Review

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Diagnostics for neonatal sepsis: current approaches and future directions

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Progress has been made in the reduction of morbidity and mortality from neonatal sepsis. However, diagnosis continues to rely primarily on conventional microbiologic techniques, which can be inaccurate. The objective of this review is to provide the clinician with an overview of the current information available on diagnosing this condition. We review currently available diagnostic approaches for documenting neonatal sepsis and also describe novel approaches for diagnosing infection in neonates who are under development and investigation. Substantial progress has been made with molecular approaches and further development of non-culture-based methods offer promise. The potential ability to incorporate antimicrobial resistance gene testing in addition to pathogen identification may provide a venue to incorporate a predominantly molecular platform into a larger program of neonatal care.

The gold standard for establishing a diagnosis of neonatal sepsis is through culture. However, several factors, including the small blood volumes obtained from neonates, the presence of low or intermittent bacteremia, as well as maternal intrapartum antimicrobial exposure, can make the confirmation of sepsis in a neonate a diagnostic challenge (1,2). Given that the clinical diagnosis of infection in a neonate is unreliable (3) and that excessive, unnecessary empiric antimicrobial therapy for the treatment of suspected sepsis can promote antimicrobial resistance, there is a heightened need for an accurate and sensitive diagnostic tool to confirm the diagnosis of early-onset neonatal sepsis (EOS), variably defined as sepsis occurring in the preterm neonate in the first 3 days of life or in the term neonate in the first week of life (4), and late-onset neonatal sepsis (LOS), defined as sepsis occurring in the following period up to 3 months of life. Diagnostics with a faster turnaround time would not only improve surveillance in all settings but also facilitate timely management. The objective of this review is to present information on advances in the diagnostics of neonatal sepsis, which remains one of the leading causes of neonatal death (5). Given that some areas presented herein are relatively new, with limited data specifically regarding EOS and LOS, we also extrapolate relevant concepts from the wider field of sepsis in general.

CHALLENGES IN THE DIAGNOSIS OF NEONATAL SEPSIS

History and Physical Examination

History and physical examination is the cornerstone of clinical practice; however, some clinical manifestations in neonates are not a reliable indicator of illness (3). Many early signs of infection in neonates are nonspecific and may also be simply associated with prematurity or the transition to extrauterine life. Conversely, asymptomatic presentation does not completely rule out infection in the high-risk setting, as seen in a study of 5,135 early-onset neonatal sepsis (EOS) evaluations in neonates ≥ 37 weeks where positive blood cultures were identified in 0.5% of asymptomatic infants, compared with 3.2% of symptomatic infants (6).

Several clinical features are associated with the early stages of late-onset neonatal sepsis (LOS), including primarily feeding intolerance and apnea, along with bradycardia and desaturations (7–9). Heart rate variability has also been identified as a potential physiomarker (10), although other studies did not find elevated heart rate characteristics to improve detection of bloodstream infections, (11) and in studies predominantly among premature very low birth weight infants, LOS hypotension has been noted to be a strong independent predictor (8). Increasingly, abnormal changes have been noted in the 12–24 h prior to an infant's abrupt clinical deterioration, with reduced baseline variability and subclinical short-lived decelerations in heart rate (sepsis skewness from the mean of -0.59 ± 0.10, compared with -0.10 ± 0.13 for controls over the 24 h before to 24 h after diagnosis) (12). When this was prospectively studied among two validation cohorts in neonatal intensive care units (NICUs) totaling 435 neonates, impending sepsis and sepsis-like illness was not only significantly associated with the heart rate characteristic index, but reduced heart rate variability and decelerations also significantly aided in the prediction of sepsis and sepsis-like illness (13).

Natural History of Changes in Neonatal Blood Counts

As the neonate's physiological circulation transitions after birth, other hematologic indices also adapt during this period.

