DETECTION OF SPECIFIC ANTIBODIES TO *PLASMODIUM FALCIPARUM* IN BLOOD BANK DONORS FROM MALARIA-ENDEMIC AND NON-ENDEMIC AREAS OF VENEZUELA

CARMEN ELENA CONTRERAS, ALENA PANCE, NORKA MARCANO, NANCY GONZÁLEZ, AND NICOLÁS BIANCO

Instituto de Inmunología, Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela

Abstract. Malaria antibody detection is valuable in providing retrospective confirmation of an attack of malaria. Blood bank screening is another area were malaria serology is potentially useful. In the present study, we tested the presence of antibodies to *Plasmodium falciparum* in sera from blood bank donors of non-endemic and malaria-endemic areas of Venezuela. Sera from 1,000 blood donors were tested by an indirect immunofluorescent antibody (IFA) assay and an IgG-ELISA for the presence of malaria antibodies using a synchronized *in vitro*-cultured Venezuelan isolate of *P. falciparum* as the antigen source. A selected group of positive and negative sera (n = 100) was also tested by a dot-IgG-ELISA. Positive results (reciprocal titer ≥ 40) were found in 0.8% and 3.8% of blood donors when tested by the IFA assay and in 0.8% and 2% (optical density ≥ 0.2) when tested by the IgG-ELISA in Caracas (non-endemic area) and Bolivar City (endemic area), respectively. The presence of anti-malarial antibodies in some sera from non-endemic areas such as Caracas reflects the increased potential risk of post-transfusional malaria in those areas due to the mobility of the blood donors. The data obtained indicate the need to implement new blood donor policy in blood banks in developing areas. Our results also indicate that the IFA assay is the most reliable test to use in malaria serodiagnosis.

The long-standing concern about transfusion-transmitted hepatitis and the intense response to potential human immunodeficiency virus (HIV) infection by transfusion have overshadowed the fact that other diseases spread by transfusion of blood components.[1,2] Transfusion-transmitted malaria was first reported in 1911,[3] and all four *Plasmodium* species that cause human malaria (*P. falciparum, P. vivax, P. ovale,* and *P. malariae*) may be transmitted by blood transfusion.[4] Moreover, all blood components, including red blood cells, platelet concentrates, white blood cells, cryoprecipitates, and fresh plasma, can transmit malaria. The fact that malarial parasites can survive in red blood cells at refrigerator temperatures (2–6°C) for days or weeks lead to the original exclusion of all blood donors who could represent a potential risk.[5]

Malaria is still one of the most prevalent parasitic diseases, particularly in tropical and subtropical undeveloped countries.[6,7] In Venezuela, malaria transmission became a minor health problem after a successful eradication program in the 1950s, which included massive antimalarial drug administration, environmental sanitary measures, and intensive indoor spraying program with residual insecticides.[8] However, an increasing number of new malaria cases associated with an augmented transmission rate has been detected between 1969 and 1974 and after 1983.[8] Uncontrolled gold mining in Bolivar State (located in southern Venezuela) has been one of the principal inciting factors. Thus, 67% of the diagnosed new cases nationwide have been generated in this area (Venezuelan Ministry of Health 1988–1997, unpublished data). Therefore, blood banks in Venezuela face the problem of transfusion-transmitted malaria. Moreover, increases in international exchanges and migration from endemic areas enhances the possibility that blood donors might have been in contact with malaria parasites.[9]

The prevention of transfusion-transmitted malaria relies primarily on the exclusion of asymptomatic carriers of *Plasmodium* sp. parasites by means of accurate donor histories of potential exposure to areas where malaria is endemic. In addition, laboratory diagnosis using Giemsa-stained blood smears is commonly used for detection of *Plasmodium* sp. Unfortunately, this technique is time-consuming and may be unsuitable for routine screening of blood donors. Thus, prevention of blood-transmitted malaria is in need of standardized, suitable, and fast serologic identification of circulating malarial antigens and antimalarial antibodies[10] that may be applied in both blood banks and ambulatory patient care facilities in heavily infected areas.

