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Is neutrophil CD11b a special marker for the early diagnosis of sepsis in neonates? A systematic review and meta-analysis

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ABSTRACT

Objectives Our study aimed to synthesise and analyse the early diagnostic value of neutrophil CD11b (nCD11b) for neonatal sepsis.

Design Systematic review and meta-analysis.

Methods Pubmed, Embase, the Cochrane Library and Web of Science Databases were searched up to June 2018. We used Stata software (V.14.0) to conduct the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic OR (DOR), pretest probability, post-test probability and summary receiver operating characteristic (SROC) curve for diagnostic efficiency of n CD11b.

Results Nine studies, accounting for 843 neonates, were included. The overall pooled sensitivity, specificity, PLR, NLR, DOR, post-test positive probability and post-test negative probability and the area under the SROC curve were 0.82 (95% CI 0.71 to 0.90), 0.93 (95% CI 0.62 to 0.99), 11.51 (95% CI 1.55 to 85.62), 0.19 (95% CI 0.10 to 0.36), 59.50 (95% CI 4.65 to 761.58), 74%, 5% and 0.90, which had accuracy in diagnosing neonatal sepsis.

Conclusion The present evidence indicated that nCD11b is a promising biomarker for the early diagnosis of neonatal sepsis.

Strengths and limitations of this study

► First, we assessed the diagnostic performance of neutrophil CD11b (nCD11b) for neonatal sepsis by a random effects model. The results showed that nCD11b, as a new diagnostic biomarker, had a high diagnostic value to discriminate neonatal sepsis.

► We made a systematic search strategy to find the best quality evidence, and Quality Assessment of Diagnostic Accuracy Studies tool-2 was used to assess each included study.

► Only studies written in English and Chinese were included, which might cause the true accuracy of nCD11b tests to be lower than that reported herein.

► Because of the small numbers of studies and patients, we could not conduct the meta-regression analysis to explore the sources of heterogeneity.

INTRODUCTION

Neonatal sepsis is a serious systemic disease that is among the main causes of neonatal deaths worldwide, and neonates with severe sepsis have a morbidity of 15%–50% in developing countries.[1,2] Although the management of newborns has improved in recent years, the incidence of neonatal sepsis is 1–10 per 1000 live births.[3,4] It is easy to misdiagnose neonatal sepsis because the early clinical signs are variable and include weight loss and sleepiness.[5] A positive blood culture is the gold standard for diagnosing neonatal sepsis. However, the results of blood cultures do not produce results for 24–72 hours, and cultures are less sensitive with a positive rate of 19.2%.[6,7] In addition, there is evidence indicating that making an early diagnosis of neonatal sepsis is more difficult because of the antibiotic prophylaxis administered during deliveries.[7] Therefore, considering some limitations of blood cultures and the importance of making a correct diagnosis, a new diagnostic biomarker that can enable early diagnose neonatal sepsis should be developed.

Neutrophil CD11b (nCD11b), belonging to the β-integrin adhesion proteins, is important for neutrophil migration to the sites of infection.[8] The nCD11b expression increases on the neutrophil surface within no more than 5 min of exposure to bacteria.[9] Recently, nCD11b has been reported as a diagnostic biomarker for neonatal sepsis because it can be detected quickly by flow cytometry using 0.05 mL of blood sample, with a high sensitivity (96%) and specificity (100%).[9,10] Accordingly, nCD11b might become an effective, rapid biomarker for diagnosing neonatal sepsis.

Many studies about the value of nCD11b test have been reported; however, the diagnostic results vary. Therefore, we conducted a systematic review and meta-analysis to

systematically evaluate the diagnostic performance of nCD11b for neonatal sepsis.

METHODS

Literature search

We conducted our study in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria. A computer-aided literature search was performed in Pubmed, Embase, the Cochrane Library and Web of Science databases for relevant citations up to June 2018. Our search terms included 'neonate,' 'newborn,' 'infant,' 'sepsis,' 'septicemia,' 'bacteremia,' 'nCD11b' and 'neutrophil CD11b.' The search strategy used for databases was as follows: ('nCD11b' OR 'Neutrophil CD11b') AND ('Sepsis' OR 'Septicemia' OR 'Bacteremia') AND ('Infant' OR 'Newborn' OR 'Neonate' OR 'Neonatal'). The complete search strategy for one database was presented in online supplementary appendix 1. Additionally, we manually searched the references of the included studies and relevant reviews to find possibly eligible studies.

