Effluent biomarkers in peritoneal dialysis: A captivating symphony from the peritoneal membrane
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CURRENT STATUS AND PRACTICAL USE OF EFFLUENT BIOMARKERS IN PERITONEAL DIALYSIS PATIENTS

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SUMMARY

Long-term peritoneal dialysis therapy can lead to alterations in the function and morphology of the peritoneal membrane. Assessment of the peritoneal dialysis membrane usually is done by investigating the transport of small solutes and fluid. Assessment of morphologic alterations and their development would require repetitive peritoneal biopsies that usually are not feasible. Peritoneal tissues are bathed in dialysis solutions during peritoneal dialysis and may secrete or shed substances that can be recovered in peritoneal effluent. These molecular effluent biomarkers may give insight into morphologic changes. In this review, established and emerging candidate biomarkers in peritoneal dialysis are discussed. Additionally, requirements, challenges, and clinical applications of effluent biomarkers in peritoneal dialysis are addressed.
BACKGROUND

Biological markers (biomarkers) can be used as clinical tools to identify patients at risk and manage treatment. They are measured in plasma, serum, and sometimes in urine. Peritoneal dialysis (PD) offers the unique possibility to investigate peritoneal effluent. This effluent contains low-molecular-weight solutes, electrolytes, and macromolecules that have diffused from the circulation, but also various peptides and proteins that are released locally from peritoneal tissues.

Several functional and morphologic peritoneal alterations may occur as PD treatment progresses over time. Functionally, this is reflected by loss of ultrafiltration capacity. In rare cases, long-term PD treatment can lead to encapsulating peritoneal sclerosis (EPS). Assessment of functional abnormalities is simple, but a peritoneal biopsy is needed to assess morphologic changes. However, these have not been performed serially in individual patients because of ethical reasons. It is an invasive procedure that may lead to temporary discontinuation of PD therapy. Furthermore, uncertainty exists about sampling errors, reproducibility, and the risk of scarring. Hence, effluent biomarkers which represent the morphology of the peritoneal tissues in dialysis patients need further investigation. These effluent biomarkers alone or in combination could provide an assessment of mesothelial cell mass, peritoneal fibrosis and inflammation.

This review will present not only current developments in the discovery of effluent biomarkers and their clinical application in PD care, but also an overview of established, recently investigated and promising effluent biomarkers in PD.

CASE VIGNETTE

A 30-year-old Caucasian man started PD therapy for treatment of end-stage kidney failure due to Alport syndrome. After six years on PD therapy, he developed intermittent bowel obstruction. Computed tomography of the abdomen showed typical signs of EPS. During the previous six years, he experienced five episodes of peritonitis, two caused by coagulase-negative staphylococci and three by *Staphylococcus aureus*. All episodes were uncomplicated and treated successfully with antibiotics. Peritoneal function, including effluent cancer antigen 125 (CA125), was determined at two months and annually thereafter by means of a standard peritoneal permeability analysis using a 3.86% / 4.25% glucose-based dialysis solution. It appeared that ultrafiltration failure developed after three years, but the patient strongly opposed the transfer to hemodialysis therapy or kidney transplantation for ethical reasons. The results of peritoneal transport parameters
are listed in Table 1. The results of effluent CA125 and other biomarkers, measured retrospectively, are listed in Table 2.

**Table 1.** Peritoneal transport parameters of the case vignette.

<table>
<thead>
<tr>
<th>PD duration (months)</th>
<th>MTAC creatinine (mL/min)</th>
<th>Glucose absorption (%)</th>
<th>Net ultrafiltration (mL/4-hrs)</th>
<th>Free water transport at 60 min (% net UF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>12</td>
<td>69</td>
<td>545</td>
<td>45</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>79</td>
<td>784</td>
<td>33</td>
</tr>
<tr>
<td>24</td>
<td>17</td>
<td>79</td>
<td>570</td>
<td>38</td>
</tr>
<tr>
<td>36</td>
<td>18</td>
<td>85</td>
<td>348</td>
<td>6</td>
</tr>
<tr>
<td>47</td>
<td>15</td>
<td>78</td>
<td>274</td>
<td>9</td>
</tr>
<tr>
<td>59</td>
<td>16</td>
<td>77</td>
<td>270</td>
<td>17</td>
</tr>
</tbody>
</table>

**Normal values**

5 - 14  48 - 86  > 400  13 - 87

PD: peritoneal dialysis; MTAC: mass transfer area coefficient; UF: ultrafiltration.

