Aspartate aminotransferase to platelet ratio index (APRI) for differentiation of primary and secondary infection by dengue virus

Aplicação do índice de relação aspartato aminotransferase sobre plaquetas (APRI) na diferenciação de infecção primária e secundária pelo vírus da dengue

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ABSTRACT

Introduction: Dengue virus (DENV) infection has been considered a major public health problem in tropical countries. The unavailability of serologic testing in public health centers might adversely impact patients' outcome. **Objective:** This study aimed to evaluate the accuracy of mean platelet volume (MPV) and aspartate aminotransferase (AST) to platelet ratio index (APRI) as laboratory markers of DENV infection that could be used to differentiate primary and secondary infections. **Methods:** We assessed laboratory results from 503 patients with positive rapid test for DENV infection. **Results:** Severe thrombocytopenia and increased liver involvement were observed in patients with DENV heterotypic secondary infection. Our data suggest that APRI was able to distinguish patients with primary and secondary infection ($p = 0.006$) with a relevant sensitivity (75%), specificity (76%) and a cut-off of 1.06. A total of 80 out of 105 (76%) patients with primary DENV infection had APRI ≤ 1.06, and 12 (75%) with secondary DENV infection had APRI > 1.06. On the other hand, MPV did not show significance in the differentiation of types of infection, coming up with poor area under the receiver operating characteristic (ROC) curve (0.61). **Conclusion:** APRI seems to be a powerful tool for early identification of DENV secondary infection cases in health centers.

Key words: dengue; aspartate aminotransferases; diagnosis; platelet count.

INTRODUCTION

Dengue virus (DENV) infection is a global public health problem and a human vector-borne viral disease. According to the World Health Organization (WHO), cases across the Western Pacific, South-East Asia and Americas reached over 3.2 millions in 2015. Brazil had the highest number of infection episodes in 2015, with most cases being DENV 1 infection. That figure was approximately three times higher than that in 2014[1-3]. Besides, the total cases that require hospitalization increase each year, and many of them evolve to death[4].

Dengue is an arboviral infection represented by four distinct serotypes (DENV 1 to 4) and transmitted by *Aedes* ssp., mainly *Aedes aegypti*, broadly found in tropical and subtropical regions[1]. The immune response arising from one serotype can confer life-long protection against that same serotype, but not against the others, leading to the possibility of secondary infections. Clinically, dengue infection may vary from asymptomatic infection to severe dengue clinical presentation and even death[5, 6]. The more severe types of dengue infection, known as dengue hemorrhagic fever/ dengue shock syndrome (DHF/DSS), are commonly caused by secondary infection, when an individual with a previous history of dengue is subsequently infected with a second virus serotype. DHF/ DSS is characterized by coagulopathy, increased vascular fragility, and loss of fluid due to capillary permeability that may progress to hypovolemic shock[7].

The DENV nonstructural glycoprotein 1 (NS1) plays an important role in replication, pathogenesis and modulation of immune response, although its functions are not completely

understood. The NS1 antigen is released abundantly in circulation, and its presence is detected in blood from the 1st to the 9th day of infection[8]. Anti-NS1 antibodies [immunoglobulin class M (IgM) type] may develop cross-reaction with platelets in dengue infection and, consequently, contribute to a transient thrombocytopenia. Furthermore, it was shown that infection by DENV in hematopoietic cells may impair megakaryocytopoiesis in mice[9]. Serum levels of NS1 antigen provide a preliminary indication of DENV and represent a new approach to the diagnosis of acute dengue infection. However, its role as an early predictor of severe dengue infection remains unclear.

The viral culture assay and viral ribonucleic acid (RNA) detection by reverse transcriptase polymerase chain reaction (RT-PCR) are considered more specific tests to confirm infection, as they detect viral dengue particles. However, these tests require highly trained staff and sophisticated equipment, besides being time consuming and costly, what limits their routine applicability in health units of developing countries[10, 11]. For this reason, cheaper and simpler tests are urgently needed so they can be available in health services of such countries.

Alternative laboratory tests have been used in screening and monitoring the evolution of viral infection, such as tourniquet test, blood count, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[12]. These tests are cheaper and widely available in health services when compared with the gold standard diagnostic tests.

