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Optimal combination of early biomarkers for infection and sepsis diagnosis in the emergency department: The BIPS study

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SUMMARY

Objective: : To define the best combination of biomarkers for the diagnosis of infection and sepsis in the emergency room.
Methods: : In this prospective study, consecutive patients with a suspicion of infection in the emergency room were included. Eighteen different biomarkers measured in plasma, and twelve biomarkers measured on monocytes, neutrophils, B and T-lymphocytes were studied and the best combinations determined by a gradient tree boosting approach.
Results: : Overall, 291 patients were included and analysed, 148 with bacterial infection, and 47 with viral infection. The best biomarker combination which first allowed the diagnosis of bacterial infection, included HLA-DR (human leukocyte antigen DR) on monocytes, MerTk (Myeloid-epithelial-reproductive tyrosine kinase) on neutrophils and plasma metaloproteinase-8 (MMP8) with an area under the curve (AUC) = 0.94 [95% confidence interval (IC95): 0.91;0.97]. Among patients in whom a bacterial infection was excluded, the combination of CD64 expression, and CD24 on neutrophils and CX3CR1 on monocytes ended to an AUC = 0.98 [0.96;1] to define those with a viral infection.
Conclusion: : In a convenient cohort of patients admitted with a suspicion of infection, two different combinations of plasma and cell surface biomarkers were performant to identify bacterial and viral infection.

Introduction

The recent update in sepsis definitions have reinforced sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to infection.[1] Because clinical signs at admission are often non-specific, sepsis biomarkers have been intensively investigated in order to improve sepsis identification and to promote the implementation of early therapeutic strategies.[2] In this very active field of research, the most studies have been conducted in the critical care setting. However, most patients with sepsis are admitted at hospitals through emergency departments (ED).[3] The ED has therefore a crucial role for the early identification of sepsis but above all, the identification of bacterial and viral infection, which is the pre-requisite for sepsis suspicion. An inflammatory biological profile is observed in many ED clinical situations. Therefore, identification of patients who really do have infection is challenging. Currently, due to complex and multimodal pathophysiological pathways, no individual biomarker of infection and/or sepsis is sufficiently discriminating to allow proper diagnosis.[4] The aim of the Biomarqueurs d'Identification Précoce du Sepsis aux urgences (BIPS) study was to measure in patients suspected of having infection or sepsis in the emergency department, a panel of

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biomarkers (covering several distinct pathophysiological pathways) and to identify the best combination that could provide high specificity and sensitivity for bacterial infection, viral infection and sepsis diagnosis. The originality of the BIPS study is that in comparison with intensive care units, the patients investigated for suspected sepsis in the ED are seen earlier in their medical management and can provide blood samples before any therapeutic intervention (such as fluid resuscitation, antibiotics, vasopressors) that could potentially interferes with several biomarkers of interest.

Patients and methods

Setting and inclusion criteria

The BIPS study was conducted in the ED of an adult academic urban tertiary 1700-bed hospital, which receives 69,000 visits a year. In order to gather the main situations where ED physicians suspect infection or sepsis (and may benefit from the added value of biomarkers) we used a DELPHI method applied to a panel of 10 national emergency medicine experts, to define the clinical or biological criteria of inclusion.[5,6] Two rounds were necessary to reach a consensus (>75% agreement) on the following criteria of inclusion (at least one requested for inclusion): Temperature >38 °C, chills, marbling, systolic blood pressure <90 mmHg or mean arterial pressure <70 mmHg, cough, purulent sputum, unilateral thoracic altered sound on auscultation, abdominal defence or contracture, monoarthritis, unilateral red swelling leg, unilateral facial oedema, urine test strip positive for leukocytes and/or nitrite, white blood cells count <4000 or > 12,000/mm^3, lactate > 2 mmol/L.

Patients aged from 18 years were prospectively recruited and signed an informed consent before inclusion. The BIPS study was approved by the Ethics Committee (Comité de protection des personnes-Ile de France VI) and has been registered in Clinical Trials (NCT02707718).

Exclusion criteria

We excluded patients aged of less than 18 years, patients with no social insurance, homeless people without possible follow-up, pregnant women, those who refusal to participate, prisoners, and patients with dementia and/or cognitive impairment precluding informed consent.

