Serum concentrations of TFF3, S100-A11 and AIF-1 in association with systemic inflammatory response, disease stage and nodal involvement in endometrial cancer

Abstract: To compare preoperative intestinal trefoil factor 3 (TFF3), allograft inflammatory factor-1 (AIF-1) and calcigizzarin (S100-A11) serum levels in patients with endometrial cancer, endometrial hyperplasia and in healthy female controls. Serum levels of TFF3, S100-A11 and AIF-1 were analyzed in 98 consecutive patients with histologically verified endometrial cancer, in 43 patients with endometrial hyperplasia diagnosed during hysteroscopy and 24 controls with benign disease. Results were correlated with urinary neopterin/creatinine ratio, serum kynurenine, tryptophan, retinol, alpha-tocopherol, vitamin D, citrulline, C-reactive protein, interleukin-6 and clinical characteristics. S100-A11, and AIF-1 levels were higher in endometrial hyperplasia patients than in controls, and also significantly higher in endometrial cancer than in patients with endometrial hyperplasia. Serum concentrations of TFF3 and S100-A11 were associated with tumor stage and lymph node status. TFF3 exhibited positive correlation with age, IL-6, vitamin D, kynurenine, urinary neopterin/creatinine ratio and kynurenine/tryptophan ratio. S100-A11, as well as AIF-1 correlated positively with IL-6 and TFF3. TFF3, S100-A11 and AIF-1 represent potential biomarkers in patients with endometrial cancer. TFF3 and S100-A11 increase with tumor stage and lymph node involvement, reflecting higher tumor mass that is also associated with increased concentration of biomarkers of immune dysfunction.

Keywords: AIF-1; endometrial cancer; inflammatory response; nodal involvement; S100-A11; TFF3.

Introduction

Endometrial cancer (EC) is the most common cancer of the female genital system [1].

Approximately 75% of cases are diagnosed when the tumor is confined to the uterine corpus, but 15%-20% of these patients experience recurrence after primary surgery with limited response to systemic therapy [2].

Surgical staging as part of the treatment paradigm for endometrial cancer was first supported by the findings from a large prospective surgical-pathological study of patients with clinical stage I and II endometrial carcinoma, conducted by the Gynecologic Oncology Group (GOG) [3]. These findings led to a change in the staging system. In 1988, FIGO replaced an inaccurate clinical staging...
system with a more accurate surgical staging, which was most recently revised in 2009 [4]. A complete pelvic and paraaortic lymphadenectomy allows for an accurate evaluation of the disease extent. The information defining the extent of disease enables the physician to recommend adjuvant therapy, minimizing the risk of over- or undertreatment. However, lymphadenectomy is not without risks. Post-operative complications occur more frequently and the extent of lymphadenectomy also impacts on post-operative morbidity rates [5]. An improved identification of high risk patients prior to primary surgical treatment could allow to the omission of lymph node dissection among low risk patients, thus lower risk for surgical complications.

Obesity associated with low-grade chronic inflammation represents an important risk factor for developing endometrial cancer [6]. As the world faces an obesity epidemic and an aging population, the number of cases is expected to rise. The management of cancer patients is increasingly dependent on biomarkers. Circulating proteins are the most commonly investigated cancer biomarkers. For example, among the three mammalian trefoil peptides (TFF1, TFF2, and TFF3) identified so far [7-9], TFF3 was found as the top highly expressed gene in endometrial cancer [10]. Based on published studies TFF3 might prove as marker in monitoring disease course both in neoplastic and inflammatory conditions [11]. Recently, calgizzarin (S100-A11) a novel and relatively unknown member of large family of S100 proteins was found to be highly expressed in uterine tumors as well as in pressure ulcer samples [12, 13]. Allograft inflammatory factor-1 (AIF-1) is a highly conserved inflammation responsive protein [14, 15]. Besides association with acute and chronic inflammation, AIF-1 is also involved in carcinogenesis [16].

Surgical intervention is followed by an inflammatory response that induces oxidative stress. In a prior study, we have demonstrated that surgical approaches inducing different tissue trauma and blood loss are associated with differential response of biomarkers of inflammatory response, oxidative stress and nutritional status. An inverse correlation between the concentrations of citrulline, a biomarker of bowel mass, and inflammatory response was also demonstrated [17].

The aim of this study was to compare preoperative TFF3, AIP and S100-A11 serum levels in patients with endometrial cancer, endometrial hyperplasia and in healthy female controls. In addition, in patients with endometrial cancer an association with tumor grade, stage and nodal status. Furthermore, we analyzed an association between TFF3, S100-A11, AIP-1 and biomarkers of inflammatory response, oxidative stress and nutritional balance in endometrial cancer patients treated with three surgical approaches including open (laparotomy), laparoscopic and robotic surgery as well as in patients operated for benign disorders.

Materials and methods

The present prospective study was performed at the University Hospital Olomouc between October 2012 and June 2015.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors’ institutional review board or equivalent committee.

