JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2006, p. 3021–3024 Vol. 44, No. 8
0095-1137/06/$08.00+0 doi:10.1128/JCM.02207-05
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False-Positive Results of Enzyme Immunoassays for Human Immunodeficiency Virus in Patients with Uncomplicated Malaria

Anne F. Gasasira,[1] Grant Dorsey,[2] Moses R. Kamya,[1] Diane Havlir,[2] Moses Kiggundu,[1] Philip J. Rosenthal,[2] and Edwin D. Charlebois[2]*

Department of Medicine, Makerere University Medical School, Kampala, Uganda,[1] and Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California[2]

Received 20 October 2005/Returned for modification 26 January 2006/Accepted 8 June 2006

Malaria may impact upon human immunodeficiency virus (HIV) test results. We evaluated two HIV enzyme immunoassays (EIAs) by testing 1,965 Ugandans with malaria. We found poor positive predictive values (53% and 76%), particularly with younger age. Combining EIAs eliminated false positives but missed 21% of true positives. Performance of HIV EIAs in malaria may be unsatisfactory.

Same-day human immunodeficiency virus (HIV) testing utilizing simple enzyme immunoassay (EIA) test kits is rapidly becoming the standard method for diagnosing HIV infection in sub-Saharan Africa (7, 11, 12, 16, 20, 24). These methods reduce laboratory costs and obviate the need for patients to return at a later date for test results. However, these methods typically use repeated EIAs in place of the more specific Western blot assay for confirmation of initial HIV-positive EIA results, which could be problematic. It is known that a number of infectious and autoimmune diseases can produce false-positive EIA results (2, 15, 18, 23). In particular, acute malarial infection may be associated with false-positive EIA results for HIV (4, 8–10, 17). This interaction is thought to be driven by marked immunological stimulation and hypergammaglobulinemia that occur with malaria, especially in children with limited antimalarial immunity (3, 13, 14). Given the wide overlap of the HIV and malaria epidemics, the potential for misdiagnosis of HIV infection with combination EIA algorithms in regions where malaria is endemic may be significant.

The aims of this study were to (i) estimate the prevalence of false-positive HIV EIA results in children and adults with acute uncomplicated malaria, (ii) determine the positive predictive values (PPVs) of single HIV EIA results and a combination EIA algorithm similar to those in widespread use in sub-Saharan Africa, and (iii) assess the relationship between age and false-positive HIV EIA results in persons with acute uncomplicated malaria.

To estimate the prevalence of false-positive HIV EIA results in children and adults with uncomplicated acute malaria, we tested blood samples from 1,965 subjects aged 18 months or older with a history of fever in the last 24 h or an axillary temperature of >37.5°C, a positive thick blood smear for malaria parasites, and no evidence of a concomitant febrile illness. Subjects with evidence of other concomitant febrile illnesses or with fever without smear positivity for malaria were excluded from the study. Study subjects were participants in a trial conducted at seven sites in Uganda between December 2002 and February 2004 who were treated for uncomplicated malaria and followed up for 28 days (1, 25). Finger prick dried blood spot samples were collected on filter paper (Whatman 3MM) at the time of malaria diagnosis, air dried, and stored at ambient temperature in sealed plastic bags containing silica desiccant. HIV testing was performed with EIA kits compatible with dried blood spot testing for antibodies to HIV type 1 (HIV-1), i.e., the Genetic Systems rLAV EIA (Bio-Rad Laboratories, Hercules, CA) and the Vironostika HIV-1 Plus O Microelisa System (BioMerieux, Inc., Durham, NC). The rLAV EIA contains target antigens from HIV-1 strain LAV propagated in a CEM cell line and purified recombinant gp41 expressed in *Escherichia coli*. The Vironostika HIV-1 Plus O Microelisa System contains antigens from inactivated, purified HIV-1 (clades A to H) viral lysate proteins, purified viral envelope proteins, and a synthetic peptide with an amino acid sequence corresponding to that of the transmembrane immunodominant domain of the HIV-1 group O (ANT 70) isolate. We also employed a confirmatory Western blot assay (Genetic System HIV-1 Western Blot; Bio-Rad). EIAs and Western blot testing were performed in accordance with the manufacturers' recommendations. Specimens testing positive by either the rLAV or the Vironostika HIV EIA were subsequently tested with the other EIA kit and evaluated by Western blotting. Specimens testing negative by both EIA kits were assumed to be negative for antibodies to HIV and were not tested by Western blotting. Specimens identified as false positive and a

TABLE 1. Diagnostic performance of EIA for HIV[a]

Test(s) or algorithm	No. of specimens tested for HIV				PPV (%)
	Positive	Negative	True positive	False positive	
Both EIAs plus WB	96	1,869	NA	NA	NA
GS rLAV EIA	146	1,819	78	68	53
Vironostika EIA	123	1,842	93	30	76
Serial algorithm[b]	75	1,890	75	0	100

[a] Abbreviations: NA, not applicable; WB, Western blotting. A total of 1,965 specimens were tested.
[b] See text for details. Briefly, the two EIAs were used in a serial testing algorithm.