Therefore, we designed a research protocol in which blood bank donors from malaria non-endemic and endemic areas in Venezuela were studied for the presence of antibodies to *P. falciparum* using serologic techniques[10–14] such as an ELISA,[10,11] a dot-ELISA,[13] and an indirect immunofluorescent antibody (IFA) test[14] using a Venezuelan isolate of *P. falciparum* adapted to *in vitro* culture conditions.[15]

MATERIALS AND METHODS

Serum samples. Four groups of Venezuelan sera were collected. All sera included in this study were obtained from volunteers who provided informed consent in accordance with the Ethical Committee on Human Research of the Institute of Immunology, School of Medicine, Central University (Caracas, Venezuela). The first group included 500 sera from the University Hospital Blood Bank in Caracas (non-endemic area for malaria). The second group included 500 sera from the Blood Bank of the Ruiz Paez Hospital in Bolivar City, Bolivar State (highly endemic area for malaria). Blood donors were mostly males (90%) between 18 and 45 years of age and had normal results on a physical examination and negative serologic test results for syphilis, viral hepatitis, HIV infection, and Chagas disease (analyzed by conventional serologic techniques). The third group included 27 patients with a thick blood film diagnosis of *P. falciparum* and/or *P. vivax* malaria. In addition, a fourth group of sera from patients with leishmaniasis, schistosomiasis, trypanosomiasis (*T. cruzi*) was included as a control. Freshly

collected sera were aliquoted and stored at −20°C until tested.

Antigen preparation. A Venezuelan *P. falciparum* strain isolated at the Venezuelan Amazonian Research Center (Puerto Ayacucho, Amazonas, Venezuela) was maintained in our laboratory using RPMI 1640 medium supplemented with 10% human serum, under reduced oxygen tension, according to the methods of Trager and Jensen.[15] Synchronous cultures were produced by treatment with 5% sorbitol,[16] and schizont infected red blood cells were obtained and concentrated by gelatin flotation.[17] Mature schizonts from synchronous cultures at a parasitemia of approximately 4% (25% hematocrit) were used for the IFA test. A soluble antigen preparation for the ELISA and dot-ELISA techniques was obtained as follows: a 95% enriched culture of young schizonts (30–33 hr) was diluted to 40% with RPMI 1640 medium without human serum (wash medium) and immediately resuspended in a mixture of protease inhibitors (Sigma, St. Louis, MO): 0.12 mM N-tosyl-L-lysyl chloromethylketone, 0.25 mM N-tosyl-L-phenylalanyl chloromethylketone, 2 mM phenylmethylsulfonyl fluoride, 0.23 U/mg of aprotinin, 50 μg/ml of chymostatin, 50 μg/ml of leupeptin, and 1 mM EDTA. After gently mixing, 2% Triton X-100 was added to the final mixture, followed by ultracentrifugation at 100,000 × *g* at 4°C for 45 min. The supernatant was removed, and aliquots were stored at −70°C or in liquid nitrogen. Uninfected O+ red blood cells were treated under similar conditions and used as a negative control. Western blots[18,19] using a positive immune serum from a *P. falciparum*-infected adult showed several polypeptides bands with apparent molecular weight ranging from 30 kD to 195 kD. In contrast, no polypeptide bands were observed when normal human serum or uninfected O+ red blood cells were used.

Enzyme-linked immunosorbent assay. The ELISA was performed using a soluble antigen preparation obtained as described previously.[11] Affinity-purified, goat anti-human IgG (Fc fragment) conjugated to alkaline phosphatase and goat anti-human IgM (μ chain specific) conjugated to alkaline phosphatase (Atlantic Antibodies Scarborough, ME) were used at a 1:1,000 dilution. After addition of the substrate (4-nitrophenylphosphate in diethanolamine buffer; Sigma), the absorbance was read at 415 nm in an ELISA plate reader (Molecular Devices, Palo Alto, CA). A positive reference serum with confirmed *P. falciparum* infection and a negative reference serum from a donor never exposed to malaria were used for antigen titration. A 1:6,400 antigen dilution (50 ng of protein/well) gave a strong reading (an optical density [OD] > 1.0 at 415 nm) with the positive serum and a low reading (OD < 0.1) with the negative serum. The latter was used at a 1:200 dilution in this assay.

