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SUMMARY

During a sero-survey for lymphotropic retroviruses, seven screening tests were performed for the three lymphotropic retroviruses of relevance in Djibouti, East Africa (HIV-1, HIV-2 and HTLV-1). Of the 82 subjects whose sera reacted in at least one retroviral screening assay, about one third could be followed, and their sera were re-examined after a 5-month interval, and then after an additional 3-month interval. Six selected individuals are reported here, whose retroviral serologies presented important and often unexplained changes over an eight-month period. The six cases summarize prototypic situations and present serological results in a style appropriate to stimulate thought on the significance and interpretation of lymphotropic viral serologies. Each case study is followed by a set of questions that formulate pertinent serological concepts.

INTRODUCTION

Infection by lymphotropic retroviruses can be documented by demonstrating specific viral antibodies in human serum. Results of serological assays tend to be objective, indicating either an evident infection, or the apparent absence of an infection. In a significant number of cases, however, results are much less definite, and test interpretation can become a genuine medical challenge. Correct interpretation of test outcomes is critical, nevertheless, since the diagnosis of a lymphotropic retroviral infection greatly and unequivocally influences the life of the person involved.

During a national sero-survey of lymphotropic retroviruses in high risk populations in the East African city of Djibouti, seven screening tests were performed for three lymphotropic retroviruses of relevance to human health in Africa: human immunodeficiency virus types 1 and 2 [(HIV-1 and HIV-2(1,2)] and human T cell lymphotropic virus type 1 [(HTLV-1(3)]. Of the 82 subjects whose sera reacted in at least one retroviral screening assay, about one third could be followed, and their sera were re-examined after a 5-month interval, and then after an additional 3- month interval.

The following report describes six selected individuals, whose retroviral serologies presented important changes over an eight-month period. The six cases summarize prototypic situations and present serological results in a style appropriate to stimulate thought on the significance and interpretation of lymphotropic viral serologies. Each case study is followed by a set of questions that formulate pertinent serological concepts.

MATERIALS AND METHODS

A total of 600 sera were collected in the city of Djibouti during a national HIV survey in June 1988 from individuals at high risk for acquiring HIV-1. All sera were screened for antibodies to the 3 lymphotropic retroviruses HIV-1, HIV-2, and HTLV-1. Screening of sera for HIV-1 was accomplished by 5 separate assays: the ABBOTT (North Chicago, IL) recombinant ELISA screening assays, The ELAVIA assay (Diagnostics Pasteur, France), The SERODIA gelatin particle agglutination test (Fujirebio, Inc, Tokyo, Japan), the RECOMBIGEN HIV Latex agglutination test (Cambridge Bioscience Corp., Worcester, MA) and the DUPONT HIV-CHEK (EI Dupont de Nemours, St. Paul, Minn.). HIV-2 infection was investigated by an ELISA screening assay (Genetic Systems, Seattle, WA.) and screening for HTLV-1 was accomplished through an agglutination assay (SERODIA, Fujirebio, Inc. Tokyo, Japan).

Each serum that gave a reactive results by a screening assay was retested, and the result was required to be reproducible before the serum was labelled positive. Each serum yielding a negative screening result by an assay, while being positive by confirmatory assays, was also retested to ensure that technical error did not occur. Each serum that was reactive in any of the 7 screening assays, even if not reproducible, was tested by western blot for antibody confirmation. HIV-1 western blot (DuPont, Biotech, Wilmington, DE.) positivity was defined by reactivities to at least p24, gp41 and gp120 or 160 gene products. Confirmation of HIV-2 infection by western blot (Diagnostics Pasteur, Paris, France) was achieved through demonstrating specific antibodies to envelop glycoproteins of HIV-2 (gp105). Western blot seropositivity for HTLV-1 (DuPont) was defined by the presence of antibody bands to the p24 and p19 kilodalton (kDa) *gag* viral proteins, or to the p19 protein in addition to antibodies to other relevant antigens, in particular antibodies to gp46. In addition, a neutralization ELISA (Abbott) to detect circulating HIV antigen in sera was performed on all samples.

