IJC Heart & Vasculature 11 (2016) 12–16

Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: http://www.journals.elsevier.com/ijc-heart-and-vasculature

Cardiovascular changes in patients with non-severe *Plasmodium vivax* malaria☆

Aristoteles Comte Alencar-Filho [a,*], Joao Marcos Bemfica Barbosa Ferreira [b], Jorge Luis Salinas [c], Camila Fabbri [d], Wuelton Marcelo Monteiro [b,e], Andre Machado Siqueira [f], Katashi Okoshi [g], Marcus Vinicius Guimaraes Lacerda [b,h], Marina Politi Okoshi [g]

[a] *Amazonas Federal University, Manaus, Brazil*
[b] *Amazonas State University (UEA), Manaus, Brazil*
[c] *Emory University, Atlanta, United States*
[d] *North University Center, Pharmacy School, Manaus, Brazil*
[e] *Tropical Disease Center "Dr. Heitor Vieira Dourado", Manaus, Brazil*
[f] *National Institute of Infectology Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*
[g] *Botucatu Medical School, UNESP, Botucatu, Brazil*
[h] *Research Center Leonidas and Maria Deane, Fundação Oswaldo Cruz, Manaus, Brazil*

ARTICLE INFO

Article history:
Received 4 January 2016
Accepted 4 March 2016
Available online 10 March 2016

Keywords:
Echocardiogram
Ventricular function
Myocardial injury
Prognosis
Pulmonary artery pressure
Right ventricle

ABSTRACT

Background: Cardiovascular system involvement in patients with *Plasmodium vivax* malaria has been poorly addressed. The aim of this study was to evaluate cardiac structures and function, and serum markers of cardiovascular injury in patients with the non-severe form of vivax malaria in Manaus, Amazonas State, Brazil.
Methods and results: We prospectively evaluated 26 patients with vivax malaria in an outpatient referral hospital and compared results with a control group of 25 gender- and age-matched healthy individuals. Patients underwent clinical evaluation, laboratory tests, and transthoracic echocardiography at first evaluation (day zero, D0) and seven days (D7) after malaria diagnosis. At D0 echocardiography showed higher left ventricular (LV) systolic diameter (28.8 ± 2.82 vs 30.9 ± 4.03 mm; $p = 0.037$) and LV diastolic volume (82.4 ± 12.3 vs 93.8 ± 25.9 ml; $p = 0.05$), and lower LV ejection fraction (Teicholz method: 73.2 ± 6.59 vs 68.4 ± 4.87%; $p = 0.004$) in patients compared to controls. Right ventricle (RV) fractional area change (54.7 ± 5.11 vs 50.5 ± 6.71%; $p = 0.014$) was lower, and RV myocardial performance index (0.21 ± 0.07 vs 0.33 ± 0.19; $p = 0.007$), and pulmonary vascular resistance (1.13 ± 0.25 vs 1.32 ± 0.26 Woods unit; $p = 0.012$) were higher in patients than controls. Patients presented higher serum levels of unconjugated bilirubin (0.24 ± 0.15 vs 1.30 ± 0.89 mg/dL; $p < 0.001$), soluble vascular cell adhesion molecule–1 (sVCAM-1; 453 ± 143 vs 1983 ± 880 ng/mL; $p < 0.001$), N-terminal prohormone brain natriuretic peptide (0.59 ± 0.86 vs 1.08 ± 0.81 pg/mL; $p = 0.045$), and troponin T (861 ± 338 vs 1037 ± 264 pg/mL; $p = 0.045$), and lower levels of plasma nitrite (13.42 ± 8.15 vs 8.98 ± 3.97 μM; $p = 0.016$) than controls. Most alterations had reversed by D7.
Conclusion: Patients with non-severe *Plasmodium vivax* malaria present subclinical reversible cardiovascular changes.

1. Introduction

Malaria, a common parasitic disease affecting humans, is one of the most important public health issues in developing countries [1]. In 2013, there were 104 countries with endemic malaria, with approximately 198 million people affected and an estimated 584 thousand deaths [1].