Dot-ELISA. The dot-ELISA was performed as previously reported[13] with minor modifications. Antigen disks were prepared by dotting 1 μl of soluble *P. falciparum* antigen on nitrocellulose strips (Bio-Rad Laboratories, Hercules, CA). Nonspecific sites were blocked with phosphate-buffered saline (PBS) containing 2% gelatin and 0.1% Tween 20. Strips were incubated with two-fold serial dilutions of tested sera and washed three times with PBS-Tween 20. A second immune reaction was performed by adding a 1:1,000 dilution of affinity-purified goat anti-human IgG (Fc fragment specific) conjugated to alkaline phosphatase for 30 min at room temperature with agitation. After washing three times with PBS and once with distilled water, a freshly prepared substrate solution (Protoblot Immunoblotting System Human, P3910; Promega, Madison, WI) was added. Color developed within 15 min and positive reactions appeared as clearly defined blue-purple dots.

To standardize the dot-ELISA, several antigens concentrations (50 ng–300 μg) were tested against increasing serum dilutions (1:50–1:60,000). The optimal antigen amount to dot on the nitrocellulose strips was found to be 100 ng at an optimal serum dilution of 1:500.

Indirect immunofluorescent antibody assay. Malarial antibody levels were also measured using the IFA method.[14,20] Commercially produced fluorescein-labeled goat anti-human immunoglobulins (G, M, A, D, and E) were used to detect bound malarial antibodies, and fluorescence was examined by ultraviolet microscopy.

Statistical analysis. Means and standard deviations were estimated for those variables with a normal distribution. Median, 75th, and 25th percentiles were estimated for those variables without a normal distribution. The gold standard for the operative characteristics of each test was the Giemsa-stained thick blood film.[21–23] Two by two tables were used to calculate the sensitivity, specificity, and the positive [sensitivity/(1 − specificity)] and negative [(1 − sensitivity)/specificity] likelihoods. Likewise, a receiver operator characteristic curve (ROC) comparing different cut-off points was constructed. The Spearman rank correlation coefficient and its 95% confidence interval (two-tailed test) was used to measure the reliability of the ELISA. The Pearson correlation coefficient was also calculated for the different techniques.

RESULTS

Sensitivity, specificity, and reliability of the performed tests. The presence of antibodies to *P. falciparum* was investigated in sera from non-endemic (Caracas, n = 500) and endemic (Bolivar City, n = 500) areas. The IFA test median values expressed as reciprocal titer were 20 in both blood banks (ranges = Caracas 10–640 and Bolivar City = 10–320). The mean ± SD values for the IgG-ELISA and IgM-ELISA for sera from Caracas were 0.075 ± 0.040 and 0.245 ± 0.119 OD units, respectively, while the values for sera from Bolivar City were 0.072 ± 0.053 and 0.250 ± 0.112 OD units, respectively.

Several cut-off points were considered for each of the assays (Table 1) and the positive and negative likelihoods were calculated. Subsequently, an ROC curve was established for each test. As shown in Figure 1, the IFA assay was shown be the best test for screening, which at the cut-off point associated with a reciprocal titer of 40, had a specificity of 0.99 and a sensitivity of 0.80, followed by the IgG-ELISA, which at a cut-off point of 0.18, showed a specificity of 0.98 and a sensitivity of 0.68.

In addition, 50 sera that were positive by at least one of these methods (IFA assay or IgG-ELISA) and 50 negative sera (from the non-endemic area) were analyzed for the presence of antibodies to *P. falciparum* by the dot-IgG-ELISA. The median reciprocal titer for the dot-IgG-ELISA for the entire group (n = 100) was 500 (25th percentile = 500, 75th

TABLE 1
Likelihood values for the performed tests

Test	Cut-off point	Likelihood*	
		Positive	Negative
IFA†	20	22.0	0.13
	40	80.0	0.20
IgG ELISA‡	0.10	4.6	0.10
	0.13	10.9	0.26
	0.15	34.0	0.33
	0.18	34.0	0.33
IgM ELISA‡	0.250	2.0	0.21
	0.300	2.9	0.28
	0.400	10.4	0.51
	0.480	8.8	0.59
Dot-ELISA†	500	4.1	0.36
	1,000	8.1	0.38

* Positive = sensitivity/(1 − specificity). Negative = (1 − sensitivity)/specificity.
† IFA = indirect immunofluorescent antibody (test). Reciprocal titer (dilution).
‡ Optical density at 415 nm.

percentile = 2,000). The median reciprocal titer for sera from Caracas (n = 50) was 500 (25th percentile = 500, 75th percentile = 500).

