Performance of 11 host biomarkers alone or in combination in the diagnosis of late-onset sepsis in hospitalized neonates: the prospective EMERAUDE study

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Performance of 11 host biomarkers alone or in combination in the diagnosis of late-onset sepsis in hospitalized neonates: the prospective EMERAUDE study

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Abstract

Background: Despite the high prevalence of late-onset sepsis (LOS) in neonatal intensive care units (NICUs), a reliable diagnosis remains difficult. The time needed to obtain laboratory results of biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) and blood culture explains why an unjustified antibiotic use is observed in numerous hospitalized neonates. This results in an increased frequency of antibiotic resistance, microbiota modification, and neonatal complications.

The objective of EMERAUDE study was to identify biomarkers (alone or in combination) to early exclude the diagnosis of LOS in neonates with suggestive clinical signs.

Methods: A prospective, multicenter cohort study (EMERAUDE)was conducted in 2 French NICUs. The participants were hospitalized neonates at ≥7 days of life with signs of suspected LOS enrolled from November 2017 to November 2020. Serum samples were collected during the venipuncture prescribed for blood culture. Eleven biomarkers were measured using customized multiplexed assays in the ELLA Automated Immunoassay System (ProteinSimple, San Jose, CA, USA) for PCT, IP-10, IL-6, IL-10, NGAL, PTX3, presepsin and LBP, and using conventional ELISA for calprotectin (R&D Systems, Minneapolis, MN, USA), gelsolin(Elabsciences, Houston, TX, USA) and IL-27(R&D Systems, Minneapolis, MN, USA). An independent adjudication committee, blind to biomarkers, assigned each patient to either infected, not infected or unclassified groups. Performances of biomarkers were assessed considering a sensitivity of at least 0.898.

Results: A total of 230 patients were analyzed. They were mainly preterm (80%) with a median gestational age of 27 weeks and a median birth weight of 940 grams. The adjudication committee classified 22% of patients (51/230) as infected and all of these received antibiotics. Among patients of the not infected group, 27% (42/153) also received antibiotics. The best biomarkers alone were IL-6, IL-10 and NGAL; the area under the curve [95%CI] was, respectively, 0.864 [0.798-0.929], 0.845 [0.777-0.914], and 0.829 [0.760-0.898]. Combinations of up to 4 biomarkers were analyzed and the best were PCT/IL-10, PTX3/NGAL, and PTX3/NGAL/gelsolin. The best models of biomarkers could avoid up to 64% of unjustified antibiotics.

Conclusions: At the onset of clinical suspicion of LOS, the dosing of additional biomarkers could help the clinician in identifying not infected patients.

Trial registration: ClinicalTrials.gov ID: NCT03299751. Registered 3 October 2017.

Background

Late-onset sepsis (LOS) is frequent in neonatal intensive care units (NICUs) especially in the most preterm and lowest birth-weight infants and can lead to life-threatening issues.[1] The diagnosis of LOS at onset is a challenge since it relies mainly on clinical signs that are not specific nor constant, including respiratory distress, temperature instability, as well as neurological or hemodynamic disorders,[2] and in this population it is difficult to differentiate signs of infection from clinical signs related to other medical conditions, especially in very low birth weight (< 1500 grams) infants. Blood culture is considered as the gold standard for the diagnosis of LOS, however the time-to-result is long (48h), in line with the time needed for culture.[3] In this context, and in the absence of a test with high negative predictive value (NPV) providing immediate results, antibiotics are frequently administered to neonates suspected of having LOS before the result of the blood culture is available in order to avoid a rapid clinical deterioration.[4] This leads to unnecessary exposure to antibiotics; for example it is reported in a Canadian NICU that 85% of very low birth

weight infants were exposed to antibiotics during their hospitalization, among whom 75% were not infected.[5] This is worrying given the negative impact of even short antibiotic exposure at the early stage of life on the gut microbiota at the time of its implementation and the associated risk of developing asthma, allergic diseases, and metabolic disorders.[6,7]

The use of biomarkers could help clinicians recognize true infections in neonates and thus decrease the prescription of unjustified antibiotics. Several studies have been published concerning the value of biomarkers in neonatal sepsis;[8] in particular, C-reactive protein (CRP) has been widely used for many years but has poor performance for the diagnosis of LOS at the onset of clinical signs, probably because of both the delay between the onset of sepsis and the rise of CRP level as well as the numerous other situations in which CRP increases.[9] This is illustrated by a recent meta-analysis that found that in an hypothetical cohort of 1000 neonates, assessing serum CRP level alone would miss 152 cases of infection (false-negative result) and wrongly diagnose 156 cases (false-positive result).[10] Furthermore, studies investigating biomarkers evaluate the performance of these in the early diagnosis of LOS, but not the ability of biomarker-based protocols to rule-out the diagnosis of LOS.[4] To avoid the prescription of antibiotics in non-infected patients, a biomarker with excellent sensitivity and negative predictive value is therefore needed. The primary objective of the present study was to identify the best combination of biomarkers or single biomarker, among 11 host biomarkers, that can exclude early-on the diagnosis of LOS in hospitalized neonates with a clinical suspicion of LOS.

