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Presepsin as a novel diagnostic biomarker for differentiating active pulmonary tuberculosis from bacterial community acquired pneumonia

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ABSTRACT

Background: The expression of presepsin in active pulmonary tuberculosis (APTB) is unknown. We observed the expression of presepsin in APTB, and to evaluate the value for discriminating between APTB and bacterial community acquired pneumonia (BCAP).

Methods: Consecutive APTB patients who were accurately diagnosed by sputum culture and BCAP patients were enrolled from August 2013 to July 2015. Clinical data were collected, and plasma presepsin concentrations were tested. Receiver operating characteristic (ROC) curves were performed for diagnostic analysis.

Results: In all, 133 healthy individuals, 103 APTB and 202 BCAP patients were enrolled. Presepsin concentrations in APTB group (218.0 [146.0, 368.0] pg/ml) were higher than those in the healthy control group (128.0 [101.5, 176.5] pg/ml, $P < 0.001$), and lower than the concentrations measured in the BCAP group (532.0 [364.0, 852.3] pg/ml, $P < 0.001$). Simple APTB and miliary tuberculosis patients showed no significant differences in presepsin concentrations. Compared with both Gram-positive and negative bacteria, *Mycobacterium tuberculosis* caused a limited increase of presepsin. With the cut-off value set at 401 pg/ml, presepsin demonstrated high positive predictive value, allowing initial discriminating between APTB and BCAP. Presepsin combined with CURB-65 score could significantly improve the discrimination ability.

Conclusions: Presepsin concentrations in APTB patients were slightly increased, and may be helpful for initial discrimination between APTB and BCAP.

1. Introduction

Fever, cough, acute dyspnea, and changes in white blood cell count are common nonspecific clinical manifestations of respiratory system infection in emergency departments. Bacteria are common pathogens of community acquired pneumonia (CAP); therefore, early identification of pathogens is essential for targeted therapy. Bacterial culture detection from blood and sputum usually takes at least several days, while positive detection rate is low, especially for blood samples [1,2]. *Mycobacterium tuberculosis* is a special kind of bacteria which causes tuberculosis, the second leading cause of death from infectious disease worldwide. For the detection of *M. tuberculosis*, routine direct microscopic examination and molecular detection are associated with high false positive rates [3]. Culture-based diagnosis of active pulmonary tuberculosis (APTB) is not widely available, because *M. tuberculosis* culturing requires specific conditions, and nearly 2 weeks are needed to obtain results [3,4]. Therefore, the identification of a biomarker that is an early indicator, and allows for the discrimination between *M. tuberculosis* and other common bacteria, is necessary for early diagnosis and the determination of the initial therapy.

Currently, the new biomarker presepsin is well known for general bacterial infections, and demonstrates good diagnostic and prognostic value for bacterial CAP (BCAP) [5–10]. Immunity against microorganisms relies primarily on the activity of monocytes and macrophages, which recognize pathogen-associated molecular patterns, partly via cluster-of-differentiation marker protein 14 (CD14), which mediates an immediate response against lipopolysaccharides (LPS) [11]. Following the binding of LPS to CD14 through LPS-binding protein, a subtype of soluble CD14 (sCD14) is released into the blood [5]. The proteolysis of sCD14 by circulating cathepsin D leads to the generation of a small soluble peptide (64 amino acids, 13 kDa), designated as presepsin. Presepsin concentrations have been shown to be significantly higher in CAP patients [6–8]. To date, no previous studies have investigated presepsin expression in APTB.

