Cancer antigen 125 concentrations in patients on chronic peritoneal dialysis: relationship with dialysis quality and membrane transport properties

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Abstract: The aim of this study was to evaluate longitudinal changes in drained dialysate cancer antigen 125 (dCA-125) levels and to assess relationships between dCA-125 and dialysis quality, peritoneal membrane transport rates, dialysate glucose load, peritonitis and use of erythropoiesis stimulating agents (ESA), angiotensin-converting-enzyme inhibitors (ACEi) and statins in patients with end-stage renal failure during the first 6 months of peritoneal dialysis (PD) treatment. This prospective study included 20 patients (11 males and 9 females; mean age 62.90±12.69 years) who were followed-up during the first 6 months of PD using conventional low pH glucose-based dialysis fluids. The concentration of dCA-125 was measured in all patients, and the peritoneal equilibration test (PET), peritoneal dialysis treatment adequacy (Kt/V), normalized protein catabolic rate (nPCR), and total, peritoneal and residual clearances of urea and creatinine were calculated. Information on peritonitis occurrence, the use of ESA, ACEi and statins were collected. Data were analyzed by the Mann-Whitney test, Wilcoxon matched pairs test and Spearman’s rank correlation. The concentration of dCA-125 significantly decreased during the follow-up (p=0.016). After 6 months of PD treatment, the concentration of dCA-125 decreased significantly (p=0.016) in all patients. The decrease was present in all patients, but was statistically significant in patients on ACEi therapy (p=0.006) and in patients not using statins (p=0.005) or ESA (p=0.012). No correlation was found between dCA-125 and glucose load, but a statistically significant negative correlation between dCA-125 and the PET for creatinine was observed (p=0.013). These findings challenge the role of dCA-125 in predicting mesothelial cell integrity in PD patients.

Key words: peritoneal dialysis; cancer antigen 125; ACE inhibitors; erythropoietin; statins

INTRODUCTION

Cancer antigen125 (CA-125) or carcinoma antigen 125 or carbohydrate antigen 125 is a transmembrane mucin-type glycoprotein. In humans, it is encoded by the MUC16 gene, also known as mucin 16 (MUC16) [1-3]. CA-125 was first detected in 1981 [4]; however, its physiological function is still unknown. Some authors have suggested that this massive glycoprotein forms a lubricating barrier against particles and infectious agents on mucosal surfaces [5]. Its function may be to protect the apical surface from pathogen adhesion due to its negatively charged structure and ligand properties for specific receptors on certain cells [6,7].

CA-125 is expressed on the surface cells of fetal tissues (the amnion, coelomic and Müllerian epithelia) and in adult tissues derived from the coelomic epithelium (pleura, epicardium, fallopian tubes, endometrium, endocervix) [8]. Small amounts of CA-125 are found in other epithelia (pancreas, colon, biliary tract, stomach, mammary and apocrine sweat glands). CA-125 is not expressed in normal ovaries and in the squamous epithelium. Human peritoneal mesothelial
cells also express CA-125 and are even more potent producers than ovarian cancer cells [9]. The presence of fluid in the serosal cavities may stimulate CA-125 production. During chronic PD, mesothelial cells come into direct contact with the dialysate and participate in the overall response. The concentration of CA-125 in the drained dialysate (dCA125) was long considered a mesothelial cell mass marker in stable PD patients, since it tends to decline in parallel with the duration of peritoneal dialysis, suggesting a loss in mesothelial cell mass due to the depletory influence of conventional PD fluid [10,11]. However, authors have reported a lack of correlation between the concentration of CA-125 and the number of mesothelial cells in peritoneal drained dialysate [12]. Even more so, the concentrations of dCA-125 were observed to be elevated during peritonitis episodes. This has put into question whether this marker might actually reflect mesothelial cell damage or even death, rather than mesothelial cell health [13]. All things considered, there is still much uncertainty regarding the physiologic and diagnostic significance of dCA-125 in peritoneal dialysis patients.

