



Peritoneal membrane fast transport status in peritoneal dialysis: characterization, evolution and therapeutic options

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Dissertação de doutoramento em Ciências Médicas

2007

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characterization, evolution and therapeutic options**

Dissertação de Candidatura ao grau de Doutor em Ciências Médicas, submetida ao Instituto de Ciências
Biomédicas de Abel Salazar da Universidade do Porto.

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I General Introduction

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I. Introduction

I.I.Peritoneal transport: the problem of fast transport status

Peritoneal dialysis (PD) is a successful end stage renal disease treatment which has been used increasingly over the last 20 years [1;2]. The peritoneal transport rate of solutes and the ultrafiltration capacity are the main characteristics of the peritoneal membrane in patients treated by peritoneal dialysis, conditioning treatment prescription and clinical outcomes [3-5].

Much is already known about the physiology and structure of peritoneal transport but many questions still remain open to research [6]. Patients with a fast transport rate of small solutes deserve a special focus because they represent a clinical challenge in peritoneal dialysis treatment [7]. Its determinants and prognosis are not fully known and appropriate therapeutic strategies are also under debate [8].

I.I.1.The structure and function of peritoneal membrane

The peritoneum is a continuous serous membrane consisting of a monolayer of mesothelium, the submesothelial interstitium, the lymphatics and the capillary bed [9]. Within the interstitium reside the capillaries, the main barrier to peritoneal transport.

The peritoneum acts as a complex biological membrane with a semipermeable heteroporous structure, involving simultaneous mechanisms of diffusion, convection, ultrafiltration, sieving and absorption. Peritoneal solute transport is size selective. The transport of small solutes is mainly diffusive while that of higher molecular weight solutes depends more on convection. Only the perfused peritoneal membrane in contact with the dialysis solution participates in the solute and fluid transport. This means the effective capillary surface depends not only on the anatomic number of capillaries but also on the vessels which are perfused and in some instances recruited or dilated, variably distributed in the interstitium. This conditions the intrinsic permeability of the membrane.

Different and progressively improved mathematical models [10-13] have been developed to allow prediction of solute and fluid transport through this dialyser. After considerable refinements the three pore model [14] is currently the accepted design of peritoneal membrane.

The three pore theory predicts that small solute transport occurs without much restriction, mainly through the small pores (radius 40-50 Å), the most abundant, and likely represented by intercellular gaps between capillary endothelial cells. An increase of the number of perfused capillaries will be reflected in an increase of small pores available for the

transport. On the other hand, macromolecules pass through larger pores (150 Å). The corresponding anatomic equivalent is not known with certainty. This passage is probably also conditioned by the interstitial structure.

The ultra small pore (<5 Å) is a specific endothelial water channel known to be represented by aquaporin-1. Evidence for the functional role of aquaporin-1 and peritoneal localization of this transcellular pore has been consistently presented [15-19]. Studies with aquaporin-1 knockout mice confirmed that this channel provides a major route for osmotically driven water transport [20]. Water is removed due to an osmotic pressure gradient, provided by the instilled dialysis solution. The osmotic agent is usually glucose, which due to its small size of 2.9 Å shows a reflection coefficient of only 0.02-0.05 through the small pores, meaning it is only able to exert its crystalloid effect through the ultra small pores, permeable only to water.

The function of these ultra small pores can be estimated with sodium sieving [21]: the decrease of dialysate sodium concentration due to aquaporin-1 mediated water transport during the early phase of the exchange with a hypertonic dwell, when the osmotic pressure gradient is maximum.

Absorption of macromolecules from the peritoneal cavity to the circulation through the lymphatic system occurs linearly in time, independently of size or concentration. Fluid lymphatic absorption counteracts transcapillary ultrafiltration, making net ultrafiltration the result of these opposite fluid processes [22].

The role of the interstitium in the peritoneal transport characteristics is still not completely known [23], but it looks increasingly relevant [24]. Hyaluronan is a matrix component involved in the restriction of proteins. An increase in the restriction coefficient to macromolecules [25] has been documented with time in PD, probably induced by alterations such as submesothelial fibrosis. A decrease in osmotic conductance may depend not only on capillary but also interstitial changes known to occur with long-term PD [23].

On the other hand, the mesothelium is not an active transport barrier [26] although its indirect role in transport alterations is a matter of current investigation [27]. Recent studies have progressively shown that mesothelial cells are not bystander cells but constitutively produce a number of vasoactive and growth factors, with an active role in peritoneal defence, repair and transport.

Effective peritoneal surface area, intrinsic peritoneal permeability and selective transcellular water transport are the main properties in this updated concept of the peritoneal dialysis membrane [28].

I.1.2. Methods of peritoneal membrane transport evaluation

The capacity of the membrane for the transport of a given solute is the product of the permeability of the membrane to that solute and its effective surface area, called the mass transfer area coefficient (MTAC). This measures the theoretical maximal diffusive transport rate at time zero, in the beginning of the dwell before diffusion and ultrafiltration has actually begun [12;13].

Simplified models for MTAC calculation, such as the Garred and Krediet formulas, have been developed to be accessible in clinical practice [10;29]. Values of MTAC for low molecular weight solutes can be more easily calculated. The introduction of the peritoneal equilibration test (PET) [30], later, the standard permeability analysis (SPA) [31], and recently the personal dialysis capacity (PDC) test [32] have offered the clinician simplified methods to monitor membrane function.

The PET is the most widely used standardized form of peritoneal transport assessment also useful for longitudinal evaluation of patients [33]. Dialysate:plasma ratio of creatinine (D/P creatinine) at the end of 4 hours standardized dwell closely correlates with MTAC creatinine. The small differences related to the use of a more complex method are smaller than the differences induced by clinical collection and laboratory measurements errors. In addition, inter and intraindividual changes also occur when more rigorous calculation methods are used [34-36]. A PET limitation is that it does not allow the diagnosis of ultrafiltration failure (UFF) due to lymphatic absorption. Although increased lymphatic absorption is one of the causes of ultrafiltration capacity failure [37], lymphatic absorption does not seem to change with the duration of peritoneal dialysis [38]. Another method for more accurate peritoneal transport assessment is the SPA [37;39;40], which includes the evaluation of an intraperitoneal marker, dextran, disappearance rate as an index of peritoneal membrane absorption. This, however, remains a research domain test, with sophisticated calculations.