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These patterns are unique to gestational age and should be taken into account when interpreting likelihood of neonatal sepsis. An analysis of 30,354 complete blood counts obtained in the first 72 h following birth demonstrated that preterm neonates with <28 weeks' gestation have blood neutrophil concentrations that peak around 24 h after birth, compared with neonates having ≥28 weeks' gestation whose counts peak between 6 and 8 h after birth (14). Maternal labor and female gender were also associated with higher average neutrophil values (14). A cross-sectional study of 67,623 newborns ≥34 weeks' gestation comparing blood counts with blood cultures obtained ≤72 h of age found that using interval likelihood ratios for the newborn's age in hours improved interpretation of culture-confirmed neonatal sepsis (15). White blood counts (WBC) and absolute neutrophil counts (ANC) were most informative when low (WBC <5,000 and ANC <1,000 at ≥4 h had likelihood ratios of 81 and 115, respectively) (15), whereas elevated counts were less helpful (WBC ≥20,000 and ANC ≥10,000 at ≥4 h had likelihood ratios of 0.16 and 0.31, respectively) (15), and no test was very sensitive. Neutrophil counts can also be affected by maternal (hypertension and fever), intrapartum (asphyxia, meconium aspiration syndrome, and type of delivery), and perinatal (periventricular hemorrhage, reticulocytosis, hemolytic disease, and pneumothorax, among others) factors (16). Proper interpretation of the neonatal blood count based on gestational age, and in combination with other hematologic indices, may aid in the early recognition of neonatal sepsis as well as may increase the sensitivity for diagnosis (17). However, multiple factors can alter neutrophil dynamics limiting the ability to make a definitive diagnosis based on these markers alone.

Currently Available Laboratory Diagnostics

Conventional microbiological methods. Blood culture is the gold standard for diagnosis but it is insensitive. Antimicrobial and hospitalization management decisions are additionally affected by the 48–72 h turnaround time for culture results. Maternal antimicrobial treatment may lead to false-negative culture results in infants (2). Inadequate volumes of blood provided for culture further diminish the yield (1), and, in cases of pediatric sepsis, they can miss up to 75% of cases among those who meet sepsis terminology guidelines (18).

Biomarkers. Given the insensitivity of physical examination and culture, diagnosis of EOS and LOS, as well as pediatric sepsis more broadly, tends to rest on a combination of clinical signs in association with laboratory markers that include blood counts and acute-phase reactants (**Table 1**). Despite extensive research, no single marker has a significant advantage over others in determining the diagnosis of neonatal sepsis. Furthermore, variation in the onset of elevation, duration of elevation or half-life of markers may also influence the test characteristics of these markers across studies.

The most extensively studied acute-phase reactants have been C-reactive protein (CRP) and procalcitonin (PCT). Serum CRP increases within 6–10 h and peaks 2–3 days after infection onset (19). Serum PCT rises within 4 h after onset and attains maximum serum concentrations at 18–24 h (20). In a study of 401 newborn infants of <28 days of age with suspected sepsis, CRP on adjusted analysis was independently predictive of a positive blood culture (8). In a study of 176 newborns >1,500 g, CRP was calculated to have a negative predictive value of 99% (21). This demonstrated the potential usefulness of this biomarker to guide early discontinuation of antimicrobial therapy in suspected early-onset neonatal bacterial infection (21). In a systematic review and meta-analysis, PCT in cord blood had high likelihood ratio (5.72) and sufficient sensitivity (82%) and specificity (86%) to be considered a reliable rule-in and rule-out test (22). Although some studies have suggested improved sensitivity and specificity of PCT for identification of LOS (23), given the current evidence and the availability of tests in clinical laboratories, serial CRP measurements are commonly used in most NICUs to identify infants with LOS.

Other studied biomarkers include cytokines and chemokines such as interleukins and cell adhesion molecules, although data in their kinetics are generally unavailable. Considerable variations exist, however, in the sensitivity and specificity of these markers. These can be explained by the different definitions of sepsis, test methodologies, reference values, cutoff points, sampling procedures, and inclusion criteria used among different studies, in addition to different approaches to stratifying groups by either gestational age or birth weight. Combinations of biomarkers as well as serial measurements lead to improved sensitivity and accuracy, and have contributed to antimicrobial management decisions (24).