Literature selection

Studies obtained from the literature were independently reviewed by two investigators (XQ and JL) to ensure high accuracy. Inclusion criteria were as follows: (1) assessment of the diagnostic accuracy of nCD11b for diagnosing neonatal sepsis; (2) reports on neonates within 28 days of birth (population); (3) provision of nCD11b in the blood as the index test; (4) use of blood cultures as the gold standard in diagnosing neonatal sepsis; (5) inclusion of sensitivity, specificity or sufficient information to construct the 2×2 tables (outcome); and (6) more than five patients reported to meet the inclusion criteria. Reviews, case reports, conference abstracts, animal experiments and meta-analyses were excluded from the study.

Data extraction

Extraction of data from the selected articles was independently performed by two investigators (XQ and JL). Disagreements were resolved by discussing and reaching a consensus. The data included the following: author; year of publication; study regions; selected time; sample size; term or preterm neonates; diagnostic gold standard; measurement method; cut-off value; type of sepsis; and sensitivity, specificity, true positive (TP), false positive (FP), false negative (FN) and true negative (TN) of nCD11b for diagnosing neonatal sepsis.

Quality assessment

We used the Quality Assessment of Diagnostic Accuracy Studies tool-2 (QUADAS-2) to assess the methodological quality of the studies.[11] Two reviewers (XQ and JL) independently performed the quality assessment. Four domains (patient selection, index test, reference standard and flow and timing) were evaluated for the risk of bias, and three domains (patient selection, index test

and reference standard) were evaluated based on applicability. Spectrum bias (neonatal sepsis alone or with other diseases) and selection bias (whether the selection criteria are clearly described, including a consecutive or random enrolment of patients) were related to patient selection. Information bias (whether sufficient details of nCD11b test have been included) was related to the index test. Partial verification bias (whether a reference standard of the selected samples is included), differential verification bias (whether the same reference standard is used for all patients) and disease progression bias (whether there is enough time between the reference test and nCD11b test) were related to the reference standard. Excluded data bias (whether the test results reported were interpretable) was related to the flow and timing. Signalling questions were asked to help estimate the risk of bias.[11] For unresolved disagreements, a third reviewer (YT) was consulted. Revman software (V.5.3, Cochrane Collaboration) was used for quality assessment.

