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Review

Diagnostic value of neutrophil CD64 combined with CRP for neonatal sepsis: A meta-analysis

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ABSTRACT

Background: Sepsis is the leading cause of morbidity and mortality in newborns. CD64 combined with c-reactive protein (CRP) could improve the sensitivity and specificity of neonatal sepsis diagnosis, but the results were still controversial. Therefore, this meta-analysis was conducted to clarify the importance of CD64 combined with CRP in the diagnosis of neonatal sepsis.

Methods: The researches published as of December 24, 2018 were comprehensively searched in PubMed, Embase (included Embase and Medline), the Cochrane Library and Web of Science. Totally, 8 articles were included, involving 1114 objects. Statistical calculations were performed using Stata14.0 and Review Manager 5.3.

Results: The diagnostic accuracy of all included studies was pooled as follows: sensitivity, 0.95 (95% CI: 0.86–0.98); specificity, 0.86 (95% CI: 0.74–0.93); positive likelihood ratio (PLR), 6.8 (95% CI: 3.50–13.20); negative likelihood ratio (NLR), 0.06 (95% CI: 0.02–0.18); diagnostic odds ratio (DOR), 118.0 (95% CI: 25.00–549.00), and the area under the curve (AUC) was 0.96 (95% CI: 0.94–0.97). It was found that heterogeneity was not caused by threshold effect ($P = 0.16$), but the results of sensitivity ($I^2 = 87.57\%$) and specificity ($I^2 = 89.07\%$) analyses indicated significant heterogeneity between studies.

Conclusions: The combined application of CD64 and CRP improved the accuracy of neonatal sepsis diagnosis.

1. Introduction

World Health Organization (WHO) estimates for 195 countries indicate that neonatal bacterial infections cause about 680,000 neonatal deaths each year, or about a quarter of all neonatal deaths [1,2], and sepsis is the leading cause of morbidity and mortality in newborns [3-5]. In routine clinical practice, it is difficult to diagnose neonatal sepsis rapidly and accurately due to various reasons. Therefore, the accuracy of diagnostic tests should be improved [6]. At present, commonly used domestic infection detection indicators included blood routine c-reactive protein (CRP), Procalcitonin (PCT), blood culture, etc., but these traditional detection indicators generally have problems such as low sensitivity and low specificity and long detection cycle time.

The traditional gold standard for the diagnosis of sepsis is blood culture, but the test needs at least 2 days to get the result [7,8]. CRP began to increase 24–48 h after bacterial infection [9,10], that it is a late indicator of infection. However, some studies have shown that CRP increases physiologically three days after birth, which is prone to misdiagnosis [11]. Other diagnostic methods such as PCT also have some limitations [12]. As a novel cytokine in recent years, CD64 has significant value in the diagnosis of bacterial infection and sepsis [13,14]. Dai et al. and Shi et al. revealed that the detection of neutrophil CD64 alone had some problems such as low sensitivity and specificity [15,16]. Thus, it is better to combine with another serum biomarker. Literature review showed that CD64 combined with CRP could improve the sensitivity and specificity of neonatal sepsis diagnosis, but the results were still controversial. Therefore, this meta-analysis was conducted to clarify the importance of CD64 combined with CRP in the diagnosis of neonatal sepsis.

2. Methods

2.1. Search strategies

We systematically searched (updated to December 24, 2018) PubMed, Embase (included Embase and Medline), the Cochrane Library, and Web of Science for studies that assessed the accuracy of neutrophil CD64 combined CRP for the diagnosis of neonatal sepsis, used the following key words and Mesh terms, such as ("sepsis" or "septicemia" or "septicaemia" or "infection") and ("CD64" or "neutrophil CD64, nCD64" or "nCD64") and ("neonatal" or "newborn"). We further manually searched the included references to avoid missing articles that might be included.

