Monitoring of the peritoneal membrane

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Abstract

Background. Indirect methods can be used to provide valuable information about peritoneal structure and function for the indirect analysis of peritoneal membrane.

Methods. The focus of this paper will be on the commonly available tools for this purpose. First, the value and clinical relevance of CA125 as a marker of mesothelial cell mass in peritoneal effluent will be evaluated. Thereafter, monitoring the peritoneal membrane by using its properties to transport solutes and water will be discussed.

Results. The data obtained can be useful for tailoring dialysis adequacy, analysis of clinical problems such as ultrafiltration failure or to predict the development of peritoneal sclerosis.

Keywords: peritoneal membrane; peritoneal transport; peritoneal equilibrium test; fluid kinetics; CA125

Introduction

The peritoneal membrane consists of living tissue. It has variable properties influenced by endogenous and exogenous factors. So it is important to monitor its functional characteristics with respect to time. The data obtained can be useful for tailoring dialysis adequacy, analysis of clinical problems such as ultrafiltration failure or to predict the development of peritoneal sclerosis.

Although peritoneal tissue can be obtained and analysed, in the absence of an easy and safe procedure, this is only done during surgical procedures for various other indications. However, various other methods can be used to provide valuable information about peritoneal structure and function for the indirect analysis of peritoneal membrane. The focus of this paper will be on the commonly available tools for this purpose. First, the value and clinical relevance of cancer antigen 125 (CA125) as a marker in peritoneal effluent will be evaluated. Thereafter, monitoring the peritoneal membrane by using its properties to transport solutes and water will be discussed.

The mesothelium

It reduces friction between abdominal organs and prevents the formation of adhesions. During peritoneal dialysis (PD), mesothelial cells are involved in local host defence [1]. The currently used PD solutions are toxic to cultured mesothelial cells [2]. They reduce cell viability [3], inhibit the synthesis of various cytokines [4] and induce apoptosis [5]. Peritoneal biopsies show that PD leads to signs of mesothelial degeneration and regeneration [6–12], including replacement of the mesothelial cell layer by a thick fibrous band in long-term PD patients [12–14]. Also, acute infectious peritonitis can result in discontinuity or denudation of the mesothelial layer [8,12–15]. Remesothelialization occurs after the infection has been cured but it might be incomplete [8,13,16]. Mesothelial cell cultures from effluents during PD show various morphologic features ranging from a cobblestone-like appearance to fibroblast-like cells or mixed cell populations [17].

CA125 as a marker of mesothelial cell mass

CA125 can be used to indirectly measure mesothelial cell mass or cell turnover in stable CAPD patients [18,19]. It is a glycoprotein with a molecular weight exceeding 200 000 Dalton [20]. CA125 is expressed in coelomic epithelium during embryonic development [21]. In adult tissues, CA125 has been demonstrated on the epithelium of the female genital tract and on mesothelial cells in the pleura, pericardium and peritoneum [21]. Its function remains unknown.

Release of CA125 by cultured human peritoneal mesothelial cells

In two previous studies, the CA125 concentration in the supernatant increased with the duration of culture and was proportional to the amount of cells brought into the culture [18,19]. This increase was exponential before confluence and linear after that time point [19], consistent with a constant production in time per cell. In contrast, in a recent
study, no relation was found between the number of mesothelial cells after lysis with trypsin and CA125 in the supernatant [22]. Only one study showed a limited increase in CA125 release after stimulation with cytokines on Day 5 of the culture [23], but this was not found in two other studies using the same cytokines on Day 8 of the culture [19] or after reaching confluence [22]. One can hypothesize that stimulation with cytokines has some effect on CA125 production only when the confluence of the monolayer is not perfect. Whether the *in vitro* experiments can be extrapolated to human situation remains questionable, as studies with more biocompatible dialysate solutions, but still containing glucose, almost universally demonstrated an increase in CA125 concentration in time (see below). CA125 is almost undetectable in lymphocytes, monocytes, granulocytes and fibroblasts [24], making peritoneal mesothelial cells the most likely source for local CA125 release during PD. In view of the constitutive release after confluence, it can be concluded that CA125 released from mesothelial cells can probably be used for follow-up of mesothelial cell mass in individuals.

