

Serum lactate as predictor and diagnostic biomarker of plasma leakage in adult dengue patients

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ABSTRACT

BACKGROUND

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are differentiated by the occurrence in DHF of plasma leakage into the interstitial space as shown by pleural and peritoneal effusion, hemoconcentration, and intravascular hypovolemia. Perfusion dysfunction causes anaerobic metabolism, which leads to increased serum lactate. This study was to determine serum lactate as prognostic predictor and diagnostic biomarker of plasma leakage in adult dengue patients.

METHODS

A cross-sectional retrospective cohort study was conducted on 57 adult dengue patients hospitalized in the internal medicine ward of Cipto Mangunkusumo Hospital and Persahabatan Hospital in Jakarta. Serum lactate was examined to determine its mean difference between DF and DHF. The data was analyzed by independent t-test and the cut-off points were identified for presence as well as absence of plasma leakage, then the receiver operating characteristics (ROC) curve was used to determine sensitivity and specificity.

RESULTS

Mean serum lactate was significantly higher in DHF than in DF. From the ROC curve, the cut-off point for serum lactate as prognostic predictor on day 3 of fever was ≥ 2.65 mmol/L with AUC of 0.626 (95% CI 0.480-0.772; $p=0.108$). The cut-off point for diagnostic biomarker of plasma leakage on day 5 of fever was ≥ 2.55 mmol/L with sensitivity 66.6%, specificity 54.2%, and AUC 0.668 (95% CI 0.550-0.826; $p=0.016$).

CONCLUSION

There was a significant difference in serum lactate between DF and DHF. In the critical phase, serum lactate of ≥ 2.55 mmol/L could be used as plasma leakage diagnostic marker of low accuracy.

Keywords: Dengue virus infection, plasma leakage, serum lactate, adult

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INTRODUCTION

Dengue infection remains a global health problem. Over half of the world population is thought to live in areas at risk of transmission, and recent estimates suggest that around 400 million infections occur annually, of which 100 million are clinically apparent.⁽¹⁾ In 2010, the Indonesian Ministry of Health reported 150,000 cases of dengue hemorrhagic fever (DHF) with mortality of 1317 cases.⁽²⁾ In 2013, more than 110,000 DHF cases with mortality of 871 cases were reported.⁽³⁾

Plasma leakage is the main process in DHF of which clinicians should be aware.⁽⁴⁾ Plasma leakage usually occurs when fever is decreased in the critical phase and reaches its peak on the fifth day after the onset of fever.⁽⁵⁻⁷⁾ In the critical phase, plasma leakage can lead to worse conditions that can cause shock and death. Since plasma leakage is the main characteristic of DHF or dengue shock syndrome (DSS), it will be a great indicator for determining DHF severity.⁽⁸⁾ According to the WHO a case of DHF is defined if there is plasma leakage which can be detected by hematocrit elevation of >20% from baseline and or hematocrit reduction from baseline of at least 20% after fluid resuscitation, accompanied by signs of fluid accumulation in the interstitial space, such as pleural effusion and ascites.⁽⁹⁾ The latter can be detected through chest radiography and abdominal USG, which however requires expertise and is expensive. Alternatively, plasma leakage can also be seen from laboratory tests for hypoalbuminemia.⁽¹⁰⁾ Regrettably, in daily practice hematocrit baseline values are not readily available and calculation of the degree hematocrit elevation is difficult. Furthermore, baseline hematocrit level may also be affected by conditions such as anemia, which can lead to a misleading diagnosis and inadequate therapy.^(11,12)

Serum lactate may reportedly be used as a marker of tissue hypoxia caused by systemic hypoperfusion.⁽¹³⁾ Serial serum lactate measurement can predict shock and multi-organ failure (MOF), and high lactate levels can be used

as predictors of survival or mortality in patients with trauma or sepsis.⁽¹⁴⁾ However, studies regarding the use of serum lactate level as dengue severity predictor and diagnostic biomarker of plasma leakage are still scarce especially in adult patients, therefore further studies are needed. Studies about the role of serum lactate in diagnosing dengue fever (DF) and predicting plasma leakage have been conducted in children.^(15,16) Incidence rates of dengue shock syndrome (DSS) are higher in children than in adults. The key factor that distinguishes this condition is associated with vascular fragility.⁽¹⁷⁾

