PATHFAST Presepsin assay for early diagnosis of bacterial infections in surgical patients. Preliminary study.

G. Novelli; V. Morabito; G. Ferretti; F. Pugliese; F. Ruberto; M. Rossi; P.B. Berloco

Department of General Surgery and Organ Transplant; Sapienza University of Rome

INTRODUCTION

Surgical patients are vulnerable to infectious complications during hospitalization because of several factors. Sepsis seems to be a common complication in the postoperative period, and prompt recognition and early intervention are effective ways of reducing mortality in this condition. Various biomarkers have been studied for diagnosing bacterial infections with aim to stop sepsis cascade. Presepsin, which is approximately 13 kDa, has been identified as a protein whose levels increase specifically in the blood of sepsis patients. Additionally, the measurement of presepsin concentrations is useful for evaluating the severity of infection and also for monitoring the clinical responses to therapeutic interventions. In this study, we evaluated the analytical and clinical performance of PATHFAST Presepsin assay system based on the chemiluminescent enzyme immunoassay (CLEIA) principle and its usefulness in the early diagnosis of infection in surgical patients.

MATERIALS AND METHODS

We studied 30 adult patients who underwent surgery between November 2011 and January 2012. During this period 17 organ transplants with a graft from cadaveric donor (8 liver transplant, 8 kidney transplant and 1 lung transplant) and 13 abdominal surgical patients were included in this study. Mean age of patients was 53,3 years[range 19-70 yrs], 13 female and 17 male (Table 1). All patients received prophylactic antibiotics (ampicillin sodium and the beta-lactamase inhibitor sulbactam sodium 3g/die) for 24 hours peri-operative. The heparinized whole blood for PATHFAST Presepsin (PFP) assay was used in the evaluation at 48 hours after surgery [T0]. The PFP was repeated at 48h [T1]; at 96h [T2]; at 144h [T3] than at 15 days [T4] for monitoring the clinical responses to therapeutic interventions. Blood cultures were performed in all patients at moment that PFP test was performed. Transplant recipients received a triple regimen as primary immunosuppressive therapy, including calcineurin inhibitor, corticosteroids and mycophenolate mofetil.

PATHFAST Presepsin (PFP)

The PFP assay contains magnetic particles coated with mouse monoclonal antibodies and alkaline phosphatase (ALP)-labeled rabbit polyclonal antibodies. Presepsin in the specimen binds to the anti-presepsin antibodies to form an immunocomplex with the ALP-labeled antibodies and the antibody-coated magnetic particles. After removal of the unbound ALP-labeled antibodies, a chemiluminescent substrate was added to the immunocomplex. After a short incubation period, the luminescence generated by the enzyme reaction was detected in order to calculate the concentration of presepsin in the samples. The assay time was 15 min using a sample volume of 100 µl. The entire procedure was automatically performed on the PATHFAST analyzer. Heparinized whole blood samples were collected from 30 surgical patients and immediately assayed with the PATHFAST Presepsin assay. ***A value > 377 pg/mL was considered positive..***

TABLE 1. BACKGROUND OF PATIENTS AND UNDERLYING CAUSES OF SURGERY

	Underlying surgery	PFP (pg/mL)	WBC (10^3cells/mm^3)	Sintomatology
pt 1	Lung transplant	1245	16268	present
pt 2	Liver transplant	2376	3700	absent
pt 3	Liver transplant	1710	10152	absent
pt 4	Kidney transplant	2592	5560	absent
pt 5	Kidney transplant	2967	9980	absent
pt 6	Liver transplant	10068	7790	present
pt 7	Kidney transplant	4335	12995	present
pt 8	Kidney transplant	1971	7100	absent
pt 9	Kidney transplant	1718	5800	absent
pt 10	Kidney transplant	1495	5130	absent
pt 11	Liver transplant	958	10920	absent
pt 12	Liver transplant	3251	16970	present
pt 13	Liver transplant	894	7590	absent
pt 14	Kidney transplant	991	20430	absent
pt 15	Liver transplant	1380	8630	absent
pt 16	Kidney transplant	9544	22580	present
pt 17	Liver transplant	950	14890	present
pt 18	Hepatic Resection*	*255*	3510	/
pt 19	Hepatic Resection*	*350*	4012	/
pt 20	Hepatic Resection*	*370*	8790	/
pt 21	Hemycolectomy	*295*	7810	/
pt 22	Hemycolectomy	*275*	5225	/
pt 23	Hepatic Resection^	916	13255	present
pt 24	Hemycolectomy	523	10880	present
pt25	Splenectomy	495	9880	absent
pt 26	Hepatic Resection*	578	6985	present
pt 27	Pancreaticoduodenectomy	1005	13225	present
pt 28	Pancreaticoduodenectomy	1615	14125	present
pt 29	Nefrectomia$^{\surd}$	20000	22680	present
pt 30	Hepatic Resection§	6066	2970	absent

