Predictive Values of Serial C-reactive Protein in Neonatal Sepsis

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Abstract

Background : Infection is one of the major problems in neonates. The diagnosis of neonatal septicemia is difficult to establish based on the clinical criteria alone. However, empirical treatment should not be delayed because of the high mortality. Laboratory tests used to support diagnosis have shown variable predictive values. C-reactive protein (CRP), an acute phase protein, increases in inflammatory disorders and tissue injury. Serial CRP have been shown to be more useful than a single measured CRP in the diagnostic evaluation of neonates with suspected infection.

Objectives : 1. To evaluate the diagnostic accuracy of serial CRP in neonatal sepsis.
2. To compare the diagnostic values between CRP and leukocyte index from a complete blood count (CBC).

Method : A prospective observational study included newborn infants, aged > 3 days and diagnosed with clinical sepsis, who were admitted in the newborn intensive care unit and special care nursery at Ramathibodi Hospital during a 14-months period. Newborn infants who received antibiotics prior to septic work up were excluded. CRP levels were measured initially at the time of septic work-up and at 24-48 hours later. Investigations for infection included CBC, blood culture and urine culture. Radiological study and lumbar puncture were performed if clinically indicated. Based on clinical and biological data, diagnosis of infants can be categorized into 4 groups as follows; (1) proven sepsis with positive culture, (2) localized infection with negative culture, (3) probable infection (clinically consistent with sepsis, negative culture without localized infection), and (4) no infection (findings not consistent with sepsis and antibiotics were discontinued within 3 days). Diagnosis was made before the CRP results were known.

Results : Of 76 newborn infants with 90 episodes of clinical sepsis, there were 24 episodes of proven sepsis, 11 episodes of localized infection with negative culture, 18 episodes of probable infection and 37 episodes of no infection. Serial CRP had better predictive values than those of CBC. The sensitivity, specificity, positive predictive value, and negative predictive value of CRP for proven sepsis and localized infection at cutoff point ≥ 5 mg/L were 100 per cent, 94 per cent, 91.6 per cent and 100 per cent respectively. False positive CRP were found in post-operative patent ductus arteriosus ligation, intracerebral hemorrhage, and post resuscitation with chest compression. To improve the

predictive value of CBC, analysis of the receiver operating characteristic (ROC) curve showed that the predictive value of CBC for sepsis would be enhanced by using abnormal leukocyte index ≥ 2 parameters.

Conclusions : Predictive value of CRP could be enhanced by serial rather than a single measurement. Serial CRP showed very high predictive values for diagnosis of neonatal sepsis and were better than those of leukocyte indices of CBC.

Key word : C-reactive Protein, Predictive Value, Neonatal Sepsis

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Neonatal infection is one of the major problems in the newborn period. The diagnosis of neonatal sepsis is difficult to establish based on the clinical picture alone. However, treatment should not be delayed because of high morbidity and mortality. Fanaroff et al[1] reported that signs and symptoms associated with neonatal sepsis were non-specific and had low positive predictive values of only 13-20 per cent, resulting in high use of empirical therapy. It has been estimated that 30 cases would receive empirical therapy to treat only one case of true sepsis leading to high medical costs and increased prevalence of drug resistant microorganisms in the nursery [2,3]. There have been many attempts to develop screening tests that can identify infected infants, however, laboratories used to support the diagnosis of neonatal sepsis have shown low predictive values such as leukocyte index from complete blood count (CBC) or erythrocyte sedimentation rate (ESR) which showed a sensitivity of only 42-58 per cent[4].

C-reactive protein (CRP) is an acute phase reactant[5,6], synthesized by hepatocyte in response to inflammation or infection. After the onset of inflammation, CRP synthesis increases within an hour and peaks at 24-48 hours with a half life of 6-19 hours [7]. Peak level may be 100-fold higher than the normal value and then rapidly declines after infection or inflammation are under controll[7-9]. Predictive values of CRP for neonatal sepsis were reported to be approximately 75-85 per cent for sensitivity and specificity[10,11]. However, Pourcyrous et al[12] reported that predictive values could be enhanced by obtaining serial CRP with 91-92 per cent sensitivity and specificity for gram negative sepsis. The data from Benitz et al found that serial CRP every 24 hours for 3 days were very useful in the diagnostic evaluation of neonates with suspected infection with a sensitivity and specificity of 96-98 per cent for late onset sepsis[13].