Case reports were prepared from ten interviews and laboratory results of six individuals, whose sera exhibited initial reactivities that changed over time. Sequential serological test outcomes are presented for the six cases after a 5-month interval in November 1988, and after an additional 3-month interval in February 1989. During these follow-ups, the six subjects had a 10 ml venous blood sample drawn on anticoagulants. Mononuclear cells were separated and processed for phenotypic analysis and polymerase chain reaction (PCR). PCR was carried out using a primer pair (SK 38/39) specific for the p24 nucleotide sequence of HIV-1. During the follow-ups, western blot testing for the 3 retroviruses was performed on patients' plasma

RESULTS

Case 1: A false-positive HIV-1 screening assay in a male with STD: The patient was a 22 year old unmarried heterosexual male, working in a dairy plant and sexually active since the age of 15. He recalled one episode of gonorrhoea. In June 1988, he felt in good general health and had no abnormal clinical signs on general examination. Beta-lactamase producing *Neisseria gonorrhoeae* were grown from a purulent urethral discharge, while syphilitic serologies were negative.

When screened for antibodies to lymphotropic retroviruses, the patient's serum tested negative by 6 tests, but positive by the ELAVIA, which gave one non-repeatable reactive result for HIV-1. The HIV-1 western blot revealed a solitary 17 Kda band at that time. After 5 months, the western blot profile had progressed and showed antibody bands against the 15, 31, 41 and 55 Kda antigens. A concomitant PCR analysis was strongly positive and made an actual infection by HIV-1 highly probable, despite the still indeterminate profile of the western blot. The patient could not be located for follow-up in February 1989.

Diagnostic challenge number 1: Was the assay, that gave one single (false?) positive result on initial screening, perhaps the only assay that detected an early HIV-1 infection? Should western blot profiles that include reactions to *gag*, *pol*, and *env* gene products, be considered positive, even though they do not meet the criteria proposed by the kit manufacturer? Since the proteins p15, p17 and p24 are cleavage products of the precursor molecule p55, why was the p24 band absent on both test occasions. Likewise, why did the patient lack detectable antibodies to other *env* and *pol* gene products? Or in the end, might the PCR result have been a false positive?...

Case 2: Triple sero-conversion and co-infection in a female with an indeterminate HIV-1 western blot: This was a young prostitute working in a bar in Djibouti. She was in good general health and had a normal clinical examination in June 1988. On initial screening for antibodies to lymphotropic retroviruses, her serum was negative by 6 test, while repeatedly reactive for HIV-1 by the ABBOTT recombinant ELISA. The corresponding HIV-1 western blot showed only a weak 24 kDa band. After 5 months the western blot profile had progressed, but was labelled indeterminate since it showed antibody against the 15, 17, 24, 55 and 66 kDa proteins only. After an additional 3-months, the serum finally converted to strongly positive by the HIV-1 western blot. In addition, both the treponemal rapid plasma reagin (RPR) test and the HTLV-1 western blot assay, both negative previously, now showed positive reactivity; i.e. this woman, in addition to HIV, had seroconverted for syphilis and HTLV-1.

Diagnostic challenge number 2: Was this triple seroconversion (HIV-1, HTLV-1, syphilis), occurring over an 8 month period in a high risk individual, truly heralded by the initial positive HIV-1 ABBOTT ELISA? Should guidelines recommend to monitor patients with a positive HIV screening test but a negative blot; or was the subsequent sero-positive just a coincidence? Could this concomitant syphilis/HIV-1/HTLV-1 co-infection have been acquired at the same time, even from the same client? What is the probability of co-infections being acquired in a population with a low prevalence of the two viruses? Which attributes might be responsible for an increase sensitivity of a recombinant assay over a viral lysate assay? And finally, why was there no reaction to the p55 band during the initial western blot?

Case 3: Another prostitute, with HIV western blot sero-progression (or sero-repression ?): This 28 year old, uneducated, healthy female of Ethiopian origin worked in Djiboutian bars for the last 10 years. When screened for HIV in June 1988, her serum produced positive reactivity only by the ELAVIA ELISA and the CAMBRIDGE agglutination tests. Initially, western blot analysis revealed reactive bands at 24 and 55 kDa proteins only, and the serum tested positive for HIV antigen, but was not confirmed by neutralization. When tested after 5 months, additional bands appeared on the HIV-1 western blot; bands 31 and 41 (both weak), and bands 51 and 66; and an assay for HIV-1 (PCR) produced an equivocal reaction. When tested after 8 months, the western blot again showed strong 24 and 55 kDa reactions, but this time no other bands, except for a weak reaction to 41.