Methods

Study design

A prospective multicenter cohort study, named EMERAUDE (Evaluation of bioMarkErs to Reduce Antibiotics Use in hospitalizeD nEonates), was conducted in 2 French NICUs (Hôpital Femme Mère Enfant, Hospices Civils de Lyon, Bron; Centre Hospitalier Universitaire de Nantes, Nantes) between November 19, 2017 and November 20, 2020.

Eligibility Criteria

Hospitalized neonates of ≥ 7 days of life with suggestive signs of LOS and requiring a blood culture were consecutively included. Suspected LOS was defined as the presence of any of the following criteria: fever > 38°C, tachycardia > 160 beats per minute, capillary refill time > 3 seconds, grey and/or pale skin complexion, apnea or bradycardia events, abdominal bloating, rectal bleeding, hypotonia or lethargy, seizures without other obvious cause, increased respiratory support and/or increased FiO2, cutaneous rash, inflammation at the needle-puncture site of the central venous catheter. A consent form signed by at least one parent/ legal representative was also mandatory to include the patient. Exclusion criteria were treatment with antibiotics for a bacteriologically confirmed infection during the previous 48 hours prior to inclusion as well as surgery or vaccination during the 7 days prior to inclusion. Patients with invalid inclusion criteria were excluded from the study, as well as those without analyzable blood samples.

Data Collection

The characteristics of patients at the time of inclusion and between 48 and 72 hours were collected, including demographics, medical history, disease history, physical examination, and results of the blood culture. Results of

other tests that could have been performed for routine care (chest X-ray, bacteriological samples, CRP, white blood cell count, absolute neutrophil count) were also collected, as was the decision whether or not to treat the patient with antibiotics, which was at the discretion of the physician.

Sample Collection And Biomarker Measurement

For each included patient, at the time of the venipuncture prescribed for standard care, up to 0.4 mL blood was collected in BD™ Microtainer™ Serum Separating Tubes (Becton Dickinson, Franklin Lakes, NJ, USA; reference BD365968). After 2 hours clotting at room temperature and centrifugation at 2,500 g for 10 minutes, sera were aliquoted and stored frozen at -80°C until the measurement of 11 biomarkers (procalcitonin [PCT], interferon gamma inducible protein 10 [IP-10], interleukin 6 [IL-6], interleukin 10 [IL-10], neutrophil gelatinase-associated lipocalin [NGAL], pentraxin 3 [PTX3], presepsin [CD14], lipopolysaccharide-binding protein [LBP], gelsolin, calprotectin, and interleukin-27 [IL-27]), as detailed in the Additional file 1 Methods. The selection of these biomarkers was based on the results of previous studies about their value in the context, as well as the absence of variation related to gestational or postnatal age, and an increase in case of infectious disease.[11–21]

Outcome

The primary outcome was the diagnosis of LOS determined by an independent expert panel, composed of 3 neonatologist experts, independent of the management of neonates in the study centers. This independent adjudication committee classified the patients into the following categories: infected patients, not infected patients or unclassified patients. Classification by each adjudication committee member was based on the clinical and microbiological data as well as the CRP level collected at inclusion and after 48 hours, blinded to the values of the study biomarkers and to the decision of their peers. Final diagnosis was determined by panel majority agreement (at least 2 out of 3 concordant classifications); if this was not attained the 3 experts arrived at a consensus by discussion.

The diagnostic performance of the biomarkers combination and of the clinical signs were based on the classification of the adjudication committee.

Statistical analysis

Continuous variables were described by the median and range, and qualitative variables by count and percentage. Comparisons between groups were made using the Kruskal-Wallis or Wilcoxon tests for continuous variables, and Chi-Squared or Fisher's exact test for qualitative variables.

The diagnostic accuracy of biomarkers and of clinical signs was assessed in the groups of infected and not infected patients. Univariate logistic regression was used to assess the association between clinical signs and confirmed infection; the association was quantified by odds ratio (OR) with 95% confidence intervals [95%CI]. Clinical signs with a *P*value < 0.20, with low collinearity, were included in a multivariate model.

Biomarkers were combined through logistic regression models to predict the infection status, considering an additive effect on the logistic scale. Logarithmic transformations were applied when necessary to fulfill the hypotheses of the model. Predictions of the model (predicted probabilities of infection) were then used as a new marker.

Receiver operating characteristic curves (ROC) were built to estimate the performance of the clinical signs, biomarkers, or combination of biomarkers for the diagnosis of infection. The area under curve (AUC) and partial AUC (part of the curve for which the sensitivity is ≥ 0.898) were then calculated.[22] For each biomarker (or combination of biomarkers), the threshold with the highest specificity and a sensitivity of ≥ 0.898 was estimated (for combination of biomarkers, the threshold of predicted infection probability), with the associated specificity, positive and negative predictive value, and positive and negative likelihood ratios. A cut-off of at least 0.898 was defined for the sensitivity in order to identify the best biomarker alone or in combination to exclude early-on the diagnosis of LOS in symptomatic neonates. The optimism, that means the fact that the model gives better predictions on the data used to build the model than on independent datasets, was assessed by 20-times 5-fold cross validation.[23]

Statistical analyses were performed using R software, version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria) and SAS Institute software, version 9.4 (Cary, CN, USA).

A heatmap was generated by scaling and centering $\log_{10}$-transformed biomarkers concentrations and the dendogram was drawn based on hierarchical clustering analysis (Euclidean distance matrix with Ward's method) using Partek® Genomics Suite® software version 7.0 (Partek Inc., St. Louis, MO, USA).