The objectives of this study were to

1. determine the diagnostic accuracy of serial CRP in neonatal sepsis.

2. compare predictive values of serial CRP to leukocyte index from CBC for the diagnosis of neonatal sepsis.

METHOD

The prospective observational study was conducted at the neonatal intensive care unit and special care nursery at the Ramathibodi Hospital from June 2000 to August 2001.

The population included all newborn infants, aged more than 3 days, who presented with signs and symptoms of neonatal sepsis, eg. lethargy, apnea, respiratory distress, temperature instability, abdominal distension, increasing oxygen requirement or respiratory support, metabolic derangement or hematologic abnormalities. Infants who had received antibiotics before septic work-up and blood culture were excluded. Infants who met the criteria had septic

work-up done including CBC, blood culture, urine culture, and cerebrospinal fluid culture. Radiographic study for localized infection was performed as clinically indicated. One and a half mL of blood was required for a serum CRP measurement which was performed by the enhanced immunonephelometry technique using a commercial kit [CRP (Latex) US, Roche Diagnostics Corporation, Indianapolis, IN, USA] with the coefficient varience of 10 per cent. CRP level was obtained at the time of initial septic work-up and again 24-48 hours later. Physicians who took care of these infants did not know the CRP results. CRP level ≥ 5 mg/L was considered abnormal. Final diagnosis, based on clinical findings and culture results, were categorized into 4 groups: (1) proven sepsis with positive culture defined as an infant who had signs and symptoms compatible with sepsis and positive culture from sterile specimens; (2) localized infection with negative culture defined as an infant who had localized infection, eg. pneumonia, necrotizing enterocolitis with negative cultures; (3) probable infection defined as an infant who had signs and symptoms compatible with sepsis, but negative culture results, no localized infection and had received antibiotics for more than 5 days; and (4) no infection defined as an infant who had no evidence of infection and antibiotics were discontinued within 3 days; initial signs and symptoms of suspected sepsis could be explained by other causes. The norms of leukocyte count for infants were based on previous studies(14-16).

Statistical analysis

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated to determine the predictive values of CRP and leukocyte index of CBC. Data were expressed in mean ± standard deviation or median (range) as applicable. The receiver operating characteristic (ROC) curve was used to determine the parameters from CBC that would show the best set of predictive values.

RESULTS

Seventy-six infants (44 males, 34 females) with 90 episodes of suspected sepsis were enrolled in the study with a mean birth weight of 2011 ± 942 g and a mean gestational age of 33 ± 4.9 weeks. The median age at the time of enrollment was 11 days. Most of the infants had a single episode of suspected sepsis except for 12 infants; 11 infants (6 males) had 2 episodes and one male had 4 episodes. Common presentations leading to evaluate for sepsis were poorfeeding, apnea, increased oxygen requirement, desaturation, and temperature instability. All of the infants had more than one presentation. The number of episodes in each group are shown in Table 1.

In 24 episodes of proven sepsis, 12 were gram negative bacteria [Escherishia coli (n=9) and Klebsiella pneumoniae (n=3)], 8 were gram positive (Staphylococcus epidermidis (n=5), Staphylococcus aureus (n=2), and group B Steptococci (n=1)], 3 were Candida and 1 was Bacillus cereus. There were 11 episodes of localized infection; 3 pneumonia, 4 necrotizing enterocolitis (NEC) stage II, 2 NEC stage III, and 2 NEC stage I, respectively.

In the proven sepsis group, all infants had abnormal CRP of both specimens. Similarly, in the localized infection group, 90 per cent of infants had abnormal CRP at the time of initial septic work-up and 24-48 hours later all infants in this group had abnormal CRP. Approximately, 66-67 per cent of infants in the probable sepsis group had abnormal CRP (Fig. 2). Only three infants had abnormal CRP but without infection (false positive CRP). The conditions of these 3 infants were; one with post patent ductus arteriosus (PDA) ligation, one with post resuscitation with chest compression and one with intracranial hemorrhage.