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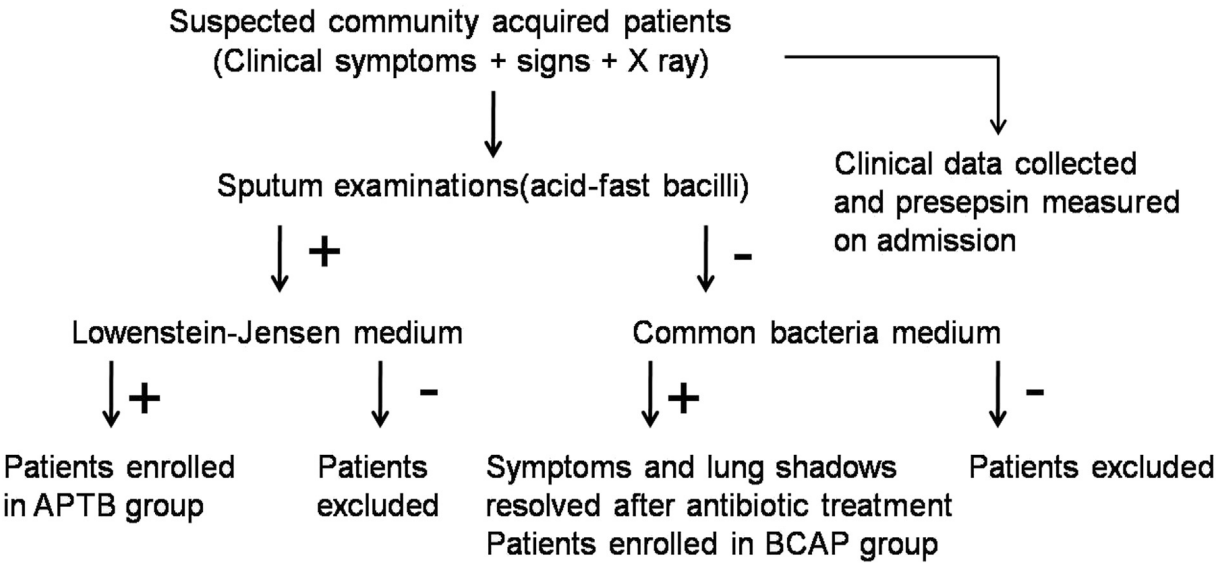

Fig. 1. Flow chart of patient inclusion.

2. Materials and methods

2.1. Patient inclusion and exclusion criteria

This study was conducted in the Emergency Departments of Beijing Chao-Yang Hospital and Beijing Tuberculosis Research Institute. China. From August 2013 to July 2015, patients who fulfilled the APTB criteria as defined by the World Health Organization (2013) and whose sputum cultures yielded positive results were enrolled [14], together with BCAP patients whose sputum examination yielded positive results for bacteria. Exclusion criteria were as follows: patients < 18 y; terminal stage of disease (malignant cancer of any type, acquired immunodeficiency syndrome, or end-stage liver or renal disease); or patients (or their relatives) who did not provide consent for participation in the study. Additionally, 133 healthy individuals with normal chest X-ray findings, were enrolled in the healthy control group, and their serum or plasma samples, remaining after routine tests, were collected. Fig. 1 shows the flow chart of patient inclusion in the study. This study was approved by the Beijing Chao-Yang Hospital Ethics Committee and Beijing Tuberculosis Research Institute Ethics Committee, and was performed in accordance with the ethical standards outlined in the Declaration of Helsinki and its later amendments. Written informed consents were obtained from all enrolled patients.

2.2. Diagnostic criteria

APTB diagnostic criteria were defined as follows [12]: one or more initial sputum smear examinations (Ziehl-Neelsen staining and direct smear microscopy) positive for acid-fast bacilli, plus chest X-ray abnormalities consistent with APTB. Meanwhile, the culture of *M. tuberculosis* in Lowenstein-Jensen medium yielded positive results in the following days.

BCAP was diagnosed when patients had clinical symptoms of pneumonia and new focal chest signs [1]. Meanwhile, chest X-ray demonstrated new lung shadows, and these resolved with antibiotic treatment. For the microbiological evaluation of patients with BCAP, we performed sputum or protective specimen brush Gram staining and culturing. Sputum smear examinations yielded negative results after Ziehl-Neelsen staining.

The criteria for sputum smear examination and sputum culture [13] were: Sputum samples were considered of good quality if they had < 10 squamous epithelial cells and > 25 leukocytes per low power field, or the ratio between the 2 is < 1:2.5. Other samples were that did not meet this criteria were excluded from the evaluation. The following test results were considered important references for etiological diagnosis: (1) Significant growth of dominant bacteria in qualified lower respiratory tract samples (except for normal flora); (2) Small amount of bacterial growth in qualified lower respiratory tract samples, but results were consistent with smear microscopy results; (3) Apparent bacterial phagocytosis by neutrophils was seen in smear microscopy of qualified lower respiratory tract samples. The presence of many morphologic microorganisms without an identifiable predominant morphotype was considered as polymicrobial flora.