Note. PFP : Pathfast Presepsin test. *A value < 377 pg/mL was considered negative (5 patients);* WBC : white blood count ; *Hepatic resection for metastasis; ^ Hepatic resection for Bile Duct Cancer; § Hepatic resection for hepatocellular carcinoma in cirrhosis Virus C related; $^{\surd}$ nefrectomia for polycystic disease.

RESULTS

At [T0] the mean Presepsin level (Pl) in the 30 patients was 3062,77 pg/mL[range 255-20000 pg/mL] (table 2). In particular, in transplant patients Pl was 3034,43± 2880,791 pg/mL[range 894-10000 pg/mL]. All the transplant patients resulted positive at PFP test. Meropenem (2g/die) and vancomicine (2g/die) were administered to the 25 test positive patients as initial empiric antibiotic treatment because in our clinical experience Pseudomonas A., Escherichia Coli and Enterococcus represent the most frequently occurring pathogens. When the test was performed, 65% transplant patients showed no signs or symptoms of infection. Presepsin level at [T1], [T2] and [T3] remained stable in six transplant patients. While, in five abdominal surgical patients, the PFP test resulted negative [mean value 325 pg/mL; range (255-370 pg/mL)]. These data were confirmed by negative blood coltures. The PFP test was positive in the eight remaining patients with Pl mean value 4963±7653,88 pg/mL [range 578-20000 pg/mL] with absence of signs or symptoms of infection in 25% of patients. Presepsin level at [T1], [T2] and [T3] remained stable in three abdominal surgical patients. Nine (36%) of these 25 patients did not respond to this treatment and after antibiogramme results, the antibiotic therapy was modified. Microbiological findings confirmed the presence of bacterial infections within 69±2,5h from enrolment. In the remaining eight patients more time [T4] was necessary to low Pl below threshold levels of 337 pg/mL. In the other 16 (64%) patients, where initial empiric antibiotic therapy was maintained, Pl values began to decrease at [T1] and fell below threshold level at [T3]. One lung transplant [Pt1] and one kidney transplant patient[Pt16] died of sepsis where Pl value had increased.

TABLE 2. PRESEPSIN LEVEL (PG/ML) DURING THE PERIOD OF EVALUATION AND OUTCOME AT 15 DAYS

Patient	PFP (T1)	PFP (T2)	PFP (T3)	PFP (T4)	Outcome
Pt 1	2845	/	/	/	*dead*
Pt 2	971	920	350	/	alive
Pt 3	1599	961	375	/	alive
Pt 4	2671	2591	2527	355	alive
Pt 5	2307	1786	295	/	alive
Pt 6	12384	7635	2550	360	alive
Pt 7	4200	4260	3970	370	alive
Pt 8	1220	658	325	/	alive
Pt 9	952	458	225	/	alive
Pt 10	1291	995	370	/	alive
Pt 11	935	725	325	/	alive
Pt 12	3255	2850	2255	347	alive
Pt 13	764	550	317	/	alive
Pt 14	781	560	330	/	alive
Pt 15	1055	788	415	200	alive
Pt 16	9987	9955	9588	10500	*dead*
Pt 17	625	485	274	/	alive
Pt 23	444	275	/	/	alive
Pt 24	488	399	250	/	alive
Pt 25	421	388	244	/	alive
Pt 26	518	389	266	/	alive
Pt 27	615	504	395	145	alive
Pt 28	1022	970	528	277	alive
Pt 29	19888	20577	9844	365	alive
Pt 30	6000	5998	2015	310	alive

CONCLUSIONS

Early diagnosis is essential to improving the results of treatment of infections in particular in transplant recipients where infection represents one of the primary barriers to successful organ transplantation. PFP test highlighted a complete sensitivity(100%) in showing the presence of infection in a very short time (15 min), confirmed by the results of positive blood cultures. A greater number of patients is necessary to confirm these data