

## Accuracy of plasma interleukin-6 and C-reactive protein as markers of sepsis in preterm neonates with respiratory distress syndrome

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**ABSTRACT:** *Aim:* To evaluate the diagnostic accuracy of plasma interleukin-6 (IL-6) and serum CRP in detecting blood culture proven sepsis in preterm neonates with respiratory distress syndrome (RDS).

*Patients and Methods:* 140 preterm neonates, 62 with RDS and 78 without RDS, who developed clinical signs of sepsis were prospectively studied. Plasma IL-6 and serum CRP levels were measured. The ROC curves, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios (LR) were calculated for IL-6 ( $> 20$  pg/mL and  $> 80$  pg/mL) and CRP ( $> 10$  mg/L).

*Results:* Positive blood cultures were found in 64 neonates (37 with RDS and 27 without RDS). Neonates with RDS had higher IL-6 ( $p < 0.0001$ ) as compared to neonates without RDS. The ROC curves showed that in neonates without RDS both markers were accurate in diagnosing sepsis with an AUC of 0,914 and 0,863 for IL-6 and the CRP, respectively. However, in neonates with RDS the AUC was 0,642 for IL-6 and 0,808 for CRP. In addition, the sensitivity, specificity and LR for IL-6  $> 20$  pg/mL was 0,93, 0,65 and 2,68, respectively, in neonates without RDS, and 0,92, 0,16 and 1,09, respectively, in neonates with RDS. The sensitivity, specificity and LR of CRP  $> 10$ mg/L were 0,86, 0,71 and 2,97, respectively, in neonates without RDS and 0,73, 0,68 and 2,28, respectively, in neonates with RDS.

*Conclusions:* In neonates without RDS both the IL-6 and CRP are equally accurate markers in diagnosing sepsis, whereas in those with RDS the diagnostic value of IL-6 is limited.

*Key Words:* Preterms, Respiratory distress syndrome, Sepsis, Sensitivity, Specificity.

### INTRODUCTION

Neonatal sepsis is a life threatening complication of prematurity needing prompt diagnosis and treatment. Blood cultures are the most specific tests for diagnosing sepsis. However, previous studies suggest that the sensitivity of blood cultures may be low in neonates<sup>1</sup>. In addition, it may take several days to obtain a definite answer for a negative blood culture. For this reason, biomarkers yielding reliable results within a few hours are desirable in order to support quick decisions about antibiotic treatment. Serum C-reactive protein (CRP) is the most widely used diagnostic marker for sepsis with a high diagnostic accuracy, especially af-

ter the 1<sup>st</sup> day of the disease<sup>2-9</sup>. During recent years, several cytokines, such as interleukin-6 (IL-6) and interleukin-8 (IL-8)<sup>7-10</sup>, and soluble receptors (soluble TNF-R, soluble IL-2R) have been evaluated for early detection of neonatal sepsis<sup>2-9,11</sup>. IL-6 is the most thoroughly evaluated sepsis parameter, with a sensitivity up to 99% on first day of neonatal sepsis<sup>4-9,11,12</sup>.

Respiratory distress syndrome (RDS) is the most common disease of the preterm neonates associated with high morbidity and mortality. Preterm neonates with RDS receiving intensive care may deteriorate due to various complications either septic or not (i.e. air leak and patent ductus arteriosus). In cases where no

obvious etiology can be identified, clinicians should be able to assess if sepsis is the reason for a clinical deterioration. Although surfactant deficiency is the main cause of RDS in preterm neonates, a growing body of evidence suggests that inflammatory response is involved in the pathogenesis of lung damage and may influence the levels of various immunological parameters, including IL-6<sup>13,14</sup>. Several observations in bronchoalveolar fluid point to an important contributing role of activated neutrophils and cytokines in the pathogenesis of RDS and progression to chronic lung disease<sup>14,15</sup>. In neonates with RDS elevated concentrations of IL-6 have been found in the plasma<sup>16</sup> and tracheobronchial lavage early in the course of the disease<sup>17</sup>. Moreover, elevation of IL-6 and IL-8 precedes the influx of neutrophils in the bronchial secretions of preterm infants with RDS who progress to chronic lung disease<sup>18</sup>. Based on these data, we assumed that plasma IL-6 levels may not be an accurate predictor of septic complications in neonates suffering from RDS.