Statistical analysis

Stata software (V.14.0, Stata Corporation) was used to draw funnel plots to analyse the publication bias. We calculated the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic OR (DOR), pretest probability and post-test probability.[12 13] The heterogeneity of eligible studies was evaluated by the Cochrane Q test and I^2 statistic.[14] I^2 could be calculated from the formula of $I^2=100\%\times(Q–df)/Q$. If I^2 is <50% or the p value is >0.1, a fixed effects model is used for pooling the data; whereas, if I^2 is >50% or the p value is <0.1, then there is more heterogeneity among studies, and a bivariate random effects model is used for pooling the data; if I^2 is <50% or the p value is <0.1, a fixed effects model could be used; if I^2 is >50% or the p value is >0.1, a bivariate random effects model could be used. On the basis of the sensitivity and specificity, we further constructed the summary receiver operating characteristic (SROC) curve.[15] The area under the curve (AUC) was also calculated to show the diagnostic performance of nCD11b, and if it was close to 1, it would indicate that nCD11b is a good diagnostic tool.[16 17] Additionally, we conducted the Galbraith plot analysis, sensitivity analysis and subgroup analyses. We used the Deek's funnel plot asymmetry test to assess for publication bias, when we included more than 10 studies.[18]
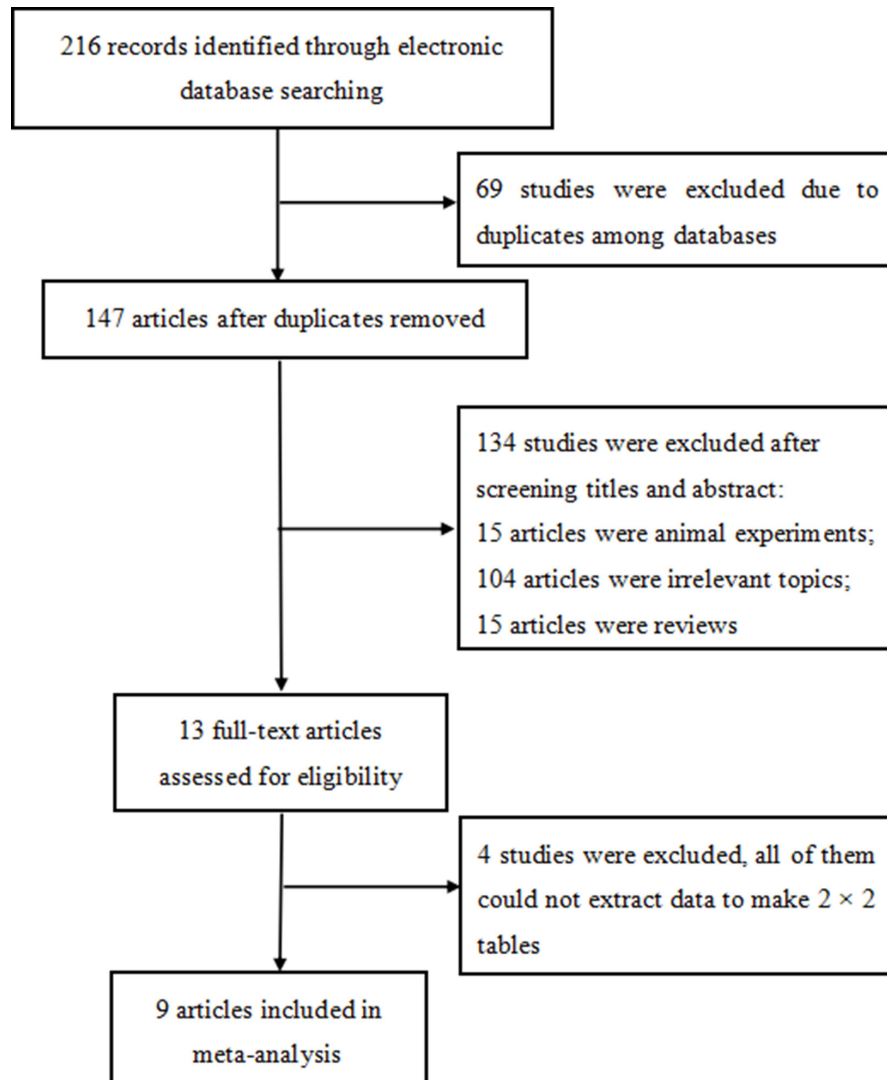
Patient and public involvement

All analyses were conducted based on previously published studies. Accordingly, there was no patient or public involvement in this study.

RESULTS

Literature research

As shown in figures 1, 216 literature citations were identified from database searches (PubMed: 50; Embase: 39; the Cochrane Library: 3; Web of Science: 124). First, 69

Figure 1 Flow chart of the process of the identified and included articles.

duplicate articles were removed automatically. Later, by reading the titles and abstracts, 134 articles were excluded as they did not meet the inclusion criteria; 15 articles were excluded as they were animal experiments; 104 studies were excluded as they reported on irrelevant topics (such as neonatal brain injury, pneumonia, intrauterine infection and necrotising enterocolitis); and 15 articles were excluded as they were reviews. Ultimately, nine articles met the inclusion criteria and were included in this study.[9 19–26]

Characteristics of the eligible studies

The characteristics of the nine eligible studies are listed in table 1. All studies were published between 1998 and 2017. There were 843 participants involved in our meta-analysis. Six of the eligible studies were performed in Asia, one in the USA and two in Europe. Seven studies included both term and preterm neonates, and two studies included only preterm neonates. The diagnostic gold standard included a microbiological test-blood culture and/or routine clinical examination and biochemical laboratory investigations. The nCD11b level was detected by flow cytometry. nCD11b cut-off levels were not reported in five studies.[9 22–24 26] The sensitivity, specificity, TP, FP, FN and TN of nCD11b in each article are also completely shown in table 1.

Quality assessment

QUADAS-2 was used to assess the methodological quality of eligible studies. As shown in figure 2 and online supplementary table 1, two-thirds of the studies were deemed to have low bias for patient selection, and one-third of the studies had unclear bias (ie, the selection criteria was not clearly described). Only two studies showed low bias for index tests, and the rest had unclear bias (ie, no sufficient details on nCD11b test). All nine studies were deemed to have low bias for reference standard and flow and timing. Regarding applicability concerns in patient selection, five studies showed low concerns, three studies showed high concerns and one study showed unclear concerns (demographic features were not available). Concerning applicability concerns of the index tests, seven studies showed unclear concerns (conduct and interpretation of nCD11b test were not available), and two studies had low