2.3. Data collection

Patients' data including age, gender, and vital signs were recorded on admission. Laboratory examinations, including white blood cell counts, microbiological detection, and X-ray scans, were carried out within 24 h after admission. CURB-65 score (confusion, serum urea > 7 mmol/l, respiratory rate ≥ 30/min, systolic blood pressure < 90 mm Hg and/or diastolic blood pressure ≤ 60 mm Hg, and age ≥ 65 y) was calculated to evaluate severity of pneumonia [1,14]. Venous blood samples were obtained, collected in tubes containing EDTA and centrifuged, and the supernatant plasma samples were collected for rapid analysis. Plasma presepsin concentrations were determined using a compact automated immunoanalyzer (PATHFAST; Mitsubishi Chemical Medience Corp.) based on a chemiluminescent enzyme immunoassay [7] with lower and upper detection limits of 20 pg/ml and 200,000 pg/ml, respectively with a reference range of 60–365 pg/ml.

2.4. Statistical analysis

All data were analysed using SPSS ver 22.0. For normally distributed data, continuous variables were presented as mean ± SD. One-way analysis of variance and least significant difference test were applied for multi-group comparisons. For skewed-distribution data, variables were expressed as the median (25th to 75th percentiles). Kruskal-Wallis test was applied for multi-group comparisons, and independent sample test was performed for 2-group comparisons. Qualitative parameters were analysed using a 2 × 2 contingency table

Table 1
Patient characteristics on admission.

	Healthy control group (n = 133)	APTB group (n = 103)	BCAP group (n = 202)	P-value
Age (y)	68.0 (63.0,73.5)	51.0 (36.0–65.0)*	71.0 (59.0,76.0)#	0.000
Male/female	76/57	66/37	130/72	NS
Laboratory test				
WBC ($\times 10^9$/l)	6.5 (5.4, 7.2)	8.8 (6.3,11.7)*	11.4 (7.7,16.2)*,#	0.000
Neutrophils (%)	61.0 (54.7,66.6)	81.4 (74.2,85.8)*	90.7 (85.1,94.7)*,#	0.000
Albumin (g/l)	44.8 ± 3.3	31.1 ± 6.8*	27.2 ± 7.1*,#	0.000
Total bilirubin (μmol/l)	10.8 (8.6,13.6)	10.1 (8.2,15.4)	13.3 (9.1,20.6)*,#	0.000
BUN (mmol/l)	5.6 (4.3,6.3)	4.0 (2.7,5.8)*	8.0 (5.1,12.0)*,#	0.000
Creatine (μmol/l)	66.5 (56.0,77.4)	52.2 (41.0, 65.0)*	91.3 (69.5124.9)*,#	0.000
Comorbidities, n (%)				
Cerebrovascular disease		1 (1.0%)	12 (5.9%)	NS
COPD		6 (5.8%)	19 (9.4%)	NS
Coronary heart disease		3 (2.9%)	7 (3.5%)	NS
Diabetes		11 (10.7%)	8 (4.0%)	0.022
Others		13 (12.6%)	10 (5.0%)	0.016
CURB-65 score		1.0 (0.0,1.0)	3.0 (2.0,4.0)	0.000

APTB: Active pulmonary tuberculosis; BCAP: Bacterial community-acquired pneumonia; COPD: Chronic obstructive pulmonary disease. Other comorbidities include gastroduodenal ulcer (n = 5), chronic gastritis (n = 4), anemia (n = 4), Parkinson's disease (n = 3), rheumatoid arthritis (n = 2), postoperative fracture of femoral shaft (n = 1), near drowning (n = 1), hypertrophic cardiomyopathy (n = 1), and motor neuron disease (n = 1), Guillian-Barrie syndrome (n = 1).

* Compared with healthy control group $P < 0.05$.

