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Increased concentrations of plasma growth arrest-specific 6 and its soluble tyrosine kinase receptor sAxl in Taiwanese women with pelvic inflammatory disease

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ABSTRACT

Backgrounds: To investigate the concentrations of plasma growth arrest-specific protein 6 (Gas6) and its soluble tyrosine kinase receptor sAxl in women with pelvic inflammatory disease (PID) and their association with clinical outcomes of PID.
Methods: Blood specimens were consecutively collected from the 64 patients with PID before and after treatment and 70 healthy women in university hospital. Concentrations of plasma Gas6 and sAxl were detected using enzyme-linked immunosorbent assay.
Results: The concentration of plasma Gas6 and sAxl was significantly increased in the patients with PID compared to the healthy controls, and then reduced significantly after treatment. Gas6 was significantly correlated with sAxl. When we selected 7.5 and 15.2 ng/ml as the cutoff concentration of plasma Gas6 and sAxl to detect PID respectively, the sensitivities of Gas6 and sAxl were 76.6% and 75.0%. When Gas6 and sAxl were combined, the sensitivity rose to 92.2%. They were not related to the incidences of tuboovarian abscesses and surgery, which were, however, significantly associated with length of hospital stay.
Conclusions: Novel application of Gas6 or sAxl in combination had a high sensitivity to detect PID and is important in order to prevent severe sequelae.

1. Introduction

Pelvic inflammatory disease (PID) is one of the most common infections in reproductive women [1]. It is defined as acute infection of the upper genital tract structures, which involves the uterus, fallopian tubes, and ovaries, and often affects the neighboring pelvic organs. Pelvic pain is the main presenting symptom, whereas the pain may be rather subtle and easily ignored by the patient herself. If PID is not treated adequately and timely, its sequelae can be severe. An infectious blended mass of ovary and fallopian tube, termed as tuboovarian abscess (TOA), often arises as a consequence of PID, which may subsequently lead to severe sequelae such as tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. Prevention of these sequelae relies on clinicians alert to make an early diagnosis and initiate aggressive treatment. Therefore, it is of value to investigate biomarkers that can aid in making an early diagnosis of PID.

Growth arrest-specific protein 6 (Gas6) was initially identified as a protein produced by growth arrested fibroblasts [2]. It belongs to the family of plasma vitamin K-dependent proteins. Gas6 is structurally related to the anticoagulant protein S and it shares 44% of amino acid identity. However, protein S is present approximately at a 1000-fold higher concentration than Gas6 in plasma [3]. Gas6 is expressed in many tissues including vessel endothelial cells, bone marrow cells, lungs and ovaries [2]. It has been demonstrated to be important for the activation of endothelium and the invasion of inflammatory cells into the vessel walls [4]. Gas6 has also been reported to act as an

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acute-phase reactant and to have increased concentrations during sepsis and chronic inflammatory demyelinating polyneuropathy [5–7].

Tyro3, Axl and Mer are members of the TAM family of receptor tyrosine kinases, and Gas6 has growth factor-like properties through its interaction with receptor tyrosine kinases of the TAM family [8,9]. Soluble Axl (sAxl) has a higher affinity with Gas6 compared to Tyro3 and Mer [10]. The function of this family of receptors has been recognized to act as regulators of inflammation [4]. The Axl receptor was originally discovered in patients with chronic myelogenous leukemia [11]. It is expressed in many tissues and cell lines such as primary hematopoietic tissues and breast cell lines [12]. Membrane-bound Axl can be shed from the cell membrane, cleaved by ADAM10, and therefore released into circulation as a soluble form, sAxl [13].

We hypothesized that the concentrations of plasma Gas6 and its receptor sAxl would be increased in women with PID and related to the incidence of TOA and surgery as well as the length of hospital stay. To date, no report has delineated the concentration of plasma Gas6 and sAxl in PID. Therefore, the objectives of this study were to compare the concentrations of plasma Gas6 and sAxl between women with PID and healthy women, and to determine the cutoff concentration to distinguish them. In addition, we correlated their plasma concentration with the clinical outcomes of PID.

2. Materials and methods

2.1. Subjects and sample collection

We consecutively collected blood samples from 64 patients with PID and 70 healthy women without PID. The patients with PID were enrolled into this study from the Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taiwan, between April 2006 and September 2007. Meanwhile, the healthy women were recruited to achieve a ratio of normal to PID women of approximately 1 to 1 from the Department of Family and Community Medicine where they received health examinations. They were matched in sociodemographic and clinical data including ethnicity, occupation, cigarette smoking and alcohol drinking.