Clinical and biological data, follow-up and adjudication

The data collected were those registered during ED visits and comprised: age, sex, vital signs at nurse triage, ongoing anti-infective treatment, immune status (immunodeficiency defined by at least one among: cancer on chemotherapy, immunosuppressive treatment, >15 mg/day prednisolone, human immunodeficiency virus infection, solid organ transplant) the routine blood tests results (total cell blood count, renal, liver and coagulation function tests), final diagnosis, disposition decision (outpatient, medical wards or intensive care unit hospitalization) and anti-infective treatment administered. Patient care was totally at the discretion of the treating emergency physician. Microbiological investigations during hospital stay and ward medical charts were also recorded. A follow-up phone call was organized at day 30 to assess vital status and confirm the diagnosis for the patients discharged home. All anonymized data were gathered into an Excel sheet and for each patient included were submitted to an adjudication committee of 3 physicians: one emergency physician, one intensivist and one infectious disease specialist. Each adjudicator independently had to classify the patients into the pre-specified sepsis-2 and sepsis-3 criteria and in case of infection into bacterial, parasitic or viral infection.[1,7] The diagnosis, site and type of infection was adjudicated based on the retrospective chart review of tests performed, on all clinical data available as well as follow-up phone call. Briefly, all test results were extracted from the records 7–10 days later, including bacterial cultures (blood, urinary, pleural effusion, cerebro-spinal, ascites, articular liquid, as drived by the clinical presentation), viral serology, molecular tests (e.g., polymerase chain reaction on respiratory samples and antigens), relevant imaging, and tissue pathology where appropriate. The patients were classified as infection if they had an infectious source and fulfilled neither sepsis-2 (≥2 SIRS criteria) nor sepsis-3 (≥ 2 points of SOFA) definition. In cases where no consensus could be reached, a fourth independent physician opinion was requested to arbitrate.

Sampling

Blood collection for biomarkers measurement (3 × 4 ml EDTA tubes) was performed by the ED nurses before any significant therapeutic intervention. The patients were screened and enrolled on week days only on morning, to allow sufficient time for sample processing on the same day (cytometric analyzes without fixation).

Immediately after collection, two tubes were kept at +4 °C until transportation on ice every day to Institut Pasteur, where expression of cell surface markers was assessed directly on whole blood by flow cytometry, and plasma isolated by centrifugation was stored at −80 °C until plasma biomarkers measurement. One tube was kept at room temperature in the ED during two hours for human neutrophil lipocalin (HNL) dosage (as recommended by the manufacturer) then plasma was isolated by centrifugation and stored at +4 °C before transportation on ice to the Institut Pasteur every day. Plasma was transferred into 0.5–1 mL aliquots tubes (Eppendorf Biopur) and stored at −80°C until assayed.

Biomarker's selection

The selection of biomarkers was screened by the scientific committee of the BIPS study, after a systematic literature review on the potentially promising biomarkers of sepsis and/or infection. First, we selected biomarkers that had been reported at least twice in the literature.[8–25] Second, a final selection took into account the technical constraints and research team skills, and the scientific committee adjudication. Eighteen different biomarkers measured in plasma, and twelve biomarkers measured on monocytes, neutrophils, B and T-lymphocytes were included; as well as a bacterial biomarker (endotoxin linked to leukocytes) (Fig. 1).

Methods of dosage

Plasma biomarkers were measured using an enzyme linked immunosorbent assay or fluorescence immunoassay: ELISA HNL/NGAL Diagnostics Development (Uppsala, Sweden), ELISA Angiopoietin2 Abcam (Cambridge, United Kingdom), ELISA Human MMP-8 R&D Systems (Minneapolis, USA), ELISA suParnotics AUTO Flex ELISA kit ViroGates (Birkerod, Denmark), Human Cytokine Assays-Bio-Plex Pro Assays BioRad (Hercules, USA), with an immunoassay analyzer. Interferon-alpha measurement was performed as described previously.[26] All measurements were performed according to the manufacturer's instructions.

Expression of cell surface markers was assessed directly on whole blood by flow cytometry (MACSquant Miltenyi Biotec). The gating strategy is represented in additional file 1. Anti-CD14-VB, anti-CD16-PE, anti-HLADR-PE/Vio770, anti-CX3CR1-AlexaFluor674; anti-CD64-VB, anti-CD66abce-FITC, anti-CD24-PE, anti-MerTK-AlexaFluor674, anti-CD3-VB, anti-CD4-FITC, anti-CD19-PE/Vio770, anti-BTLA (CD272)-APC were obtained from Miltenyi Biotec (CD14, CD16, HLADR, CD64, CD66abce, CD24, CD3, CD4, CD19: Bergisch

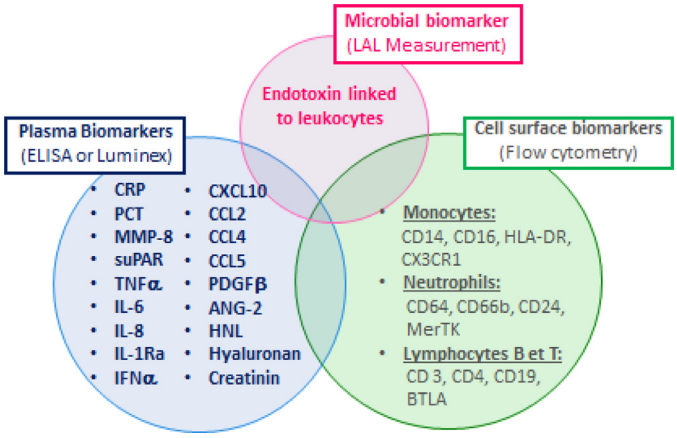

Fig. 1. List of biomarkers simultaneously investigated in this study (Abbreviation: CRP : C reactive protein, PCT : procalcitonin ; MMP8 : metalloproteinase-8 ; suPAR : soluble utokinase-type plasminogen activator receptor ; TNFa: tumor necrosis factor-a; IL-: interleukin ; IFN: interferon; CCL : Chemokine CCL ; CXCL : Chemokine CXCL ; PDGFb: platelet growth factor-b; ANG-2 : angiopoietin-2 ; HNL : human neutrophil lipocalin.