PD therapy was discontinued after the diagnosis of EPS, the patient was referred to haemodialysis therapy and treatment with tamoxifen was started. This had a moderate positive effect on his abdominal symptoms. Kidney transplantation was performed after ten months on haemodialysis therapy. One year later a laparotomy was done due to a bowel strangulation in which the diagnosis of EPS was confirmed. After extensive adhesiolysis, a focal stenosis was resected. Recovery was uneventful.

**Table 2.** Longitudinal analysis of molecular biomarkers of the case vignette.

<table>
<thead>
<tr>
<th>PD duration (months)</th>
<th>CA125 (kU/L)</th>
<th>IL-6 (pg/mL)</th>
<th>VEGF (pg/mL)</th>
<th>MMP-2 (ng/mL)</th>
<th>PAI-1 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20.0</td>
<td>-</td>
<td>-</td>
<td>18.6</td>
<td>0.2</td>
</tr>
<tr>
<td>12</td>
<td>9.9</td>
<td>-</td>
<td>-</td>
<td>25.6</td>
<td>6.0</td>
</tr>
<tr>
<td>24</td>
<td>3.9</td>
<td>16.4</td>
<td>45.5</td>
<td>65.5</td>
<td>15.1</td>
</tr>
<tr>
<td>36</td>
<td>2.8</td>
<td>21.5</td>
<td>11.3</td>
<td>61.1</td>
<td>2.4</td>
</tr>
<tr>
<td>47</td>
<td>3.2</td>
<td>16.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>59</td>
<td>2.0</td>
<td>20.0</td>
<td>8.0</td>
<td>33.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**Assumed normal values**

> 11  < 40  < 30  < 20  < 0.9

PD: peritoneal dialysis; CA125: cancer antigen 125; IL-6: interleukin-6; VEGF: vascular endothelial growth factor; MMP-2: matrix metalloproteinase-2; PAI-1: plasminogen activator inhibitor-1.
PATHOGENESIS

Functional and Morphologic Studies

Long-term PD treatment may lead to damage to the peritoneal membrane and functional abnormalities. Although no formal pathogenesis of peritoneal membrane damage has been described to date, a conceptual framework is presented in Figure 1.

Peritoneal transport studies provide information about the vascular peritoneal surface area. The development of late ultrafiltration failure is the most frequently encountered functional abnormality, occurring in 12% of unselected patients after at least two years of dialysis. In the same population, EPS, the most serious peritoneal abnormality, occurred in 3% of the patients. Morphologic studies on the development of peritoneal alterations are sparse. These studies show loss of mesothelium, neoangiogenesis of vessels with diabetiform alterations such as reduplication of basement membranes and subendothelial hyalinosis of both arterioles and venules. The epithelio-to-mesenchymal transition of mesothelial cells is an early event which may initiate other abnormalities. The development of interstitial fibrosis does not only lead to thickening of the submesothelial zone, but is also present more diffusely. The neoangiogenesis and amount of fibrosis are related. Also, deposition of advanced glycosylation end products is present, both submesothelial and perivascular. Moreover, colocalization of advanced glycosylation end products with vascular endothelial growth factor (VEGF) has been demonstrated. Because of difficulties with longitudinal morphologic studies of humans, the main source of morphometric data has been provided by animal studies. However, key issues in most of these animal models are the short duration of exposure or follow-up and inability to generate a chronic uremic state. Additionally, in vitro research with various cell cultures has reported data, which could be interesting in themselves, but their results and measured effects are frequently not representative for the condition of the human peritoneal membrane in dialysis patients.

