Identification of a panel of serum protein markers in early stage of pediatric sepsis and its validation in a cohort of patients

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Sepsis is defined as a life-threatening organ dysfunction caused by a deregulated host response to microbial infection. Despite advances in health care, sepsis remains a major problem due to its high prevalence and mortality. Early diagnosis and initiation of treatment are considered critical to reduce mortality. The current biomarkers used in clinical practice (Procalcitonina, C reactive protein, among others) are not sensitive enough for sepsis diagnosis since they are usually deregulated in other inflammatory diseases. New biomarkers can help in the early diagnosis of sepsis and predict prognosis. With the aim of finding proteins associated with sepsis, serum protein profile was compared between patients and healthy donors.

METHODS

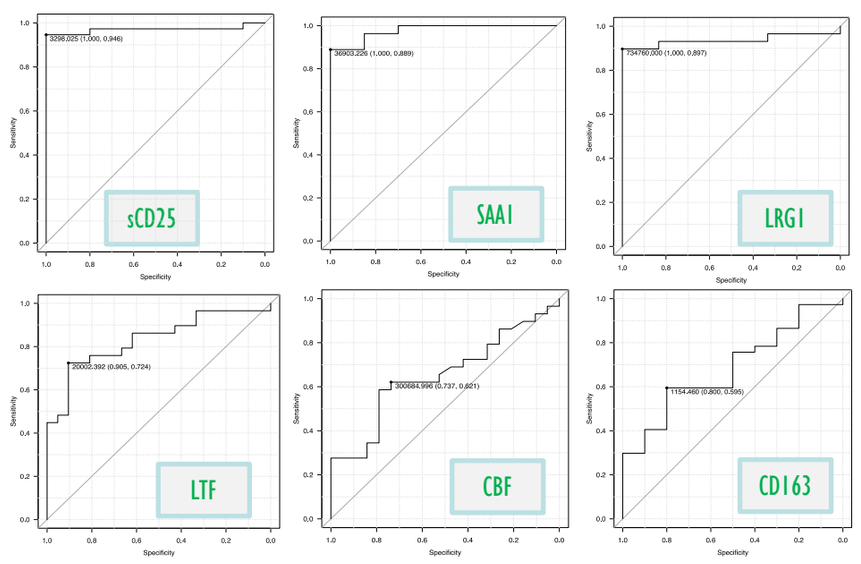
Prospective study of a single center. Patients with sepsis or septic shock admitted to PICU and healthy donors were selected.
Patients with immunological diseases (oncologic or transplanted patients) were excluded from the study.
Identification of the proteins was carried out by mass spectrometry and their validation was performed by Enzyme-linked Immunosorbent Assay (ELISA).
Only proteins with at least two peptides and an ANOVA p-value <0.05 and a ratio > 2 (Max fold change) in either direction were selected for further analyses.
Next step was to know in which biological processes identified proteins were involved. For this purpose, STRING search engine (http://string-db.org) and Gene Ontology with the highest confidence (0.9) were used. The proteins that participated in immunological or infectious processes were selected to carry on. Then, a bibliographic review was made to select the proteins to validate.
Bibliographic search was conducted in PubMed.
This study was approved by the Ethics Committee of the Basque Country and written informed consent was obtained.

RESULTS

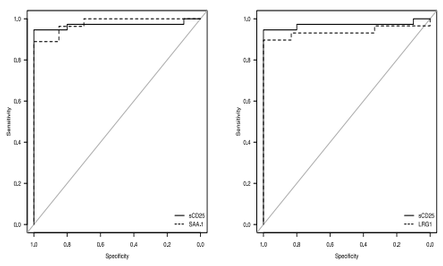
Forty pediatric patients diagnosed with sepsis or septic shock (45% male and 55% female) and twenty-four healthy donors (50% male and 50% female) were selected for this study.
For the proteomic analysis, 15 serum samples were selected to work with in this study: 10 from septic patients and 5 from healthy donors.
Mass-spectrometry analysis revealed 44 significant deregulated proteins between patients and healthy donors with an ANOVA p-value < 0.05 and Max fold change > 2. Table I
After applying the selection criteria, 4 proteins were selected for the validation process: Lactoferrin (LTF), Serum Amyloid A1 (SAA-1), Leucine-Rich alpha-2-Glycoprotein (LRG1) and Complement Factor B (CFB).
Moreover, interleukin-2 receptor a-chain (CD25) and scavenger receptor cysteine-rich-type-1 (CD163) were analyzed in the same cohort of patients and healthy donors.
All these proteins were upregulated in septic patients´ serum when they were compared with healthy donors´ serum with the exception of CFB. Further analysis revealed that SAA-1, LRG1 and sCD25 have high sensitivity and specificity with a high area under the receiver operating characteristic that make them promising biomarkers in the diagnosis of pediatric sepsis. Figure I