Ethics Statement

Written informed consent was obtained from at least one of the parents or legal guardians. The study was approved by a French ethics committee (*Comité de Protection des Personnes* [CPP Sud-Ouest et Outremer III]) under the registration number 2017-A02492-51, and was conducted according to the recommendations of Good Clinical Practice and the Declaration of Helsinki. The study was registered in ClinicalTrials.gov.fr under the number NCT03299751.

Results

Cohort characteristics

A total of 234 hospitalized neonates with suspicion of LOS were included and 230/234 had analyzable samples (Additional file 1 Fig. S1). These were mainly boys (59.6%), the median (range) gestational age was 27 (23–41) weeks, and the median (range) birth weight was 940 (450–4660) grams (Table 1). Suspicion of LOS occurred at a median (range) of 14 (7-178) days of age. The most frequent signs related to suspicion of LOS were tachycardia (124/230, 53.9%), bloating/rectal bleeding (120/230, 52.2%), apnea or bradycardia events (111/229, 48.5%) and increased respiratory support and/or FiO2 (107/230, 46.5%).

Table 1
Patient characteristics.

	All patients (N = 230)	Infected (N = 51)	Not Infected (N = 153)	Unclassified (N = 26)	*P* value	Adjusted *P* value[a]
Demographic characteristics						
Sex, male No. (%)	137 (59.6)	39 (76.5)	86 (56.2)	12 (46.2)	0.011	0.231
Gestational age (in weeks), Median (range)	27.0 (23.0–41.0)	27 (24–41)	28 (23–41)	26.5 (24–38)	0.610	1.000
Birth weight (g), Median (range)	940.0 (450.0–4660.0)	960 (530–3400)	930 (450–4660)	902.5 (490–3430)	0.692	1.000
Birth weight <1500g No. (%)	184 (80.0)	39 (76.5)	126 (82.4)	19 (73.1)	0.393	1.000
Apgar Score at 5 minutes, Median (range)	8 (1–10)	9 (1–10)	8 (1–10)	8 (4–10)	0.850	1.000
Small for gestational Age No. (%)	67 (29.1)	10 (19.6)	52 (34.0)	5 (19.2)	0.074	1.000
C-section birth, No. (%)	152 (66.1)	27 (52.9)	109 (71.2)	16 (61.5)	0.051	0.816
Histological chorioamnionitis, No. (%)	36 (16.5)	7 (14.6)	26 (17.8)	3 (12.5)	0.816	1.000
Congenital malformations, No. (%)	41 (17.8)	13 (25.5)	25 (16.3)	3 (11.5)	0.260	1.000
Surgery prior to inclusion, No. (%)	35 (15.2)	17 (33.3)	15 (9.8)	3 (11.5)	0.001	0.028
Time from surgery to inclusion(in days), Median (range)	15.0 (4.0–63.0)	16 (6–63)	15 (6–43)	6 (4–52)	0.566	1.000
Clinical features at inclusion						
Calculated age (in days), Median (range)	14.0 (7.0–178.0)	11 (7–159)	15 (7–178)	14 (7–69)	0.637	1.000
Fever > 38°C, No./N. (%)	84/229 (36.7)	25/51(50)	103/153 (67.3)	17/26 (65.4)	0.087	1.000
Tachycardia > 160 bpm, No./N. (%)	124/230 (53.9)	33/51 (64.7)	74/153(48.4)	17/26 (65.4)	0.065	0.975
Capillary refill time > 3 seconds, No./N. (%)	18/226 (8.0)	10/51 (19.6)	5/150 (3.3)	3/25 (12.0)	0.001	0.028
Grey and/or pale skin complexion, No./N. (%)	56 /227 (24.7)	18/51 (35.3)	29/152 (19.1)	9/24(37.5)	0.020	0.360