In total 98 consecutive patients with histologically verified endometrial cancer, aged (mean ±standard deviation) 65±10 (range 33-88) years, who underwent hysterectomy with bilateral salpingoophorectomy, pelvic and paraaortic lymphadenectomy were included in the study. The surgery was performed by open (laparotomy), robotic or laparoscopic approach. The control group was comprised of 24 patients, aged 55±12 (range 36-80) years, who had elective total hysterectomy for non-malignant disorder and were randomly allocated to either abdominal hysterectomy or laparoscopic assisted hysterectomy for benign disease. Samples of peripheral venous blood were collected on the day of surgery before skin incision. The samples were separated and the sera were stored at -80°C until analysis. Urinary samples with the same timing were collected and stored at -20°C until analysis for urinary neopterin/creatinine ratio determination. Other clinical or laboratory parameters evaluated included patient age, body mass index (BMI), number of lymph nodes obtained, grade, stage of the disease and nodal involvement. BMI was calculated using the standard formula (body weight/height²). The results of changes and correlations of biomarkers of inflammatory response and antioxidant balance in the present cohort were published earlier [18]. The present study expanded the analysis to the measurement of TFF3, S100-A11 and AIP-1 serum concentrations in collected samples. Furthermore, preoperative blood samples were taken in 43 patients aged 63±10 (range 44-97) years diagnosed with endometrial hyperplasia during hysteroscopy. Ninety-one patients, aged 65±9 (range 40-87) years, with negative hysteroscopic
findings represented a control group. The samples, as well as endometrial cancer patient samples from the day 0 (before skin incision) were separated and the sera were all stored at -80°C until analysis for TFF3, S100-A11 and AIF-1. Urinary neopterin/creatinine ratio, serum kynurenine, tryptophan, retinol, alpha-tocopherol and vitamin D were determined by high-performance liquid chromatography as described. Citrulline was measured by flow injection mass spectrometry, serum C-reactive protein (CRP) and interleukin-6 (IL-6) were determined on Cobas 8000 analyzer (Hitachi, Japan) as described.

The assay for TFF3, S100a11 and AIF-1 was developed by Biovendor (Brno, Czech Republic). All parameters above were measured with sufficient analytical precision; coefficient of variation (CV) < 5%, CV between series <8%; and analytical characteristics were superior than those in laboratory sheet. All measurements were made in the same time, using biorobotic analyzer DS2 (Dynex, USA), after centrifugation (aliquoted serum, cooled centrifuge, 4 C, 3500/4 min).

**Statistics**

Results were quantitatively assessed as mean, median, standard deviation, minimal and maximal value. Differences in age between groups were analyzed using the nonparametric Friedman ANOVA test. Other data were analyzed using the Mann-Whitney with Bonferroni’s correction and Kruskal-Wallis tests. The level of significance was set at 5%.

**Results**

TFF3 serum levels were significantly higher in endometrial cancer patients when compared to controls (Figure 1). S100-A11, and AIF-1 levels were higher in endometrial hyperplasia patients than in controls, and also significantly higher in endometrial cancer than in endometrial hyperplasia patients (Figures 2 and 3). The serum concentrations of TFF3 (Figures 4 and 6), S100-A11 (Figures 5 and 7), but not AIF-1 (data not shown) were associated with tumor stage and lymph node status. The serum concentrations of TFF3, S100-A11 and AIF-1 were not associated with tumor grade (data not shown).

Table 1 shows the correlations between TFF3, S100-A11, AIF-1 and biomarkers of oxidative balance and inflammatory response at in patients with endometrial cancer. TFF3 exhibited positive correlation with age, IL-6,
Table 1: Correlations between laboratory and clinical parameters at baseline in patients with endometrial carcinoma (EC), (n=98), BMI (body mass index), CRP (c-reactive protein), IL-6 (interleukin – 6), TFF3 (intestinal trefoil factor 3), AIF-1 (allograft inflammatory factor-1), S100-A11 (calgizzarin).

<table>
<thead>
<tr>
<th>EC (n = 98)</th>
<th>Age</th>
<th>BMI</th>
<th>Leuko-</th>
<th>Platelets</th>
<th>CRP -1</th>
<th>IL-6 -1</th>
<th>Citrulline</th>
<th>Vitamin D-1</th>
<th>Alpha- tocopherol -1</th>
<th>Retinol</th>
<th>Kynurenine</th>
<th>Urinary neopterin/ creatinine ratio -1</th>
<th>Tryptophan -1</th>
<th>Kynurenine/ Tryptophan ratio -1</th>
<th>TFF3 (ng/ ml)</th>
<th>S100-A11 (ng/ml)</th>
<th>AIF-1 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFF3</td>
<td>Corr.</td>
<td>0.371</td>
<td>0.141</td>
<td>0.088</td>
<td>-0.028</td>
<td>0.176</td>
<td>0.291</td>
<td>0.191</td>
<td>-0.279</td>
<td>-0.073</td>
<td>0.004</td>
<td>0.312</td>
<td>0.215</td>
<td>-0.047</td>
<td>0.294</td>
<td>1.000</td>
<td>0.311</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.0002</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.004</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.002</td>
<td>NS</td>
<td>0.034</td>
<td>NS</td>
<td>0.004</td>
<td>0.002</td>
<td>0.17</td>
</tr>
<tr>
<td>S100-A11</td>
<td>Corr.</td>
<td>0.044</td>
<td>0.110</td>
<td>0.130</td>
<td>0.027</td>
<td>0.136</td>
<td>0.200</td>
<td>-0.167</td>
<td>-0.103</td>
<td>-0.037</td>
<td>-0.105</td>
<td>0.017</td>
<td>0.122</td>
<td>-0.090</td>
<td>0.082</td>
<td>0.311</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.049</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AIF-1</td>
<td>Corr.</td>
<td>0.061</td>
<td>0.185</td>
<td>0.181</td>
<td>-0.035</td>
<td>0.213</td>
<td>0.253</td>
<td>-0.146</td>
<td>-0.050</td>
<td>-0.117</td>
<td>-0.065</td>
<td>0.098</td>
<td>0.198</td>
<td>-0.080</td>
<td>0.157</td>
<td>0.240</td>
<td>0.778</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.012</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