Compared with APTB group $P < 0.05$.

and χ^2 or Fisher's exact tests were used for further analysis. Receiver operating characteristic (ROC) curves were constructed and the areas under the ROC curves (AUCs) were determined. Based on optimal thresholds determined according to ROC curve analysis, prognostic parameters (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], positive likelihood ratio [LR^+], and negative likelihood ratio [LR^-]) were also calculated. The cut-off value was calculated by largest Youden index which is equal to sensitivity plus specificity - 1. All statistical tests were 2-tailed, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patients' characteristics

A total of 133 healthy individuals, 103 APTB and 202 BCAP patients, were enrolled. Compared with BCAP patients, APTB patients were younger, and diabetes was an important comorbidity. There were no significant differences in gender among the groups. The white blood cell counts, percentage of neutrophils, albumin, total bilirubin, blood urea nitrogen, and creatinine concentrations in the 3 groups are presented in Table 1. The median CURB-65 score of the BCAP group was higher than that of the APTB group.

3.2. Determination of presepsin concentrations

Presepsin concentrations in the APTB group (218.0 [146.0, 368.0] pg/ml) were shown to be increased compared to those in the healthy control group (128.0 [101.5, 176.5] pg/ml, $P < 0.001$), while lower than the concentrations measured in the BCAP group (532.0 [364.0, 852.3] pg/ml, $P < 0.001$) (Fig. 2).

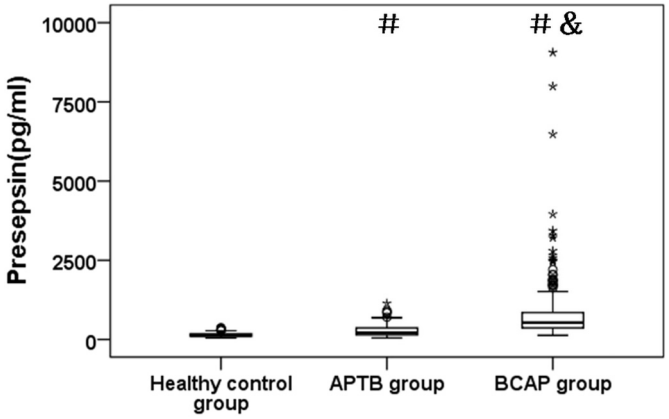

Fig. 2. Presepsin levels in 3 groups. APTB: active pulmonary tuberculosis; BCAP: bacterial community acquired pneumonia. #Compared with healthy control group ($P < 0.001$); & Compared with APTB group ($P < 0.001$).

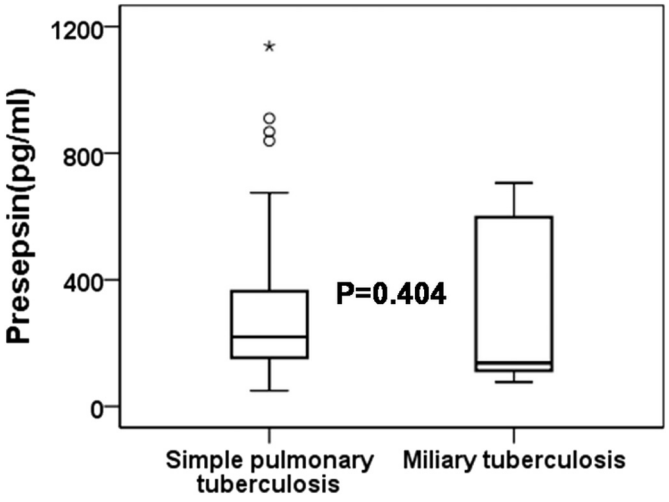

Fig. 3. Presepsin levels in simple pulmonary tuberculosis and miliary tuberculosis groups. No statistical difference was shown between the 2 groups (P = 0.404).

When the APTB patients were divided into simple pulmonary tuberculosis and miliary tuberculosis groups, there was no significant difference in presepsin concentrations between the 2 groups (P = 0.404) (Fig. 3).

3.3. Presepsin concentrations in different pathogens

For BCAP patients, *Streptococcus pneumonia, Staphylococcus aureus, and Enterococcus faecalis* were the main gram-positive bacteria identified (n = 100, 49.5%). *Haemophilus influenzae*, *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Moraxella catarrhalis* were the main gram-negative bacteria (n = 88, 43.6%). Meanwhile, mixed bacterial infections were seen in 6.9% of all infections (n = 14). Compared with *M. tuberculosis* infection, presepsin showed higher expression in patients with Gram-positive, Gram-negative and mixed bacterial infection, especially in patients with Gram-negative bacterial infection (Fig. 4).

