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False-Negative HIV Antibody Test Results

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Ideally HIV antibody tests have to be both extremely sensitive and able to recognize all known HIV subtypes. Three patients whose sera failed to react with a synthetic oligopeptide-based HIV antibody test are described in this report. The patients were a Pakistani male infected recently, an Australian male infected for several years, and a Ugandan woman with AIDS. The presence of anti-HIV antibodies was confirmed by means of a standard algorithm with different assay formats. All three sera failed to react in one antiglobulin enzyme-linked immunosorbent assay (ELISA) (Bioelisa HIV-1+2, Biokit SA). No single underlying reason could be identified for the assay failure in the three cases. The first patient, probably infected recently when first tested, was strongly positive by the same assay a year later, confirming the relative insensitivity of oligopeptide assays reported previously for detecting the early antibody response. The other two patients appear to have been infected for several years. Although unlikely to have been infected with a non-clade B virus, the sample from patient 2 lacked detectable antibody to the transmembrane glycoprotein (gp41), the site of the synthetic oligopeptides. Patient 3, of Ugandan origin, was found to be infected with a non-clade B virus. Although her serum reacted strongly to subtype B gp41 in Western blot, it failed to react in the antiglobulin ELISA. Since there appears to be no single common explanation for these three failures there is little opportunity to identify prospectively those situations where testing using assays employing synthetic oligopeptides on the solid phase is likely to fail. ***J. Med. Virol. 60:43–47, 2000.*** © 2000 Wiley-Liss, Inc.

INTRODUCTION

Since the discovery of HIV in the early 1980s, testing for HIV-specific antibodies, the standard marker of infection, has improved markedly. Typical modern HIV antibody tests (often using the enzyme-linked immunosorbent assay technology) have a sensitivity in excess of 99.9% [UNAIDS/WHO, 1997] and detect antibodies against all known subtypes of HIV-1 and HIV-2. Most assays employ recombinant proteins and/or synthetic peptides representing defined viral epitopes rather than crude viral lysate preparations.

As there is an inevitable trade-off between sensitivity (the ability to detect true positives) and specificity (the ability to avoid false-positives), the reactivity of samples on initial screening must be confirmed by further testing. Therefore, the diagnosis of HIV infection employs both screening and confirmatory tests for HIV antibodies, often in the form of an algorithm [UNAIDS/WHO, 1997]. These tests need to be evaluated carefully in each setting to assess their performance in terms of sensitivity and specificity; the failure of any single component jeopardizes the accuracy of that algorithm. Assay performance may be compromised by factors such as "unusual" HIV subtypes (not well represented by the antigen profile contained in the assay), recent infection (with low antibody levels against HIV antigens), and problems inherent to the assay, due to its format and design [Evans et al., 1997]. In particular, some modern assays employing synthetic peptides as antigens have previously been shown to lead to false-negative results with certain samples [McAlpine et al., 1995]. We present here three cases where sera gave false-negative HIV antibody test results with a commercially available antiglobulin enzyme-linked immunosorbent assay (ELISA) based on synthetic peptides.

MATERIALS AND METHODS

Patients

Patient 1 was a man from Karachi, reported to be HIV antibody-positive after local testing. A serum sample was received by the Department of Medical Virology, University College London Hospitals, for confirmatory HIV antibody testing in October 1996. Patient 2 was a 44-year-old man, diagnosed as HIV antibody-positive in Australia in 1992. He came to the U.K.

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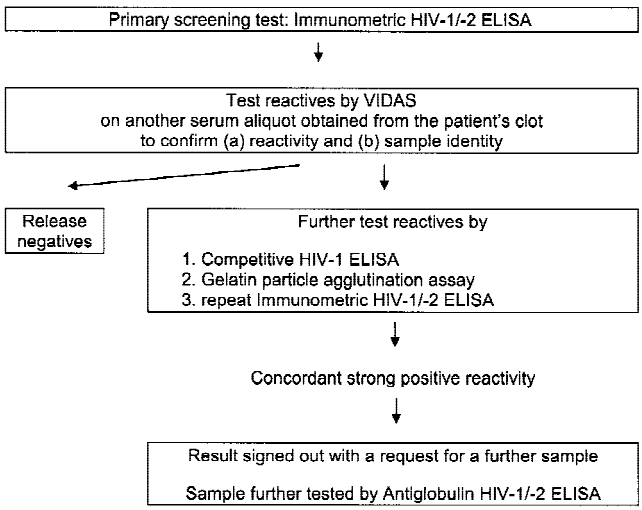

Fig. 1. Diagnostic algorithm for an adult HIV-1 infection at the Department of Virology, UCLMS (since 1994).

in June 1997 for advice on his antiretroviral treatment options, and a blood sample was taken for confirmation of his HIV antibody status. Patient 3 was a 30-year-old Ugandan woman with AIDS, who had a proven *Pneumocystis carinii* pneumonia in April 1998. A serum sample was taken in May 1998 when she was admitted to University College London Hospitals with a *Salmonella enteritidis* septicaemia, as initial HIV testing had been done elsewhere.

