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Abstract
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Reference

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Management of resistant mucocutaneous herpes simplex infections in AIDS patients: a clinical and virological challenge

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Background
The use of highly active antiretroviral therapy (HAART) has been associated with a marked decrease in the prevalence of opportunistic infections in HIV-infected patients. However, chronic mucocutaneous herpes simplex virus (HSV) infection remains a difficult clinical challenge.

Objective
The aim of the study was to optimize the diagnosis and follow-up of chronic HSV-2 infection in HIV-infected patients and to correlate clinical data with CD4 cell count, in vitro HSV virological resistance and histology.

Methods
A retrospective case series was collected from a specialist out-patient clinic providing consultations to patients with infectious skin diseases. Clinical, biological, virological and histological data were analysed.

Results
Seven HIV-infected patients with genital and perianal herpes simplex infection were followed over 10 years. Ulcerative and pseudo-tumoral forms were observed. Lesions occurred at various stages of immune suppression (CD4 counts from 1 to 449 cells/μL). Clinical resistance to conventional anti-herpetic drugs was correlated with the in vitro resistance of HSV in 70% of cases.

Conclusions
Chronic mucocutaneous HSV infection in AIDS patients remains a rare but regularly observed infection in very immunosuppressed patients or those with unstable immunity during HAART. Virological results obtained from mucocutaneous samples were in most cases found to be correlated with clinical evolution and should therefore be used in making decisions on treatment. Despite efficient antiviral therapy, mucocutaneous healing is slow in the majority of cases.

Keywords: chronic herpes, cutaneous ulceration, HIV, pseudotumoral, resistance

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Introduction
Mucocutaneous herpes simplex virus (HSV) infections are very common in HIV-infected patients. They are usually recurrent and heal spontaneously or under acyclovir (ACV) treatment within a few days [1]. Nevertheless, some of these recurrent infections become chronic. According to the Centers for Disease Control and Prevention (CDC) definition of AIDS-related illnesses, chronic herpes is a herpetic infection lasting for more than 4 weeks that does not resolve with first-line anti-herpetic treatment.

In the highly active antiretroviral therapy (HAART) era, it was expected that chronic herpes would no longer exist, but experience suggests that HSV infection does not require severe immunosuppression to persist and may even worsen under HAART in patients experiencing the so-called immune reconstitution syndrome [2].

The fact that there are few reported cases of chronic and resistant mucocutaneous herpes infections suggests that this form is uncommon. Systematic correlation studies of clinical presentation, evolution, HSV in vitro sensitivity to anti-herpetic drugs and histopathology have not yet been performed.

We systematically analysed several cases of chronic mucocutaneous herpes simplex type 2 infection associated with AIDS and examined correlations among clinical type,
clinical evolution, histopathology, HSV detection and HSV sensitivity to anti-herpetic drugs.

**Methods**

Cases were analysed retrospectively. All patients with chronic HSV infection associated with HIV infection seen between 1997 and 2007 in our specialist skin and HIV clinic were included in the study. Six of seven patients were participating in the Swiss HIV Cohort Study requiring their informed consent for prospective and retrospective studies.

To be included in the analysis, patients had to fulfill the following criteria.

1. A clinical diagnosis of chronic herpes was made according to the CDC definition and resistance to at least 4 weeks of appropriate valacyclovir (valACV) treatment (500 mg twice a day) was observed clinically.
2. HSV-2 was detected in mucocutaneous smears at the time of clinical diagnosis: chronic lesions (>4 weeks) or an incomplete clinical response to appropriate ACV or valACV oral treatment was noted. Results of previous cultures for HSV-2 obtained from similar lesions were disregarded.

For detection of HSV, two different cell types were used for culture, namely human fibroblasts cultivated in Dulbecco’s modified Eagle’s minimal essential medium (DMEM; containing 4.5 g/L glucose, 2 mM L-glutamine, 25 mM HEPES) and A549 human lung carcinoma cells [CCL185; American Type Culture Collection (ATCC), Rockville, MD, USA] in Hams F-12 medium with 2 mM HEPES, without glutamine (Amimed reference number 1-14F04-I, Bioconcept, Allschwill, Switzerland). Both culture media contained 10% fetal bovine serum as well as penicillin, streptomycin and gentamycin. HSV from positive cultures was tested for its sensitivity to ACV, foscarnet and cidofovir (CFV) using a plaque reduction assay.

