Variability of effluent cancer antigen 125 and interleukin-6 determination in peritoneal dialysis patients

Deirisa Lopes Barreto1, Annemieke M. Coester1, Marlies Noordzij2, Watske Smit1,3, Dirk G. Struijk1,3, Susan Rogers3, Dirk R. de Waart4 and Raymond T. Krediet1

1Division of Nephrology, Department of Internal Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, 2Department of Medical Informatics, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, 3Dianet Foundation Amsterdam-Utrecht, The Netherlands and 4Department of Experimental Hepatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Correspondence and offprint requests to: Deirisa Lopes Barreto; E-mail: d.lopesbarreto@amc.uva.nl

Abstract

Background. Cancer antigen (CA) 125 is a glycoprotein that provides data on the state of the peritoneal membrane in peritoneal dialysis (PD). Interleukin-6 (IL-6) acts as a mediator in acute-phase responses. The study evaluated the usefulness of CA125 and IL-6 in random effluent samples, by assessing their variability in individual patients during clinical practice at the outpatient department.

Methods. This longitudinal prospective study was conducted from 2007 till 2009. Participants included 52 stable PD patients aged ≥18 years. Effluent samples were obtained during regular outpatient visits and appearance rates (ARs) were calculated. Inter- and intra-individual variability was determined by the coefficient of variation (CV). A linear mixed model was used to analyse time courses. Furthermore, release patterns of these effluent markers were studied.

Results. CA125-AR of short-term patients (≤24 months) ranged from 39.2 to 766.7 U/min and IL-6-AR from 15.5 to 220.0 pg/min. Long-term patients (≥25 months) had a CA125-AR of 7.3–1534.0 U/min and IL-6-AR of 6.9–956.4 pg/min. Overall, CVintra was 15% in effluent CA125-AR and 28% in IL-6-AR. Intermediate sampling during a 4-h dwell showed a linear increase of CA125 and IL-6 effluent concentrations. The trend of CA125-AR was different (P = 0.001) between short- and long-term patients. A negative relationship was found between CA125 (r = −0.44, P = 0.02) and PD duration.

Conclusions. The clinical relevance of effluent CA125 determinations from an unstandardized dwell during every outpatient visit is reasonable, as judged from the CVintra. The inferior CVintra values of ARs as compared to effluent values indicate that ARs should only be calculated under standardized conditions. A single IL-6 measurement, as a predictor of outcome, should be interpreted cautiously.

Keywords: biomarkers; cancer antigen 125; interleukin-6; mesothelial cells; peritoneal dialysis

Introduction

Cancer antigen (CA) 125 is a high molecular weight (220 kDa) glycoprotein that is constitutively produced by peritoneal mesothelial cells in vitro [1]. The number of mesothelial cells in peritoneal effluent of stable peritoneal dialysis (PD) patients is proportional to peritoneal dialysate CA125 concentration [2]. Therefore, it can be regarded as a marker for the mesothelial cell mass in stable PD patients and thus provides data on the state of the peritoneal membrane in vivo [3]. Extremely low CA125 concentrations have been found in effluent of patients preceding the diagnosis of peritoneal sclerosis [4]. This is supportive to the contention that a loss of mesothelial cells is implicated in the pathogenesis of peritoneal sclerosis, a serious complication of long-term PD [5]. The small day-to-day
variability of CA125, which was found to average 7.7%, supports its reliability as a marker for mesothelial status [6], at least when measured under strictly standardized conditions.

Inflammatory changes are often seen in the peritoneum, even in the absence of peritonitis, indicating that the peritoneum of a PD patient may be chronically inflamed [7]. Interleukin 6 (IL-6) is a multifunctional protein produced by a wide array of cells such as lymphoid and non-lymphoid cells and by normal and transformed cells, including T cells, monocyte/macrophages, fibroblasts, mesothelial cells and vascular endothelial cells [8–10]. Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine. IL-6 is one of the most important mediators in the acute-phase response, which makes it an interesting protein in the early diagnosis of inflammation. Especially, because an increase is present in effluent IL-6 concentrations shortly before the onset [11] of and during peritonitis [12], suggesting its local production and reflecting an intraperitoneal inflammatory state. A recent study performed by our group [13] indicates the potential use of effluent IL-6 and CA125 for an early diagnosis of encapsulating peritoneal sclerosis.