The role of serum lactate as diagnostic biomarker in adult dengue patients was reported previously by Thanachartwet et al.⁽¹⁸⁾, who found that a cut-off value at plasma venous lactate levels of ≥ 2.5 mmol/L, with sensitivity and specificity of 65.0 % and 96.2 %, respectively, could identify severe dengue in adult patients. As prognostic factor, they suggested the combination of procalcitonin level ≥ 0.7 ng/ml and serum lactate ≥ 2.5 mmol/L for predicting DSS.⁽¹⁹⁾ The present study was intended to re-evaluate the role of serum lactate as predictor of dengue severity and diagnostic biomarker of plasma leakage in adult Indonesian dengue patients.

METHODS

Research design

This retrospective cohort study used secondary data (medical records) of adult dengue patients who were enrolled in ongoing research related to dengue fever in Cipto Mangunkusumo Hospital and Persahabatan Hospital in Jakarta. The study was conducted between April 2015 and March 2016.

Study subjects

Data collection was performed by consecutive non-random sampling. The inclusion criteria were patients with a diagnosis of dengue virus infection through positive NS1 antigen (a non structural protein of the dengue virus) tests on the first and second day of fever, above 14

years of age, and hospitalized in the Internal Medicine Ward of Cipto Mangunkusumo Hospital and Persahabatan Hospital between 2014 and 2015. The exclusion criteria were pregnant patients and those with incomplete medical records. Dengue hemorrhagic fever patients were defined by hematocrit elevation of $>20\%$ on the fifth day as compared to baseline and or the presence of pleural effusion or ascites by abdominal USG.

We used the sample size calculation method for the comparison of two independent means and the diagnostic test formula.^(20,21) For sample size calculation by the two independent means formula, we used the study by Santosa et al.,⁽⁸⁾ who reported a mean and standard deviation of 2.81 ± 0.62 in the plasma leakage group (n=19) and of 2.36 ± 0.46 in the non-plasma leakage group (n=30). In our study, the calculated sample size per group was 17, which was estimated to be adequate to detect a difference in means of 0.44 between the two groups using a two-tailed test, an alpha of 0.05 and power of 90%. Therefore the total minimum sample was 35 patients. Using the formula for diagnostic test,⁽²¹⁾ we found that the total minimum sample size was 57 patients, which was estimated to be adequate for a sensitivity of 90%, precision of 10% and dengue prevalence of 0.61.⁽²²⁾ From the results of the two formulas, we used the largest calculated minimum sample size, which was 57 patients.

Measurements

Data taken at the time of admission were the patients' identity such as name, age, gender, date of birth and address. From the patients' history we retrieved data about onset of fever and other clinical symptoms such as nausea, vomiting, heartburn, headache and myalgia. Vital signs such as blood pressure, heart rate, respiratory rate, and temperature were obtained through physical examination.

Data taken during hospitalization were bleeding manifestations, such as positive tourniquet tests, petechiae, gingival bleeding, epistaxis, menometrorrhagia, hematemesis, and melena. From the physical examination we

obtained the presence or absence of hepatomegaly. The presence or absence of ascites and pleural effusion was determined by USG.

Laboratory analysis

This study measured serial complete blood counts and serum lactate, aspartate transaminase (AST) and alanine transaminase (ALT) on the third and fifth days after the onset of fever.

Statistical analysis

All data was inserted into the patient forms and processed according to the aim of this study. Subject characteristics were descriptively displayed in table form with SPSS (Statistical Package for the Social Sciences) version 20.0. Independent t-test was used to analyze the differences in mean serum lactate on the third and fifth days and in mean basic laboratory test results between DF and DHF patients. An analysis to determine the diagnostic and prognostic role of serum lactate was done by using the receiver operating characteristics (ROC) curve and calculating the area under the ROC (AUC) with 95% confidence interval. The serum lactate cut-off points for diagnosis and prognosis of plasma leakage were obtained by determining the highest sensitivity and specificity values from the ROC. To determine the accuracy of the diagnostic test, we also calculated the sensitivity, specificity, positive and negative predictive value (PPV and NPV), as well likelihood ratios. Prognostic ability was calculated by absolute risk (AR) and relative risk (RR) at 95 % CI for plasma leakage in dengue patients with serum lactate above the cut-off point.

