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Interleukin-6 ELISA in the Early Diagnosis of Neonatal Sepsis: Feasibility Within a Poor Setting in Bafoussam, Cameroon

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Abstract: Introduction: Neonatal sepsis is a leading cause of mortality in Cameroon. Diagnosis still relies heavily on the detection of C reactive protein (CRP) levels, whereas other biomarkers like interleukin 6 (IL-6), could improve the early diagnosis of neonatal sepsis in comparison to CRP. This study aimed to assess the efficacy and feasibility of IL-6 ELISA as an early diagnostic tool within a Cameroonian context with the hope of its applicability in other poor income settings and in other diseases like COVID-19. Methods: We enrolled thirty-two (32) neonates equally distributed between a septic group (including infants with risk factors and clinical signs of sepsis) and a control group (infants without clinical signs of infections) in the study. We performed Full Blood Count, C-reactive protein and IL-6 ELISA on all blood samples. Thirty-five (35) medical personnel were interviewed in order to assess acceptability, practicality (cost and duration) and a limited-efficacy of IL-6 ELISA testing at the Bafoussam regional Hospital, Cameroon. Results: The mean age of participants was 2.81 days. IL-6 ELSIA showed a sensitivity, specificity, positive predictive value and negative predictive value of 56.20%, 100%, 100% and 69.56% respectively while CRP was reported to be highly specific (81.25%). Despite a longer testing time of IL-6 ELISA compared to CRP ($p = 0.0385$), the IL-6 was acceptable ($p = 0.008$), affordable ($p = 0.006$) and could be promising for use within this poor setting. Conclusion: Though we did not see a strong correlation between its levels and the apparition of disease, IL-6 ELISA testing was feasible as a highly specific marker for an early diagnosis neonatal sepsis in Bafoussam, and could acceptably be used as an early diagnostic marker for other diseases like COVID-19 within that context.

Keywords: Interleukin-6 ELISA, Early Diagnosis, Neonatal Sepsis, Feasibility

1. Introduction

Neonatal hospital mortality is still high and accounted for by three main pathologies; complications of preterm births, birth asphyxia and neonatal sepsis [1]. Neonatal sepsis is defined as a whole-body inflammatory state (systemic inflammatory response syndrome) following an infection and that occurs in the first 28 days of life. It remains a challenging issue for Clinicians worldwide. One of its most complex aspect is to determine if a newborn who is clinically unstable is truly infected [2]. Knowing that the outcome may easily be dramatic, early identification and treatment are of a

major importance. However, the diagnosis of neonatal sepsis may be difficult because clinical presentations are often nonspecific, bacterial cultures are time-consuming and other laboratory tests lack sensitivity and specificity [3]. To support the diagnosis by Clinicians, blood culture, C-reactive protein (CRP) and full blood count (FBC) are commonly performed concurrently.

Until now, blood culture has been considered the *gold standard* method of diagnosis of neonatal sepsis, which involves isolating the pathogen from blood. While this test is the most reliable, it has as drawback a long turnover time (it can take up to 7 days to obtain the results) [4]. On the other hand, CRP is one of the most widely available tests to rule-out other infections and follow the neonatal sepsis course. However, it provides limited sensitivity when determined during the early phases of the disease, especially at the initial presentation [4].

To improve the ability to accurately detect sepsis, research has been conducted assessing the use of various biomarkers. The role of procalcitonin (PCT), interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha (TNF-alpha) have also been used in establishing the diagnosis and evaluating the prognosis of neonatal sepsis [3, 5, 6]. Inflammatory markers like interleukins especially interleukin-6 (IL-6), are thought to be promising tools for an early diagnosis of sepsis because of its rapid increase after a stimulus [3, 7, 8]. Interleukin-6 is secreted by macrophages and T cells to stimulate the immune response which occurs during infection and after trauma [2]. It is a proinflammatory cytokine that triggers acute phase proteins secretion and compared to CRP, IL-6 levels peak 2 hours after the initiation of the inflammatory cascade. Numerous studies have been conducted in developed countries to demonstrate the effectiveness of IL-6 in the diagnosis of neonatal sepsis [6, 7]. However, little has been done so far in the Cameroon health care settings. Therefore, the present study was aimed at assessing the reliability of IL-6 testing and its feasibility in the early identification of neonatal sepsis at the Bafoussam hospital in Cameroon. The ultimate goal is to set a premise for its applicability in other poor income settings and in other diseases like COVID-19 for which IL-6 has shown great discriminatory potential between severe and non-severe disease.