Significant correlations were observed when comparisons were made between the IFA test and the IgG-ELISA (n = 1,000) (r = 0.609, 95% confidence interval [CI] = 0.568–0.648, *P* < 0.05) and the dot-IgG-ELISA and the IgG-ELISA (n = 98) (r = 0.687, 95% CI = 0.201–0.842, *P* < 0.01). A nonsignificant correlation was observed between the dot-IgG-ELISA and the IFA test. To determine the reliability of the ELISA, a sample of 54 sera were retested and the following results were obtained: IgG-ELISA (r = 0.987, 95% CI = 0.97–0.99, *P* < 0.0001); IgM-ELISA (r = 0.969, 95% CI = 0.95–0.98, *P* < 0.0001).

Presence of antibodies to *P. falciparum* in blood bank donors. Once the cut-off values for the techniques used were established, we determined the presence of antibodies to *P. falciparum* in blood bank sera from Caracas and Bolivar City. As shown in Table 2, specific antibodies were detected in 0.8% (n = 4) of the Caracas blood donors by the IFA test and the IgG-ELISA. In Bolivar City, 3.8% (n = 19) and 2% (n = 10) of the sera showed specific antibodies by both techniques, respectively.

Presence of antibodies to *P. falciparum* in malaria-infected patients. Patients with a diagnosis of malaria (n = 27; *P. falciparum* n = 15 and *P. vivax* n = 12) were investigated for the presence of antibodies to *P. falciparum* by the three described techniques. The median titer was 640 (reciprocal titer range = 20–5,120) for the IFA test and 10,000 (reciprocal titer range = 500–80,000) for the dot-IgG-ELISA. The mean ± SD value for the IgG-ELISA was 0.42 ± 0.41 OD units. A strong correlation was found between the IgG-ELISA and the dot-IgG-ELISA (r = 0.911), while a slight correlation was observed between the IFA test and the dot-IgG-ELISA.

Comparison between Western blot analysis and serologic antibody detection. Serum samples from negative and positive blood donors as well as *P. falciparum*- and/or *P. vivax*-infected patients and sera from patients with different parasitic infections were analyzed by Western blot. As shown in Figure 2, polypeptide bands with molecular weights ranging between 27 kD and 195 kD were observed
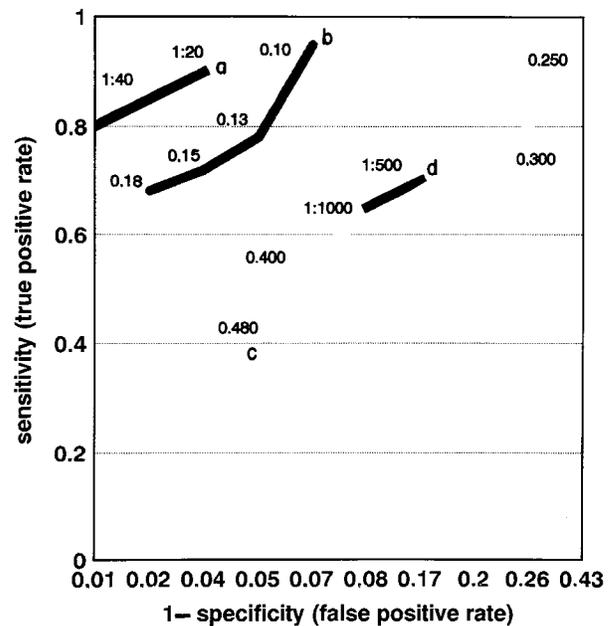

