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Association of Inconclusive Sera for Human Immunodeficiency Virus Infection with Malaria and Epstein-Barr Virus Infection in Central Africa

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Among 464 sera from adults in Cameroon, 56 (12.1%) gave inconclusive HIV serology. All were negative for HIV-1 DNA; 44.6% ($n = 25$) were significantly associated with *Plasmodium* (42.8%) or Epstein-Barr virus (EBV) (17.8%) infections. In Central Africa, sera giving inconclusive results for HIV are frequently associated with malaria, EBV infection, or both.

Serum tested for HIV should respond consistently and identically whatever the serological test used and should be either negative or positive. A serum sample can be classified as "inconclusive" when it yields a questionable or indeterminate result for a serological HIV test or when it gives discrepant results in different HIV tests used when a given algorithm for assessing serum for HIV infection serodiagnosis is followed.

Despite the increase in the quality of serodiagnostic HIV tests, it has become apparent that the serology of HIV infection remains particularly difficult in Central Africa. Thus, the frequency of inconclusive sera varies depending on definitions, ranging from 3.4% in the Central African Republic (1), to 8.4% (2) and 9% (3) in Cameroon, and even to 10.5% in the Democratic Republic of Congo (4). Reported causes of indeterminate or discrepant rapid HIV test results include early HIV infection (5, 6) and false-positive reactions due to a variety of conditions associated with autoimmunity (7), pregnancy (8), and vaccinations against influenza (9), hepatitis B (10), and rabies (11), as well as concurrent infection by other pathogens. Several associations have been found between indeterminate HIV serologies and a number of infectious diseases, such as uncomplicated malaria (11, 12), sleeping sickness due to *Trypanosoma brucei gambiense* (13), schistosomiasis (14), leishmaniasis (15), syphilis (16), and dengue (11).

(Preliminary results of the study were presented at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, 10 to 13 September 2013, Denver, CO [17].)

A total of 464 volunteers were prospectively included during HIV screening campaigns by mobile units in northwestern Cameroon. For each volunteer, informed consent was obtained; a blood sample was collected in dry tubes, and two aliquots of decanted serum were conserved. One aliquot was used for immediate HIV screening testing in the mobile unit, as described previously (18). The second was sent to the Laboratoire National de Santé Hygiène Mobile and frozen at −20°C. In addition, dried blood spot (DBS) samples were prepared using Whatman 903 filter paper (Schleicher & Schuell, Whatman, Versailles, France) and stored at −30°C, as described previously (19). The study protocol was approved by the Cameroonese Ethical Committee.

The combination of the test results obtained in parallel by Alere Determine Test HIV-1/2 Ag/Ab Combo (Alere Inc., Waltham, MA) and ImmunoComb II HIV 1 & 2 CombFirm (Alere Inc.) gave a high frequency of inconclusive results for the 464 collected sera, including 382 (82.3%) negative, 26 (5.6%) positive, and 56 (12.1%) inconclusive sera.

The DBS samples from all individuals with positive and inconclusive sera and from 1 randomly selected individual out of 5 with negative sera were further assayed by PCR for HIV-1 DNA, cytomegalovirus (CMV) DNA, human herpesvirus 6 (HHV-6) DNA, Epstein-Barr virus (EBV) DNA, and DNA from *Plasmodium* spp. Total DNA was extracted from two blood circles cut from the DBS samples, as described previously (19). The HIV-1 LTR (long terminal repeat) gene was amplified by in-house real-time PCR (20), the *UL122* (*IE-1*) CMV gene by the Abbott CMV PCR kit (Abbott Diagnostics, Des Plaines, IL), the *BXLF1* thymidine kinase EBV gene by the Argene PCR assay (EBV R-gene quantification kit; Argene SA, Verniolle, France), the *U11* HHV-6 gene by a commercial kit (HHV6 LightMix kit/TIB MolBio; TIB MolBio GmbH, Berlin, Germany), and the mitochondrial *coxI* gene of *Plasmodium* spp. parasites by an in-house PCR (21).

