

Serum thymosin β 4 levels in patients with hepatitis B virus-related liver failure

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Abstract

AIM: To investigate whether serum thymosin β 4 can provide diagnostic or prognostic information in liver failure patients caused by chronic hepatitis B virus (HBV) infection.

METHODS: Serum thymosin β 4 levels were measured in 30 patients with acute-on-chronic liver failure (ACLF), 31 patients with chronic liver failure (CLF), 30 patients with compensated liver cirrhosis (CR) and 32 patients with chronic hepatitis B and 30 healthy controls. Serum thymosin β 4 levels were measured by enzyme-linked immunosorbent assay and Child-Pugh and model for end-stage liver disease (MELD) scores were calculated for each patient on admission.

RESULTS: Compared with healthy controls, serum thymosin β 4 levels in ACLF, CLF, CR and chronic hepatitis B patients were significantly lower, 6.5047 (4.7879-10.5314) μ g/mL vs 0.4632 (0.2759-0.8768) μ g/mL, 0.6981 (0.5209-1.2008) μ g/mL, 1.8053 (0.8110-2.3397) μ g/mL, 3.7803 (1.8570-6.4722) μ g/mL, respectively ($P < 0.001$). The levels of thymosin β 4 in liver failure (ACLF or CLF) patients were markedly lower than that in CR ($P < 0.001$), and a difference was also found between CLF and ACLF patients ($P = 0.038$). In patients with chronic liver disease, there was a positive relationship between thymosin β 4 levels and albumin, choline esterase, and platelet ($P < 0.001$), and negative relationship with alanine aminotransferase ($P = 0.020$), aspartate aminotransferase, total bilirubin, international normalized ratio of prothrombin time, and Child-Pugh and MELD scores ($P < 0.001$). Of the 61 liver failure patients, the thymosin β 4 levels of non-survivors were significantly lower than that of survivors ($P = 0.007$). Receiver operating characteristics analysis identified a thymosin β 4 cutoff level of 0.5708 μ g/mL for predicting poor prognosis in all liver failure patients. The serial thymosin β 4 values were observed in 13 liver failure inpatients. Lower initial values were observed in the death. While greater improvement in thymosin β 4 value was found in those who recovered from the disease.

CONCLUSION: Serum thymosin β 4 can be used as an important potential predictor for liver failure caused by chronic HBV infection.

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Key words: Thymosin β 4; Liver failure; Serum; Hepatitis B virus; Biochemistry

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains one of the most challenging public health problems in Asia. Acute-on-chronic liver failure (ACLF) and chronic liver failure (CLF) are common serious conditions with a high mortality among chronic HBV infected patients. The prognosis is clearly related to the stage of the disease and early diagnosis has resulted in a significant reduction in mortality^[1-4]. Unfortunately, a differential diagnosis from non-liver failure patient is sometimes very difficult to make since the current biomarkers used in clinical diagnosis are still lack of sensitivity and reliability^[5,6]. Thus, it is very important to discover new biomarkers for liver failure diagnosis.

Thymosin β 4 is a 4.9-kDa polypeptide widely distributed in human tissues, which is considered as the major G-actin sequestering protein^[7-9]. Thymosin β 4 has been shown to have multiple biological activities involved in a variety of physiologic and pathologic processes. For example, thymosin β 4 is known to promote wound healing, tumor metastasis and angiogenesis^[10-12]. Recently thymosin β 4 has been found to stimulate cardiac cell migration and survival related to cardiac repair. It was also considered to play an important role in healing hypoxic injury in the heart by preventing apoptotic cell death of cardiomyocytes and reducing scarring^[13], meanwhile, hypoxia and oxidative stress also play a key role in the pathophysiology of liver failure. Recent findings suggested that thymosin β 4 could be beneficial for the treatment of chronic liver disease (CLD)^[14]. These data have attracted more consideration of whether thymosin β 4 plays an important role in liver failure. However, the levels of thymosin β 4 in liver failure patients are still unknown.

This present study aims to determine serum thymosin β 4 levels in CLD patients with HBV infection, and to assess the potential usefulness of thymosin β 4 in diagnosis and prognosis prediction of HBV-related liver failure, including ACLF and CLF.