FIGURE 1. Receiver operator characteristic curves of the techniques used. Each point represents the relationship between the true-positive rate (sensitivity) and false-positive rate (1 − specificity). The best test is the one located at the top of the figure. The best cut-off point is the one located nearest to the top left of the respective curve. **a,** indirect immunofluorescent assay; **b,** IgG-ELISA; **c,** IgM-ELISA; **d,** dot-ELISA).

in the sera of positive blood donors (Caracas and Bolivar City) and in *P. falciparum*- and *P. vivax*-infected patients. As expected, high levels of antibodies to *P. falciparum* were also found in the corresponding samples by the three described techniques, as shown in Table 3. In contrast, no polypeptide bands were observed in normal blood donor serum or in serum from patients with three other parasitic infections.

DISCUSSION

Malaria is a devastating and uncontrolled disease and among the most prevalent parasitic infections in both tropical and subtropical underdeveloped countries. Although malaria in Venezuela was nearly eradicated in the early 1950s,[8] increasing poverty, uncontrolled gold and mineral exploitation, and dissemination into the country of malaria cases from neighboring countries have resulted in a progressive re-establishment of this disease in the last 15 years.

Transmission of malaria by blood or blood products re-

TABLE 2
Presence of anti-*Plasmodium falciparum* antibodies in areas of Venezuela non-endemic and endemic for malaria

	Caracas (Non-endemic) (n = 500)	Bolivar City (Endemic) (n = 500)
IFA*	0.8%	3.8%
IgG ELISA†	0.8%	2%

* IFA = indirect immunofluorescent antibody (test). Positive values >40 (reciprocal titer).
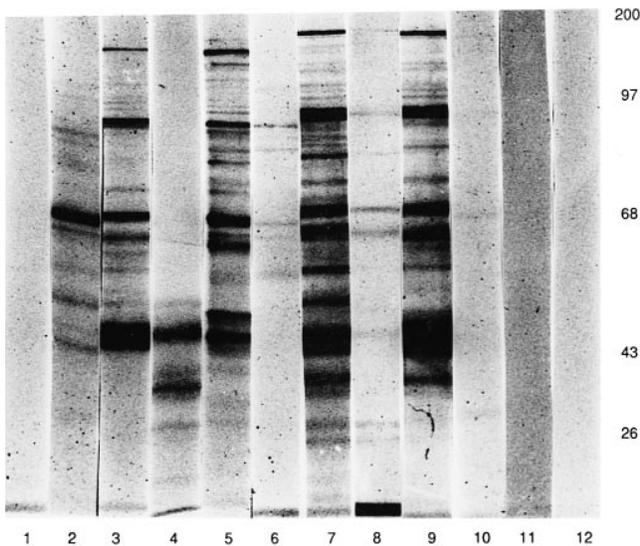† Positive values >0.2 optical density units.

FIGURE 2. Western blot analysis of sera from the study groups. Soluble *Plasmodium falciparum* antigens (5 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose. The filters were probed with the following: lane 1, normal human serum; 2, Caracas blood donor's positive serum; 3, Bolivar City blood donor's positive serum; 4–6, *P. vivax*-infected patient's sera; 7–9, *P. falciparum*-infected patient's sera; 10, *Trypanosoma cruzi* pooled infected patient's sera; 11, *Schistosoma mansoni*–infected patient's serum; 12, *Leishmania donovani*–infected patient's serum. The positions of molecular weight markers are indicated on the right: myosin (200 kD), phosphorylase B (97 kD), bovine serum albumin (68 kD), ovalbumin (43 kD), and α chymotrypsinogen (26 kD).

TABLE 3
Anti-*Plasmodium falciparum* antibody titers in sera analyzed by Western blot

Lane no.*	IFA†‡	IgG ELISA§	Dot ELISA‡
1. Seronegative blood donor	10	0.07	<500
2. Seropositive blood donor¶	640	0.380	5,000
3. Seropositive blood donor#	340	0.575	10,000
4. *P. vivax* patient's serum	640	0.258	5,000
5. *P. vivax* patient's serum	2,560	0.505	40,000
6. *P. vivax* patient's serum	80	0.117	<500
7. *P. falciparum* patient's serum	5,120	0.980	40,000
8. *P. falciparum* patient's serum	1,280	0.184	10,000
9. *P. falciparum* patient's serum	10,240	1.175	80,000
10. *Trypanosoma cruzi* pooled sera	10	0.180	<500
11. *Schistosoma mansoni* patient's serum	20	0.107	<500
12. *Leishmania donovani* patient's serum	10	0.071	<500