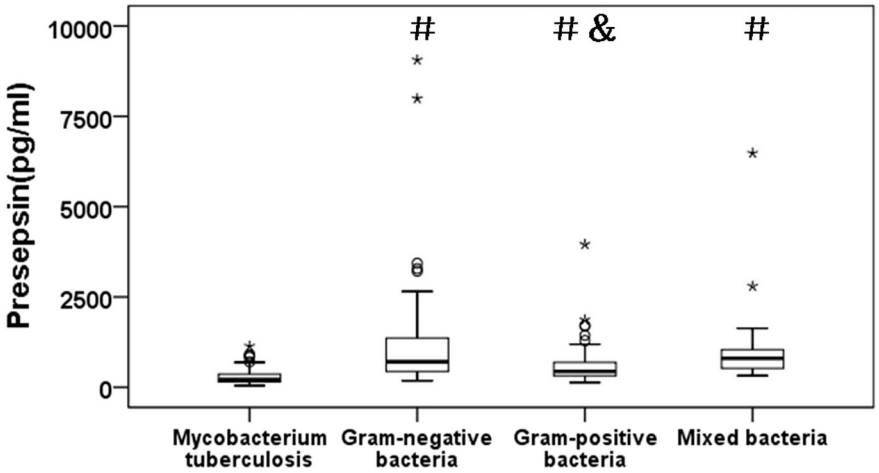

Fig. 4. Presepsin levels in patients with infection by different pathogens. [#]Compared with *Mycobacterium tuberculosis* ($P < 0.05$); [&]Compared with Gram-negative bacteria ($P < 0.05$).

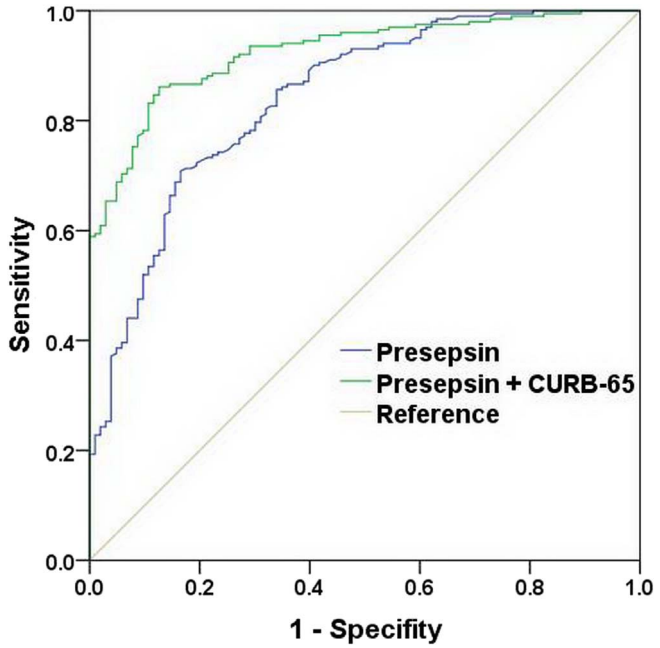

Fig. 5. ROC curves generated in order to discriminate between APTB and BCAP. Areas under the ROC curve: presepsin (purple), 0.841 (95% CI: 0.795–0.888); presepsin + CURB-65 (green), 0.925 (95% CI: 0.896–0.953). APTB: Active pulmonary tuberculosis; BCAP: Bacterial community-acquired pneumonia.

3.4. The value of presepsin in discriminating between APTB and BCAP

The ROC curves of presepsin are shown in Fig. 5. The AUC of presepsin was moderate (0.841). Using a presepsin cut-off value of 401 pg/ml, the sensitivity, specificity, PPV, NPV, and predictive accuracy of using these data for the discrimination between APTB and BCAP, are presented in Table 2. Additionally, combined presepsin and CURB-65 results can significantly improve the obtained results.