2. Methods

2.1. Study Design and Inclusion/Exclusion Criteria

The transversal case/control study was conducted from November to December 2015 at the Bafoussam Regional Hospital's intensive care unit, West region of Cameroon. Fourthy-one (41) newborns (up to 28 days of life) whose parents gave their consent were enrolled in the study. Newborns with a presumptive diagnosis of sepsis (based on clinical grounds) as well as those without clinical signs were included in the study. Medical histories of mothers during pregnancy and on clinical signs of the neonates were taken through an inquiry form. Neonates with potential confounding factors (malformation, congenital heart anomalies, prematurity, birth Apgar score < 7) of their IL-6 plasma levels and those who already received an antibiotic treatment were not included. Also, those with mismatched clinical and biological signs were excluded.

2.2. Blood Collection and Laboratory Analysis

After enrollment in to the study, 3.5 ml of blood was collected from each newborn at the femoral vein by qualified nurses in an aseptic environment. Those 3.5 ml were dispatched in 3 different tubes (2 EDTA tubes for FBC and IL-6 analyses and 1 dry tube for CRP measurement) and the tests carried out. Full blood count was immediately performed on the first sample (first EDTA tube: 1ml blood) using the MINDRAY BC-2800 Auto Hematology Analyzer. Alongside clinical features, leukocytosis, thrombocytopenia and granulocytosis were presumptive of sepsis. The second sample (dry tube: 1ml blood) was centrifuged for 5 minutes at 3000 rpm following the laboratory standard procedure and 200ul of serum was then separated to measure plasma CRP levels by the Roche's COBAS C111 spectrophotometer. An inbuilt calibrated normal reference value of the newborn (normal value < 3.2 mg/L) was considered. In parallel, the third sample (second EDTA tube: 1.5 ml blood) was centrifuged for 15 minutes at 1000 x g as prescribed by the IL-6 kit manufacturer. Plasma was aliquoted and stored at 2-8°C within 30 minutes of collection. IL-6 assay was run weekly by a quantitative sandwich enzyme-linked immunosorbent assay (CUSABIO's Human Interleukin-6 ELISA kit - CSB-E04638h). A standard curve was drawn using the professional soft Curve Expert 1.3 and IL-6 concentrations were obtained from that curve. A cut-off value of 4.30 pg/mL was considered.

2.3. Sample Size Determination

In the absence of published data on CRP levels in neonatal sepsis in Cameroon, we planned our study based on 6 months hospital records of CRP levels of neonates (with and without fever) from the Bafoussam regional hospital. The difference in the experimental and control means was 4.0 while the standard deviation of 3.9 was recorded in the group with fever. We assumed normal distribution within each subject group and used the PS software to determine that we will need to study 16 experimental subjects and 16 control subjects to be able to reject our null hypothesis that the population means of the experimental and control groups are equal with power of 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

2.4. Statistical Considerations

The distribution of the study population (age and sex) was expressed in means and frequencies. The Pearson correlation was calculated to assess the correlation between the markers and the presence of the disease or initial diagnosis. Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 16.0. A p-value of

0.05 was considered significant. Graphpad Prism v8.2.1 was used to plot graphs for the simplistic feasibility assessments that focused on three aspects namely acceptability, practically and limited efficacy perception (using a few criteria established by Bowen *et al.,* [9].

2.5. Feasibility Assessment

Thirty-five (35) medical personnel at the Bafoussam hospital were approached during two sets of key-informant interviews performed 7 days apart during the last week of the study. For acceptability, we basically tried to understand how IL-6 testing would fit within daily-life diagnosis and management of neonatal sepsis (acceptable /unacceptable). Practicality examined predicted cost and duration of IL-6 testing (appropriate / not appropriate). Limited efficacy answered the question "Does IL-6 testing show promise of being successful within the Bafoussam regional hospital? Yes/No options for this response was a first step towards the possibility of expanding IL-6 testing to other health facilities with the goal to be able to determine the regional/national cut-off value for IL-6.

2.6. Ethics and Consent to Participate

This study was approved by the Institutional Ethical Committee of the School of Health Sciences, Yaounde, Cameroon. No: 2015/0220/CEISH/ESS/MIM of September 25, 2015. An assent form was provided to each parent prior to the enrollment of their child in the study.

3. Results

The septic group included newborns with a presumptive diagnosis of sepsis and hematological parameters suggestive of septicemia (leukopenia/cytosis, thrombopenia, granulocytosis), whereas the control group included neonates with no clinical signs and normal hematological parameters.