* As in Figure 2.
† IFA = indirect immunofluorescent antibody (test).
‡ Reciprocal titer.
§ Absorbance at 415 nm.
¶ Caracas.
# Bolivar City.

mains a real risk in most countries with this disease. Since blood donors are not routinely screened for malaria infection, the true incidence of post-transfusional malaria is unclear. Prevention of malaria by blood transfusion depends largely on the awareness of asymptomatic carriers previously exposed to the disease. The blood donor may have a discrete infection of 1–2 parasites/ml that cannot be detected by ordinary microscopy, yet a recipient of one unit of this blood would receive approximately half a million parasites.[9]

Estimation of the rate of risk of transfusion-transmitted malaria has been done in both developed and underdeveloped countries. Such comparative rates are much lower in developed countries (0.22 cases per million units of blood in the United Kingdom,[24] 0.25 cases per million units of blood in the United States,[25] 1–2 cases per million units of blood in France[24]) compared with 79 cases per million units of whole blood drawn in a non-endemic country such as Kuwait.[26] The presence of antibodies to *P. falciparum* detected by the ELISA was observed in 8.6 per thousand units of blood at the Hospital Militar Central in Bogota, Colombia.[27] Three cases of transfusion-induced malaria were confirmed during the same study.

The availability of molecular biological techniques for parasitologic diagnosis has already produced important improvements in detection of malaria parasites and is likely to have a major role in studies of malaria epidemiolgy.[10] DNA or RNA probes, as well as radioimmunoassays for antigen detection with improved sensitivity, may be valuable tools for malaria diagnosis. However, these techniques are not currently available for massive screenings such as for blood

bank donors. The most sensitive way to confirm a diagnosis of malaria is still thick or thin blood films examined by a competent specialist.[10] Likewise, in malaria serodiagnosis the most widely used technique is the IFA test. In fact, simplicity and satisfactory sensitivity are the main advantages of the IFA test. This test shows positive results as early as a day or two after parasitemia appears and decreases rapidly after treatment. Moreover, the test results become negative 3–6 months after the infection. However, this test also has disadvantages: it cannot be automated, which limits the number of sera that can be studied daily, readings can be influenced by subjectivity for weak positive results, and a fluorescent microscope is required.

Among other serologic techniques, the ELISA appears most promising due to its high sensitivity, which depends mainly on the purity of the reagents used. Furthermore, it can be automated. However, the range and limits of the ELISA are not well known, mainly because of the different types of soluble antigens used worldwide and geographic strain variability.

The present study is the first that attempted to detect *P. falciparum* carriers in the blood banks of Venezuela using serologic techniques as a preliminary step in prevention of post-transfusional malaria. In our investigations, we included a Venezuelan *P. falciparum* strain as a source of antigen to detect circulating antibodies to *Plasmodium* by an ELISA[11,28] and dot-ELISA[13] and compared their performance against an IFA technique.[14,20]

Sera were collected from blood banks located in areas of Venezuela with low (Caracas) and high (Bolivar State) malarial endemicity. Donors screening complied with the standards recommended by the American Association of Blood Banks.[29] Antibodies to *P. falciparum* were detected by the IFA test in 0.8% and 3.8% of the Caracas and Bolivar City blood donors, respectively. Similar results (0.8% in Caracas and 2% in Bolivar City) were observed with the IgG-ELISA. The presence of specific antibodies in 2% of the donors in Bolivar State represents a considerable risk of post-transfu-

sional malaria. Moreover, a 0.8% positive rate for the donors from a non-endemic area such as Caracas is surprising and reflects the mobility of the blood donors, the increasing risk of post-transfusional malaria in non-endemic areas, and the imminent need to measure the risk of transfusion-transmitted malaria in Venezuela or any other country.