[a]Holm's adjusted p-values, NA: Not appropriate

	All patients	Infected	Not Infected	Unclassified	*P* value	Adjusted *P* value[a]
	(N = 230)	(N = 51)	(N = 153)	(N = 26)		
Apnea or bradycardia events, No./N. (%)	111/229 (48.5)	23/51 (45.1)	78/153 (51.0)	10/25 (40.0)	0.534	1.000
Digestive disorders (abdominal bloating or rectal bleeding), No./N. (%)	120/230 (52.2)	26/51 (51.0)	81/153 (52.9)	13/26 (50.0)	0.938	1.000
Hypotonia or lethargy, No./N. (%)	38/229 (16.6)	14/51 (27.5)	17/153 (11.1)	7/25 (28.0)	0.006	0.132
Increased ventilatory support and/or increased FiO2, No./N. (%)	107/230 (46.5)	26/51 (51.0)	63/153 (41.2)	18/26 (69.2)	0.022	0.374
Cutaneous rash, No./N. (%)	5/230 (2.2)	1/51 (2.0)	3/153(2.0)	1/26 (3.8)	0.782	1.000
Presence of a central venous catheter, No./N. (%)	146/229 (63.8)	44/50 (88.0)	86/153 (56.2)	16/26 (61.5)	0.001	0.028
	All patients	Infected	Not Infected	Unclassified	P value	Adjusted P value[a]
	(N = 230)	(N = 51)	(N = 153)	(N = 26)		
Antibiotics at 48h, No. (%)						
No	117 (50.9)	0 (0)	111 (72.5)	6 (23.1)	0.001	0.028
Yes	113 (49.1)	51 (100)	42 (27.5)	20 (76.9)	NA	NA
Vancomycin	98 (42.6)	48 (94.1)	36 (23.5)	14 (53.8)	NA	NA
Amikacin	80 (34.8)	35 (68.6)	32 (20.9)	13 (50.0)	NA	NA
Cefotaxime	41 (17.8)	20 (39.2)	13 (8.5)	8 (30.8)	NA	NA
Other betalactams	18 (7.8)	8 (15.7)	5 (3.3)	5 (19.2)	NA	NA
Metronidazole	2 (0.9)	1 (2.0)	1 (0.7)	0 (0)	NA	NA
Other	20 (8.7)	9 (17.6)	6 (3.9)	5 (19.2)	NA	NA
Duration of exposure (days), median (range)	3 (1–26)	10 (2–21)	2 (2–26)	3 (2–21)	NA	NA
Antibiotic exposure > 2 days	64 (66)	48 (94)	7 (17)	9 (45)	NA	NA
Laboratory values						
C-reactive protein, mg/L, (n = 187) Median (range)	1.0 (0.0–207.0)	13.5 (0–207)	1 (0–30.0)	5.6 (0–165.9)	0.001	0.028

[a]Holm's adjusted p-values, NA: Not appropriate

	All patients	Infected	Not Infected	Unclassified	*P* value	Adjusted *P* value[a]
	(N = 230)	(N = 51)	(N = 153)	(N = 26)		
White blood cell count, G/L, (n = 133) Median (range)	13.3 (2.30–40.12)	14.48 (2.30–40.12)	12.65 (2.94–38.05)	16.67 (5.67–33.3)	0.205	1.000
Neutrophils, G/L, (n = 106) Median (range)	5.13 (0.93–22.45)	6.50 (0.95–22.07)	4.61 (0.93–22.45)	4.70 (1.01–21.98)	0.015	0.285
Lymphocytes, G/L, (n = 106) Median (range)	5.06 (0.77–14.90)	3.81 (0.77–6.73)	5.39 (1.14–14.90)	5.01 (1.17–7.93)	0.012	0.240
Blood cultures No./N. (%)						
Not done	2/230 (0.9)	0/51 (0)	2/153 (1.3)	0/26 (0)	0.001	0.028
Sterile	180/230 (78.0)	8/51 (15.7)	148/153 (96.7)	24/26 (92.3)	NA	NA
Positive	48/230 (20.9)	43/51(84.3)	3/153 (2)	2/26 (7.7)	NA	NA
Staphylococcus aureus (n = 228)	8/228(3.5)	8/51 (15.7)	0/151 (0)	0/26 (0)	NA	NA
Coagulase-negative *staphylococci* (n = 228)	35/228(15.4)	30/51 (58.8)	3/151 (2.0)	2/26 (7.7)	NA	NA
Gram-negative bacilli (n = 228)	3/228(1.3)	3/51 (5.9)	0/151 (0)	0/26 (0)	NA	NA
Other Gram-positive organisms (n = 228)	2/228(0.9)	2/51 (3.9)	0/151 (0)	0/26 (0)	NA	NA
Candida albicans (n = 228)	1/228(0.4)	1/51 (2.0)	0/151 (0)	0/26 (0)	NA	NA

[a]Holm's adjusted p-values, NA: Not appropriate

Demographics And Microbiological Characteristics According To Infection Status

The adjudication committee classified 51 (22.2%) neonates as infected, 153 (66.5%) as not infected and 26 (11.3%) neonates were unclassified (Table 1). In univariate analysis, signs significantly more frequent in the infected group than in not infected group were a capillary refill time > 3 seconds, hypotonia or lethargy, grey and/or pale skin complexion, fever and tachycardia (Fig. 1A). In multivariate analysis, capillary refill time > 3 seconds was the only sign that was significantly associated with an infection (adjusted OR: 4.02, 95%CI [1.15–15.18], *P* value 0.029). This sign was present in only 10/51 patients of the infected group so its sensitivity was of 20%. A model combining tachycardia, capillary refill time > 3 seconds, and hypotonia or lethargy showed a partial AUC of 0.517 (95%CI [0.502–0.551]) for the diagnosis of infection (Fig. 1B).

Half of the neonates were treated by antibiotics (49.1%) including all subjects (100%) classified as infected and 27% of those classified as the not infected. Vancomycin was the most prescribed drug (42.6% of the total population),

followed by amikacin (34.8%) and cefotaxime (17.8%; Table 1).

The median CRP values were significantly higher in infected patients (13.5 mg/L, range: 0-207) than in not infected patients (1 mg/L, range 0–30, *P* value 0.001). Blood culture was positive in 43/51 (84.3%) patients classified as infected, *Staphylococcus spp* represented 88.4% (38/43) of identified pathogens. Among the 8 patients with a sterile blood culture but classified as infected, pathogens were detected in the tracheal suctioning culture in 4 patients, and 2 had a positive blood culture (for either *Pseudomonas aeruginosa* or *Staphylococcus epidermidis*) the day following their inclusion. In the not infected group, 3/153 (2%) patients had a positive blood culture; for these 3 patients, coagulase negative staphylococci was identified.