The rapid test for DENV infection has been largely used in Brazilian health units as an alternative diagnostic test. It is based on a qualitative and differential diagnosis with detection of IgM and IgG DENV antibody in human serum or plasma, enabling the detection of primary and secondary infection, respectively[13].

Once DHF/DSS are associated with severe thrombocytopenia, bleeding and increased vascular permeability, the decreasing platelet counts have been found to predict the severity of the disease. Platelet count is also used as a recovery parameter for these conditions[14]. Mean platelet volume (MPV) is a geometric measure that assesses the size of platelets circulating in the plasma. It is often used to indicate platelet activation and, indirectly, bone marrow activity[15, 16]. Not much work has been done on this parameter related to DENV infection. AST to platelet ratio index (APRI) includes AST and platelet count as variables. It is calculated as (AST/upper limit of normal range)/platelet count (10^9/l) $\times$ 100. This index was firstly proposed to predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C[16]. Once viral replication can affect liver function, and DENV infection is associated with thrombocytopenia, APRI test

could be useful for early infection diagnosis. No previous studies have been performed that relate APRI and DENV infection.

Despite the several studies investigating DENV and its multiple sites of infection, such as endothelial cells, liver cells, bone marrow, spleen, kidneys and lungs, the pathophysiology of this disease is still not fully understood[17-19]. Due to variation of clinical manifestations in DENV infections and the multifactorial nature of the host response to the pathogen[1], it becomes necessary to find new biomarkers that can indicate the infection severity and the involvement of other organs. Such biomarkers could guide better management approach and result in better outcomes for patients. In addition, due to the increasing number of cases of DENV infection and the low availability of gold standard diagnostic tests in health units of developing countries, cheaper and simpler tests are urgently needed. In this context, we aim to evaluate the accuracy of MPV and APRI as laboratory markers of DENV infection that could be used to differentiate primary and secondary infections with a better cost-benefit than current gold standard tests.

METHODS

Ethical aspects

This study was approved by the Ethics Committee of Universidade Federal de Minas Gerais (UFMG), Brazil (CAAE 61330316.6.0000.5149), and it was conducted in accordance with the Helsinki Declaration. The research protocol did not interfere with any medical recommendations or prescriptions.

Study design

We assessed laboratory results from 503 patients with positive rapid test for DENV infection. These patients were treated in outpatient units of a reference hospital in the metropolitan area of Belo Horizonte (MG), Brazil, from January to July 2015.

Rapid tests for dengue were performed with an immunochromatographic kit (Dengue fever test duo – Bioeasy® Diagnostica Ltda. – Belo Horizonte, Brazil), according to the manufacturer's instructions to measure NS1 antigen levels (sensitivity: 92.8%, specificity: 98.8%) and IgM/IgG levels (sensitivity: 99.4%, specificity 93%). These biomarkers are commonly used in clinical practices at health units, according to the Technical Guides on dengue of the Brazilian Ministry of Health[13].

Considering the results of these rapid tests, all participants were distributed into two groups: group I – patients with DENV

primary infection ($n = 473$), and group II – patients with DENV heterotypic secondary infection ($n = 30$).

Inclusion criteria

• Group I (primary test infection): positive for NS1 antigen or positive NS1 and IgM DENV.

• Group II (heterotypic secondary infection): positive test for NS1 antigen and IgG DENV or positive NS1, IgM and IgG DENV.

Non-inclusion criteria

Negative immunochromatographic tests for the three parameters (NS1, IgM and IgG DENV).

Positive tests of IgM or/and IgG without the presence of NS1 antigen. Positive test, but no request for additional biochemical or hematological analysis.

Laboratory parameters assays

Laboratory parameters as red blood cells, hematocrit, total leukocyte count, absolute neutrophil count, absolute lymphocyte count, platelets, MPV, AST and ALT were assessed and compared between the two studied groups. APRI was calculated as (AST/upper limit of normal range)/platelet count (10^9/l) $\times$ 100.

