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A preliminary study of paraoxonase-1 in infected patients with an indwelling central venous catheter

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ABSTRACT

Objectives: Identification of biochemical markers to diagnose bloodstream infections in patients with a central venous catheter (CVC) inserted is an active research pursuit. Paraoxonase-1 (PON1) is an enzyme participating in the innate immune system protecting against toxic substances and infectious agents. We investigated the relationships between serum PON1 alterations and the characteristics of infection in a group of patients with a CVC implant.

Methods: Patients (n = 114) who had had an inserted CVC removed because of infection or because the usefulness was at an end, and 407 healthy volunteers were recruited. In all participants we measured serum PON1 lactonase and paraoxonase activities, PON1 concentration and genetic polymorphisms, together with levels of the chemokine (C–C motif) ligand 2 (CCL2), procalcitonin and C-reactive protein (CRP).

Results: Patients with an acute concomitant infection (ACI) had higher CCL2, CRP and procalcitonin concentrations than the control group, together with lower paraoxonase and lactonase activities and specific activities. The areas under the curve of the receiver operating characteristic plots for paraoxonase and lactonase specific activities in the discrimination between patients with or without and ACI were 0.81 (0.73–0.89) and 0.81 (0.71–0.89), respectively, indicating the high diagnostic accuracy of these parameters.

Conclusion: This preliminary study suggests that the measurement of PON1 may be useful as a tool for the diagnosis of ACI in patients with an indwelling CVC.

1. Introduction

The use of central venous catheters (CVC) is ubiquitous in hospital care worldwide. These devices provide vascular access for the extraction of blood samples for analyses, infusion of intravenous drugs, parenteral nutrition, access for hemodialysis, and for hemodynamic monitoring. However, their use carries the risk of bloodstream infection, which is associated with increased morbidity, mortality and healthcare costs [1]. The conventional approach to the management of such infections involves decision-making regarding the removal of the indwelling catheter and the implementation of intravenous antibiotic therapy. Unfortunately, diagnosis of bloodstream infection is often complicated by symptoms such as fever, chills and hypotension, which are non-specific [2]. Therefore, to identify biochemical markers able to diagnose bloodstream infections accurately in patients with a CVC inserted is imperative, and constitutes an active line of research. Several studies have proposed C-reactive protein (CRP) or procalcitonin as useful markers of sepsis. However, their utility varies depending on the clinical setting, and this is still an unresolved issue [3].

The paraoxonases (PON) are a group of three lactonases (PON1, PON2, and PON3) ubiquitously expressed in human tissues, with antioxidant and anti-inflammatory properties. While PON2 is exclusively intracellular, PON1 and PON3 are also found in the circulation bound to high-density lipoproteins. PON1 degrades oxidized lipids in low-density lipoproteins and inhibits the synthesis of the chemokine

Abbreviations: ACI, acute concomitant infection; AUROC, areas under the curve of receiver operating characteristic; CCL2, chemokine (C–C motif) ligand 2; CVC, central venous catheter; CRI, catheter-related infection; CRP, C-reactive protein; DTNB, 5,5′-dithio-bis-2-nitrobenzoic acid; EDTA, ethylene diamine tetraacetate; ELISA, enzyme-linked immunosorbent assay; HICPAC-CDC, Healthcare Infection Control Practices Advisory Committee of the Center for Disease Control and Prevention; ICU, intensive care unit; PON, paraoxonase; ROC, receiver operating characteristic; SPSS, Statistical Package for Social Sciences; TBBL, 5-thiobutyl butyrolactone.

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(C–C motif) ligand 2 (CCL2), a pro-inflammatory molecule that attracts monocytes to the inflammatory sites and induces their differentiation to macrophages [4]. Schweikert et al. [5] suggested that PON2 and PON3 are an important part of our innate defense system against, for example, *Pseudomonas aeruginosa*. Moreover, PON2-deficient mice have a higher sensitivity to bacterial infection than wild-type mice [6,7]. Several lines of evidence suggest that PON1 participates in the protection conferred by high-density lipoproteins against different infectious agents, including bacteria [8,9] and viruses [10,11]. Overall, these results indicate that the PON family of proteins can be considered part of the innate immunity system [12].

Our study sought to characterize the alterations of PON1 levels in the circulation in patients with an implanted CVC, to relate them to their clinical and biochemical characteristics, and to investigate the potential utility of these parameters as biomarkers for the diagnosis of infection.

2. Materials and methods

2.1. Ethics approval

The study was approved by the Ethics Committee (Institutional Review Board) of the Hospital Universitari de Sant Joan. All the participants provided written informed consent to participation in the study on the understanding that anonymity of data was guaranteed.

