

Beyond the Complete Blood Cell Count and C-Reactive Protein

A Systematic Review of Modern Diagnostic Tests for Neonatal Sepsis

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Objective: To systematically review the accuracy of modern laboratory tests for the diagnosis of serious bacterial infection in newborns.

Methods: The MEDLINE, EMBASE, and Cochrane Library databases were searched using the keywords newborn, infection, sepsis, and diagnosis. We included studies published from 1995 through 2001 that included infants younger than 90 days with proven bacterial growth in a sample from a sterile site. Whenever possible, relevant data were extracted to calculate likelihood ratios (LRs) for whether each test can diagnose a serious bacterial infection. Two independent reviewers selected and reviewed the articles (interobserver agreement, $\kappa=0.80$). All disagreements were resolved by consensus.

Results: Of the 137 citations we retrieved, 37 articles met the inclusion criteria; 17 studies, evaluating 11 different tests, met the highest methodological criteria. The most commonly evaluated test was interleukin 6 (IL-6)

level ($n=7$ studies). The remaining tests were each evaluated in no more than 3 studies. Positive LRs ranged from 1.5 to ∞ . Six individual tests examined in 8 studies had LRs of more than 10 (range, 12.5- ∞). Combined tests also had a wide range of LRs (3.4-9.9). All studies were performed in single medical centers and had small sample sizes, making recommendations according to gestational age criteria difficult.

Conclusions: We found few methodologically rigorous studies of the accuracy of laboratory tests for the diagnosis of bacterial infection in newborns; in a significant proportion of studies, the accuracy of the tests could not be independently determined because of a lack of adequate data. There was marked heterogeneity in sample selection and cutoff levels for diagnosis of neonatal sepsis. A few tests showed promising accuracy, but there are insufficient data to support their confident use as clinical tools.

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CLINICIANS ARE frustrated by the limitations in the diagnosis of neonatal sepsis and would benefit from reliable tests to diagnose sepsis early in its course. Currently, no single test fulfills the criteria of an ideal diagnostic test.^{1,2} In neonatology, tests using hematological indices or acute-phase reactants, such as C-reactive protein (CRP), remain in widespread use despite continuing concerns about their reliability. These concerns largely stem

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from the demonstrated marked variations in the predictive accuracy of hematological parameters.¹ We wished to assess the validity of several newly available immunological markers, including acute-phase reactants other than CRP, and inflammatory mediators, levels of which have been claimed to assist in the diagnosis of neonatal sepsis (**Table 1**).³ Therefore, we

examined studies of various diagnostic tests with reference to their methodological rigor.

METHODS

SEARCH STRATEGY

We identified all relevant articles on bacterial infection in newborns using the following MeSH headings: newborn, infection, sepsis, and diagnosis. We searched the National Library of Medicine's PubMed database, EMBASE, and the Cochrane Library's Cochrane Controlled Trials Register for the years 1995 through 2001. Our search was limited to English-language articles with human data; editorials, commentaries, letters, and reviews were excluded. We searched the bibliographies of review articles, and we also attempted to obtain pertinent missing data from authors.

In addition, we used the following criteria and definitions to select relevant articles. Inclusion criteria were that (1) the diagnostic tests being evaluated were considered to be "new" tests (ie, excluding hematological parameters, such

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Table 1. Clinical Characteristics of Modern Diagnostic Tests for Neonatal Sepsis

Test	Mechanism	Laboratory Time Required	Volume of Blood Required	Type of Test
Cytokine levels (eg, IL-6, IL-8, IL-1 receptor antagonist, and TNF)	Intercellular messengers that act via specific receptors on target cells and mediate inflammatory response	<2 h	200 μ L	ELISA
Fibronectin level	Acute-phase reactant that plays a role in opsonization	2-3 h	1-2 mL of plasma	ELISA
Neutrophil elastase inhibitor level	Neutrophil elastase is released from granulocytes in response to infection; in turn, it forms a complex with the inhibitor	1 h	4 μ L	Turbidimetric assay
Neutrophil CD11b level	Adhesion proteins that form on the surface of neutrophils in response to bacterial toxins	30-60 min	0.1 mL of whole blood	Flow cytometry
PCR	Detection of known nucleic acid sequences of bacteria	1-4 h	200 μ L	Nucleic acid extraction, amplification, and analysis
Procalcitonin level	Increases in response to bacterial infections	Quantitative, 2 h; semiquantitative, 30 min	Quantitative, 20 μ L; semiquantitative, 200 μ L	Semiquantitative and quantitative immunoluminometric assay
TNF receptor p55 and p75 levels	Receptors on target cells; increase in response to infection	4-6 h	200 μ L	ELISA
Soluble intracellular adhesion molecule level	Adherence of leukocytes to endothelial cells; increases in response to infection	2 h	200 μ L	ELISA