Gladbach, Deutschland), BioLegend (CX3CR1, MERTK, San Diego CA, USA), and BD Biosciences (BTLA, San Jose, CA, USA). Whole blood (100uL) was processed by FCR blocking (Miltenyi −10 min of incubation in the dark) and was stained with antibody (20 min of incubation in the dark); subsequently, 3 mL of lysis buffer (BioLegend) was added to samples to lyse erythrocytes. After a 10 min incubation at +4 °C and centrifugation, the supernatant was treated by Viability Dye-eFluor780 (Invitrogen/eBioscience-ThermoFisher, CarlsBad, CA, USA) and after a new step of incubation at +4 °C and centrifugation the supernatant was removed and 300uL of MACS buffer was added to cell. The expression of surface markers was immediately measured by flow cytometry. Data analysis was performed using FlowJo software. Settings of the flow cytometer were maintained constant during the whole study. Values were expressed as mean fluorescence intensity (MFI) or percentage of expression.

For the endotoxin assay, test QCL1000- Limulus Amebocyte Lysate Biowhittaker-Lonza (Basel- Switzerland) was applied for the dosage of endotoxin binding on neutrophils and mononuclear cells. The LAL assay was used according to manufacter's recommendation.

Statistical methods

Clinical and biological data are described as frequencies and percentages for categorical variables and as means and standard deviations or medians and interquartile ranges for continuous variables, as appropriate. To identify the biomarkers which may discriminate pre-defined groups of patients (bacterial infection, viral infection, sepsis) a gradient boosting tree approach (xgbTree function from the caret R package v6.0.3.81, http://cran.r-project.org) was applied. Gradient Boosting Tree is a machine learning approach which maximizes the accuracy of the prediction by progressively training more complex models. All the models are combined to obtain the predictions. This process helps to reduce bias and variance. The final estimation depends on a set of hyperparameters which are tuned according to the accuracy (default option in caret package).[27] For each combination of biomarkers, the gradient boosting tree approach provides a score (posterior probability between 0 and 1). This score is used to determine the group in which the patient is classified. The score depends on the value of all the biomarkers of the combination and on the model parameters (estimated with the cohort). We selected the 15 most discriminating markers (including clinical and routine biological variables) using a Mann Whitney test as a preliminary filtering step. The size of the training and testing sets was 262 and 29, respectively. All the combinations of the 15 most discriminating markers (2^{15}-1 combinations) were explored to determine the best one according to the receiver operating characteristic area under the curve (AUC) criterion. To avoid over - fitting, we performed a 10-fold cross-validation process. All the AUCs were calculated on the test samples. We aimed at identifying a biomarker or combination of biomarkers with an AUC >0.9 for the main criteria of judgment. Estimating that one third of included patients would fulfill this criteria, 280 patients had to be recruited with an alpha risk of 5% and to obtain an AUC's 95% confidence interval of 0.1.

Results

From March 2016 to July 2017, 308 patients with suspicion of infection or sepsis were included: 6 patients were excluded for missing blood samples, 3 for acute appendicitis and 8 for malaria. Further analysis was performed on the 291 remaining patients. Median age was 60 years (interquartile range IQR, 32) and 53.6% were women. Baseline characteristics of patients are summarized in Table 1. Bacterial infection was adjudicated for 148/291 (51%) patients, and viral infection for 47/143 (33%) patients with no bacterial infection, including 11 respiratory infections (influenzae virus: 10, metapneumovirus: 1), 1 acute cytomegalovirus hepatitis, 1 HIV meningitis primo-infection. The remaining viral infections were adjudicated based on clinical presentation (flu-like symptoms, acute gastro-enteritis symptoms) together with negative systematic bacterial cultures and spontaneous recovery without antibiotics.

70/291 (24%) patients were adjudicated as sepsis-2 and 16 (5%) as sepsis-3 (all met sepsis-2 criteria). The inter-rater reliability for the different endpoints (bacterial infection, viral infection, sepsis) was good (Kappa: 0.69) and a 4th adjudicator (arbitrator) was requested in 3.2% of cases. Thirty-day mortality rate was 5.2%.

Biomarker values distribution according to the group of adjudication (bacterial infection, viral infection, no infection, sepsis-2, sepsis-3) are represented on Fig. 2. The statistical combinatory approach showed the association of HLA-DR on monocytes (defined as CD14+ cells), MerTk (Myeloid-epithelial reproductive tyrosine kinase) on neutrophils (defined as CD66+ cells) and plasma metalloproteinase-8 as the best combination for bacterial infection identification among the whole cohort, with an AUC of 0.94; [95% confidence interval (IC95): 0.91;0.97] (Fig. 3A). The respective strength of each biomarker in the combinatory approach is represented on additional file 2. Of note, the association of both HLA-DR and MerTK had an AUC = 0.91 [0.88;0.94]. The addition of a third biomarker improved only moderately the performance (see additional file 3).