<table>
<thead>
<tr>
<th>PD duration (months)</th>
<th>MTAC creatinine (pL/min)</th>
<th>Normal values (pL/min)</th>
<th>Absorption (absorption (%))</th>
<th>IL-6 (pg/mL)</th>
<th>CA125 (kU/L)</th>
<th>VEGF (ng/mL)</th>
<th>MMP (ng/mL)</th>
<th>Glucose (glu/mL)</th>
<th>Free water transport at 60 min (%)</th>
<th>Effluent Bioarkers in PD</th>
<th>PATHOGENESIS</th>
<th>Functional and Morphologic Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>47</td>
<td>36</td>
<td>24</td>
<td>12</td>
<td>&gt; 11</td>
<td>&lt; 30</td>
<td>&lt; 20</td>
<td>&lt; 40</td>
<td>1</td>
<td></td>
<td>PATHOGENESIS</td>
<td>Functional and Morphologic Studies</td>
</tr>
<tr>
<td>Longitudinal analysis of molecular biomarkers of the case vignette.</td>
<td><strong>Figure 1:</strong> The development of interstitial fibrosis does not only lead to thickening of the submesothelial zone, but is also present more diffusely. The neoangiogenesis and amount of fibrosis are related. Also, deposition of advanced glycosylation end products is present, both submesothelial and perivascular. Moreover, colocalization of advanced glycosylation end products with vascular endothelial growth factor (VEGF) has been demonstrated. Because of difficulties with longitudinal morphologic studies of humans, the main source of morphometric data has been provided by animal studies. However, key issues in most of these animal models are the short duration of exposure or follow-up and inability to generate a chronic uremic state. Additionally, in vitro research with various cell cultures has reported data, which could be interesting in themselves, but their results and measured effects are frequently not representative for the condition of the human peritoneal membrane in dialysis patients.</td>
<td>PATHOGENESIS</td>
<td>Functional and Morphologic Studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Figure 1. A conceptual framework: pathophysiology and morphologic alterations of the peritoneal membrane. A representation of a conceptual framework of the pathophysiology and morphological modifications that may occur in some PD patients is given. The upper horizontal blocks present an indicative course of the clinical presentation of peritoneal membrane physiology. The blocks shown below are the morphological alterations, which could arise with duration of PD treatment and indicates the potential application of biomarkers in routine clinical practice.

Requirements for a Peritoneal Effluent Biomarker

The discovery of effluent biomarkers in PD usually is hypothesis driven and based on the pathology of the peritoneal membrane. Emphasis is on the local integrity of the peritoneal membrane rather than systemic contributions. Therefore, serum markers in PD are not addressed in this review.

For standardization and comparison, the most clinically relevant specimen is the peritoneal effluent obtained after a predefined dwell, such as a peritoneal function test.\textsuperscript{12,13} One of the pivotal characteristics of an effluent biomarker is that it is derived from intraperitoneal production. When the dialysate level exceeds the serum concentration of the studied biomarker, the possibility of peritoneal transport by diffusion can be neglected and local production can be acknowledged. Mathematically, an
individual transport line can be generated based on the least square regression of dialysate to plasma ratios of β2-microglobulin, albumin, immunoglobulin G and α2-macroglobulin. After interpolation of the molecular weight of the biomarker, the positive discrepancy between predicted and measured levels is attributed to local production.\textsuperscript{14,15} Obviously, not all substances present in the effluent are produced locally. Moreover, some proteins that are present in the drained dialysate require prior concentration by positive pressure ultrafiltration before they can be measured accurately. This method requires stringent laboratory conditions and is not always feasible due to the large volume of drained effluent, which needs to be acquired under standardized conditions. Furthermore, the concentration factor is determined by means of the pre and post concentrations of dialysate albumin. For other substances, diffusion across the peritoneum is responsible for their presence in effluent and are therefore not discussed. These substances comprise nitrate, secretory phospholipase A2, hydroxyproline and various complement factors.\textsuperscript{16} Studies also have investigated biomarkers during PD-induced peritonitis. Throughout peritonitis, elevated levels of various proteins are measured in peritoneal effluent, such as CA125,\textsuperscript{17} cytokines,\textsuperscript{18,19} factors of coagulation and fibrinolysis,\textsuperscript{17,20} hyaluronan,\textsuperscript{17,21} and phospholipids.\textsuperscript{17} This temporary elevation often persists for a maximum of one week during the treatment of peritonitis.\textsuperscript{17} Therefore, effluent biomarkers should be measured exclusively after resolution of peritonitis to avoid falsely high results.