2.2. Participants

We prospectively recruited, between March 2011 and June 2013, a total of 114 patients who had had an indwelling CVC removed because of infection, or because it was no longer needed according to the criterion of the attending physician. Exclusion criteria were: <18 years of age, severe alcoholism, psychiatric diseases, or liver impairment. Our protocol for management of an indwelling CVC is based on the recommendations of the Healthcare Infection Control Practices Advisory Committee of the Center for Disease Control and Prevention (HICPAC-CDC, 2002) [13]. At the time of catheter removal, a blood sample was obtained for biochemical and genetic analyses, and the catheter tip was cultured for microbiological analyses. The participants' medical records were reviewed and pertinent demographic data, comorbidities, bacteriologic and therapeutic data were recorded. Data on the patients' local and general infection-related clinical manifestations were also collected, as well as the presence of other acute or chronic infections. The type of treatment received in the 24 h before the present study and the appearance of neoplasia after a 6 months follow-up, were recorded. The McCabe classification [14] and the Charlson comorbidity index [15] were recorded in all patients. Of the participants, 38 (33.3%) were hospitalized for surgery-related reasons and 22 (19.3%) for an infectious disease. The location of the catheter tip was brachial in 51 patients (44.7%), subclavian in 51 (44.7%), jugular in 9 (7.9%), and femoral in 3 (2.6%).

Patients with an acute concomitant infection (ACI) were those suffering from an infection (abdominal abscess, pneumonia, etc.) that was not related to an infected catheter. Twenty-two (19.3%) patients had an ACI without a catheter-related infection (CRI), 9 (7.9%) patients had a CRI without an ACI, and 14 (12.3%) patients had both infections simultaneously.

The control group consisted of 407 healthy volunteers who participated in an epidemiological study conducted in our geographical area, the details of which have been previously reported [16]. These subjects had no clinical or biochemical evidence of renal insufficiency, liver disease, neoplasia or neurological disorders.

2.3. Biochemical analyses

The physiological substrate, or substrates, for PON1 have not, as yet, been identified. Since PON1 has lactonase and esterase activities [4], we decided, in the present study, to measure the catalytic activity of PON1 using two different substrates: 5-thiobutyl butyrolactone (TBBL, a synthetic lactone) and paraoxon (an ester), as previously described [17]. Briefly, TBBLase activity was measured in an assay reagent containing 1 mM $CaCl_2$, 0.25 mM TBBL and 0.5 mM 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) in 0.05 mM Tris–HCl buffer, pH = 8.0. The change in absorbance was monitored at 412 nm. Activities were expressed as U/L (1 U = 1 mmol of TBBL hydrolyzed per minute). Serum PON1 paraoxonase activity was determined as the rate of hydrolysis of paraoxon at 410 nm and 37 °C in a 0.05 mM glycine buffer, pH 10.5 with 1 mM $CaCl_2$ [16]. Activities were expressed as U/L (1 U = 1 μmol of paraoxon hydrolyzed per minute). Serum PON1 concentrations were determined by an in-house enzyme-linked immunosorbent assay (ELISA) with a rabbit polyclonal antibody generated against the synthetic peptide CRNHQSSYQTRLNALREVQ which is a sequence specific for mature PON1 [18]. PON1 specific activities were calculated as the ratio between the activity and the corresponding concentration. The serum concentration of C-reactive protein (CRP) was measured using a high sensitivity method (Horiba ABX, Montpellier, France). The serum concentration of procalcitonin and the EDTA-plasma concentration of CCL2 were measured by ELISA (Biovendor, Brno, Czech Republic, and Prepotech, London, UK, respectively).

2.4. PON1 genotyping

Serum PON1 paraoxonase activity is strongly determined by the enzyme genotype [17]. Several polymorphisms in the promoter and the coding regions of the *PON1* gene have been described and, in the present study, we chose to analyze the polymorphisms Arg/Gln at position 192 ($PON1_{192}$, with two alleles termed Q and R), and the polymorphism Leu/Met at position 55 ($PON1_{55}$, with two alleles termed L and M). $PON1_{192}$ is strongly associated with the enzyme's activity and $PON1_{55}$ is a surrogate of the $PON1_{-108}$ promoter polymorphism [4]. For polymorphism analyses, genomic DNA was obtained from leukocytes (Puregene DNA Isolation reagent set, Gentra Systems Inc., Minneapolis, MN, USA), and the chosen polymorphisms were analyzed by the Iplex Gold MassArray™ method (Sequenom Inc., San Diego, CA, USA).

2.5. Microbiology analyses

Catheter tips were cultured by the semi-quantitative method of Maki et al. [19]. Patients were classified according to clinical and microbiological criteria [20,21] as follows: “uninfected”, when there was no evidence of infection and catheter cultures were negative (CRI = 0); “localized catheter colonization”, when a significant growth of a microorganism (>15 colony-forming units) from the catheter tip was positive but without clinical evidence of infection (CRI = 1); “exit site infection” when there was erythema or induration within 2 cm of the catheter exit site (CRI = 2); “catheter-related bloodstream infection”, when catheter culture and blood culture tested positive for the same microorganism (CRI = 3). Subsequently, the identification and susceptibility testing of the culture isolate was performed by automated microdilution (MicroScan WalkAway®, Siemens Healthcare, Erlangen, Germany) and/or disk diffusion method and complementary biochemical tests, depending on the type of microorganism.

2.6. Statistical analyses

All calculations were performed with the SPSS 22.0 statistical package (SPSS Inc., Chicago, IL, USA). Differences between two groups were assessed with the Mann–Whitney *U* test, since most of the studied variables had non-parametric distributions. Differences between more than two groups were analyzed by the Kruskal–Wallis test. We fitted two binary logistic regression analyses to identify those variables independently associated with the presence of an ACI. One of the models included paraoxonase specific activity, and the other included TBBLase