* Corresponding author. Mailing address: Department of Medicine, University of California, San Francisco, AIDS Research Institute, 50 Beale Street, 13th Floor, San Francisco, CA 94105. Phone: (415) 597-9301. Fax: (415) 597-9213. E-mail: Edwin.Charlebois@ucsf.edu.

TABLE 2. Concordance of EIAs for HIV

Vironostika EIA result	No. of specimens tested by GS rLAV EIA[a]	
	Negative	Positive
Negative	1,771	71[b] (3)
Positive	48[b] (18)	75 (75)

[a] Values in parentheses are numbers of specimens that were confirmed to be positive by Western blotting.
[b] Discordant results. A total of 119 specimens had results that were discordant.

random group of 10 Western blot-confirmed positive samples were subsequently tested for the presence of HIV-1 DNA by using Chelex resin extraction of DNA from dried blood spots and the Roche AMPLICOR HIV-1 DNA test, version 1.5, as described by Fischer et al. (6). The observed PPV was calculated as the ratio of the number of individuals testing positive for antibodies to HIV who were confirmed by Western blotting (true positives) to the sum of the number of individuals testing positive for antibodies to HIV who were confirmed by Western blotting (true positives) plus the number of individuals testing positive for antibodies to HIV who were not confirmed by Western blotting (false positives). Data were analyzed in the STATA statistical analysis package (STATACorp, College Station, Texas). This study was approved by the University of California, San Francisco, Committee on Human Research; the Makerere University Research Ethics Committee; and the Uganda National Council on Science and Technology.

Results from EIAs of the 1,965 specimens indicated that 1,771 (90%) were negative by both EIAs, 75 (4%) were positive by both EIAs, and 119 (6%) produced discordant results with the rLAV and Vironostika EIAs (Tables 1 and 2). Among the discordant specimens, 21 (18%) were confirmed by Western blotting to contain antibodies to HIV. Overall, the prevalence of HIV in this population of acute uncomplicated malaria patients was 4.9% (95% confidence interval [CI], 3.9% to 5.8%). Western blot assays of the false-positive specimens

