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(RESEARCH ARTICLE)

Neonatal infections in Lubumbashi, in the Democratic Republic of Congo: Correlation between C-reactive protein (CRP), interleukin-6 (IL-6) and procalcitonin (PCT)

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Abstract

Introduction: The study focuses on Neonatal Infections in Lubumbashi, DRC , a major public health concern due to their high prevalence and impact on newborn health. Early diagnosis is crucial , but difficult due to non-specific symptoms and the dilemma of antibiotic therapy. Biological markers of inflammation, which must be early, sensitive, specific, and inexpensive , are essential to aid diagnosis. C-Reactive Protein (CRP), Interleukin-6 (IL-6), and Procalcitonin (PCT) are three markers used, each with a different kinetic of action (IL-6 very early, PCT early/specific to bacteria, CRP later/for follow-up). This research aims to assess the correlation between these three markers in newborns suspected of infection.

Materials and Methods: This was a prospective cohort study conducted at the Dee Service medical laboratory in Lubumbashi. The sample included 75 newborns (0 to 28 days) hospitalized in Lubumbashi and presenting with suspected or confirmed neonatal infection. Serum concentrations of CRP, IL-6, and PCT were measured from blood samples using immunofluorescence analyzers (SK-1000, Ichroma 2, Fine care Wondfo) with specific kits. Statistical analysis used the Pearson correlation coefficient and Student's t-test, with R Studio and Excel software.

Results: The mean serum concentrations were 46.46±37.23 mg/L for CRP, 25.61±23.72 mg/L for IL-6, and 8.01±6.28 mg/L for PCT. Correlation analysis revealed a poor correlation between CRP and PCT (*rxy*=0.57). The correlation between PCT and IL-6 was inverse (*rxy*=−0.17). Finally, the correlation between CRP and IL-6 was also poor (*rxy*=0.10).

Conclusion: The correlation coefficients suggest an overall poor correlation between CRP, IL-6, and Procalcitonin in infected newborns in Lubumbashi, with results ranging from poor to inverse. These results emphasize that, although individually useful in diagnosis, these markers have distinct induction mechanisms and kinetics. The study confirms the relevance of integrating multiple markers to optimize the diagnosis and monitoring of neonatal infections , as the inflammatory syndrome is not specific to a precise cause.

Keywords: Neonatal Infections; CRP, IL-6; Procalcitonin; Lubumbashi; Correlation

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1. Introduction

In the Democratic Republic of Congo (DRC), the management of neonatal infections is particularly important due to the high prevalence of these infections and their consequences on the health of newborns in the country. In the neonatal period, the main cause of inflammation is infection. A distinction is made between early neonatal infections (or maternal-fetal) and secondary neonatal bacterial infections. Although the implicated germs, modalities, and times of infection occurrence are different for these two entities, the crucial problem of their diagnosis remains identical(Coly et al., 2021). We distinguish between maternal-fetal infections (MFI), which can be acquired before, during, or after birth, and nosocomial infections, the occurrence of which can be early (from the first day of life) and which pose crucial problems(Chiesa et al., 2015)

The physician in charge of newborns suspected of infection faces a dilemma: delaying the start of antibiotic therapy while awaiting a definitive diagnosis can endanger the life of a truly infected newborn ; conversely, starting the same treatment at the slightest doubt often leads to unjustified antibiotic therapy , which is a source of discomfort and selection pressure on the bacterial ecology of the newborn and that of the neonatology unit. The tools usually used to establish this diagnosis of neonatal infection do not always prevent these two pitfalls(Khassawneh et al., 2007).

A biological marker, also called a biomarker, is a measurable characteristic that indicates a normal or pathological biological process. The identification of new biological markers of a disease is very important for monitoring the evolution of a disease and the effectiveness of new treatments, whether these markers are physiological (change in blood pressure, heart rate, etc.) or molecular (change in protein expression) .(Durand & Beaudeux, 2011).