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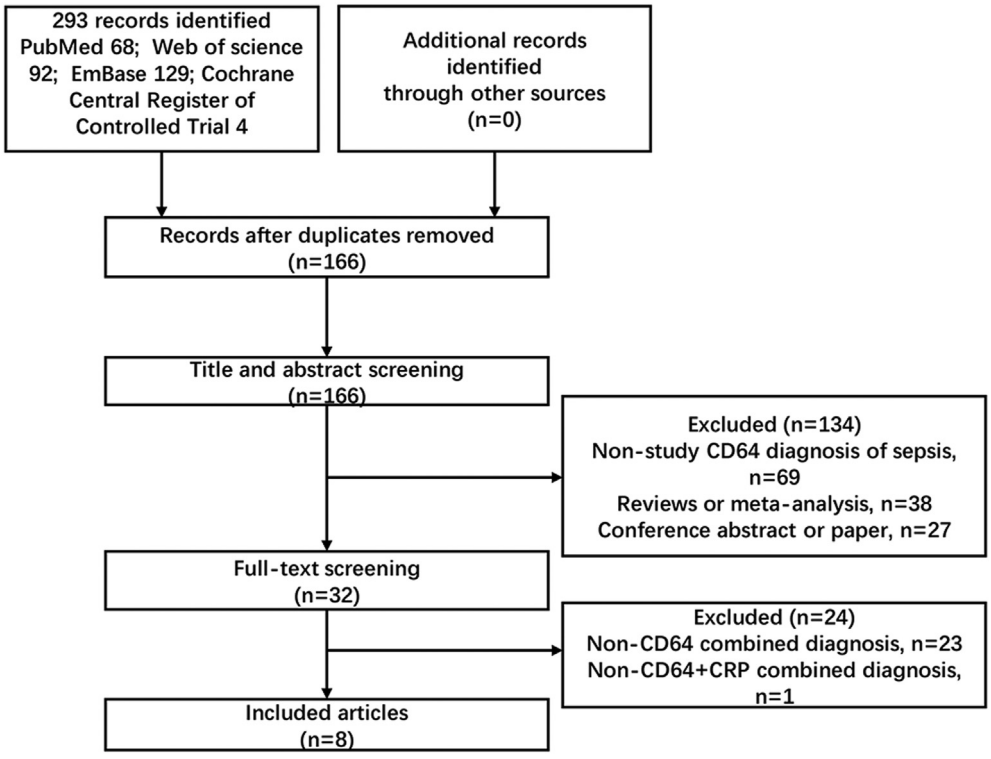

Fig. 1. Flow diagram of study selection process.

2.2. Selection criteria

If the following inclusion criteria were met, relevant studies were included: 1) To investigate the diagnostic value of nCD64 combined with CRP in neonatal sepsis; 2) providing the golden standard of blood culture; 3) the number of true positive (TP), false positive (FP), true negative (TN) and false negative (FN) can be obtained by calculation. The exclusion criteria in this study were as follows: 1) Eliminated the conference abstract or report, review or meta-analysis, and republished articles; 2) studies with insufficient data to extract were also excluded.

2.3. Data extraction and quality assessment

Two independent authors performed the initial search, imported EndNote and deleted the duplicate record automatically or manually, screened the titles and abstract, recognized the potentially studies, and got the full text. The two authors independently determined the included references and extracted the data, and the differences were resolved by a third author. The following data were extracted from each study: surname of the first author, publication year, country, number of infected and non-infected groups, the type of sepsis, nCD64 analysis method, analysis cutoff, sensitivity, specificity, TP, FP, FN, TN. The quality assessment of diagnostic research methodologies was conducted in accordance with QUADAS-2 tool guidelines [17].

2.4. Statistical analysis

STATA 14.0 and Review Manager 5.3 were used to perform the meta-analysis. In the diagnostic meta-analysis, considering that the combined CD64 test and the single CD64 test may have different diagnostic effects on neonatal sepsis, and the single test has been studied [15,16], we collected the individual data of CD64 combined CRP

Table 1
Characteristics of the included studies.

Author	Year	Country	Infected/noninfected	Diagnosis standard	Type of sepsis	Infants	ncd64 analysis	Analysis cutoff	Sensitivity (%)	Specificity (%)	TP	FP	FN	TN
Qin	2017	China	37/21	Clinical or proven	c	N	FCM	2.58 CD64 index	97.29	76.19	31	4	6	17
Shimi	2016	Egypt	60/60	Clinical or proven	c	Preterm + term	FCM	91.1 CD64 index	100	100	60	0	0	60
Yang	2015	China	60/60	Clinical or proven	b	Preterm + term	FCM	2.5 CD64 index	88.64	87.8	53	7	7	53
Du	2014	China	88/70	Clinical	a	Preterm	FCM	1010 PE-molecules bound/cell	77.27	90	68	7	20	63
Genel	2012	Turkey	49/35	Clinical or proven	c	Preterm + term	FCM	3.05 MFI	89	71	44	10	5	25
Dilli	2010	USA	35/74	Clinical or proven	c	Preterm + term	FCM	4.93 CD64 index	97.1	68.9	34	23	1	51
Ng	2004	China	115/223	Clinical or proven	a	Term	FCM	6136 PE molecules bound/cell	97	71	112	65	3	158
Ng	2002	China	37/90	Proven	b	Preterm	FCM	4000 PE-molecules bound/cell	100	90	37	9	0	91