In this study IL-6 and CRP levels were measured in preterm neonates with and without RDS who developed clinical signs suggestive of sepsis aiming at evaluating the accuracy of these two markers in detecting sepsis and how it is influenced by the presence of RDS.

## PATIENTS AND METHODS

### *Study population*

In a prospective study, 140 preterm neonates, 62 with RDS and 78 without RDS, who were hospitalized in the 1<sup>st</sup> Neonatal Department of the Aristotle University of Thessaloniki and developed clinical evidence of sepsis were included. Clinical signs of suspected sepsis were lethargy, hypotonia, reduced motor activity, pallor, hypotension, decreased peripheral circulation, deterioration of respiratory distress, increasing demands for ventilatory support, feeding intolerance, abdominal distension, vomiting and bloody gastric aspirates or stools. Diagnosis of RDS was made in neonates with respiratory distress and radiological findings consistent with RDS. Neonates with positive blood cultures were defined as septic. The study was approved by the ethical committee of our institution. Blood samples were collected as soon as clinical signs of sepsis emerged.

### *Laboratory investigation*

Laboratory investigation included a full sepsis screen

(complete blood count and differential, platelet count and CRP measurement), measurement of plasma IL-6 and blood and urine cultures for bacteria and fungi. Chest radiographs were routinely performed while cerebrospinal fluid cultures and abdominal radiographs were performed when clinically indicated. CRP was measured by using nephelometry. IL-6 was measured by a solid phase quantitative enzyme immunoassay with appropriate commercial available kits (Quantikine-R&D systems, British Biotechnology, Ltd) according to the manufacturer's instructions. The lower limit of detection was 0.36 pg/mL and 3.5 mg/L for IL-6 and CRP, respectively.

### *Statistical analysis*

Values of IL-6 and CRP were not normally distributed (Kolmogorov-Smirnov test) and differences between groups were analyzed using the Mann Whitney *U*-test. Comparisons of dichotomous variables were performed using the Fisher exact test. A two-sided significant level of 0,05 or less was considered statistically significant.

To test the validity of these markers in diagnosing sepsis two receiver operating characteristic (ROC) curves were obtained for IL-6 and CRP, each one in the RDS and the non RDS groups. The area under the curve (AUC) was used as an estimator of the overall diagnostic accuracy<sup>19</sup>. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the likelihood ratios (LR) for detecting sepsis were calculated for CRP using the level of 10 mg/L as cut-off value<sup>20</sup> and for IL-6 using two different cut-off values, the 20 pm/mL and the 80 pg/ml based on published data<sup>3,20-22</sup>. Statistical analyses were performed using the softwares SPSS 16.0 for WINDOWS® και GraphPad InStat 3®.

## RESULTS

### *Population characteristics*

Blood cultures were found positive in 64 neonates (37 with RDS and 27 without RDS) and negative in 76 (25 with RDS and 51 without RDS) (Table 1). The two groups of neonates with RDS (septic and non-septic) did not differ significantly with regard to the clinical characteristics and the incidence of the commonest neonatal problems. Also, similar numbers among the two groups were on mechanical ventilation, on total parenteral nutrition and presence of umbilical arterial catheters. Comparison between the two groups of ne-

**Table 1.** Clinical of the studied population.

GROUPS	NEONATES WITH RDS			NEONATES WITHOUT RDS		
	NON- SEPTIC	SEPTIC	p	NON-SEPTIC	SEPTIC	p
n	25	37		51	27	
Gestational age (wks, x±SD, range)	30,1 ± 3,3 (25-37)	30,8 ± 2,4 (27-37)	0,39	31,5 ± 2,7 (26-36)	32,2 ± 3,2 (26-37)	0,044
Birth weight (g, x±SD, range)	1434 ± 511 (650-2700)	1546 ± 490 (800-2820)	0,43	1703 ± 512 (850-2940)	1718 ± 636 (720-2730)	0,91
Age at study entry (d)	5,0 ± 5,2 (1-21)	7,6 ± 5,0 (0-24)	0,075	5,3 ± 6,7 (0-28)	7,8 ± 5,0 (1-22)	0,65
sex (M/F)	17/8	23/14	0,79	29/22	13/14	0,16
Perinatal asphyxia (n, %)	3 (12)	5 (14)	1	8 (16)	3 (11)	0,74
Mechanical ventilation (n, %)	18 (72)	35 (95)	0,21	21 (41)	19 (70)	0,018
Umbilical arterial catheter (n, %)	23 (92)	35 (95)	1	9 (18)	7 (26)	0,39
Patent ductus arteriosus (n, %)	2 (8)	7 (19)	0,29	-	1 (4)	0,42
Necrotizing enterocolitis (n, %)	-	3 (8)	0,266	-	3 (11)	0,07
Intra-peri-ventricular haemorrhage grade III-IV (n, %)	3 (12)	6 (16)	0,73	-	-	N.S.