Table 1 Main characteristics of studies included in the meta-analysis

Author	Year	Region	Selected time	N	Neonates	Assay method	Cut-off	Sepsis diagnosis	Type of sepsis	Sensitivity(%)	Specificity(%)	TP	FP	FN	TN
Weirich[9]	1998	USA	1994.8–1995.1	106	Term+preterm	FCM	Unclear	Culture	Early onset	96	100	23	0	1	82
Cui[19]	2003	China	1999.7–2001.6	66	Term+preterm	FCM	600 MFI	Culture	Early/late onset	86	100	44	0	7	15
Nupponen[20]	2001	Finland	1998.1–1999.6	47	Term+preterm	FCM	≥150 RFU	Culture	Early onset	100	100	35	0	0	12
Genel[21]	2012	Turkey	2006.3–2007.4	119	Term+preterm	FCM	12.6 MFI	Culture	Early/late onset	66	71	32	20	17	50
Adib[22]	2007	Iran	Unclear	77	Term+preterm	FCM	Unclear	Culture	Early/late-onset	75	100	49	0	16	12
Du[23]	2014	China	2010.11–2011.6	158	Preterm	FCM	Unclear	Culture	Early-onset	75	64	66	25	22	45
Ng[24]	2002	China	Unclear	147	Preterm	FCM	Unclear	Culture	Late-onset	70	72	26	31	11	79
Turunen[25]	2005	Finland	2000.12–2002.4	23	Term+preterm	FCM	137 RFU	Culture	Late-onset	100	56	7	7	0	9
Aydin[26]	2017	Turkey	2014.3–2015.6	100	Term+preterm	FCM	Unclear	Culture	Early/late-onset	72	68	36	16	14	34

FCM, flow cytometry; FN, false negative; FP, false positive; MFI, mean fluorescence intensity; RFU, relative fluorescence unit.; TN, true negative; TP, true positive.

concerns. Regarding the reference standard, nine studies were rated as low concerns.

Pooled analysis

Nine articles met the inclusion criteria. The heterogeneity and inconsistency existed according to the χ^2 and I^2 analysis (Q value=6.255, I^2=68%, 95% CI 28 to 100, p=0.022). We used a random effects model to investigate the use of nCD11b for diagnosing neonatal sepsis. Eight hundred and forty-three cases were included. The sensitivity ranged from 0.65 to 1.00 (pooled sensitivity: 0.82, 95% CI 0.71 to 0.90); whereas, specificity ranged from 0.56 to 1.00 (pooled specificity: 0.93, 95% CI 0.62 to 0.99) (figure 3). The pooled PLR of nCD11b was 11.51 (95% CI 1.55 to 85.62), and pooled NLR of nCD11b was 0.19 (95% CI 0.10 to 0.36) (see online supplementary figure 1). Additionally, the pooled DOR of nCD11b was 59.50 (95% CI 4.65 to 761.58) (see online supplementary figure 2). DOR, as a single indicator, could be used to assess the accuracy, which showed that the discriminatory effect in our study was comparatively good. The pretest probability of nCD11b was 20%, and the positive and negative post-test probability of nCD11b was 74% and 5%, respectively (see online supplementary figure 3). The SROC curve reflected the overall performance of the nCD11b, from the SROC in figure 4, it is quite close to the top left-hand corner of the graph, which indicates good accuracy of nCD11b; in our case, the AUC was 0.90 (95% CI 0.87 to 0.93), which represented perfect discriminatory ability.

Sensitivity analysis and subgroup analysis

Since a significant heterogeneity was detected (I^2=68%, p=0.022), the potential sources of heterogeneity were investigated by the Galbraith plot (see online supplementary figure 4) which identified one outlier study.[20] On performing the sensitivity analysis, after excluding this outlier study by Nupponen *et al*, the pooled sensitivity, specificity, PLR, NLR, DOR and AUC of nCD11b for neonatal sepsis were 0.77 (95% CI 0.70 to 0.84), 0.88 (95% CI 0.61 to 0.97), 6.7 (95% CI 1.6 to 28.4), 0.25 (95% CI 0.16 to 0.40), 26.20 (95% CI 4.16 to 165.05) and 0.84, respectively. The sensitivity analysis indicated that the outlier study had no significant influence on the results of our meta-analysis.