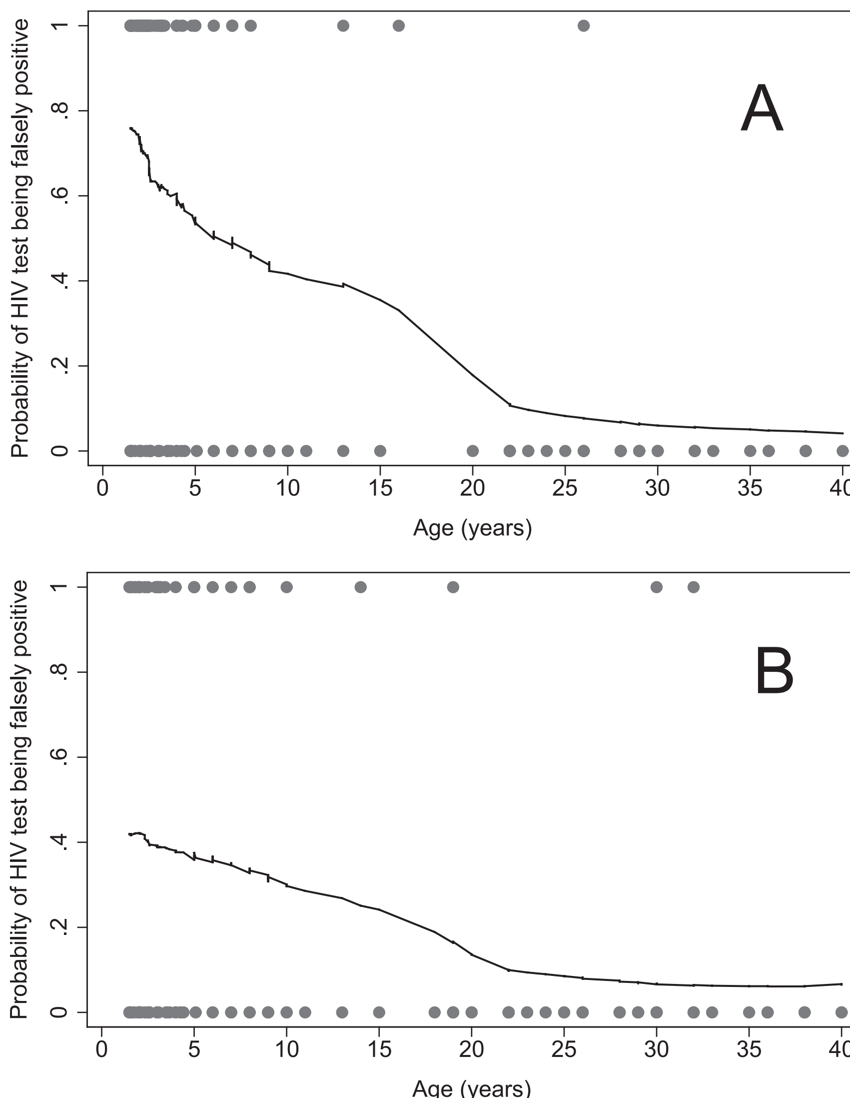

FIG. 1. Relationship between the probability of a false-positive HIV-1 EIA result and increasing age among subjects witha positive HIV-1 EIA test result, determined using locally weighted scatter plot smoothing (LOESS). A, rLAV test; B, Vironostika test.

showed that the majority were reactive with p51 and p41 in both EIAs, with a mixture of bands for gp160 and p24. All (10/10) of the randomly selected EIA-positive, Western blotting-confirmed samples were also positive for HIV DNA, and 100% (98/98) of the EIA false-positive samples tested negative for HIV DNA.

We next applied a serial HIV EIA-reporting algorithm to our results. In this algorithm, a single negative EIA is reported as negative and a positive test is confirmed with a second EIA kit from a different manufacturer. If the confirmatory EIA is positive, the test is reported as positive. If the confirmatory EIA is negative (and thus, the two tests are discordant), the test is reported as negative. Regardless of which EIA was used first in the serial algorithm, the combination of the two tests would not have misdiagnosed any individuals as HIV infected. However, 20 individuals would have been incorrectly identified as not being HIV infected (false negatives). Considering the ages of the test subjects, we found that younger age was significantly associated with a higher prevalence of false-positive EIA results (Fig. 1). In a multivariate logistic regression analysis, there was a strong association between age and the odds of having a false-positive EIA result (per 5-year increase in age, the rLAV test odds ratio was 0.39 [95% CI, 0.25 to 0.60; $P < 0.001$] and the Vironostika test odds ratio was 0.61 [95% CI, 0.47 to 0.80; $P < 0.001$]) after controlling for gender, baseline temperature, and pretreatment parasite density.

We found a high prevalence of false-positive EIA results for HIV among acute uncomplicated malaria patients. The PPV of an HIV EIA in this population was substantially lower than that previously reported for HIV EIA in sub-Saharan Africa (5, 11, 19, 21, 22). Two pieces of evidence argue against acute HIV infection as a reason for false-positive HIV test results in this setting. First, nucleic acid testing of the false-positive samples failed to detect the presence of HIV-1 DNA. The second piece of evidence that argues against acute HIV infection as the cause of false-positive HIV EIA results is that the observed patterns of the Western blot assays were inconsistent with the typical pattern seen with acute HIV infection. The association of false-positive results with young age as depicted in Fig. 1 argues that this finding is related to the immune response to acute malaria. We postulate that younger individuals with a less developed immune response to malaria are more likely to exhibit nonspecific B-cell stimulation, producing antibodies that cross-react with HIV-1 antigens in the absence of HIV infection. Although the rLAV and Vironostika EIAs are commonly used on dried blood spots for seroepidemiology studies and quality control, direct extrapolation of this study's results to clinical rapid HIV EIA kits used on whole blood is limited by the nature of the study specimens (dried blood spots) and the use of nonrapid HIV EIAs. While this study reports an unexpectedly high prevalence of false-positive EIA results in the setting of acute malaria, it does not provide definitive proof of malaria as the causal factor as a cohort of treated subjects with malaria does not contain negative control subjects without malaria. However, the proportion of false-positive EIA HIV results among the positive results in this population with malaria (98/194 or ~50%) is substantially higher than published EIA HIV results ranging from 0% to 6% false positives out of all EIA positives from adults in neighboring Kenya (7). Further investigation of the specificity of the clinical rapid EIA HIV kits in use in sub-Saharan Africa, in particular for patients with malaria over time, is warranted.

We thank the study participants and the study team directly involved in this study (Moses Musinguzi, Moses Kiggundu, Felix Jurua, Maxwell Kilama, and Oswald Byaruhanga), the study team involved in the clinical trials (Joy Bbosa, Nelson Budaka, Dorothy Kirunda, Betty Nanzigu, Godfrey Buyinza, Isaac Kigozi, Joanita Nankabirwa, Fred Kizito, Sam Balikowa, and Grace Musimenta), and the administrative team (Sara Kibirango and Kenneth Mwebaze). We also thank Robert Downing, CDC Entebbe, for expert advice and support of this study.

This work was supported by the National Institutes of Health (AI62677) and the Centers for Disease Control and Prevention. P.J.R. is a Doris Duke Charitable Foundation Distinguished Clinical Scientist.

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