Ethical clearance

This study used secondary data from medical records therefore informed consent was not necessary. This study has been approved by the Health Research Ethics Committee, Faculty of Medicine, University of Indonesia, and the Cipto Mangunkusumo National Central General Hospital (RSUPN Cipto Mangunkusumo) under no. 412/UN2.F1/ETIK/2015. All data were kept confidential by the researchers.

Table 1. Distribution of characteristics and clinical features of subjects (n=57)

C haracteristic	n (%)
Age (years), median (IQR)	23 (11)
Male	35 (61.4)
Duration of fever before admission	
2 days	15 (26.3)
3 days	42 (73.7)
Accompanying symptoms	
Nausea	45 (78.9)
Vomiting	36 (63.2)
Epigastric pain	25 (43.9)
Headache	38 (66.7)
Myalgia	32 (56.1)
Blood pressure mmHg, median (IQR)	
Systolic	110 (10)
Diastolic	70 (10)
Bleeding manifestation	
Positive tourniquet test	34 (59.7)
Petechiae	25 (43.8)
Gingival bleeding	1 (1.8)
Epistaxis	2 (7.0)

* Patients may have reported more than one symptom

** IQR : interquartile range

RESULTS

A total of 57 adult dengue patients were enrolled in the study. The median age of the patients was 23 years (interquartile range [IQR]: 11) and 35 (61.4%) were male. The most common complaint besides fever was nausea (78.9%), while the most common bleeding manifestation was a positive tourniquet test (59.7%). The characteristics of the subjects are summarized in Table 1.

During monitoring, we found that laboratory findings such as hemoglobin, hematocrit, leukocytes, and platelets on days 3 and 5 corresponded to the course of dengue. However, we found no significant differences in basic laboratory workup results between DF and DHF patients, except the leukocyte count on day 5 and AST level on day 3 (Table 2). On day 3, we found that mean serum lactate level was statistically higher in DHF patients than in DF patients (2.88

Table 2. Distribution of mean of laboratory blood parameters between dengue fever and dengue hemorrhagic fever

Variable^a	Group		p
	Dengue Fever (n=22)	Dengue Hemorrhagic Fever (n= 35)	
Hemoglobin (g/dl)			
Day 3	13.65 ± 1.69	14.37 ± 1.45	0.104
Day 5	13.66 ± 1.91	14.42 ± 1.90	0.155
Hematocrit (%)			
Day 3	39.63 ± 4.16	41.69 ± 4.68	0.107
Day 5	40.22 ± 4.72	42.22 ± 5.59	0.168
Leukocyte count (x 10 ³ /μL)			
Day 3	3.36 ± 1.31	3.15 ± 1.38	0.656
Day 5	2.20 ± 1.15	3.82 ± 1.87	0.033
Platelet count (x 10 ³ /μL)			
Day 3	115.09 ± 32.55	109.36 ± 40.69	0.595
Day 5	70.00 ± 38.43	56.72 ± 34.35	0.168
AST (U/L)			
Day 3	36.91 ± 14.65	84.18 ± 40.01	0.003
Day 5	73.35 ± 58.78	140.87 ± 44.66	0.101
ALT (U/L)			
Day 3	32.64 ± 25.55	45.20 ± 26.11	0.279
Day 5	58.21 ± 13.95	75.50 ± 23.25	0.504
Lactate level (mmol/L) ^a			
Day 3	2.55 ± 0.43	2.88 ± 0.59	0.028*
Day 5	2.40 ± 0.55	2.84 ± 0.55	0.005*

^aData presented as mean±standard deviation

Table 3. Diagnostic value of serum lactate levels for dengue severity

	Dengue Severity		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR(+) (95% CI)	LR(-) (95% CI)
	DF	DHF						
Lactate level Day 3 cut-off ≥ 2.65 mmol/L	24	33	42.5 % [0.44-0.79]	50.0 % [0.29-0.71]	62.50 % [0.44-0.79]	54.2 % [0.29-0.71]	1.25 [0.77-2.02]	0.75 [0.41-1.37]
Lactate level Day 5 cut-off ≥ 2.55 mmol/L	24	33	66.6 % [0.50-0.80]	54.2 % [0.35-0.72]	67 % [0.50-0.80]	54 % [0.35-0.72]	1.45 [0.88-2.39]	0.62 [0.34-1.13]

± 0.59 mmol/L vs. 2.55 ± 0.43 mmol/L; $p=0.028$). Serum lactate in DHF patients remained higher than in DF patients on day 5 (2.84 ± 0.55 mmol/L vs. 2.40 ± 0.55 mmol/L; $p=0.005$) (Table 2).