onates without RDS showed that the septic neonates had a higher GA of borderline significance, obviously due to the fact that in the absence of RDS, sepsis is the main reason of clinical deterioration of preterm neonates with higher GA. Also, septic neonates without RDS had significantly higher incidence of necrotizing enterocolitis, ventilatory support and total parenteral nutrition in comparison with the non-septic-non-RDS neonates, as expected (Table 1). Microorganisms isolated from blood cultures included *klebsiella spp.* (n = 23), *coagulase negative staphylococcus spp.* (n = 17), *coagulase positive staphylococcus* (n = 5), *fungi* (n = 5), *streptococcus spp* (n = 2), *acinetobacter* (n = 5), *enterobacter* (n = 3), *pseudomonas spp* (n = 2), *E. Coli* (n = 1) and *enterococcus* (n = 1).

#### **Plasma IL-6 levels**

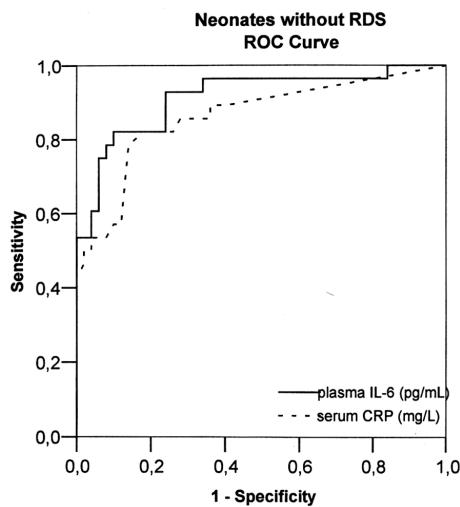
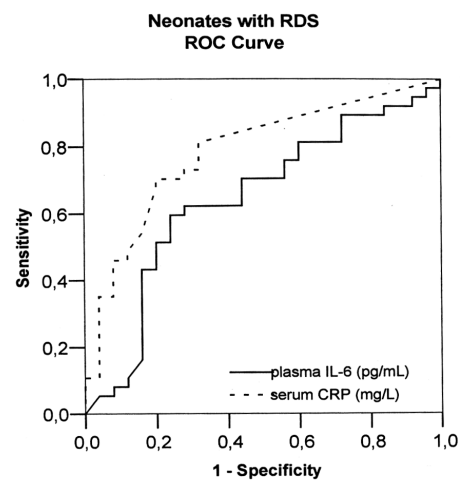
Among the neonates without RDS, those with sepsis had significantly higher IL-6 levels than the non-septic ones. In neonates with RDS, IL-6 levels did not differ significantly between the septic and non-septic ones. Comparison between the two groups without sepsis showed neonates with RDS had significantly higher IL-6 than those without RDS ( $p < 0,0001$ ). No significant difference was found between the two groups of septic neonates ( $p = 0,25$ ) (Table 2).

#### **Serum CRP levels**

Among the two groups without RDS, those with sepsis had significantly higher CRP levels in comparison with neonates without sepsis. Similarly, of the neonates with RDS, neonates with sepsis had significantly higher CRP levels than those without sepsis (Table

**Table 2.** Plasma IL-6 and serum CRP levels in the groups studied.

GROUPS	NEONATES WITH RDS			NEONATES WITHOUT RDS		
	NON-SEPTIC	SEPTIC	p	NON- SEPTIC	SEPTIC	p
n	25	37		51	27	
IL-6 (pg/mL, median-range)	129 (13-480)	326 (12-480)	0,059	10,0 (1-372)	305 (3-480)	< 0,0001
CRP (mg/L, median-range)	3,5 (3,5-99)	23 (3,5-150)	< 0,0001	3,5 (3,5-45)	44 (3,5-132)	< 0,0001

**Figure 1.** The ROC curves for CRP and IL-6 in neonates without RDS and those without RDS.**Figure 2.** The ROC curves for CRP and IL-6 in neonates without RDS and those with RDS.