Biochemical parameters (AST and ALT) were measured in the integrated device Abbott Architect C4000i1000SR- Illinois, USA, by turbidimetric and colorimetric methods. Hematologic parameters analyses (red blood cells, hematocrit, total leukocyte count, absolute neutrophil count, absolute lymphocyte count, platelets, MPV) were performed on the XS-1000i™- Sysmex Corporation, by flow cytometry and impedance.

Statistical analysis

Statistical analyses were made using SPSS software (version 19.0). Data normality was tested with Shapiro-Wilk test. Nonparametric variables (AST, ALT, APRI, leukocyte, neutrophil, lymphocyte and age) were presented as median and 25th-75th percentiles (interquartile range). Data on biomarkers obtained for each group were compared by Mann-Whitney method. Student t test was used to compare parametric continuous variables [red blood cell (RBCs), hematocrit, MPV, platelets], shown as mean and standard deviation. The comparison of categorical variable (sex) was performed using the Pearson's chi-square test. The MPV and APRI values were assessed by the area under the receiver operating characteristic (ROC) curves. The best model derived

from the training set then was applied to the validation set to test for accuracy by measuring the areas under the ROC curves (AUC). Values of $p < 0.05$ were considered statistically significant.

RESULTS

The two groups showed similar age and sex distribution ($p > 0.05$; **Table**). Therefore, potential differences of variables studied between the groups are likely to reflect the different nature of DENV infection. For hematological tests (RBCs, hematocrit, total leukocyte count, absolute neutrophil count, absolute lymphocyte count, platelets, MPV), a higher count of absolute neutrophil and platelet was found ($p = 0.004$ for both parameters; Table) in group I than in group II. On the other hand, absolute lymphocytes count and MPV were lower in group I than in group II ($p < 0.001$ and $p = 0.042$, respectively; Table).

For biochemical parameters, higher aminotransferases (AST and ALT) serum activity and APRI index were found in secondary DENV infection compared to primary ($p = 0.005$; $p = 0.008$ and $p = 0.006$, respectively). Only 117 patients had aminotransferases results, being 105 with primary infection (group I) and 12 with secondary infection (group II).

TABLE – Comparison of demographic and laboratorial variables between the groups

Variables	Group I – primary DENV infection ($n = 473$)	Group I – secondary DENV infection ($n = 30$)	p
Age[a] (years)	36 (33)	45 (32)	0.112
Sex[1] (%)			
Male	209 (44%)	14 (47%)	0.791
Female	264 (56%)	16 (53%)	
Laboratory parameters			
no. RBCs[b] $\times 10^6$/mm^3	4.69 (0.51)	4.8 (0.57)	0.312
Hematocrit[b] (%)	39.7 (4.1)	40.5 (4)	0.29
Global leukocyte[a]/mm^3	3,985 (2,588)	4,260 (1,755)	0.988
Neutrophils absolute[a] value/mm^3	2,357 (2,265)	1,607 (1,01)	0.004*
Lymphocytes absolute[a] value/mm^3	791 (522)	1,288 (1,093)	< 0.001*
Platelet count[b] $\times 10^3$/mm^3	163 (74)	127 (95)	0.004*
MPV[b]	10.6 (1.3)	11 (1.75)	0.042*
AST U/l[a]	35 (28)	65 (111)	0.005*
ALT U/l[a]	30 (28)	70 (76)	0.008*
APRI[a]	0.63 (0.6)	1.87 (2.17)	0.006*

*RBCs: red blood cells; MPV: mean platelet volume; AST: aspartate aminotransferase; ALT: alanine aminotransferase; APRI: AST to platelet ratio index; DENV: dengue virus; [1]Chi-square Pearson asymptotic test; [a]non-parametric data expressed as median (interquartile range) (Mann-Whitney); [b]normally distributed data are presented as mean ± standard deviation (t-student); *p < 0.05.*