Malaria pathophysiology has been extensively studied. However, since the first reports by Laveran [2] in 1884 describing myocardial and coronary changes in patients dying from malaria, few studies have carefully evaluated the cardiovascular system in malaria. These clinical and experimental studies have suggested that acute infection is accompanied by parasite sequestration and obstruction in microvascular coronary and myocardial injury caused by parasite released proteins as well as inflammatory cytokines and anemia [3–8]. More recently, falciparum malaria patients were shown to present endothelial

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

* Corresponding author at: Tropical Disease Center "Dr. Heitor Vieira Dourado", Av. Pedro Teixeira, n° 25. Dom Pedro, Manaus, AM 69040-000, Brazil.
E-mail address: aristoteles.caf@gmail.com (A.C. Alencar-Filho).

http://dx.doi.org/10.1016/j.ijcha.2016.03.004

dysfunction with impaired vascular nitric oxide bioavailability and increased pulmonary artery pressure [4,9].

All studies on cardiovascular involvement in malaria have been performed on *Plasmodium falciparum* malaria, which is related to the most severe form of the disease affecting several organs and systems [10]. Of the various Plasmodium species, *Plasmodium vivax* was previously considered to cause a benign non-fatal infection. However, in the last decade several reports have linked *P. vivax* to systemic complications involving the central nervous system, renal and respiratory failure, abnormal bleeding, anemia, and jaundice [11–16]. To the best of our knowledge, there are no studies analyzing the cardiovascular system during *P. vivax* malaria. In this study, we evaluated cardiac structures and function by Doppler-echocardiogram and plasmatic markers of cardiovascular injury in patients with the non-severe form of *P. vivax* malaria in Manaus, Amazonas State, Brazil.

2. Materials and methods

2.1. Study subjects

In a case–control study, we prospectively evaluated outpatients with *P. vivax* malaria attending the Dr. Heitor Vieira Dourado Tropical Medicine Foundation (FMT-HVD), in Manaus, Brazil, between December 2012 and March 2013. The FMT-HVD is a tertiary care center for infectious diseases, where patients can either seek attention directly or be referred for specialized care in neighboring municipalities. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by FMT-HVD Research Ethics Committee. All individuals signed the informed consent.

Patients aged 18–60 years were eligible to participate if they had no known illness and were diagnosed with *P. vivax* malaria to be treated out of hospital (*P. vivax* group, n = 26). Age and gender-matched individuals testing negative for malaria comprised the Control group (n = 25). The controls had similar economic conditions and lived in the same neighborhood as the patients. Exclusion criteria included electrocardiographic changes suggesting regional abnormalities, heart valve disease, and previously diagnosed severe diseases such as stage C heart failure, renal or liver insufficiency, cancer, pregnancy, malaria other than *P. vivax*, or severe malaria needing in-hospital treatment according to the World Health Organization [1]. All patients had positive thick blood smear and real-time qPCR assay for *P. vivax* malaria. Controls tested negative in both tests. Patients were treated with a combination of chloroquine and primaquine or a combination of artesunate and amodiaquine.

All individuals were subjected to the following: medical history evaluation, physical examination, 12-lead resting electrocardiogram, transthoracic Doppler-echocardiogram, and Laboratory investigation. In patients, clinical and laboratory evaluation was performed before treatment (day zero, D0) and seven days after starting treatment (day seven, D7). Medical history and physical examination were performed to assess general health and to clinically exclude diseases or conditions described in the exclusion criteria. Blood pressure was measured by the auscultatory technique with a conventional mercury sphygmomanometer.

2.2. Echocardiographic evaluation

A standard echocardiography system (General Electric Medical Systems, Vivid 3) was used to measure cardiac structures as previously described and following American Society of Echocardiography recommendations [17–20]. All echocardiograms were performed by the same examiner (JMBBF). With individuals positioned in left lateral decubitus position and monitored with an electrocardiographic lead, the following echocardiographic cuts were performed: short parasternal axis to measure ventricles, aorta and left atrium; apical 2, 4 and 5 chambers to evaluate ventricular cavities and systolic and diastolic function. The average of three measurements was calculated for each variable. The following left ventricular (LV) structures were measured by two-dimensional guided M-mode images: diastolic and systolic diameters (LVDD and LVSD, respectively), and diastolic and systolic volume (LVDV and LVSV, respectively). LV systolic function was evaluated by measuring ejection fraction according to the Teicholz index, endocardial fractional shortening, and myocardial performance index (Tei index) [21]. Right ventricle (RV) was structurally evaluated by measuring diastolic and systolic areas. RV systolic function was evaluated by fractional area change (FAC), and Tei index. Pulmonary vascular resistance (PVR) was estimated by Doppler echocardiography [22] according to the formula: PVR = tricuspid regurgitation peak velocity/right ventricular outflow tract velocity time integral) X 10 + 0.16. Pulmonary artery systolic pressure (PASP) was estimated by Doppler echocardiography using the modified Bernoulli equation [22]: PASP = 4 X (tricuspid regurgitation peak velocity) [2] + right atrial pressure. Right atrial pressure was estimated from inferior vena cava diameter [22].