These markers must meet several requirements to be considered interesting and usable in neonatology: increase early ; be specific (identification of newborns free of infection) ; be sensitive (identification of infected newborns) ; have high negative predictive values (probability of being free of infection when the marker's assay value is below the threshold chosen for diagnosis) and positive predictive values (probability of being infected when the marker is above the chosen threshold) ; benefit from a rapid, reliable, and inexpensive assay technique(Granner, 2003).

Among the biochemical markers of the inflammatory syndrome, a distinction must be made between tests that provide useful information for diagnosis (sedimentation rate, complete blood count, serum protein electrophoresis, inflammation proteins) and tests reserved for clinical research protocols that are not yet validated (assay of eicosanoids, cytokines, and chemokines, etc.) (Magny et al., 2000).

The concentrations of inflammation reaction proteins (IRPs) vary during this IR. Some are called positive IRPs because their synthesis is stimulated by cytokines such as IL-1, IL-6, and TNF-α. Others are called negative IRPs because their catabolism is greater than their synthesis. Their assay is performed by immunonephelometry. Among these proteins whose concentration varies during the IR, a small number can be used as biochemical markers of inflammation. Indeed, the latter must present a particular kinetic (rapid, intermediate, or slow) , their concentration variation must be proportional to the degree of the IR , and their assay must be precise, rapid, and standardizable(Magny et al., 2000).

C-Reactive Protein (CRP) is one of the acute phase proteins of inflammation whose level increases in serum or plasma during a reaction to infections or non-infectious inflammatory events. Normal CRP values are less than 5 mg/l. This threshold is often exceeded within four to eight hours after an acute inflammatory event , with CRP values that can reach approximately 20 to 500 mg/l , and the increase is on average significantly greater during bacterial infections than during viral infections. Since high values are always associated with pathological changes, CRP assay provides useful information for the diagnosis, therapy, and monitoring of inflammatory diseases(Alima Yanda et al., 2015).

Procalcitonin (PCT) is a biochemical marker specific to bacterial infection , but a serum PCT concentration <0.25 ng/ml does not exclude a local bacterial infection. A value >2 ng/ml is strongly suggestive of a septic state. PCT assay is therefore an emergency test ; moreover, the serum PCT concentration is higher the more severe the infection is (Rapid kinetic). CRP and procalcitonin have different induction mechanisms in response to bacterial infection, suggesting that they might present complementary expression profiles(Chemsi et al., 2012)..

IL-6 is a cytokine involved with IL-1 beta and tumor necrosis factor in the acute phase of inflammation. IL-6 is a key cytokine in the regulation of acute and chronic inflammation and plays a messenger role between the cells involved in this process. The interest of Interleukin 6 assay lies in the fact that it is a pleiotropic molecule with a rapid response and is therefore capable of being used as a rapid diagnostic tool. IL-6 is a very early marker of inflammation. Its level rises 1-2 hours after the start of inflammation but normalizes within 24 hours maximum. It is a fleeting marker ; however, the CRP-IL6 pair constitutes a more sensitive marker of inflammation.(Mihara et al., 2012)

This study aims to evaluate the correlation of CRP, IL-6, and procalcitonin. More specifically, it seeks to: Determine the serum concentrations of CRP, IL-6, and Procalcitonin. Evaluate the correlation between CRP, IL-6, and Procalcitonin during neonatal infections.

2. Materials and Methods

2.1. Research Setting

The Dee Service medical laboratory in Lubumbashi was selected as the experimental site for this study. The choice of this location is based on several geographical and environmental factors. Kambove is located at approximately −10.87◦ South latitude and 26.60◦ East longitude, with an average altitude of 1,386 meters. The city is located in the south of the DRC, in the Haut-Katanga province. It is located about 147 km northwest of Lubumbashi, the provincial capital. By choosing the Dee Service medical laboratory in Lubumbashi, both the geographical and environmental characteristics of the region were taken into account. These factors contribute to the relevance and representativeness of the results obtained within the framework of this scientific study. (Guellord et al., 2021)