Among patients identified as non-bacterial infection with the first biomarker combination, it was possible to define those with a viral infection by the combination of CD64 expression (%), CD24 (MFI) on neutrophils (among CD66+ cells) and CX3CR1 (%) on monocytes (defined as CD14+ cells): AUC = 0.98 [0.96;1] (Figs. 2A and 3B). Of note, the AUC of CD64 and CX3CR1 was already 0.96 [0.93;0.99] (see additional file 4).

The best combination to define patients with sepsis-2 was HLA-DR, PCT and IL-6 (AUC = 0.89 [0.85;0.93]) while the best combination to define patients with sepsis-3 was HLA-DR, Hyaluronidase and creatinine: AUC = 0.92 [0.87;0.97] (Figs. 2B and C, 3C and D). Other possible combinations are given in additional files 5 and 6.

The statistical performances of the best combinations are represented on Table 2.

Table 1
study participants baseline characteristics and outcome, according to the different endpoints. N: number. IQR: interquartile range.

	All (n=291)	Bacterial (n=148)	Viral (n=47)	No infection (n=96)	*p* value	Sepsis2-0 (n=70)	Sepsis3-0 (n=16)	Infection no sepsis (n=78)
Sex (%)					0.058			
Men	46.4	39.9	57.4	51		38.6	25	47.2
Women	53.6	60.1	42.6	49		61.4	75	52.8
Age, y Median (IQR)	60 (42-73)	60 (40-73)	58 (42-72)	58 (50-75)	0.591	62 (48-73)	65 (57-72)	59 (39-73)
Systolic blood pressure, median (IQR), mm Hg	130 (114-147)	123 (108-143)	140 (126-150)	140 (121-151)	<0.001	123 (105-143)	101 (88-139)	131 (114-144)
Heart rate, median (IQR), mm Hg	95 (82-108)	98 (87-110)	98.5 (85-106)	99 (76-104)	0.006	104 (94-118)	104 (82-115)	94 (83-104)
Temperature, median (IQR),°C	37.1 (36.5-38.0)	37.4 (36.8-38.2)	37.8 (36.6-38.2)	37.8 (36.4-37.2)	<0.001	37.9 (36.9-38.6)	37.3 (36.9-38.2)	37.3 (36.6-38.0)
Immuno-compromised No. (%)	52 (17.9)	27 (18.2)	9 (19.1)	16 (16.7)	0.923	17 (24.3)	5 (31.2)	12 (15.2)
White blood cell count, Giga/L	10.37 (7.75-14.5)	13.0 (9.98-17.11)	7.9 (6.65-11.89)	8.88 (7.03-11.73)	0.578	14.08 (10.43-18.2)	15.97 (13.0-19.15)	10.6 (7.8-13.8)
Polymorphonuclear Giga/L	7.99 (5.31-11.63)	10.39 (7.20-14.46)	5.69 (4.38-9.49)	6.53 (4.50-8.21)	0.587	11.27 (8.84-15.72)	13.83 (11.47-17.94)	7.88 (5.24-11.58)
Lymphocytes Giga/L	1.14 (0.76-1.73)	1.08 (0.69-1.64)	1.03 (0.62-1.44)	1.36 (0.97-1.97)	0.741	0.92 (0.60-1.45)	0.61 (0.51-0.78)	1.18 (0.74-1.72)
Creatinin microgr/L	74 (59-91)	78 (63-97)	68 (57-88)	70 (56-85)	0.022	82 (66-126)	151 (114-201)	69 (56-87)
Patient's course after ED visit No. (%)					<0.001			
Non admitted	120 (41.2)	39 (26.4)	22 (46.8)	59 (61.5)		12(17.1)	0 (0.0)	30 (38.5)
ICU	22 (7.6)	10 (6.8)	2 (4.3)	10 (10.4)		8 (11.4)	6 (37.5)	3 (3.8)
Medical or surgical wards	149 (51.2)	99 (66.9)	23 (48.9)	27 (28.1)		50 (71.4)	10 (62.5)	45 (57.7)
Deceased at day-30 No. (%)	15 (5.2)	11 (7.4)	1 (2.1)	3 (3.1)	0.195	8 (11.4)	4 (25)	3 (3.8)
Hemoculture No. (%)					<0.001			

(*continued on next page*)

Table 1 (*continued*)

	All (n=291)	Bacterial (n=148)	Viral (n=47)	No infection (n=96)	*p* value	Sepsis2-0 (n=70)	Sepsis3-0 (n=16)	Infection no sepsis (n=78)
Positive	26 (8.9)	19 (12.8)	0	0		14 (20)	5 (31.2)	37 (4.8)
Gram +	14 (53.8)	9 (47.4)	-			6 (43)	2 (40)	25 (66.7)
Gram –	11 (42.3)	9 (47.4)	-			7 (50)	2 (40)	12 (33.3)
Yeast	1 (3.8)	1 (5.2)	-			1 (7)	1 (20)	0
Negative	130 (44.7)	81 (54.7)	29 (61.7)	27 (28.1)		42 (60)	11 (68.8)	42 (53.6)
Not performed	135 (46.4)	48 (32.4)	18 (38.3)	69 (71.9)		14 (20)	0	32 (41.6)
Main sites of infection No. (%)								
Respiratory	84 (28.9)	49 (33.1)	35 (74.5)			19 (27.1)	3 (18.7)	30 (38.5)
Urinary	44 (15.1)	44 (29.7)	0			22 (31.4)	6 (37.5)	16 (20.5)
Pelvi-abdominal	35 (12.0)	28 (18.9)	7 (14.9)			15 (21.4)	4 (25.0)	13 (16.6)
Cutaneous	22 (7.6)	21 (14.2)	1 (2.1)			8 (11.4)	3 (18.7)	3 (3.8)
Head and neck	6 (2.0)	3 (2.0)	3 (6.4)			3 (4.3)	0	0
Neuro-meningeal	1 (0.3)	0	1 (2.1)			0	0	0
Endocarditis	3 (1.0)	3 (2.0)	0			3 (4.3)	0	0