At present, the CA125 determinations are standardized to be performed at the end of the 4-h standard peritoneal permeability analysis (SPA), while IL-6 has not yet been included in this standard assessment. At our centre, SPA’s are done yearly on a voluntary basis in each PD patient. The variability of effluent CA125 measurements on a more frequent basis than once yearly is not known. The variability of effluent CA125 measurements was determined by means of the CV. Inter-individual variability (CV in inter) is defined as the CV within the short-term or long-term study population and calculated as the SD within the groups divided by the mean and multiplied by 100%. Intra-individual variability (CV in intra) is defined as the variability within one patient and was calculated as the overall SD divided by the mean of all experiments and multiplied by 100%. The overall SD was calculated as the square root of the mean of the squares of the SDs of each patient.

Coefficient of variation. Inter- and intra-individual variability was determined by means of the CV. Inter-individual variability (CV in inter) is identified as the CV within the short-term or long-term study population and calculated as the SD within the groups divided by the mean and multiplied by 100%. Intra-individual variability (CV in intra) is identified as the variability within one patient and was calculated as the overall SD divided by the mean of all experiments and multiplied by 100%. The overall SD was calculated as the square root of the mean of the squares of the SDs of each patient.

Materials and methods

This was a prospective longitudinal, observational open-label study in which patients were included between 2007 and 2008. On average, PD patients visit the outpatient department every 6–8 weeks. Therefore, to obtain at least six consecutive effluent CA125 and IL-6 measurements, follow-up duration was at least 1 year. The patients were divided into a short-term and a long-term PD group, where short-term is defined as patients with a PD duration between 0 and 24 months, and long-term patients with a PD duration of ≥25 months.

Patients

All prevalent stable chronic renal failure patients treated with PD and with a minimum age of 18 years were eligible to participate in this study. Patient characteristics such as primary kidney disease, APD/CAPO treatment at the start of PD, and initial regimen were collected. Baseline characteristics of the patients were assessed at the first visit at the outpatient clinic. Patients with a clinically significant diagnosis of peritonitis, malignancy or unstable patients with severe oedema were excluded. Furthermore, transfer to haemodialysis, renal transplantation, patient preference or physician’s discretion were reasons for withdrawal from the study. In addition, patients that underwent a 4-h standardized dwell were selected for analysis of CA125 and IL-6 release patterns.

Data collection

At every visit, PD regimen, glucose load, iodextrin use, peritonitis episodes and body weight were registered. The patients were asked to keep their collection bag of the last long day or night dwell, regardless of the dialysis solution used. This was done because in a previous study, it was shown that the glucose concentration of the dialysis solution did not influence CA125 concentration [6]. In order to analyse proteins release patterns, intermediate effluent was obtained in a subgroup at time point 0’, 10’, 20’, 30’, 60’, 120’ and 240’ during a 4-h standardized dwell. All samples were centrifuged at 1710 g for 10 min and frozen at −26°C until analysis.

CA125 and IL-6 assays

CA125 was determined by using a microparticle enzyme immunoassay in combination with a monoclonal antibody OC125 on an E170 autoanalyser (Roche Diagnostics, Basel, Switzerland). The total coefficient of variation (CV) for this determination is 1.6–3.0%. The concentration of IL-6 was measured with an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA) with a sensitivity of 0.7 pg/mL.

Calculations and statistical analysis

Appearance rate dialysate. Dwell times and volumes of the bag were recorded in order to calculate the appearance rate (AR) of CA125 and IL-6. The AR is the amount of CA125 or IL-6 present in the total drained effluent divided by the duration of the dwell in minutes, according to the following equation: AR = ([protein]×volume effluent)/dwell time.

Coefficient of variation. Inter- and intra-individual variability was determined by means of the CV. Inter-individual variability (CV in inter) is identified as the CV within the short-term or long-term study population and calculated as the SD within the groups divided by the mean and multiplied by 100%. Intra-individual variability (CV in intra) is defined as the variability within one patient and was calculated as the overall SD divided by the mean of all experiments and multiplied by 100%. The overall SD was calculated as the square root of the mean of the squares of the SDs of each patient.