Correction for the drained effluent volume is desired and achieved by calculating appearance rates of the biomarker of interest. This correction may be applied only when a biomarker is known to increase linearly during a predefined dwell time. In case this criterion is not met, use of absolute concentrations is commonly used. Moreover, coefficients of variation should be evaluated for each biomarker to determine its dispersion and reliability. With regard to pathologic pathways, it follows from the aforementioned considerations that an effluent biomarker has to meet considerable criteria. In brief, an ideal effluent biomarker for PD should encompass the following properties:

1. Detectable in peritoneal effluent
2. Local release/production within the peritoneal cavity
3. Involved in pathology of the peritoneal membrane
4. High sensitivity and specificity for the clinical outcome of interest (e.g. EPS)
RECENT ADVANCES

Individual Biomarkers

At present, integration of effluent biomarkers in the routine clinical practice of PD is still modest. A concise synopsis is given on CA125 and interleukin-6 (IL-6) which both can easily be measured in unconcentrated effluent. This will be followed by a short review of other cytokines, growth factors and candidate biomarkers of peritoneal tissue remodeling, coagulation and fibrosis. Although not exhaustive, Figure 2 and Table 3 represent an overview of established and candidate effluent biomarkers in PD.

Cancer Antigen 125

Effluent CA125 is the most extensively studied biomarker in PD. It is a high-molecular-weight glycoprotein of 220 kDa, which represents the amount of mesothelial cells. Serum CA125 was discovered as a biomarker of ovarian neoplasia and currently is the gold standard for surveillance of ovarian carcinomas. In vitro research showed constitutive synthesis of CA125 solely by human peritoneal mesothelial cells. Moreover, dialysate CA125 correlates with the number of mesothelial cells in effluent, suggesting that local production can be assessed. Effluent CA125 levels show a linear increase during a 4-hour dwell, irrespective of the dialysis solution. The day-to-day variability within a patient is 7.7% under standardized conditions. However, intra-individual variability increased to 15% when the appearance rate was measured in day or overnight dwells from random outpatient clinic visits.

In general, longitudinal follow-up of effluent CA125 levels shows a gradual decrease, which is in accordance with morphologic data. A CA125 appearance rate below 33.0 U/min is capable of differentiating patients who develop EPS with a sensitivity of 70% and specificity of 89% when combined with an appearance rate of IL-6 above 350.0 pg/min. These results require confirmation by other studies. It follows from these considerations that a sudden decline in dialysate CA125 levels may indicate severe mesothelial cell damage, transdifferentiation, or increased risk of the development of EPS.

The influence of biocompatible dialysis solutions on the peritoneal membrane has been studied, especially in prevalent patients. Studies of less acidic dialysis solutions have shown a reduced concentration of glucose degradation products and an increase in effluent CA125 levels after two years. This effect was less pronounced when incident patients were investigated. In accordance with previous longitudinal studies,
CA125 levels still decreased with the duration of PD in patients treated with biocompatible solutions. Nevertheless, higher effluent levels are present at baseline and remain greater during longitudinal follow-up as compared to patients treated with conventional dialysis solutions.\textsuperscript{62}

Including effluent CA125 measurements in routine follow-up of PD patients could provide insight into the volume and quality of the peritoneal mesothelium. However, further investigations are needed with regard to CA125 level as a predictor of PD technique survival or as a potential risk factor in the development of EPS.

\textbf{Figure 2.} Potential effluent biomarkers in peritoneal dialysis. An overview is given on morphological peritoneal membrane alterations and where several biomarkers might play a role in the pathophysiology and reflect the condition of the peritoneal membrane. CA125: cancer antigen 125; CCL18: CC chemokine ligand 18; GDPs: glucose degradation products; HA: hyaluronan; IL-6: interleukin-6; MMP-2: matrix metalloproteinase-2; PAI-1: plasminogen activator inhibitor-1; TGF-β: transforming growth factor-β; TNF-α: tumor necrosis factor-α; VCAM-1: vascular cell adhesion molecule-1; VEGF: vascular endothelial growth factor.
Table 3. Overview of candidate effluent biomarkers in peritoneal dialysis.

