Vox Sanguinis

Vox Sang 1999;77:237–238

Malaria Antibody ELISA Insufficiently Sensitive for Blood Donor Screening

Gerhard Mertens[a], Tony Vervoort[b], Sandra Heylen[a], Ludo Muylle[a]
[a]Bloedtransfusiecentrum Antwerpen, Edegem, and
[b]Instituut voor Tropische Geneeskunde, Antwerpen, Belgium

The paper of Chiodini et al. [1] prompted us to evaluate the same commercial malaria antibody ELISA. We examined 49 sera of patients from a malaria-endemic area, containing plasmodium antibodies found with a reference indirect fluorescence antibody test (IFAT) by the Institute for Tropical Medicine, Antwerpen. Serological specificities were *Plasmodium falciparum* in 42 cases, *Plasmodium vivax* in 4 cases and *Plasmodium ovale* in 3 cases. The antibody titers ranged from 80 to 40,960 (median 1,280). These sera were tested at the Blood Transfusion Centre, Edegem, with the Malaria IgG CELISA (Cellabs, Brookvale, Australia) and the Falciparum-Spot IF (bioMérieux, Marcy-l'Etoile, France). This commercial IFAT, applied for donor screening since 1985, uses *Plasmodium falciparum* as a single antigen. To destroy IgM antibodies, sera with a positive IFAT and a negative CELISA were treated with dithiothreitol (DTT). Equal volumes of serum and 0.01 *M* DTT were mixed and incubated for 30 min at 37 °C, after which the IFAT was performed. As a control for the effect of the 1:2 dilution of the serum with DTT, an aliquot of each untreated original serum was diluted 1:2 with saline and also subjected to the IFAT. The CELISA was not performed on the DTT-treated samples. With the CELISA, all samples were tested in duplicate, then retested in duplicate using microplates from different production lots.

All 49 sera had a positive bioMérieux IFAT. Twenty-eight (57%) sera were reactive in the CELISA, with an OD/cut-off ratio between 1.24 and 11.7 (median 5.29). The ratios of the CELISA-nonreactive sera showed a continuous distribution between 0.26 and 0.89. Repeat testing showed the CELISA to be reproducible. Sensitivity of the CELISA for the different *Plasmodium* species was 27/42 (64%) for *Plasmodium falciparum*, 0/4 for *Plasmodium vivax* and 1/3 (33%) for *Plasmodium ovale*. After DTT treatment, the IFAT became negative for 7 (33%) of the 21 'IFAT-positive/CELISA-negative' sera, with a positive IFAT in the corresponding saline dilution.

While in the study of Chiodini et al. [1] the CELISA detected 93% of the samples found positive by IFAT, we observed a sensitivity of only 57% of the CELISA as compared to the reference IFAT. Our result is partly explained by the presence of fluorescein-labeled total anti-human globulin in the IFAT, while only horseradish-peroxidase-labeled anti-IgG is used in the CELISA. The CELISA thus cannot detect IgM malaria antibodies. We have shown that this situation occurs in our samples. There might also be less cross-reactivity of the purified *Plasmodium falciparum* antigen coated on the CELISA microplate than of the *Plasmodium falciparum* preparation applied on the IFAT slides. Finally, the relatively low ratios of the CELISA-reactive sera suggest a suboptimal analytical sensitivity. The possibility of false-positivity of the 49 reference sera is excluded, firstly by the fact that all samples also reacted positively with the commercial IFAT, and secondly by the elevated antibody titers with the reference IFAT. It seems otherwise improbable that the reference material would have lost its reactivity during storage in the frozen state or during subsequent thawing. If the sera had deteriorated, we would not have found 49 out of 49 positive with the bioMérieux IFAT, which was performed in parallel with the CELISA.

Though one can argue the meaning of our finding in the setting of blood donor screening, we feel the highest safety level is attained with a screening test detecting IgG as well as IgM antibodies. It should be stressed that the preventive strategy of Chiodini et al. [1] includes 6 months of exclusion from donation after returning from a risk area. This period might be considered sufficient for an eventual development of malaria IgG antibodies, but, in our opinion, security would still be greater if the CELISA were adapted by adding an anti-IgM conjugate. Then, a negative test result would more truly reflect the absence of plasmodium antibodies.

Reference

1 Chiodini PL, Hartley S, Hewitt PE, Barbara JAJ, Lalloo K, Bligh J, Voller A: Evaluation of a malaria antibody ELISA and its value in reducing potential wastage of red cell donations from blood donors exposed to malaria, with a note on a case of transfusion-transmitted malaria. Vox Sang 1997;73:143–148.

Dr. G. Mertens
Bloedtransfusiecentrum Antwerpen
Wilrijkstraat 8
B–2650 Edegem (Belgium)
Tel. +32 3 829 00 00, Fax +32 3 829 01 61

Author's Reply

P.L. Chiodini, S. Hartley, P.E. Hewitt, J.A.J. Barbara, K. Lalloo, J. Bligh, A. Voller
North London Blood Transfusion Centre, London, UK

The rationale behind the screening system which we propose is based upon a combination of exclusion by time and by antibody testing. The period of 6 months (which others have advocated even without antibody screening) allows enough time for nonimmune individuals incubating malaria to develop a clinical illness which will lead to an antibody response. Semi-immune candidate donors, who may be asymptomatic despite harbouring a malaria parasitaemia and who thus carry a risk of transmitting malaria by transfusion of their blood, characteristically have high levels of antimalarial antibody. In acute malaria, the IgG response occurs early (within a week), and there is an additional safeguard in nonimmune potential donors in that they will be symptomatic when parasitaemic and thus excluded on the basis that they are unwell at the time of the donor session.

In both scenarios outlined above, detection of specific IgG will be adequate for the purpose of detecting antimalarial antibody and thus excluding potential donors whose blood might have the potential to transmit malaria. The indirect immunofluorescent antibody test (IFAT) used in our study and against which the commercial enzyme-

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 1999 S. Karger AG, Basel
0042–9007/99/0774–0237 $ 17.50/0

Accessible online at:
www.karger.com/journals/vox