NOVEL DIAGNOSTIC APPROACHES TO NEONATAL SEPSIS

Testing on Non-Neonatal Specimens

Cord blood. Cord blood, of which the cell composition is similar to peripheral blood composition of the fetus during the last stage of gestation, is the first hematologic source from the neonate and does not require an invasive procedure nor the infliction of pain; thus, specimen collection avoids iatrogenic stress and procedural complications. Larger volumes of blood may also be obtained, thereby increasing the potential of organism recovery without risking hemodynamic instability in the newborn. A study evaluating 350 pairs of samples from umbilical cord and peripheral venous samples found that WBC and platelet counts significantly correlated between them (correlation coefficients $r=0.683$ and $r=0.54$, respectively), but with a lower correlation for hemoglobin ($r=0.36$) (25). This may be explained because cord blood from a premature infant may not be identical to peripheral blood in a preterm infant. Although no cases of EOS were detected, contamination rates were higher in umbilical cord versus peripheral blood (12% vs. 2.5%) (25). This rate of contamination in cord blood samples was not seen in another study involving 200 neonates, where the contamination rate was 0.5% (26). This study demonstrated similarity in hematocrit, platelet, and elevated WBC and ANC between cord and peripheral blood (26). A

Table 1. Markers of neonatal sepsis

Marker	Performance				Comments
	Sensitivity	Specificity	Positive predictive value	Negative predictive value	
Blood counts					
Total leukocyte counts	EOS: 0.3(62)–18%(62) LOS: 0.1(63)–23%(63)	EOS: 79(62)–99%(62,63) LOS: 80(63)–99%(63)	EOS: 36%(64) LOS: 13(65)–100%(17)	EOS: 94(64)–>99.8%(62) LOS: 74(17)–96%(65)	Need to correlate value with newborn age in hours. Unreliable indicator of infection during first few hours of EOS as infants with proven bacteremia can have normal levels at the time of initial evaluation(66).
Absolute neutrophil count	EOS: 0.8(62)–68%(67) LOS: 2.4(63)–5%(63)	EOS: 95(62)–99%(62) LOS: 34(17)–98%(63)	LOS: 14(17)–21%(68)	LOS: 74(17)–96%(65)	Low neutrophil counts have been noted among ELBW neonates at a rate five times that of a general NICU population, and have been associated with neonates born SGA and those with maternal hypertension(69).
Neutrophil ratio: Immature-to-mature neutrophil ratio (I:M ratio); immature-to-total neutrophil ratio (I:T ratio)	EOS: 22(62)–62%(67) LOS: 33(17)–54%(63)	EOS: 74(62)–96%(62) LOS: 62(63)–100%(17)	EOS: 2.5(62) LOS: 12(65)–100%(17)	EOS: 99%(62) LOS: 66(17)–96%(65)	Low band counts caused by exhaustion of marrow can produce misleadingly low ratios in the presence of serious/overwhelming infection(70–72).
Platelet count	EOS: 0.8(62)–4%(62) LOS: 8(63)–48%(17)	EOS: 97(62)–99%(62) LOS: 89(63)–98%(63)	EOS: 13(73)–14%(73) LOS: 9%(65)	LOS: 94%(65)	Thrombocytopenia may accompany viral infections, as well as complications associated with umbilical catheter placement, birth asphyxia, maternal hypertension, mechanical ventilation, meconium aspiration, multiple exchange transfusions, and necrotizing enterocolitis(66,74,75).
Acute-phase reactants					
C-reactive protein	EOS: 9(76)–89%(77) LOS: 29(78)–94%(79)	EOS: 59(19)–87%(77) LOS: 78(79)–100%(80)	EOS: 33(76)–96%(81) LOS: 66(82)–100%(80,83)	EOS: 50(76)–94%(84) LOS: 38(78)–90%(80)	CRP on adjusted analysis was independently predictive of a positive blood culture(8). Nonspecific physiological 3-day increase is affected by non-infectious perinatal and maternal factors(85).
Procalcitonin	7(86)–100%(87)	35(87)–99%(86)	33(87,88)–90%(89)	91(88)–100%(87)	Increased serum levels can be seen in non-infectious episodes such as respiratory distress syndrome, perinatal asphyxia, intracranial hemorrhage, hemodynamic failure, pneumothorax, resuscitation, and fetal distress(19).
Serum amyloid A	23(90)–75%(91)	44(91)–93%(90)			Acute-phase reactant synthesized in the liver, regulated by proinflammatory cytokines (IL-6 and TNF-α), and involved in chemotaxis, immunomodulation, and tissue regeneration.
Lipopolysaccharide-binding protein	94(92)–100%(87)	70(88)–94%(87)	37(88)–80%(87)	92(88)–100%(87)	Synthesized primarily by hepatocytes as well as epithelial and muscular cells. Does not differentiate between infectious and non-infectious systemic inflammatory response syndrome.
Cytokines and chemokines					
IL-1β	EOS: 74(93)–83%(94) LOS: 95%(82)	EOS: 70(93)–86%(94) LOS: 59%(82)	EOS: 71%(94) LOS: 35%(82)	EOS: 94%(94) LOS: 97%(82)	Elevated in cord plasma specimens from infants born after induced vaginal or urgent cesarean delivery.
IL-6	EOS: 54(81)–84%(93) LOS: 44(80)–100%(82)	EOS: 70(93)–100%(81) LOS: 74(82)–93%(80)	EOS: 38(95)–100%(81) LOS: 40(82)–86%(96)	EOS: 59(81)–97%(97) LOS: 74(96)–100%(82)	Elevated in the presence of chorioamnionitis and delivery room intubation, but depressed in the presence of maternal hypertension.
IL-8	EOS: 44(98)–96%(99)	EOS: 70(100)–94%(101)	EOS: 43(84)–91%(94)	EOS: 83(98)–98%(99)	A neutrophil-activating agent produced by activated phagocytes, which result in development of systemic inflammation.
TNF-α	EOS: 75%(94) LOS: 60(102)–100%(82)	EOS: 88%(94) LOS: 79(82)–86%(83)	EOS: 67%(94) LOS: 54(82)–82%(83)	EOS: 51%(94) LOS: 78(102)–100%(82)	Involved in systemic inflammation and a member of a group of cytokines that stimulate the acute-phase reaction.