<table>
<thead>
<tr>
<th>Effluent Biomarker</th>
<th>Local Production (+/-)</th>
<th>Postulated Role</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coagulation and Fibrinolysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrin monomers</td>
<td>+</td>
<td>Coagulation and fibrinolysis</td>
<td>Decreasing tendency of FV in first 2 years of PD treatment[32]</td>
</tr>
<tr>
<td>PAI-1</td>
<td>+</td>
<td>Fibrosis</td>
<td>Linear increasing tendency during 4-hour peritoneal equilibration test[13]; during peritonitis expression is augmented[24]; elevated levels in homogenates of peritoneal tissue of patients with intra-abdominal adhesions[25,26]</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>+</td>
<td>Pro- and anti-inflammation, induces synthesis of hepatic acute-phase proteins</td>
<td>Increased plasma concentrations during acute-phase reactions are associated with mortality in haemodialysis and PD patients; intra-individual coefficients of variation of 28%; variable results with peritoneal transport; longitudinal[28,31] and conventional vs. biocompatible PD fluids[24,26]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-</td>
<td>Angiogenesis, fibrosis, acute-phase inflammation</td>
<td>Signs of local production during acute phase peritonitis; no difference between patients treated with biocompatible vs. conventional fluids[37]</td>
</tr>
<tr>
<td><strong>Endothelial Dysfunction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-Selectin</td>
<td>+/-</td>
<td>Cell adhesion</td>
<td>Correlation between percentage of free water transport and E-Selectin attributed to local production (DLB., unpublished observations 2012)</td>
</tr>
<tr>
<td><strong>Growth Factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTGF</td>
<td>+</td>
<td>Angiogenesis, cell adhesion and fibrosis</td>
<td>In vitro induction of extracellular matrix expression; relationships with peritoneal transport of low-molecular-weight solutes[30,39]</td>
</tr>
<tr>
<td>TGF-β</td>
<td>+</td>
<td>Angiogenesis, fibrosis and stimulation extracellular matrix formation[40]</td>
<td>Present in inactive form; induction of EPS-like peritoneal alterations after adenoviral vector gene transfer[41]; no difference in levels between conventional vs. biocompatible PD fluids[42]</td>
</tr>
<tr>
<td>VEGF</td>
<td>+</td>
<td>Neoangiogenesis &amp; increases vascular permeability</td>
<td>An increasing tendency with duration on PD[43]; not a predictor of EPS[44]</td>
</tr>
<tr>
<td><strong>Matrix and Tissue Remodeling</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL18</td>
<td>-</td>
<td>Fibrosis</td>
<td>Elevated in EPS patients[45]</td>
</tr>
<tr>
<td>HA</td>
<td>+</td>
<td>Connective tissue turnover</td>
<td>Elevated in the acute phase of peritonitis[17] and in patients with a fast transport status[46,47]; not a predictor of EPS, but low levels of effluent hyaluronan may predict survival of CAPD patients[48]</td>
</tr>
<tr>
<td>MMP-2</td>
<td>+</td>
<td>Epithelial-to-mesenchymal transition, fibrosis, tissue remodeling</td>
<td>High values of MMP-2 in patients with EPS were found in multicenter study[49]; positive relationship with PD duration and with tissue inhibitor of metalloproteinase 1 has been reported[50]; negative association with free water transport in prevalent PD patients[50]; associated with the amount of peritoneal fibrosis in EPS-like rat model[50]</td>
</tr>
<tr>
<td>Procollagen peptides</td>
<td>+</td>
<td>Fibrosis</td>
<td>Modest elevation during peritonitis[31]; exposure to biocompatible PD fluids either led to an increase of effluent procollagen peptides[52] or had no effect[37]</td>
</tr>
<tr>
<td><strong>Mesothelium</strong></td>
<td>+</td>
<td>Mesothelial cell mass</td>
<td>Long-term PD may be associated with loss of mesothelial cell mass[8] and may be absent or severely reduced in patients with abundant fibrosis[8]; exposure to biocompatible PD fluids for up to 2 years led to an increase in effluent CA125[37,42,52-55]</td>
</tr>
</tbody>
</table>
Interleukin-6

IL-6 is a pleiotropic cytokine produced by various cell types, including T cells, activated monocyte/macrophages, fibroblasts, mesothelial cells and vascular endothelial cells.\textsuperscript{38,32,63} Although it has both pro- and anti-inflammatory properties,\textsuperscript{64} IL-6 induces synthesis of hepatic acute-phase proteins. Plasma IL-6 concentrations are increased during acute-phase reactions and associated with mortality in both hemodialysis and PD patients.\textsuperscript{55} IL-6 has a molecular weight of 26 kDa and is produced locally in the peritoneal cavity during PD, as indicated by effluent concentrations that exceed those in serum.\textsuperscript{27-29,66} Effluent IL-6 concentrations increase linearly during a peritoneal function test.\textsuperscript{27} The within-patient coefficient of variation of effluent IL-6 is 28%.\textsuperscript{27} Therefore, a single effluent IL-6 measurement should be evaluated carefully when applied in clinical practice.