Biomarkers

The distribution of concentration of each biomarker is presented for the 3 groups of patients in Fig. 2A and Additional file 1: Table S1. Concerning IL-27, due to the high proportion of missing data (94/230) related to the serum volume requirement of 100 µL, we decided to not calculate its performance for infection diagnosis. Considering patients classified as infected and not infected, the AUC were calculated for each biomarker alone (Fig. 2B). IL-6, IL-10, and NGAL had the best AUC (> 0.8 for all). In line with the clinical context and the need to identify a biomarker useful to exclude the presence of an infection in symptomatic neonates, partial AUC focusing on a high sensitivity were then calculated to evaluate the performance of each biomarker and of all combinations of 2 to 4 biomarkers (Additional file 1: Table S2). No added value was obtained when combining 4 rather than 3 biomarkers; combinations of more than 4 biomarkers were therefore not tested (Additional file 1: Fig. S2). Focusing on partial AUC, the best performance was found for IL-6, IL-10, and NGAL alone, as well as the combinations PCT/IL-10, PTX3/NGAL, and PTX3/NGAL/gelsolin (Fig. 2C). Of note, the combination of biomarkers with clinical signs did not improve performance (Additional file 1: Fig. S3). As illustrated in the heatmap, unsupervised analysis revealed a cluster characterized by high plasmatic levels of IL-10, IL-6, NGAL, that was mainly composed of infected neonates (73%) or unclassified neonates (23%; Fig. 3). This indicates that the biomarker profile of patients in the unclassified group were close to the one of the infected group (Fig. 2A).

Application Of The Best Models To The Cohort

We assessed the reclassification of patients using the identified biomarkers alone and in combination. Using the 6 models with highest partial AUC (Fig. 2C), 5/51 (9%) patients of the infected group were reclassified as not infected; this was consistent with the sensitivity of each model that was preset to about 90%. The 5 patients reclassified as not infected varied depending on the model. The 6 models were able to identify as not infected up to 64.3% (27/42) of neonates of the not infected group who had received unjustified antibiotics (Table 2).

Table 2
Reclassification by selected models of patients treated by antibiotics

	PATIENTS WHO RECEIVED ANTIBIOTICS	
Selected models	Patients from the **Infected group, reclassified as Not infected** using biomarker models	Patients from the **Not infected group, also classified as Not infected** using biomarker models
IL-6	5/51 (9.8%)	10/42 (23.8%)
IL-10	5/51 (9.8%)	26/42 (61.9%)
NGAL	5/49 (10.2%)	25/42 (59.5%)
PCT/IL-10	5/51 (9.8%)	26/42 (61.9%)
PTX3/NGAL	5/49 (10.2%)	27/42 (64.3%)
PTX3/NGAL/gelsolin	5/49 (10.2%)	23/41 (56.1%)
Data represent the proportion of patients reclassified using the best selected models among patients treated by antibiotics		
(51 and 42 neonates classified by adjudication committee as patients with infected and not infected status respectively).		
There are missing data for 3 biomarkers' detection.		

Discussion

In the present study we identified biomarkers alone or in combination that had high performance to identify non-infected neonates among symptomatic patients; it was estimated that using these biomarkers could avoid nearly two-thirds of unjustified antibiotic use.

Among the 6 models that had the best performance, half were combinations of biomarkers (PCT/IL-10, PTX3/NGAL, and PTX3/NGAL/gelsolin). As far as we know, this is the first time these combinations have been tested in a neonatal population with suspected LOS. The use of combinations of biomarkers seemed an interesting idea since such combinations benefit from the performance of each biomarker, that could have, individually, different advantages and limits. However, the combinations tested herein did not show significantly better performance than biomarkers alone, and we therefore focused the rest of the discussion on biomarkers used alone. IL-6, IL-10, and NGAL showed the best performance. Contrarily to IL-10 and NGAL, and despite high AUC and sensitivity, IL-6 surprisingly failed to correctly identify as not infected the patients who received unjustified antibiotics. This is likely to be due to the close relationship between IL-6 and CRP, since the former is a cytokine of the early immune response that directly stimulates the hepatic production of CRP.[24] Thus we hypothesize that the input of IL-6 in the reclassification of these patients is moderate because the choice of treating or not patients was made by the clinicians on the basis of the CRP value that increased in parallel with that of IL-6. In contrast, IL-10 seems very interesting since it could have avoided unjustified antibiotics for two-thirds of patients. This is consistent with that reported in a previous study exploring the performance of IL-10 for the diagnosis of LOS in in a population of full-term neonates;[25] and the reason is likely to be related to the immune response during neonatal period, notably in preterm infants, being polarized towards an anti-inflammatory response (T helper 2 lymphocytes), involving an increased production of cytokines such as IL-10.[26] In addition, NGAL is another biomarker that had good

performance to identify not infected neonates herein. NGAL is a protein produced by neutrophils that inhibits bacterial growth by blocking the access of bacteria to iron, the production of which is activated by different stimuli to that of cytokines.[27] It has been proposed as a promising early biomarker of invasive neonatal sepsis in a previous study including both term and preterm infants.[28] However it can be influenced by other neonatal conditions including respiratory distress and acute kidney injury (AKI).[28,29] More studies are needed to thoroughly investigate the performance of this biomarker in patients suffering from AKI, and to evaluate whether a different threshold value for plasmatic NGAL concentration can be proposed to differentiate AKI from LOS. Another point of note is that there was no improvement in performance observed by combining clinical and biomarker models.