2.3. Laboratorial analysis

Venous blood samples were obtained after a 12–15 h overnight fast in EDTA-coated tubes. Plasma was frozen at −80 °C in tubes containing 5 μL/mL antioxidant butyl hydroxytoluene (BHT, 20 μM), proteases inhibitor (aprotinin, 2 mg/mL), phenylmethylsulphonyl fluoride (PMSF, 1 mM), and benzamidine (2 mM). Nitrite plasma concentration was quantified by colorimetry using a commercially available nitric oxide assay kit (Cayman, Chemical Company, AnnArbor, Michigan, USA). Concentrations of N-terminal prohormone brain natriuretic peptide (NT-proBNP) and troponin T were measured by ELISA using commercially available kits (USCN Life Science Inc., Houston, Texas, USA). Soluble vascular cell adhesion molecule (sVCAM)-1 concentration was analyzed by immunoassay using a commercially kit (R&D Systems, Inc., Minneapolis, Minnesota, USA). All kits used in this study are available for laboratory research use only, not for human diagnostics.

Subjects were tested for malaria by thick blood smear. Parasite density was calculated by the arithmetic mean of two concordant readings; the white blood cell count was obtained from total blood count analysis as previously described [23]. In case of discordance (species-specific, or in the density quantification whenever a discrepancy was higher than 10%), a third reading was performed by a senior investigator (WMM). Real-time qPCR was performed as previously described [24] to confirm *P. vivax* malaria.

2.4. Statistical analysis

Variables are presented as mean and standard deviation or median and minimum and maximum values. Comparisons between periods were performed by Student's *t* test for dependent data and comparisons between groups were performed by unpaired Student's *t* test. Categorical parameters were compared by Fisher's exact test. The association between variables was assessed with Pearson's correlation coefficient. The level of significance was 5%. Statistical analyses were performed using IBM SPSS Statistics software Version 21.

3. Results

Baseline characteristics for controls and patients at day zero (D0) are presented in Table 1. Heart rate, although within the normal range, was higher in *P. vivax* group than Controls.

The *P. vivax* group had a mean peripheral parasitemia of 2844 ± 3286 parasites/mm^3, ranging from 87 to 11,806 parasites/mm^3. Laboratory data are shown in Table 2. *P. vivax* D0 had increased plasma concentrations of unconjugated bilirubin, troponin T, NT-proBNP, and sVCAM-1 and decreased platelet count and nitrite levels compared to Controls. At D7, unconjugated bilirubin, troponin T, NT-proBNP, and sVCAM-1 were lower, and platelet count and nitrite levels higher than D0. Sixty

Table 1
Baseline characteristics at day zero (D0).

	Control (n = 25)	*P. vivax* (n = 26)	P-value*
Age, years	44.5 ± 8.43	41.7 ± 13.7	0.37
Female, %	44.0	30.8	0.39*
Height, m	1.67 ± 0.07	1.67 ± 0.08	0.99
Body weight, kg	79.3 ± 15.0	79.0 ± 13.9	0.87
BMI	28.3 ± 4.54	28.2 ± 5.07	0.93
Systolic blood pressure, mm Hg	121 ± 11.6	121 ± 12.2	0.98
Diastolic blood pressure, mm Hg	79 ± 8.6	78 ± 8.1	0.69
Heart rate, bpm	66 ± 7.6	76 ± 13.2	0.02

Data are mean and standard deviation or frequency. BMI: body-mass index (weight in kilograms divided by the square of height in meters); and bpm: beats/min. Unpaired Student's *t* test or Fisher's exact test (*).

five percent of malaria patients had less than 150,000 platelets/μL at D0; no patient presented clinical bleeding or severe thrombocytopenia (<50,000 platelets/μL).