Infectious peritonitis causes a dramatic increase in the local production of this cytokine.\textsuperscript{67,68} Relationships between effluent IL-6 and peritoneal solute transport have been described in clinically uninfected PD patients,\textsuperscript{28-31} with longitudinal studies showing variable results.\textsuperscript{32,33} This also accounts for executed studies with biocompatible dialysis solutions.\textsuperscript{34-36} It follows from the discussed studies that effluent IL-6 probably is a biomarker for local peritoneal inflammation in the absence of clinical peritonitis.

Other Cytokines and Growth Factors

Other cytokines and growth factors, including tumor necrosis factor α (TNF-α), transforming growth factor β (TGF-β), VEGF and connective tissue growth factor (CTGF), are summarized in Table 3.

TNF-α secretion is stimulated in specific circumstances, for instance, with endotoxin.\textsuperscript{69} Accordingly, its concentrations in the peritoneal effluent of stable PD patients can be explained fully by transport from the circulation.\textsuperscript{15} In the Euro-Balance trial, no differences in effluent TNF-α levels were found between patients treated with conventional dialysis solutions and those exposed to biocompatible ones.\textsuperscript{37}

TGF-β is a growth factor that is secreted \textit{in vitro} by fibroblasts in an inactive form.\textsuperscript{70} In the circulation, TGF-β is coupled to α2-macroglobulin where it remains biologically inactive.\textsuperscript{70} Peritoneal effluent acidification was necessary before it could be measured.\textsuperscript{40} Despite local production and release, no relationship is noted with the duration of PD.
VEGF is a glycoprotein which is secreted primarily in a soluble form. VEGF increases vascular permeability, as illustrated by the relationship of VEGF in ocular fluid of diabetic patients with the presence of proliferative diabetic retinopathy. VEGF can be measured in peritoneal effluent, is produced locally and is related to peritoneal transport of low-molecular weight solutes. Studies comparing VEGF levels in patients treated with conventional or biocompatible dialysis fluids with a long-term follow-up are not available.

CTGF is a cysteine-rich peptide that has angiogenic properties. CTGF messenger RNA expression is higher in peritoneal tissue of PD patients with ultrafiltration failure than in pre-PD kidney failure. In two separate studies, peritoneal effluent contained CTGF in elevated concentrations, pointing to local production. The same studies show relationships with peritoneal transport of low-molecular-weight solutes. No data are available for longitudinal follow-up, long-term PD therapy or effects of biocompatible solutions.

*Candidate Biomarkers of Peritoneal Tissue Remodeling, Coagulation and Fibrosis*

The interstitium contains high levels of coagulation and fibrinolytic factors in stable PD patients, as well as those with peritonitis. The exact contribution of the interstitial tissue to peritoneal transport is not clear, but with extended PD duration, deposits of collagen and fibrosis are found in the peritoneal interstitium. Characteristics of these biomarkers are summarized in Table 3.

Hyaluronan is a major constituent of connective tissue. Besides diffusion from the circulation, local peritoneal production likely occurs. Associations between hyaluronan and inflammatory markers have been observed. Duration of PD has no effect on effluent hyaluronan and it also is not a predictor of EPS. Studies with biocompatible dialysis solutions have almost all shown lower effluent hyaluronan concentrations than obtained with the conventional solutions. Only in one study in which the first weeks were investigated in incident PD patients an increase in effluent hyaluronan was found.

Procollagen peptides are released during the synthesis of collagen I and III. Peritoneal effluent concentrations exceed those in serum, pointing to local synthesis and release. One patient with high effluent levels of collagen propeptides and EPS has been described, but we were unable to confirm this in a larger group of patients. Local
production also has been found for other factors of coagulation and fibrinolysis, such as fibrin monomers, D-dimer, fibrin degradation products, fibrinopeptide A, thrombin antithrombin complexes, tissue-plasminogen activator and its inhibitor. Although these markers might reflect the quantity of connective tissue in the submesothelium, replication of the study is warranted.

CC chemokine ligand 18 (CCL18) is a cytokine produced by monocytes and macrophages and involved in pulmonary fibrosis. One study has investigated its potential use in PD, but the reported concentrations are likely caused by diffusion from the circulation.