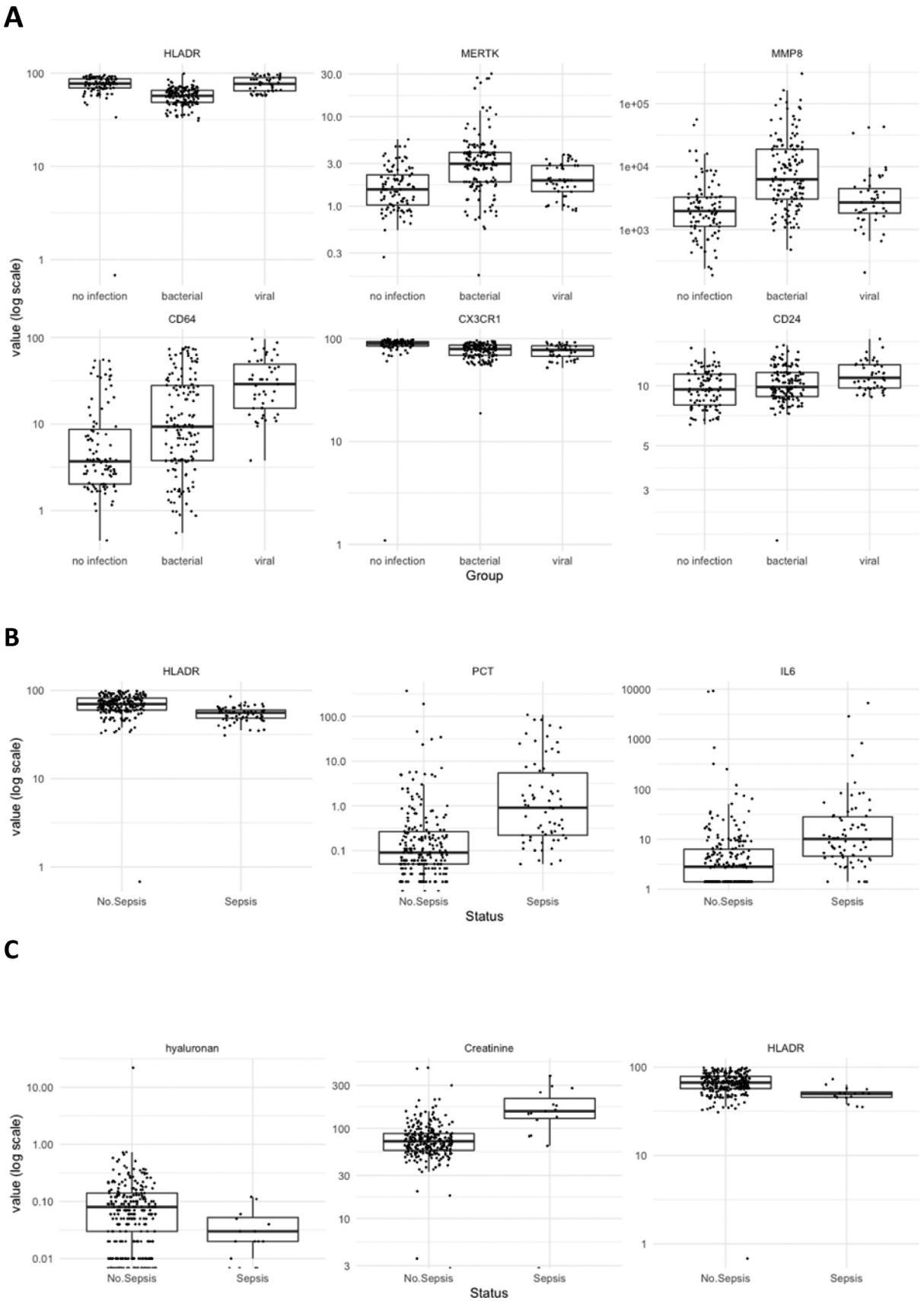

Fig. 2. Individual values of the most relevant biomarkers according to the group of interest: (A) bacterial infection, viral infection, no infection (B) Sepsis-2,no sepsis, (C) Sepsis-3, no sepsis . Box plots indicate the distribution of values and dots the individual values.

Table 2
Statistical performances of different combinations according to each population of interest.
PPV: positive predictive value. NPV: negative predictive value. LR+: positive likelihood ratio, LR-: negative likelihood ratio.

