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Rapid detection and ruling out of neonatal sepsis by PCR coupled with Electrospray Ionization Mass Spectrometry (PCR/ESI-MS)

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ABSTRACT

Background: Sepsis is an important cause of morbidity and mortality in neonates and clinicians are typically required to administer empiric antibiotics while waiting for blood culture results. However, prolonged and inappropriate use of antibiotics is associated with various complications and adverse events. Better tools to rapidly rule out bacterial infections are therefore needed.
Aims: We aimed to assess the negative predictive value of PCR coupled with Electrospray Ionization Mass Spectrometry (PCR/ESI-MS) compared to conventional blood cultures in neonatal sepsis.
Study design: Prospective observational study.
Subjects: All consecutive neonates (<28 days old) with clinical suspicion of sepsis. Samples for PCR/ESI-MS analysis were collected at the same time as samples for the blood culture, before the initiation of antibiotics.
Outcome measures: Our primary objective was to evaluate the negative predictive value of PCR/ESI-MS for the detection of bacteria in the bloodstream of newborns with suspected sepsis. Our secondary objective was the evaluation of the sensitivity, specificity and positive predictive value of the PCR/ESI-MS in such a neonatal population.
Results: We analysed 114 samples over 14 months. The median age and weight were 32 weeks + 3 days and 1840 g, respectively. Two patients had negative PCR/ESI-MS results, but positive blood cultures. Overall, the negative predictive value was 98% (95%CI: 92% to 100%).
Conclusions: Based on these results, PCR/ESI-MS analysis of blood samples of neonates with suspected sepsis appears to have a very good negative predictive value when compared to blood cultures as gold standard. This novel test might allow for early reassessment of the need for antibiotics.

1. Introduction

Sepsis is an important cause of morbidity and mortality in neonates, especially in premature infants [1]. A rapid and accurate diagnosis is essential to prevent severe and life-threatening complications. However, diagnosis is often difficult in routine clinical practice. Biomarkers are also not sufficiently specific [2,3]. As a result, clinicians frequently administer empiric antibiotics to symptomatic or "at risk" infants while waiting for blood culture results, which are currently the gold standard to identify bloodstream infection. Experts agree in stating that blood culture, despite being the gold standard in the microbiological diagnosis of neonatal sepsis, suffers from multiple disadvantages [4,5]. Amongst other disadvantages, blood cultures require a substantial amount of blood to reach adequate sensitivity and the one to 2 mL blood required are often difficult to obtain in very small premature infants. Furthermore, blood cultures are very "time expensive" as results are available several days after clinical suspicion. During this time clinicians continue empirical antibiotics in neonates who may not be infected.

On the other hand, prolonged and inappropriate use of antibiotics is associated with various and well documented complications and adverse events: [6,7] increased risk of death, necrotizing enterocolitis [8], late onset sepsis [9], alteration of gut colonization, increased risk of *Candida* colonization and subsequent invasive candidiasis [10], and increased bacterial antibiotic resistance [11]. Therefore, it is mandatory

Abbreviations: PCR/ESI-MS, PCR coupled with Electrospray Ionization Mass Spectrometry; NICU, Neonatal Intensive Care Unit; CBC, complete blood count; CRP, C-reactive protein; IQR, interquartile range; NPV, negative predictive value; PPV, positive predictive value; CI, confidence interval; PDA, patent ductus arteriosus; NEC, necrotizing enterocolitis; CPAP, continuous positive airway pressure.

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not only to rapidly treat a suspected neonatal sepsis, but also to rule out the infection as soon as possible, in order to stop unnecessary antibiotics.

Molecular methods have the potential to overcome many limitation of blood culture [5].

PCR/ESI-MS (IRIDICA®, Abbott Laboratories, Abbott Park, IL, USA) is a novel culture-independent technology coupling broad amplification by PCR and detection of the PCR-amplified products by electrospray ionization mass spectroscopy (ESI-MS). PCR/ESI-MS can detect over 600 different species of bacteria in a single assay, and in approximately 6 h. The concentration of pathogens in neonatal bacteraemia appears to be highly variable: despite many organisms occur in high concentrations, low-density bacteraemia is also recorded for most pathogens [4, 12]. Therefore, one could hypothesize that DNA analysis could be more sensitive than standard blood cultures, thanks to the detection of circulating DNA from dead or non-growing bacteria.

Our primary objective was to evaluate the negative predictive value of PCR/ESI-MS for the detection of bacteria in the bloodstream of newborns with suspected sepsis. Our secondary objective was the evaluation of the sensitivity, specificity and positive predictive value of the PCR/ESI-MS in such a neonatal population.

2. Patients and methods

This prospective observational study was conducted in the Neonatal Intensive Care Unit (NICU) at the University Hospitals of Geneva, a tertiary perinatal centre in Switzerland. The study was approved by the local ethics committee. Informed written consent was obtained from the parents before inclusion.