NOTE: a, early-onset; b, late-onset; c, early and late-onset; FCM, flow cytometric technology; MFI, mean fluorescence intensity; TP, true positive; FP, false positive; TN, true negative; FN, false negative.

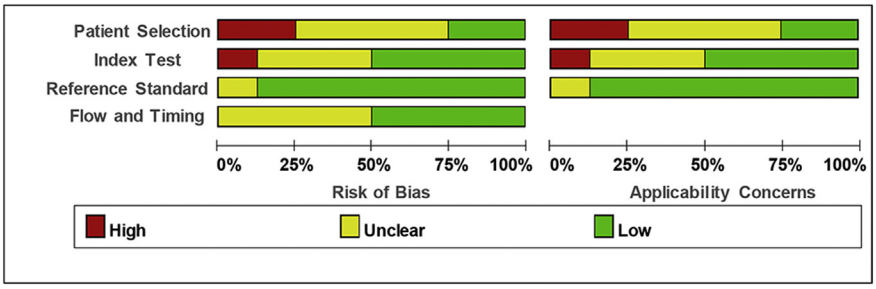

Fig. 2. Quality assessment of diagnostic accuracy for the included studies.

diagnostic markers from the included studies. Therefore, we calculated the pooled sensitivity, specificity, PLR, NLR, DOR and 95% CI of CD64 combined CRP diagnosis using a bivariable meta-analysis model [18]. We constructed the symmetric receiver operator characteristic curve (SROC), calculated the area under the SROC curve, and evaluated the overall performance of CD64 combined with CRP in the diagnosis of neonatal sepsis. Spearman correlation coefficient was used to test the inter-study heterogeneity caused by threshold effect [19]. The Cochran Q test and I^2 statistics have always been used to evaluate non-threshold effects [20]. Fagan's nomogram was used to investigate the clinical value of CD64 combined with CRP in the detection of neonatal sepsis. The Deek's funnel plot method was used to explore publication bias ($P < 0.05$) [21]. The sensitivity analysis was performed by excluding one study at a time and recalculating the risk effect.

3. Results

A total of 293 studies were found through literature search, of which 127 studies were excluded due to duplicates and 134 studies were excluded after we reviewed the retrieved literature's titles and abstracts. After reading the full-text of the remaining 32 studies, we further eliminated 24 studies, of which 23 were identified as CD64 alone and one as non-CD64 combined with CRP. Finally, 8 studies were included in this meta-analysis [22-29]. The flow chart of the meta-analysis is represented in Fig. 1. The basic information and quality evaluation results included in the study are shown in Table 1 and Fig. 2.

3.1. Diagnostic accuracy of CD64 + CRP for neonatal sepsis

The pooled results for diagnostic accuracy of all included studies were as follows: sensitivity, 0.95 (95% CI: 0.86–0.98); specificity, 0.86 (95% CI: 0.74–0.93); PLR, 6.8 (95% CI: 3.50–13.20); NLR, 0.06 (95% CI: 0.02–0.18); and DOR, 118.0 (95% CI: 25.00–549.00), and the AUC was 0.96, (95% CI: 0.94–0.97) (Figs. 3 and 4).

Nomogram of Fagan was regarded as a graphical tool for digging out the clinical diagnostic values of CD64 + CRP in neonatal sepsis detection. When 20% value was selected as the pre-test probability, the positive results of CD64 + CRP showed the post-test probability of correctly diagnosing neonatal sepsis would rise to 63%, while negative results of CD64 + CRP indicated the post-test probability would drop to 1%, as demonstrated in the Fagan plot in Fig. 5.