The aim of this investigation was to evaluate the changes in the concentrations of drained dialysate dCA-125 in end-stage renal failure patients during the first six months of PD, and to study the relationships between dCA-125 and PD quality, exposure of the peritoneum to glucose from the dialysate in peritonitis, as well as the relationships between dCA-125 and statin, erythropoietin and ACE-inhibitor use.

MATERIALS AND METHODS

Patients

The study was approved by the Ethical Committee of the Faculty of Medicine, University of Belgrade, and all patients provided informed consent for participation. This prospective study included 20 end-stage renal failure patients (11 males and 9 females; mean age 62.90±12.69 years, body surface area 1.81±0.15 m², body weight 69.99±8.97 kg, body height 170.6±7.96 cm), assessed at the beginning and after six months of peritoneal dialysis treatment. The patients were free of clinical and laboratory signs of infection within 4 weeks before enrollment. They used at least 8 L of conventional low-pH, glucose-based dialysis fluids (glucose concentration ranged from 1.25-2.5%) per day and drained a larger amount of dialysate than instilled liquid (Table 1). The underlying kidney disease was diabetes mellitus-linked in 8 patients, nephroangiosclerosis was due to long-lasting hypertension in 3, glomerular disease in 1, obstructive nephropathy in 1, and chronic undetermined nephropathy in 7 patients. During the follow-up period, 3 patients developed peritonitis; however, none of them had sclerosing peritonitis.

**Blood sample analyses**

At the beginning and after six months of PD, fasting morning blood, urine and dialysate samples were taken to determine urea and creatinine. CA-125 concentrations were measured in the drained dialysate after the overnight dwell time. Data concerning statins, ACEi and ESA usage, glucose load and occurrence of peritonitis were collected. PD quality and peritoneal membrane transport properties were assessed at baseline and after 6 months follow-up.

The concentrations of dCA-125 were determined by a two-step immunoassay using CMIA technology of ARCHITECT CA-125 II (Mannheim, Germany). In the first step of the assay, the sample and microparticles coated with antibody OC125 were combined. Reactive determinants of CA-125 present in the sample bind to the OC125-coated microparticles. In the second step after washing, an MII acridinium-labeled conjugate was added, followed by pre-Trigger and Trigger solutions to the reaction mixture. The resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship was observed between the amount of CA-125 reactive determinants in the sample and detected RLUs. The analytical sensitivity of the ARCHITECT CA-125 II assay is ≤1.0 U/mL: the referent serum values of Ca-125 are 35 U/mL. Normal baseline CA-125 values for the dialysate have not yet been established. PD was performed continuously, and dialysis quality was assessed at 7-day intervals [14] by calculating total weekly Kt/V and total weekly creatinine clearance (ClCr), which are the sum of peritoneal and residual renal components. Peritoneal and residual renal Kt/V and ClCr are calculated based on concentrations of urea and creatinine in previously collected 24-h urine
and drained dialysate samples, and concentrations of urea and creatinine in serum assessed during the same time interval. Kt/V is normalized to the volume of total body water, which approximately reflects the volume of urea distribution and is calculated using the Watson formula [15]. ClCr is normalized by the body surface area, calculated using the DuBois-DuBois formula [16]. Fasting venous blood samples to assess serum levels of glucose, urea, creatinine and albumin were taken in biochemistry vacutainer vials and centrifuged at 3000 rpm for 10 min. Concentrations of glucose, urea, creatinine and albumin were assessed in samples of 24-h urine and drained dialysate collections. All parameters were determined using the Biochemical analyzer ARCHITECT ci8200 (Abbott Diagnostics, Wiesbaden, Germany). Residual renal function was calculated as a mean value of residual clearance of urea and residual clearance of creatinine. The protein catabolic rate (PCR) and normalized protein catabolic rate (nPCR) were calculated using the Bergstrom or Randerson formula [17]. Peritoneal membrane transport characteristics were examined using the standard peritoneal equilibration test (PET) according to Twardowski [18]. After an 8-h night dwell, 2 L of conventional dialysate with glucose concentration between 2.2 and 2.5% were instilled for 4 h. Glucose and creatinine concentrations in blood and in drained dialysate samples were assessed at the start of the test, and again after 2 and 4 h. Peritoneal membrane transport characteristics for glucose and creatinine were calculated based on D/D0 and D/P ratio at the beginning of the test, after 2 and 4 h of instillation of dialysis solution.