Out of 41 newborns enrolled, 9 were excluded because they did not meet the criteria of either group, while 32 neonates were retained in the study. The septic group had 56.25% (9/16) of males and 43.75% (6/16) of females. The sex ration male/female was 1.5. Participants had a mean age of 2.87 days with a minimum of 2 and a maximum of 5 days. The control group on the other hand was made of 37.5% (6/16) male and 62.5% (10/16) female. The sex ration male/female was 0.6. Participants had a mean age of 2.75 days with a minimum of 1 and a maximum of 6 days. The mean of age was 2.81 days.

Of the 16 newborns in the septic group, seven (7) of them had elevated CRP levels while 9 had elevated IL-6 levels. The control group also included 16 newborns, 3 presented with high CRP levels and none with high IL-6 levels (Figures 1 and 2). The Pearson correlation showed minimal correlation, though not statistically significant, between IL-6 and the initial sepsis diagnosis with r = 0.429 (*p = 0.014*) as well as between CRP (r = 0.367, p = 0.039) and initial sepsis diagnosis (Table 1).

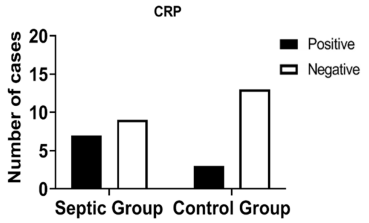

Figure 1. *Distribution of cases according to CRP.*

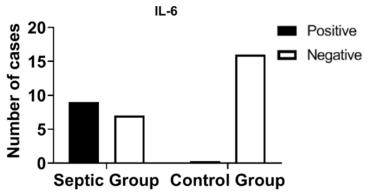

Figure 2. *Distribution of cases according to IL-6.*

Table 1. *Pearson correlations between initial Sepsis diagnosis and levels of CRP and IL-6.*

	Correlations			
	Interleukin 6		CRP	
Initial sepsis diagnosis	r	*p-value*	r	*p-value*
	0.429	0.014	0.367	0.039

Table 2 summaries the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of IL-6 and CRP relative to group's separation criteria. IL-6 has maximum sensitivity, specificity, PPV and NPV compared to CRP.

Table 2. *Comparative parameters: sensitivity, specificity, PPV and NPV.*

Tests	Sensitivity	Specificity	PPV	NPV
IL-6	56.25%	100%	100%	69.56%
CRP	43.75%	81.25%	70%	59.1%

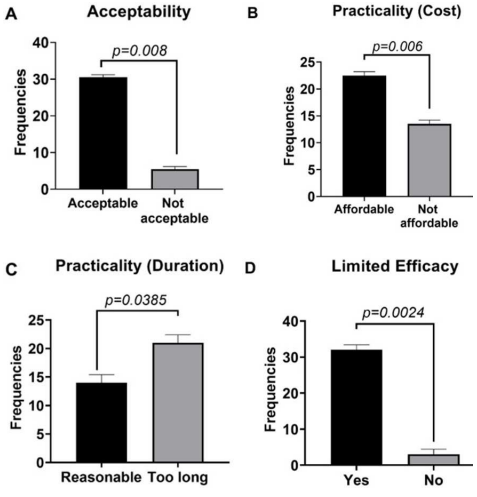

Figure 3. *Feasibility of IL-6 ELISA in Bafoussam Regional Hospital.*

Legend: Responses form 35 medical personal interviewed twice (7 days apart) during the last week of the study about IL-6 ELISA feasibility in the Bafoussam regional hospital. (A) Acceptability, (B) Practicality with respect to the cost, (C) Practically with respect to the duration of the test and (D) Limited efficacy to test if IL-6 ELISA testing could be promising within this setting. Participants' responses were compared by two-tailed unpaired t-test with Welch's correction in GraphPad Prism V8.2.1 Software. Error bars represent Standard Error of the Mean (SEM).

In order to ascertain the responses from the medical personal about the feasibility of IL-ELISA testing in the Bafoussam Regional Hospital, we interviewed each of the 35 medical personal twice (7 days apart) during the last week of the study. The results for acceptability, practicality and limited efficacy (with respect to the "Does IL-6 testing show promise of being successful within the Bafoussam regional hospital) are presented in Figure 3.