We used ROC analysis as a test for the association between serum lactate on the third day and the presence of plasma leakage on the fifth day to determine the role of serum lactate as a predictor of plasma leakage. The results of the analysis showed that serum lactate cannot be used as predictor of plasma leakage (AUC 0.626; 95% CI 0.480-0.772; $p=0.108$) (Figure 1). The cut-off point of serum lactate on the third day of ≥ 2.65 mmol/L had sensitivity of 42.5%, specificity 50.0%, PPV 62.5%, NPV 54.2%, which was also associated with a 1.20-fold increased risk (95% CI 0.76-1.91) to develop plasma leakage in the

critical phase, but this finding was not statistically significant (Table 3).

However, the ROC on serum lactate and the presence of plasma leakage on the fifth day showed that serum lactate on the fifth day can be used as diagnostic tool for plasma leakage in DHF patients (AUC 0.688; 95% CI 0.550-0.826; $p=0.016$) (Figure 2). The cut-off point of serum lactate on the fifth day of ≥ 2.55 mmol/L had the highest sensitivity (66.6%; 95% CI 0.50-0.80) and specificity (54.2%; 95% CI 0.35-0.72). This cut-off value had PPV 67% (95% CI 0.50-0.80), NPV 54% (95% CI 0.35-0.72), positive likelihood ratio LR (+) 1.45 and negative likelihood ratio LR (-) 0.62 (Table 3). Therefore it can be concluded that serum lactate had a low diagnostic accuracy.

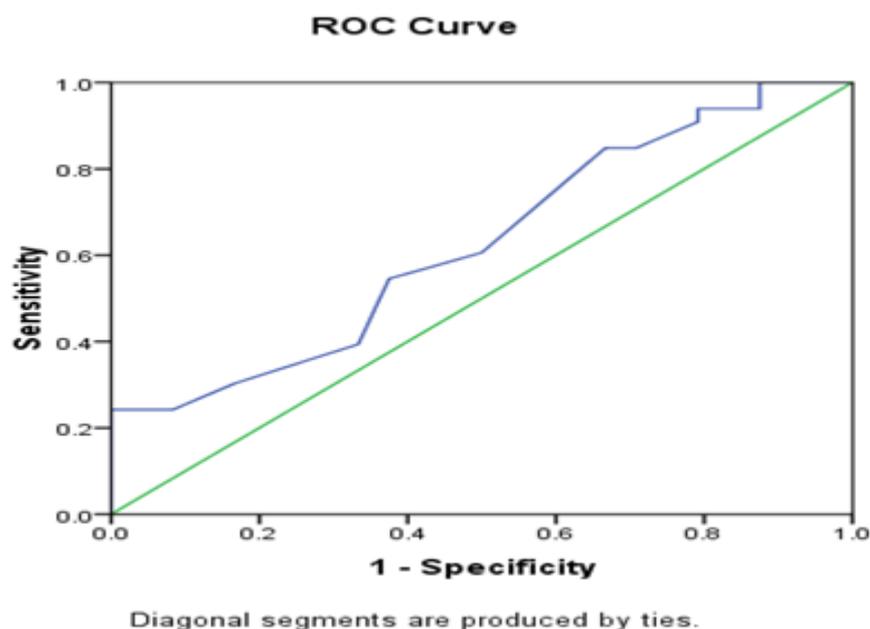


Figure 1. Area under the receiver operating characteristic curve (AUC) for serum lactate level ≥ 2.65 mmol/L on day 3 as predictor of plasma leakage was 0.626 (95% CI: 0.480-0.772; $p=0.108$)