Predictive values for proven sepsis or localized infection of the initial CRP (CRP 1) and the second CRP (CRP 2) are shown in Table 2. It clearly showed that serial CRP 24 to 48 hours after the onset of infection had very high predictive values with a

Table 1. Number of episodes according to final diagnosis.

Final diagnosis	No. of episodes	%
Proven sepsis with positive culture	24	26.7
Localized infection with negative culture	11	12.2
Probable infection	18	20
No infection	37	41.1

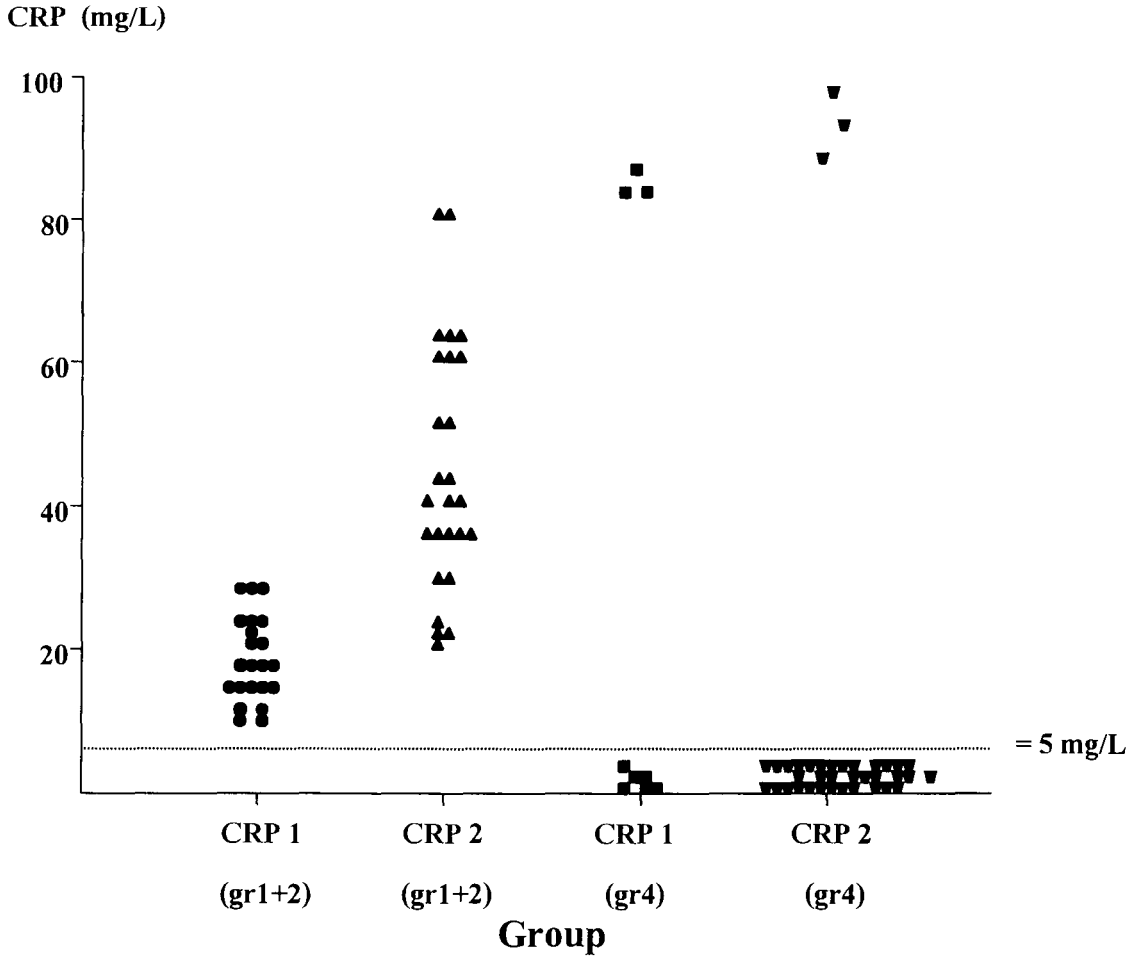

Fig. 1. Scattogram shows CRP level by diagnostic group. CRP level of ≥ 5 mg/L demonstrates the best cut-off point to differentiate infected from non-infected infants. (CRP1 = initial CRP, CRP2 = second CRP 24-48 hours later, gr1+2 = proven sepsis and localized infection group and gr4 = non-infected group).

sensitivity of 100 per cent and negative predictive value of 100 per cent.