2.2. Material

- Immunofluorescence Analyzer SK-1000
- Immunofluorescence Analyzer Ichroma 2
- Immunofluorescence Analyzer Fine care Wondfo
- EDTA tubes
- Racks
- Syringes
- Refrigerator
- Centrifuge
- Pipettes of 10-100 μl and 100-1000 μl
- Cuvettes
- Tips
- Cotton wool
- Tourniquet
- Test tubes
- CRP Kit
- PCT Kit
- IL-6 Kit

2.3. Study Subjects

A Prospective Cohort Study was conducted. The sample size was 75 newborns , including 42 female and 33 male subjects, aged between 0 and 28 days. The selection of study subjects was performed randomly without distinction of race, tribe, or social class among newborns in Lubumbashi. The results obtained were recorded and statistically analyzed to evaluate the correlation of the parameters under study. Data analysis was performed using specialized software such as R Studio and Excel , considering confidence intervals and appropriate statistical significance thresholds(Bouyer, 2017).

2.4. Inclusion and Exclusion Criteria

2.4.1. Inclusion Criteria:

- Newborns whose parents provided free and informed consent.
- Newborns hospitalized in Lubumbashi presenting with suspected neonatal infection (fever, leucopenia, respiratory distress, etc.).
- Newborns aged between 0 and 28 days.
- Newborns whose clinical or biological diagnoses of neonatal infection are suspected or confirmed.

2.4.2. Exclusion Criteria:

- Newborns whose parents did not provide free and informed consent.
- Newborns who received antibiotic treatment before sample collection.

- Newborns presenting with a severe non-infectious congenital disease (e.g., heart disease, cerebral malformation).
- Newborns who died before the necessary blood samples for the study were taken.

2.5. Sampling and Sample Processing

Blood collection was carried out in the morning between 8:00 AM and 10:00 AM in the study subjects. The blood was collected in test tubes without anticoagulant. The samples were centrifuged at 2,500 revolutions per minute for 10 minutes to obtain the serum, which was collected and stored at 4◦C until the time of analysis, which was carried out on the same day.

2.6. Laboratory Analyses

- **CRP Assay Principle:** The CRP antigen in the sample combines with the fluor-labeled monoclonal CRP antibody conjugate , then continues to move and combine with another monoclonal CRP antibody fixed on the nitrocellulose membrane to form a double-antibody sandwich immune complex at the detection line of the nitrocellulose membrane(Grandjean et al., 2006). Quantitative detection results are obtained by dry assay.(Bossuyt & Boeynaems, 2001a).
- **Procalcitonin (PCT) Assay Principle:** The Finecare PCT quantitative test is based on fluorescence. The Finecare PCT rapid quantitative test uses a sandwich immunodetection method. When the sample is added to the sample well of the test cartridge, the fluorescence-labeled PCT antibody detector on the sample pad binds to the PCT antigens in the blood samples and forms immune complexes(Hausfater, 2007). As the complexes migrate on the nitrocellulose matrix of the test strip by capillary action, the detector antibody and PCT complexes are captured by immobilized PCT antibodies on a test strip. Thus, the more PCT antigens in the blood sample, the more complexes accumulate on the test strip. The fluorescence signal intensity of the detector antibodies reflects the amount of captured PCT antibodies(Bossuyt & Boeynaems, 2001a).
- **IL-6 Assay Principle:** The test uses a sandwich-type immunodetection method. Detector antibodies present in the buffer combine with sample antigens, forming antigen-antibody complexes , and migrate to the nitrocellulose matrix to be captured by another streptavidin immobilized on a test strip. More antigens in the sample will form more antigen-antibody complexes, leading to a more intense fluorescence signal from the detector antibodies, which is processed by the ichroma probe instrument to indicate the concentration of IL6 in the sample. (Bossuyt & Boeynaems, 2001b)
- **Kit Validation:** Kit validation is generally documented by manufacturers by demonstrating good correlation with standard laboratory methods (CLIA, ELFA reference methods, or other quantitative latex agglutination tests) for the same analytes. These kits generally have the CE marking for *in vitro* diagnostic (IVD) use, certifying that they comply with European regulatory requirements for health and safety. Precision is measured by repeatability (intra-series precision) and fidelity (inter-series precision). The manufacturer provides data on the Coefficient of Variation (CV%) which should be low in the measuring range to confirm the reliability of the results. (Giannoli et al., 2019).
- **Integrated Cartridge Control:** Each test cartridge contains an integrated Internal Control (often a distinct control line) that is activated during the test. This control serves to confirm that:
    - The volume of sample added was sufficient.
    - Reagent migration on the cartridge membrane occurred correctly.
    - The reading device read the cartridge correctly.
- **Analytical Sensitivity (Limit of Detection, LoD):** This is the smallest concentration of analyte that can be reliably detected.
- **Traceability:** Traceability is ensured by the ID chip system and lot numbers:
- **Identification Chip (ID Chip):** Each kit lot comes with a calibration chip. The analyzer must read this chip before using a new batch of cartridges. This chip contains information on the reagent lot number and the calibration curves specific to that lot, ensuring the reading adjustment.
- **Automatic Recording:** The analyzer automatically records the lot number information (read via the ID chip) with the patient results, ensuring the traceability of each measurement. (Pascal & Beyerle, 2006).