LV echocardiographic data are shown in Table 3. At D0, the *P. vivax* group presented higher LV systolic diameter and lower ejection fraction and endocardial fractional shortening than Controls. LV variables did not significantly differed between D7 and D0 in the *P. vivax* group. RV data are shown in Table 4. At D0, the *P. vivax* group had higher RV diastolic and systolic area, Tei index, and pulmonary vascular resistance, and lower fractional area change than Controls. In *P. vivax* patients, fractional area change was higher and pulmonary vascular resistance lower in D7 than D0. At D0, NT-proBNP levels positively correlated with RV diastolic ($r = 0.409$; $P = 0.038$) and systolic ($r = 0.435$; $P = 0.026$) areas and with Tei index ($r = 0.493$; $P = 0.01$) and pulmonary vascular resistance positively correlated with RV Tei index ($r = 0.646$; $P < 0.001$) in malaria patients.

4. Discussion

In this study we evaluated cardiac structures and function by Doppler echocardiography and systemic markers of cardiovascular injury in patients with the non-severe form of *P. vivax* malaria at the beginning of infection and seven days later.

Malaria was diagnosed by examining thick blood smears and *P. vivax* malaria was confirmed by real-time qPCR assay. As jaundice is a diagnosis criteria for severe malaria, no icteric patient was included in this study. Nonetheless, mean unconjugated bilirubin values were higher at D0 in *P. vivax* than Controls suggesting a low degree of hemolysis. Malaria patients had a slight decrease in platelet count at D0, which is commonly observed in vivax malaria and may be related to platelet phagocytosis [25,26]. Platelet counting often normalizes after treatment [25].

As markers of myocardial injury, we evaluated plasma concentrations of troponin T and NT-proBNP, which were higher at D0 in *P. vivax* than Controls and D7 *P. vivax*. The troponin T levels in our malaria patients suggest a slight degree of myocardial injury. NT-proBNP concentration is often used for heart failure diagnosis and prognosis. More recently, it has also been considered a good biomarker for pulmonary arterial hypertension and right ventricular dysfunction [27]. The higher NT-proBNP levels in D0 *P. vivax* than Controls is probably related to acute dilation of the left and right ventricles.

Table 3
Left ventricular echocardiographic data.

	Control (n = 25)	*P. vivax* D0 (n = 26)	*P. vivax* D7 (n = 26)	P-Value	
				P. vivax D0 X Control	*P. vivax* D7 X D0
LVSD, mm	28.8 ± 2.82	30.9 ± 4.03	30.1 ± 3.04	0.037	0.46
LVDV, mL	82.4 ± 12.3	93.8 ± 25.9	91.1 ± 19.8	0.050	0.67
EF, %	73.2 ± 6.59	68.4 ± 4.87	70.3 ± 4.78	0.004	0.15
EFS, %	42.8 ± 5.81	38.4 ± 3.28	40.2 ± 4.07	0.003	0.11
Tei index	0.31 ± 0.64	0.36 ± 0.12	0.35 ± 0.10	0.88	0.70

Data are mean and standard deviation. D0: day zero; D7: day seven; LVSD: left ventricular (LV) systolic diameter; LVDV: LV diastolic volume; EF: ejection fraction; EFS: LV endocardial fractional shortening. Unpaired Student's *t* test *P. vivax* D0 vs Control; and paired Student's *t* test *P. vivax* D7 vs *P. vivax* D0.

Soluble vascular cell adhesion molecule-1 contributes to endothelial activation and dysfunction, which are involved in the pathogenesis of several infectious diseases [28]. In hemolytic conditions such as sickle-cell anemia and malaria, sVCAM-1 levels are usually increased [28]. It facilitates adhesion of infected and non-infected blood red cells to endothelium, resulting in fibrin and platelet deposition, and microvascular sequestration and obstruction [29–31]. Expression of sVCAM-1 may be induced by proinflammatory cytokines and nitric oxide depletion [4,32]. Nitric oxide depletion also impairs vasodilation [33]. In this study, plasma concentration of sVCAM-1 was higher and nitrite levels lower in D0 *P. vivax* than Controls. Similar results were found in children with falciparum malaria [4] and endothelial activation was previously observed in uncomplicated vivax malaria [34]. Nitrite is considered as a physiological storage pool of nitric oxide that can be reduced to bioactive nitric oxide in hypoxic conditions to mediate physiological responses in blood and tissue [35]. D7 sVCAM-1 was lower and nitrite higher than at D0 in malaria patients.