ROC curves of MPV and APRI for discriminate primary and secondary DENV infection were plotted in the **Figure**. The AUC was 0.613 ($p = 0.042$) and 0.744 ($p = 0.744$), respectively.
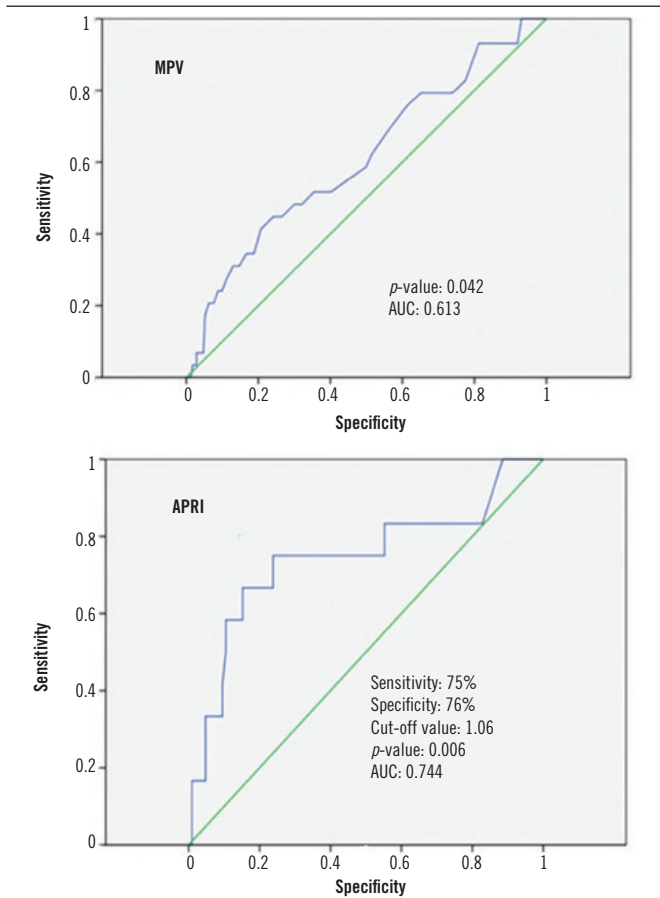

FIGURE – *ROC curves of MPV and APRI in the prediction of significant discrimination of primary and secondary DENV infections. An AUC of 1 is characteristic of an ideal test, whereas an AUC of 0.5 or less indicates a test of no diagnostic value*

ROC: receiver operating characteristic; MPV: mean platelet volume; APRI: aspartate aminotransferase to platelet ratio index; DENV: dengue virus; AUC: area under the curve.

DISCUSSION

Dengue fever is a growing public health concern in most tropical countries. Brazil is one of the countries with the highest number of cases reported in the latest years with predominance of DENV 1 infection[1-3]. The present study showed a small number of patients in group II, composed of patients with secondary infections, what can be explained by the epidemiological DENV infection profile in Brazil in recent years. The predominance of DENV 1 serotype, which is not related to the severe form, and the low incidence of secondary infection by other serotypes justify the reduced size of group II.

Our data show that the number of circulating platelets was lower in group II. It is known that thrombocytopenia by DENV infection can be caused by cross-reactivity of antibodies to platelets[20, 21] by infection of hematopoietic cells and rich circulation of environment cytokines[22, 23]. In addition, viral replication in the liver, which disturbs thrombopoietin synthesis, can contribute to the thrombocytopenia observed in group II[19].

The markers of liver function showed a higher AST and ALT activity in group II versus group I. Elevated liver enzymes suggest that the liver is affected, but the role of hepatic damage in coagulopathy and disease severity remains to be established[19]. Therefore, AST and ALT monitoring during DENV infection seems to be essential for the prognosis of liver infection.

As our data showed a good relationship between change in AST activity and platelet count in secondary DENV infections, the analysis of APRI by ROC curve was carried out. The first study developed and published using APRI as a marker of fibrosis and cirrhosis in patients with hepatitis C longed for a biomarker that easily identified fibrosis and cirrhosis with good significance in this condition[24]. Wai *et al*. (2003)[16] developed a formula based on the good relationship between AST and platelets related to abnormal liver (fibrosis/cirrhosis).

Our findings showed that APRI was able to distinguish patients with primary and secondary infection ($p = 0.006$) with a relevant sensitivity (75%) and specificity (76%) and a cut-off of 1.06. A total of 80 out of 105 (76%) patients with primary DENV infection had APRI ≤ 1.06, and nine out of 12 (75%) patients with secondary DENV infection had APRI > 1.06. We did an exhaustive literature search looking for APRI results in DENV infection, however nothing was found. Our data strongly suggest that APRI can have other applications, besides fibrosis and cirrhosis in patients with hepatitis C. It can help discriminate primary or secondary DENV infection, since patients with secondary dengue showed a higher degree of hepatic changes (APRI median: 1.84) in comparison to patients with primary dengue (APRI median: 0.6).

