TITLE: Development of a molecular marker for diagnosis and monitoring of neonatal bacterial sepsis.

AUTORS: Inês Stranieri 1, Kelly Aparecida Kanunfre 2, Thelma Suely Okay 3, Patricia Palmeira 4 , e Maria Isabel Santos Valdomir 5.

INSTITUTION: 1 Laboratory of Pathology, Division of Microbiology, Julio Muller University Hospital, Federal University of Mato Grosso, Cuiaba ´, Brazil. **2 e 3** Laboratory of Seroepidemiology and Immunobiology, Institute of Tropical Medicine, University of São Paulo, SP, Brazil. **4** Laboratory of Medical Investigation (LIM-36), Children's Institute, Clinical Hospital, School of Medicine, University of São Paulo, SP, Brazil. **5** Department of Pediatrics, Federal University of Mato Grosso (UFMT), Cuiaba ´, Brazil,

ABSTRACT

Bacterial sepsis constitutes one of the most frequent causes of neonatal deaths and its diagnosis is difficult due to the lack of a definitive laboratorial approach. The present study developed a bacterial 16S rDNA-based quantitative real time polymerase chain reaction (qPCR) both to the diagnosis of neonatal sepsis and to evaluate if qPCR is capable of monitoring antimicrobial treatment. For enrollment, the newborn (NB) should present, at least, two signs/symptoms suggestive of sepsis, and two abnormal laboratory parameters. Blood samples were collected on day zero (suspected sepsis), 48 hours and 7 days after the initiation of antibiotic therapy. Seventy-three newborns with suspected sepsis were recruited (21 term NB and 52 preterm NB), blood culture was positive in 32 (43.8% - confirmed sepsis) and negative in 41 (56.2% - clinical sepsis), while qPCR was positive in 65 (89.0%) and negative in 8 cases (11.0%). Considering the group of 32 NB with confirmed sepsis (11 TNB and 21PTNB), qPCR was positive in 30 (30/32 - 93.7%). Neutrophilia was found in 22 NB (68.75%), elevated CRP in 21 (65.62%), thrombocytopenia in 15 (46.87%) and leukopenia in 14 (43.75%). Of the 73 cases, taking into account the three collected samples (day zero, 48h and 7 days), 200 samples were analyzed, with 36 positive blood culture (18.0%) and 135 positive qPCR (67.5%). Of the 36 positive blood cultures, there were 38 bacterial isolations. Gram-positive bacteria were found in 32 samples (84.21%) and Gram-negative in 6 (15.78%). Coagulase-negative Staphylococcus was predominant in the Grampositive group (75.0%). In 14 cases, qPCR anticipated the diagnosis when compared with blood culture, and was positive in 22 cases on day zero (68.75%), whereas blood culture was positive in 11. Among the 41 cases of clinical sepsis, qPCR was positive in 35 (85.4%); of these 26 (74.3%) on day zero. McNemar test found discordance between the results of blood cultures and qPCR ($p<0.0001$, CI of 95%), indicating superiority of qPCR. There were nine deaths in the casuistic, all with positive blood culture and qPCR. In six of the nine deaths only the third blood culture was positive, while qPCR was positive in five cases already on day zero, and was still positive in the third sample in 6 cases. The qPCR employed the touchdown technique, with annealing temperatures decreasing from 66 to 62oC, detection threshold between 1-10 CFU/ml. Bacterial loads were generally low (<50 CFU/ml), even in those cases with confirmed sepsis and deaths, however when bacterial load medians on day zero were compared between confirmed (37.1 CFU/ml) and clinical (24.49 CFU/ml) sepsis groups, a statistically significant difference was found ($p = 0.0402$). The study concluded that qPCR can detect more cases of neonatal sepsis than blood culture, anticipating the diagnosis in most of them. Regarding the monitoring of treatment, qPCR was associated with success or treatment failure, became negative in cases that progressed favorably, remained positive in the majority of the deaths, however these data need to be confirmed.

Descriptors: Sepsis; Infant, newborn; Bacterial infections; Real Time Polymerase Chain Reaction; RNA, ribosomal, 16S.

Development Agencies: FAPEMAT, CNPQ USP, UFMT.