3.2. Heterogeneity and publication bias

It was found that heterogeneity was not caused by threshold effect ($P = 0.16$), but the results of sensitivity ($I^2 = 87.57\%$) and specificity

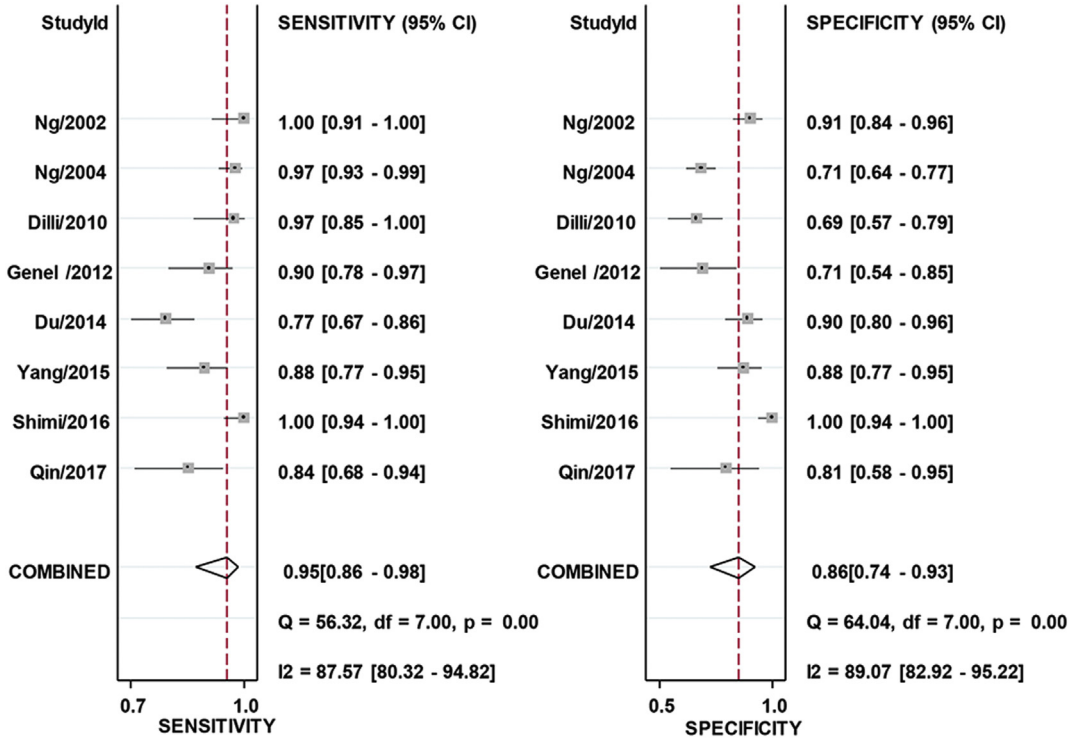

Fig. 3. Sensitivity and specificity forest of CD64 combined with CRP in the diagnosis of neonatal sepsis.

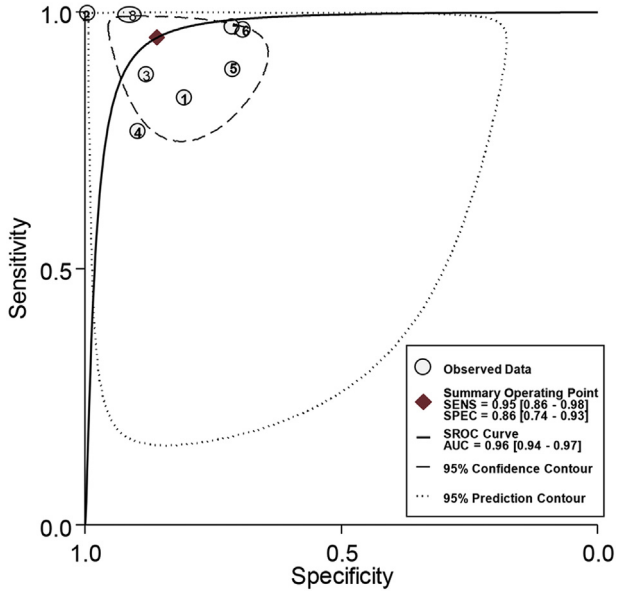

Fig. 4. SROC curves of CD64 combined with CRP in the diagnosis of neonatal sepsis.

($I^2 = 89.07\%$) analysis indicated significant heterogeneity between studies (Fig. 3). In addition, Deeks' funnel plot showed no significant publication bias in our study ($P = 0.960$) (Fig. 6).