The modified PET test with 3.86% solution is the recommended tool [41] for peritoneal transport categorization and ultrafiltration failure (UFF) investigation because it gives similar information about small solute transport but is more sensitive for the definition and investigation of UFF causes, allowing the calculation of sodium sieving at 60 min as an estimate of free water transport.

Very recent research has been carried out to propose correction of sodium sieving for sodium diffusion [42], because a fast transport rate with rapid sodium diffusion may mask the initial decrease of intraperitoneal sodium due to ultrafiltration. This correction is also applicable to the PET [43]. In addition, new clinically accessible formulas have been proposed to more directly measure free water transport capacity at 60 min dwell, during a fast-fast PET [44]. These efforts come from the definitive clinical relevance of fluid and sodium removals in the management of PD patients [45].

I.I.3. Relationship between solute transport and UF

There is a large variability in membrane transport categories at the start of PD [28;46-50]. It is not fully known why this occurs. Differences in effective capillary surface area probably account for such large inter-patient variability. Ultrafiltration (UF) profile also presents enormous variability. While we would expect a strong and significant negative correlation between small solute transport rate and ultrafiltration, differences in solute transport only account for 5-10% of the variability of UF capacity [51], meaning that categorization of peritoneal small solute transport based on ultrafiltration capacity is unreliable. UF has a high error in reproducibility of measurement, typically 20-25% [28;52]. It depends on the intrinsic membrane function, such as hydraulic conductance and glucose reflection coefficient, and also results from the sum of fluid absorption, instilled volume and intraperitoneal pressure effect. As such, it is not proportional to small solute transport.

Additionally, the relative importance of water transport through the transcellular and intercellular pathways is a matter of current investigation [52;53]. Simple methods of water transport evaluation for population studies are under scrutiny [44].

I.I.4. The clinical problem: fast transport rate

From a clinical point of view, fast peritoneal transport rate (as expressed by D/P creatinine levels higher than 0.81 or currently by D/P creatinine higher than the average plus standard deviation levels of the studied population [30]) would be expected to permit higher dialysis clearances. However, more rapid dissipation of the osmotic gradient due to glucose absorption induces reduction of ultrafiltration capacity with less fluid removal. This is a high risk condition for metabolic consequences of glucose absorption and fluid overload. This means that a fast transport rate is an immediate relevant problem, to be managed in day to day clinical practice [8;47].

This status may be present either from the beginning or be acquired during long-term PD treatment. Technical failure, comorbidity and higher mortality have previously been associated to acquired fast transport [4;5;54-56]. It is controversial if intrinsic baseline fast transport status brings uniformly similar threats [50;57;58]. Whichever the case, timely detection, characterization and adequate therapeutic strategies are fundamentally important for the outcome of these patients. In the absence of sufficient residual renal function, this status usually implies individualization of prescription [59-61] with shorter dwell times, increased use of hypertonic glucose solutions or an alternative agent with colloid-osmotic properties, such as icodextrin. Acquired fast transport state is also a risk factor and an early marker of encapsulating peritoneal sclerosis [62;63], a rare although serious and life threatening PD complication [64].

I.I.5. Determinants of fast transport state

1. Baseline fast transport status

Link with systemic inflammation

The effective peritoneal surface area is determined by the number of perfused peritoneal capillaries that are in contact with the dialysate. Therefore peritoneal transport rate is only weak and inconsistently correlated to patient body surface area. Splanchnic blood flow through the peritoneum averages 70-100 ml/min [65;66] but this is influenced by conventional peritoneal solutions which cause generalized microvascular dilation [67]. Studies demonstrating that nitric oxide mediated vascular tone and permeability changes are involved in the loss of ultrafiltration and transient fast transport state during peritonitis [68] suggest that an inflammatory acute situation induces a fast transport state through variations in splanchnic blood flow and volume. The effect of inhibition of nitric oxide (NO) synthesis on peritoneal transport during peritonitis also confirms this pathophysiological process: the NO inhibitor N-nitro-L-arginine methyl ester (L-NAME), given intraperitoneally, prevented the expected fall in ultrafiltration and increase in small solute transport [69]. A number of many other vasoactive and growth factors, among them prostaglandins, cytokines, hyaluronic acid, are released from resident and infiltrating cells during peritonitis, modulating the peritoneal membrane transport and response to the insult. Acute episodes of systemic infection also temporarily induce a state of fast transport [70].

Taking these examples of acute transient increase in peritoneal transport rate into account, extrapolation was done to baseline PD patients and the most recent appealing theory points to a causal link between systemic chronic inflammation and peritoneal fast transport rate. It is hypothesized though not yet confirmed that systemic inflammation, such as in uraemia or diabetes mellitus [71-73], could also induce increased effective capillary surface at start of PD. The peritoneal Biopsy Study Group has reported vascular changes and submesothelial fibrosis in the peritoneal membrane of uraemic patients even before PD treatment [74]. There are also studies supporting the higher prevalence of diabetes [75], comorbidity [76;77] or serum markers of systemic inflammation such as IL-6 [78] in baseline fast transporters. Others, however, refute such associations [50;57;79-81].

Controversies from epidemiological studies

There are very few large clinical studies researching into and explaining the variability in solute transport at the start of PD and its impact on patient outcomes. The multicentre study, Canada-USA (CANUSA) Peritoneal Dialysis Study Group [4] concluded that fast peritoneal

membrane transport is associated with decreased patient and technique survival for continuous peritoneal dialysis (CAPD) patients. Australian registry data [82] also showed similar worse outcomes in Australian and New Zealand peritoneal dialysis fast transporters. No details of dialysis prescription in these patients were given. When analyzing predictors of baseline fast transport in this population [79], fast transport status was associated with older age but was not independently predicted by sex, diabetes, other comorbid diseases, smoking, previous hemodialysis therapy or transplantation or residual renal function. Similar results were found when peritoneal permeability was modeled as a continuous variable (D/P creatinine). In the STROKE PD study [52] independent demographic factors associated with higher solute transport at baseline were male gender and higher residual urine volume with comorbidity or serum albumin not independently predictive. In a cohort of Spanish patients [50] no relationship between baseline transport parameters and gender, age, patient size, or diabetes was observed. As pointed out by Davies [28] these epidemiological variables account for very little of the between patient variability in solute transport and there is very little that we identify that explains the variability in solute transport in patients commencing PD.