Table 1 Continued

Marker	Performance				Comments
	Sensitivity	Specificity	Positive predictive value	Negative predictive value	
Soluble CD14	67(87)–83%(87)	67(87)–88%(87)			May be affected by duration of labor and chronological age.
CD64	70(17)–96%(103)	62(17)–98%(104)			Neutrophil surface marker, extensively expressed on the surface of neutrophil granulocytes during bacterial infection(105–107).
Mannose-binding lectin	62%(108)	66%(108)			Genetic variations in the promoter region of MBL are posited to correlate with infection risk in neonates(108,109).
Hepcidin	76%(110)	100%(110)	100%(110)	87%(110)	Acute-phase reactant expressed on neutrophils and macrophages, synthesized by hepatocytes and stimulated by lipopolysaccharides and IL-6 in response to microorganisms.
Molecular diagnostics					
Broad-range conventional PCR	63(111)–100%(47,112)	87(111)–95%(47)	37(111)–77.2%(47)	82.8(111)–100%(47)	16S rRNA PCR increased the sensitivity in detecting bacterial DNA in newborns with signs of sepsis, allowed a rapid detection of the pathogens, and led to shorter antibiotic courses. However, uncertainty about the bacterial cause of sepsis was not reduced by this method(47,48). PCR has the potential to detect bacteria in culture-negative samples even after the initiation of intravenous antibiotics(113).
Real-time PCR	59(114)–96%(45)	53(114)–97%(115)			
Multiplex PCR	61(116)–75%(116,117)	87(117)–92%(116)			
Species- and genus-specific PCR	69(118)–100%(119)	97(119)–100%(118)	100%(118)	98%(118)	
PCR followed by post-PCR processing	41(50)–100%(120)	94(121)–98%(50,120)	50(121)–96.8%(121)	80.4(121)–99.2%(50)	PCR coupled with mass spectrometry technology led to the additional identification of pathogens that were not found by conventional methods(121).
Hybridization	72%(122)	100%(122)	99.3%(122)	90.4%(122)	Some bacteria may be identified only at the genus level because no species-specific probes are available(123).
Mass spectrometry	76(124)–80%(125)	96–100%(123)	99.2%(124)		Species that do not differ sufficiently in their ribosomal protein sequences, such as *Shigella* spp., *Escherichia coli*, *S. pneumonia,* and *S. oralis/mitis* group cannot be distinguished by MALDI-TOF MS(52).