2.6.1. Statistical Analysis

The correlation between the concentrations of CRP, IL-6, and PCT was evaluated using the **Pearson correlation coefficient**. The correlation coefficient is always between −1 and 1. **Student's t-test** was used to determine the degree of significance(Bouyer, 2017). R Studio and Excel software were used for descriptive and inferential statistics (Kaur et al., 2018).

3. Results

Table 1 Mean serum concentrations of CRP, IL6, PCT in newborns

Parameters	Mean – standard deviation
PCT	8.01±6.28 mg/L
CRP	46.46±37.23 mg/L
IL 6	25.61±23.72 mg/L

3.1. Correlation Coefficient and Graphical Representation between CRP and PCT in Newborns

rxy=0.57 *t*=−0.57

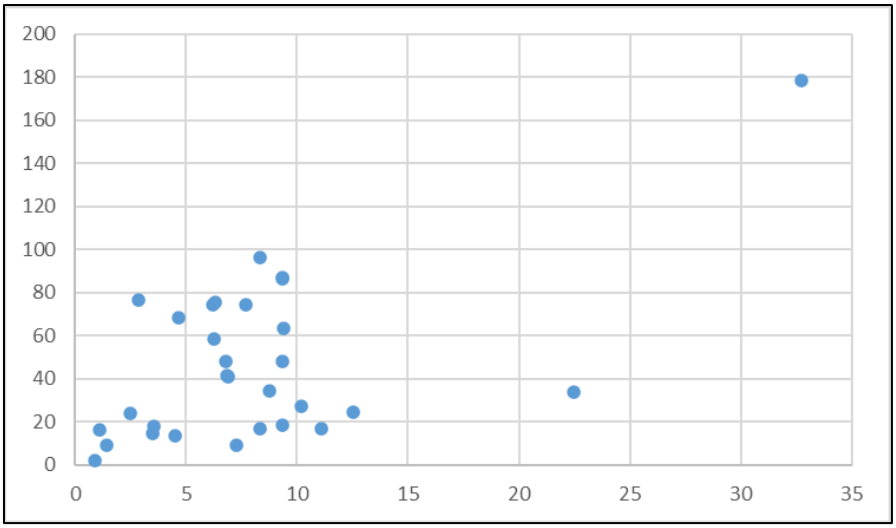

Figure 1 Graphical Representation between CRP and PCT in Newborns