Therefore controversy still remains concerning the causes and clinical characteristics of intrinsic peritoneal fast transport status in the beginning of PD.

2. Acquired long-term peritoneal fast transport status

More consistent studies were able to shed some light on the determinants of longitudinal acquired changes of peritoneal function. These are known to be mainly expressed as an increase in low-molecular weight solute transport and coincident decrease in ultrafiltration [83]. The onset of UFF with fast transport usually occurs after three or four years on PD with variable prevalence rates of 23% [31] and 30.9% [84], possibly higher in long-term PD patients.

Peritonitis

Severe and recurrent peritonitis episodes are important causes of peritoneal damage [50;63]. Peritonitis induces an acute inflammatory situation and although the relationship between long-term changes such as fibrosis and peritonitis is not clear, profibrotic interleukines are up-regulated in peritonitis.

Bio-incompatible solutions: up-regulation of neoangiogenic and fibrotic agents

Acquired fast transport status may occur in the absence of infectious insults. Patients with earlier loss in residual renal function exposed to significantly more hypertonic glucose during the first 2 years of treatment showed an increased transport rate later [85]. Selgas' group also verified that diabetic state and a higher glucose requirement to obtain adequate UF are risk factors for developing early UFF with fast transport, meaning the use of hypertonic glucose PD solutions is associated with increase in solute transport [86].

Ex vivo studies [87-89] and animal models also demonstrated the effects of bio-incompatible solutions on mesothelial cells, showing the detrimental effect of glucose, glucose degradation products, lactate buffer or acidic pH on peritoneal membrane cells viability and function, but glucose degradation products (GDP) seem to be the main vectors of lesion [90]. Glucose degradation products are reactive carbonyl compounds produced in peritoneal dialysate during heat sterilization and are more potent inducers of advanced glycation end-products (AGEs) than glucose itself [91]. Interaction of AGEs and RAGE (receptor for AGE) leads to secretion of inflammatory cytokines and growth factors such as vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)- β .

There is evidence of accumulation of AGEs in the peritoneal membrane in uraemia, exposure to high glucose concentrations and exposure to glucose degradation products [91]. These AGEs correlate with peritoneal membrane fibrosis [92] and also with solute transport and ultrafiltration dysfunction [93], just as they co-localize with VEGF in peritoneal vessels [94]. Local peritoneal production of VEGF was found to increase with glucose PD solution and decrease with glucose free PD solutions [86]. Reduction of the number of vessels with anti-angiogenic therapy also induced improvement in net ultrafiltration in an animal model [95]. On the other hand, high glucose upregulates TGF- β 1 [96] and fibronectine [97] expression by human peritoneal mesothelial cells. These cells also secrete more VEGF and procollagen III N-terminal [98] after exposition to dialysis solutions.

Neoangiogenesis is also associated with the nitric oxide [94] system: this is upregulated in animal models of peritonitis [68] and uraemia [16] but inversely regulates TGF- β , preventing fibrosis. Other cytokines such as platelet derived growth factor (PDGF), connective tissue growth factor (CTGF), fibroblast growth factor (FGF) and plasminogen activator inhibitor (PAI)-1 are involved on fibrogenic processes [99;100].

The use of low-GDP two chambered solutions is expected to reduce these effects [61]. However it is not known whether the use of alternative solutions will translate into better peritoneal function preservation.

3. Peritoneal sclerosis: correlation with increased solute transport

Biopsy studies revealed important data [74]: average submesothelial thickness was 50 μm in control non uraemic patients; uraemia alone was associated to 140 μm , but increase to 700 μm submesothelial thickness was observed in patients on PD for more than 8 years. Angiogenesis and vasculopathy was also observed, mainly in UFF patients. Therefore progressive thickening of the submesothelial compact collagenous zone occurs with time on dialysis [95;100-102]. Interstitial collagen deposition and vascularization correlate with α -smooth muscle actin (α SMA) positive myofibroblasts.

Peritoneal fibrosis occurs as a normal response to a variety of insults including bioincompatible dialysis solutions, peritonitis, uraemia, and chronic local inflammation. Increased submesothelial thickness also correlates with increased solute transport, as measured by the D/P creatinine [103] and loss of mesothelial cells [74].

A role for peritoneal mesothelial cells: epithelial-mesenchymal transdifferentiation

TGF- β plays a central role in fibrogenesis [95;104]. It induces fibroblast activation, collagen deposition, inhibition of fibrinolysis through PAI-1 maintenance of fibrosis and inhibition of matrix metalloproteinase (MMP), and angiogenesis. TGF- β also mediates conversion of epithelial cells into myofibroblasts, this means epithelial-to-mesenchymal transition [105;106]. A recent study demonstrated that peritoneal mesothelial cells suffer this transdifferentiation with expression of mesothelial markers in stromal α SMA positive myofibroblasts. Mesothelial cells migrate toward the interstitium and acquire a myofibroblast phenotype. This myofibroblastic conversion of mesothelial cells was confirmed *in vivo* by injection of an adenovirus vector that transferred active TGF β 1 in rat peritoneum [101]. Inhibition of RAGE induces less TGF- β production and less submesothelial and interstitial fibrotic tissues, as expressed by less α SMA and cytokeratin staining [107]. It is not clear that measurement of TGF- β in peritoneal fluid, which is found mostly in an inactive state, bound to latency associated protein, reflects tissue levels of active TGF- β .

In parallel to fibrosis, an increase in capillary number also occurs. Angiogenesis may be induced as part of the fibrotic response, as many key fibrogenic cytokines are also strongly angiogenic. Fibrotic tissue may support and preserve angiogenesis [100]. VEGF is a main vector of neoangiogenesis, with mesothelial cells being its important source. It seems that mesothelial cells that have undergone epithelial-to-mesenchymal transition produce more VEGF than epithelioid mesothelial cells [27;50], suggesting a link between mesothelial cell transdifferentiation, fibrosis and acquired fast transport [95].