CBC, complete blood count; CRP, C-reactive protein; ELBW, extremely low birth weight; EOS, early-onset neonatal sepsis; IL, interleukin; LBP, lipopolysaccharide-binding protein; LOS, late-onset neonatal sepsis; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MBL, mannose-binding lectin; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; PCR, polymerase chain reaction; PCT, procalcitonin; SAA, serum amyloid A; SGA, small for gestational age; TNF, tumor necrosis factor.

review of 15 studies evaluating over 2,000 episodes of suspected neonatal infection identified interleukin-6 (IL-6) and PCT in cord blood as having high positive (9.47 and 5.72, respectively) and negative likelihood ratios (0.10 and 0.20, respectively) (22). Another study evaluating 40 neonates with two or more risk factors for EOS found that, compared with peripheral venous samples, cord blood had 100% sensitivity and 95% specificity (27). Cord blood CRP, however, was negative in all 11 neonates who screened positive for sepsis (27).

Maternal serum. Maternal serum has been examined as another potential noninvasive source for early identification of neonatal sepsis. Modest systemic inflammation in maternal serum is associated with histological chorioamnionitis, considered a risk factor for EOS. However, these serum inflammatory markers may also increase postnatally, diminishing their utility in diagnosing EOS (28). A review and meta-analysis of inflammatory markers in maternal serum found that only IL-6 accurately compared with, and had a high positive likelihood ratio (5.47) in, cases of EOS (22).

Novel Biomarkers

Biomarkers identified from proteomic and metabolomic studies have been promising. Proteomics is the large-scale study of proteins from organisms, tissues, and cells, and this approach provides an opportunity for detecting fetuses at risk of sepsis (29). Metabolomics is the study of the metabolic profile of an individual, and increases in metabolites can be seen in septic patients, thereby carrying with it a diagnostic potential (30,31). On the basis of proteomic analyses, a mass-restricted scoring strategy has been devised using relevant proteomic biomarkers. These measurements of amniotic fluid have provided information regarding the fetal response to intra-amniotic inflammation, and have successfully predicted EOS with $>92\%$ accuracy (32,33). Altered protein expression patterns have been identified in proteomic analysis of cord blood. A proteomics approach identified biomarkers that were validated in a prospective cohort study and identified two promising biomarkers in proapolipoprotein CII and a des-arginine variant of serum amyloid A (34). When infants were stratified by risk category based on a score computed using these two concentrations, these markers were effective in directing antimicrobial management decisions and excluded sepsis with 100% negative predictive value (34).

Although not specifically studied in neonatal sepsis, the use of metabolomics and nuclear magnetic resonance imaging or mass spectrometry to measure concentrations of metabolites has demonstrated significantly higher concentrations of acylcarnitines and glycerophosphatidylcholines in adult sepsis patients compared with those with systemic inflammatory response syndrome. This approach may have value in differentiating infectious from non-infectious systemic inflammation (35). The ability to use nuclear magnetic resonance imaging may be limited to the critically ill neonate who is too unstable to leave the NICU. Furthermore, the urinary metabolomics profile of adult pneumococcal pneumonia has been found to be distinctly different from the profiles for viral and other bacterial forms of pneumonia, with the concentrations of 27/61 metabolites significantly increased and 6/61 significantly decreased in *S. pneumoniae*-infected subjects (36). This indicates that evaluation of urinary metabolite profiles may be useful for effective diagnosis of community-acquired pneumonia, and that this can be extrapolated to aid management decisions for other infectious processes, including neonatal sepsis.

However, these techniques are limited by the inability to definitively distinguish whether the proteins identified correlate with sepsis, in addition to the difficulty of detecting low levels in the blood (29). This limitation is reflected in one study that explored the serum proteome and metabolome longitudinally in 10 preterm infants with necrotizing enterocolitis (NEC) or LOS that was matched to 9 controls. Four hundred and forty-seven unique proteins and 24,153 metabolites were detected (37). The result of the proteomic and metabolomics profiles of serum identified eight proteins that were associated with NEC and four that were associated with LOS. However, no single protein or metabolite was detected in all cases, which was absent from controls (37). As these approaches can rapidly screen and identify potential markers to aid diagnosis, future research should be focused on independently and prospectively validating markers in larger sample cohorts (35).