3.2. Correlation Coefficient and Graphical Representation between PCT and IL 6 in Newborns

rxy=−0.17 *t*=−0.17

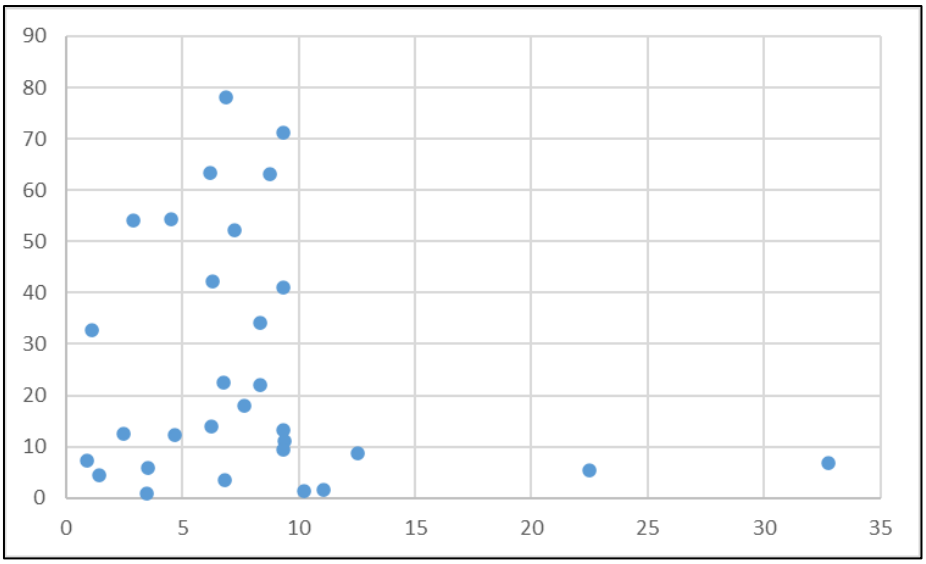

Figure 2 Graphical Representation between PCT and IL 6 in Newborns

3.3. Correlation Coefficient and Graphical Representation between CRP and IL 6 in Newborns

rxy=0.10 *t*=0.10

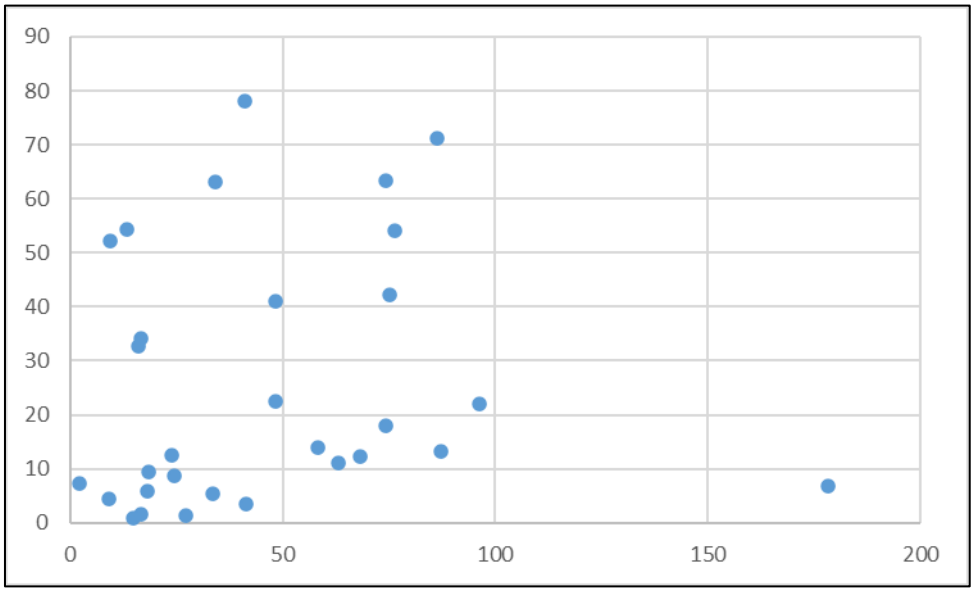

Figure 3 Representation between CRP and IL 6 in Newborns

The examination of Table 1, the calculation of the correlation coefficient, the graphical representation in the form of scatter plots, and the use of the Student's t-test led to the following observations:

The mean serum concentrations of PCT, CRP, and IL-6 are 8.01±6.28 mg/L, 46.46±37.23 mg/L, and 25.61±23.72 mg/L.