MATERIALS AND METHODS

Patients

The study included 61 patients with liver failure (44 males, 17 females), of whom 30 (23 males, 7 females) were diagnosed as having ACLF, the other 31 (21 males,

10 females) patients as having CLF. And 30 (20 males, 10 females) patients with Child-Pugh A cirrhosis, and 32 (26 males, 6 females) patients with chronic hepatitis B were also enrolled. All patients had chronic HBV infection. Those with concurrent hepatocellular carcinoma (HCC), splenectomy, fatty liver, and hepatitis C or alcohol-related liver diseases were excluded. ACLF was defined as acute deterioration of liver function within 4 wk in CLD, with severe jaundice [serum total bilirubin (TBIL) \geq 171 μ mol/L or an increase of TBIL \geq 17.1 μ mol/L per day] and coagulopathy [international normalized ratio of prothrombin time (INR) $>$ 1.5 or PTA $<$ 40%]. CLF was defined as chronic decompensation of an end-stage liver disease, complicated with refractory ascites and/or encephalopathy and hepatorenal syndrome^[3]. The diagnosis of liver cirrhosis (CR) was based on the clinical manifestation, physical examination, biochemical, endoscopic and ultrasound findings and/or liver biopsy, with features of liver fibrosis, portal hypertension and hypersplenism. All patients infected with HBV were recruited from January to July 2009 at Tianjin Third Central Hospital. Thirty healthy volunteers served as controls, including 20 males and 10 females, aged 41 (31-49) years [median (inter-quartile range, IQR)].

The study was approved by Tianjin Third Central Hospital Ethics Committee. Informed consent was obtained from either the patients or their immediate family members.

Collection of clinical data

INR, platelets (PLT), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), choline esterase (CHE), albumin (ALB), TBIL and renal function were tested. Child-Pugh and model for end-stage liver disease (MELD) scores were calculated on admission.

Measurement of serum thymosin β 4 levels

All the samples were collected in the morning when the patients were admitted to our hospital. Blood collected without anticoagulant was spun at $2600 \times g$ for 15 min, and the serum obtained was immediately stored at -80°C until thymosin β 4 measurement. Thymosin β 4 was measured with a newly developed commercial enzyme-linked immunosorbent assay (ELISA) (Immunodiagnostik AG, Bensheim, Germany). The test principle was based on a competition between antigen in the sample or standards and the antigen coated on the wells of microplate. A peroxidase-conjugated antibody was used for detection and quantification, and tetramethylbenzidine as a peroxidase substrate. The enzymatic reaction was terminated by acidic stop solution. The results were obtained by measuring the absorbance at 450 nm in an ELISA reader.

Statistical analysis

Kruskal-Wallis and Mann-Whitney *U* tests were used. Correlations were analyzed by Spearman rank test. Results were expressed as median and IQR. Cutoff values for the identification of non-survivors with liver failure (ACLF

Table 1 Clinical characteristics of the patients [median (IQR)]

	ACLF	CLF	CR	Chronic hepatitis B	Healthy subjects
<i>n</i>	30	31	30	32	30
Age (yr)	54 (48-61)	50 (46-55)	50 (37-55)	39 (31-45)	41 (31-49)
Gender (M:F)	23:7	21:10	20:10	26:6	20:10
ALB (g/L)	30.9 (26.3-33.2)	25.7 (22.6-28.9)	42.2 (38.4-45.7)	46.7 (42.0-49.1)	39.3 (38.1-41.1)
ALT (IU/L)	174.0 (104.3-653.0)	38.0 (27.5-49.5)	35.5 (24.0-52.8)	57.5 (25.0-75.5)	30.5 (25.1-34.7)
AST (IU/L)	274.5 (74.0-521.0)	34.0 (21.0-71.0)	19.0 (9.3-32.3)	15.0 (10.0-20.5)	23.3 (19.2-31.3)
TBIL (μ mol/L)	409.7 (208.1-474.7)	170.9 (98.2-215.2)	14.3 (12.5-18.1)	15.1 (12.7-20.5)	9.7 (8.75-13.2)
CHE	2699 (1760-3286)	1586 (1129-2035)	3526 (3287-4529)	4938 (4168-5465)	6784 (6553-7463)
INR	2.29 (1.94-2.70)	2.52 (2.27-2.85)	1.08 (1.01-1.13)	1.02 (1.01-1.04)	1.01 (0.95-1.02)
PLT	72.5 (44.5-104.0)	44.0 (30.5-79.0)	89.0 (78.0-99.8)	197.5 (178.0-225.0)	197.5 (187.0-222.3)
MELD	23.75 (19.81-29.55)	22.97 (19.16-27.98)	3.52 (2.39-5.23)	2.65 (1.72-4.44)	2.65 (2.05-3.70)
Child-Pugh score	11 (10-12)	12 (11-12)	6 (5-7)	5 (5-6)	5 (5-6)