**CA125 in peritoneal dialysate in stable PD patients**

Mesothelial cells in peritoneal effluent are CA125 positive when investigated with immunohistochemistry [18,25]. The median percentage of CA125-positive cells was 92%, but ranged between 0% and 100%. Values between 75% and 100% were found in 80% of patients [25]. In most of the studies, a relationship was found between the number of mesothelial cells in peritoneal effluent of PD patients and effluent CA125 concentration [18,19,25,26]. Radioimmunoassay for the measurement of CA125 was not validated in peritoneal effluent and has been reported to be unreliable for low concentrations [27].

In 24 patients on continuous ambulatory PD, CA125 concentrations in the effluent of the overnight dwell yielded values ranging between 5.2 and 76 U/mL, median 18 U/mL [18]. Later it was shown that CA125 dialysate concentrations increased linearly during a 4-h dwell [28,29], even in dwellsw exceeding 4 h [30]. Due to the effect of time on CA125 appearance, low values have been found when measured after a 4-h standardized dialysis dwell [31]. This implies that either the method is accurate in low range or longer dwells should be used. To compare CA125 in samples with various dwell times, it was advised to calculate the appearance rates of CA125 [30]. Due to the limited number of patients included in the cross-sectional studies, and the various methodologies used, normal baseline values for dialysate CA125 have not yet been established.

**CA125 and peritoneal transport**

Mesothelium is unlikely to be directly involved in the transport of solutes from the circulation to the dialysate [32]. An indirect effect of mesothelial cells can be expected as cultured mesothelial cells produce various cytokines, chemokines and prostaglandins, some of which are vasoactive [33] and involved in the changes in peritoneal permeability that occur during peritonitis [34–36]. A positive relation between dialysate-to-plasma ratios (D/P) of creatinine and dialysate CA125 has been found in some studies [26,37–39], but was absent in others [29,31,40–42]. This positive relation was especially found in the early phase of the dialysis treatment and might be explained by cytokines and vasoactive substances produced by mesothelial cells [38,39]. The increase in peritoneal transport after long duration PD has been explained by revascularization in the peritoneal membrane [43], resulting in disappearance of the initial positive relation between CA125 and peritoneal solute transport.

**Dialysate CA125 and duration of PD**

A decrease in CA125 appearance rate has been found during longitudinal analysis [28,44,45]. Low values have been found in peritoneal sclerosis patients [46,47]. Due to the large interindividual variability of dialysate CA125 [31], a single low CA125 appearance rate is difficult to interpret. Serial longitudinal observations showing a decrease suggest loss of mesothelial cell mass.

Peritoneal resting has been investigated in PD patients with peritoneal membrane failure [48,49]. Patients treated with peritoneal resting later on during their treatment had lower dialysate CA125 levels than those not needing temporary discontinuation [29]. Although experience is limited, peritoneal resting might lead to an increase in effluent CA125 [47]. One study suggested that no increase in the CA125 concentration after withdrawal of PD was predictive of peritoneal sclerosis development [47].

**Dialysate CA125 as marker of biocompatibility of dialysis solutions**

Peritoneal membrane alterations during long-term PD are most probably due to continuous exposure to currently used bioincompatible dialysis solutions. Loss of mesothelial cells is one feature of these alterations. Follow-up of dialysate CA125 in patients during treatment with more biocompatible dialysis solutions could provide information on their biocompatibility *in vivo*, at least with respect to the mesothelium. In majority of clinical studies, CA125 in the dialysate increased when using more biocompatible dialysate solutions, while it decreased after switching to the standard solutions [46,50–60]. It appears from these studies that dialysate CA125 is a useful marker for *in vivo* biocompatibility assessment of dialysis solutions, at least with respect to their effect on mesothelium.