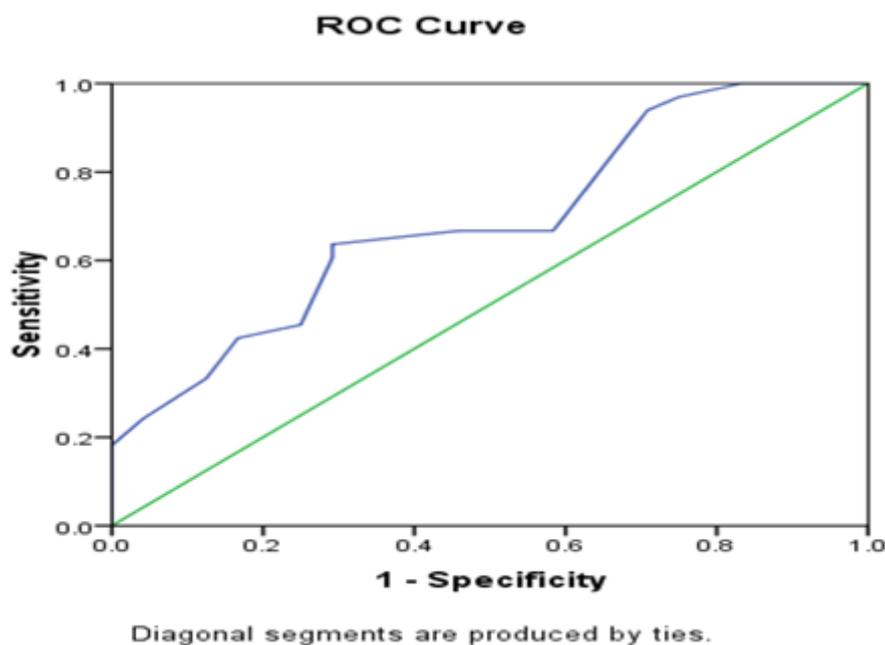


Figure 2. AUC for serum lactate level ≥ 2.55 mmol/L as diagnostic biomarker of plasma leakage on day 5 was 0.668 [CI 95% 0.550-0.826; $p=0.016$]

DISCUSSION

Lactate is a marker for tissue hypoxia that may be the result of anaerobic metabolism mainly due to hypoperfusion.⁽¹³⁾ Serum lactate laboratory tests had been incorporated in Surviving Sepsis Campaign (SSC) Guideline 2012 as important workup for all suspected patients with sepsis.⁽²³⁾ The SSC used as cut-off value a serum lactate level of 4 mmol/L as marker for severe sepsis. Hyperlactatemia is associated with increased mortality in septic patients, therefore it can be used as prognostic factor. Normalization of lactate level also has been suggested as one of the therapeutic goals of sepsis management.⁽²³⁾ While the role of serum lactate in septic patients had been well established, the role of serum lactate as biomarker in dengue patients was understudied, especially in adult patients. In DHF patients the main characteristic finding is plasma leakage from increased vascular permeability, resulting in interstitial edema, pleural effusion, ascites, hemoconcentration, and intravascular hypovolemia. These conditions cause tissue hypoperfusion, which lead to impaired oxygen supply and activation of anaerobic metabolism.⁽¹⁸⁾ Based on dengue pathogenesis, we assessed the

role of serum lactate in predicting and diagnosing plasma leakage in adult dengue patients.

In this study, we included 57 adult dengue patients consisting of 22 DF cases and 35 DHF cases. Most of our patients were young male adults, who reported nausea as the most common chief complaint and positive tourniquet test as the most common bleeding manifestation. There are many studies assessing the factors associated with severe dengue or DSS.^(24,25) The WHO proposed a list of warning signs, such as abdominal pain, persistent vomiting, mucosal bleed, and lethargy, for predicting severe dengue and the need for strict observation. However, a study by Thanachartwet et al found that the sensitivity and specificity of the 2009 WHO warning signs (except the presence of clinical fluid accumulation) were too low for identifying severe dengue at admission.⁽¹⁸⁾ In our study, among the laboratory parameters we found no significant differences between DF and DHF patients, except leukocyte count on day 5 and AST level on day 3. This finding may have been caused by the adequate fluid resuscitation received by the patients.