The correlation coefficient between CRP and PCT in newborns is poor (*rxy*=0.57; *t*=−0.57). The graphical representation in the form of scatter plots shows a poor correlation.

The correlation coefficient between PCT and IL-6 in newborns is inverse (*rxy*=−0.17; *t*=−0.17). The graphical representation in the form of scatter plots shows an inverse correlation.

The correlation coefficient between CRP and IL-6 in newborns is poor (*rxy*=0.10; *t*=0.10). The graphical representation in the form of scatter plots shows a poor correlation.

4. Discussion

In the Democratic Republic of Congo (DRC), the management of neonatal infections is particularly important due to the high prevalence of these infections and their consequences on the health of newborns in the country. In the neonatal period, the main cause of inflammation is infection. A distinction is made between early neonatal infections (or maternal-fetal) and secondary neonatal bacterial infections. Although the implicated germs, modalities, and times of infection occurrence are different for these two entities, the crucial problem of their diagnosis remains identical. (Magny et al., 2000)

A biological marker, also called a biomarker, is a measurable characteristic that indicates a normal or pathological biological process. The identification of new biological markers of a disease is very important for monitoring the evolution of a disease and the effectiveness of new treatments, whether these markers are physiological (change in blood pressure, heart rate, etc.) or molecular (change in protein expression) (Durand & Beaudeux, 2011).

CRP, IL-6, and PCT, taken individually, have variable diagnostic performance. CRP, for example, has slower kinetics and may be low at the beginning of infection. IL-6, although early, is often transient and less specific(Shahkar et al., 2011). PCT, although more specific for bacterial infections, can be influenced by other non-infectious factors in the newborn (respiratory distress, asphyxia, etc.). (Nouri-Merchaoui et al., 2009)

After data grouping and laboratory analysis, the results are as follows:

- The mean serum concentrations of PCT, CRP, and IL-6 are 8.01±6.28 mg/L, 46.46±37.23 mg/L, and 25.61±23.72 mg/L.
- The correlation coefficient between CRP and PCT in newborns is poor (*rxy*=0.57; *t*=−0.57). The graphical representation in the form of scatter plots shows a poor correlation.
- The correlation coefficient between PCT and IL-6 in newborns is inverse (*rxy*=−0.17; *t*=−0.17). The graphical representation in the form of scatter plots shows an inverse correlation.
- The correlation coefficient between CRP and IL-6 in newborns is poor (*rxy*=0.10; *t*=0.10). The graphical representation in the form of scatter plots shows a poor correlation.

Given the great clinical polymorphism and the long response time of bacteriological tests , biological markers of inflammation play a very important role in the early management of these bacterial infections. The inflammatory syndrome indicates the presence of an organic pathology but is not specific to any precise cause. It is a marker of the activity of many diseases(Pratt et al., 2019). Among the biochemical markers of the inflammatory syndrome, a distinction must be made between tests that provide useful information for diagnosis (sedimentation rate, complete blood count, serum protein electrophoresis, inflammation proteins) and tests reserved for clinical research protocols that are not yet validated (assay of eicosanoids, cytokines, and chemokines, etc.). (Magny et al., 2000)

Neonatal infections represent a major cause of morbidity and mortality in newborns. Early diagnosis is crucial for effective management. However, clinical signs in newborns are often non-specific. This is why the use of biological markers of inflammation is essential. (*Infections Néonatales*, 2015). Early identification of infections in newborns is crucial but often difficult due to non-specific symptoms. Inflammatory markers play an important role in aiding diagnosis. CRP, IL-6, and procalcitonin are three of these markers, each having distinct kinetic characteristics that influence their clinical utility(Alima Yanda et al., 2015).