Our results indicate that the IFA test is best for screening purposes.[10,14] As previously mentioned, this test at a reciprocal titer of 40 showed a specificity of 0.99 and a sensitivity of 0.80. The IgG-ELISA may be useful at two different cutoff points: one for screening (OD = 0.10) and another for diagnosis (OD = 0.2). However, among donors in the endemic area, an IgG-ELISA OD of 0.2 detected only 0.2% of the seropositive samples compared with 3.8% detected by the IFA test. These differences could be quite significant in a large population of donors. Conversely, both the IgM-ELISA and the dot-IgG-ELISA are not useful for diagnosis or for screening (Table 2) because of their calculated likelihoods at different cut-off points.

To confirm the specificity of the antibodies detected, selected sera were studied by Western blot. Sera of blood donors with confirmed antimalarial antibodies detected by the IFA test, IgG-ELISA, and dot-IgG-ELISA showed polypeptide bands in he presence of the *P. falciparum* antigenic preparation.[30] Similar bands (27–195 kD) were also detected in patients infected with either *P. falciparum* or *P. vivax,* indicating cross-reactivity that could be considered as an advantage since the purpose of donor screening is to detect all types of malaria parasites. In Bolivar State, the prevalence of *P. vivax* is higher than that of *P. falciparum* (62.3% versus 37.6%) and the patients could have had mixed infections at the time of the diagnosis. No cross-reactivity was observed when sera from patients infected with *T. cruzi, Schistosoma mansoni,* or *Leishmania donovani* were analyzed.

In conclusion, the combination of a screening process such as the one we established and an adequate medical history would represent an advance in transfusion safety, especially for patients with a compromised immune system or post-surgical patients in whom a transfusion-transmitted malaria could be fatal.[31] The establishment of standard guidelines for each technique is rather difficult and depends on the complexities of each situation. However, blood banks will continue to face the challenge of finding an acceptable balance between the limitation of transfusion-transmitted malaria and the assurance on an adequate supply of high-quality blood.[32] The inclusion of antigen detection[33] as part of the screening process for donated blood in the near future will hopefully reduce the risk of transfusion-transmitted diseases such as malaria.

Authors' address: Carmen Elena Contreras, Alena Pance, Norka Marcano, Nancy González, and Nicolás Bianco, Instituto de Inmunología, Apartado Postal 50109, Caracas 1050A, Venezuela.