Bacterial infection						
$N = 148$	HLADR-MERTK-MMP8	HLADR-MERTK-CX3CR1	HLADR-MERTK-IL6	HLADR-MERTK-WBC	HLADR-WBC	HLADR-MMP8
Optimal cut-off	0.434	0.356	0.477	0.593	0.393	0.328
Sensitivity	0.88 (0.81–0.93)	0.89 (0.82–0.93)	0.86 (0.79–0.91)	0.80 (0.72–0.86)	0.84 (0.77–0.89)	0.90 (0.84–0.94)
Specificity	0.85 (0.78–0.90)	0.81 (0.74–0.87)	0.85 (0.78–0.90)	0.89 (0.83–0.93)	0.76 (0.69–0.83)	0.76 (0.69–0.83)
PPV	0.85 (0.78–0.90)	0.82 (0.75–0.88)	0.85 (0.78–0.90)	0.88 (0.81–0.93)	0.77 (0.70–0.84)	0.79 (0.71–0.85)
NPV	0.88 (0.81–0.93)	0.88 (0.81–0.93)	0.86 (0.79–0.91)	0.82 (0.75–0.87)	0.83 (0.76–0.89)	0.89 (0.82–0.94)
LR+	5.93 (4.01–8.76)	4.69 (3.35–6.59)	5.79 (3.91–8.56)	7.37 (4.61–11.80)	3.55 (2.63–4.78)	3.82 (2.84–5.12)
LR-	0.14 (0.09–0.22)	0.14 (0.09–0.22)	0.16 (0.11–0.25)	0.23 (0.16–0.32)	0.21 (0.14–0.3)	0.13 (0.08–0.21)
Viral infection $n = 47$	CD64-CX3CR1-CD24	CD64-CX3CR1	CD64-IP10			
Optimal cut-off	0.644	0.713	0.716			
Sensitivity	0.86 (0.79–0.93)	0.76 (0.66–0.84)	0.78 (0.68–0.86)			
Specificity	0.89 (0.76–0.96)	0.96 (0.84–0.99)	0.98 (0.87–1.00)			
PPV	0.94 (0.87–0.98)	0.97 (0.90–0.99)	0.99 (0.92–1.00)			
NPV	0.78 (0.64–0.88)	0.66 (0.54–0.77)	0.69 (0.56–0.79)			
LR+	8.22 (3.58–18.90)	17.87 (4.58–69.68)	36.72 (5.27–256)			
LR-	0.14 (0.08–0.24)	0.25 (0.17–0.36)	0.22 (0.15–0.33)			
Sepsis 2.0 $n = 70$	HLADR-PCT-IL6	IL6-PCT-CRP	HLADR-PCT	HLADR-IL6	HLADR-CRP	HLADR-MERTK
Optimal cut-off	0.779	0.761	0.803	0.769	0.765	0.814
Sensitivity	0.76 (0.70–0.81)	0.76 (0.69–0.8)	0.73 (0.66–0.79)	0.74 (0.67–0.79)	0.75 (0.68–0.80)	0.71 (0.65–0.77)
Specificity	0.87 (0.77–0.94)	0.77 (0.65–0.86)	0.86 (0.75–0.93)	0.89 (0.78–0.95)	0.80 (0.68–0.88)	0.84 (0.73–0.92)
PPV	0.95 (0.90–0.98)	0.91 (0.86–0.95)	0.94 (0.89–0.97)	0.95 (0.91–0.98)	0.92 (0.87–0.96)	0.94 (0.88–0.97)
NPV	0.54 (0.44–0.63)	0.50 (0.41–0.59)	0.50 (0.41–0.59)	0.52 (0.42–0.61)	0.50 (0.41–0.59)	0.48 (0.39–0.57)
LR+	5.91 (3.20–10.93)	3.31 (2.14–5.12)	5.10 (2.86–9.10)	6.45 (3.35–12.45)	3.73 (2.32–6.00)	4.52 (2.61–7.83)
LR-	0.28 (0.21–0.35)	0.32 (0.24–0.41)	0.32 (0.25–0.401	0.30 (0.23–0.38)	0.32 (0.25–0.41)	0.34 (0.27–0.43)
Sepsis 3.0 $n = 16$	hyaluronan-Creatinine-HLADR	hyaluronan-Creatinine	hyaluronan-HLADR			
Optimal cut-off	0.953		0.969	0.949		
Sensitivity	0.81 (0.76–0.85)	0.73 (0.67–0.78)	0.10 (0.07–0.14)			
Specificity	0.94 (0.68–1.00)	0.94 (0.68–1.00)	0.94 (0.68–1.00)			
PPV	1.00 (0.97–1.00)	1.00 (0.97–1.00)	0.96 (0.80–1.00)			
NPV	0.22 (0.13–0.34)	0.17 (0.10–0.27)	0.06 (0.03–0.09)			
LR+	12.92 (1.94–86.24)	11.70 (1.75–78.12)	1.57 (0.23–10.84)			
LR-	0.21 (0.16–0.27)	0.29 (0.23–0.36)	0.96 (0.84–1.10)			