This can progress to encapsulating peritoneal sclerosis after a triggering event such as a late peritonitis or serious staph. aureus, pseudomonas or fungal peritonitis, abdominal bleeding or abdominal surgery. These could possibly enhance collagenous tissue proliferation and TGF- β expression. Inflammatory cytokines and growth factors secreted by mesothelium, endothelial and interstitial cells up-regulate fibrogenic processes with increase of TGF- β . Increased VEGF is also able to increase neoangiogenesis and plasma exsudation with fibrin formation. Reduced fibrinolytic activity of the peritoneal membrane adds to these pro-sclerosis steps.

Mesothelial cell denudation with a decrease in effluent cancer antigen-125 (CA125), a mesothelial cell mass marker, occurs with time on PD and it has been monitored in patients before transfer to haemodialysis due to UF failure [108-110]. It has been concluded that CA125 was very low in patients who developed peritoneal sclerosis and a lack of response to a peritoneal rest also predicted peritoneal encapsulating sclerosis after PD discontinuation [111].

I.II Aim and Outline of the Thesis

The evaluation of peritoneal transport is an obligatory clinical task for characterizing our patients at baseline, tailoring the PD prescription, understanding their natural history on PD and detecting signs of peritoneal membrane deterioration.

Some knowledge has been gathered on long-term peritoneal membrane changes, since both molecular biology and histological studies point to neoangiogenic and fibrogenic processes as the key factors of acquired fast transport with ultrafiltration failure. The clinical expression of these alterations is an increase in D/P creatinine with long-term PD, although others report a relatively stable membrane function, at least in the first 2-3 years on PD [112]. Less is known about the characteristics of baseline fast transporters and the explanations for early peritoneal membrane function profiles.

The aim of this thesis was to investigate the clinical and functional characteristics of patients with fast peritoneal transport rate, in early and late-term PD, also evaluating the results of rescue therapies in acquired status.

In **chapter II** the epidemiological context of our long-term PD programme was analysed. The need for implementation of routine peritoneal transport evaluation was reinforced.

In **chapter III.I** characterization of peritoneal membrane function and status was investigated with a modified PET 3.86% test with corrected sodium sieving calculation as an estimate of free water transport. Information obtained from CA125 effluent measurement was

studied. We also evaluated the information on peritoneal solute transport rate obtained from 1 hour D/P creatinine in comparison with standard 4hours dwell categorization.

In **chapter III.II** we cross-sectionally studied the relationship between peritoneal transport and effluent markers of mesothelial cell mass (CA125), angiogenesis (VEGF) and inflammation (IL-6), with a focus on early and late-term PD fast transporters.

In **chapter IV.I** the impact of baseline fast transport status on patient survival was investigated. Relationships of fast transport, in this early PD stage, with clinical variables such as comorbidity, atherosclerosis, residual renal function and serum IL-6 were studied.

In **chapter IV.II** further characterization of early and late fast transporters was done: differences in free-water transport and the marker of systemic inflammation IL-6 were studied; we also evaluated the relationship between transcellular water transport evaluated by the corrected Na dip and time on peritoneal dialysis (PD).

Chapter IV.III reports the results of a prospective study in a cohort of PD patients in search for the time-dependent determinants of peritoneal transport rate, as measured by D/P creatinine.

In **chapter V.I** the results of rescue therapy with peritoneal rest in acquired fast transport status are reported. A successful case of immunosuppressive therapy in sclerosing peritonitis (**chapter V.II**) highlights the severe inflammatory process under such complication.

Chapter VI includes a general discussion and summary of results. Also future perspectives are discussed.

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II Epidemiology of peritoneal dialysis in Hospital General Santo Antonio

*Long-term peritoneal dialysis experience in Portugal. Int J Artificial
Organs 2006,vol 29,12: 1109 - 116*

Artificial Kidney and Dialysis

Long-term peritoneal dialysis experience in Portugal

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ABSTRACT: Peritoneal dialysis (PD) penetration varies widely. Since the beginning of this therapy, indications have changed and outcomes have improved. In Portugal, PD still remains clearly underutilized.

The results of a 20 year PD programme were evaluated: 312 cumulative patients, 48±16 years, 27% >60 years old, 27% diabetic, 59% with prior hemodialysis (HD). The main reason for admission was vascular access failure (48.7%). Admission due to patient preference has increased significantly between first and second decades of the programme (33% vs 47% ($P<0.001$)); 98 patients (31.4%) were treated with automated PD but this prescription increased to 43% of the active patients.

A total of 376 Tenckhoff catheters were surgically implanted, recently by the Popovich-Moncrief technique (77 catheters): the cumulative survival was 82%, 64% and 50% at 1, 3 and 5 years, respectively. A better catheter survival was found in the last decade (85.7%, 69.6%, 54.8% versus 77.3%, 55.5%, 40.2%, at 1, 3 and 5 years, respectively ($P=0.007$)).

The patient and technique cumulative survivals were 91, 74, 55% and 85, 67, 41%, at 1, 3, and 5 years, respectively.

The main drop-out was to hemodialysis (35.8%), followed by death (23.7%), and transplantation (21.5%). Peritonitis and access-related infections caused 35% of the transfer to HD. Cardiovascular events caused 58% of deaths. The median PD retention was 35.5 months. The rate of peritonitis has decreased to one episode /30 patient months. Hospital admission has also decreased to 4.8 days/patient year.

This is a first report on long-term PD experience in Portugal. It has been an effective modality of renal replacement therapy, reflected by the growing patient preference in our PD programme. Experience, knowledge and new technical solutions have improved the outcomes. (*Int J Artif Organs* 2006; 29: 1109-16)

KEY WORDS: Peritoneal dialysis, Outcomes, Survival

INTRODUCTION

Since its early stages, peritoneal dialysis has developed new connectology systems, new therapy protocols and new dialysis solutions. Increasing experience and investigation in the field of peritoneal dialysis has enabled nephrologists to propose PD as a good alternative and, usually, a better first option modality of chronic renal therapy (1).