Identification of Sepsis Through Gene Expression Profiling

Gene expression microarray-based testing has been evaluated in two neonatal studies. The first had high sensitivity (95%) and specificity (60%), which could be used to reduce inappropriate antimicrobial usage (38). When compared with the culture, RNA biosignatures among 279 febrile and 19 afebrile infants ≤ 60 days attending the Emergency Department, 10 classifier genes distinguished bacteremic infants with 94% sensitivity and 95% specificity (39). Evaluation of gene expression profiling has been shown in pediatric studies to predict sepsis (40), and clinically relevant phenotypes have been identified within subclasses of pediatric septic shock (41). Repression of genes corresponding to zinc-related biology has been linked to patients with septic shock (42). A prospective observational double-cohort study comparing genome-wide expression profiles of 17 very low birth weight infants with bacterial sepsis with 19 matched controls identified distinct clusters of gene expression patterns among those with Gram-positive versus Gram-negative sepsis, and controls (43). Although a gene-expression-based classification method has been developed that could potentially be used to make therapeutic decisions in the clinical setting (44), this has yet to be utilized prospectively in the neonatal setting.

Molecular Diagnostics

Molecular pathogen detection methods predominantly evaluated in neonatal studies have used amplification methods such as PCR, rather than hybridization-based methods or

mass spectrometry techniques. Molecular diagnostics carry the promise of more rapid and sensitive results, particularly, as molecular assays can be completed in under 12 h, and would be of utility in settings where there is pretreatment with antimicrobials, where low-density bacteremia occurs and culture-negative sepsis is common. Of the various amplification methods studied, broad-range conventional and real-time PCR have both been documented to provide a higher diagnostic sensitivity and specificity compared with other assays, although other methods have been insufficiently studied (45). Universal PCR, identifying conserved regions of the 16S ribosomal RNA gene common to all bacteria, has been an area of interest for researchers. As bacterial viability is not a necessary requirement, this approach has the potential to detect pathogens in patients with previous antimicrobial exposure; unfortunately, this has not been consistently demonstrated (46). One study that prospectively evaluated several hundred neonates before and after starting antimicrobial therapy found that several neonates were culture-negative but PCR-positive at the onset of antimicrobial therapy, and remained PCR-positive 12 h into therapy, although none remained PCR-positive after 24 h of therapy (46).

As PCR has increased the sensitivity in detecting bacterial DNA in cases of suspected neonatal sepsis, leading to rapid detection of pathogens and subsequently short antimicrobial courses, the discordance between conventional culture and PCR results has led to continued uncertainty about the bacterial etiology of sepsis using this methodology. In one study, blood cultures in over 700 neonates were positive in 13.5%, whereas PCR in this group was positive in 17.4%, leading to a sensitivity and negative predictive value both of 100% (47). However, another study evaluated 48 infants and detected bacterial DNA by PCR in 10 of them, missed 2 of the 6 specimens positive on culture, with a resulting sensitivity of 67% and negative predictive value of 75% (48). Hence, although molecular diagnostics provided a more sensitive method to identify a pathogen, the increased detection was not significant, and it was not the basis for the immediate clinical management, which was informed by the neonate's irritability, feeding difficulty and a marked rise in CRP (48). False positives can be seen when a high cycle threshold is used in a PCR reaction, thereby opening the possibility for nonspecific amplification and low reproducibility (49). In addition, presence of PCR inhibitors, DNA degradation, or low DNA quality can also contribute to erroneous results (49).

Furthermore, studies evaluating molecular diagnostics varied in the extraction methodologies, the processing utilized to break down cell wall structures and the type of PCR used. Sensitivity of molecular assays is dependent on the yield of DNA from the extraction process and the presence of inhibitors, and low sensitivity has been noted in studies where DNA was extracted from whole blood, whereas high sensitivity was noted in studies where specimens were preincubated before PCR processing (50). The quality of the molecular assay is also affected by contamination, either through other organisms or within the laboratory environment, and in situations of low-level bacteremia (51). A systematic review and meta-analysis of molecular assays in the diagnosis of neonatal sepsis concluded that molecular diagnostics had value as adjunctive tests with an overall sensitivity of 90% and specificity of 96% (45). However, these estimates would mean that two cases of sepsis in a cohort of 1,000 neonates would be missed, and 39 patients without sepsis would be unnecessarily treated (45). The overall sensitivity and specificity would arguably not be regarded as sufficient to replace the currently available diagnostics.