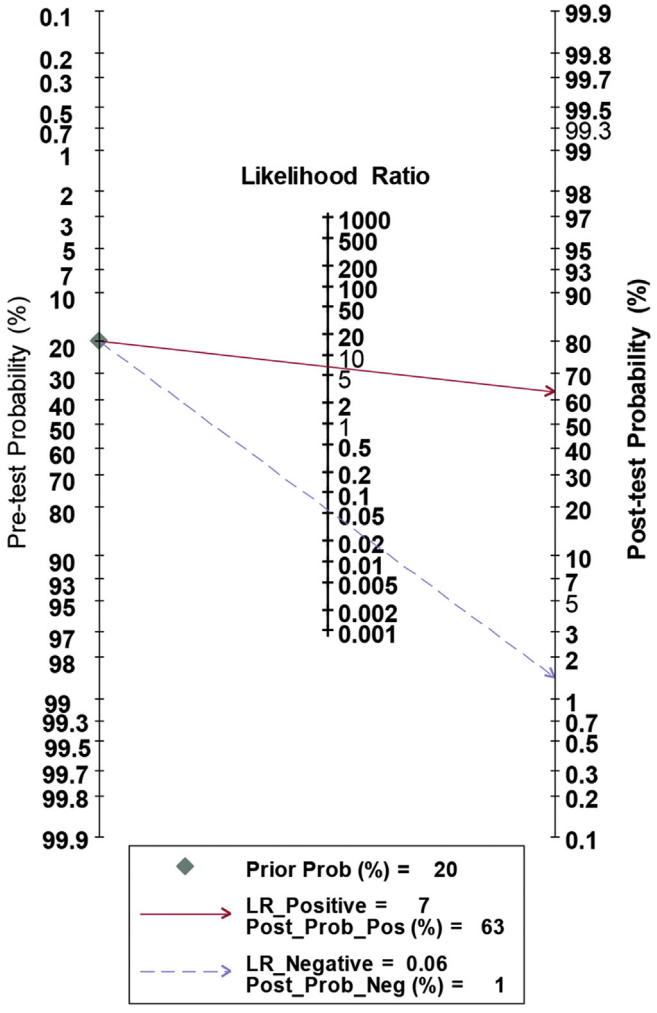

Fig. 5. Nomogram of Fagan describes the probability CD64 combined with CRP to confirm or exclude neonatal sepsis patients.

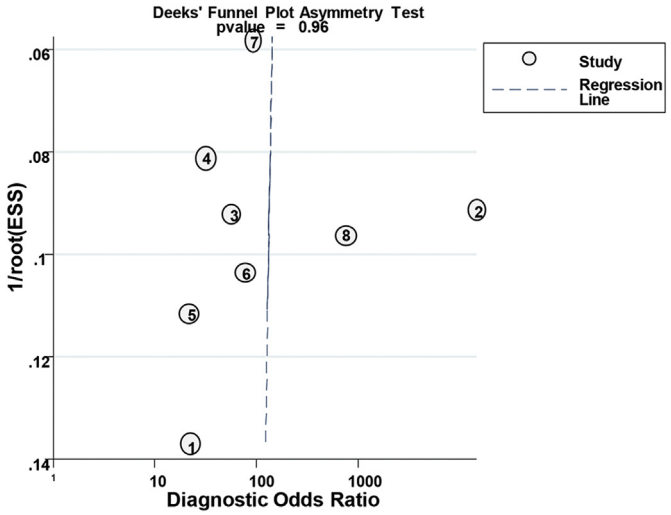

Fig. 6. Funnel plots for the assessment of potential diagnosis bias in CD64 combined with CRP assays.

3.3. Sensitivity analysis

An outlier study was found through the sensitivity analysis and outlier detection (Fig. 7A, B). After the abnormal study was excluded, the combined results were sensitivity 0.92 (95% CI: 0.85–0.96), specificity 0.82 (95% CI: 0.74–0.88), PLR 5.20 (95% CI: 3.50–7.60), NLR 0.09 (95% CI: 0.05–0.18), DOR 56.00 (95% CI: 27.00–115.00), AUC 0.93 (95% CI: 0.91–0.95), however, the results were a change from previous results. Further analysis found that there was still outlier in the study, and the combined results after elimination had minimal changes: sensitivity 0.91 (95% CI: 0.83–0.95), specificity 0.80 (95% CI: 0.72–0.86), PLR 4.60 (95% CI: 3.30–6.40), NLR 0.11 (95% CI: 0.06–0.20), DOR 41.00 (95% CI: 24.00–71.00), AUC 0.91 (95% CI: 0.89–0.94). The heterogeneity was reduced after removing the literature one by one (sensitivity $I^2 = 81.95\%$, specificity $I^2 = 81.21\%$; sensitivity $I^2 = 79.35\%$, specificity $I^2 = 72.94\%$).

