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

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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 regime 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.

RESULTS
At [T0] the mean Presepsin level (PI) in the 30 patients was 3062.77 pg/mL [range 255-20000 pg/mL] (Table 2). In particular, in transplant patients PI was 3034.43± 2880.79 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 cultures. The PFP test was positive in the eight remaining patients with PI 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 PI below threshold levels of 337 pg/mL. In the other 16 (64%) patients, when initial empiric antibiotic therapy was maintained, PI 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 PI value had increased.

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.