Cutaneous biopsies were stained with haematoxylin-eosin and specific stains such as Ziehl–Nielsen, Gram acid Schiff. Immunohistochemistry with specific HSV antibodies (rabbit anti-HSV types 1 and 2; Dako A/S, Glostrup, Denmark) was carried out in four patients. For detection of cytomegalovirus (CMV), immunostaining with anti-human CMV immediate early antigen antibodies (Argène Biosoft, Verniolle, France) was used.

**Results**

Between 1997 and 2007, seven patients were regularly followed and provided enough clinical and biological data for analysis. Table 1 summarizes their characteristics and follow-up data.
Five patients were of African origin, one was Asian and one was Caucasian. Three were women. The mean age at diagnosis was 41 years. All the patients had recurrent suspected or confirmed genital or perianal herpes before the diagnosis of chronic herpes and were repeatedly treated with ACV, famciclovir (FCV) or valACV. On average, chronic herpes was diagnosed approximately 6.5 years after a confirmed positive HIV test, with a range from 3 months to 14 years. The mean CD4 count at diagnosis was 214 cells/µL (range 1–449 cells/µL). Three patients were not on HAART when chronic herpes was diagnosed: patient 1 was not on HAART because she had HIV2 infection, and she died a few months after the diagnosis of chronic herpes from a nonherpetic complication; patient 3 refused HAART despite a long, painful evolution of herpes infection; and patient 7 initiated HAART and foscarnet (PFA) treatment soon after the herpes diagnosis and achieved complete healing without any recurrence. HAART was ineffective because of multiple resistance in patient 2, and poor compliance was noted for patient 5. Finally, patients 4 and 6, who had the hypertrophic form of herpes, started HAART 1 month before developing chronic herpes. Patient 4, who discontinued HAART several times (and then switched to a different HAART regimen), presented a hypertrophic chronic herpetic relapse 2 to 3 weeks after each reinitiation of HAART.

Clinical presentation was ulcerations in five patients (Fig. 1; patient 2 is shown) and tumour-like lesions in two patients (Figs 2 and 3). Chronic pain was always associated with the lesions, but its intensity varied from slight for the hypertrophic forms to unbearable with functional disability for the ulcerated forms.

Healing of the lesions under different successive antiviral treatments took between 2 months and 5 years after diagnosis of chronic herpes. Three patients were in poor general condition and suffered from malnutrition and anaemia.

Treatments for HSV infection consisted of oral and intravenous (i.v.) ACV, oral FCV, topical and i.v. PFA, topical and i.v. CFV and thalidomide. Topical imiquimod was used in three patients (patients 2, 3 and 5) but was not well tolerated (burning sensation) and ineffective.

The histological features of the four biopsies taken are summarized in Table 1. Immunostaining for HSV-1 and HSV-2 was negative in all patients except patient 4, for whom coexistence of the two viruses was observed in giant epithelial cells.

Virus cultures for HSV-2 were positive in all patients. Sensitivity testing using a plaque reduction assay showed that HSV-2 was ACV-resistant in four patients, PFA-resistant in two patients and CFV-resistant in three patients (Table 1).

Although some patients (patients 1, 2 and 4) were clinically resistant to ACV, cultures at the time of chronic HSV-2 infection did not show in vitro resistance to this drug.

**Discussion**

Our study illustrates the clinical, virological and histological features of chronic mucocutaneous HSV-2 infection in HIV-positive patients.