Statistical analysis. Results are presented as median and ranges, unless stated otherwise. The Mann–Whitney U-test was used to compare the baseline characteristics of the short-term and long-term PD patients because of asymmetrically distributed data. A linear mixed model with a repeated statement was used to analyze the time courses of the effluent markers CA125 and IL-6 (dependent variables). This method takes into account the correlation between repeated measurements within the same patient. All available visits were included in the analysis. The multivariate model contained the time (number of visits) as categorical variable and PD duration, both as fixed effects. Several covariance structures were compared using the log likelihood ratio test with Restricted Maximum Likelihood estimation methods. Based on this comparison, Toeplitz heterogeneous appeared to be the best covariance structure for our data and was chosen for the analyses. To evaluate possible relationships, correlation analysis was performed by Spearman rank correlation analysis. In this analysis, only the last measure point of every patient was included. All statistical analyses were performed using SPSS version 16.0.

Results

Peritoneal effluent was collected from 52 patients. Included patients had at least two samples available with a maximum interval of 6 months and were omitted when an exclusion criteria was met. Exclusion of patients during the study was due to transfer to haemodialysis, renal transplantation and patients with less than two available samples (Figure 1).
Furthermore, measure points were lost due to values that were outside of the reference standard curve derived from CA125 and IL-6 assays, peritonitis episodes or missing dwell times and dwell volumes, resulting in the inability of calculating ARs. Therefore, our final study population consisted of 30 PD patients with two or more samples available. Baseline characteristics of the patients are presented in Table 1.

**Effluent concentration and AR**

Table 2 shows the results of the effluent concentrations and AR of CA125 and IL-6. Long-term patients had significantly lower CA125-AR (P < 0.001) when compared with short-term patients. Furthermore, significantly higher values of IL-6-AR (P = 0.05) between the long-term patients were found as compared to the short-term patients.

**Coefficient of variation**

Results of the CV_intra in short-term, long-term patients and the total study population, are presented in Table 3. The median interval between the visits was 2 months. There was no difference in the median interval between the short-term and long-term group. CV_intra defined as the variability within one patient of the CA125-AR ranged from 0 to 37% in short-term PD patients and 2 to 37% in long-term PD patients (Table 4). Much higher values were found for IL-6-AR. IL-6 CV_intra ranged from 2 to 98% in short-term patients and in long-term PD patients from 7 to 82%. Furthermore, the ARs of both markers showed higher CV_intra values as compared to effluent concentrations.

**CA125 and IL-6 release pattern with the duration of a dwell**

For elucidation of the release patterns, we selected eight stable PD patients who underwent a 4-h SPA. A linear relationship with the duration of the dwell was present both for CA125 and IL-6 effluent concentrations (Figure 2).

**CA125 and IL-6 time courses**

CA125 in the short-term patients was higher than in the long-term ones (P = 0.001). During the follow-up period, also the time-course of CA125-AR in short-term patients was different from that in long-term patients, with a slight decrease at the end of follow-up (Figure 3A). The time course of IL-6-AR was not different (Figure 3B). Furthermore, the time courses of effluent concentrations were relatively similar.

**Correlation analysis between the effluent markers and PD duration**

To study the relationship between the effluent markers and PD duration, only the last measure point of every patient was included in this analysis. A negative correlation was present between PD duration and CA125 effluent concentrations (Figure 4A), whereas no correlation was present between PD duration and effluent concentrations of IL-6 (Figure 4B). Also no correlation was found between CA125 and IL-6 (r = 0.108, P = 0.35).

**Discussion**

PD induces peritoneal and morphological alterations and can lead to ultrafiltration failure with time on PD. Therefore, biomarkers in peritoneal effluent may be useful for the detection of abnormalities in peritoneal tissue. Yet, the variability of biomarkers is unknown. The routine measurement of effluent CA125 was included in the standard peritoneal permeability analysis (SPA) in our patient population since 1998, which is performed once yearly [14]. The present study aimed to evaluate the value of CA125 and IL-6 measurements in prospectively collected random effluent samples at the outpatient department. The use of non-standardized dwell times as was the case in the present study can be overcome by using the AR of a biomarker. However, this can only be done when the increase in effluent concentration is linear in time. This was previously shown for CA125 during a 4-h dwell [6], and beyond [15], but no data on IL-6 could be traced. The present study shows that a linear increase in effluent IL-6 was also present during a 4-h dwell.