Diagnostic challenge number 3: How accurate were the initial screening tests in this case?, and what was the significance of the strong 24 and 55 bands?; do these strong bands always suggest early HIV infection? Why would a serum be positive for HIV antigen, but non-neutralizable? Importantly, should this person be considered HIV positive at the 5 month follow-up, when considering the western blot and PCR results? Would the diagnosis need to be revised after the results of the 8-month western blot? And finally, why did the other screening assays not identify the serum on the initial screening?

Case 4: Delayed western blot sero-conversion in a female with an early positive HTLV-1 screening test: This 20 year old prostitute of Ethiopian lived in Djibouti for the last year and worked in a "low-class" bar. Her clinical examination was unremarkable in June 1988, but Chlamydia antigen was detected by ELISA on a sample of vaginal secretions. Her serum test negative by all screening assays for HIV but an agglutination tested for HTLV-1

was repeatedly reactive; the HTLV-1 western blot was negative and showed only one 24 kDa band. During the two serological follow-ups at 5-and 8- months, HTLV-1 infection was confirmed, since in addition to the agglutination test, the western blot assay now had become positive. On both occasions, the western blot profile comprised antibody bands against the 19 (weak), 24, 36, and 46 (weak) kDa antigens.

Diagnostic challenge number 4: This case describes standard progression of a western blot profile characteristic on an ongoing HTLV-1 infection. But, was the outcome of the initial screening test a false positive result, or was the agglutination assay initially more sensitive than the western blot for diagnosing an early HTLV-1 infection? What was the diagnostic significance of the solitary p24 antibody band on the early HTLV-1 western blot? Should all subjects with a p24 band be monitored for future seroconversion for HTLV-1?, for HIV? In fact, do antibodies to p24 cross react between HTLV-1 and HIV antigens? And is it possible that a PCR test done for HIV may detect proviral DNA from an individual with HTLV-1 infection?

Cases 5 and 6: Two female prostitutes of Ethiopian origin, with antibodies against either envelope or polymerase antigens alone: One was 25 years old and worked in a bar in Djibouti for the last 3 years. She claims to use condoms rarely, and recalled previous episodes of syphilis and gonorrhoea. The clinical examination was normal in June 1988, and her serum tested negative by 6 retroviral screening assays, but was reactive by the SERODIA HIV agglutination test. The HIV western blots were negative both in June and in November 1988, but in February 1989, the HIV-1 western blot displayed antibody bands against the 41 and the 120 kDa antigens.

The other was 35 years old and worked in bars in Djibouti over 12 years. She claimed to use condoms regularly, and to have no history of sexually transmitted diseases. She frequently drank alcohol and used the oral drug *khat*. Clinical and gynaecological examinations were normal in June 1988; her serum tested negative by 5 screening assays, but was reactive by the SERODIA HIV agglutination test and the ELAVIA test. At this time the HIV-1 western blot displayed strong antibody bands against the 31 and the 66 kDa proteins only. After 8 months follow-up, this HIV-1 western blot profile remained essentially the same.

Diagnostic challenge number 5: Case five describes a western blot profile with antibody bands against HIV-1 envelope proteins solely, while Case 6 describes a western blot with solitary antibody bands against the retroviral enzymes: endonuclease and reverse transcriptase (polymerase gene products). What is the significance of the initial screening tests, in particular the SERODIA test, which was reactive in both instances, which is notorious for false positive reaction(3)? What is the diagnostic significance of solitary HIV-1 envelope bands in Case 5?, and the persisting polymerase antibodies in the total absence of *env* and *gag* antibodies in Case 6? Is it possible to have antibodies to polymerase antigens in the absence of a retroviral infection? Or could it be that Case 6 was infected by a novel retrovirus?

DISCUSSION

We have presented six case reports and twenty six diagnostic problems related to the three retroviruses. These problems represented actual situations encountered in diagnostic laboratories. The essential questions have been asked, the challenges have been pose, yet the answers have not been provided. At this time, there are no easy and clear-cut answers for explaining most of our puzzling test results. Based on the number and variety of different tests that we incorporated to determine the true status of subjects, we had expected results to yield conclusive results. However, the final conclusion leads to additional, and even more fundamental questions: How and when, if at all, should our patients be monitored in the future? How, and by which tests should the physician best screen and follow his patients? And mainly, what should the caring physician tell the person with a questionable lymphotropic serology?

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