Two types of clinical presentation were found: ulcerative and pseudo-tumoral. The ulcerative form has previously been reported in both heavily and mildly immunosuppressed patients, both on HAART and not on HAART [2].
Pseudo-tumoral lesions have been already described [3–7], and the reported cases describing either hypertrophic or granulomatous forms of herpes may be grouped in a same entity as pseudo-tumoral lesions. We took into account that the probable nosological variation used as pseudo-tumoral, hypertrophic, granulomatous forms of HSV-2 represent the same entity. As the clinical presentation of herpes can be misleading, the overall incidence of chronic herpes may be underestimated and lead to a delay in the initiation of appropriate treatment. Histology can be disappointing in some cases because it is nonspecific and of little diagnostic value. Nevertheless, it allows one to rule out other opportunistic infections or tumours such as squamous cell carcinoma [6,7]. Only one patient was positive for HSV using immunohistochemistry. However, immunostaining for HSV does not distinguish between HSV-1 and HSV-2. The control of HSV infection depends on individual immunity. In immunosuppressed HIV-negative individuals, chronic herpes is also observed [8–10]. The host hypothesis may help to explain the occurrence of chronic herpes, its various clinical presentations and its response to antiviral therapies [11]. Despite a close follow-up for HIV control with HAART, the clinical response of HSV infection is long (several months in the majority of patients) and require a perfect HIV control.

A patient who had high viraemia (patient 3) and a patient known to have poor adherence to HAART (patient 5) had the longest healing times. The two cases of pseudo-tumoral presentation were in patients on HAART. Patient 4 (Fig. 2) had a history of multiple interruptions of HAART because of poor compliance and travelling. In 2 years he received three different antiretroviral regimens, which produced good virological and immunological responses (aviraemia and an increase in CD4 count from about 200 to 400 cells/µL), but he experienced a recurrence of inguinal pseudo-tumoral herpes 2 or 3 weeks after each new HAART initiation. Patient 6 (Fig. 3) developed a chronic, stable, tumour-like genital lesion following the initiation of HAART, and this lesion persisted for 2 years despite well-controlled HIV viraemia (under the laboratory detection limit at that time), but the CD4 count remained at approximately 150 cells/µL. Complete healing occurred after intravenous treatment with PFA. Patient 4 clearly met all the clinical and biological criteria for an immune restoration syndrome. Immune restoration syndromes usually occur in the first 3 months after HAART initiation and have previously been described for cutaneous herpes simplex and various other skin infections such as flare-ups of molluscum contagiosum, human papilloma virus warts and Kaposi sarcoma [2,12].

All the patients were tested for anti-herpetic drug resistance, and four of the seven patients showed in vitro resistance to ACV which correlated well with clinical resistance. Previous drug exposure has been found to be the main explanation for the development of resistance [13]. These patients had received repeated treatments and/or long durations of treatment with ACV-type drugs. Clinical resistance was partially counteracted using higher drug doses: valACV 3 g/day or famciclovir (FCV) 1.5 g/day for 2 or 3 weeks with renal function control. As several viral populations are known to coexist in such chronic lesions, the risk of selecting a resistant viral population is high, and the use of prolonged high dosages is not recommended. A switch to a drug with a different antiviral target, such as foscarnet (PFA), is recommended. Moreover, in our study, in vitro primary resistance was detected in patients never
Exposed to the tested drugs: patients 4 and 5 showed viral resistance to CFV and PFA, respectively. To our knowledge, no such primary resistance in a clinical sample has previously been reported. However, a strain profile of resistance obtained using a genotyping method is lacking in our series. The choice of anti-herpetic drugs thus requires careful clinical evaluation guided by virological tests: to summarize, when the lesion does not heal despite prolonged treatment with oral valACV or FCV (10–14 days) and/or the use of higher posology, i.v. ACV may be given as soon as the diagnosis is confirmed. We recommend the use of a second-line anti-herpetic drug only after laboratory confirmation of the diagnosis. This may require a simple smear and sometimes a mucous or cutaneous deep biopsy. HSV isolation is essential for drug sensitivity evaluation. When strains of ACV-resistant HSV are detected, i.v. PFA remains the drug of choice. Ten days of treatment with PFA is sufficient to heal a true ACV-resistant herpetic lesion. If the lesion does not heal, on the clinical side, the patient’s general condition and HIV evolution should be checked, and, on the laboratory side, PFA- and CFV-specific sensitivity testing should be carried out. CFV may be tried in the case of PFA resistance or intolerance. When choice is possible, CFV is more convenient to use than PFA. Topical ACV, PFA and CFV are not efficient and could theoretically induce viral resistance; we applied these treatments in well-defined cases and observed a transient improvement only in patient 1 [14]. The suppression by valACV or FCV is started as soon as the lesion is completely healed (see algorithm in Fig. 4 and Gilbert et al. [15]).

