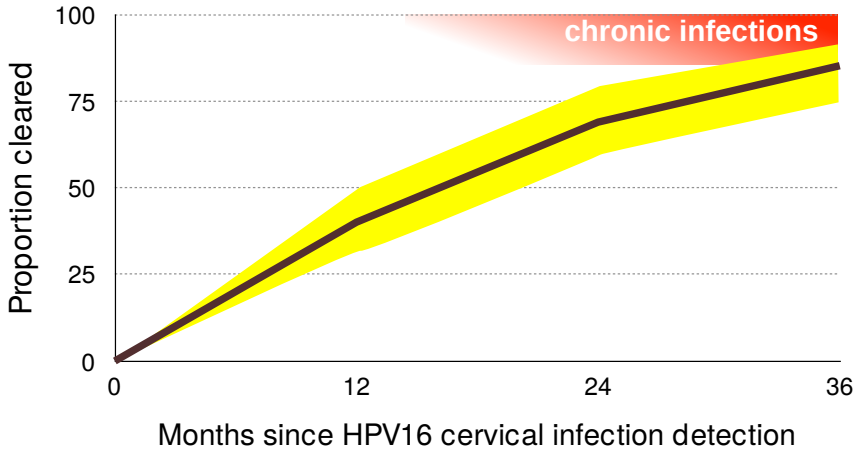


Figure 2: **Proportion of HPV16 infections cleared 12, 24 and 36 months after the first positive visit.** 95% confidence intervals are shown in yellow. Acute infections result in clearance or chronic infections, shown in red (note that chronic infections may clear as well). Data on clearance proportions originate from Table 4 in [8] and was obtained on 895 women aged 16 to 23 in the United States of America.

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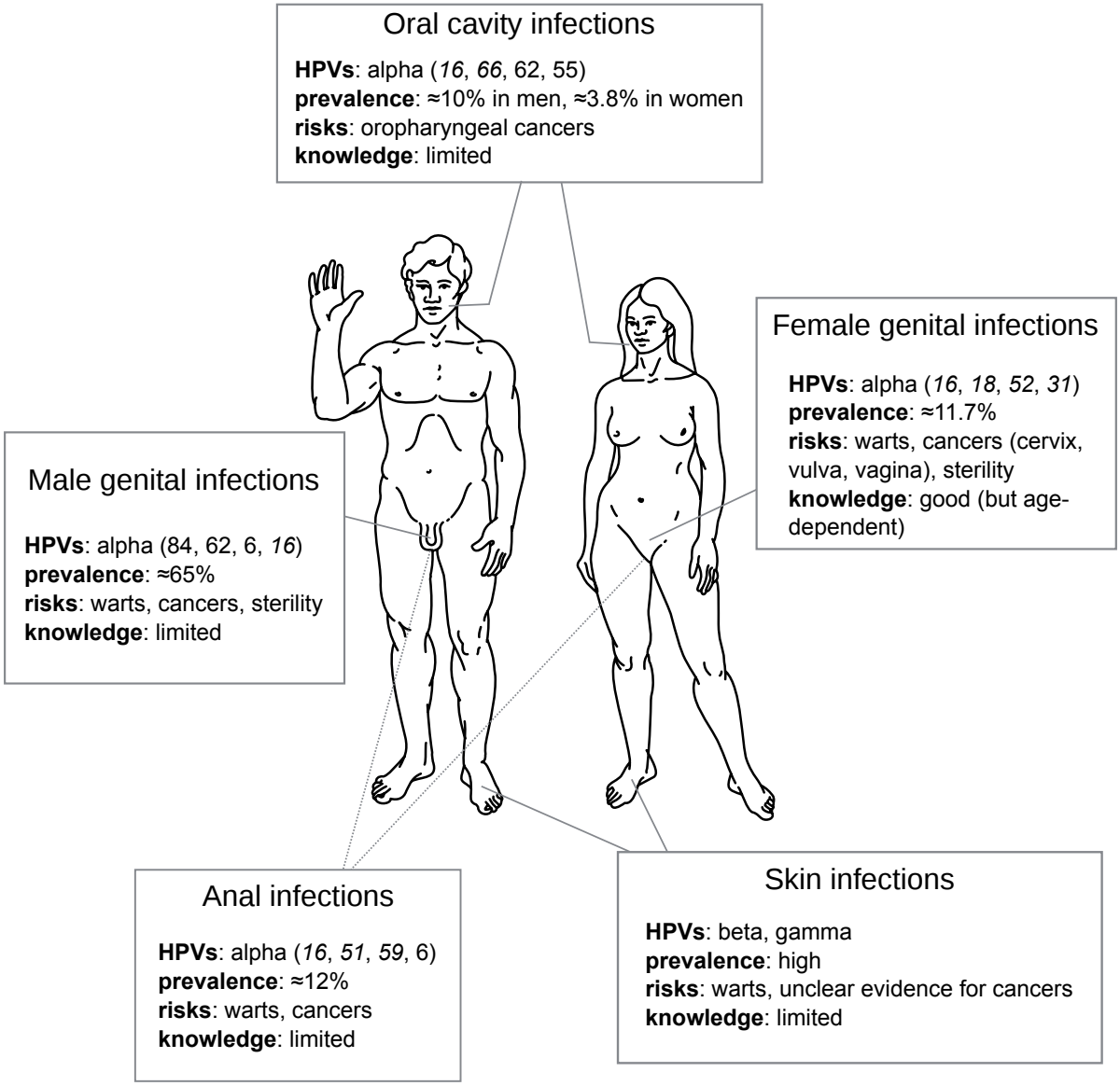


Figure 3: **Challenges of HPV acute infections per anatomical location.** For each location, HPV types are ordered per prevalence and high risk types are in italic.