**Monitoring the peritoneal membrane function**

**Solute transport**

Peritoneal transport of solutes is determined by the effective surface area as well as the intrinsic permeability of the membrane. The effective surface area is either determined by the number and flow within capillaries [61,62] or by the splanchnic volume [63]. The mesothelium is not a significant barrier to small solute transport [64]. However, changes found in the interstitium after CAPD treatment may be important [10,65,66]. Although the interstitium cannot act as
a mechanical barrier to solutes due to its large gaps [67], it might be a diffusive barrier to solutes [68–71]. Hyaluronan, which is highly negatively charged, could be primarily involved in the restriction of proteins [72]. As the peritoneal capillary represents the major barrier in blood to peritoneal transport [73,74], changes in solute transport might reflect ultrastructural changes of these vessels.

The transport of low- and middle-molecular-weight solutes is only size dependent [75–79] and their transport mainly depends on the effective peritoneal surface area. Stagnant fluid layers are not considered to be important because first, it is very unlikely that these stagnant fluid films will change in time, and second, the permeability tests are performed under standardized conditions.

The transport of macromolecules is size-selectively restricted either by restricted diffusion [80] or by convection through large pores [81]. Thus, it is likely that clearances of serum proteins are dependent both on effective surface area and permeability. As proteins in the dialysate are usually not measured in clinical practice, the interpretation of changes in macromolecules during PD are beyond the scope of this paper.

In conclusion, changes in low-molecular-weight solute transport are explained by changes in vascularization of the peritoneal membrane.

Fluid transport

Water transport through the peritoneal membrane is possible due to differences in osmotic and hydrostatic pressures between the peritoneal capillaries and the dialysate. This pressure difference is exerted over small pores and through the water channels in the endothelium of peritoneal capillaries and vessels resulting in transcapillary ultrafiltration (TCUF). The transendothelial water channels have been identified morphologically as aquaporin-1 [82–84]. As the aquaporin-1 channel is impermeable to solutes, crystalloid osmotic-induced free water transport occurs through them. Free water transport is especially important with the use of hyperosmolar solution, as small pores are influenced by tonicity only to a limited extent due to their very low reflection coefficient to glucose. In contrast, solutions with low osmolarity will induce little free water transport [85]. Fluid within the peritoneal cavity can disappear either through the peritoneal membrane or through the peritoneal lymphatics. The magnitude of lymphatic transport during a short dialysis dwell with a hypertonic solution is still a matter of debate [86,87].

The difference between TCUF and fluid loss from the peritoneal cavity is the net ultrafiltration (NUF). The International Society of Peritoneal Dialysis Committee on ultrafiltration failure has advised to standardize the definition of ultrafiltration failure to <400 mL after a 4-h dwell test with 3.86%/4.25% glucose [88].

In conclusion, changes in ultrafiltration volume can be caused by various mechanisms. Usually, it is the result of changes in the vascular surface area leading to either slower or faster dissipation of the osmotic gradient [89], but changes in aquaporin-mediated water transport, either by loss of aquaporins or functional impairment, could also be responsible [90,91]. Furthermore, it could be caused by fluctuations in fluid resorption from the peritoneal cavity [92].

Commonly used tests for the measurement of solute and fluid transport

The Peritoneal Equilibrium Test (PET)

The principle of this test was proposed by several authors [93–96]. Since its introduction by Twardowski et al. in 1987 [97], it is the most widely used test to assess peritoneal transport in CAPD patients probably due to its simplicity. Numerous papers have been published using this test in paediatric [98] and adult patients [99]. Following a long dwell, the PET is performed during a 4-h dwell using glucose 2.27%/2.5% dialysate. Dialysate and serum is sampled and low-molecular-weight solutes (sodium, potassium, urea, creatinine, glucose) and total protein are measured. Peritoneal solute transport is calculated by the D/P ratio of sodium, potassium, urea, creatinine and total protein and the dialysate240/initial dialysate ratio of glucose (D/D0). Residual volume can be calculated using the dilution of solutes present in the effluent. NUF is calculated as the difference between the drained and the instilled volume. NUF can be corrected for the calculated residual volume before and after the test.