4. Discussion

Neonatal sepsis is one of the important causes of high neonatal mortality. Therefore, accurate diagnosis and appropriate medicine are particularly important for improving adverse outcomes. However, numerous studies have shown that clinical signals, non-specificity, and laboratory tests, including blood cultures, are not always reliable [30]. Up to now, many markers for the diagnosis of neonatal sepsis have been proposed, such as c-reactive protein, PCT, Il-6, etc. However, the identification of NS by a single biomarker is not reliable enough at present. More researchers focused on the combination of different biomarkers in different clinical environments, hoping to obtain clearer conclusions [31,32]. Thus, we conducted this meta-analysis to investigate the important role of CD64 combined with CRP in the diagnosis of neonatal sepsis. The main finding of this meta-analysis was that CD64 combined with CRP could improve the accuracy of neonatal sepsis diagnosis.

A meta-analysis of the diagnostic value of ncd64 in the independent diagnosis of neonatal sepsis was performed in the two studies, and a large difference was found between the results of the two studies, which may be caused by the different inclusion and exclusion criteria of the two studies. The integrated sensitivity, specificity, PLR, NLR, DOR and AUC of Dai's and Shi's studies were 80% (95% CI: 69–88%), 83% (95% CI: 71–90%), 4.6 (95% CI: 2.5–8.6), 0.24 (95% CI: 0.14–0.41), 19 (95% CI: 6–57), 0.88 (95% CI: 0.85–0.91); 0.77 (95% CI: 0.74–0.79), 0.74 (95% CI: 0.72–0.75), 3.58 (95% CI: 2.85–4.49), 0.29 (95% CI: 0.22–0.37), 15.18 (95% CI: 9.75–23.62), respectively [15,16]. Our study found that the value of CD64 combined with CRP in the diagnosis of neonatal sepsis was superior

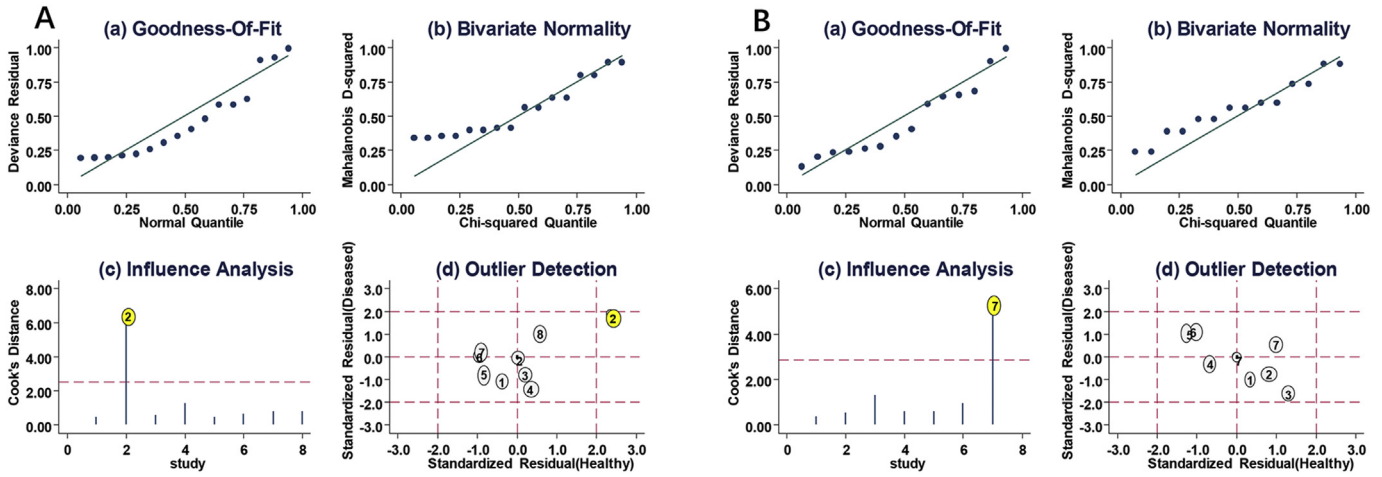

Fig. 7. Influence analysis and outlier detection. A and B, (a) goodness of fit, (b) bivariate normality, (c) influence analysis, and (d) outlier detection.

to the independent diagnosis of CD64. In addition, it was found that CD64 + CRP had higher diagnostic value by comparing other diagnostic markers such as IL-6, PCT, CRP, PCT + CRP in the meta-analysis [33-36].