The theoretical advantages were confirmed in several

population studies (2) and debate about outcomes and costs (3) have clearly shown that this therapy should be part of an integrated therapy, side by side with hemodialysis and transplantation (4, 5). In reality, therapy allocation to PD or HD varies widely. The extreme disparity of PD prevalence in a single country and among other countries can not be explained by medical factors (6-9). In Portugal, paralleling other European countries, although medical knowledge about the role and benefits of PD has grown, there is still a minority of

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patients who are allowed to do PD, less than 4% of the total dialysed patients. Besides cultural and scientific bias, hospitals and doctors usually face organizational, structural and logistic difficulties to implement a programme (10).

Our programme has been developing in Hospital Geral Santo Antonio since late 1985. Over two decades, the efforts of a team of doctors, nurses and surgeons has managed to overcome significant structural deficiencies. This programme remains the most representative of PD modality in Portugal. We analyse here the results of a cohort of patients enrolled in the programme during these past 20 years.

PATIENTS AND METHODS

This study was conducted to evaluate the patients' characteristics, rates of PD transfer to hemodialysis, technique failure, and mortality among 312 cumulative incident dialysis patients. Adults who initiated dialysis between October 1985 and May 2005 were included.

In the very first years PD patients were admitted to the Unit as a rescue therapy when hemodialysis was not feasible due to vascular exhaustion or cardiopathy. The rate of peritonitis was high. With cumulative experience, better results were able to persuade our colleagues to expose their patients to the modality. Therefore more patients had the opportunity to choose the therapy according to their preference. In the last few years a pre-dialysis visit was developed to timely refer patients and offer them a panel of renal therapies. Counselling is done by a nurse and a doctor.

Our aim was also to analyze data on 376 chronic peritoneal dialysis (CPD) catheters implanted between 1985 and 2005 in adult patients enrolled in our PD programme by comparing two different time periods 1985-1995 and 1996-2005.

Statistics

Continuous symmetrically distributed data is expressed as mean $\pm$ standard deviation. Asymmetrically distributed data is expressed as median and range. Categorical data are expressed as absolute number and percentages. Chi-square test and the Mann-Whitney test were used accordingly to evaluate differences between the patients admitted in the first and last decades.

Life tables were used to analyse actuarial patients and technique survival. For patient survival, an as-treated analysis was performed, in which only death occurring during or shortly after hemodialysis transfer was taken into account. Therefore, death within 30 days after transfer to HD was attributed to peritoneal dialysis. Survival times were censored at the following events: switch to HD, transplantation, loss to follow-up, recovery of renal function.

For technique survival switch to HD was considered the final event and all other observations were censored (death, transplantation, loss to follow-up, recovery of renal function).

Both death and transfer to HD were events for the analysis of the combined patient and technique survival.

Follow-up was censored at the time of transplantation, transfer to HD, patient withdrawal or at 31 May 2005.

Causes of transfer to HD were grouped into broad categories as follows: infection (peritonitis and exit site infections), catheter problem, inadequate dialysis (dose/ultrafiltration failure/fluid management issues), psychosocial (psychological, insufficient support and loss of auto dialysis capacity), surgical complications not related to PD (acute abdomen).

The Kaplan Meier method was used to compare actuarial survivals between the cohorts.

Statistical analysis was performed using SPSS 13.0 for Windows operating system. A p value less than 0.05 was considered statistically significant.

Peritonitis and hospitalization rates were evaluated.

RESULTS

Patient characteristics

Since October 1985 up to 31 January 2005, 312 cumulative patients were admitted to our PD programme (Fig. 1). At entry the average age of the patients was 48 years old (range 15 to 83), 27% were older than 60, 37% male, 27% diabetic, 17% presented ischemic heart disease, 59% were previously treated with hemodialysis (HD) or renal transplantation for a median time of 28.8 months (Tab. I); 29% patients were anuric at the start of PD. The main reason for admission was vascular access failure (48.7%); patient preference occurred in 41.7%, and has increased significantly between the first and second decades of the programme (33% vs 47% ($P < 0.001$)). Fifty

-five patients were on active treatment by January 2005.

Comparisons of patient characteristics between the first and second decades of PD programme are presented in Table II.

CAPD systems

The first CAPD system used was a spike system, but, since 1990, it has changed to Y-set systems and twin bag disconnecting systems. The introduction of Y-set systems system, incorporating the flush- before- fill procedure was clearly associated with a drop in peritonitis rate (Fig. 5).

Catheters and implantation technique

Straight double cuffed Tenckhoff catheters were used until 1993, implanted surgically (11) by surgeons. Since then, the catheter adopted was the double cuffed coiled Tenckhoff catheter, implanted by Seldinger technique (11), after antibiotic prophylaxis. After two post-implantation complications due to intestinal perforation, the implantation technique was reviewed and it is consistently being done by mini-laparotomy (11). Five Missouri catheters were electively implanted in obese patients with a high risk for leaking.

TABLE I - GENERAL DEMOGRAPHICS OF THE TOTAL ENROLLED POPULATION

Patients (N, total)	312
Age at entry (M±SD, years)	48±15.9
Gender (N(%), male)	116 (37.2%)
Admission due to patient preference (N (%))	130 (41.7%)
Time of previous renal therapy (median (range), months)	30.36 (91.1)
Diabetes (N (%), yes)	84 (26.9%)
APD (N (%))	98 (31.4%)

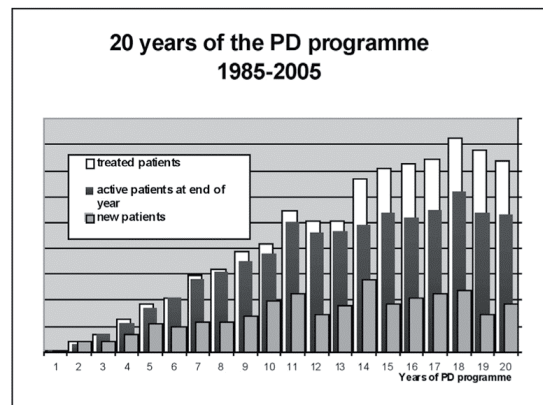


Fig. 1 - The activity of the PD programme over 20 years.

In the last decade, nephrologists have become committed to the procedure, using the Moncrief-Popovich technique (12).