Serial CRP in the present study provided a much better predictive value compared to leukocyte index from CBC including total white blood cell count, absolute neutrophil count, immature to total neutrophil ratio and platelet count. (Table 3). A single parameter index from CBC showed a sensitivity range of 37-57 per cent. Because of the low predictive value of single leukocyte index from CBC, the authors used the ROC curve to figure out how many abnormal indices from CBC would provide a more reliable value to predict infection. The analysis (not illustrated here) showed that a combination of any two abnormal indices from CBC provided better values with a sensitivity of 39 per cent, a specificity of 89 per cent, a positive predictive value of 69 per cent, and a negative predictive value of 70 per cent, but these were still not as good as the CRP values.

DISCUSSION

Infection is one of the major causes of death during the neonatal period. To establish the diagnosis is also difficult due to non-specific symptoms and

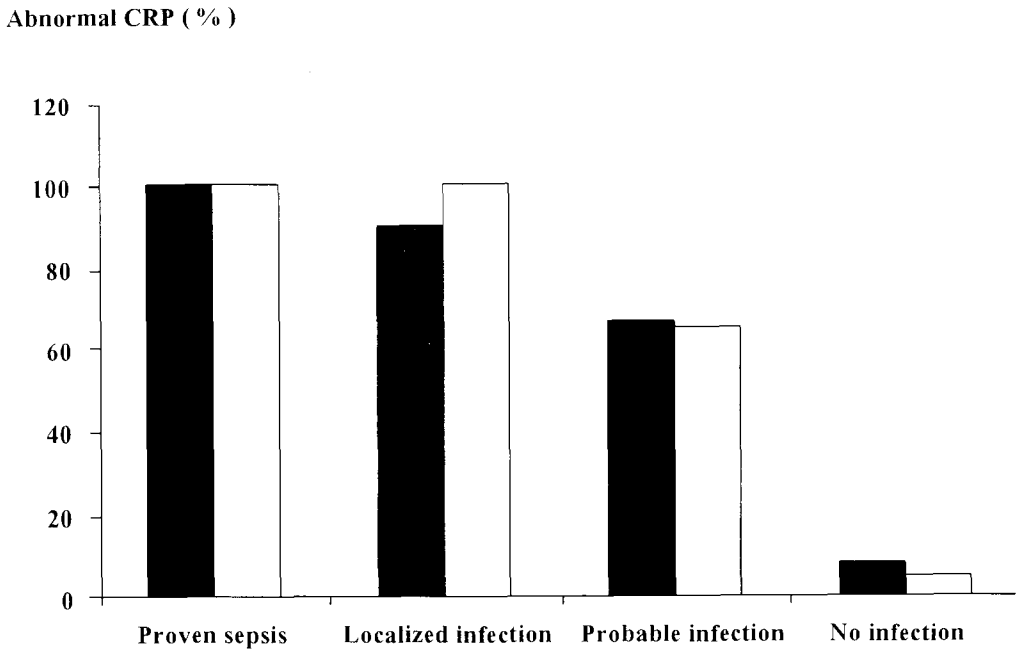

Fig. 2. Percentage of abnormal CRP (≥ 5 mg/L) according to diagnostic groups. ■ represents initial CRP and represents CRP obtained 24-48 h later.

Table 2. Predictive values of the initial CRP (CRP1) and the second CRP (CRP2) (cut-off point ≥ 5 mg/L).

Predictive values	CRP1 (%)	CRP2 (%)
Sensitivity	94.2	100
Specificity	91.8	94
Positive predictive value	91.6	91.6
Negative predictive value	94.4	100

signs and lack of sensitive diagnostic tests[1,2]; these reasons lead to initiate treatment with empirical antibiotics as soon as suspected infection is of concern [3]. This clinical practice can result in an increased incidence of antibacterial resistant organisms and high medical costs, therefore a sensitive diagnostic test which can differentiate infected from non-infected infants would be very helpful. In the present study, serial CRP showed a very high sensitivity and demonstrated very high positive and negative predictive values for proven sepsis and localized infection. The advantages of CRP over the other acute phase proteins are that it does not depend on gestational age, post-natal age, hemoglobin level, or red blood cell mass, and because it does not cross the placenta, thus increased CRP level indicates the response of the infants by themselves to infection or inflammation[11].