4. Discussion

IL-6 testing either singly [10-15] or in combination with other parameters such as CRP levels [16], IL-8 [10, 17], procalcitonin (PCT) [4], and TNFα [18] have been used widely to improve the accuracy of diagnosis of neonatal sepsis in many localities. However, we still do not see the deployment and use of IL-6 in many poor settings in Cameroon. Against this backdrop, we conducted this study on a population of 32 newborns to pilot the feasibility of IL-6 ELISA within such resource-poor setting. Sepsis diagnosis was based on presence of clinical signs and abnormal hematology, thus lack real gold standard. Nevertheless, when the disease state is unknown in the clinical setting, the predictive value of the tests used becomes of great importance to Clinicians because they must decide how likely it is that a positive test result reflects true disease. In the case of high-risk diseases such as neonatal sepsis, high sensitivity and predictive values are desirable because newborns who have false-negative results and are not treated could have fatal outcomes. Studies have used several methods to detect IL-6 levels including but not limited to bioassay for IL-6 measurements (which is not clinically practical), used ELISA to measure IL-6 [19, 20]. This method takes a few hours to perform and may be feasible in clinical medicine, but an automated method for clinical assessments should be developed. Because some values are missing, these data were analyzed by fitting a mixed model, rather than by repeated measures ANOVA (which can't handle missing values).

IL-6 values of positive versus negative is significantly different. The spread is quite substantial for the positive in the septic group. Note that the data table contains missing values. These mixed model results will be meaningful only if those values are missing for completely random reasons. If the reason that a value is missing is related to what value might have been, then these results are misleading.

Despite the small sample size, results for CRP sensitivity and specificity of 43.75% and 81.25% respectively, as well as NPV of 59.10% and PPV of 70% were similar to that reported by Franz *et al.,* who worked on a broader sample (1386 neonates) i.e. sensitivity, specificity, NPV and PPV of 41%, 97%, 79% and 84% respectively [17]. Contrary to our findings however, highly sensitive CRP (84.21%) but low specific (28.57%) was reported by Sonawane *et al.,* in an Indian cohort of 40 babies with sepsis [21]. They tested it at 12-24 hours of newborns lives while there is generally a delay between the onset of symptoms and the rise in serum level of CRP.

Concerning IL-6, our analysis revealed sensitivity, specificity, NPV and PPV of 56.20%, 100%, 69.54% and 100% respectively. In a study conducted in North Jordan, Khassawneh and collaborators presented higher IL-6 sensitivity, of 87% compared to our study [22]. However, they recorded specificity of 50% which is half what we report in our study, and NPV and PPV of 41.3% and 87.1% respectively, which were far lower than that obtained in this study, using a cut off value of 20 pg/mL. The difference in results might be because all newborns were included in this study without considering the presence of congenital malformation or diseases during the pregnancy. These are factors that raise CRP levels and that is why we excluded them from our work. However, we did not exclude newborns of mothers who may have exposed their babies to other stressors (alcohol and drugs use) which could have also influenced the levels of IL-6. The mean IL-6 levels in the umbilical cord blood of newborns with a history of crack/cocaine exposure during pregnancy was reported to be significantly higher that of unexposed newborns [23]. An IL-6 level in umbilical cord is elevated in the newborns suffering from early onset of sepsis (EOS) [24]. IL-6 as a biomarker shows 87-100% sensitivity with 93-100% negative predictive value [20]. IL6 has a very short half-life and hence, shows decline in sensitivity within 24-48 h [25]. IL6 levels are combined with the CRP levels in order to improve the accuracy of diagnosis [26].

The accuracy of IL-6 as a marker for late-onset infection has been determined in several studies. Chiesa *et al.,* [27] found IL-6 to be the ideal marker for the diagnosis of late onset infection (48 h), with a sensitivity and specificity of 100%. This is in contrast with the findings of Ng *et al.,* [28] and Küster *et al.,* [11] who measured IL-6 in very low birth weight infants aged 48 and 72 h, respectively, and reported fewer ideal sensitivities (Table 1). The 95% CIs for the ideal figures reported by Chiesa *et al.,* were 81 to 100% for the sensitivity and 93 to 100% for the NPV [27].