However, there was significant statistical heterogeneity in some analyses that required further explanation. Threshold effect, publication bias, sensitivity analysis and other methods were used to identify the sources of heterogeneity. First, the analysis results showed that the Spearman correlation coefficient and *P* value between the 8 studies were 0.40 and 0.16, indicating that the heterogeneity was not caused by threshold effect. Second, Deek's funnel plot asymmetry test showed no publication bias ($P = 0.96$). Third, sensitivity analysis found that heterogeneity decreased after the exclusion of abnormal studies, which may be one of the reasons for the high heterogeneity in this study. In addition to the above reasons, we carefully read the content of the article and found that the cutoff values included in the study were all different, which may be one of the reasons for the high heterogeneity. Furthermore, the type of sepsis (early-onset, late-onset, or early and late-onset) and the type of patients (preterm, term, or preterm + term) included in the study may be the cause of heterogeneity.

There were also several limitations to this meta-analysis. First, as the number of the included literatures was <10, meta-regression quantitative analysis of heterogeneous sources was not conducted. Second, the sensitivity analysis results showed change as a result and poor stability after eliminating outlier literature, although the merged results (such as sensitivity, specificity, and AUC) still had relatively high diagnostic value of CD64, high-quality diagnostic experiments are still needed to further validate the important role of CD64 combined with CRP in the diagnosis of neonatal sepsis.

In conclusion, the combination of CD64 and CRP improves the accuracy of the diagnosis of neonatal sepsis. However, further studies are required to confirm these findings.

Conflict of interests

All authors declare that they have no conflict of interests.

Acknowledgments

None.

References

[1] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet 2015;385(9966):430–40.
[2] Fitchett EJA, Seale AC, Vergnano S, et al. Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI): an extension of the STROBE statement for neonatal infection research. Lancet Infect Dis 2016;16(10):e202–13.
[3] Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L. Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem 2004;50(2):279–87.
[4] Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. Jama 2004;292(19):2357–65.
[5] Boo NY, Nor Azlina AA, Rohana J. Usefulness of a semi-quantitative procalcitonin test kit for early diagnosis of neonatal sepsis. Singapore Med J 2008;49(3):204–8.
[6] Ugarte H, Silva E, Mercan D, De Mendonca A, Vincent JL. Procalcitonin used as a marker of infection in the intensive care unit. Crit Care Med 1999;27(3):498–504.
[7] Lynema S, Marmer D, Hall ES, Meinzen-Derr J, Kingma PS. Neutrophil CD64 as a diagnostic marker of sepsis: impact on neonatal care. Am J Perinatol 2015;32(4):331–6.
[8] Hoffmann JJ. Neutrophil CD64 as a sepsis biomarker. Biochem Med (Zagreb) 2011;21(3):282–90.
[9] Kipfmueller F, Schneider J, Prusseit J, et al. Role of neutrophil CD64 index as a screening marker for late-onset sepsis in very low birth weight infants. PLoS One 2015;10(4):e0124634.
[10] Delanghe JR, Speeckaert MM. Translational research and biomarkers in neonatal sepsis. Clin Chim Acta 2015;451(Pt A):46–64.
[11] Bhandari V. Effective biomarkers for diagnosis of neonatal Sepsis. J Pediatric Infect Dis Soc 2014;3(3):234–45.
[12] Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clin Microbiol Rev 2014;27(1):21–47.
[13] de Jong E, de Lange DW, Beishuizen A, van de Ven PM, Girbes AR, Huisman A. Neutrophil CD64 expression as a longitudinal biomarker for severe disease and acute infection in critically ill patients. Int J Lab Hematol 2016;38(5):576–84.
[14] Pradhan R, Jain P, Paria A, et al. Ratio of neutrophilic CD64 and monocytic HLA-DR: a novel parameter in diagnosis and prognostication of neonatal sepsis. Cytometry B Clin Cytom 2016;90(3):295–302.
[15] Dai J, Jiang W, Min Z, et al. Neutrophil CD64 as a diagnostic marker for neonatal sepsis: meta-analysis. Adv Clin Exp Med 2017;26(2):327–32.
[16] Shi J, Tang J, Chen D. Meta-analysis of diagnostic accuracy of neutrophil CD64 for neonatal sepsis. Ital J Pediatr 2016;42(1):57.
[17] Shidfar A, Costa FF, Scholtens D, et al. Expression of miR-18a and miR-210 in normal breast tissue as candidate biomarkers of breast cancer risk. Cancer Prev Res (Phila) 2017;10(1):89–97.
[18] Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 2005;58(10):982–90.
[19] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21(11):1539–58.
[20] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. Bmj 2003;327(7414):557–60.
[21] Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol 2005;58(9):882–93.
[22] Qin DJ, Tang ZS, Chen SL, Xu XM, Mao SG, Zhang SF. Value of combined determination of neutrophil CD64 and procalcitonin in early diagnosis of neonatal bacterial infection. Chinese Journal of Contemporary Pediatrics 2017;19(8):872–6.
[23] El Shimi MS, Abou Shady NM, Hamed GM, Shedeed NS. Significance of neutrophilic CD64 as an early marker for detection of neonatal sepsis and prediction of disease outcome. Journal of Maternal-Fetal and Neonatal Medicine 2017;30(14):1709–14.
[24] Yang AP, Liu J, Yue LH, Wang HQ, Yang WJ, Yang GH. Neutrophil CD64 combined with PCT, CRP and WBC improves the sensitivity for the early diagnosis of neonatal sepsis. Clin Chem Lab Med 2016;54(2):345–51.
[25] Du J, Li L, Dou Y, Li P, Chen R, Liu H. Diagnostic utility of neutrophil CD64 as a marker for early-onset sepsis in preterm neonates. PLoS ONE 2014;9(7).

