Is Western blot alone sufficient to confirm a reactive result of a fourth-generation HIV screening assay?

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Introduction

Despite the fact that today over 40 million individuals worldwide are living with HIV, fewer than 1,000 cases have been diagnosed in the first month of infection [1]. Due to clinical reasons, such as the lack of a specific and easily recognizable acute retroviral syndrome and the tendency of acute retroviral syndromes to mimic many common febrile illnesses, (including infectious mononucleosis, influenza, malaria, and rickettsial diseases), an HIV diagnosis is rarely considered at an initial patient encounter [2, 3]. The diagnostic challenge in acute HIV infection is made more difficult by the fact that even routine third-generation HIV antibody tests, which replaced the first- and second-generation HIV assays several years ago in Europe, usually remain negative for 10–15 days beyond the onset of acute retroviral symptoms. Fourth-generation EIAs, which detect both antibodies and p24 antigen, additionally reduce the diagnostic window for primary HIV-1 infection in comparison with third-generation antibody screening tests [4, 5].

According to the 2004 guidelines of the Federal Office for Public Health (HIV-Test concept 2004: New guidelines for Screening) the shortening of the diagnostic window after the introduction of more sensitive fourth-generation EIA tests and confirmation from the first sample should enable earlier detection of an HIV-infection [6]. In spite of this very important improvement in the HIV diagnostic concept in Switzerland acute HIV infections may still be missed.

Case report

In our case a 30-year-old male presented to us in April 2005, with a massive inflammatory skin condition on his face associated with raised nodules with a pustular head on an erythematous base and recidivous oral ulcers. Seborrhoeic dermatitis, bacterial folliculitis, syccosis barbae, eosinophilic folliculitis, prurigo nodularis, acne vulgares and disseminated cryptococcosis were considered as differential diagnoses. Although a variety of skin disorders and abnormalities of the oral cavity can occur throughout the course of HIV infection, an HIV test was not performed at that point because the patient reported that he had previously been tested (in September 2004) and found HIV negative in another practice setting.

The patient appeared again in June 2005 with recidivous skin disorders and oral ulcers. Laboratory data showed normal leukocyte (5,200 cells/µl), erythrocyte ($5{,}21\times10^{12}$ cells/l) and platelet (266×10^{9} cells/l) count and elevated C reactive protein (CRP, 60 mg/l). Based on these facts a differential diagnosis of HIV infection was made. HIV EIA fourth-generation screening tests performed in our laboratory gave reactive results (table 1). From the same sample, according to the HIV concept, HIV-1 infection was confirmed by Western blot in an external confirmatory laboratory. A second sample collected a few days later also gave a positive result so a mistake in sample collection was excluded.

In a case history investigation we realized that a serum sample from the same patient had been submitted to our laboratory in September 2004 by another physician. At that time the reason for clinical consultation was recidivating stomatitis aphtosa and elevated CRP value with normal leukocyte, erythrocyte and platelet counts. At that point the physician already suspected an HIV-infection and submitted the serum in September 2004 to our laboratory for HIV-screening. HIV fourth-generation antigen/antibody test (Combo, AxSYM, Abbott) gave a reactive result in our laboratory and we sent the sample to an external confirmatory laboratory. A second sample, taken one day later, also gave a reactive result. The confirmatory laboratory obtained a negative result by Western blot test and reported the following comment to us: "The Western blot can not confirm the questionable/reactive screening result. Most probably there is a non-specific screening test result, e.g. HLA antibodies, elevated IgG concentration, autoantibodies or many other test-related causes. An HIV infection is highly improbable. It is recommended the patient with this questionable screening test result be tested again in 2–6 months." Knowing that fourth-generation EIA measures both p24 antigen and antibodies and that the Western blot test can be false negative in an early phase of HIV infection, we recommended to the practitioner that an immediate PCR test and serology follow up in two weeks should be carried out. The practitioner decided to carry out only a serology follow up but the patient did not appear for the consultation.

In a retrospective PCR testing in June 2005, which was possible because we keep all patient samples frozen in our serum bank for at least one year, we found an HIV viral load of 384,000 copies of RNA per millilitre of plasma already present in the second patient sample, approximately nine months before the current evaluation. The results of all tests performed are presented in the table 1.

Discussion and conclusions

Many countries have recently placed significant emphasis on the identification of people with acute HIV infection [1, 7–9]. This is important for several reasons. Firstly, acute HIV provides a unique view of HIV transmission and pathogenesis, including early host-virus interactions and these require further studies. Secondly, prevention strategies directed at subjects with acute HIV infection could have significant impact. Thirdly, very early recognition may allow for HIV treatment that could alter the natural progression of disease [1]. The standard HIV antibody screening test (third-generation) has therefore been replaced by the more sensitive fourth-generation and new serological testing algorithms for recent HIV seroconversion have been introduced [7, 9]. Recently, some laboratories have also begun adding HIV nucleic acid amplification testing to HIV diagnostic testing algorithms so that acute (antibody-negative) HIV infections can be routinely detected within the first 1–3 weeks of exposure in about 50% of patients and thereafter up to 80 days in the remaining ones [8, 10].

In our case we have shown that, despite clinical suspicion, an early HIV infection can still be missed using Western blot as the sole

Table 1
HIV case history investigation.

Collection place	Collection date	HIV 4th generation Antigen/Antibody test Combo AxSYM, Abbott*	Immunoblot Inno-Lia HIV-Confirmation, Innogenetics	Cobas Amplicor HIV-1 Monitor Test Version 1.5, Roche Diagnostics
Practitioner A	September 23, 2004	4.33	no antibodies detectable	no material available
Practitioner A	September 24, 2004	4.32	not done	384,000 copies / mL**
Practitioner B	June 14, 2005	17.66	positive (p17, p24, p31, gp41, gp120)	33,100 copies / mL

* cut off 1.0
** retrospectively performed on June 14, 2005

confirmatory method in patients with an early HIV infection in whom only the HIV antigen is detectable and antibodies have not yet been produced [4, 8, 11]. In addition, comments about possible non-specific reaction with the suggestion of a control at a later time can be misunderstood and consequently mistakenly ignored by both the physician and the patient. Following this, the patient can disappear and reappear later in a different setting with all the complications of disease progression, late therapy, possible unrecognized transmission of a resistant virus and infection of the environment and contacts.

Specifically, reactive fourth-generation HIV screening tests should be confirmed properly in the first sample and all possible diagnostic tests need to be exploited at this point as the patient may later be lost to follow up. This case leads us to recommend that in patients with reactive HIV EIA fourth-generation, with or without clinical illness, a negative immunoblot result should be followed by nucleic acid amplification testing or a p24 antigen immunoassay with confirmatory ability using the neutralization principle. This would optimize early HIV diagnosis without a significant cost increase considering the benefits accruing in terms of opportunities for earlier treatment, source identification and introduction of preventive measures.

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