In this study, the *P. vivax* group presented slight left ventricular dilation with reduced systolic function compared to Controls at D0. Left ventricular echocardiographic parameters did not significantly differ between D7 and D0 in the *P. vivax* group. At D0, *P. vivax* group presented right ventricular dilation, characterized by increased diastolic and systolic areas, and right ventricular dysfunction, characterized by reduced fractional area change and increased Tei index, with increased pulmonary vascular resistance. Fractional area change has been used to evaluate right ventricular systolic function, presenting a good correlation with ejection fraction estimated by cardiac magnetic resonance imaging [36]. Furthermore, in this study, right ventricular diastolic and systolic area and Tei index positively correlated with NT-proBNP levels. Alterations in right ventricle function and pulmonary vascular resistance were reversible after treatment, as suggested by the higher fractional area change and the lower pulmonary vascular resistance at D7 compared to D0. Right ventricular dilation may be the first indicator of an acute increase in pulmonary vascular resistance; and increased pulmonary vascular resistance can be related to vasoconstriction, inflammation, and obstruction of pulmonary arteries in malaria [22,37].

Table 2
Laboratorial data.

	Control (n = 25)	*P. vivax* D0 (n = 26)	*P. vivax* D7 (n = 26)	P-Value	
				P. vivax D0 X Control	P. vivax D7 X D0
UCB, mg/dL	0.24 ± 0.15	1.32 ± 0.89	0.33 ± 0.15	<0.001	<0.001
Platelets, X 1000 cells/mm^3	281 ± 64	145 ± 60	341 ± 77	<0.001	<0.001
Troponin T, pg/mL	861 ± 338	1037 ± 264	784 ± 249	0.045	<0.001
NT-proBNP, pg/mL	0.59 ± 0.86	1.08 ± 0.81	0.63 ± 0.51	0.045	0.027
sVCAM-1, ng/mL	453 ± 143	1983 ± 880	849 ± 467	<0.001	<0.001
Nitrite, μM	13.4 ± 8.15	8.98 ± 3.97	12.3 ± 5.30	0.016	0.014

Data are mean and standard deviation. D0: day zero; D7: day seven; UCB: unconjugated bilirubin; NT-proBNP: N-terminal prohormone brain natriuretic peptide; sVCAM-1: soluble vascular cell adhesion molecule; unpaired Student's *t* test *P. vivax* D0 vs Control; and paired Student's *t* test *P. vivax* D7 vs *P. vivax* D0.

Table 4
Right ventricular echocardiographic data.

	Control (n = 25)	*P. vivax* D0 (n = 26)	*P. vivax* D7 (n = 26)	P-Value	
				P. vivax D0 X Control	*P. vivax* D7 X D0
DA, cm^2	13.0 ± 3.19	15.3 ± 2.96	15.1 ± 2.55	0.009	0.762
SA, cm^2	6.41 ± 1.27	7.45 ± 1.46	6.96 ± 1.41	0.009	0.220
FAC, %	54.7 ± 5.11	50.5 ± 6.71	55.7 ± 6.90	0.014	0.007
Tei index	0.21 ± 0.07	0.33 ± 0.19	0.27 ± 0.10	0.007	0.170
PVR, Wood units	1.13 ± 0.25	1.32 ± 0.26	1.17 ± 0.19	0.012	0.031
PASP, mm Hg	19.2 ± 2.95	19.7 ± 3.05	19.8 ± 4.19	0.576	0.922

Data are mean and standard deviation. D0: day zero; D7: day seven; DA: diastolic area; SA: systolic area; FAC: fractional area change; PVR: pulmonary vascular resistance; PASP: pulmonary artery systolic pressure. Unpaired Student's *t* test *P. vivax* D0 vs Control; and paired Student's *t* test *P. vivax* D7 vs *P. vivax* D0.

We have not identified any other studies in literature evaluating cardiac structures and function in patients with vivax malaria. Our data suggest that outpatients with *P. vivax* malaria present subclinical cardiovascular changes and allow us to hypothesize that events previously described in severe falciparum malaria [4,9,38,39] also occur in non-severe vivax malaria. Products from low grade hemolysis of parasitized red blood cells induce a reduction in plasma nitrite, which is related with nitric oxide bioavailability. Increased expression of cellular adhesion molecules such as sVCAM-1 facilitates adherence to vascular endothelium and destruction of infected reticulocytes and non-infected erythrocytes. Nitric oxide depletion decreases vasodilation therefore increasing pulmonary vascular resistance and impairing right ventricular function. Additionally, left ventricular dilation and dysfunction may result from infection, depleted nitric oxide-induced increase in afterload, and/or microvascular obstruction. The increase in NT-proBNP, suggesting dilation of cardiac chambers, and troponin T, suggesting myocyte injury, reinforces myocardial injury during *P. vivax* malaria. Most alterations are reversible seven days after treatment.