Previous studies have already explored the performance of biomarkers for the diagnosis of LOS in hospitalized neonates, but with methods different from those used herein. First, some studies compared biomarker levels in infected versus healthy neonates;[12,15,16,21,30] however, we consider that comparing with healthy neonates is not relevant in clinical practice since the real difficulty is to differentiate infected from not infected neonates among those with clinical signs. Second, studies about biomarkers are frequently either descriptive about mean and distribution of biomarker levels in a specific population, or focused on the overall performance of the biomarker via the measurement of AUC, specificity, sensitivity.[11–13, 15,16] However, in the practice the daily issue is not to confirm LOS but to rule-out this diagnosis at the onset of clinical signs. This is illustrated herein as all infected patients had been properly identified by clinicians given that all had received antibiotics. In this context, we decided to use an original approach: we determined the best partial AUC considering a minimal sensitivity of 0.898, which seems acceptable from a clinician point of view to avoid missing the diagnosis of LOS. This innovative approach explains why the threshold value for the biomarkers of the present study differed from the ones reported elsewhere; for example the cut-off for IL-10 in our study was 2.5 to 4.5-fold lower than the ones proposed in previous studies.[25,31]

Another point of note is that the study of 11 biomarkers was possible by the use of ELLA Automated Immunoassay System that requires only 25 µL of serum for the quantification of 4 proteins,[32] such a low volume of blood being a prerequisite in the specific population of neonates and very low birth weight infants to avoid blood depletion. To the best of our knowledge this is the first time this method was used in neonates and this opens new prospects for future research, but this technique is not able to be applied to clinical use. The next step before being able to use these biomarkers in a clinical decision rule is to develop a rapid point of care test, because a quick result is essential to impact the decision to prescribe or not antibiotics, as described in a previous study.[33] The second step will be to evaluate whether having the biomarker value in neonates suspected of LOS will decrease unjustified antibiotic prescription without missing LOS. The impact on microbiota of hospitalized neonates and on emergent multidrug resistant bacteria in NICU settings will also be an essential outcome to evaluate in future studies.

The present study does have some limitations. The first is the heterogeneity of the included patients. However, the aim was to include all neonates with a suspicion of LOS, in order to be able to extrapolate the results to the whole population of hospitalized neonates without restriction. Second, although published data suggests that it could be promising for the diagnosis of LOS,[34] it was not possible to evaluate the performance of IL-27 due to the blood volume required for the test. This cytokine is not currently measurable using ELLA but could be in the future. Third, it was not possible to evaluate the performance of CRP in since this biomarker was used in routine, therefore the adjudication committee was not blinded to CRP values, and precluded the comparison of the present study with those investigating CRP performance for LOS diagnosis.

Conclusion

The present study found that the diagnosis of LOS can be improved by the use of new biomarkers. The next step will be to evaluate if including these biomarkers in a decision rule could have a positive impact on the adequate prescription of antibiotics in hospitalized neonates.

List Of Abbreviations

LOS	Late-onset sepsis
CRP	C-reactive protein
PCT	Procalcitonin
IP-10	IFN-γ-induced protein 10
IL-6	Interleukin 6
IL-10	Interleukin 10
NGAL	Neutrophil gelatinase-associated lipocalin-2
PTX3	Pentraxin 3
LBP	Lipopolysaccharide-binding protein
IL-27	Interleukin 27
ELISA	Enzyme-linked immunosorbent assay
IQR	Interquartile range
CI	Confidence interval
AUC	Area under the curve
ROC	Receiver operating characteristic (curve)

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from at least one of the parents or legal guardians. The study was approved by a French ethics committee (Comité de Protection des Personnes [CPP Sud-Ouest et Outremer III]) under the registration number 2017-A02492-51, and was conducted according to the recommendations of Good Clinical Practice and the Declaration of Helsinki. The study was registered in ClinicalTrials.gov.fr under the number NCT03299751.

Consent for publication

Not Applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interests

SP,LG and KBP are bioMérieux employees.

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Authors' contributions

MB,SP,STA and KBP contributed to the literature search ,study design and data interpretation. EC,AB,SG,CF,AC,FP ,AP and OC carried out patient recruitment . MB was principal investigator of the study. LG and SP designed and performed the experiments. FS ,FA, verified and analysed the data. SP,STA and MB revised the manuscript critically for important intellectual content. SP constructed the figures. All authors read and approved the final manuscript.