Regarding the types of sepsis, 311 neonates had early-onset sepsis, 362 had early/late-onset sepsis and 170 had late-onset sepsis. The diagnostic accuracy of nCD11b was higher in neonates with early-onset sepsis than in those with early/late-onset and late-onset sepsis. The sensitivity of nCD11b was higher in neonates with early-onset sepsis than in those with early/late-onset and late-onset sepsis (92% and 74%, respectively). The specificity of nCD11b was higher in neonates with early-onset sepsis than in those with early/late-onset and late-onset sepsis (99% vs 83%, p=0.001).

With regard to the regions, 667 cases were from Asia and 176 were from Europe or the USA. The sensitivity of nCD11b was higher in Europe or the USA than in
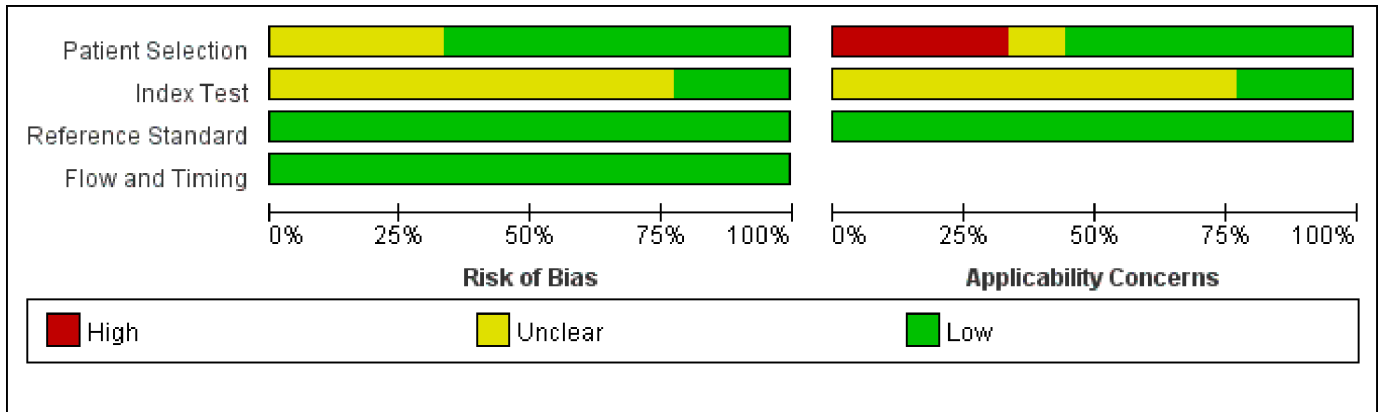
Figure 2 Methodological quality of the identified and included articles.

Asia (98% vs 75%, p=0.001). Similarly, the specificity of nCD11b was also higher in Europe or the USA than in Asia (98% vs 87%, p=0.01).

With regard to the types of neonates, 305 neonates were preterm neonates, and 538 were preterm/term neonates. The sensitivity of nCD11b was higher in preterm/term neonates than in preterm neonates (84% and 74%, respectively). The specificity of nCD11b was higher in preterm/term neonates than in preterm neonates (96% vs 68%, p=0.01).

Regarding the participants, 213 cases were included from the eligible studies that each had less than 100 participants, and 630 cases were included from the eligible studies that

each study had more than 100 participants. The sensitivity and specificity of nCD11b were lower in studies with less than 100 participants than in those with more than 100 participants (78% and 86% and 86% and 98%, respectively).