Testing

All patient sera were tested initially by our standard HIV antibody screening immunometric ELISA which contains recombinant HIV-1 antigens (core and envelope) and synthetic HIV-2 antigen (envelope) as coating on the solid phase and as conjugate. Being reactive on initial screening, the sample reactivity was then confirmed by a standard confirmatory algorithm, shown in Figure 1. This involves another four commercially available assays. These use a combination of different assay methodologies and formats (enzyme-linked fluorescent assay, competitive enzyme immunoassay, gelatin particle agglutination assay, and antiglobulin enzyme immunoassay) and HIV antigens (recombinant proteins, viral lysates and synthetic peptides) (see Table I).

In addition, an EDTA whole blood sample was also available from patient 3. After preparation of genomic DNA from 200 μl of EDTA blood using a commercial guanidinium lysis procedure (Qiagen), the proviral gp120 sequence was amplified, cloned and sequenced as previously described [Lewis et al., 1998]. Sequences obtained were compared with the Genbank database using a BLASTN algorithm to determine HIV subtype.

RESULTS

All three samples were reactive with our standard immunometric HIV antibody screening assay (Immunometric HIV 1+2 ELISA, Murex) and were confirmed by competitive ELISA (Wellcozyme HIV Recombinant, Murex), gelatin particle agglutination assay (Serodia HIV-1/2, Fujirebio), and enzyme-linked fluorescent assay (VIDAS HIV 1/2 new, BioMérieux SA). All three sera, however, failed to react by the manufacturer's criteria in the antiglobulin format ELISA (Bioelisa HIV-1+2, Biokit SA). To rule out assay run- or lot-specific problems leading to non-reactivity, all three samples were retested, using both the same and a different lot number of the antiglobulin ELISA. These retests confirmed the initial results. Samples from patients 2 and 3 were also retested at different dilutions (1:3, 1:10, 1:30, 1:100) in normal human serum and in phosphate-buffered saline; again no reactivity was observed in any of the diluted samples. Therefore, prozone-like phenomena could be excluded.

In addition, all three sera were tested by HIV-1 antibody Western blot (Cambridge Biotech) to investigate their reactivity against specific HIV-1 antigens. While the samples from patients 1 and 3 reacted against all ten HIV-1 antigens by Western blot, the serum from patient 2 did not contain detectable antibodies against either the transmembrane glycoprotein gp41 or the gag-precursor protein p55. The results of the different HIV antibody tests on the three patients' samples are summarised in Table II.

A second serum sample obtained eight months later from patient 1, in August 1997, was fully reactive by all tests, including the antiglobulin ELISA (Bioelisa HIV-1+2). Sequencing of the proviral gp120 sequence obtained from patient 3 showed that she was infected with an HIV-1 clade D virus (see Table III).

DISCUSSION

Three patients are described with a confirmed HIV-1 infection whose sera failed to react on a synthetic antiglobulin ELISA. Previous examples of failures of tests used widely to identify correctly anti-HIV containing samples have been ascribed to subtype divergence, recent infection [McAlpine et al., 1995] and, in a recent incident, to inherent susceptibility to false reactions of the assay format [Evans et al., 1997]. Our results lead us to conclude that the lack of reactivity by the synthetic peptide-based assay in the three cases described above has a diverse aetiology: Patient 1 appears to have undergone a relatively recent infection at the time the first sample was taken; this is inferred from the fact that a subsequent sample obtained one year later was fully reactive by the same antiglobulin ELISA. Previous studies have demonstrated the relative insensitivity of synthetic peptide-based assays with sera from recent seroconverters [McAlpine et al., 1995]; probably because the antibody repertoire of these seroconverting patients is still limited and fails to recognise the epitopes represented by the test's antigens. However, the other two patients appear to have been infected for a relatively long time, and the possibility of