All DBS samples from individuals with inconclusive sera were negative for HIV-1 LTR gene DNA (Table 1). The rate of detection of CMV DNA was 10.1% (47/464): 8.9% of DBS samples from individuals with inconclusive sera were positive for CMV DNA, compared to 9.9% and 15.3% of DBS samples from HIV-negative and HIV-positive persons, respectively. The rate of detection of HHV-6 DNA was 0.2% (1/464): only one (3.8%) DBS sample from an HIV-positive individual was positive. The rate of detection of EBV DNA was 5.2% (24/464); 17.8% of DBS samples from individuals with inconclusive sera showed positivity for EBV DNA, whereas only 2.9% and 11.5% of DBS samples from individuals with HIV-negative and HIV-positive sera, respectively,

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TABLE 1 Distribution of positive results for HIV-1, cytomegalovirus, human herpesvirus 6, Epstein-Barr virus, and *Plasmodium* DNA in dried blood spot samples

Organism	No. (%) of samples yielding result			
	HIV negative ($n = 382$ [82.3%])	HIV positive ($n = 26$ [5.6%])	Inconclusive ($n = 56$ [12.1%])	P[a]
HIV[b]	0 (0)	26 (100)	0 (0)	NA
Cytomegalovirus	38 (9.9)	4 (15.3)	5 (8.9)	NS
Human herpesvirus 6	0 (0)	1 (3.8)	0 (0)	NS
Epstein-Barr virus	11 (2.9)[c]	3 (11.5)	10 (17.8)[d]	<0.03
Plasmodium spp.	11 (2.9)	2 (7.6)	24 (42.8)	<0.01

[a] Determined by the χ^2 test. A P value of <0.05 is considered significant. NA, not applicable; NS, not significant.
[b] For HIV-1, the use of the NEC152 and NEC131 primer set and the NEC-LTR probe in the long terminal repeat gene allowed us to detect accurately the majority of HIV-1 subtypes of group M circulating in sub-Saharan Africa, including subtypes A, B, C, D, and G, as well as circulating recombinant forms 02 and 11 (20).
[c] Two Epstein-Barr virus-positive DBS samples from HIV-negative individuals were also positive for *Plasmodium* spp.
[d] Nine Epstein-Barr virus-positive DBS samples from individuals with inconclusive sera were also positive for *Plasmodium* spp.

were positive ($P < 0.03$). The rate of detection of DNA from *Plasmodium* spp. was 7.9% (37/464); 42.8% of DBS samples from individuals with inconclusive sera were positive, whereas only 2.9% and 7.6% of DBS samples from individuals with HIV-negative and HIV-positive sera, respectively, were positive ($P < 0.01$). Nine of 10 (90%) of samples from EBV-positive individuals with inconclusive sera were also positive for *Plasmodium* spp., whereas only 2 of 11 (18%) of samples from EBV-positive, HIV-negative individuals were positive for *Plasmodium* spp. ($P < 0.01$). Finally, 25 of 56 (44.6%) inconclusive sera were associated with either *Plasmodium* spp. or EBV, compared to only 20 of 382 (5.2%) and 5 of 26 (19.2%) HIV-negative and HIV-positive individuals, respectively ($P < 0.01$).

Acute malarial infection may be associated with false-positive enzyme-immunoassay results for HIV (12, 22), as similarly reported for the retrovirus human T-cell leukemia virus type 1 (HTLV-1) (23). This interaction is thought to be driven by marked immunological stimulation, strong nonspecific polyclonal B-cell activation, hypergammaglobulinemia, and production of autoantibodies and of circulating immune complexes, which are prominent features of malaria infection (22, 24–27). Given the wide overlap of the HIV/AIDS and malaria epidemics, the potential for misdiagnosis of HIV infection with algorithms combining HIV serological testing in regions where malaria is endemic may be significant.

Detection of circulating EBV genomes, likely reflecting EBV reactivation, was the second identified association. EBV infection is well known to be associated with polyclonal B cell activation (28), which may lead to nonspecific cross-reactivity with HIV antigens. It may also be hypothesized that *Plasmodium* spp. and EBV infections contribute additively to inconclusive sera in coinfected patients. Indeed, complex interactions exist between *P. falciparum* and EBV infection, leading to endemic Burkitt lymphoma, the most frequent lymphoma in children living in central equatorial Africa (29). *Falciparum* malaria has been shown to be a powerful polyclonal B-cell mitogen inducing hypergammaglobulinemia and production of autoantibodies (24, 30).

In conclusion, inconclusive serodiagnosis of HIV infection is frequent in central equatorial Africa. In our series, nearly half of inconclusive sera were not associated with *Plasmodium* spp. infection or EBV infection, indicating other possible causes. These could include other endemic infectious diseases, as well as conditions associated with autoimmunity. Whether circulating natural polyreactive antibodies whose production is likely genetically controlled (31) can recognize HIV-1 antigens, leading to indeterminate or equivocal reactivities, warrants further investigation.

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