Interpretation of the test

Patients are categorized into four groups of low, low-average, high-average and high transporters according to the values of solute transport. This classification into transport categories based on D/P ratios may be confusing as patients with a high D/P ratio of creatinine may in fact have a low mass transfer and clearance of this solute [100]. It has been proposed to rename the categories either to high, high average, low-average and low D/P ratio to very large, large, medium and small surface area [88] or according to the speed of transport into very fast, fast, slow and very slow transport. Recommendations can be given on the mode and quantity of PD according to the transport status of the patients [97,101]. The dip in the D/P of sodium gives an impression of free water transport [90].

Drawbacks

Theoretically there are many drawbacks of the PET, but in clinical practice the errors are mostly unimportant. Although the D/Pcr is influenced by convective transport from the circulation to the peritoneal cavity [102,103], no differences were found for the D/P ratios of urea and creatinine between a PET using 1.36/1.5% and 3.86/4.25% [104] or a PET with 2.27/2.5% and 3.86/4.25% [105,106]. Despite the advice that the PET should be performed after a long dwell, D/P ratios of low-molecular-weight solutes are not influenced by a short preceding dwell [107–109]. Only a dry day [107] or the use of polyglucose [110] for the long dwell can result in higher D/P ratios of small solutes and protein. It has to be realized that NUF is a composite measurement of TCUF and fluid reabsorption. It is important to
correct for overfill volume. If this is not done it will result in overestimation of NUF [111,112]. Finally, the residual volume at the start and end of the dwell may vary [113] that may lead to either overestimation or underestimation of NUF.

The PET can be enhanced by either correcting the sodium dip for sodium diffusion [114] or measuring the intraperitoneal volume after 1 h followed by reinfusion. The latter allows calculation of free water transport by the method of La Milia without influencing the results of solute transport and NUF [115].

Fast PET

To reduce the costs and the time commitment for the test, a simplification of his PET test was proposed by Twardowski [116]. As expected, a good correlation between the PET and the fast PET was found [117]. The fast PET was performed during a 4-h dwell using glucose 2.27%/2.5% dialysate. Dialysate and serum were sampled only at the end of the test. Only urea and creatinine were measured in these samples.

How frequently should the peritoneal membrane be monitored?

The European Best Practice Guidelines on Peritoneal Dialysis do not give recommendations on this theme. As long-term follow-up data are lacking, no evidence-based advice can be given. However, in order to follow CA125 in time, monitoring of the peritoneal membrane once every 3–4 months seems advisable. Solute and fluid transport does not change that fast, so monitoring once every year, starting at least 1 month after the start of PD, is probably enough. The patient should regularly report ultrafiltration data during outpatient visits and the PET repeated earlier when clinical complaints develop.

Summary

Changes in dialysate CA125 over time probably indicate changes in peritoneal mesothelial cell mass in non-infected PD patients. It is advisable to either standardize the duration of the dwell or express CA125 production as appearance rate. Giving the large interindividual variability, caused by differences in the number of cells expressing CA125 and in the amount of CA125 produced per cell, a single measurement is often not informative, especially when a low value is found. Thus, follow-up of dialysate CA125 in individual patients is essential, where a decline points to loss of mesothelial cell mass and failure to increase after peritoneal restig might predict the development of peritoneal sclerosis. CA125 can also be used as an in vivo marker of biocompatibility in the evaluation of new dialysis solutions. Still more research is needed, especially in the field of morphological functional relationships.

Changes in the vascular surface area are reflected by changes in the transport of low-molecular-weight solutes, and repeated PETs can monitor fluid removal. The question remains how to monitor changes in the interstitial tissue of the peritoneal membrane, which might help to predict the development of peritoneal sclerosis.

Conflict of interest statement. None declared.

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