Plasminogen activator inhibitor-1 (PAI-1) is a single chain glycoprotein. As the most important inhibitor of tissue plasminogen activator, the main sites of production are endothelial cells and vascular smooth muscle cells. Elevated PAI-1 levels are present in the peritoneal tissue of patients with intra-abdominal adhesions, and PAI-1 is distributed extensively in submesothelial tissue. Additionally, intraperitoneal production has been established in both pediatric and adult PD patients.

Matrix metalloproteinase-2 (MMP-2) is produced in vitro by mesothelial cells. An in vivo study in rats with TGF-β1-induced peritoneal fibrosis suggested a role for MMP-2 in epithelial-to-mesenchymal transition. Together with other matrix metalloproteinases, MMP-2 is able to degrade all extracellular matrix components. A multicenter study suggested local synthesis and showed increased values of MMP-2 in patients with EPS. We recently found that effluent MMP-2 was related to peritoneal fibrosis in a rat model with peritoneal fibrosis.

**Discovery-Based Research**

The discovery of effluent biomarkers frequently involves cell culture with the use of commercially available kits, such as enzyme-linked immunosorbent assays and western blots. High-throughput laboratory technologies in peritoneal effluent are emerging, along with genomics, metabolomics and proteomics. The number of proteomic studies with peritoneal effluent is still limited. To date, proteomic analyses in PD are focused on the characterization and identification of the peritoneal dialysate in adult and pediatric PD patients. Additionally, studies have been performed in patients with peritonitis and to detect differences in diabetic dialysate and membrane transport status. Challenges in proteomic analysis of the effluent include the dynamic range in which the ability to detect...
and identify low-abundance proteins can be problematic. Overall, the proteins found in the peritoneal effluent generally are plasma-derived and exist merely as extracellular proteins.\textsuperscript{92,93} Because the peritoneal proteome of PD patients is likely to be susceptible to posttranslational modifications by patient-related and external factors, a priori definitions of clinical outcomes and homogeneous populations in proteomic studies should be well specified. In brief, studies on the indiscriminate use of proteomics in effluent have not given workable results up to now. However, use of these powerful techniques on peritoneal effluent has the potential to shed light on the underlying pathogenic mechanisms of peritoneal membrane deterioration and may lead to novel biomarkers for diagnostic and therapeutic applications after more exploration and calibration.

**SUMMARY**

Today, the available biomarkers in peritoneal effluent have been developed from pathogenic concepts. These solutes together with their advantages and limitations have been discussed in the present article. Implementation of effluent biomarkers in patient care is a goal for the near future as peritoneal transport studies provide insufficient information on the development of peritoneal membrane alterations, including the development of EPS. A feasible approach could include some biomarkers in routine clinical assessment of peritoneal function. This will provide more information than currently available. Optimally, the number of selected biomarkers should be limited to two or three that can be easily measured in effluent. Currently, CA125, reflecting mesothelial cell mass and IL-6, representing peritoneal inflammation, are at a stage at which their measurement can be included easily in peritoneal function tests. Until now, no good biomarker of the extent of peritoneal fibrosis has been identified with certainty. It follows from these considerations that assessment of the peritoneal membrane should focus on the detection of ultrafiltration failure and its possible causes and the transport of low-molecular weight solutes representing the effective peritoneal surface area. The use of effluent biomarkers may give further insight in the development of morphologic alterations.

In the patient described in the case vignette, it is obvious that ultrafiltration failure developed after three years of PD therapy without a change in small-solute transport. In retrospect, this was preceded by a marked decrease in peritoneal appearance rates of CA125 and an increase in MMP-2 and PAI-1. The results of the functional measurements led us to discuss the possibility of transfer to hemodialysis therapy.
Because the patient wanted to continue PD treatment, we agreed. If we had known the results of the biomarker assessments, it is likely that the discussion with the patient would have been different, with much more emphasis on the risk of developing EPS. This may have protected him from the severe morbidity, which he consequently experienced.

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REFERENCES


REFERENCES


22. Sakurada T, Kojima S, Oishi D et al. Prothrombin fragment 1,2 (F1,2) in effluent is a useful marker for peritoneal permeability in peritoneal dialysis patients using neutral dialysate. Adv Perit Dial 2011; 27: 2-5.


77. Le Poole CY, Welten AG, ter Wee PM et al. A peritoneal dialysis regimen low in glucose degradation products results in increased cancer antigen 125 and peritoneal activation. Perit Dial Int 2012; 32: 305-315.