Conversely, concern has also been raised that molecular diagnostics may be too sensitive. Prospective studies that utilized PCR for the diagnosis of neonatal sepsis found that despite the increased sensitivity and rapid detection of the pathogens that this allowed, uncertainty about whether the bacteria present was the cause for the sepsis symptoms in a specific patient was not reduced (48). A study evaluating 48 infants with suspected and culture-proven sepsis revealed that the combination of PCR with blood culture identified bacteria in only 35% of patients diagnosed with sepsis (48). As advances in molecular diagnostics clearly hold promise, studies need to evaluate the impact of using this diagnostic approach on clinical management and outcomes, before it can be adopted widely.

Newer molecular platforms using mass spectrometry such as matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) have identified pathogens. As this process remains dependent on culture, and hence some delay is unavoidable, these spectrometers can shorten the diagnostic process to within 30 min of a positive identification of a blood culture (52). An additional benefit of this technology is that it can detect antimicrobial resistance. Newer developments have included broad-range PCR amplification with electrospray ionization mass spectrometry (PCR/ESI-MS) that enables diagnosis directly from a specimen without need for culture (53).

FUTURE DIRECTIONS

The goal for developing diagnostics for use by providers and patients to improve clinical outcomes must align with broader societal goals of enhancing data on the etiologies of neonatal sepsis, particularly in low- and middle-income countries where GBS diagnosis has been surprisingly absent (54), as well as in preventing the emergence of antimicrobial resistance by limiting excessive and unnecessary antimicrobial use.

The focus of future research should be on identifying, developing and refining rapid, sensitive and specific diagnostic tools that reliably screen for and identify all pathogens relevant in neonatal sepsis, regardless of prior antimicrobial exposure and not limited by small blood volumes obtained. Novel techniques such as analysis of volatile organic compounds in the breath has been demonstrated to be reasonably sensitive and specific (55) and capable of distinguishing sepsis from inflammation in rat models (56),

but they have yet to be validated in human studies. Expression of damage-associated molecular pattern (DAMP) molecules has been noted to be significantly increased at sites of tissue damage in animal models, indicating that these molecules may be important mediators of cellular injury (57). However, many of the technological concepts and approaches to diagnosis are still years away from deployment into bedside practice. Additional steps are required to move to a point-of-care testing in the clinical realm, including the standardization of technologies and various platforms, the demonstration of clinical benefit either in management process or on patient outcome, the improvement of workflow and efficiency, and demonstrated cost-effectiveness. New biomarkers will need to be independently and prospectively validated, ideally enrolling large cohorts in multicenter studies. Optimization of molecular assays that do not require growth of the organism on culture, as well as the development of assays that additionally evaluate for pathogen virulence and antimicrobial resistance will be a contribution.

There is also growing recognition that improvement of diagnostics exists within a larger framework that is composed of a number of stakeholders, from funding and regulatory bodies, public health agencies, diagnostics industry, healthcare systems, professional societies, and to individual clinicians (58). These interested parties all need to work in a coordinated fashion to facilitate innovative approaches to the diagnosis of neonatal sepsis and implementation of algorithms to manage neonates with suspected sepsis.

CONCLUSION

Overall approaches to neonatal sepsis management will incorporate a combination of measures beyond technology, including clinical suspicion, coordination between laboratory staff and healthcare providers, robust infection control, and strong antimicrobial stewardship (59,60). Microbiological diagnosis for neonatal sepsis has historically relied on culture; however, novel diagnostic tools—from biomarkers to molecular diagnostics—offer promise for more rapid and sensitive detection of disease. It may be that a combination of a host response biomarker (such as that derived from gene expression, proteome, and/or metabolome) plus an advanced molecular tool could prove to be the ultimate diagnostic combination (61), which could confirm that a detected pathogen is truly eliciting a host response suggestive of sepsis. Until such a combination has been made available, the evaluation and workup of a neonate with suspected sepsis should include consideration of the patient's risk factors combined with a comprehensive physical exam. Any workup should include careful thought regarding the diagnostic sensitivity and specificity, and positive and negative predictive value of the test used. Better understanding of the predictive value of the diagnostic tools will also aid providers in making a more informed interpretation of the results to guide management. The potential ability to incorporate antimicrobial resistance gene testing in addition to pathogen identification may provide a venue to fully transition to a molecular platform, and form part of a larger program of neonatal care.

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