Statistical analysis

Data are presented as means±standard deviation (SD) or median where appropriate. Differences between groups were assessed using the non-parametric Mann Whitney test and Wilcoxon matched pairs test. Correlational analysis was performed using the Spearman test.

RESULTS

The concentration of dCA-125 decreased significantly (p=0.016) from 28.83±25.35 at baseline to 16.56±14.65 U/mL after 6 months of PD (Fig. 1A). Residual diuresis and mean values of total weekly clearances of urea and creatinine were in agreement with the recommendations for dialysis quality (Table 1). Mean values of normalized protein catabolic rate (nPCR) were lower than 1 g/kg/day at baseline and after 6 months, and they did not differ significantly (Table 1). The PET for glucose and creatinine did not differ significantly at the beginning and after six months of peritoneal dialysis (Table 1). We observed that the concentrations of dCA-125 did not correlate with the nPCR, total, peritoneal and residual clearances of urea and creatinine, neither at the beginning nor after 6 months of chronic PD (Table 2). At the beginning of chronic PD, the concentrations of dCA-125 did not correlate significantly with PET<sub>glucose</sub> and PET<sub>creatinine</sub>. Residual diuresis (RD) and residual renal

Table 1. Parameters of dialysis quality at the beginning (0) and after six months (6) of PD.
function (RRF) (Table 3). After 6 months of chronic PD, no significant correlation was found between dCA-125 concentration and PETglucose, residual diuresis (RD) and RRF (Table 3), while a statistically significant inverse correlation (R=-0.546; p=0.013) was found between dCA-125 concentration and PETcreatinine (Fig. 1B).

In our study cohort, there was no significant correlation between dCA-125 and peritoneal glucose exposure (at baseline: R=-0.195, p=0.409, and after 6 months it was R=-0.262, p=0.265). The concentrations of dCA-125 did not differ significantly between patients with and without peritonitis during the follow-up (Table 4). During the 6-month follow-up, the concentrations of dCA-125 decreased in all patients, the difference being statistically significant in patients not treated with ESA or statins and in patients receiving ACE inhibitors (Table 5).

### DISCUSSION

Highly glycosylated CA-125 creates a hydrophilic environment on the epithelial cell apical membrane, acting as a lubricating barrier against foreign particles and infectious agents in different epithelia, such as the ocu-
Elevated CA-125 has served as a tumor marker in gynecological malignancies, lung cancer, mediastinal teratoma and even in non-Hodgkin’s lymphoma [20], and serum CA-125 levels are helpful in monitoring, risk stratification and prognosis after treatment of ovarian cancer [21]. Elevated CA-125 levels can be seen in endometriosis, acute pancreatitis, peritonitis, inflammatory pelvic disease, ascites of nonmalignant origin, cirrhosis, as well as in physiological conditions, including menstruation and early pregnancy [6]. During the last decade, CA-125 has been accepted as a marker of the integrity and/or regeneration of mesothelial cells in in vitro conditions [12,19,20], and dCA-125 can be considered a marker of mesothelial cell mass in stable PD patients [10]. During chronic PD, the peritoneal membrane is continuously exposed to bioincompatible dialysate solutions and sporadically to peritonitis episodes. These conditions cause adverse structural and functional changes that limit the membrane’s efficacy as a dialyzing organ [21,22]. In vitro studies have shown that conventional dialysis solutions are toxic to cultured mesothelial cells: the combination of low-pH lactate with high glucose and glucose-degradation product (GDP) concentrations and hyperosmolarity reduce cell viability, inhibit the synthesis of interleukins and prostaglandins and induce apoptosis [22,23]. Chronic peritoneal dialysis treatment is accompanied by a decrease in dCA-125, which points to a loss of mesothelial cell mass [10]. We observed that dCA-125 concentrations significantly decreased from 28.83±25.35 to 16.56±14.65 IU/mL after 6 months of PD, which is in agreement with literature data. Several cross-sectional studies reported a significant relationship between dCA-125 and the duration of PD; however, some researchers did not confirm this finding, probably because of the large interindividual variability. In a cross-sectional study including 31 patients, the mean dCA-125 concentration was 13.8 U/mL, range 1.1-96.5 U/mL. In a stable subgroup of 26 patients during the second year of PD, the mean dCA-125 concentration was 22.1±2.1 U/mL, with a calculated decreasing annual trend of 2.2% [24]. The application of theoretically more-biocompatible PD solutions with a low content of GDPs and a higher pH is associated with an increase in dCA-125 as compared to treatment with conventional fluid [22-25]. In a 3-month longitudinal follow-up study of 35 patients treated with neutral pH and a low-GDP PD fluid, a significant increase in dCA-125 from 12 (ranging from 3.93-85) U/mL to 48 (ranging from 14-373) U/mL was shown. Another study that followed 36 peritoneal dialysis patients treated with conventional dialysis fluid showed a decrease in dCA-125 from 38 (ranging from 3.42-122) U/mL to 13 (ranging from 4-44) U/mL [21]. However, after the follow-up period of 12 months, no significant differences were observed between dCA-125 levels at the beginning and at the end of the study (8.26±5.52 U/mL vs. 9.08±6.8 U/mL) [26].