False positive CRP occurred in 3 infants who already had tissue injury: one with post-operative PDA ligation, one with intracranial hemorrhage and one with post resuscitation with chest compression. With these circumstances, physicians can predict the events associated with elevated CRP and can be clinically aware of the possibility of false positive CRP before interpreting the results. In the present study, the authors elected not to include early onset sepsis since the yield of positive bacterial culture is low and most of the infants are asymptomatic and being treated due to maternal risk factors.

The authors selected the positive cultures from sterile specimens in suspected septic infants (proven sepsis) or localized infection group as a gold standard for calculation of predictive values. The probable infection group was not included for calculation of predictive values, because it was difficult to assess whether the good clinical responses in this group were due to given antibiotics or not, since they usually received more than one medical intervention

Table 3. Comparison of the predictive values between serial CRP and CBC.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Serial CRP	100	94.4	91.6	100
White blood cell count	37	72	56	55
Absolute neutrophil count	54	75	67	63
Immature:total neutrophil ratio	57	89	83	67
Platelet count	52	97	92	61

at the time of septic work-up, such as increased respiratory support, blood transfusion, increased inspired oxygen concentration, or holding feeding, etc. However, if probable infection was recruited into the proven sepsis group, the predictive values would have a sensitivity of 89.8 per cent, a specificity of 91.6 per cent, a positive predictive value (PPV) of 93.6 per cent, and a negative predictive value (NPV) of 86.8 per cent.

Comparison of diagnostic values between serial CRP and CBC, this study showed much better predictive values of serial CRP than those of CBC. This finding supports the previous reports of low sensitivity and non-specificity of CBC in neonatal sepsis[4]. The ROC analysis indicated that combined more than one abnormal parameter or leukocyte index would enhance the specificity and predictive values of CBC.

In the present study, the second CRP using a cutoff point $\geq$ 5 mg/L obtained 24-48 hours apart from the initial septic work-up, showed a very high predictive value with a sensitivity of 100 per cent, specificity of 94.4 per cent, PPV of 91.6 per cent and NPV of 100 per cent. This finding supports the use of serial CRP instead of a single measurement[12, 13]. In addition, due to very high predictive values of the second CRP, physicians might obtain only one CRP measurement 24 to 48 hours after the onset of suspected sepsis as a diagnostic test especially in a limited budget situation or in a non-reliable bacterial culture facility.

Another application is that, due to a high NPV of almost 100 per cent, one might consider using a negative CRP together with clinical evaluation to discontinue the empirical antibiotics[17], this would help to reduce medical cost and decrease the chance of developing anti-microbial resistant organisms in the nursery. Further studies are required concerning the possibility and safety of using CRP to determine the duration of antibiotic treatment.

In summary, serial CRP showed a very high predictive value which was better than that of CBC in neonatal sepsis, and due to a very high NPV, it could be used to rule out bacterial infection or localized infection.

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ความแม่นยำของ Serial C–reactive Protein ต่อการทำนายภาวะติดเชื้อในทารกแรกเกิด

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ภาวะติดเชื้อเป็นปัญหาที่พบบ่อยในทารกแรกเกิด แต่การวินิจฉัยทำได้ยาก เนื่องจากอาการและอาการแสดงต่าง ๆ ที่สงสัยว่าสัมพันธ์กับการติดเชื้อในผู้ป่วยทารกนั้น ไม่มีความจำเพาะเจาะจง นอกจากนี้ผลการตรวจทางห้องปฏิบัติการต่าง ๆ ที่นำมาใช้ช่วยวินิจฉัยภาวะติดเชื้อในทารก เช่น leukocyte index จาก complete blood count (CBC) หรือ erythrocyte sedimentation rate (ESR) นั้น มี sensitivity เพียงประมาณร้อยละ 60 เท่านั้น จึงมีการศึกษาหา marker อื่นที่จะนำมาใช้ช่วยในการวินิจฉัยภาวะนี้ C–reactive protein (CRP) เป็นหนึ่งใน acute phase reactant การศึกษาถึงความแม่นยำของ CRP ในภาวะติดเชื้อน่าจะเป็นประโยชน์อย่างมากในการดูแลผู้ป่วย