Serum lactate values on day 3 and day 5 were found to be significantly higher in DHF patients than in DF patients. A higher lactate level

may reflect the degree of severity of plasma leakage in DHF patients compared to DF. Sirikutt et al.⁽¹⁶⁾ also reported that serum lactate was found to be elevated in children with DHF and/or DSS. Hyperlactatemia, in addition to being caused by anaerobic metabolism due to hypoperfusion from plasma leakage, may also be caused by impaired lactate clearance from hepatic impairment.^(16,26) Therefore, serum lactate may be considered as a marker for the severity of adult dengue patients.

We analyzed the sensitivity and specificity of various cut-off values using ROC to determine the best cut-off point for serum lactate on the third day as a plasma leakage predictor. Serum lactate on the third day of ≥ 2.65 mmol/L was shown to be nonsignificant in predicting plasma leakage on the fifth day, having a sensitivity of 42.5%, specificity 50.0%, PPV 62.5%, and NPV 54.2%. It can be concluded that serum lactate is nonsignificant as a predictor of plasma leakage in the critical phase. A prospective observational study from Thailand found that the combination of procalcitonin level of ≥ 0.7 ng/ml and serum lactate level of ≥ 2.5 mmol/L resulted in a sensitivity of 81.2%, specificity of 84.4%, PPV 56.5%, NPV 94.7% to predict DSS and/or organ failure.⁽¹⁹⁾ The diagnostic accuracy can be improved if combined with the finding of clinical fluid accumulation, resulting in a sensitivity of 90% and specificity of 87.6%. These differing findings were presumably the result of differences in the degree of plasma leakage caused by differences in the timing of blood sample collection for serum lactate measurement. The abovementioned study in Thailand used the lactate level at admission for diagnostic accuracy assessment,⁽¹⁹⁾ but we uniformly take blood samples on day 3 which is generally thought as the beginning of plasma leakage. This is presumably done because the degree of plasma leakage is still minimal on day 3, since plasma leakage in the form of third space fluid accumulation on ultrasound is mostly detected on the fourth and fifth days.⁽²⁷⁾ Another possible explanation is that because our study was of retrospective design, the patients may have received fluid therapy so that the intravascular

volume had been replaced and met the needs for microcirculation. When perfusion is maintained the lactate level may decrease.⁽²⁸⁾

According to the 1997 WHO guideline, plasma leakage is determined by the presence of hemoconcentration as reflected by increased hematocrit level of $\geq 20\%$ and/or thrombocytopenia of $< 100,000/\text{mm}^3$. A limitation to these criteria is that baseline hematocrit values are commonly not available, and may be misleading in the presence of anemia or hemorrhage and during the early phase of resuscitation.^(11,12) Based on ROC, the cut-off point of serum lactate ≥ 2.55 mmol/L was determined as plasma leakage diagnostic, with sensitivity of 66.6%, specificity 54.2%, PPV 67% and NPV 54%. Our cut-off value was slightly different from that reported in the prospective observational study in Thailand which found that the cut-off value of serum lactate level of ≥ 2.5 mmol/L was optimal to identify severe dengue, having a sensitivity of 65%, specificity of 96.2%, PPV of 76.5%, and NPV of 93.5%.⁽¹⁸⁾ The difference in cut-off values can be explained by the different timing of serum lactate sampling, since we used the serum lactate value on day 3 while the study from Thailand used the lactate value on day 5.

One limitation of our study was its retrospective cohort design which may be associated with a risk of bias and confounding factors. We suggest that a prospective study be conducted, with blinding of the treating physicians and investigators to the results, to minimize the risk of missing data and bias. For further research, we recommend serial daily serum lactate measurement starting before the third day of the onset of fever, and six-hourly serum lactate measurement during the critical phase until the recovery phase. The clinical implication of our study is that according to the supporting evidence, serum lactate level on the fifth day can be used as diagnostic biomarker of plasma leakage, apart from radiological workup. Therefore early identification and treatment of severe dengue can be made, thus resulting in decreased mortality rates.

CONCLUSIONS

We found that serum lactate level on the third and fifth days of fever was higher in DHF patients compared to DF patients. Serum lactate level on the third day cannot be used for predicting plasma leakage in the critical phase. Serum lactate on the fifth day can be used for diagnosis of plasma leakage in the critical phase with low accuracy.

CONFLICT OF INTEREST

The authors declare no competing interest.

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