PD prescription

All patients were treated with lactate -buffered, conventional dialysis solutions. Icodextrine was introduced in 1997, and it has been electively prescribed in patients with ultrafiltration capacity failure.

In the last two years, low- glucose degradation products (GDPs) two- chambered solutions have been prescribed in new patients, and are being used now in 50% of the patients; 22% use lactate/bicarbonate solutions.

Automated peritoneal dialysis (APD) has also been prescribed since 1998. In the beginning, due to financial constraints, only anuric patients transferred from

TABLE II - COMPARISON OF THE TWO COHORT'S DEMOGRAPHIC CHARACTERISTICS

	First decade (1985-1995)	Second decade (1996-2005)	P
Total	132	180	
Male (N (%))	51 (38.6%)	65 (36.1%)	0.64
Aged (patients >60 years, N (%))	35 (26.5%)	49 (27.2%)	0.88
DM (N (%))	40 (30.3%)	44 (24.4%)	0.24
New to dialysis (N (%))	50 (37.9%)	79 (43.9%)	0.28
PD patient preference (N (%))	44 (33.3%)	86 (47.8%)	0.011
Time of previous renal therapy (median(range), months)	24.7 (91.2)	32.4 (89.6)	0.98
APD (N (%))	14 (10.6%)	84 (46.7%)	<0.001
Transfer to HD (N (%))	54 (40.9%)	49 (39.2%)	0.80

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hemodialysis and patients with ultrafiltration capacity failure were allowed to do APD. Later, patient preference and logistic/professional reasons were also indications for APD prescription. Due to similar economic issues, alternative solutions have not been used yet for APD, except electively in patients reporting pain with standard solutions infusion.

Since 1978 a cumulative number of 98 patients (31.4%) were treated with automated PD, but this prescription increased to 43% of the actual active patients.

Adequacy was regularly checked according to international guidelines. Adequest software is being routinely used in the unit with registered levels of 2.1 ± 0.78 total KT/V.

Transfer to hemodialysis

The drop-out included: 107 (35.8%) transferred to hemodialysis, death (23.7%), and transplantation (21.5%). Fifty-five patients were under active treatment by January 2005. Recovery of residual renal function allowed the stopping of PD in 9 patients.

The main reasons for change of modality were peritonitis and access related infections (35% of transfer to HD), followed by inadequate ultrafiltration (25.5%). Figure 2 shows the causes of transfer to HD in the two time periods.

Mortality

The causes of death were classified as cardiovascular, infectious, or various other reasons. Cardiovascular events caused 58% of deaths, followed by infections (20.3%).

The patient cumulative survival was 91, 74 and 55% at 1, 3, and 5 years, respectively (Fig. 3).

Technique failure

The cumulative technique survival was 85, 67 and 41%, at 1, 3, and 5 years, respectively (Fig. 3). The median PD retention time was 35.5 months which is a combined patient and technique survival of 78%, 49% and 23% at 1, 3 and 5 years respectively.

Higher PD retention was observed from the first to the second decade of the programme: median, 29.1 to 37.4 months, however it did not reach statistical significance ($P=0.22$). In active patients, 16/55 (29%) were on PD for more than 4 years (65.3 ± 10.56 months).

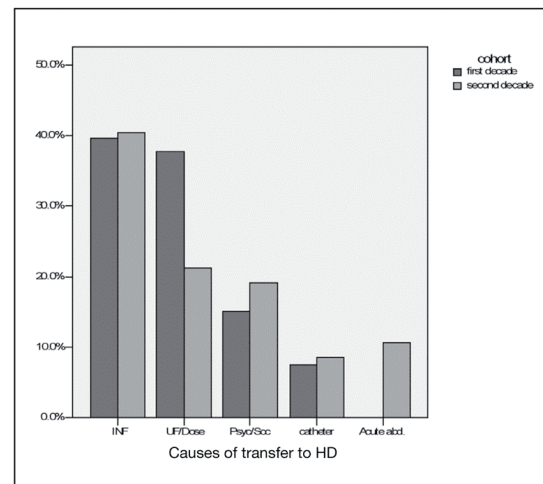


Fig. 2 - Causes of transfer to HD in the two cohorts (Infection refers to peritonitis and access related infections, UF/Dose refers to inadequate dialysis including ultrafiltration and dose inadequacy); Psyc/soc reasons include burn-out and loss of auto dialysis capacity without assistant helper; Catheter refers to mechanical complications as leak or obstruction; acute abd. refers to non-dialysis related surgical complications).

Catheter survival

A total of 376 Tenckhoff catheters have been surgically implanted; in the last two years the Popovich-Moncrief technique has been used with 77 catheters.

Kaplan Meier comparison of catheter survival curves in the two decade cohorts are shown in Figure 4. It has improved from 77.3%, 52.3%, 40.2% to 85.7%, 69.6%, 54.8%, at one, three and five years, respectively (Log Rank $P=0.007$) (Fig. 4).

Rate of peritonitis and hospital admission

The rate of peritonitis has decreased in the last period to 1 episode /30 patient months (Fig. 5).

Hospital admission has also decreased to 4.8 days/patient year.

DISCUSSION

The evaluation of more recent PD incident cohorts has shown improved patient survival, higher technique success, and an increasing use of cycled-based PD (13).

This review of a 20-year PD single center programme goes along with such evidence and has allowed us to document better outcomes with the increasing experience of our PD team. Integrated care with pre-dialysis information about the modalities increased optional admission in our center. We have observed that PD is an effective therapy, achieving patient survival and outcomes similar to other single center reports (14-16) and larger European and American PD population studies (17-19) results.

The patient cumulative survival was 91, 74 and 55% at one, three and five years, respectively. These were better results than the global data from the EUA cohort of 30000 patients starting PD in 1999 (82.5% and 57.9% at one and three years respectively) (13) and similar to the Europeans (20).

It is well-known that a number of baseline population characteristics condition global survival rates (2, 21-23), but median age and prevalence of diabetes, as examples, were very similar to other PD centers (24), although lower than in hemodialysis. There is probably a positive selection of patients for PD, mainly because auto-dialysis capacity has been a prerequisite in the majority of our Center's admissions. Besides, according to our recent review (25) residual renal function is still present in many patients, averaging 4 ml/min/1.73 m². On the other hand, almost 30% of the patients were anuric when they began PD, and half of the patients were admitted because a vascular access failure and therefore transfer from hemodialysis not only carried associated time on dialysis related comorbidity, but also did not allow the majority of them to benefit from the most important PD advantage in new patients - residual renal function preservation.