PID was diagnosed based on the guidelines of the Centers for Disease Control and Prevention (CDC) when the patients suffered from lower abdominal pain or pelvic pain of no other origin, with one of the following criteria: uterine tenderness, adnexal tenderness, or cervical motion tenderness [14,15]. Women who were breast feeding, pregnant, taking antibiotics for other inflammatory diseases or infections, suspected to have tumors originating from any organ such as the cervix or ovaries, or had undergone a gynecologic operation within 3 weeks before admission were excluded from the study. In patients with PID and a palpable adnexal mass, the diagnosis of TOA was verified by ultrasound. All patients with PID received treatment protocols based on the CDC guidelines. They were given antibiotics intravenously for at least 3 days, or for an additional 24 h after they were afebrile. Thereafter, oral antibiotics were given until day 14 of treatment. We collected plasma samples from the 70 healthy women and 64 patients with PID before and 3 days after they received the treatment. All samples were analyzed for Gas6, sAxl and C-reactive protein (CRP) concentrations, and for white blood cell (WBC) and neutrophil counts. These parameters were measured by clinical laboratory staff and technicians. The Chung Shan Medical University Hospital Institutional Review Board approved our study protocol (CSMUH CS11153), and informed consents were obtained from all patients.

2.2. Measurements of plasma Gas6 and sAxl concentration by enzyme-linked immunosorbent assay (ELISA)

The concentrations of plasma Gas6 and sAxl were analyzed by human Gas6 (Quantikine Human Gas6 Immunoassay; R&D Systems) and sAxl (Aviscera Bioscience) ELISA kits in all blood samples. For each plasma sample, 100 μl was directly transferred to the microtest strip wells of the ELISA plate and subsequently incubated for 2 h at room temperature. After 4 washing steps, the detection antibody was added, and the reaction mixture was incubated for 2 h at room temperature. Antibody binding was detected with streptavidin-conjugated horseradish peroxidase and developed with a substrate solution. The reaction was stopped, and optical density was determined using a microplate reader set at 450 nm. The Gas6 and sAxl concentrations were quantitated by a calibration curve using a human Gas6 and sAxl standard, respectively. Each plasma sample was assayed according to the instructions of the manufacturers, and the values were within the linear portion of the standard curve.

2.3. Statistical analysis

A Mann-Whitney U test was used to test statistical differences among pretreatment plasma Gas6, sAxl and CRP concentration, and WBC and neutrophil counts in the 64 patients with PID and those in the 70 healthy individuals. Differences in certain parameters were also statistically analyzed between pretreatment and posttreatment plasma samples of the same PID patients using the Wilcoxon signed rank test. The Spearman rank correlation coefficient r was used to correlate these parameters.

Odds ratios (ORs) with their 95% confidence intervals (CIs) of the concentrations of plasma Gas6, sAxl and CRP as well as counts of WBC and neutrophil for PID risk were estimated using the chi-square test. A logistic regression model was used to evaluate the adjusted odds ratios (AORs) with their 95% CIs of these parameters.

We then plotted receiver-operating characteristic curves (ROCs) to select the cutoff concentration of plasma Gas6 and sAxl to distinguish patients with PID from normal individuals. These cutoff concentrations were determined according to the best Youden's index (maximum [sensitivity + specificity − 1]) for the concentration of pretreatment plasma Gas6 and sAxl in the 64 patients with PID and their concentration in the 70 healthy women [16]. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy as well as the likelihood ratios of positive and negative results (LR^+ and LR^-) were calculated.

In addition, we correlated the concentration of plasma Gas6 and sAxl with disease severity and clinical course of PID. Severe infectious signs included TOA, sepsis, and organ failure. However, no cases of sepsis or organ failure were found in the PID patients. The Mann-Whitney U test was used to test statistical significance among plasma Gas6 and sAxl as well as the incidences of TOA and surgery. The Spearman rank correlation coefficient analysis was applied to correlate the concentration of plasma Gas6 and sAxl with the length of hospital stay. We then adjusted variable factors to define the relationship of the length of hospitalization with these factors using a multiple linear regression model.

All statistical analyses were processed using the SPSS statistical software program (version 11.0; SPSS, Inc., Chicago, IL). A P value of less than 0.05 was considered to be statistically significant.

3. Results

We analyzed the concentration of plasma Gas6 and sAxl in the patients with PID and healthy women by non-parametric tests. The median and range of the age of the patients with PID and healthy women were 33.5 (16–68) and 41 (22–67) y, respectively ($P = 0.008$). The plasma Gas6 level was significantly raised in the patients with PID before they received treatment compared with that in the healthy women (11.0 [2.9–21.0] vs. 6.7 [3.0–11.0] ng/ml, $P < 0.001$; Fig. 1 and Table 1). Moreover, the Gas6 concentration of pretreatment plasma in the patients with PID was significantly increased compared with that of posttreatment plasma (11.0 [2.9–21.0] vs. 6.4 [2.7–14.8] ng/ml; $P < 0.001$). The plasma sAxl level was also significantly increased in the patients with PID before they received treatment compared with that in the healthy subjects (19.8 [5.8–86.8] vs. 14.5 [4.7–24.2] ng/ml;