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Figures

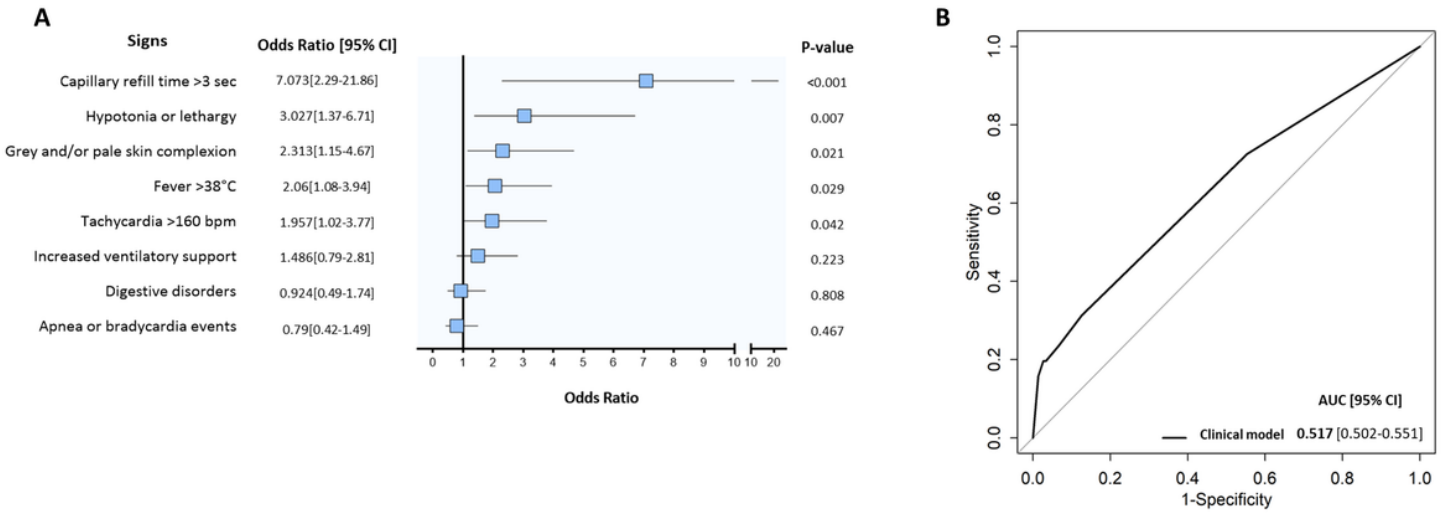

Figure 1

Performance of clinical signs to discriminate infected and not infected neonates.

A. Forest plot showing Odds Ratio [95%CI] relative to each clinical sign for infection diagnosis. The squares represent Odds Ratio and bars indicate the 95% confidence interval.

B. ROC curve of best performing model combining tachycardia, capillary refill time >3 seconds and hypotonia/lethargy. Partial AUC [95%CI] is calculated for the diagnosis of confirmed infection.