Baseline peritoneal transport was not available for analysis because a peritoneal equilibration test was not routinely performed until 1998, when it was then included in a baseline standardized evaluation. Therefore characterization of the global incident patient's peritoneal transport and investigation of its impact on survival (26) was only done in a subgroup of more recent patients (25).

Our study did not aim to investigate comparisons with hemodialysis. But a number of studies allow us to conclude that significant disparity of the prevalence of the two dialysis modalities in our country cannot be explained by medical reasons. As it has been recently highlighted (2), survival differences between HD and PD are not constant but vary substantially according to the underlying cause of ESRD, such as diabetes, age and

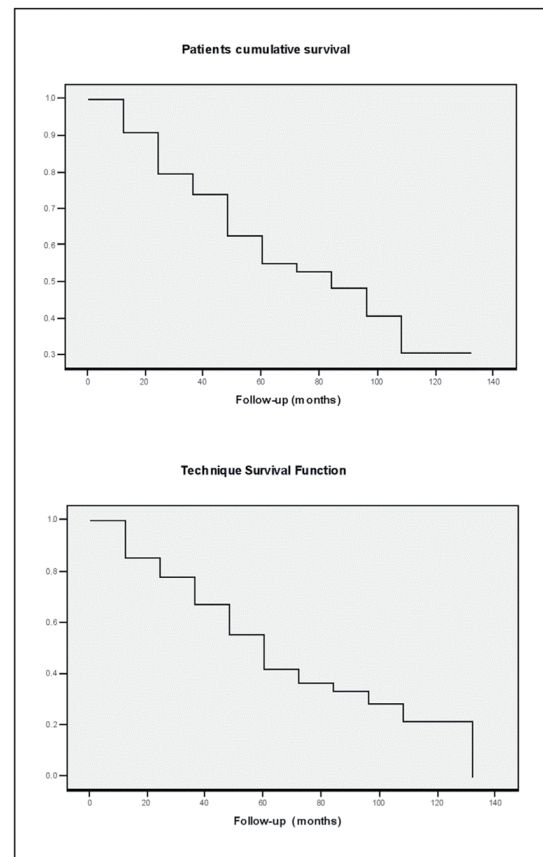


Fig. 3 - Patient and technique cumulative survivals: median patient survival time was 79,2 months and median technique survival was 52,6 months (switch to HD was considered the final event and all other observations -death, transplantation, loss to follow-up, and recovery of renal function- were censored).

level of baseline comorbidity. However, except from aged diabetic patients with baseline comorbidity who show lower survivals with PD there is a clear survival advantage for a vast number of uremic patients, who are not allowed an option. Indeed, adjusted mortality rates in non-diabetics with no baseline comorbidity were higher in HD than in PD and a survival advantage was also documented with PD in younger diabetic patients with no baseline comorbidity. Within the group of non-diabetic patients with baseline comorbidity, similar survivals were found with HD and PD. These results are similar to those

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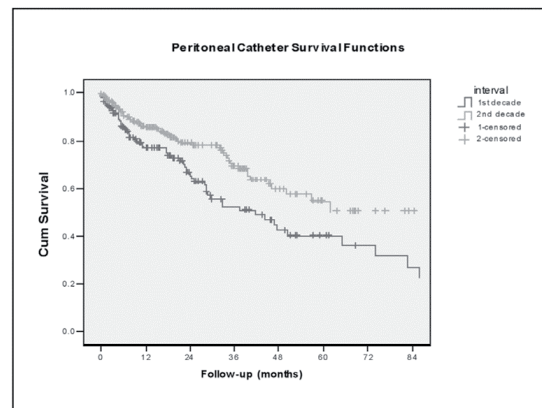


Fig. 4 - Catheter survival in the two time periods analyzed.

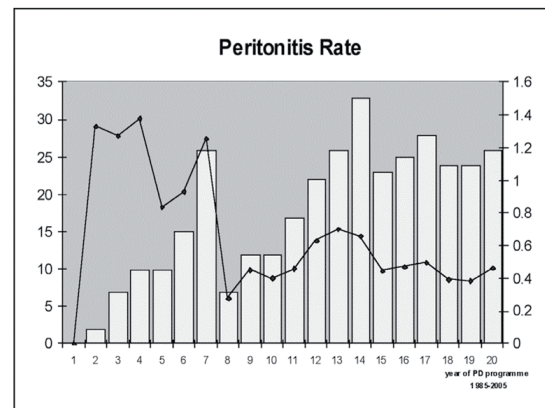


Fig. 5 - Peritonitis rate.

of other previous studies (27, 28)

It also appears that patients on the two therapies have different mortality patterns over time which makes survival analysis vulnerable to the length of follow-up (29), favoring PD mainly within the first years of treatment.

Due to lower technique survival, PD retention is lower than in HD, but global survival, in an intention-to-treat analysis, may still be able to show higher survival for patients beginning PD as the first modality of chronic renal dialysis (30).

Favorable costs analysis has also been shown even considering transfer to hemodialysis (31): compared to “hemodialysis, no switch” subgroup, both “peritoneal dialysis, no switch” and “peritoneal dialysis, with at least one switch” showed a significantly lower expenditure. After adjusting for patient characteristics, annual Medicare expenditure was still significantly lower for patients with peritoneal dialysis as an initial modality. There is no published report on PD costs in Portugal, and it was also out of the scope of this descriptive review. However, it is known that the overall structure of health care, and particularly of renal care, in an individual country, is an important determinant of the under-use of PD (32, 33). In Portugal the healthcare system is mainly public, but private hemodialysis clinics entirely assure chronic renal therapy to the vast majority of patients. Reduction of PD fluids and disposables costs could possibly make PD more financially attractive to private investors.

Similarly to what happens in other countries, this also

explains the discrepancy between dialysis allocations in Portugal with under use of PD.