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IL, interleukin; PCR, polymerase chain reaction; TNF, tumor necrosis factor.

as immature-total neutrophil ratio, or the commonly used acute-phase reactant CRP); (2) the postnatal age of the infants studied was younger than 90 days; and (3) studies were focused on serious bacterial infections, and true infections were proven by a criterion standard. Because of the uncertainty surrounding the concept of clinical sepsis in neonates, we focused only on studies in which the criterion standard for diagnostic tests was unequivocal proof of bacterial infection (ie, bacterial growth) in cultures of blood or cerebrospinal fluid (CSF) samples from sterile body sites. (We did not include urinary tract infections because they are an uncommon cause of sepsis in newborns unless they are accompanied by general sepsis and because for validity, these samples must be obtained by invasive measures that many neonatologists are reluctant to employ.) We defined clinical sepsis as sepsis not meeting the definition of true infection. If studies reported positive bacterial growth from endotracheal tube aspirates, with or without changes on chest radiographs, in the absence of blood or CSF cultures positive for bacterial growth, we considered this to be clinical sepsis.

We excluded studies that examined antenatal tests, including amniotic fluid tests, and studies in which data for antenatal and neonatal infection could not be separated.

ARTICLE REVIEW AND DATA EXTRACTION

Two of us (A.M. and C.P.S.H.) assessed each article for methodological quality independently to establish that all selected studies allowed us to distinguish true bacterial infections from clinical sepsis. These 2 authors also attempted to extract data for an independent calculation of sensitivity, specificity, and likelihood ratio (LR). Each article was then independently assessed by 2 of us (A.M. and C.P.S.H.). Adjudication (H.K.) and subsequent consensus resolved all disagreements regarding the inclusion of studies and the extracted data.

ANALYSIS

If studies provided adequate data, 2×2 tables were created to calculate sensitivity, specificity, positive predictive value, negative predictive value, and LRs. Following the recommenda-

tions of Sackett et al,² an a priori rule was defined for this study whereby an LR of less than 10 was considered unlikely to affect clinical diagnosis. Confidence intervals were recorded for the LRs in studies that presented adequate data to perform an independent calculation of pretest probabilities and LRs (StatsDirect statistical software, version 1.9.8; StatsDirect Ltd, Sale, England; Confidence Interval Analysis, version 2.0, Wilson Method, T. Bryant, 2000). If we were not able to independently extract raw data, LRs were calculated from sensitivity and specificity values provided by the authors.

RESULTS

LITERATURE REVIEW

The literature search generated 137 citations. Of these, 49 were potentially relevant for inclusion based on a review of the abstract. More detailed review resulted in good interobserver agreement between the 2 reviewers regarding eligible articles; full agreement was reached for 42 articles, and consensus discussion was required for only 7 articles (Cohen $\kappa=0.80$). We excluded one study that reported on 548 blood samples but did not specify the number of infants from whom they were drawn,⁴ making it impossible for us to determine whether multiple samples were from single subjects. Another article⁵ also included multiple samples from single subjects; however, this article provided the sample size from which the specimens were drawn. Although this limits our ability to fully interpret the data, we chose to include this article because the denominator was provided. In total, 37 articles met the inclusion criteria and were assessed for methodological quality.