NS: not significant.
vitamin D, kynurenine, urinary neopterin/creatinine ratio and kynurenine/tryptophan ratio. S100-A11, as well as AIF-1 correlated positively with IL-6 and TFF3. In controls (Tab 2), S100-A11 correlated positively with AIF-1 ($r_s=0.874$, $p=0.0001$).

Discussion

The present data demonstrate an increase of serum TFF3, S100-A11 and AIF-1 concentrations in patients with endometrial carcinoma compared to control groups with endometrial hyperplasia or normal endometrium. Moreover, the concentrations of TFF3 and S100-A11 were associated with tumor stage and lymph node status. These findings indicate that these molecules could serve as biomarkers in the diagnosis, pre-operative work up and possibly follow up of patients with endometrial cancer.

The determination of biomarkers is essential in the management of cancer patients [19]. In addition to identification of additional tumor biomarkers in endometrial cancer, present data provide further insight into the interaction between the tumor mass, immune and inflammatory responses. TFF3, S100-A11 and AIF-1 concentrations exhibited correlation with biomarkers of inflammatory response. Interestingly, a significant correlation with IL-6 was observed for all these three biomarkers, while only TFF3 correlated with urinary neopterin, serum kynurenine and kynurenine/tryptophan ratio, and none of the biomarkers correlated with CRP. These correlations may be explained by an association of inflammatory response with the tumor mass, in correspondence to the observed association between TFF3...
and S100-A11 concentrations and tumor stage and lymph node status. Low vitamin D concentrations are predictive of poor prognosis in different primary tumors and an inverse association with TFF3 is not surprising.

Tumor growth and progression is associated with an inflammatory response. In fact, this inflammatory response promoting tumor progression is now considered to represent one of the hallmarks of cancer [20]. Macrophages may represent important effectors of antitumor response, but also contribute to tumor growth and progression [21, 22]. Neopterin is a product of activated macrophages. Although increased serum or urinary neopterin concentrations are encountered in different disorders leading to the stimulation of macrophages by interferon-gamma [23, 24], and neopterin as a biomarker is therefore non-specific, chronic macrophage stimulation leading to increased neopterin concentrations is characteristic of advanced tumors across the spectrum of different malignancies [25, 26]. Moreover, increased neopterin has been shown to be a negative prognostic biomarker in different tumors, including gynecological cancers [25, 26]. High neopterin concentrations have been associated with the presence of laboratory parameters of immune dysfunction both in the tumor microenvironment [27, 28] and in the circulation [29]. Kynurenine is produced from tryptophan in a reaction catalyzed by another enzyme induced by interferon-gamma, indoleamine 2,3-dioxygenase (IDO). Although kynurenine, in high concentrations, has cytotoxic activity against tumor cells [30], and IDO induction has been implicated in the mechanism of antitumor activity of interferon-gamma [31], the predominant effect of tryptophan depletion and kynurenine itself is the suppression of the immune response [32]. Present data illustrate that higher tumor mass reflected in increased concentration of biomarkers of immune dysfunction like neopterin, kynurenine or kynurenine/tryptophan ratio.

In future studies, all these biomarkers should be investigated simultaneously to determine how therapeutic interventions are reflected in changing concentrations of these parameters. The studies should also include biomarkers of toxicity of systemic therapy, a largely neglected topic. With the exception of hematologic toxicity, other side effects of chemotherapy and radiation are assessed mostly by history or clinical examination while reliable laboratory biomarkers for monitoring and prediction of toxicity are still lacking [33-35]. Interestingly, in patients with rectal cancer an association between neopterin concentrations and complications of radiation and chemotherapy, two methods also commonly used in endometrial cancer has been recently reported [36].

In conclusion, TFF3, S100-A11 and AIF-1 represent potential biomarkers in patients with endometrial cancer. TFF3 and S100-A11 increase with tumor stage and lymph node involvement, reflecting higher tumor mass that is also associated with increased concentration of biomarkers of immune dysfunction.

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References