ACLF: Acute-on-chronic liver failure; CLF: Chronic liver failure; CR: Cirrhosis; ALB: Albumin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TBIL: Total bilirubin; CHE: Cholinesterase; INR: International normalized ratio of prothrombin time; PLT: Platelet; MELD: Model for end-stage liver disease.

Table 2 Correlation coefficients (*r*) and *P* values of thymosin β 4 values vs biochemical parameters in chronic liver disease patients

Characteristics	<i>r</i> value	<i>P</i> value
ALB	0.536	< 0.001
ALT	-0.210	0.020
AST	-0.553	< 0.001
TBIL	-0.581	< 0.001
CHE	0.656	< 0.001
INR	-0.605	< 0.001
PLT	0.541	< 0.001
Child-Pugh score	-0.629	< 0.001
MELD	-0.587	< 0.001

and CLF) and survivors were determined using the receiver operating characteristic (ROC) analysis. A *P* value of < 0.05 was considered to be statistically significant. All analyses were performed with SPSS 13.0 software.

RESULTS

Group comparison

The clinical characteristics of CLD patients in the study are listed in Table 1.

Thymosin β 4 levels in the groups of ACLF, CLF, CR, chronic hepatitis B (CHB) and healthy controls are shown in Figure 1. Patients in ACLF, CLF, CR and CHB groups had significantly lower median (IQR) thymosin β 4 levels than healthy controls, 0.4632 (0.2759-0.8768) μ g/mL, 0.6981 (0.5209-1.2008) μ g/mL, 1.8053 (0.8110-2.3397) μ g/mL, 3.7803 (1.8570-6.4722) μ g/mL vs 6.5047 (4.7879-10.5314) μ g/mL, respectively (*P* < 0.001). The thymosin β 4 in ACLF and CLF patients was markedly reduced as compared with that in CR patients (*P* < 0.001). A difference was also found between ACLF and CLF (*P* = 0.038).

Relationship between thymosin β 4 and biochemical parameters in liver failure patients

Table 2 shows the relationship between thymosin β 4 values and biochemical parameters in CLD patients

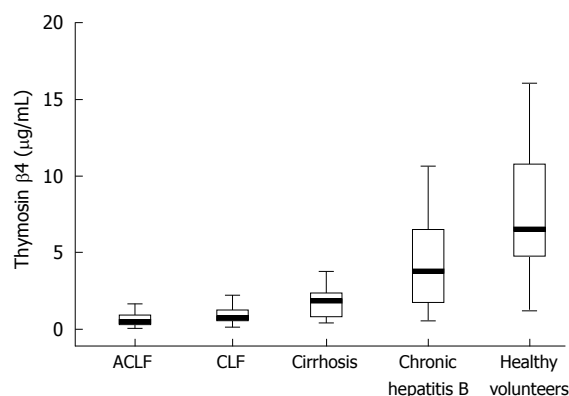


Figure 1 Comparison of thymosin β 4 levels (μ g/mL) between ACLF, CLF, cirrhosis, chronic hepatitis B patients and healthy volunteers. ACLF: Acute-on-chronic liver failure; CLF: Chronic liver failure.

(including ACLF, CLF, CR and CHB). There was a positive relationship between thymosin β 4 levels and ALB, CHE, and PLT (*P* < 0.001). And in CLD patients, thymosin β 4 was negatively correlated with ALT (*r* = -0.210, *P* = 0.020), AST (*r* = -0.553, *P* < 0.001), TBIL (*r* = -0.581, *P* < 0.001), INR (*r* = -0.605, *P* < 0.001), Child-Pugh (*r* = -0.629, *P* < 0.001) and MELD scores (*r* = -0.587, *P* < 0.001).

