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Interleukin-6 serum level assessment using a new qualitative point-of-care test in sepsis: A comparison with ELISA measurements

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Abstract

Objectives: Interleukin-6 (IL-6) is involved in inflammatory diseases, provides prognostical information, and allows the early identification and monitoring of septic patients. We investigated whether IL-6 can be assessed using a new densitometric point-of-care (POC) bedside assay.

Design and methods: 392 samples were prospectively collected from 57 intensive care unit patients (38 male, age: 45.2 ± 16.9years, APACHE II score: 25.4 ± 4.8). Blinded IL-6 measurements were performed using conventional semiautomatic enzyme-linked immunosorbent assays (ELISA) and the POC test.

Results: Mean IL-6 levels were 102.9 ± 388.6pg/mL (ELISA) and 97.0 ± 535.5 (POC). A significant correlation was found ($p < 0.0001$, $r = 0.92$). The sensitivity/specificity for sepsis was 82.6%/86.5% (ELISA, AUC: 0.881), and 76.4%/95.0% (POC, AUC: 0.868).

Conclusions: Here we demonstrate significant correlations of IL-6 levels determined using a POC test and semiautomatic ELISA. ROC analyses revealed no significant differences between the two tests. With a turn-around time of 20min, the bedside IL-6 test is a new tool that may help to initiate early goal-directed therapy.

Keywords: Inflammation; Immune system; Tissue injury; Severe sepsis; Septic shock

Introduction

Sepsis is a leading cause of death in the critically ill. Incidence rates of severe sepsis and septic shock are rising, and mortality rates still exceed 30% [1–4]. Comorbidities further aggravate these devastating numbers. Pathogenetically, endogenous and exogenous products trigger cascades of immunological host responses [5]. Usually, the first systemic response consists of excess pro-inflammatory cytokine release [2,5,6], which eventually amplifies the inflammatory process.

Interleukin-6 (IL-6), a potent pleiotropic cytokine, is considered a key player in the septic scenario and is among the earliest mediators released. IL-6 is a member of the glycoprotein (gp) 130 receptor ligand family [7] whose cDNA was identified in 1986 [8] and is expressed mainly by monocytes/macrophages, endothelial cells, keratinocytes, and fibroblasts [9]. IL-6 is crucial for the initiation of an innate immune response and propagates acquired immune responses through induction of T-cell activation and proliferation as well as B-cell differentiation [8,9]. Furthermore, IL-6 is a potent pyrogen that stimulates a wide spectrum of acute phase proteins in human hepatocytes [8,9]. IL-6 has been shown to be rapidly released with peak serum levels being reached within 2h under experimental conditions [8]. Thus, IL-6 serum levels are often elevated before the onset of clinical symptoms and before routine laboratory tests such as measurement of high-sensitivity C-reactive protein (hsCRP) turn positive [10,11]. Owing to its

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short half-life, human serum IL-6 levels change rapidly [12]. Therefore, IL-6 may also be a useful adjunct to indices such as procalcitonin and white blood cell count in the longitudinal monitoring of patients with sepsis.

Clinically, a growing body of data suggests that early identification and early administration of supportive therapy are of pivotal importance in the management of septic patients [13,14]. This approach has been termed early goal-directed therapy (EGDT) and includes appropriate source control, target-oriented haemodynamic support, early start of antimicrobial therapy, and glycemic control [1,13–18]. In addition, early identification of hospitalised patients at risk for septic complications also seems to be crucial, especially in paediatric patients, where time is even more critical. As early identification of septic patients is often impeded by the fact that classic diagnostic tools such as white blood count (WBC), hsCRP, and blood sedimentation rate (BSR) do not respond until the full clinical manifestation of sepsis, measurement of serum IL-6 may be used as a highly sensitive diagnostic tool for the early identification of sepsis in both newborns and adults [10,11,19–24].

In this analysis, we investigated whether densitometric IL-6 POC measurement using a newly developed bedside assay might provide a new approach for the determination of IL-6 serum levels. The test results were compared to IL-6 levels determined using the gold standard (enzyme-linked immunosorbent assay, ELISA). Between tests comparison was performed using ROC curve and Bland–Altman analyses.