STUDIES THAT MET INCLUSION CRITERIA

Of the 37 studies that were included, 17 (46%) clearly distinguished clinically septic infants from those who had

Table 2. Included Studies (n = 17)*

Test Evaluated	Source	Cumulative No. of Participants, Sepsis/Total Enrolled
Cytokine levels IL-6	Kallman et al, ⁶ 1996	92/616
	Messer et al, ⁷ 1996	
	Panero et al, ⁸ 1997	
	Kuster et al, ⁹ 1998	
	Silveira and Procianoy, ¹⁰ 1999	
	Bhartiya et al, ¹¹ 2000	
IL-8	Kashlan et al, ¹² 2000	72/676
	Franz et al, ¹³ 1999	
	Franz et al, ¹⁴ 1999	
	Nupponen et al, ¹⁵ 2001	
IL-1 receptor antagonist TNF- α	Kuster et al, ⁹	21/101
	Atici et al, ¹⁶ 1997	65/209
	Silveira and Procianoy, ¹⁰ 1999	
Fibronectin levels	Kocak et al, ¹⁷ 1997	15/45
	Fischer et al, ⁹ 2000	15/133
Neutrophil elastase inhibitor level	Weirich et al, ¹⁸ 1998	9/145
Neutrophil CD11b level	Nupponen et al, ¹⁵ 2001	
PCR	Laforgia et al, ¹⁹ 1997	4/33
Procalcitonin level	Gendrel et al, ²⁰ 1996	56/373
	Chiesa et al, ²¹ 1998	
	Franz et al, ¹⁴ 1999	
	Messer et al, ⁷ 1996	
TNF receptor p55 and p75 levels	Kuster et al, ⁹ 1998	21/101
Soluble intracellular adhesion molecule level		

Abbreviations: IL, interleukin; PCR, polymerase chain reaction; TNF, tumor necrosis factor.

*All included studies met the criterion standard, the ability to distinguish clinical sepsis from true bacterial infection.

true bacterial growth according to our criteria (**Table 2**).⁵⁻²¹ The cumulative sample sizes of these studies were small, even for the most frequently applied diagnostic tests. Because we deliberately chose bacterial growth as a criterion standard, the remaining 20 studies could not be further analyzed. A list of references found but that did not meet inclusion criteria can be obtained from the corresponding author (H. K.).

Included Studies

The 17 studies that met our methodological criteria assessed 11 new tests and a total of 299 septic infants. Of these studies, 7 (41%), enrolling a total of 68 septic infants, provided adequate raw data to allow independent calculation of sensitivities, specificities, and LRs along with confidence intervals (**Table 3**). In 10 studies, we could only use the values as originally calculated by the authors. From the study by Messer et al,⁷ we could extract data for interleukin (IL) 6 levels but not for levels of the tumor necrosis factor (TNF) receptors p55 and p75. Similarly, the study by Silveira and Procianoy¹⁰ examined 3 diagnostic tests (IL-6, TNF- α , and IL-1 β levels), but no data are provided for IL-1 β levels. The authors concluded that "IL-1 β is not a good marker of neonatal sepsis."^{10(p650)} In the absence of numeric data, we have omitted it from Table 3. Three studies reviewed more than 1 diagnostic test used in combination in an attempt to enhance diagnostic accuracy (**Table 4**). In the 17 included studies, the most common new reported test was IL-6 level, which was examined by 7 separate studies that enrolled a total of 92 sep-

tic and 524 nonseptic infants. The remaining tests were assessed by no more than 3 studies each (cumulative range, 4-72 septic infants).

Cutoff Values Used by the Studies

The cutoff laboratory values that were chosen to distinguish between the presence and absence of infection appear to be unique to each study. Franz et al¹³ employed 2 separate cutoff values for IL-8 in 2 separate study periods, which allowed a comparison of these values (Table 3). In a later study, Franz et al¹⁴ provided more data using the second cutoff value. Franz et al^{13,14,22} also reported on similar data sets employing IL-8 and procalcitonin levels. We included 2 of these studies^{13,14} because we could not determine how much they overlapped. We annotated data from all 3 subsets of infants described by these researchers (Table 3 and Table 4). We used the authors' own cutoff values whenever available. Gendrel et al,²⁰ who examined 13 septic infants, did not specify any cutoff level to demarcate between infected and noninfected newborns. However, the authors did provide a scattergram that allowed us to make a distinction. In order to use the study by Gendrel et al,²⁰ we employed the cutoff value for procalcitonin used by Franz et al¹⁴ in their similar study.