REFERENCES

1. Bove JR, 1990. Transfusion-transmitted diseases other than AIDS and hepatitis. *Yale J Biol Med 63:* 347–351.
2. Klein HG, 1995. Allogenic transfusion risks in the surgical patient. *Am J Surg 170:* 215–265.
3. Woolsey G, 1911. Transfusion for pernicious anaemia: two cases. *Ann Surg 53:* 132–135.
4. Wylie BR, 1993. Transfusion transmitted infection: viral and exotic diseases. *Anaesth Intens Care 21:* 24–30.
5. Sazama K, 1991. Prevention of transfusion-transmitted malaria: is it time to revisit the standards? *Transfusion 31:* 786–788.
6. OPS, 1986. Control de la malaria en las Américas: análisis crítico. *Bull Oficina Sanit Panam 101:* 522–539.
7. WHO, 1997. World malaria situation in 1994. Part I. Population at risk. *Wkly Epidemiol Rec 72:* 269–274.
8. Gabaldon A, 1986. Malaria eradication in Venezuela: doctrine, practice and achievements after 20 years. *Am J Trop Med Hyg 32:* 203–211.
9. International Forum. 1987. Which are the appropriated modifications of existing regulations designed to prevent the transmission of malaria by blood transfusion in view of the increasing frequency of travel to endemic areas? *Vox Sang 52:* 138–148.
10. Chiodini PL, Moody AH, 1989. Techniques for the detection of malaria parasites. *J R Soc Med 17:* 41–43.
11. Voller A, Biowell B, Holdt G, Engvall E, 1974. A microplate method of enzyme-linked immunosorbent assay and its application to malaria. *Bull World Health Organ 51:* 209–211.
12. Voller A, Draper CC, 1982. Immunodiagnosis and seroepidemiology of malaria. *Br Med Bull 38:* 173–177.
13. Lodner MV, Revel A, Rosen G, Shehata MG, Kemawi MA, El Said S, Spira DT, 1988. Dot-ELISA, a potential immunoassay for the detection of *P. falciparum* antibodies. *Trans R Soc Trop Med Hyg 82:* 686–688.
14. Voller A, 1976. Serodiagnosis of malaria. Cohen S, Saoum S, eds. *Immunology of Parasite Infections.* Oxford: Blackwell, 107.
15. Trager W, Jensen JB, 1976. Human malaria parasites in continuous culture. *Science 193:* 673–675.
16. Lambros C, Vanderberg JP, 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J Parasitol 65:* 418–420.
17. Jensen JB, 1978. Concentration from continuous culture of erythrocytes infected with trophozoites and schizonts of *Plasmodium falciparum. Am J Trop Med Hyg 27:* 1274–1276.
18. Fibanks G, Steck T, Wallach D, 1971. Electrophoretic analysis of the major polypeptides of the human erythrocytes membrane. *Biochemistry 10:* 606–624.
19. Towbin H, Staehelin T, Gordon J, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA 76:* 4350–4354.
20. Contreras CE, Santiago JI, Jensen JB, Udeinya IJ, Kennedy DD, Druilhe P, 1988. RESA-IFA assay in *Plasmodium falciparum* malaria, observations on relationships between serum antibody titers, immunity and antigenic diversity. *J Parasitol 74:* 129–134.
21. Mayewski RJ, Mushlin AI, Griner PF, 1989. Principles of test selection and use in clinical diagnosis and the laboratory. Griner PF, Panzer RJ, Greenland P, eds. *Logical Strategies for Common Medical Problems.* Chapters 1 and 2. Chicago: Year Book Medical Publishers, Inc., 1–16 and 17–35.
22. Sackett D, Hayness RB, Tugwell P, eds. 1985. *Clinical Epidemiology. A Basic Science for Clinical Medicine.* Toronto: Little Brown and Company.
23. Daniel W, 1987. *Biostatistics: A Foundation for Analysis in the Health Sciences.* New York: John Wiley and Sons.
24. Bruce-Chwatt LT, 1982. Transfusion malaria revisited. *Trop Dis Bull 79:* 827–840.

25. Guerrero IC, Weniger BC, Schultz MG, 1983. Transfusion malaria in the United States, 1972–1981. *Ann. Intern Med 99:* 221–226.
26. Hira PR, Siboo R, Al-Kandari S, Behbehani K, 1987. Induced malaria and antibody titers in acute infections and in blood donors in Kuwait. *Trans R Soc Trop Med Hyg 81:* 391–394.
27. Espinal CA, Morales P, 1984. Indirect ELISA test for malaria in blood donors. *Trans R Soc Trop Med Hyg 78:* 645–647.
28. Aznar C, Liegeard P, Mariette C, Lafon S, Levin MJ, Hontebeyrie M, 1997. A simple *Trypanosoma cruzi* enzyme-linked immunoassay for control of human infection in nonendemic areas. *FEMS Immunol Med Microbiol 18:* 31–37.
29. Widman FK, ed., 1981. *Technical Manual.* Eighth edition. Arlington, VA: American Association of Blood Banks.
30. Holder AA, Freeman RR, 1982. Biosynthesis and processing of a *Plasmodium falciparum* schizont antigen recognized by immune serum and a monoclonal antibody. *J Exp Med 156:* 1528–1538.
31. Talabiska DG, Komar MJ, Wytock DH, Rubin RA, 1996. Posttransfusion acquired malaria complicating orthotopic liver transplantation. *Am J Gastroenterol 91:* 376–379.
32. Nahley BL, Lobel HO, Cannon SE, Campell CC, 1991. Reassessment of blood donor selection criteria for the United States travelers to malarious areas. *Transfusion 31:* 798–804.
33. Choudry N, Jolly JG, Ganguly NK, Mahajan RC, Dubey ML, 1991. Plasmodial antigen detection by monoclonal antibody as a screening procedure for blood donors in transfusion medicine. *J Indian Med Assoc 89:* 334–336.