ORIGINAL ARTICLE

Combination of three laboratory data as predictor of severe dengue in adults : a retrospective cohort study

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ABSTRACT

BACKGROUND
Dengue infection is a worldwide health problem. Treatment is symptomatic, since no specific treatments are available which are effective in early detection. The roles of various clinical and laboratory findings were studied to predict severity of dengue infection. However, risk factors for severe dengue have not been fully investigated. Thus, the aim of the study was to develop a combination of risk factors based on patient routine laboratory investigations to predict dengue infection severity in adults.

METHODS
This retrospective cohort study was conducted at a private hospital in Jakarta, Indonesia, from September 2010 to April 2016. Complete blood count, AST ratio, and albumin were recorded. Logistic regression was used to analyze the data. The receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off values of each parameter.

RESULTS
A total of 191 patients with non-severe dengue and 20 patients with severe dengue were included in this study. A multivariate analysis showed that hemoconcentration $\geq 15.625\%$ (adjusted OR, 6.28; 95% CI, 2.01-19.57), platelet count $\leq 15,500$/mL (adjusted OR, 3.18; 95% CI, 1.08-9.38), and AST ratio ≥ 5.01 (adjusted OR, 3.93; 95 % CI, 1.36-11.35) were associated with severe dengue. The ROC of the logistic regression model was 85.9% (95% CI 77.8-94.0).

CONCLUSION
The combination of hemoconcentration, thrombocytopenia, and an elevated AST ratio is a risk factor for severe dengue in adults. The application of these findings may help to optimize the allocation of resources, which leads to a more appropriate and effective for the management of patients with severe dengue.

Keywords: Hemoconcentration, thrombocytopenia, AST ratio, severe dengue

INTRODUCTION

Severe dengue is a serious complication in dengue-infected patients and is associated with increased mortality. A previous study observed patients for 5 years and showed that of 12,321 patients with severe dengue, 1,062 patients had died.[1] According to the World Health Organization (WHO), severe dengue is defined by severe bleeding, dengue shock syndrome, acute respiratory distress syndrome, aspartate aminotransferase (AST), alanine aminotransferase (ALT) ratio ≥1000 U/L, or organ dysfunction.[2] This dengue guideline proposed criteria for diagnostic warning signs that are risk factors for severe dengue. The warning sign criteria include clinical syndrome (pain in the abdomen, unrelenting vomiting, fluid accumulation, bleeding in the mucosa, lethargy, restlessness or hepatomegaly more than 2 cm) and laboratory parameters (increment of hemoconcentration simultaneous with a rapid decrease of thrombocytopenia).[2] One of these criteria, fluid accumulation, can be detected in clinical practice using abdominal USG.[3] However, this risk factor was determined in a group of pediatric patients.[4,5] In addition, an increment of hemoconcentration simultaneous with a rapid decrease of thrombocytopenia was not defined.[6]

A recent study in adult patients with dengue showed that four laboratory parameters such as hematocrit, platelets, albumin, and increased AST ratio at a critical phase could be used to identify which patients are at risk for developing plasma leakage. An increase in AST provides better association with plasma leakage compared with ALT.[7,8] These four variables are included in routine laboratory tests used in clinical practice, and are diagnostic predictors of plasma leakage.[7] However, the association between these four laboratory parameters and severe dengue has not been investigated. Knowledge of severe dengue risk factors is crucial in identifying patients who are at risk for developing severe dengue. Therefore, we conducted a study to investigate the association of hematocrit, platelets, albumin, and AST ratio with severe dengue in adult patients.

METHODS

Research design

A retrospective cohort study was conducted at Pondok Indah Hospital, Jakarta from September 2010 to April 2016.

Research subjects

Adult dengue patients 14 years of age or older who had a positive non-structural protein 1 (NS-1) antigen test result were included in this study. Patients' medical records from September 2010 to April 2016 were reviewed in this study. The exclusion criterion was incomplete data in medical records. The sample size was calculated using the formula for the difference between two population means by estimating the hematocrit population variance of 1.64,[9,10] assuming a difference in the population mean of 0.5. With 80% power to detect a 5% difference, the minimum total sample size required was 206 patients.