Methods

Study patients and serum samples

Between January 2003 and August 2005, 392 serum samples were collected from 57 consecutive patients (38 male) at risk for sepsis hospitalised in the intensive care units (ICUs) of the Charité University Medicine, Campus Virchow Clinic, Berlin, Germany. On a daily basis, patients were analysed whether severe sepsis or septic shock was present based on the consensus definition of the American College of Chest Physicians and the Society of Critical Care Medicine (ACCP/SCCM) [25]. Sepsis was diagnosed when at least two systemic inflammatory response syndrome (SIRS) criteria were present: core body temperature ≥ 38 or ≤ 36°C, heart rate $\geq$ 90bpm except in those patients with a medical condition or medication known to increase or to prevent an increase in heart rate, respiratory rate $\geq$ 20bpm or PaCO2 $\leq$ 32mm Hg or need for mechanical ventilation for an acute respiratory process, or white cell count $\geq$ 12,000/mm^3 or $\leq$ 4000/mm^3 or differential count showing $\geq$ 10% immature neutrophils. Known or suspected infection as evidenced by one or more of the following was furthermore necessary to establish the diagnosis of sepsis: neutrophils in a normally sterile body fluid, perforated viscus, radiographic evidence of pneumonia in association with purulent sputum, a syndrome associated with a high risk of infection (e.g. ascending cholangitis). Patients were excluded from the study when any of the following criteria were met: age < 18years or > 90years, pregnancy, presence of acute leukemia or malignoma, refusal to sign consent, or participation in other clinical trials. On the ICU, serum samples were drawn on seven consecutive days (every 24h) on every morning from every study patient beginning on the day of study inclusion. Two data analyses were performed: one including all IL-6 samples (total sample analysis) and one analysis including only the baseline samples (samples drawn within the first 72h after study inclusion). All serum samples were drawn from central venous lines and stored at − 40°C until assay. The study was approved by the local *Ethics Committee On Human Research* and was performed in accordance with the *Declaration of Helsinki*. Written informed consent was obtained from the study patients or authorized representatives.

Analysis of serum samples

IL-6 levels (pg/mL) were assessed from central venous blood samples in a blinded fashion using a lateral flow immunoassay point-of-care (POC) test (Milenia QuickLine® IL-6 (MQL6 1); Milenia Biotec, Bad Nauheim, Germany) and a chemiluminescent enzyme immunometric assay. The POC IL-6 test was performed on a test strip enclosed within a credit card sized plastic housing. 100μL of a patient's serum was transferred to the test unit and after 15min of incubation, 50μL of a chase buffer was added. In the first step, IL-6 present in the sample binds to a monoclonal anti-human IL-6 antibody (Ab) conjugated to gold particles. The complex of IL-6 and the conjugate continues to migrate through the analytical membrane. A test line and a control line are printed on this membrane. The test line is coated with a second anti-IL-6 Ab directed against a different epitope than the conjugate Ab. Once the complex of IL-6 and the conjugate passes the test line, it forms a sandwich with the coated IL-6 Ab. A band becomes visible whose intensity correlates with the IL-6 concentration of the sample being tested. The area of the control band is coated with an Ab against the conjugate Ab. Thus, a band is formed that shows that sample flow and conjugate quality correspond to the manufacturer's quality recommendations. An excess sample continues to migrate to a wicking pad where liquid is soaked up. Results are available after 20min and can be interpreted both visually and by densitometry. The IL-6 concentration of the sample correlates with the intensity of the test band. IL-6 concentrations were analysed using a PicoScan® densitometry system which is about 30 × 40cm in size (Milenia Biotec, Bad Nauheim, Germany). This system (sensitivity range 50–10,000pg/mL of IL-6) quantitatively measures the intensity of the test band and calculates IL-6 concentrations according to a standard curve. Furthermore, all results were visually checked. Visual interpretation was performed using an evaluation card enclosed in the kit. The colour intensity of the test band is compared to the range of colour intensities printed on the card. Four ranges of IL-6 concentration can be distinguished: IL-6 < 100pg/mL, $\geq$ 100pg/mL, $\geq$ 300pg/mL, and $\geq$ 1000pg/mL. According to the manufacturer, mean intra-lot and inter-lot variances are 12.1% and 15.5%, respectively.

A second aliquot of each serum sample was analysed in a blinded fashion by a certified immunologic laboratory (Institute of Medical Immunology, Charité Campus Mitte, Berlin,