4. Discussion

In this study, we determined that the concentrations of presepsin slightly increased in APTB patients. Compared with other common bacterial infections, a relatively lower expression of presepsin was induced by *M. tuberculosis*, allowing the differentiation between APTB and BCAP.

To date, as a biomarker of infection, there is still no report about presepsin in APTB patients. The immune response against *M. tuberculosis*, at least in part, depends on monocyte–macrophage lineage [15]. Activation of monocytes and macrophages occurs after contact with various elements from pathogens. Examples of such elements are membrane and structural proteins, sugars, lipids, and nucleic acids. LPS which is a main membrane protein of Gam-positive bacteria can effectively mediate immune response by CD14. Following the binding of LPS to CD14, sCD14 is released into the blood, and the proteolysis of sCD14 leads to the generation of presepsin. Previous studies have shown significant increase in the expression of sCD14 or presepsin in sepsis [5–10,16], especially in Gram-negative bacterial infections [9,10]. Lipoarabinomannan (LAM) is a lipid glycoprotein cell wall component of *M. tuberculosis*, and shares many physiochemical properties with LPS. Hence, CD14 may play a role in the regulation of the inflammatory response during tuberculosis by interference with the bioavailability of LAM. In previous studies, it was demonstrated that the concentrations of sCD14, which is the precursor of presepsin, is somewhat increased in APTB patients [17–19]. In this study, the concentrations of presepsin, the complex product of sCD14 cleavage, were also slightly increased. Nevertheless, compared with either Gram-positive or Gram-negative bacteria, *M. tuberculosis* caused a limited increase in the concentrations of presepsin. We further observed the presepsin concentrations in nine patients with miliary tuberculosis which is usually accompanied by multifocal dissemination of *M. tuberculosis* and critical systematic inflammation response. Interestingly, the concentrations of presepsin did not increase significantly. Therefore, it is conceivable that the expression of presepsin in APTB patients was merely limited.

The ROC curves showed that presepsin had a moderate AUC (0.841)

Table 2
The value of presepsin in discriminating between active pulmonary tuberculosis and bacterial community-acquired pneumonia.

	AUC	SE	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	LR^+	LR^-
Presepsin	0.841	0.024	401.0	70.8%	83.5%	89.4%	59.3%	75.1%	4.29	0.35
Presepsin + CURB-65	0.925	0.015								

AUC: Area under the receiver operating characteristic curve; LR^+: Positive likelihood ratio; LR^-: Negative likelihood ratio; PPV: Positive predictive value; NPV: Negative predictive value; SE: Standard error.

which allowed the ability for discriminating between APTB and BCAP. With the cut-off value for presepsin set at 401 pg/ml, its sensitivity (70.8%) was low, but specificity (83.5%) was relatively high. Nevertheless, presepsin showed a high PPV (89.4%). Notably, CURB-65 scores are usually used for the evaluation of CAP, and presepsin combined with CURB-65 score could significantly improve the discrimination power.

Presepsin is stable in the general circulation. An automated rapid quantification is currently available for point of care testing. Presepsin is gradually being more widely used for auxiliary diagnosis for bacterial infection, especially in emergency departments, respiratory units or other infectious disease related departments. In this study, we showed a slight increase in presepsin concentrations in APTB patients. Nevertheless, presepsin still has an auxiliary value for initial discrimination between APTB and BCAP, and to guide the choice of initial antimicrobial treatment.

5. Limitations

This study has some limitations. First, we did not observe the dynamic change of presepsin after antituberculosis treatment. Dynamic observation may help to further understand the significance of presepsin in APTB. Second, for the determination of bacteriology, due to the low positivity rate and utility of blood culture in emergency departments [20,21], we took the results of sputum culture into account. Nevertheless, sputum samples of good quality have shown a good etiological value in recent studies [22,23].

6. Conclusions

This study first demonstrated that presepsin concentrations were slightly increased in APTB patients, and no difference was shown in simple APTB or miliary tuberculosis. Compared with other common bacteria, *M. tuberculosis* caused a limited increase in the concentrations of presepsin. With the cut-off value set at 401 pg/ml, presepsin demonstrated high PPV, which allowed initial auxiliary differentiation between APTB and BCAP. Presepsin combined with CURB-65 score could significantly improve the discrimination ability.

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