In our study, no significant correlation was observed between dCA-125 concentrations and total, residual and peritoneal clearances of urea and creatinine, RD and RRF during the first six months of PD. These findings could be explained by the comparatively short duration of dialysis and may suggest that structural and functional changes of the peritoneal membrane had not developed during the first months of PD, in spite of the significant changes in dCA-125 concentrations. This could suggest that changes in dCA-125 do not have a prognostic value, at least not in the early period of PD. In our study cohort, a sig-

<table>
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<th>p</th>
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N – number of patients

<table>
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<th>N</th>
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<td>16.26±12.08</td>
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<td>30.56±29.69</td>
<td>17.03±18.80</td>
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<tr>
<td>P_W</td>
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<tr>
<td>ACEi</td>
<td>Yes</td>
<td>28.15±25.32</td>
<td>14.90±12.22</td>
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<tr>
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<td>31.55±32.31</td>
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<tr>
<td>P_W</td>
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<td>Statins</td>
<td>Yes</td>
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<tr>
<td>P_W</td>
<td>0.284</td>
<td>0.161</td>
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ESA – erythropoiesis stimulating agents; ACEi – angiotensin converting enzyme inhibitors; p_W – probability Wilcoxon matched pairs test; P_W – probability Mann-Whitney test.
significant inverse correlation was found between the concentration of dCA-125 and PET_{\text{creatinine}} after the 6-months follow-up. Lower dCA-125 concentrations are related to higher creatinine transport rates, which is an unfavorable characteristic of the mesothelial layer, thus implying that a low dCA-125 concentration is associated with a poor prognosis. The majority of studies found no relationship between dCA-125 and peritoneal transport rates [10,27]. One case-control study showed that an appearance rate of dCA-125 less than 33 U/min, combined with the appearance rate of interleukin 6 (IL-6) above 350 pg/min, was predictive for the development of encapsulating peritoneal sclerosis – a deleterious complication of chronic PD occurring in 2 to 3 years, with a sensitivity of 70% and a specificity of 89%. The temporal trend of dCA-125 is a useful predictor of encapsulating peritoneal sclerosis, which has a very poor prognosis when diagnosed [28].