Performance of the Individual Tests Evaluated

The range of test sensitivity and specificity, both calculated and reported, was large (Table 3). Sensitivities ranged

Table 3. Accuracy of Diagnostic Tests*

Source	Cutoff Point	Gestational Age, wk	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Likelihood Ratio (95% CI)	
					Positive	Negative
Independent Analysis Performed						
IL-6 level						
Kallman et al, ⁶ 1996	135 pg/mL	25-40	93 (70-99)	86 (69-94)	6.5 (2.89-16.49)	0.08 (0.01-0.35)
Messer et al, ⁷ 1996	100 pg/mL	24-42	82 (52-95)	46 (39-52)	1.5 (0.95-1.86)	0.4 (0.11-1.06)
Bhartiya et al,¹¹ 2000	133 pg/mL	26-40	81 (57-93)	96 (87-99)	21.5 (6.2-79.2)	0.19 (0.07-0.4)
IL-8 level						
Nupponen et al,¹⁵ 2001	>50 pg/mL	29-41	100 (51-100)	100 (75-100)	∞	∞
Neutrophil CD11b level						
Weirich et al,¹⁸ 1998	>60	>28	100 (57-100)	100 (96-100)	∞	∞
Nupponen et al,¹⁵ 2001	>150 RFU	29-41	100 (51-100)	100 (75-100)	∞	∞
PCR						
Laforgia et al, ¹⁹ 1997	+/-	Unknown	100 (51-100)	48 (35-61)	1.9 (0.81-2.38)	∞
Procalcitonin level						
Gendrel et al,²⁰ 1996	0.5 µg/L (Assumed)	>32	100 (77-100)	92 (84-96)	12.5 (5.83-23.64)	∞
Independent Analysis Not Performed						
IL-6 level						
Panero et al,⁸ 1997	15 pg/mL	35-40	100	100	∞	∞
Kuster et al, ⁹ 1998	25 pg/mL	Unknown	86	57	2	0.24
Silveira and Procianny, ¹⁰ 1999	32 pg/mL	>33	90	43	1.6	0.23
Kashlan et al, ¹² 2000	100 pg/mL	<32	80	90	8	0.22
IL-8 level						
Franz et al, ¹³ 1999	>70 ng/mL	<30	69/80†	91/87†	7.7/6.2†	0.34/0.23†
	>53 ng/mL	<30	84/91†	85/53†	5.6/1.9†	0.19/0.17†
Franz et al, ¹⁴ 1999	>70 ng/L	23-42	83	76	3.5	0.22
IL-1 receptor antagonist level						
Kuster et al,⁹ 1998	1200 pg/mL	Unknown	100	93	14.3	0
TNF-α level						
Atici et al, ¹⁶ 1997	90 pg/mL	30-40	72	62	1.9	0.45
Silveira and Procianny, ¹⁰ 1999	12 pg/mL	>33	88	43	1.5	0.28
Kashlan et al, ¹² 2000	30 pg/mL	<32	95	78	4.3	0.06
Fibronectin level						
Kocak et al, ¹⁷ 1997	90-100 µg/mL	30-40	100	88	8.3	0
Neutrophil elastase inhibitor level						
Fischer et al,⁵ 2000	NG	25-44	80	98	40	0.2
Procalcitonin level						
Chiesa et al,²¹ 1998	Own Reference Range	27-37.4	93	98	46.5	0.07
Franz et al, ¹⁴ 1999	0.50 µg/L	23-42	57	66	1.7	0.65
TNF receptor p55 and p75 levels						
Messer et al, ⁷ 1996	6	24-42	81	77	3.5	0.25
Soluble intracellular adhesion molecule level						
Kuster et al, ⁹ 1998	425 ng/mL	Unknown	82	79	3.9	0.23

Abbreviations: CI, confidence interval; IL, interleukin; NG, not given; PCR, polymerase chain reaction; RFU, relative fluorescence units; TNF, tumor necrosis factor. *Boldface type indicates studies with a likelihood ratio of more than 10. †Virgule separates data from 2 different study periods.