2). No significant difference was found between the two groups of non-septic neonates (with and without RDS) nor between the two groups of septic ones.

#### **Diagnostic accuracy**

The ROC curves obtained from the neonates without RDS showed that both markers were accurate in diagnosing sepsis in this group of neonates with an AUC of 0,914 and 0,863 for IL-6 and CRP, respectively (Figure 1). However, the ROC curves obtained from neonates with RDS showed that the IL-6 had a low overall accuracy with an AUC 0,642 (30% lower than that in the non-RDS group), whereas the overall accuracy CRP remained high with an AUC 0,808 (fig. 2). The sensitivity, specificity, PPV, NPV and LR of IL-6 >20 pg/mL and >80 pg/mL and CRP >10mg/L in neonates with RDS and those without RDS are shown in Tables 3 and 4.

#### **DISCUSSION**

The main findings of our study were two; a) In neonates without RDS both the IL-6 and CRP are accurate diagnostic markers of blood culture proven sepsis, whereas in those with RDS IL-6 is of limited diagnostic value and only the CRP can be used as a marker of sepsis. b) In neonates with RDS IL-6 levels are increased regardless of the presence of sepsis.

Previous studies have documented increased circulating IL-6 levels soon after the onset of sepsis. For this reason, this proinflammatory cytokine has been evaluated as a diagnostic tool by several authors<sup>7,9,20,23</sup>. The reported sensitivity of IL-6 in detecting neonatal sepsis ranges from 0,80 to 1,00, the specificity from 0,43 to 1,00 and the positive LR from 1,5 to 21,5<sup>3-9,11,16,24</sup>. These discrepancies, which make the comparisons between studies difficult, can be attributed to the

**Table 3.** Diagnostic accuracy of plasma IL-6 and serum CRP in neonates without RDS.

	<b>IL-6 <math>\geq</math> 20 pg/mL</b>	<b>IL-6 <math>\geq</math> 80 pg/mL</b>	<b>CRP <math>\geq</math> 10 mg/L</b>
	Value (96% CI)	Value (96% CI)	Value (96% CI)
<b>Sensitivity</b>	0,93 (0,77-0,99)	0,71 (0,51-0,87)	0,86 (0,67-0,96)
<b>Specificity</b>	0,65 (0,51-0,78)	0,94 (0,84-0,99)	0,71 (0,57-0,83)
<b>PPV</b>	0,59 (0,43-0,74)	0,87 (0,66-0,97)	0,62 (0,45-0,77)
<b>NPV</b>	0,94 (0,81-0,99)	0,86 (0,74-0,94)	0,90 (0,77-0,97)
<b>LR</b>	2,68	12,38	2,97

PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR: Likelihood Ratio.

**Table 4.** Diagnostic accuracy of plasma IL-6 and serum CRP in neonates with RDS.

	<b>IL-6 <math>&gt;</math>18 pg/mL</b>	<b>IL-6 <math>&gt;</math>80 pg/mL</b>	<b>CRP <math>&gt;</math>10 mg/L</b>
	Value (95% CI)	Value (95% CI)	Value (95% CI)
<b>Sensitivity</b>	0,92 (0,78-0,98)	0,81 (0,65-0,92)	0,73 (0,56-0,86)
<b>Specificity</b>	0,16 (0,05-0,36)	0,40 (0,21-0,61)	0,68 (0,46-0,85)
<b>PPV</b>	0,62 (0,48-0,75)	0,67 (0,51-0,80)	0,77 (0,60-0,90)
<b>NPV</b>	0,57 (0,18-0,90)	0,59 (0,33-0,82)	0,63 (0,42-0,81)
<b>LR</b>	1,09	1,35	2,28

PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR: Likelihood Ratio.

wide range of cut-off points used (15 pg/mL - 150 pg/mL), the demographic data of the population studied and the time elapsed between the onset of sepsis and blood sampling<sup>25,26</sup>. In most studies, the cut-off values used were obtained from healthy neonates. However, for a certain population, like neonates with RDS, special cut-off values may be needed. In our study, two ROC curves were evaluated; the first, for the neonates without RDS and the second, for those with RDS. The ROC curve obtained from neonates without RDS had an AUC of 0,901 which is considered high for a diagnostic test. However, when values from neonates with RDS were used for ROC curve, the AUC was very low (0,642) indicating that IL-6 has a low overall accuracy in diagnosing sepsis in this group of neonates.