In a previous study, we found a day-to-day coefficient of intra-individual variation for effluent CA125 of 7.7% during a 4-h SPA [6]. The lack of standardization in the present study led to a doubling of the intra-individual variability but values were similar to the intra-individual CV for parameters of fluid and solute transport in CAPD patients found by Imholz et al. [16]. No significant difference between inter- and intra-individual variabilities was present for IL-6 effluent concentrations and ARs. This suggests that the linearity of the appearance in effluent may no longer be present for dwell times > 4 h.

Not all patients reached six visits. Therefore, in the linear mixed model, the number of visits was restricted to a maximum of four visits. In IL-6-AR and effluent concentration analysis, no difference between short- and long-term was found. This may indicate that the reaction to an
inflammatory stimulus was not different. However, only a limited number of patients in the long-term group had more than two samples available for IL-6 determinations. The significant difference in trend between short- and long-term patients in CA125-AR, also showing lower values in the long-term group, confirms the results of previous studies with a longer follow-up [6]. This emphasizes the importance of individual assessment of CA125 with duration of PD to evaluate mesothelial status.

Apart from the higher values of the CA125-AR, also the D/Pcreat ratio was higher in the short-term group as compared to the long-term group. Previously, a study by Parikova et al. [17] also showed higher initial values for solute transport. In incident PD patients, a clear relationship was present between small solute transport and effluent CA125. These data suggest that fast solute transport rates in incident patients may be caused by vasoactive substances produced by mesothelial cells as described previously [18]. During follow-up, effluent CA125 was consistently lower in the long-term patients as compared to the short-term patients. When the effluent concentration of CA125 was analysed with regard to the duration of PD, a negative trend was found. This is in accordance with the results of previous studies [6]. The results of IL-6 suggest that the degree of inflammation may be similar in short- and long-term patients, but a slight tendency is present to higher values with long-term PD treatment. Our results also support the contention that IL-6 acts not only as a mediator in the acute phase response but may also be influenced by angiogenesis and chronic inflammation [19]. We were unable to find a relationship between effluent CA125 and IL-6. This is probably due to the constitutive synthesis of CA125, while IL-6 production depends also on inflammatory stimuli. This is in line with a study performed by Rodrigues et al. [15]. Furthermore, CA125 is released by mesothelial cells only, while IL-6 can be produced by various cell types [19, 20].

### Table 1. Baseline characteristics of the short-term (≤24 months) and long-term (≥25 months) patients

<table>
<thead>
<tr>
<th></th>
<th>Short-term PD (n = 17)</th>
<th>Long-term PD (n = 13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female / male)</td>
<td>8/9</td>
<td>5/8</td>
<td>0.96</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55 (28–71)</td>
<td>47 (19–75)</td>
<td>0.11</td>
</tr>
<tr>
<td>PD duration at first visit (months)</td>
<td>8.9 ± 5.5</td>
<td>48.4 ± 20.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APD/CAPD</td>
<td>9/8</td>
<td>7/6</td>
<td>0.74</td>
</tr>
<tr>
<td>Initial regimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dianeo</td>
<td>0 (0%)</td>
<td>3 (23%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Physioneal</td>
<td>17 (100%)</td>
<td>10 (77%)</td>
<td></td>
</tr>
<tr>
<td>Primary kidney disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renovascular</td>
<td>4 (24%)</td>
<td>3 (23%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>1 (6%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>7 (41%)</td>
<td>2 (15%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 (29%)</td>
<td>8 (62%)</td>
<td></td>
</tr>
<tr>
<td>D/P creatinine</td>
<td>0.81 ± 0.08</td>
<td>0.70 ± 0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>Net ultrafiltration at 240 min (mL)</td>
<td>463 ± 373</td>
<td>497 ± 291</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*Values are presented as median and ranges for age, and mean ± SD for PD duration and transport parameters.