DISCUSSION
To date, neonatal sepsis is still a death-related disease.[1] Because the early symptoms and physical signs of neonatal sepsis vary, it is easy to misdiagnose it as pneumonia.[5] Although positive blood cultures can be used to make an accurate diagnosis of neonatal sepsis, they have a low sensitivity and are time consuming and ineffective.
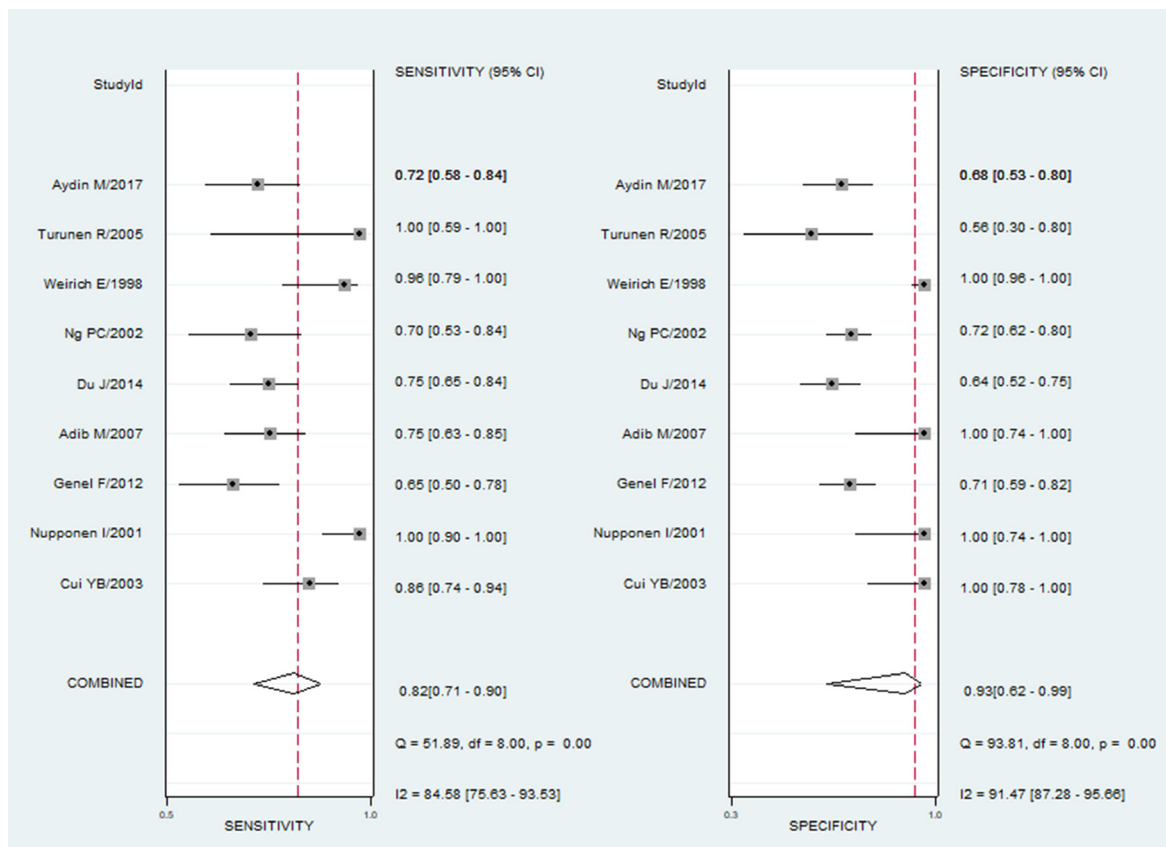
Figure 3 The forest plots of the pooled sensitivity and specificity of neutrophil CD11b to diagnose neonatal sepsis.
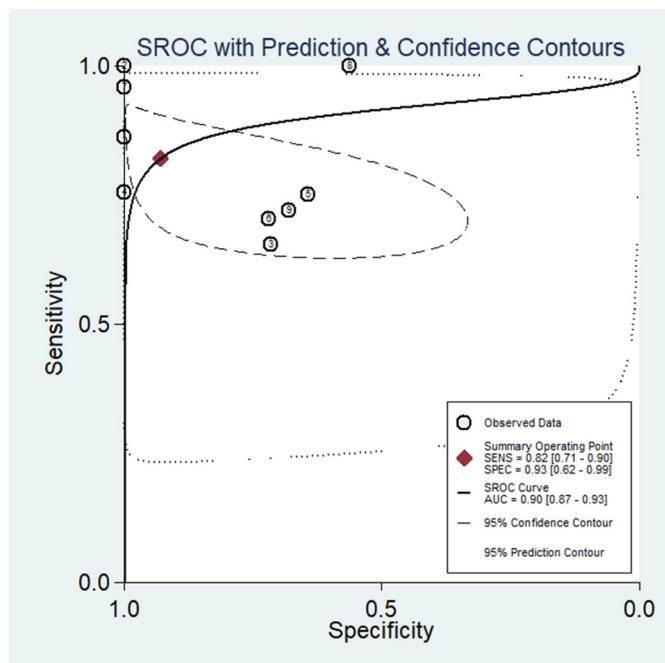
Figure 4 The summary receiver operating characteristic (SROC) curve for assessment of neutrophil CD11b to diagnose neonatal sepsis. AUC, area under the curve.

Previous studies have shown that nCD11b can promptly be detected by flow cytometry with 0.05 mL of blood and may be a promising marker to diagnose neonatal sepsis.[9 19–26] However, to date, these comparable studies have not been evaluated with a systematic approach.