[26] Genel F, Atlihan F, Gulez N, et al. Evaluation of adhesion molecules CD64, CD11b and CD62L in neutrophils and monocytes of peripheral blood for early diagnosis of neonatal infection. World J Pediatr 2012;8(1):72–5.
[27] Dilli D, Oğuz Ş S, Dilmen U, Köker MY, Kızılgün M. Predictive values of neutrophil CD64 expression compared with interleukin-6 and C-reactive protein in early diagnosis of neonatal sepsis. J Clin Lab Anal 2010;24(6):363–70.
[28] Ng PC, Li G, Chui KM, et al. Neutrophil CD64 is a sensitive diagnostic marker for early-onset neonatal infection. Pediatr Res 2004;56(5):796–803.
[29] Ng PC, Li K, Wong RPO, Chui KM, Wong E, Fok TF. Neutrophil CD64 expression: a sensitive diagnostic marker for late-onset nosocomial infection in very low birthweight infants. Pediatr Res 2002;51(3):296–303.
[30] Gerdes J. Diagnosis and management of bacterial infections in the neonate. Pediatr Clin North Am 2004;51(4):939–59 [viii-ix].
[31] Arnon S, Litmanovitz I. Diagnostic tests in neonatal sepsis. Curr Opin Infect Dis 2008; 21(3):223–7.
[32] Fan Y, Yu JL. Umbilical blood biomarkers for predicting early-onset neonatal sepsis. World J Pediatr 2012;8(2):101–8.
[33] Qiu X, Zhang L, Tong Y, Qu Y, Wang H, Mu D. Interleukin-6 for early diagnosis of neonatal sepsis with premature rupture of the membranes: a meta-analysis. Medicine (Baltimore) 2018;97(47):e13146.
[34] Yu Z, Liu J, Sun Q, Qiu Y, Han S, Guo X. The accuracy of the procalcitonin test for the diagnosis of neonatal sepsis: a meta-analysis. Scand J Infect Dis 2010;42(10): 723–33.
[35] Su H, S-S C, C-M H, et al. Inflammatory markers in cord blood or maternal serum for early detection of neonatal sepsis-a systemic review and meta-analysis. J Perinatol 2014;34(4):268–74.
[36] Ruan L, Chen GY, Liu Z, et al. The combination of procalcitonin and C-reactive protein or presepsin alone improves the accuracy of diagnosis of neonatal sepsis: a meta-analysis and systematic review. Crit Care 2018;22(1):316.