Extremely high plasma levels of IL-6 are strongly associated with a fatal outcome in COVID-19 patients as suggested by several studies. Lan *et al.,* found IL-6 levels over a certain threshold to be a powerful biomarker for disastrous outcomes in COVID-19 patients with a sensitivity of 100% and a specificity of 100% at the cut-off value of 453.85 pg/ml [29]. A meta-analysis of nine articles corroborate the importance of measuring IL-6 in COVID-19 patients but lowered the cut-off for fatal outcome at more than 80 pg/ml [30]. Similarly, Chen *et al.,* found higher IL-6 levels to be strongly associated with the COVID-19 severity [31]. Death patients showed extremely high IL-6 values in comparison to critically ill patients that in turn had IL-6 values 10 times that of severe patients. They found poor diagnosis to be associated with IL-6 levels equal or greater than 100 pg/mL [31]. IL-6 plays a major role in the activation of cytokines storms which may aggravate inflammation, impair organs functions and lead to fatal consequences. In regards to these findings, measurement of IL-6 levels may help in identifying disease progression among infected patients.

Feasibility studies are usually very broad. However, they could be down scaled to help investigators prepare for full-scale research leading to intervention. We have employed a simplistic two-response option in this study to test the acceptability, practicality with respect to the cost and the duration) as well the limited efficacy of IL-6 ELISA testing within this setting. Responses form 35 medical personal interviewed twice (7 days apart) during the last week of the study about IL-6 ELISA feasibility in the Bafoussam regional hospital (Figure 3A - D). Participants' responses were compared by two-tailed unpaired t-test with Welch's correction in GraphPad Prism V8.2.1 Software, with error bars representing Standard Error of the Mean (SEM). Responses include descriptions from basic social science (to determine the best variables to target), through methods development, to efficacy and effectiveness studies, and to dissemination research. The term *feasibility study* is used more broadly than usual to encompass any sort of study that can truly bring out any of such aspects. In this study, the feasibility of healthcare personnel to perform IL-6 measurement by ELISA (cutoff value of 20 pg/ml) rather than the usual CRP is determined within a poor setting like Bafoussam. Despite the little uptake of the bioassay for IL-6 measurements, probably due to its little clinical practicality, other investigators have used ELISA to measure IL-6 an showed its non-inferiority as an early diagnostic marker for neonatal sepsis [20, 23] and COVID-19 [30-32]. This method takes a few more hours to perform compared to CRP and may be feasible in clinical medicine even within poor settings if proper training is offered. Despite the lack of evidence of infection in the neonates, one study reported that IL-6 levels in cord plasma increased with clinical chorioamnionitis, it may not be a specific marker of infection in the newborn [33]. However, with current and growing evidence generated since the beginning of the COVID-19 pandemic, IL-6 measurement might become handy to predict severe COVID-19 infections especially in poor settings.

Limitations

The gold standard (blood culture) for sepsis diagnosis was not conducted in this study. Our classification into the study (sepsis) group or control group was based on clinical features and full blood count (FBC) reports. In fact, after collecting blood for our assays, it was unethical to collect the additional blood required for blood culture (3ml collected twice) from our study participants. Therefore, blood culture was not performed. FBC results and clinical signs were then set as our "gold standard".

5. Conclusion

Despite the poor correlation between IL-6 level and the presence of the disease, the study has shown IL-6 to be a useful marker with fair sensitivity and high predictive values compared to CRP. Hence, IL-6 use for the early diagnosis of sepsis can be considered.

However, the small sample size, the lack of an accurate diagnosis standard, and a non-local cut-off value of IL-6 limited the study. Further studies to confirm the reliability of IL-6 in the diagnosis of neonatal sepsis should be conducted in this region. Also, the assessment of others tests such as procalcitonin, immunoglobulin M and Q-PCR might revolutionize sepsis diagnosis nationally. This notwithstanding, IL-6 measurements by ELISA are acceptable, practical (with respect to the cost and duration of the test) and have an acceptable limited efficacy in Bafoussam and could be promising within this and other poor settings.

Competing Interests

The authors declare no competing interest.

Authors' Contributions

PMN and MCMN designed the study and wrote the first draft and revised subsequent drafts of the manuscript. MCMN, SDK and DK participated in the performance of the study. AHNK and IAA revised the manuscript substantially. All authors read and approved the final manuscript.

Abbreviations

CRP: C Reactive Protein; FBC: Full Blood Count; IL-6: Interleukin 6; NPV: Negative Predictive Value; PPV: Positive Predictive Value; r: Pearson correlation.

Ethics and Consents to Participate

This study was approved by the Institutional Ethical Committee of the School of Health Sciences, Yaounde, Cameroon. No: 2015/0220/CEISH/ESS/MIM of September 25, 2015. An assent form was provided to each parent prior to the enrollment of their child in the study.

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