Further analysis showed that in neonates without RDS the IL-6  $\geq$  20 pg/mL had a high sensitivity (0,93), which is very important when dealing with a disease like neonatal sepsis that could be fatal without

prompt treatment. Ideally, the specificity should also be high in order to avoid a high rate of false positive results leading to unnecessary use of antibiotics. Unfortunately, in our study the IL-6  $>$  20pg/mL had a low specificity (0,65). In addition, the LR of 2,68 is rather weakly positive for the disease, implying that, even in the absence of RDS, a neonate with sepsis is only about 2,5 times more likely to have an IL-6 over 20 than a non-septic one<sup>27,28</sup>.

Neonates suffering from RDS form a very specific group of preterm neonates, where inflammatory response is involved in the pathogenesis and progression to chronic lung disease<sup>13,14,16,18</sup>. This view is supported by the high plasma IL-6 levels in neonates with RDS found in our study. This finding also suggests that a cut-off value of IL-6 based on values obtained from neonates without RDS as referent population may not be suitable for detecting septic complications in neonates with RDS. Indeed, in our study, the use of the



level 20 pg/mL as cut-off value for the RDS group resulted in a high sensitivity (0,92) but a very low specificity (0,16). These figures imply that in neonates with RDS a level of IL-6 > 20 pg/mL can accurately detect sepsis in 92% of the cases, which is acceptable, but it has a high rate of false positive results. In addition, the extremely low LR found in this group of sick neonates (1,09) implies that IL-6 levels over 20 pg/mL are found equally in both septic and non-septic neonates with RDS<sup>29</sup>. In line with these results, Kallman et al<sup>16</sup> reported that measurement of IL-6 could not discriminate between septic neonates and those with RDS. For this reason, in the RDS group, we assessed the diagnostic accuracy of a higher IL-6 level, namely the level of 80 pg/mL which has been suggested by previous researchers<sup>3,20-22</sup>. The use of the IL-6 of 80 pg/mL as a cut-off value for the neonates with RDS resulted in lower sensitivity and a better, albeit still unacceptably low, specificity (0,40). The LR was also low (1,35) as was the NPV (0,59). From the clinical point of view these figures would not justify withholding or withdrawing treatment when neonatal sepsis is suspected only on the grounds of an IL-6 < 80 pg/mL.

Unlike IL-6, CRP levels were not influenced by the presence of RDS and thus did not differ significantly between neonates with RDS and those without RDS. On the other hand, CRP levels were significantly increased in the septic neonates as compared to the non-septic ones, regardless of the presence of RDS. Estimation of diagnostic accuracy of CRP by the ROC curves showed that the AUC was not influenced by the presence of RDS being high in both the non RDS and RDS groups (0,863 and 0,808, respectively). When the overall diagnostic accuracy of IL-6 and CRP were compared, in neonates without RDS both parameters

had a similar AUC, whereas in neonates with RDS the CRP had higher AUC than the IL-6 (0,808 vs. 0,642). The accuracy of CRP as a marker of neonatal sepsis has been well-documented in previous studies, although there is still a controversy as to whether CRP is a better predictor of neonatal sepsis than IL-6<sup>4-9</sup>. However, most authors support the view that IL-6 is a better predictor during the first 24 hours after the onset of sepsis, whereas CRP seems to be more accurate after this time<sup>6,20</sup>. Nevertheless, the time of the onset of sepsis can not always be accurately defined. In our study, blood samples were obtained at the time of clinical deterioration, but still this time point does not necessarily coincide with the onset of sepsis. Our results showed that CRP had a lower sensitivity than that of IL-6 at either > 20 pg/mL or > 80 pg/mL, but a higher specificity. The LRs of CRP (2,97) in neonates without RDS was similar to that of IL-6 > 20 (2,68) but lower than the LR of IL-6 > 80 pg/mL (12,38). However, in neonates with RDS the LR of CRP was higher than that of IL-6 at both levels.