Analysis of liver failure group

Among the 61 liver failure patients, the survival rate was 63.93% (39 patients), while the non-survival rate was 36.07% (22 patients). The thymosin β 4 level of non-survivors was significantly lower than that of the survivors (*P* = 0.007) (Table 3). ROC analysis identified a thymosin β 4 cutoff value of 0.5708 μ g/mL [area under the ROC (AUROC) 0.710] with a sensitivity of 64.1% and a specificity of 68.2% for predicting poor prognosis in all liver failure patients (Figure 2A).

To further investigate the prediction value of thymosin β 4 for different liver failure status, the 61 patients were divided into two groups: CLF and ACLF. Table 3 shows the median (IQR) thymosin β 4 values of the two groups. In the ACLF group, ROC analysis identified a thymosin

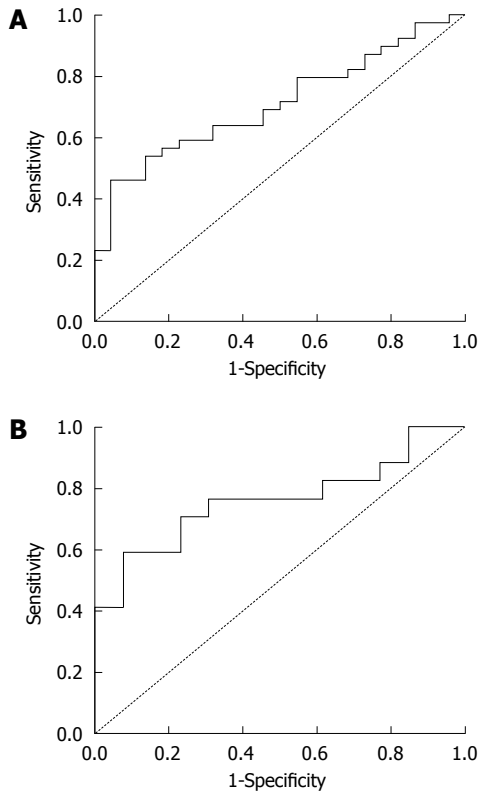


Figure 2 Receiver operating characteristics curve. A: Liver failure patients; B: ACLF patients.

Table 3 Comparison of thymosin β 4 levels between survivors and non-survivors in liver failure group [median (IQR)]				
Group	n	Survivors	Non-survivors	P value
LF	61	0.8144 (0.3979-1.1510)	0.4699 (0.2771-0.6658)	0.007
ACLF	30	0.8144 (0.4042-1.0998)	0.3656 (0.2291-0.4458)	0.016
CLF	31	0.8329 (0.4306-1.4280)	0.6581 (0.5603-0.7713)	0.317

Table 4 Median (IQR) thymosin β 4 values (μ g/mL) in 13 liver failure patients					
Group	1 wk	2 wk	3 wk	4 wk	5 wk
Survivors	0.9402	1.2146	1.2762	1.2226	1.5486
Non-survivors	0.5458	0.6765	0.5056	0.3986	0.3488

β 4 cutoff value of 0.3873 μ g/mL (AUROC 0.760) with a sensitivity of 76.5% and a specificity of 69.2 % for predicting poor prognosis (Figure 2B). In CLF group, lower thymosin β 4 levels were observed in non-survivors, but there was no statistically significant difference between the survivors and non-survivors of CLF group. Figure 3 shows the distribution of thymosin β 4 levels in the survivors and non-survivors with liver failure in the groups of ACLF and CLF.

Dynamic changes of serum thymosin β 4 values in liver failure patients

In this study, 13 liver failure patients were selected to

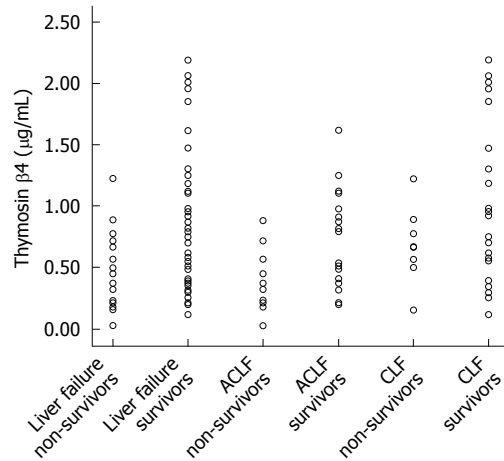


Figure 3 Thymosin β 4 levels in non-survivors and survivors of liver failure patients.