### Table 2. CA125 and IL-6 concentrations and ARs

<table>
<thead>
<tr>
<th></th>
<th>Short-term PD (n = 17)</th>
<th>Long-term PD (n = 13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent CA125 (kU/L)</td>
<td>58.0 (19.0–345.0)</td>
<td>19.0 (5.0–162.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AR CA125 (U/min)</td>
<td>152.9 (39.2–766.7)</td>
<td>58.4 (15.5–220.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Effluent IL-6 (pg/mL)</td>
<td>12.3 (3.1–322.9)</td>
<td>22.9 (4.9–394.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>AR IL-6 (pg/min)</td>
<td>31.5 (7.3–1534.0)</td>
<td>64.3 (6.9–956.4)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Values are presented as median and ranges.

### Table 3. Inter-individual variability expressed by CV of CA125 and IL-6 in effluent

<table>
<thead>
<tr>
<th></th>
<th>Effluent concentration CA125</th>
<th>AR CA125</th>
<th>Effluent concentration IL-6</th>
<th>AR IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term PD (n = 17)</td>
<td>54</td>
<td>56</td>
<td>122</td>
<td>140</td>
</tr>
<tr>
<td>Long-term PD (n = 13)</td>
<td>75</td>
<td>58</td>
<td>139</td>
<td>128</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>73</td>
<td>141</td>
<td>142</td>
</tr>
</tbody>
</table>
The clinical relevance of CA125 and IL-6 determinations from an unstandardized dwell during every outpatient visit is reasonable, as judged from the CVs. The intra-individual variability in the ARs of the effluent markers was much higher as compared to the effluent concentrations. These may have been influenced by possible systematic errors, for instance inaccurate notated dwell times. Moreover, the median dwell time was 11 h, implicating that this could also be a factor for the cause of large variabilities, which is possibly reflected by the difference between CVs calculations on the average concentration, and AR values of CA125 and IL-6. Therefore, this study illustrates that ARs should only be calculated under standardized conditions. Akman et al. [21] found that night dwells can be used as a possibility for regular assessment of effluent CA125 in PD patients. However, with duration of the dwell, there was an increase in variation of the results. Our results underline the latter finding. Potentially, the type of dialysis solution may have an influence. This has been investigated for effluent CA125 where the percentage of the glucose-based PD solution used, 1.36 versus 3.86%, did not significantly influence the CA125 levels during the dwell. Also the type of osmotic agent had no significant influence on CA125. At present, no studies have yet been performed to analyse the effect of glucose-based PD solutions on IL-6. Differences between the conventional and more biocompatible PD solution have also not been investigated yet. Therefore, it should be noted that previous studies were performed with the use of the conventional PD fluid Dianeal® and that the present study concerns an almost pure Physioneal® population.

Table 4. Intra-individual variability expressed by CV of CA125 and IL-6 in effluent

<table>
<thead>
<tr>
<th></th>
<th>Coefficient of intra-individual variation (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effluent concentration CA125</td>
<td>AR CA125</td>
<td>Effluent concentration IL-6</td>
</tr>
<tr>
<td>Short-term PD (n = 17)</td>
<td>12 (2–65)</td>
<td>13 (0–37)</td>
<td>32 (11–98)</td>
</tr>
<tr>
<td>Long-term PD (n = 13)</td>
<td>17 (0–46)</td>
<td>16 (2–37)</td>
<td>20 (5–90)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.63</td>
<td>0.69</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Values are presented as median and ranges. The P-values represent the difference between short-term and long-term groups.

Fig. 2. Linear release pattern of CA125 (panel A) and IL-6 (panel B) effluent concentrations during a 4-h standardized dwell in eight stable PD patients.

Fig. 3. Time course of CA125 and IL-6. ARs of CA125 (units per minute) and IL-6 (picograms per minute) in short-term and long-term PD patients, studied by a linear mixed model expressed in means and SD. Panel (A) shows the time course of CA125-AR, where long-term PD patients have significantly lower time course (P = 0.001). Panel (B) shows the non-significant time course of IL-6-AR (P = 0.12). The number of patients per time point are presented underneath the graphs.
In conclusion, the present study shows that a deviation of the expected effluent CA125 pattern can be of clinical relevance. Especially, with the aid of standardization of the dwells. Based on the high coefficients of intra-individual variation, the results of a single measurement of effluent CA125 is a reflection of peritoneal cell mass in CAPD patients.

Acknowledgements. This study was supported by a grant from The Dutch Kidney Foundation—C06.2186.

Conflict of interest statement. None declared.

References


Received for publication: 1.6.10; Accepted in revised form: 7.3.11