CRP is an acute-phase protein synthesized by the liver in response to inflammation. It is one of the most commonly used markers. CRP levels generally increase 12 to 24 hours after the onset of infection. Its half-life is about 19 hours. It is very useful for monitoring the response to antibiotic treatment because its levels decrease rapidly once the infection is controlled. Its late increase may limit its use for the early diagnosis of neonatal infections, which require rapid intervention. (Aseri et al., 2014)

IL-6 is a pro-inflammatory cytokine produced by various cells in response to an infectious stimulus. It is one of the earliest markers, with levels increasing within 2 to 4 hours after infection. Its half-life is very short, only a few hours. IL-6 is an excellent marker for the early diagnosis of neonatal infections. Due to its short half-life, a single measurement may not reflect the current status of the infection, and repeated measurements may be necessary. Furthermore, it is more expensive and less widely available than CRP. (« Chapter 5 Proinflammatory Cytokines in CRP Baseline Regulation », 2009)

Procalcitonin (PCT) is the precursor of the hormone calcitonin. It is produced in large quantities by various tissues in response to systemic bacterial infection. Its levels increase 4 to 6 hours after infection, faster than CRP but slightly after IL-6. It reaches its peak around 8 to 24 hours. Its half-life is about 24 hours. PCT is particularly useful for differentiating bacterial infections from viral infections, as it is much higher during severe bacterial infections. PCT levels may be physiologically elevated during the first 48 hours of life due to the stress of birth, which can complicate interpretation(Chemsi et al., 2012).

5. Conclusion

The study conducted at the Dee Service Medical Laboratory in Lubumbashi, which was a prospective cohort study involving 75 newborns aged 0 to 28 days with suspected or confirmed neonatal infection , allowed the determination of the mean serum concentrations of the three biomarkers studied.

The mean serum concentrations observed were:

- Procalcitonin (PCT): 8.01±6.28 mg/L
- C-Reactive Protein (CRP): 46.46±37.23 mg/L
- Interleukin-6 (IL-6): 25.61±23.72 mg/L

The correlation analysis between these biomarkers, performed using the Pearson correlation coefficient, led to the following observations regarding the links between the markers in the context of neonatal infections in Lubumbashi:

- CRP and PCT Correlation: It is judged to be poor (r=0.57). The scatter plot representation shows a poor correlation.
- PCT and IL-6 Correlation: It is judged to be inverse (r=−0.17). The scatter plot representation also shows an inverse correlation.
- CRP and IL-6 Correlation: It is judged to be poor (r=0.10). The scatter plot representation shows a poor correlation.

It was concluded that there is a **weak or inverse correlation** between CRP, IL-6, and Procalcitonin in this population of newborns in Lubumbashi , suggesting that these three markers of inflammation do not vary in a strongly synchronized or linear manner. This weak correlation can be explained by the different kinetics and specificities of each marker , as the difference in kinetics and induction mechanism between CRP and PCT had already suggested complementary expression profiles. The use of biological markers is considered essential for early diagnosis , particularly in the DRC where the prevalence of neonatal infections is high and clinical diagnosis is often non-specific.

Compliance with ethical standards

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Disclosure of conflict of interest

This work was carried out with impartiality and complete independence of mind. No conflict of interest was reported.

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Statement of ethical approval

The free and informed consent of each subject was obtained from the parents. Data confidentiality was guaranteed with the collected data being anonymized as soon as possible to protect the identity of the participants , and the selection of participants was fair, avoiding the exploitation of vulnerable groups.

Authors' Contributions

- **Arold Fazili** conceived and supervised the study, wrote the main manuscript, validated the final version, validated the data, contributed to the discussion, and gave final approval for the version to be submitted.
- **Cynthia BUTEKA** conceived and supervised the study, wrote the main manuscript, and validated the final version.
- **Victoire KAPINGA** conceived and supervised the study, wrote the main manuscript, and validated the final version.
- **Gloire MPANGA** conceived and supervised the study, wrote the main manuscript, and validated the final version.
- **Armand ABASI** participated in data collection, statistical analysis, and contributed to the interpretation of the results and the critical review of the manuscript.
- **Grégoire MULIMBI** ensured the bibliographic review and participated in the document formatting.
- All authors contributed to the interpretation of the results and the critical review of the manuscript.
- All authors have read and approved the final version of the manuscript.

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