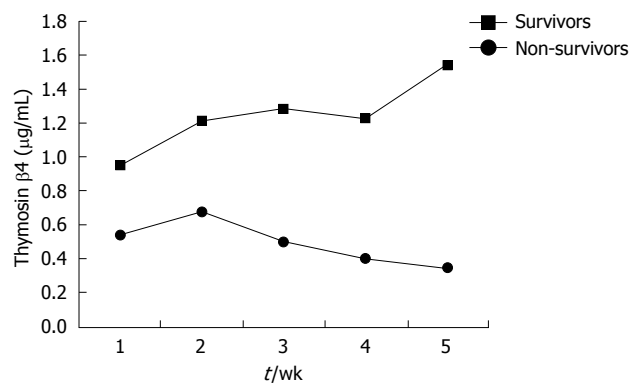


Figure 4 Median (inter-quartile range, IQR) thymosin β 4 values (μ g/mL) observed in 13 liver failure patients.

observe the dynamic changes of their serum thymosin β 4 values. Among them, 6 patients survived and the other 7 patients died. Blood samples were taken from these patients every week during their hospitalization. The median thymosin β 4 concentrations of non-survivors were significantly lower than those of survivors ($P < 0.001$, Table 4). And greater improvement in thymosin β 4 values was observed in those who recovered than in the non-survivors (Figure 4). Figure 5 shows the serial thymosin β 4 changes in 6 typical liver failure patients of the 13 patients.

DISCUSSION

In this study, we found that serum thymosin β 4 levels were significantly lower in patients with chronic hepatitis B infection. The magnitude of reduction of thymosin β 4 was closely related to the severity of the hepatic injury. Serum thymosin β 4 concentrations were most significantly decreased in ACLF and CLF. Dynamic changes of serum thymosin β 4 values could reflect the recovery or death in some liver failure patients.

Liver failure occurs in the hepatocytes with extensive injury, which represents either a failure to regenerate after

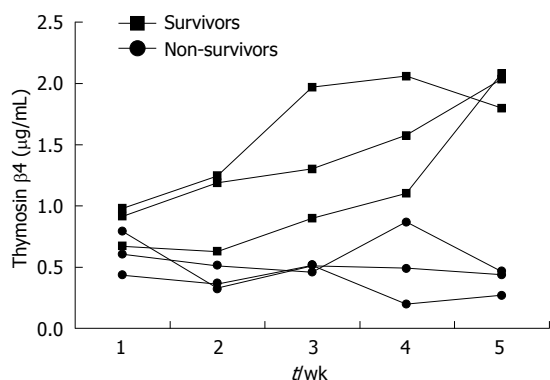


Figure 5 Serial thymosin β 4 concentrations in 6 patients with liver failure.

accelerated destruction of hepatocytes from necrosis or apoptosis^[15]. As a result, surviving hepatocytes cannot maintain adequate metabolic functions. ALB and CHE are synthesized by hepatocyte, and their content can directly reflect the state of liver function. In this study, the levels of thymosin β 4 in CLD patients had a significantly positive correlation with ALB and CHE. In addition, a negative correlation between serum levels of thymosin β 4 and other parameters (INR, ALT, AST and TBIL) was found, which reflected the severity of acute hepatocellular injury in patients with liver failure. There was a strong correlation between composite scores of CLF (Child-Pugh and MELD scores) and thymosin β 4. Thymosin β 4 significantly decreased in ACLF and CLF patients. These results suggested that serum thymosin β 4 can be used as an important potential predictor of liver failure caused by chronic HBV infection.