Proteins	ANOVA p	Max fold change	Highest mean condition	Description
LBP	2,60E-09	29,31262596	Sepsis	Lipopolysaccharide-binding protein
A2GL	3,20E-08	4,426431966	Sepsis	Leucine-rich alpha-2-glycoprotein
CRP	8,14E-08	30,41797734	Sepsis	C-reactive protein
SAA2	7,47E-07	84,27765527	Sepsis	Serum amyloid A-2 protein
IPSP	1,21E-06	5,010881524	Control	Plasma serine protease inhibitor
FINC	9,13E-06	8,758802062	Control	Fibronectin
HBB	1,11E-05	25,27836532	Sepsis	Hemoglobin subunit beta
AACT	1,88E-05	5,28365135	Sepsis	Alpha-1-antichymotrypsin
SAA1	2,97E-05	76,09708591	Sepsis	Serum amyloid A-1 protein
HBA	3,78E-05	16,39969014	Sepsis	Hemoglobin subunit alpha
CATA	5,92E-05	7,286451137	Sepsis	Catalase
HABP2	0,000159	2,179074926	Control	Hyaluronan-binding protein 2
TTHY	0,000214	2,365904777	Control	Transthyretin
CAH1	0,000233	8,950113813	Sepsis	Carbonic anhydrase 1
CFAB	0,000336	2,04889514	Sepsis	Complement factor B
LYAM1	0,000400	2,675522882	Sepsis	L-selectin
NGAL	0,000556	7,169256836	Sepsis	Neutrophil gelatinase-associated lipocalin
B2MG	0,000752	3,486170968	Sepsis	Beta-2-microglobulin
FA12	0,000830	3,237084548	Control	Coagulation factor XII
CD14	0,000870	2,784850681	Sepsis	Monocyte differentiation antigen CD14
FIBG	0,000995	5,317048543	Sepsis	Fibrinogen gamma chain
TRFL	0,001180	9,346009409	Sepsis	Lactotransferrin
ITIH3	0,001239	2,192084305	Sepsis	Inter-alpha-trypsin inhibitor heavy chain H3
PRDX2	0,001879	7,267809361	Sepsis	Peroxiredoxin-2
A1AG1	0,002062	2,941180349	Sepsis	Alpha-1-acid glycoprotein 1
FIBB	0,002142	7,926360983	Sepsis	Fibrinogen beta chain
APOA4	0,002251	3,062538098	Control	Apolipoprotein A-IV
RET4	0,002343	2,804231401	Control	Retinol-binding protein 4
PLMN	0,002494	2,124858228	Control	Plasminogen
SHBG	0,004819	2,274379563	Sepsis	Sex hormone-binding globulin
SPRC	0,005756	2,146585316	Control	SPARC
FETUB	0,007872	2,061182556	Control	Fetuin-B
HPT	0,008057	9,390239114	Sepsis	Haptoglobin
PTX3	0,008478	8,831503885	Sepsis	Pentraxin-related protein PTX3
6PGD	0,008556	3,129852553	Sepsis	6-phosphogluconate dehydrogenase, decarboxylating
ZPI	0,011271	2,795039962	Sepsis	Protein Z-dependent protease inhibitor
PLSL	0,016815	2,81289335	Sepsis	Plastin-2
ACTB	0,019984	3,0241456	Sepsis	Actin, cytoplasmic
POSTN	0,023622	2,072017036	Sepsis	Periostin
CBPN	0,026209	2,40257809	Sepsis	Carboxypeptidase N catalytic chain
APOC1	0,026858	2,093199286	Control	Apolipoprotein C-I
LDHA	0,029134	2,57915749	Sepsis	L-lactate dehydrogenase A chain
CD5L	0,033286	6,760591147	Control	CD5 antigen-like
ALDOB	0,037227	5,740725522	Sepsis	Fructose-bisphosphate aldolase B

Table I. deregulated proteins between patients and healthy donors



sCD25: 0.97 (IC95%: 0.92-1). SAA1 0.98 [(IC 95%: 0.94-1.
LRG1: 0.94 (IC 955: 0,85-1). LTF 0.83 (iC 95%: 0.71-0.94)
CBF 0.65 (IC 95%: 0.49-0.8). CD163: 0.68 (IC 95%: 0,51-0.85)



sCD25-SAA1 sCD25-LRG1

Figure I. Area under the ROC curve

CONCLUSIONS

- Mass spectrometry analysis gave a set of 44 deregulated proteins between septic patients and healthy donors
- sCD25, LRG1 and SAA-1 are upregulated in septic patients´ serum when compared with healthy donors´ serum. They show an excellent value of the area under the ROC curve
- We have identified a panel of three potential biomarkers, biologically connected and validated, in a group of pediatric patients with sepsis, whose analysis could be considered as a complementary tool for the diagnosis of sepsis