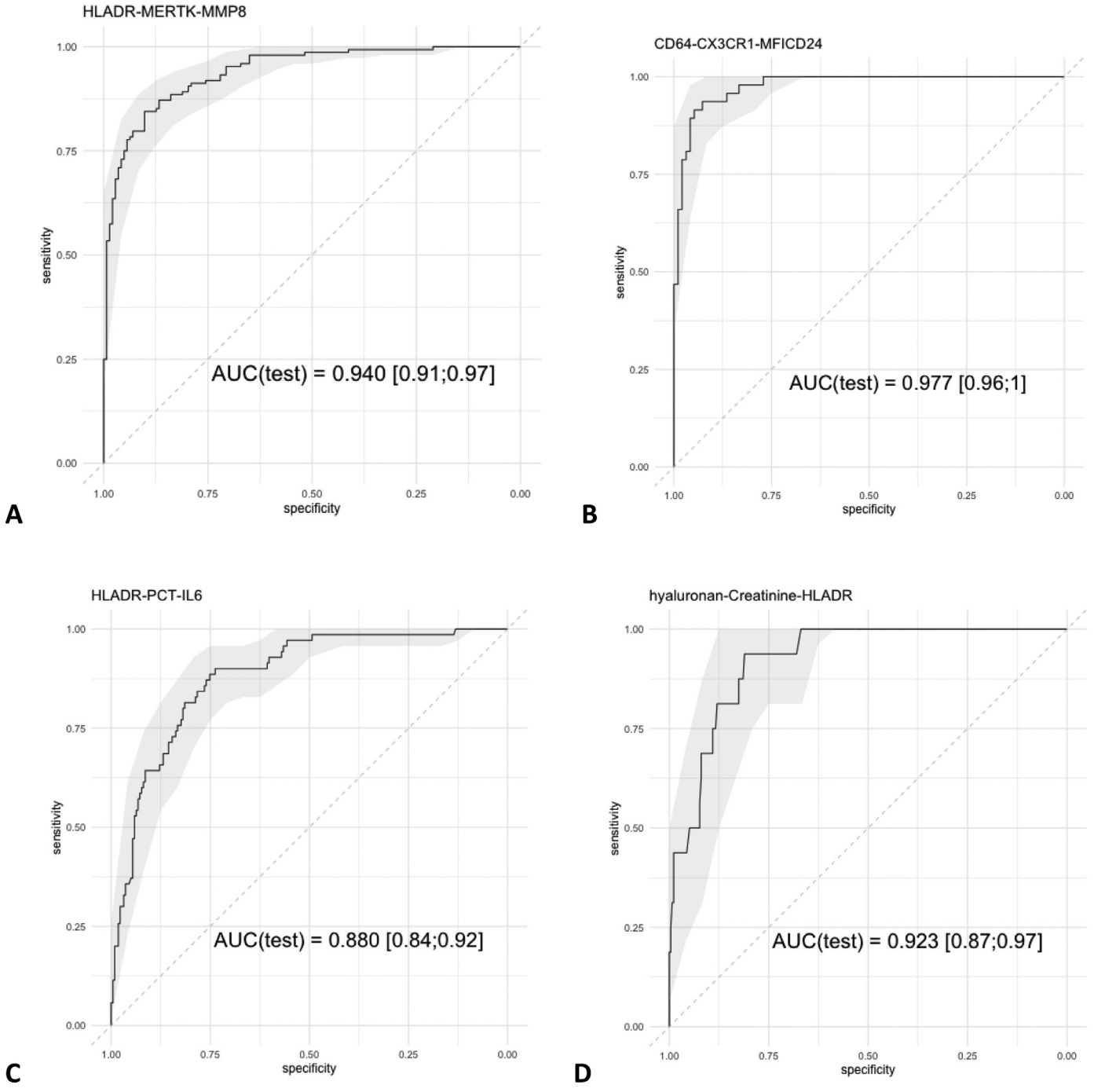

Fig. 3. Receiver Operating Characteristics (ROC) curve and Area Under The Curve (AUC) values for the combination of three biomarkers for the diagnosis of bacterial infection (A), viral infection (B), Sepsis-2 (C) and Sepsis-3 (D).

Discussion

Using a limited number of biomarkers which had previously been individually associated with sepsis diagnosis, we report here a very promising approach for the diagnosis of bacterial infection among ED patients. The association of HLA DR (% on CD14+ monocytes) and MerTK (% on CD66+ neutrophils) resulted in an AUC = 0.92 [0.89;0.95].[17,28] The addition of a third biomarker (MMP8) further improved the AUC at 0.94 [0.91;0.97] with a sensitivity of 0.88 and a specificity of 0.85 (Table 2).[10,29] Despite the fact that a large number of biomarkers have already been reported to be associated with the diagnosis of infection or sepsis, none had sufficient specificity or sensitivity to be routinely used in clinical practice in ED.[4] When all authors unanimously concluded that there was no "magic maker", a combination of biomarkers appeared promising.[2,30] For example, the association of CRP and neutrophil CD64, or the association of PCT, soluble TREM-1 and neutrophil CD64 were shown to be promising.[31,32] However, a large multicenter study including 29 plasma biomarkers, 14 cell surface biomarkers and 10 mRNA failed to find any combination use-