5. Limitations

This study evaluated a small sized sample of outpatients with vivax malaria, which allowed us to raise a hypothesis on the pathophysiological events involved in cardiovascular changes. Therefore, additional studies are needed to evaluate a larger sample of both *P. vivax* malaria in- and out-patients in order to confirm our results and to extend the understanding of vivax malaria to patients with the severe form of the disease.

In conclusion, patients with non-severe *P. vivax* malaria present subclinical cardiovascular changes.

Conflicts of interest

The authors report no conflicts of interest.

Acknowledgements

We are grateful to Colin Edward Knaggs for English editing.

Financial support was provided by CNPq (306857/2012-0, 306845/2012-1, and 479085/2013-7).

References

[1] WHO, World Malaria Report 2014, World Health Organization, Geneva, 2014.
[2] A. Laveran, Traité des fiévres palustres, Octave Doinc, Paris, 1884.
[3] S. Ehrhardt, D. Wichmann, C.J. Hemmer, G.D. Burchard, N.W. Brattig, Circulating concentrations of cardiac proteins in complicated and uncomplicated *Plasmodium falciparum* malaria, Tropical Med. Int. Health 9 (2004) 1099–1103.
[4] J.J. Janka, O.A. Koita, B. Traore, J.M. Traore, F. Mzayek, V. Sachdev, X. Wang, K. Sanogo, L. Sangare, L. Mendelsohn, H. Masur, G.J. Kato, M.T. Gladwin, D.J. Krogstad, Increased pulmonary pressures and myocardial wall stress in children with severe malaria, J. Infect. Dis. 202 (2010) 791–800.
[5] D. Franzen, J.M. Curtius, W. Heitz, H.W. Hopp, V. Diehl, H.H. Hilger, Cardiac involvement during and after malaria, Clin. Investig. 70 (1992) 670–673.
[6] K. Wennicke, F. Debierre-Grockiego, D. Wichmann, N.W. Brattig, S. Pankuweit, B. Maisch, R.T. Schwarz, V. Ruppert, Glycosylphosphatidylinositol-induced cardiac myocyte death might contribute to the fatal outcome of *Plasmodium falciparum* malaria, Apoptosis 13 (2008) 857–866.
[7] G.L. Genrich, J. Guarner, C.D. Paddock, W.J. Shieh, P.W. Greer, J.W. Barnwell, S.R. Zaki, Fatal malaria infection in travelers: novel immunohistochemical assays for the detection of *Plasmodium falciparum* in tissues and implications for pathogenesis, Am.J.Trop. Med. Hyg. 76 (2007) 251–259.
[8] A. Gunther, M.P. Grobusch, H. Slevogt, W. Abel, G.D. Burchard, Myocardial damage in falciparum malaria detectable by cardiac troponin T is rare, Tropical Med. Int. Health 8 (2003) 30–32.
[9] T.W. Yeo, D.A. Lampah, E. Tjitra, R. Gitawati, C.J. Darcy, C. Jones, E. Kenangalem, Y.R. McNeil, D.L. Granger, B.K. Lopansri, J.B. Weinberg, R.N. Price, S.B. Duffull, D.S. Celermajer, N.M. Anstey, Increased asymmetric dimethylarginine in severe falciparum malaria: association with impaired nitric oxide bioavailability and fatal outcome, PLoS Pathog. 6 (2010) e1000868.
[10] S.C. Wassmer, T.E. Taylor, P.K. Rathod, S.K. Mishra, S. Mohanty, M. Arevalo-Herrera, M.T. Duraisingh, J.D. Smith, Investigating the pathogenesis of severe malaria: a multidisciplinary and cross-geographical approach, Am.J.Trop. Med. Hyg. 93 (2015) 42–56.
[11] B.E. Barber, T. William, M.J. Grigg, U. Parameswaran, K.A. Piera, R.N. Price, T.W. Yeo, N.M. Anstey, Parasite biomass-related inflammation, endothelial activation, microvascular dysfunction and disease severity in vivax malaria, PLoS Pathog. 11 (2015) e1004558.
[12] V.R. Mendonça, L.C. Souza, G.C. Garcia, B.M. Magalhães, M.S. Gonçalves, M.V. Lacerda, M. Barral-Netto, Associations between hepcidin and immune response in individuals with hyperbilirubinaemia and severe malaria due to *Plasmodium vivax* infection, Malar. J. 14 (2015) 407.