Laboratory analysis

Parameters such as age, sex, and laboratory parameters: complete blood count, AST, and albumin were recorded. AST and albumin tests were performed during the critical phase. The AST values were then converted to the AST ratio. This was calculated by dividing the AST value by the upper reference limit (here, the AST upper reference limit was 37 U/L).[7] Hemoconcentration was calculated based on a previously published formula.[7] The criteria for severe dengue were based on dengue guidelines proposed by the WHO in 2009, and included severe bleeding, dengue shock syndrome, acute respiratory distress syndrome, AST ≥1000 U/L, or organ dysfunction.[2]

Ethical approval

This study was approved by the management of Pondok Indah Hospital and the

Table 1. Distribution of clinical characteristics of patients with non-severe dengue and severe dengue

Variable	Non-severe dengue (n=191)	Severe dengue (n=20)
Sex (n, male/female)	85/106	8/12
Age (year)	32.50 (22.00)	43 (21.00)
Hemoconcentration (%)*	10.36 (6.79)	18.38 (10.56)
Platelet count ($10^3/\mu$L)*	38,000 (60,500)	13,000 (18,500)
Albumin concentration (g/dL)*	3.3 (0.42)	3.1 (0.63)
AST ratio*	97 (129.25)	311 (929.50)

[a]data presented as median (interquartile range); * $p<0.05$ by Mann Whitney test

Health Research Ethics Committee Faculty of Medicine Universitas Indonesia Cipto Mangunkusumo Hospital.

Statistical analysis

Association between the laboratory parameter variables (i.e., hematocrit, platelets, albumin, and AST ratio) and severe dengue was analyzed using a non-parametric Mann–Whitney U test. All laboratory parameters that had a significant association with severe dengue based on a bivariate analysis were then subjected to the receiver operating characteristic (ROC) curve analysis to determine the optimal cut-off values of each variable. These cut-off values were used to classify the subjects using nominal variables. The nominal variables were then analyzed by logistic regression using a backward selection to obtain an adjusted odds ratio (OR) for the predictors of severe dengue. SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and STATA version 14.0 (STATA Corp Station, TX, USA) were used to perform the statistical analysis.

RESULTS

There were 211 infected dengue patients and 20 of these patients had severe dengue. Clinical characteristics are presented in Table 1. We found that hematocrit, platelets, albumin, and AST ratio have a significant association with severe dengue based on a bivariate analysis (Table 1). The best cut-off value for each variable based on an ROC analysis is presented in Table 2. This cut-off value was used to classify subjects using nominal variables. All nominal variables were then subjected to logistic regression analysis. We found that hemoconcentration $\geq$15.625% (adjusted OR=6.28; 95% CI 2.01-19.57), platelet count $\leq$15,500/μL (adjusted OR= 3.18; 95% CI 1.08-9.38), and AST ratio $\geq$5.01 (adjusted OR, 3.93; 95% CI, 1.36-11.35) were associated with severe dengue (Table 3).

Table 2. Receiver operating characteristic curve of the laboratory parameters as predictors of severe dengue and cut off values for optimal sensitivity and specificity

Variable	ROC (95% CI)	Cut off	Sensitivity (%)	Specificity (%)
Hemoconcentration (%)	0.78 (0.68-0.89)	$\geq$15.625	75	80
Platelet count ($10^3/\mu$L)	0.77 (0.68-0.86)	$\leq$15,500	65	77
Albumin concentration (g/dL)	0.68 (0.56-0.80)	$\leq$3.05	25	55
AST ratio	0.80 (0.69-0.91)	$\geq$5.01	70	76.4

Table 3. Multiple logistic regression of the laboratory parameters to predict severe dengue

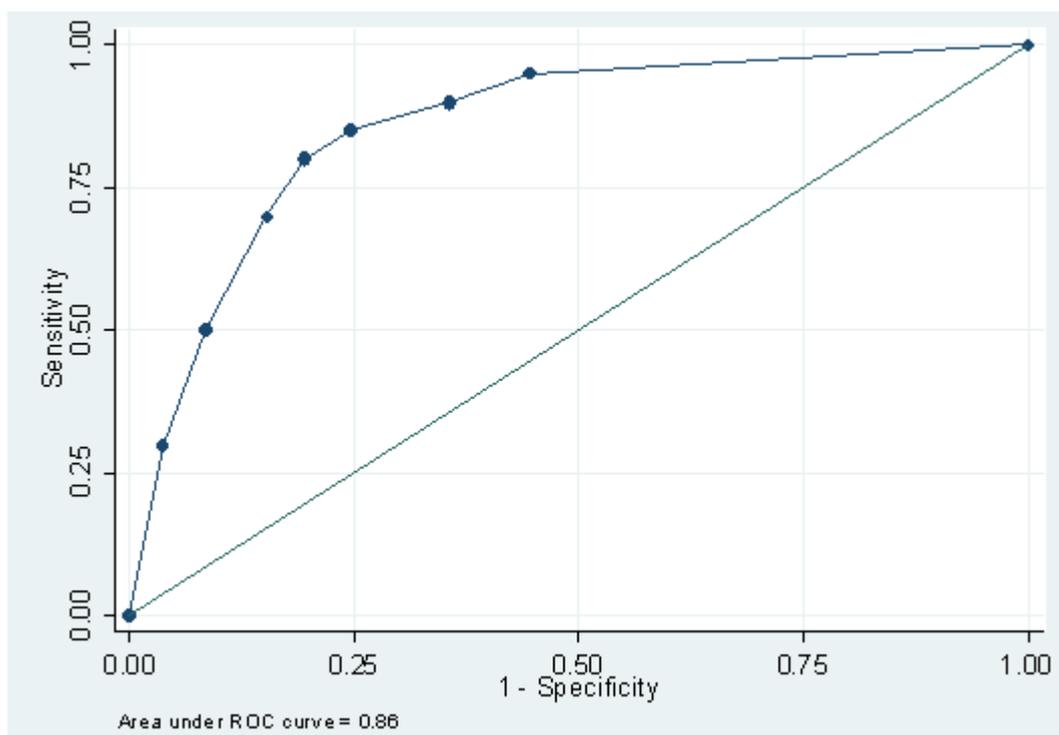| Variable	Odds ratio (95% CI)	p
Hemoconcentration =15.625 %	6.28 (2.01-19.57)	0.002
platelet count =15,500/μL	3.18 (1.08-9.38)	0.035
AST ratio =5.01	3.93 (1.36-11.35)	0.011