วัตถุประสงค์ : 1. ศึกษา predictive value ของ serial CRP ในการวินิจฉัยภาวะติดเชื้อในผู้ป่วยทารก
2. เพื่อเปรียบเทียบ predictive value ระหว่าง serial CRP กับ leukocyte index ในการวินิจฉัยภาวะติดเชื้อ

รูปแบบการศึกษา : Prospective observational study

ประชากรที่ศึกษา : ทารกแรกเกิด อายุ > 3 วัน ที่มีอาการและอาการแสดงสงสัยภาวะติดเชื้อในกระแสเลือด โดยไม่รวมผู้ป่วยที่ได้รับยาปฏิชีวนะมาก่อนที่จะทำการศึกษา ช่วงเวลาตั้งแต่เดือนมิถุนายน 2543 ถึงเดือนสิงหาคม 2544

วิธีการศึกษา : ทารกที่เข้ารับการศึกษาทุกรายจะได้รับการตรวจ CBC, CRP, เพาะเชื้อจากเลือดและปัสสาวะ สำหรับการเจาะตรวจน้ำไขสันหลังหรือการตรวจทางรังสีขึ้นกับการพิจารณาของแพทย์ผู้ดูแลรักษา หลังจากนั้น 24–48 ชั่วโมง จะส่งตรวจ CRP ครั้งที่ 2 ความเห็นของแพทย์ผู้ดูแล และผลการเพาะเชื้อสามารถนำมาแบ่งกลุ่มผู้ป่วยเป็น 4 กลุ่มคือ

1. ผลการเพาะเชื้อขึ้นใน sterile specimen (proven sepsis)
2. ผลการเพาะเชื้อไม่ขึ้น แต่มีการติดเชื้อเฉพาะที่ (localized infection)
3. ผลการเพาะเชื้อไม่ขึ้น ไม่มีการติดเชื้อเฉพาะที่ แต่อาการแสดงสงสัยภาวะ sepsis (probable sepsis)
4. ผลการเพาะเชื้อไม่ขึ้น ไม่มีการติดเชื้อ และหยุด antibiotic ก่อน 3 วัน (no infection)

ผลการศึกษา : มีทารก 76 ราย ในการศึกษานี้ โดยสงสัยมีภาวะติดเชื้อทั้งสิ้น 90 ครั้ง แยกเป็น กลุ่มที่ 1 จำนวน 24 ครั้ง, กลุ่มที่ 2 จำนวน 11 ครั้ง, กลุ่มที่ 3 จำนวน 18 ครั้ง, กลุ่มที่ 4 จำนวน 37 ครั้ง Serial CRP มีค่า predictive value สูงกว่าของ CBC ในการทำนายภาวะติดเชื้อ ค่า cutoff ที่ดีที่สุดของ CRP ในการทำนายภาวะติดเชื้อ คือ 5 มก/ล โดยมีค่า sensitivity 100%, specificity 94%, positive predictive value 91.6%, negative predictive value 100% CRP ที่ผิดปกติโดยไม่มีภาวะติดเชื้อพบในผู้ป่วย 3 ราย คือผู้ป่วยหลังผ่าตัด patent ductus arteriosus ligation, post resuscitation with chest compression และภาวะเลือดออกในสมอง Leukocyte index จาก CBC ที่มีความผิดปกติตั้งแต่หรือเท่ากับ 2 ค่าขึ้นไป พบว่าให้ค่า predictive value ที่ดีกว่าใช้ความผิดปกติเพียง 1 ค่า

สรุป : Serial CRP มีความไวและความจำเพาะที่สูง จึงเป็นการทดสอบที่ดีที่จะช่วยในการวินิจฉัยภาวะติดเชื้อและมีความแม่นยำมากกว่าการใช้ค่า leukocyte index จาก CBC

คำสำคัญ : ซี-รี แอคทีฟ โปรตีน, ความแม่นยำ, ภาวะติดเชื้อในทารก

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