from 57% to 100%, and specificities ranged from 43% to 100%. Similarly, positive LRs ranged from 1.5 to ∞ (Table 3). Six tests in 8 studies had LRs of more than 10.^{5,8,9,11,15,18,20,21} Of these studies, we were able to perform an independent verification that the positive LR was more than 10 in the study of procalcitonin by Gendrel et al²⁰; the studies of neutrophil CD11b by Weirich et al¹⁸ and Nupponen et al¹⁵; the study of IL-6 by Bhartiya et al¹¹; and the study of IL-8 by Nupponen et al.¹⁵ In total, 2 of 3 studies that evaluated procalcitonin levels^{20,21} had a positive LR of more than 10.

Combined Tests

We also assessed the accuracy of combinations of tests evaluated in 3 studies (Table 4), although these studies

did not provide adequate raw data to allow calculation of LRs. All were small studies; the largest, by Franz et al,¹³ enrolled only 26 septic infants. None of these test combinations had a positive LR of more than 10.

COMMENT

The rapidly evolving understanding of the molecular physiological processes underlying sepsis and technical advances in biochemical testing hold promise for rapid accurate diagnosis, although it should be remembered that bacterial growth requires at least 12 hours by commercial automated testing. Table 1 addresses the clinical utility of these putative new tests. However, in this systematic review of the accuracy of these newer diagnostic tests,

Table 4. Accuracy of Combined Tests

Source	Positive for Bacterial Infection	Gestational Age, wk	Sensitivity, %	Specificity, %	Likelihood Ratio	
					Positive	Negative
Franzet al, ¹³ 1999	IL-8 level >53 pg/mL and/or CRP level >10 mg/L	<30	93/100*	80/83*	4.7/5.9*	0.09/0.00*
	IL-8 level >70 pg/mL and/or CRP level >10 mg/L	<30	88/95*	87/87*	6.8/7.3*	0.14/0.06*
Franz et al, ¹⁴ 1999	IL-8 level >70 pg/mL and/or CRP level >10 mg/L	23-42	91	73	3.4	0.12
Silveira and Procianny, ¹⁰ 1999	IL-6 level >32 pg/mL and/or TNF- α level >12 pg/mL	>33	99	90	9.9	0.01

Abbreviations: CRP, C-reactive protein; IL, interleukin; TNF, tumor necrosis factor.
*Virgule separates data from 2 different study periods.

we were unable to provide a summary in a simple statistical form that clinicians could use. This reflects several methodological issues. Perhaps the most striking is that the predominance of small studies, all using such differing approaches, makes formal meta-analysis unproductive. Had studies been of large enough size and power, it would have been possible to report on test characteristics by birth weight and gestational age, because risk of neonatal sepsis is likely to be different with both.

The marked heterogeneity in studies of tests for neonatal sepsis has also been noted by authors reviewing older diagnostic tests.^{1,23} It is worth emphasizing that, among studies, enrolled subjects were heterogeneous, varying by postnatal age, gestational age, and risk factors. Each study consisted of small numbers of patients from single medical centers, which differed with regard to types of patients (eg, surgical, cardiac, medical, and inborn and out-born) and their demographic characteristics. Often, we were uncertain about the exact nature of the neonatal population in which the diagnostic test was studied because most studies reported only gestational age, sex, and birth weight. Even the prevalence of sepsis in the nurseries studied was recorded in only a small minority of articles. In addition, a wide range of cutoff values was employed, and no study used previously reported values; instead, authors chose to use unique cutoff values, making comparison of these tests difficult. Most articles (Tables 3) did not report whether blood culture and lumbar puncture were performed before infants received antibiotics. We were left to assume that these tests were performed first if there was clinical suspicion of sepsis and that antibiotic treatment was instituted afterward. Attempts to contact the authors of several studies to obtain further raw data were by and large unhelpful. These methodological problems prevented us from providing a concise statistical summary in the form of a meta-analysis.