In conclusion, in neonates without RDS both the IL-6 and CRP are acceptably accurate in diagnosing sepsis, whereas in those with RDS the diagnostic value of IL-6 is limited. The high sensitivity of plasma IL-6 at levels over 20 pg/mL in neonates with RDS could be useful for ruling out sepsis, but the low LR limits its usefulness as a diagnostic tool.

## Αξία της ιντερλευκίνης-6 and της CRP στη διάγνωση της σήψης σε νεογνά με σύνδρομο αναπνευστικής δυσχέρειας

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**ΠΕΡΙΛΗΨΗ:** Σκοπός: Η εκτίμηση της διαγνωστικής ακρίβειας της ιντερλευκίνης-6 (IL-6) πλάσματος και CRP ορού για την ανίχνευση της σήψης (θετικές καλλιέργειες αίματος) σε πρόωρα νεογνά με σύνδρομο αναπνευστικής δυσχέρειας (ΣΑΔ).

**Ασθενείς και Μέθοδοι:** Σε 140 πρόωρα νεογνά, 62 με ΣΑΔ και 78 χωρίς ΣΑΔ, τα οποία παρουσίασαν κλινική επιδείνωση ύποπτη για σήψη, μετρήθηκαν προγραμματισμένα τα επίπεδα IL-6 πλάσματος και CRP ορού. Η διαγνωστική αξία εκτιμήθηκε με καμπύλες ROC και υπολογίστηκαν η ευαισθησία, ειδικότητα, θετική προγνωστική αξία, αρνητική προγνωστική αξία και ο λόγος πιθανοτήτων (ΛΠ) για IL-6 (> 20 pg/mL και >80 pg/mL) και CRP (> 10mg/L).

**Αποτελέσματα:** Θετικές καλλιέργειες αίματος βρέθηκαν σε 64 νεογνά (37 με ΣΑΔ και 27 χωρίς ΣΑΔ). Τα νεογνά με ΣΑΔ είχαν σημαντικά υψηλότερα επίπεδα IL-6 ( $p < 0.0001$ ) συγκριτικά με τα νεογνά χωρίς ΣΑΔ. Οι καμπύλες ROC στα νεογνά χωρίς ΣΑΔ έδωσαν περιοχή κάτω από την καμπύλη (AUC) 0,914 και 0,863 για την IL-6 και CRP, αντίστοιχα. Στα νεογνά με ΣΑΔ, η AUC ήταν 0,642 για IL-6 και 0,808 για CRP. Επιπλέον, η ευαισθησία, ειδικότητα και ο ΛΠ για επίπεδα IL-6 >20 pg/mL ήταν 0,93, 0,65 and 2,68, αντίστοιχα, στα νεογνά χωρίς ΣΑΔ και 0,92, 0,16 και 1,09, αντίστοιχα, στα νεογνά με ΣΑΔ. Οι αντίστοιχες τιμές για CRP > 10mg/L ήταν 0,86, 0,71 και 2,97, αντίστοιχα, στα νεογνά χωρίς ΣΑΔ και 0,73, 0,68 και 2,28, αντίστοιχα, σε εκείνα με ΣΑΔ.

**Συμπεράσματα:** Σε νεογνά χωρίς ΣΑΔ, τόσο η IL-6 πλάσματος > 20 pg/mL όσο και η CRP > 10mg/L είναι αξιόπιστοι δείκτες σήψης, ενώ σε νεογνά με ΣΑΔ η διαγνωστική αξία της IL-6 είναι περιορισμένη.

*Λέξεις Κλειδιά:* Ειδικότητα, Ευαισθησία, Πρόωρα, Σήψη, Σύνδρομο αναπνευστικής δυσχέρειας.

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### **Abbreviations**

*AUC* area under the curve

*CRP* C-reactive protein

*IL-6* interleukin-6

*LR* likelihood ratio

*NPV* negative predictive value

*PPV* positive predictive value and

*RDS* respiratory distress syndrome

*ROC* receiver operating characteristics