Figure 1. Receiver operating characteristic (ROC) curve of the logistic regression model

The ROC of the logistic regression model was 85.9% (95% CI 77.8-94.0%; Figure 1).

DISCUSSION

In 2009, the WHO recommended criteria for warning signs in dengue-infected patients to identify patients with severe dengue.[2] The use of abdominal USG to detect pleural effusion or ascites as a marker for plasma leakage is not a routine procedure in the management of dengue infection.[11] Hematocrit, platelet count, albumin concentration, and AST ratio are laboratory parameters that are suggested to be checked as part of dengue patient management. These laboratory parameters were associated with plasma leakage.[7,12] Previous studies reported that the plasma leakage was most commonly detected in the critical phase, thus, in the present study, we collected data of the laboratory parameters measured in the critical phase, i.e., one day after defervescence.[13,14]

This study showed that a combination of hemoconcentration, platelet count, and AST ratio was associated with severe dengue. However, a previous study reported that an increase in hematocrit of ≥20% is not sensitive for detecting plasma leakage and that it occurred infrequently in severe dengue.[6,9,15] Suwarto et al.[7] showed that the cut-off point for hemoconcentration of ≥15.1% is a better predictor of plasma leakage and minimizes the risk of under-diagnosing patients who are at risk for developing severe dengue. In the present study, we suggest a similar cut-off point as the previous study,[7] which is ≥15.625%, as a risk factor for severe dengue.

We found that a platelet count ≤15,500/μL was associated with severe dengue. This result is compatible with a previous study that reported that platelet counts <20,000/mL were strongly associated with severe dengue.[15] The patients who have severe thrombocytopenia, as indicated by a platelet count <20,000/mL, have a high risk of bleeding.[16] Bivariate analysis showed that albumin levels are significantly lower in severe dengue patients compared to patients without severe dengue. However, based on multivariate analysis, this association did not persist. Several studies reported low levels of albumin in dengue-infected patients without warning signs.[17-19] This

previous finding may explain a less significant role for albumin in severe dengue.

The present study showed that an elevated AST ratio ≥5.01 was a risk factor of severe dengue. Hepatic dysfunction is known to be associated with severe dengue. The cause of elevated AST levels in severe dengue is not fully understood, and possible mechanisms include a direct effect of dengue virus, dysregulation of immune response, necrosis of the liver cell, and ischemia or hypoxia result in liver injury.[20,21] However, further study is needed to confirm the mechanism of hepatic dysfunction in dengue. Our study suggested that hemoconcentration, thrombocytopenia, and an elevated AST ratio is a risk factor for developing severe plasma leakage. This result is consistent with that of a previous study reporting that the combination of fluid accumulation that can be detected by abdominal USG, elevated AST and thrombocytopenia was associated with severe dengue.[22]

Limitations of this study include its retrospective nature, and lack of clinical data such as abdominal pain, vomiting, mucosal bleeding, lethargy, and hepatomegaly that may potentially influence the predictors of severe dengue.

CONCLUSION

This study presents the novel finding that a lower cut-off point for hemoconcentration than that suggested by WHO, the presence of severe thrombocytopenia, and an increased AST ratio ≥5.01 are good criteria to identify patients at risk of severe dengue.

COMPETING INTERESTS

No reported conflicts of interest.

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CONTRIBUTION

SS, SU and BW contributed to drafting the manuscript. SS contributed to design the research, and responsible for the final content. SS, SU and BW contributed to collected, analyzed and interpretatethe data. SS contributed to wrote the manuscript, and all authors read and approved the final manuscript.

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