Viral detection using culture was paradoxically very poor in the majority of lesions, especially those of pseudo-tumoral form (a positive culture was obtained for one of three swabs over 4 months of follow-up in patient 4, and in one of eight swabs over 19 months in patient 6). One lesion of ulcerative form also displayed very poor viral shedding (one of 17 swabs produced a positive culture over 30 months in patient 5). This suggests that, in chronic herpes, HSV viral replication is not necessarily the driving force for the formation of lesions. The pathogenesis is not understood, but we believe that one live virus, or particles from dead virus, may induce sufficient epidermal or dermal reaction and cell death to create weak inflammation and an
ulcer that heals very slowly in an immunosuppressed individual. This hypothesis is supported by the histology showing poor inflammatory reactions in three patients associated with typical scarring and granulation tissue as seen in other chronic ulcers, for instance those of vascular origin. Molecular biology using polymerase chain reaction (PCR) on a superficial smear confirmed HSV infection in two patients (patients 5 and 6). Smear samples for genotyping by PCR were not obtained for the other patients because this test was not routinely used at that time in our laboratory for mucocutaneous samples. PCR was also performed for the four fixed-block biopsies after DNA extraction and gave negative results. Since 2009, our virology laboratory has used PCR for mucocutaneous superficial smear samples as this procedure has been proved to be very sensitive. Fresh biopsy samples for HSV detection by PCR were not obtained in our series. With the developing use of PCR to diagnose HSV infection, clinical, histological and virological evaluations should be required, and particularly in tissue biopsies.

A careful, systematic approach is needed for the global management of this chronic infection in AIDS patients. We suggest the following procedure:

1. Consider a diagnosis of HSV infection when an HIV-infected patient with a low CD4 cell count or with recovering immunity under HAART presents with genital or perianal persistent ulceration or granulomatous tumefaction.
2. Confirm clinical suspicions with laboratory tests: PCR and culture.
3. Inhibit the replication of HSV with anti-herpetic agents, chosen with the help of in vitro drug sensitivity tests if necessary.
4. Treat the HIV infection and promote the recovery of general immunity with HAART.
5. Promote mucocutaneous healing by management of the patient’s general condition: treat anaemia, address nutritional issues, and treat any acute internal diseases and bacterial wound superinfections (algorithm).

We would like to emphasize the importance of confirming the diagnosis, particularly when the patient is in the immune restoration phase and the lesion could be confused with a tumour [7]. Each step backwards in the healing process should raise the question of new HSV resistance to the drug and repeated smear samples should be obtained for culture and in vitro sensitivity testing should be carried out promptly to allow the treatment to be adapted. At the same time, all possible causes of increased HIV viraemia should be borne in mind. To ensure correct diagnosis in such cases, a multidisciplinary approach should be adopted: where local expertise and laboratory facilities are available, the diagnosis can be confirmed locally; where they are not available, photographs and samples can be sent off for a remote consultation. Physicians should be encouraged to obtain advice at an early stage in order that such patients with several comorbidities can be offered optimal treatment that provides the best chance of success. As cases of chronic herpes are not common even in the largest HIV centres, therapeutic prospective and controlled clinical studies have not been conducted. A new expert consensus on HSV and HIV coinfection would be welcome, 16 years after the first algorithms were proposed during the pre-HAART era [16]. In particular, such an updated consensus could integrate the influence of HAART and the immune restoration syndrome.

To conclude, chronic mucocutaneous HSV-2 infection in HIV-positive patients remains uncommon in the HAART era. We describe its two main clinical forms, ulcerative and pseudo-tumoral, and emphasize the importance of laboratory confirmation tests not only for diagnosis but also for treatment and follow-up using culture and in vitro HSV sensitivity testing. The long evolution and active viral replication of HSV-2 are linked to dysimmunity and the development of viral resistance to anti-herpetic drugs, and patients with HIV and HSV-2 coinfection therefore require careful and specialized management.

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