Liver failure occurs when the extent of hepatocyte death exceeds the liver's regenerating capacity. Liver regeneration is considered to be suppressed in liver failure. Hepatocyte growth factor (HGF) is a promising therapeutic agent for the treatment of liver failure. HGF not only stimulates liver regeneration, but also acts as an antiapoptotic factor in experimental liver failure models^[16]. Recent findings showed that thymosin β 4 upregulates the expression of HGF and downregulates the expression of PDGF- β receptor in human hepatic stellate cells^[14]. HGF could induce apoptosis of hepatic stellate cells and hepatocyte regeneration^[17,18]. So it is conceived that thymosin β 4 protects the liver from injury. The study demonstrated that thymosin β 4 level was significantly lowered in liver failure patients, suggesting that thymosin β 4 might become a new therapeutic agent for liver failure caused by chronic HBV infection. Of course, more investigations will be needed to answer this question.

In addition, thymosin β 4 promotes wound healing and modulates inflammatory mediators in different tissue injury^[19-21]. Thymosin β 4 reduces lethality and down-regulates inflammatory mediators in endotoxin-induced septic shock^[22]. Liver failure is a systemic inflammatory reaction, which is characterized by a predominantly proinflammatory cytokine profile, causing the transition from stable clinical condition to severe deterioration in

liver function. So it is considered that the more severe the liver inflammatory reaction is, the lower level of thymosin β 4. In this study, thymosin β 4 significantly decreased in liver failure patients.

In this study, we also found that serum thymosin β 4 was an attractive parameter in the assessment of prognosis for liver failure patients. Thymosin β 4 levels were markedly lower in the non-survivors than the survivors in liver failure cohorts. ACLF group had the same result with the liver failure group. A trend toward higher thymosin β 4 levels was seen in survivors as compared with non-survivors of CLF patients. A possible explanation for the absence of a statistically significant difference could relate to heterogeneity in complications of CLF. In addition, the relatively small number of patients may not permit small differences to be detected. Dynamic changes of thymosin β 4 concentration may further help determine the prognosis of liver failure patients.

In conclusion, our study has demonstrated a clear relationship between reductions in serum thymosin β 4 level and severity of liver failure. Serum thymosin β 4 level could be used as an important potential marker for predicting the prognosis of HBV-related liver failure (ACLF and CLF) patients. Further investigations including comparison with Child-Pugh or MELD scores are needed to assess the thymosin β 4 value and explore the mechanism of lower thymosin β 4 levels in liver failure caused by chronic HBV infection. More studies in different etiologies and a larger number of subjects of liver failure should also be considered.

COMMENTS

Background

Chronic hepatitis B virus (HBV) infection remains one of the most challenging public health problems in Asian region. Acute-on-chronic liver failure (ACLF) and chronic liver failure (CLF) are common serious conditions with a high mortality among chronic HBV infected patients. The prognosis is clearly related to the stage of the disease and early diagnosis has resulted in a significant reduction in mortality. This study aims to discover a new biomarker for diagnosing liver failure caused by chronic HBV infection.

Research frontiers

Thymosin β 4 is a 4.9-kDa polypeptide widely distributed in human tissues, which is considered as the major G-actin sequestering protein. Thymosin β 4 has been shown to have multiple biological activities involved in a variety of physiologic and pathologic processes. Recent findings suggested that thymosin β 4 could be beneficial for the treatment of chronic liver diseases (CLD). However, the levels of thymosin β 4 in liver failure patients are still unknown.

Innovations and breakthroughs

In this study, the authors examined the serum thymosin β 4 level in the HBV-related CLD patients and investigated the relationship between thymosin β 4 values and biochemical parameters and observed the dynamic changes of serum thymosin β 4 in liver failure patients. The results suggested that a lower serum thymosin β 4 level could be a potential marker for predicting HBV-related liver failure (ACLF and CLF) prognosis.

Applications

Serum thymosin β 4 level could become an important potential marker for liver failure diagnosis and is helpful in predicting the prognosis of patients with liver failure caused by chronic HBV infection.

Terminology

ACLF was defined as acute deterioration of liver function within 4 wk in

CLD, with severe jaundice and coagulopathy. CLF was defined as chronic decompensation of an end-stage liver disease, complicated with refractory ascites and/or encephalopathy, and hepatorenal syndrome.

Peer review

This study is an interesting piece of work investigating whether serum thymosin β 4 can provide diagnostic or prognostic information in liver failure patients caused by chronic HBV infection.

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