2.1. Patient inclusion and exclusion criteria

All newborns <28 days-old admitted in our NICU were eligible. Patients were included if the treating physicians diagnosed a suspected sepsis and intended to treat it with antibiotics. Neonates were excluded if blood sample for blood cultures and/or PCR/ESI-MS were impossible to obtain, or if their parents declined consent. Neonates were also excluded a posteriori if the PCR/ESI-MS blood sample was not analysable (because blood sample not available).

2.2. Sepsis evaluation, specimen collection and processing

As per standard procedure, we measured complete blood count (CBC) and C-reactive protein (CRP) for all infants with suspected sepsis.

A venous or arterial blood culture was collected (a minimum of 0.5 ml EDTA whole blood) through a central line or a peripheral blood draw, and analysed by a Bactec Peds PlusF system (Becton Dickinson Diagnostics system, Sparks, MD). Results of these routine tests were transmitted to the medical team and decision regarding antibiotic treatment was left to the discretion of the physician in charge of the patient.

Simultaneously, from the same blood draw, all included patients also had another 0.2 to 0.5 mL of blood sampled in an EDTA tube. Specimens were stored at 4 °C in the unit within 30 min of collection, then transported to the central laboratory, where samples were kept at −20 °C until analysis by batch was performed.

The principle of PCR/ESI-MS (IRIDICA®, Abbott Laboratories, Abbott Park, IL, USA) can be summarized as follow: multiple pairs of primers are used to amplify carefully selected regions of the genome of the microorganisms of interest (called amplicons). Following amplification, a fully automated mass spectroscopy analysis is performed weighting such amplicons. The nucleotide composition is then deduced for each amplicon present and compared with a database, allowing for the identification of the microorganism, by relying on the analysis of several amplicons. As a result PCR/ESI-MS can detect >600 pathogens in a single assay, and in approximately 6 h. It can also identify three classes of antibiotics resistance markers (mecA, vanA/vanB and KPC). A full description of the method is reported by Ecker et al. [13]. PCR/ESI-MS also provides levels, which are semi-quantitative measurements of pathogen DNA in samples. Currently, there are no data regarding interpretation of PCR/ESI-MS levels, but higher levels may indicate higher pathogen load.

The medical team was not informed of the results of the PCR/ESI-MS analysis.

2.3. Clinical data collection

The following baseline data were collected: patient demographics (gestational age, sex, birth weight, day of life at inclusion), infectious risk factors (e.g., maternal group B *Streptococcus* carriage, maternal chorioamnionitis, prolonged membrane rupture of >18 h, neonatal central lines, mechanical ventilation) and major comorbidities (necrotizing enterocolitis, patent ductus arteriosus, intraventricular haemorrhage, malformations, or previous surgery).

Clinical and laboratory data were also recorded: vital signs, hyperthermia (defined as at least one central temperature measure > 38 °C), hypothermia (defined as at least one central temperature measure < 36 °C), and thermal instability (defined as the variation of environmental temperature > 1 °C to maintain constant skin temperature), recrudescence of apnea-bradycardia-desaturation episodes (defined as >50% of baseline), prolonged refill time (>3 s), blood gas parameters (acidosis defined as pH < 7.3, BE < −4, lactates >2 mmol/L), and laboratory inflammatory markers (CRP, white blood cells count, neutrophil and platelet count).

Finally, outcome data included death, necrotizing enterocolitis, invasive candidiasis, and renal failure (defined as diuresis < 0.5 ml/kg/h and increased plasmatic creatinine) <28 days after inclusion.

The follow-up period was for 28 days after inclusion.

2.4. Statistical analysis

Results are presented as median and interquartile range (IQR) or number and proportion. Results obtained with the PCR/ESI-MS technology for each specimen were compared with those obtained using conventional microbiology methods (blood cultures) for the same sample.

We defined a true negative result as negative blood cultures at 72 h after incubation AND negative PCR/ESI-MS results. We defined a false negative result as positive blood cultures within 72 h after incubation AND negative PCR/ESI-MS result.

We defined a true positive result as positive blood cultures AND positive PCR/ESI-MS results. We defined a false positive result as positive PCR/ESI-MS results AND negative blood culture at 72 h after incubation.

The choice of 72 h was made with the intention to be the closest to our clinical setting. Indeed, in our center, clinicians usually stop antibiotics after 72 h if the blood culture remains negative. This strategy is based on a study that showed that a 3-day incubation period is sufficient to detect all clinically important blood culture isolates using the automated system [14]. However, all blood samples were indeed kept for culture for 5 days, and the information regarding possible late positive results were available to us (data not shown).

Negative predictive value (NPV) was calculated by dividing the true negative / (true negative + false negative).

In 2012, 289 blood cultures were obtained in neonates <28-days old in our center (NICU, pediatric intensive care unit, emergency room, general pediatric wards), for 209 distinct episodes. Among these, 22 (11%) yielded one or more bacteria after standard blood cultures. Our objective was to achieve a lower margin of the NPV 95%CI ≥ 95%, therefore, we calculated a sample size of 111 patients if the proportion of false positive was only 1% and the prevalence of negative results in our population was 89%.