In this meta-analysis, we found that nCD11b could be a valuable tool for diagnosing neonatal sepsis. The PLR of 11.51 showed that neonates with sepsis have a 11.51-fold higher chance of testing nCD11b-positive than neonates without sepsis. This ratio supports a possible role for nCD11b in diagnosing neonatal sepsis.

In clinical practice, the C-reactive protein (CRP) test is the most conventional biomarker for testing neonatal sepsis, and it is as important as the assessment of symptoms and microbiological examination.[27 28] To the best of our knowledge, although the CRP test has been used for a long time, numerous original studies report poor CRP performance in diagnosing neonatal sepsis.[7 20 23] Nupponen *et al* found that the peak level of CRP for diagnosing neonatal sepsis showed relatively lower sensitivity (82%) than that of nCD11b (100%).[20] Du *et al* reported that the sensitivity of nCD11b was higher than that of CRP for diagnosing neonatal sepsis (40.91% and 75.00%, respectively).[23] Recently, interleukin-8 (IL-8) was used to test neonatal sepsis because of its short, highly efficient, non-invasive test.[29 30] A meta-analysis showed that the pooled sensitivity and specificity of IL-8 were 78% and 84%, respectively.[31] In this meta-analysis, the results of nCD11b were similar to those of IL-8. These results indicated that nCD11b is a helpful biomarker for diagnosing neonatal sepsis.

The sensitivity analysis indicated that the results of the meta-analysis were stable. Regarding the types of sepsis, the diagnostic accuracy of nCD11b was higher in neonates with early-onset sepsis; the small number of included studies might have led to this result. Therefore, a further meta-analysis that includes more studies is needed to explain this difference. Regarding the regions, the diagnostic accuracy of nCD11b was higher in Europe or the USA than in Asia. Although we tend to agree with the result, more studies that are similar to ours are needed to support this finding. Concerning the types of neonates and the number of participants, more studies are needed to explain the diagnostic accuracy of nCD11b, and the numbers included in the studies need to be larger.

Our meta-analysis has several limitations. First, nCD11b tests are usually performed along with conventional tests, but we did not address issues such as the reliability of adding the evaluation of nCD11b to other tests. Second, some studies included neonates with neonatal sepsis after treatment, whereas some studies included those without treatment. This may have affected the diagnosis. Third, publication bias was a concern. Because of the linguistic abilities of our team, only studies written in English and Chinese were included. Accordingly, the true accuracy of nCD11b tests for diagnosing neonatal sepsis may be lower than that reported herein.

CONCLUSIONS

In summary, nCD11b is a promising biomarker for making an early diagnosis of neonatal sepsis. Furthermore, large, multicentre, prospective studies are warranted to support our findings.

Contributors Conceptualisation and investigation: XY, JT, JS, XQ, YT, YQ and DM. Data curation, formal analysis, software and writing of original draft: XQ and JL. Funding acquisition: DM. Methodology and supervision: XQ, JL, XY, JT, JS, YT and YQ. Project administration: allauthors. Validation: XQ, JL and DM. Visualisation: YT and YQ. Writing-review and editing: XY, JT, JS, YT, YQ and DM.

REFERENCES

1. Weston EJ, Pondo T, Lewis MM, *et al*. The burden of invasive early-onset neonatal sepsis in the United States, 2005-2008. *Pediatr Infect Dis J* 2011;30:937–41.
2. Bryce J, Boschi-Pinto C, Shibuya K, *et al*. WHO estimates of the causes of death in children. *Lancet* 2005;365:1147–52.
3. Blencowe H, Vos T, Lee AC, *et al*. Estimates of neonatal morbidities and disabilities at regional and global levels for 2010: introduction,

methods overview, and relevant findings from the Global Burden of Disease study. *Pediatr Res* 2013;74 Suppl 1:4–16.

4. Andaluz-Ojeda D, Iglesias V, Bobillo F, *et al*. Early natural killer cell counts in blood predict mortality in severe sepsis. *Crit Care* 2011;15:R243.

5. Shi J, Tang J, Chen D. Meta-analysis of diagnostic accuracy of neutrophil CD64 for neonatal sepsis. *Ital J Pediatr* 2016;42:57.

6. Jyothi P, Basavaraj MC, Basavaraj PV. Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates. *J Nat Sci Biol Med* 2013;4:306–9.

7. Wójkowska-Mach J, Borszewska-Kornacka M, Domańska J, *et al*. Early-onset infections of very-low-birth-weight infants in Polish neonatal intensive care units. *Pediatr Infect Dis J* 2012;31:691–5.