[13] A.M. Siqueira, M.V. Lacerda, B.M. Magalhães, M.P. Mourão, G.C. Melo, M.A. Alexandre, M.G. Alecrim, D. Kochar, S. Kochar, A. Kochar, K. Nayak, H. del Portillo, C. Guinovart, P. Alonso, Q. Bassat, Characterization of *Plasmodium vivax*-associated admissions to reference hospitals in Brazil and India, BMC Med. 13 (2015) 57.
[14] M.V. Lacerda, S.C. Fragoso, M.G. Alecrim, M.A. Alexandre, B.M. Magalhães, A.M. Siqueira, L.C. Ferreira, J.R. Araújo, M.P. Mourão, M. Ferrer, P. Castillo, L. Martin-Jaular, C. Fernandez-Becerra, H. del Portillo, J. Ordi, P.L. Alonso, Q. Bassat, Postmortem characterization of patients with clinical diagnosis of *Plasmodium vivax* malaria: to what extent does this parasite kill? Clin. Infect. Dis. 55 (2012) e67–e74.
[15] D.K. Kochar, V. Saxena, N. Singh, S.K. Kochar, S.V. Kumar, A. Das, *Plasmodium vivax* malaria, Emerg. Infect. Dis. 11 (2005) 132–134.
[16] E.F. Lança, B.M. Magalhães, S. Vitor-Silva, A.M. Siqueira, S.G. Benzecry, M.A. Alexandre, C. O'Brien, Q. Bassat, M.V. Lacerda, Risk factors and characterization of plasmodium vivax-associated admissions to pediatric intensive care units in the Brazilian Amazon, PLoS ONE 7 (2012) e35406.
[17] R.M. Lang, M. Bierig, R.B. Devereux, F.A. Flachskampf, E. Foster, P.A. Pellikka, M.H. Picard, M.J. Roman, J. Seward, J.S. Shanewise, S.D. Solomon, K.T. Spencer, M.S.J. Sutton, W.J. Stewart, Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology, J. Am. Soc. Echocardiogr. 18 (2005) 1440–1463.
[18] G.N. Guirado, R.L. Damatto, B.B. Matsubara, M.G. Roscani, D.R. Fusco, L.A.F. Cicchetto, M.M. Seki, A.S. Teixeira, A.P. Valle, K. Okoshi, M.P. Okoshi, Combined exercise training in asymptomatic elderly with controlled hypertension: effects on functional capacity and cardiac diastolic function, Med. Sci. Monit. 18 (2012) CR461–CR465.
[19] A.S. Moro, M.P. Okoshi, C.R. Padovani, K. Okoshi, Doppler echocardiography in athletes from different sports, Med. Sci. Monit. 19 (2013) 187–193.
[20] A. Rodrigues, F.V. Guimarães-Filho, J.C. Braga, C.S. Rodrigues, P. Waib, A. Fabron-Junior, D.M. Tan, A.C. França, M.P. Okoshi, K. Okoshi, Echocardiography in thalassemic patients on blood transfusions and chelation without heart failure, Arq. Bras. Cardiol. 100 (2013) 75–81.
[21] C. Tei, R.A. Nishimura, J.B. Seward, A.J. Tajik, Noninvasive doppler-derived myocardial performance index: correlation with simultaneous measurements of cardiac catheterization measurements, J. Am. Soc. Echocardiogr. 10 (1997) 169–178.
[22] L.G. Rudski, W.W. Lai, J. Afilalo, L. Hua, M.D. Handschumacher, K. Chandrasekaran, S.D. Solomon, E.K. Louie, N.B. Schiller, Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography, J. Am. Soc. Echocardiogr. 23 (2010) 685–713.
[23] World Health Organization, Basic Malaria Microscopy, second ed. World Health Organization, Geneva, 2010.
[24] F. Perandin, N. Manca, A. Calderaro, G. Piccolo, L. Galati, L. Ricci, M.C. Medici, M.C. Arcangeletti, G. Snounou, G. Dettori, C. Chezzi, Development of a real-time pcr assay for detection of *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium ovale* for routine clinical diagnosis, J. Clin. Microbiol. 42 (2004) 1214–1219.
[25] M.V. Lacerda, M.P. Mourão, H.C. Coelho, J.B. Santos, Thrombocytopenia in malaria: who cares? Mem. Inst. Oswaldo Cruz 106 (2011) 52–63.
[26] H.C. Coelho, S.C. Lopes, J.P. Pimentel, P.A. Nogueira, F.T. Costa, A.M. Siqueira, G.C. Melo, W.M. Monteiro, A. Malheiro, M.V. Lacerda, Thrombocytopenia in *Plasmodium vivax* malaria is related to platelets phagocytosis, PLoS ONE 8 (2013) e63410.
[27] V. Panagopoulou, S. Deftereos, C. Kossyvakis, K. Raisakis, G. Giannopoulos, G. Bouras, V. Pyrgakis, M.W. Cleman, NTproBNP: an important biomarker in cardiac diseases, Curr. Top. Med. Chem. 13 (2013) 82–94.