TABLE I. Characteristics of Assays Employed*

Assay	Antigen	Conjugate	Result
Immunometric HIV-1/-2 ELISA (HIV 1+2 ELISA VK84/85, Murex)	Recombinant HIV-1 core and envelope (Weiss isolate); synthetic HIV-2 envelope	Same antigens:AP	O.D.
VIDAS HIV1/2 new enzyme-linked fluorescent assay (ELFA) (BioMérieux SA)	Synthetic gp41 (HIV-1) and gp36 (HIV-2), recombinant p24	Anti-human IgG:AP	TV
Competitive HUV-1 ELISA (Wellcozyme HIV Recombinant VK56/57, Murex)	Recombinant HIV-1 core and transmembrane (envelope) (Weiss isolate)	Human anti-HIV antibodies:HRPO	O.D.
Gelatin particle agglutination assay (Serodia HIV-1/2, Fujirebio)	Gelatin particle carriers sensitised with inactivated HIV-1 and HIV-2 antigens	(Specific antibodies agglutinate sensitised particles)	titre
Antiglobulin HIV-1/-2 ELISA (Biokit Bioelisa HIV 1+2)	Synthetic oligopeptides: gp41 (HIV-1) and gp36 (HIV-2)	Goat anti-human IgG:HRPO	O.D.
Western blot (HIV-1 Cambridge Biotech Western blot kit)	Electrophoretically separated antigens from partially purified inactivated HIV-1 bound on nitrocellulose strips	(1) Goat anti-human IgG biotinylated; (2) avidin:HRPO	antigen-specific bands

*AP = alkaline phosphatase; HRPO = horseradish peroxydase; O.D. = optical density; TV = test value; titre = reciprocal dilution.

TABLE II. HIV Antibody Test Results of the Three Patient Samples

Assay	Sample O.D. (optical density)	Cut-off value	Control values positive	Control values negative
Patient 1 24 October 1996				
Murex HIV 1+2 ELISA	3.625; 3.263	0.258	1.524	0.058
Serodia HIV-1	>256	16	128	<16
VIDAS HIV 1/2 new ELFA	TV 4.24	≥0.27, <0.57	≥0.57	<0.27
Wellcozyme HIV recomb.	0.175	0.900	0.070	1.809
Bioelisa HIV-1+2	0.183	0.221	2.273	0.021
(repeat)	0.135	0.223	1.760	0.023
(repeat in duplicate)	0.122; 0.120	0.227	2.244	0.027
W.b.: p15/17+, p24+, p31±, gp41+, p51±, p55+, p66+, gp120±, gp160+				
11 August 1997				
Murex HIV 1+2 ELISA	3.133	0.268	0.903	0.068
Serodia HIV-1	>256	<32	128	<16
VIDAS HIV 1/2 new ELFA	TV 22.73	≥0.27, <0.57	≥0.57	<0.27
Wellcozyme HIV recomb.	0.053	1.067	0.053	1.900
Bioelisa HIV-1+2	2.100	0.224	2.063	0.024
Patient 2 20 June 1997				
Murex HIV 1+2 ELISA	3.250	0.270	1.396	0.070
Serodia HIV-1	102,400	16	128	<16
VIDAS HIV 1/2 new ELFA	TV 0.70	≥0.27, <0.57	≥0.57	<0.27
Wellcozyme HIV recomb.	0.153	0.954	0.048	1.152
Bioelisa HIV-1+2	0.071	0.256	1.839	0.056
(repeat)	0.099	0.274	1.329	0.074
(repeat)	0.078	0.205	1.058	0.005
W.b.: p15/17+, p24+, p31+, gp41∅, p51±, p55∅, p66+, gp120±, gp160+				
Patient 3 9 June 1998				
Murex HIV 1+2 ELISA	3.427	0.352	1.068	0.152
Serodia HIV-1	>12,800	16	128	<16
VIDAS HIV 1/2 new ELFA	TV 12.61	≥0.27, <0.57	≥0.57	<0.27
Wellcozyme HIV recomb.	0.169	0.525	0.095	1.419
Bioelisa HIV-1+2	0.043	0.215	2.860	0.015
(repeat)	0.058	0.225	2.626	0.025
(repeat)	0.127	0.205	1.058	0.005
W.b.: p15/17+, p24+, p31+, gp41±, p51+, p55+, p66+, gp120+, gp160±				

*Murex HIV 1+2 ELISA: Immunometric HIV-1/-2 ELISA (Murex VK 84/85); Serodia HIV-1: Gelatin particle agglutination assay (Fujirebio); VIDAS HIV 1/2 new ELFA: enzyme-linked fluorescent assay (BioMérieux); Wellcozyme HIV recombinant: competitive HIV-1 ELISA (Murex VK56/57) Bioelisa HIV-1+2: Antiglobulin HIV-1/-2 ELISA (Biokit); W.b.: HIV-1 Cambridge Biotech Western blot kit (+ strongly, ± weakly, ∅ not reactive).