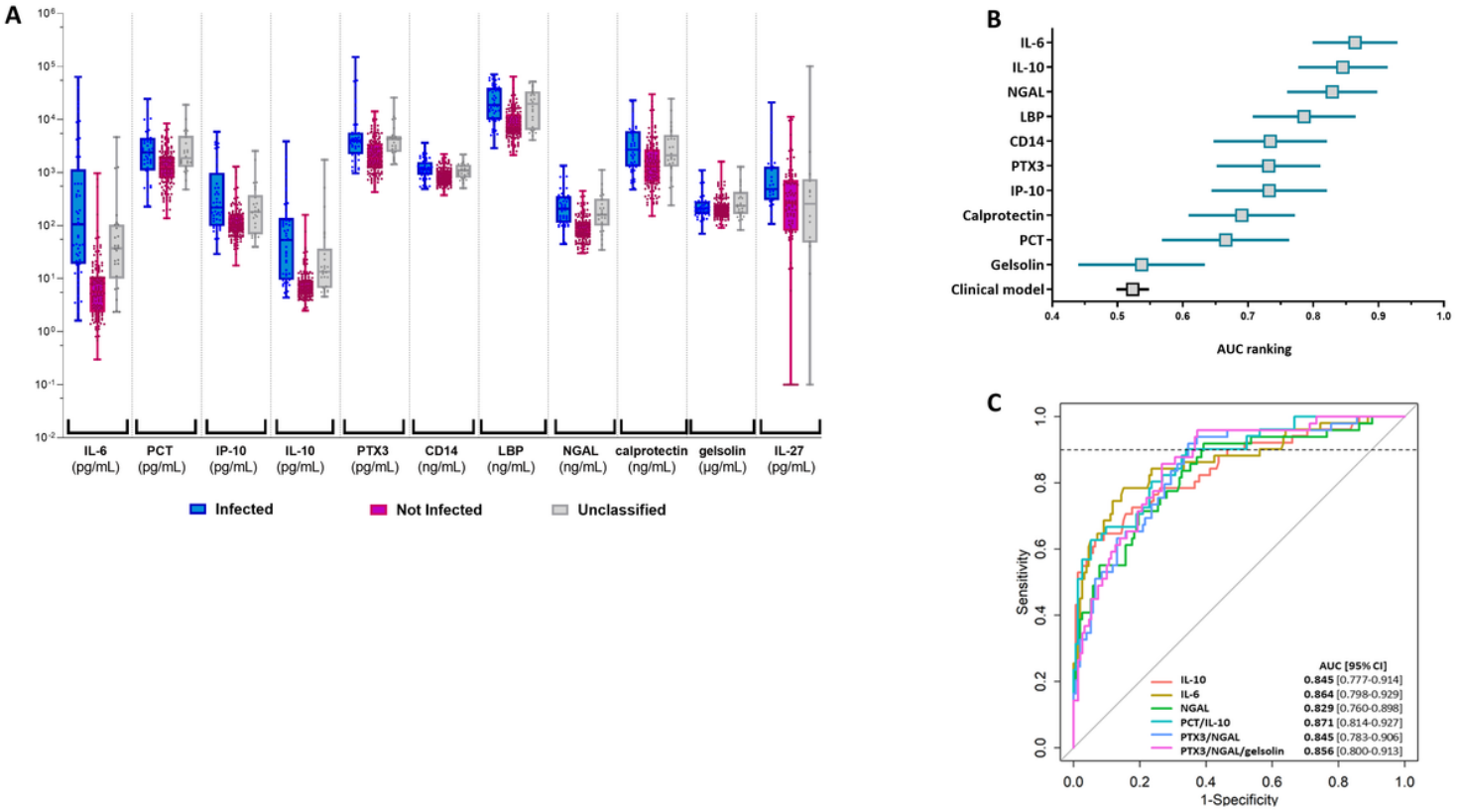

Figure 2

Description and performance of biomarkers to discriminate infected and not infected neonates.

A. Data are expressed as box-and-whiskers plots showing median (horizontal line inside the box), interquartile range (upper and lower horizontal lines of the box), minimum and maximum (whiskers), and each dot corresponds to one subject.

B. Forest plot showing AUC [95%CI] relative to each biomarker for infection diagnosis in comparison to that of clinical model. The squares represent AUC and bars indicate the 95%CI.

C. ROC curve of best performing biomarker alone or in combination for infection diagnosis. Dotted line represent a sensitivity of 0.9 established to calculate partial AUC [95%CI].

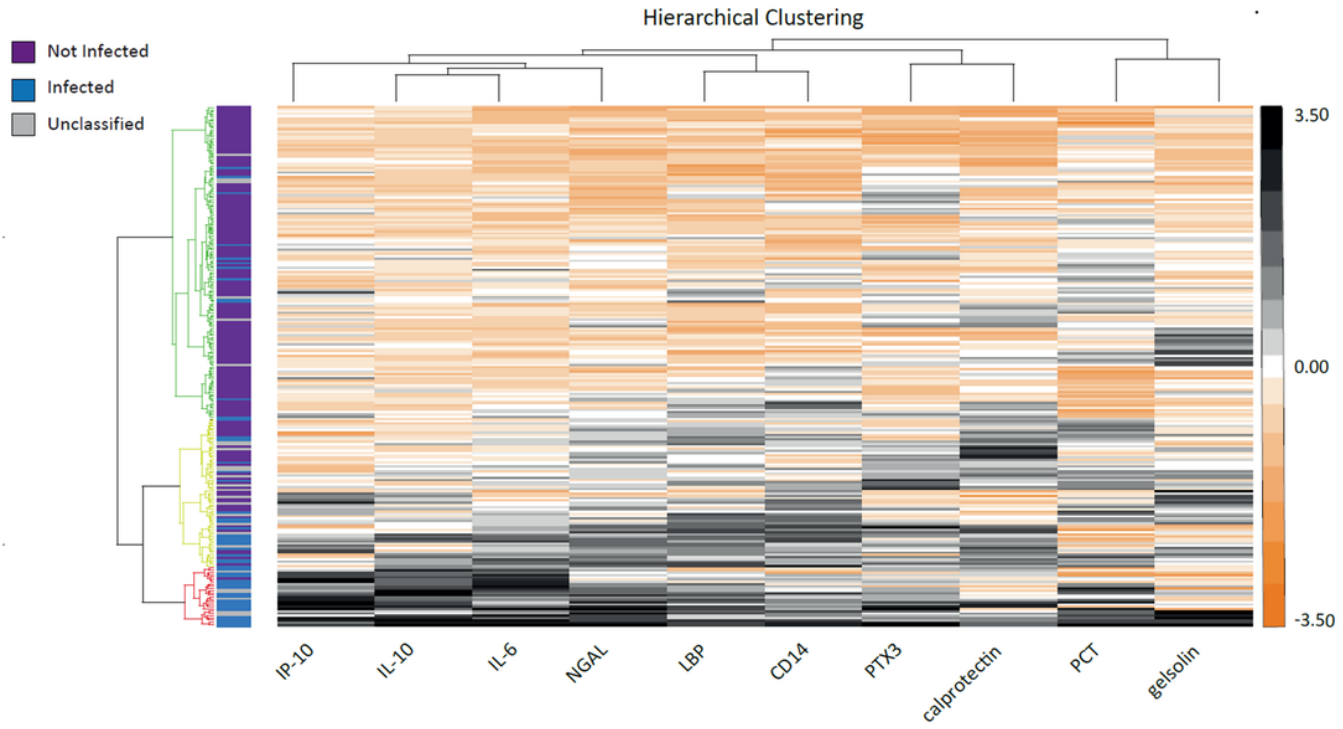

Figure 3

Biomarkers' quantification in neonates with suspected infection.

Heatmap of protein expression profiles from unsupervised analysis (Euclidean distances matrix with Ward's methods) generated by scaling and centering log10-transformed normalized protein concentrations. Protein clustering is indicated by dendrogram trees respectively on the top and on the left side of the heatmap. Clustering allows to discriminate 3 distinct clusters composed of various proportion of patients with infected (blue bars), not infected (purple bars) or unclassified status (grey bars). The biomarker profile of patients in the unclassified group were close to the one of the infected group.

Supplementary Files

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