Only 3 patients from our study group developed peritonitis during the follow-up. Concentrations of dCA-125 were assessed in patients free of peritonitis and/or signs of other infection within 4 weeks prior to the examination. The concentration of dCA-125 was slightly lower in patients who developed peritonitis during the follow-up vs. the peritonitis-free group. Some investigators reported increased levels of dCA-125 during acute peritonitis, probably due to necrosis of mesothelial cells [10]. Other studies did not find any additional diagnostic or prognostic benefit of measuring dCA-125 in patients on PD presenting acute peritonitis when compared to standard investigations, including peritoneal white cell count and serum C-reactive protein [29]. No differences in peritoneal dCA-125 concentrations were found in patients with Gram-positive, Gram-negative, fungal and polymicrobial peritonitis, nor in patients who responded successfully to intraperitoneal antibiotics and those with treatment failure [30]. No correlation was observed between dCA-125 levels and the number of peritonitis episodes, accumulated days of peritoneal inflammation and ultrafiltration capacity [31]. However, in a patient with five episodes of peritonitis, ultrafiltration failure was diagnosed; this was preceded by a gradual and marked decline in CA-125 levels during a 3-year follow-up [32,33].

In our study cohort, dCA-125 concentrations were not significantly different between the patients on ESA, ACEi and statin therapy and patients that were not receiving these medications, both at baseline and after 6 months of PD. The concentration of dCA-125 decreased in all patients during the 6-month PD treatment, and the difference was significant in patients receiving ACEi therapy and in those not receiving statins or ESA therapy. No literature data are available concerning the influence of ACEi on dCA-125 levels during PD, but it was found that ACEi play a protective role in preserving residual renal function and the transport rates of the peritoneal membrane during long-term PD, indirectly suggesting better preservation of the mesothelial membrane [34,35]. Our findings of significantly lower dCA-125 concentrations in patients on ACEi therapy after the 6-month follow-up are not in agreement with the suggested protective role of ACEi, so possibly a longer follow-up would be required to better define the correlation between ACEi therapy and dCA-125 levels.

Erythropoietin is a highly specialized growth factor. In our group of patients, ESA therapy was not significantly associated with changes in dCA-125 concentrations. However, patients not receiving ESA had significantly reduced dCA-125 levels after 6 months of PD, suggesting that ESA has a protective roll in preserving mesothelial cell mass.

In our patients who were not using statins during the entire follow-up period, the concentrations of dCA-125 were insignificantly lower than in those treated with statins. After six months of PD, in both groups the concentrations of dCA-125 were lower than at baseline, and the difference was significant in the group that was not treated with statins. While the literature data concerning this relationship are limited, it was published that the use of atorvastatin is associated with increased dCA-125 levels, which may be linked with the anti-inflammatory and antihyperlipidemic effects of the drug [36].

Concerning the protective effect of some medications on mesothelial cell mass during chronic PD treatment, it has also recently been found that spironolactone therapy is significantly correlated with higher dCA-125 concentrations [37]. This effect, however, was not investigated in our study.

Peritoneal glucose exposure from the dialysate solution was not in correlation with the dCA-125 con-
centrations in our study. Previous studies showed a significant decline in dCA-125 levels, reflecting the loss of mesothelial cell mass in children (>5 years) undergoing long-term PD, but this was not related to peritoneal glucose exposure [38]. On the other hand, the concentration of dCA125 increased significantly from baseline values in patients with a low-GDP level [39].

CONCLUSION

In our study in which we examined the effects of the use of conventional peritoneal dialysis solutions, the drained dialysate CA-125 levels decreased significantly during the first 6 months of PD treatment, they did not correlate with membrane transport rates and the quality of dialysis and they were not influenced by peritoneal glucose exposure. These results challenge the role of dCA_125 as an indicator of mesothelium integrity in chronic PD patients. The use of statins and ESA appeared to be protective for mesothelial cell mass while ACEi did not exhibit such an effect.

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Author contributions: Nataša Jovanović and Biljana Stojimirović were responsible for conceiving and designing the study and the drafting of the manuscript. Jasna Trobojević-Stanković and Dejan Nešić collected the literature. Nataša Jovanović and Biljana Stojimirović made critical revisions to the paper. Radmila Obrenović and Novica Boričić provided statistical expertise.

Conflict of interest disclosure: None to declare

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