TABLE III. HIV Envelope Sequence Obtained From Patient 3

	117	←		V 1	→	←		V 2		→	
IIIB	PCVKLTPLCV		SLKCTDLKND	TNTNSSSGRM	IMEKGEIKNC	SFNISTSIRG	KVQKEYAFFY	KLDIIPIDND	TTSYSLTSCN	TSVITQACPK
Patient 3	PCVKLTPLCV		TLNCTEWKND	TVTN--ATDL	EMKNLEMKNS	SFNVTTGLRD	KKKQVYALFY	KLDVISIDKN	SSSYRLINCN	TSAITQTCPK
–											←
IIIB	VSFEPIPIHY		CAPAGFAILK	CNNKTFNGTG	PCTNVSTVQC	THGIRPVVST	QLLLNGSLAE	EEVVIRSVNF	TDNAKTIIVQ	LNTSVEINCT
Patient 3	TTFEPIPIHY		CAPAGYAILK	CNEKNFNGTG	CKNVSTVQC	THGIRPVVST	QLLLNGSLAE	EDIIISSEKL	EDNAKIIIVQ	LNKSIPITCT
			V 3		→ 334						
IIIB	RPNNNTRKRI		RIQRGPGRAF	VTIGKIGNMR	QAHCNIS						
Patient 3	RPYNNTRQGT		RI--GPGQAY	FTTRT-GDIR	QAHCNIS						

The sequence of the HIV_{IIIB} isolate commonly used in the formulation of diagnostic tests is shown for comparison. The sequence shown is aligned to the region from amino acid 117 to 334 of subtype B gp120 (IIIB) and includes the V1, V2 and V3 domains.

an idiosyncratic host response to the virus should be considered.

Patient 2 was not a recent seroconverter, having had a positive result approximately five years previously. As he was infected in Australia, he was unlikely to be infected with a non-clade B HIV-1 subtype. His serum did not contain detectable antibody to the transmembrane glycoprotein (gp41) on Western blot, i.e. the antigen represented by the synthetic peptides in the antiglobulin ELISA. Furthermore, his serum had a comparatively low though positive reading by the VIDAS test, which also employs synthetic gp41, in addition to synthetic p24, as its HIV-1 antigens. The reason for this lack of serological reactivity against the transmembrane glycoprotein is unclear. The patient may have been infected with an unusual strain of HIV-1 with a markedly heterologous sequence in the "env" gp41 region concerned; alternatively, there may have been an idiosyncratic failure on part of the patient's immune system to mount an antibody response against "typical" gp41. Unfortunately, no sample was available for sequence analysis to assess these possibilities.

Finally, the serum from patient 3 reacted strongly against all antigens including gp41 on Western blot but failed to react by the antiglobulin ELISA. She was in a late stage of HIV infection, as evident from her clinical presentation. Not surprisingly, given her Ugandan origin, she was found to be infected with an HIV-1 subtype D virus. It is therefore possible that her HIV-1 clade D virus differs in its gp41 region from the one from which the epitopes for the antiglobulin ELISA are derived, leading to non-recognition of these clade B-derived epitopes, as described previously [Engelbrecht et al., 1994; Brennan et al., 1997]. In the latter report, the researchers failed to detect three infections by clade D viruses in a peptide-based assay. An alternative explanation would be an absorption of anti-HIV antibodies in a patient with a high viral load (this patient had a viral load of 93,700 genome equivalents/ml as determined by commercial branch DNA assay). While this has been described in numerous cases for antibodies against HIV gag protein p24, the titre of which decreases as disease progresses and the HIV p24 antigen concentration increases, these studies also showed that antibodies directed against HIV envelope glycoprotein gp41 remain at a constant level throughout the course of the disease [Portera et al., 1990]. This is therefore an unlikely explanation. A common pattern pertaining to all three sera missed by the antiglobulin ELISA in our laboratory was not identified. We consider that in the first case, a recently infected patient had not yet formed antibodies against the antigens contained in the assay; in the second, that there was either a genuine, permanent failure to mount an antibody response against gp41, including the epitopes present in the antiglobulin ELISA (although we were unable to formally eliminate the possibility of a divergent virus). In the third case, a divergent viral subtype was found which may have epitopes differing from those con-

tained in the test. In summary, no common reason could be identified for the assay failure in the three cases described above.

Although assays employing synthetic oligopeptides may be sensitive and highly specific, their "fit" with the patient's repertoire of antibody may sometimes be insufficient to lead to positive signal generation. In our experience in a London-based reference laboratory, it is estimated that this test fails to detect somewhere in the region of 1 in 500 positive individuals (data not shown). This incidence may be higher in settings with a higher prevalence of divergent viruses and may rise in the future as the prevalence of non-B viruses increases. From these results it could be argued that the test is not suitable as a first-line screening assay, though it may still have a place in a confirmatory algorithm. However, since there appears to be no coherent explanation for the three failures described here, there is little opportunity to identify prospectively those situations where this test may be susceptible to the problems associated with employing synthetic oligopeptides on the solid phase. Its use may therefore place in jeopardy the performance of any diagnostic algorithm which includes it and has been discontinued in our laboratory.

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