ful for the diagnosis of sepsis-2 among ICU patients.[33] Similarly, Lvovschi et al. failed to identify specific cytokine profiles in ED's patients suspected of sepsis.[34] In addition, a recent study analyzing numerous cell surface biomarkers concluded that no combination had clinically relevant predictive value for the diagnosis of sepsis among patients with suspected acute infection.[35] This apparent discrepancy with our results may be explained by the population studied (ED's patients suspected of acute infection, compared to no infection but also to ICU patients with sepsis), the adjudication according to sepsis-3 criteria only, and the choice of logistic regression instead of gradient boosting tree analysis. However, it is interesting to note that Shankar-Hari et al. also identified HLA-DR as part of the best combination biomarker of sepsis (although not clinically relevant), as we report here.[35]

It should be noted that most studies carried out for the diagnosis of sepsis have been performed in ICU, in patients with high inflammatory states. For these patients, the occurrence of infection could be hidden within a storm of inflammatory biomarkers and highly altered expression of cell-surface makers.

Implementing sepsis-3 definition in ED is questionable because it only identifies a small proportion of infected patients (roughly those who were classified into severe sepsis in sepsis-2) and by definition those with ongoing organ failure, who are usually already flagged by ED physicians.[36–38] Much more challenging is the accurate identification of patients with bacterial infection which is fundamental for sepsis screening and for improving antibiotic stewardship. Interestingly, among the patients classified in the non-bacterial infection group, thanks to the first combination of biomarkers, we were able to identify those who had a viral infection. This was achieved with the association of CD64 and CX3CR1 (AUC=0.95 [0.92;0.99]), two biomarkers previously known to be associated with bacterial sepsis.[16,39] The addition of a third biomarker on neutrophil (MFI of CD24)[40] produced an AUC of 0.98 [0.96;1]. The sensitivity of this combination of three biomarkers was 0.86 and its specificity 0.89 (Table 2). To our knowledge, very few studies have reported combinations that allow accurate discrimination between bacterial and viral infection. Oved and al. have reported the measurement of three plasma markers (CRP, TRAIL, and CXCL10)[41] and Shapiro et al. recently reported a high accuracy of the combination of point of care CRP and myxovirus resistance protein A measurement, to differentiate bacterial from viral acute upper respiratory infections.[42] Surprinsingly, IFN-alpha did not emerge as a biomarker of interest following the statistical combinatory approach in our study, although it had been previously reported in case of viral infection.[26,43] While the combination of gene expressions has led to numerous positive investigations for the diagnosis of bacterial infection, one study revealed that a set of seven genes could allow a robust discrimination between bacterial and viral infection.[44–46]

Since our study was planned before the Sepsis-3 definition became published, the patients were initially classified according to Sepsis-2. Seventy patients met the sepsis-2 definition. The best predictive combination included some well known biomarkers of sepsis, HLA-DR, PCT and IL-6.[13,47] Regarding the classification of patients according to the Sepsis-3 definition ($n = 16$), the best combination was HLA-DR together with hyaluronan and creatinine.[48,49] However, this result must be interpreted with cautious, due to the small number of sepsis-3 cases an need further confirmation in a larger cohort.

We acknowledge several limitations in our study. First, this is a monocentric study, and the identification of these biomarkers combinations may not be applied elsewhere. Nevertheless, our population is diverse and representative of large urban ED's recruitment. Second, the biomarker selection was not exhaustive and several other candidates as sTREM-1 and proADM may have been interesting to study. Third, as every study on infection and sepsis, we acknowledge that the perfect gold standard for adjudication does not exist and because a systematic microbiological documentation is not available, we cannot exclude misclassification despite three adjudicators and one arbitrator. Fourth, given the number of biomarker combinations studied, we cannot exclude over-fitting even with cross-validation. Fifth, there is no validation cohort in our study, but a study is planned to confirm the validity of our biomarker combinations. Lastly, if the adjudicators were blind for the almost entire panel of biomarkers tested, this was not possible for those performed routinely like CRP and PCT. However, as these two markers did not emerged in our best combinations for bacterial/viral discrimination, we think that this bias was moderate to negligible.

Conclusion

So far, this study is one of the first published that allows the diagnosis of bacterial infection and of viral infection in ED' patients. Because new technologies are rapidly developing, a combination of cell surface biomarkers and plasma biomarkers should not face technical barriers to achieve diagnosis at the bedside.[50,51]. Given the number of biomarker combinations studied in this small sample of patients from one center, it will be important to validate these findings in a subsequent population of patients.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by Comité de protection des personnes-Ile de France VI on January 16th 2016

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Authors contributions

LV, PH and JMC contributed to the study conception and design. Material preparation and data collection were performed by LV. LV and CF performed the biomarker's measurements. SV and FS were in charge of the statistical analysis. DAG, JM and GM were in charge of the review and adjudication of each patient's medical chart. The first draft of the manuscript was written by PH, LV and JMC and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

Declaration of Competing Interest

PH has received educational and congress lectures honorarium from ThermoFisher, Radiometer, Beckman Coulter and bioMérieux. FS is employed by Biorad. All other authors have no conflict interest to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.02.019.

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