Because some clinicians are more familiar with sensitivity than LRs, we provide these values. Where possible, however, we emphasize LRs because this statistic offers potential advantages compared with measures such as sensitivity and specificity.^{24,25} Likelihood ratios may be more useful than sensitivity and specificity, largely because of their independence from prevalence.^{26,27} This is relevant to our review because the prevalence of neonatal sepsis (whether nosocomial or peripartum) appears to vary considerably, from 5% to 42% in a recent study.²⁸ We could not find the true prevalence in most studies included in this review. The prevalence of sepsis varies according to

birth weight, gestational age, and the characteristics of the neonatal nursery, including its proportion of infants undergoing medical vs surgical treatment.

Sackett et al² suggest that positive LRs of less than 10 are unlikely to greatly enhance posttest probabilities. We chose to follow this rule in assessing how useful these studies are to clinical practice. Only 8 individual studies examining 6 tests had LRs outside this range. We have not emphasized negative LRs, although we also report these (Table 3 and Table 4). Taking the top prevalence range of Brodie et al²⁸ at 42%, even an extremely negative LR would likely reduce the posttest probability of the patient having sepsis to 5%, as calculated using the LR nomogram in Sackett et al.² We remain unconvinced that a clinician would accept a 5% risk in choosing not to treat.

A common diagnosis in neonatology is clinical sepsis, without confirmation from blood or CSF cultures. Combining clinical sepsis and documented infections dilutes the true sepsis rate, which should be the denominator for rates. We have avoided these possible problems by specifically examining only studies that enrolled infants with clinical signs leading to identification of an unequivocally true bacterial infection (ie, bacterial growth). Less than half the initial studies met this criterion, and less than half the remaining studies provided adequate data to enable an independent confirmation of the statistics, including the LRs.

In contrast to our insistence on an unequivocal criterion standard, Mehr and Doyle,²⁹ in reviewing selected cytokine levels as markers of bacterial sepsis in newborn infants, chose to examine data from all infants, including those with clinical sepsis. They reviewed articles examining TNF- α , IL-6, and IL-8 levels that were published from 1966 to 1999 in English-language journals listed in MEDLINE. They argued that including "probable/suspected" sepsis was more relevant to "real life" clinical circumstances. However, a strict and uniform definition of culture-negative sepsis was not adhered to in the reviewed studies. In addition, the clinical terms were often not defined, nor were the cutoff levels for various tests consistent across studies. Mehr and Doyle also raised concerns about false-positive results due to skin contaminants and the lack of stringent definitions to distinguish contaminants from true infections, leading to a falsely high positive predictive value. Although we did not attempt to address this in our review, a previous study, for which age-matched and birth weight-matched controls were specifically recruited, showed a lower rate of positive blood cul-

What This Study Adds

We reviewed all the literature on diagnostic tests for neonatal sepsis published since the last substantive review in 1995. Although many newer tests are being evaluated, this study highlights the fact that few of these tests have been evaluated with methodological rigor. The problems of significant heterogeneity in sample selection and cutoff levels for diagnosis of sepsis persist. A few tests look promising, but until larger multicenter trials are performed, they should not be employed in routine clinical practice.

tures in nonsymptomatic infants.³⁰ Mehr and Doyle's overall concerns about the methodological rigor of this field of study were similar to ours.

We recognize that there are other tests for infection, such as heart rate analysis,³¹ sepsis scores,³² and urinary tests.³³ However, we did not include these, because they were not in the scope of our review. In addition, we did not include articles examining CRP because they had previously been reviewed¹ and CRP tests appear to be as inaccurate as more standard hematological tests.

We conclude that serious methodological flaws plague current studies that aim to improve the diagnosis of neonatal bacterial sepsis with modern tests. In particular, the predominance of studies at single medical centers with small sample sizes makes it difficult to apply the tests in clinical decision making. Furthermore, diagnostic tests were not applied to differing populations with a mix of inborn and outborn infants, gestational ages, birth weights, and levels of acuity (level 1, 2, and 3 neonatal intensive care units), which made generalizability a problem. A few diagnostic tests remain promising, of which IL-6 level is the most intensively studied, probably because of its acknowledged importance as an alarm cytokine. In addition, procalcitonin levels appear to show considerable promise as a diagnostic test for neonatal sepsis. To give clinicians a firmer recommendation, studies of adequate size and using rigorous methods are now needed to enable estimates of the diagnostic accuracy of these new tests.

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