[28] A.V. Page, W.C. Liles, Biomarkers of endothelial activation/dysfunction in infectious diseases, Virulence 4 (2013) 507–516.
[29] E. Pongponratn, G.D. Turner, N.P. Day, N.H. Phu, J.A. Simpson, K. Stepniewska, N.T. Mai, P. Viriyavejakul, S. Looareesuwan, T.T. Hien, D.J. Ferguson, N.J. White, An ultrastructural study of the brain in fatal *Plasmodium falciparum* malaria, Am.J.Trop. Med. Hyg. 69 (2003) 345–359.
[30] Q. Bassat, P.L. Alonso, Defying malaria: fathoming severe *Plasmodium vivax* disease, Nat. Med. 17 (2011) 48–49.
[31] S.J. Rogerson, G.E. Grau, N.H. Hunt, The microcirculation in severe malaria, Microcirculation 11 (2004) 559–576.
[32] G.J. Kato, S. Martyr, W.C. Blackwelder, J.S. Nichols, W.A. Coles, L.A. Hunter, M.L. Brennan, S.L. Hazen, M.T. Gladwin, Levels of soluble endothelium-derived adhesion molecules in patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction, and mortality, Br. J. Haematol. 130 (2005) 943–953.
[33] P. Sobolewski, I. Gramaglia, J. Frangos, M. Intaglietta, H.C. van der Heyde, Nitric oxide bioavailability in malaria, Trends Parasitol. 21 (2005) 415–422.
[34] T.W. Yeo, D.A. Lampah, E. Tjitra, K. Piera, R. Gitawati, E. Kenangalem, R.N. Price, N.M. Anstey, Greater endothelial activation, weibel-palade body release and host inflammatory response to *Plasmodium vivax*, compared with *Plasmodium falciparum*: a prospective study in Papua, Indonesia, J. Infect. Dis. 202 (2010) 109–112.
[35] S. Shiva, Nitrite: a physiological store of nitric oxide and modulator of mitochondrial function, Redox Biol. 1 (2013) 40–44.
[36] N.S. Anavekar, D. Gerson, H. Skali, R.Y. Kwong, E.K. Yucel, S.D. Solomon, Two-dimensional assessment of right ventricular function: an echocardiographic-MRI correlative study, Echocardiography 24 (2007) 452–456.
[37] N. Galie, M. Humbert, J.L. Vachiery, S. Gibbs, I. Lang, A. Torbicki, G. Simonneau, A. Peacock, A. Vonk Noordegraaf, M. Beghetti, A. Ghofrani, M.A. Gomez Sanchez, G. Hansmann, W. Klepetko, P. Lancellotti, M. Matucci, T. McDonagh, L.A. Pierard, P.T. Trindade, M. Zompatori, M. Hoeper, 2015 ESC/ERS Guidelines for the Diagnosis and Treatment of Pulmonary Hypertension: The joint task force for the diagnosis and treatment of pulmonary hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS) endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT), Eur. Heart J. 37 (2016) 67–119.
[38] T.W. Yeo, D.A. Lampah, R. Gitawati, E. Tjitra, E. Kenangalem, K. Piera, R.N. Price, S.B. Duffull, D.S. Celermajer, N.M. Anstey, Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 17097–17102.
[39] T.W. Yeo, D.A. Lampah, E. Kenangalem, E. Tjitra, J.B. Weinberg, D.L. Granger, R.N. Price, N.M. Anstey, Decreased endothelial nitric oxide bioavailability, impaired microvascular function, and increased tissue oxygen consumption in children with falciparum malaria, J. Infect. Dis. 210 (2014) 1627–1632.