8. O'Hare FM, Watson W, O'Neill A, *et al*. Neutrophil and monocyte toll-like receptor 4, CD11b and reactive oxygen intermediates, and neuroimaging outcomes in preterm infants. *Pediatr Res* 2015;78:82–90.

9. Weirich E, Rabin RL, Maldonado Y, *et al*. Neutrophil CD11b expression as a diagnostic marker for early-onset neonatal infection. *J Pediatr* 1998;132:445–51.

10. Buhimschi CS, Buhimschi IA, Abdel-Razeq S, *et al*. Proteomic biomarkers of intra-amniotic inflammation: relationship with funisitis and early-onset sepsis in the premature neonate. *Pediatr Res* 2007;61:318–24.

11. Whiting PF, Rutjes AW, Westwood ME, *et al*. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;155:529–36.

12. Vamvakas EC. Meta-analyses of studies of the diagnostic accuracy of laboratory tests: a review of the concepts and methods. *Arch Pathol Lab Med* 1998;122:675–86.

13. Devillé WL, Buntinx F, Bouter LM, *et al*. Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002;2:9.

14. Higgins JP, Thompson SG, Deeks JJ, *et al*. Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557–60.

15. Higgins JP. Commentary: Heterogeneity in meta-analysis should be expected and appropriately quantified. *Int J Epidemiol* 2008;37:1158–60.

16. Arends LR, Hamza TH, van Houwelingen JC, *et al*. Bivariate random effects meta-analysis of ROC curves. *Med Decis Making* 2008;28:621–38.

17. Chappell FM, Raab GM, Wardlaw JM. When are summary ROC curves appropriate for diagnostic meta-analyses? *Stat Med* 2009;28:2653–68.

18. 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;58:882–93.

19. Cui YB, Du LZ, Chen YZ, *et al*. [Expression of neutrophil adhesion molecule CD11b as an early diagnostic marker for neonatal sepsis]. *Zhonghua Er Ke Za Zhi* 2003;41:348–51.

20. Nupponen I, Andersson S, Järvenpää AL, *et al*. Neutrophil CD11b expression and circulating interleukin-8 as diagnostic markers for early-onset neonatal sepsis. *Pediatrics* 2001;108:E12.

21. 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;8:72–5.

22. Adib M, Ostadi V, Navaei F, *et al*. Evaluation of CD11b expression on peripheral blood neutrophils for early detection of neonatal sepsis. *Iran J Allergy Asthma Immunol* 2007;6:93–6.

23. Du J, Li L, Dou Y, *et al*. Diagnostic utility of neutrophil CD64 as a marker for early-onset sepsis in preterm neonates. *PLoS One* 2014;9:e102647.

24. Ng PC, Li K, Wong RP, *et al*. Neutrophil CD64 expression: a sensitive diagnostic marker for late-onset nosocomial infection in very low birthweight infants. *Pediatr Res* 2002;51:296–303.

25. Turunen R, Andersson S, Nupponen I, *et al*. Increased CD11b-density on circulating phagocytes as an early sign of late-onset sepsis in extremely low-birth-weight infants. *Pediatr Res* 2005;57:270–5.

26. Aydin M, Barut S, Akbulut HH, *et al*. Application of flow cytometry in the early diagnosis of neonatal sepsis. *Ann Clin Lab Sci* 2017;47:184–90.

27. Meem M, Modak JK, Mortuza R, *et al*. Biomarkers for diagnosis of neonatal infections: A systematic analysis of their potential as a point-of-care diagnostics. *J Glob Health* 2011;1:201–9.

28. Prashant A, Vishwanath P, Kulkarni P, *et al*. Comparative assessment of cytokines and other inflammatory markers for the early diagnosis of neonatal sepsis-a case control study. *PLoS One* 2013;8:e68426.

29. Mishra UK, Jacobs SE, Doyle LW, *et al*. Newer approaches to the diagnosis of early onset neonatal sepsis. *Arch Dis Child Fetal Neonatal Ed* 2006;91:F208–F212.

30. Hotoura E, Giapros V, Kostoula A, *et al*. Pre-inflammatory mediators and lymphocyte subpopulations in preterm neonates with sepsis. *Inflammation* 2012;35:1094–101.

31. Zhou M, Cheng S, Yu J, *et al*. Interleukin-8 for diagnosis of neonatal sepsis: a meta-analysis. *PLoS One* 2015;10:e0127170.