The accuracy measured by AUC of the ROC curve for MPV was 0.613. Since the rough guide for classifying the accuracy of a diagnostic test considers an AUC between 0.6 and 0.7 poor, we could not choose a cut-off point for MPV. Recently, the results of Sharma and Yadav (2015)[25] research corroborated ours. They showed no correlation of MPV with severity, serology and treatment outcome in dengue fever[25].

Anthropometric data, as age and gender, were collected from the patients with DENV infection, and no differences were found between the two groups. Although we did not have access to clinical data, it is known that MPV is not altered by smoking, obesity, and alcohol abuse, as shown by Maluf *et al*. (2015)[26] in a Brazilian population. These authors reported influence of ethnicity in MPV, but differences related to ethnicity are hard to interpret

in our population, probably because of the five centuries of high miscegenation of Europeans, Africans, and Native Americans[27].

Regarding the blood count parameters, no differences were found in hematocrit and global leukocytes between the groups. It is important to note that a hematocrit rise can indicate a need for hydration and possible increase in capillary permeability, while leukopenia and neutropenia suggest high opsonization of leukocytes[21]. It is known that such changes are observed in greater intensity in the critical phase of DENV infection. By contrast, patients usually seek health care in the early stage of the infection, the febrile period; therefore, it is unlikely that significant changes of these parameters can be identified in this first consultation.

It should be highlighted that dengue fasting tests (NS1, IgM and IgG) are not currently available in basic health units in developing countries such as Brazil. However, conventional tests such as blood count and AST and ALT activity are more easily acquired and available in centers that perform basic hematology and biochemistry tests. Accordingly, the proposed use of APRI as a biomarker to classify dengue infection type (primary or secondary) may be applicable in these places. It is recommended that the results should be evaluated along with the previous health records and current patient clinical presentation to support medical decisions.

CONCLUSION

Our findings suggest that APRI could help distinguishing patients with primary and secondary infection. Since the APRI calculation only requires the results of simple, cheap and widely distributed tests (AST and platelet count), APRI may help improve DENV infection diagnosis in developing countries.

CONFLICTS OF INTEREST

All authors affirm the inexistence of conflicts of interest in this study.

RESUMO

*Introdução: A infecção pelo vírus da dengue (DENV) é considerada um grande problema de saúde pública nos países tropicais. A indisponibilidade de testes sorológicos em centros de saúde pública pode afetar negativamente o prognóstico do paciente. Objetivo: Este estudo teve como objetivo avaliar a precisão do volume médio de plaquetas (MPV) e o índice da relação de aspartato aminotransferase (AST) sobre plaquetas (APRI) como marcadores laboratoriais de infecção por DENV, que poderiam ser utilizados para diferenciar infecções primárias e secundárias. Métodos: Foram avaliados os resultados laboratoriais de 503 pacientes com teste rápido positivo para infecção por DENV. Resultados: Foram observadas trombocitopenia grave e disfunção hepática em pacientes com infecção secundária heterogênea por DENV. Nossos dados sugerem que o APRI foi capaz de distinguir os pacientes com infecção primária e secundária (*p = 0,006)*, com relevante sensibilidade (75%) e especificidade (76%) e corte de 1,06. Um total de 80 de 105 (76%) pacientes com infecção primária por DENV tinha APRI ≤ 1,06; e 12 (75%) com infecção secundária por DENV, APRI > 1,06. Por outro lado, o MPV não mostrou significância na diferenciação de tipos de infecção, apresentando baixo valor da área sob a curva de característica de operação do receptor (ROC) (0,61). Conclusão: APRI parece ser uma ferramenta poderosa para identificação precoce de casos de infecção secundária de DENV em centros de saúde.*

Unitermos: dengue; aspartato aminotransferases; diagnóstico; contagem de plaquetas.

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