Considering other outcomes such as hospitalization rate, 14% higher hospital admission rates per patient a year with PD, in the United States, has been reported in an earlier study (34). But there are large differences in dialysis practice conditioning high variability in hospitalization rates. In our center we have observed a decreasing rate of admissions, also because we implemented an effective strategy of catheter implantation, training and peritonitis treatment on an ambulatory regime. Besides we, as others, (35) also believe that the time the physician spends with the patient influences the quality of care.

Peritonitis rate has also been decreasing, and is in agreement with international guidelines (36).

Along with technique advances, improvement of catheter connectology and the use of adjusted treatment protocols have allowed such favorable evolution.

Mirroring this, more effective infection control was obtained. Due to the cumulative team-acquired knowledge, catheter survival has also improved from the first to the second decade. Significant quality improvement was possibly due to the articulation with a surgical theater for the procedures, allowing planned catheter interventions and avoiding the use of the Emergency Room. Besides, we attribute an important role to the nephrologists' involvement in the catheter implantation: two key factors, skill and commitment have certainly grown.

Technique survival is, however, still PD's Achilles' heel: cumulative death-censored technique survival was 85, 67 and 41%, at 1, 3, and 5 years, respectively with median PD retention of only 3 years. We still aim for a lower rate of peritonitis and catheter related infections, possibly achievable through the implementation of domiciliary nurse support and more prophylactic strategies. A lower rate of transfer to HD due to inadequate dialysis has occurred in late decade: the use of APD and icodextrine surely had a role. Investigation of more biocompatible solutions and optimization of APD, also reducing its costs may help to further increase PD retention. An opportune transfer to HD, supported by integrated care, should however be borne in mind, to not compromise global patient survival (37).

We can then conclude that our center reflects worldwide PD trends: advances in PD have resulted in improved patient outcomes but these largely depend on our center's experience and degree of specialization in PD (35, 38).

An educational intervention, during pre-dialysis visits increases the proportion of patients who intend to initiate dialysis with self-care dialysis (39, 40).

An integrated approach to the treatment of uremia and team commitment to patient care has also improved the results (35).

ACKNOWLEDGEMENTS

The authors are very grateful to the team of dedicated nurses of our PD Unit without whom these results could have hardly been presented.

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II - Epidemiology of peritoneal dialysis in Hospital General Santo Antonio

III - Characterization of the peritoneal membrane

IV - Evolution of peritoneal membrane parameters

V - Therapeutic options

VI - General Discussion

VII - Summary and Conclusions

VIII - Acknowledgement

III Characterization of the peritoneal membrane

III:I *Peritoneal membrane evaluation in routine clinical practice.(submitted)*

III:II *Evaluation of effluent markers cancer antigen 125, vascular endothelial growth factor, and interleukin-6: relationship with peritoneal transport. Adv Perit Dial 2004; 20: 8-12*

PERITONEAL MEMBRANE EVALUATION IN ROUTINE CLINICAL PRACTICE

Anabela S Rodrigues¹, Sandra Silva¹, Fernanda Bravo², Jose C Oliveira², Isabel Fonseca¹, Antonio Cabrita¹, Raymond T Krediet³

Abstract

Background: The peritoneal equilibrium test with 3.86% / 4.25% glucose solution (PET 3.86%) has been proposed for clinical assessment of peritoneal membrane transport. The aim of the present cross-sectional analysis of peritoneal equilibration tests was 4 fold: the establishment of reference values for 1) small solute transport parameters using a 3.86% glucose solution, 2) the sieving of sodium after one hour without and after correction for diffusion, 3) the CA125 appearance rate in effluent, and 4) to compare the results of the proposed fast-fast PET with a dwell time of one hour, with those obtained after 4 hour dwell, especially with regard to transport categories.

Methods: 69 prevalent patients on PD for a median time of 15.1 (range 1.3-91.3) months, were evaluated. Sodium sieving was corrected for sodium diffusion with a formula applicable to the PET, using the simplified Garred method for mass transfer area coefficient (MTAC) creatinine calculation. CA125 appearance rate was measured at the end of 4 hours dwell. Mean and 95% confidence intervals (95% CI) for PET variables were obtained in the stable population. Corrected and uncorrected values for sodium sieving, according to the transport categories, were also investigated. PET parameters were compared between patients with ultrafiltration failure (UFF) and stable patients. Expected and measured D/Pcreatinine at 60 min dwell were compared by Bland and Altman analysis.

Results: 11(17.5%) of stable patients were classified as slow transporters (D/Pcreatinine < 0.63); 19 (30%) as slow-average (D/Pcreatinine 0.63-0.73); 22 (35%) as fast-average (D/Pcreatinine 0.73-0.83) and 11 (17.5%) as fast transporters (D/Pcreatinine >0.83). Means and 95% CI for stable patients were as follows: mass transfer area coefficient (MTAC) for creatinine 9.6 (8.4-10.9) mL/min, D/D₀ glucose 0.30 (0.28-0.31), corrected dip D/P sodium 0.17 (0.15-0.18),

CA125 150 (125-176) U/min. Both corrected and uncorrected values of sodium sieving significantly differed among the transport categories. D/P creatinine at 60 min correlated positively with D/Pcreatinine at 240 min ($r=0.69$, $P<0.0001$). However, patient transport categorization was significantly different (Pearson Chi-Square $P<0.0001$) with the two methods. The Bland and Altman analysis showed an overestimation of D/Pcreatinine at one hour especially marked the higher the D/Pcreatinine.

Six long-term PD patients, on treatment for a median of 53 (range 30-58) months had UFF. In comparison with stable patients, they presented higher median levels of D/Pcreatinine (0.95) and MTAC creatinine (29.4 mL/min) with lower median levels of corrected sodium dip (0.06) and CA125 appearance rate (56 U/min), $P < 0.001$ for all the variables.

Conclusions: a 3.86% (4h) glucose PET with an additional dialysate sampling taken after one hour provides results on peritoneal function that are similar to those obtained with the much more complicated SPA. Some expected overestimation of sodium sieving was clinically acceptable, but the uncorrected sodium dip was also informative. However the percentage dip increase after correction was higher in fast transporters. Small solute transport categorization based on D/P creatinine at 60 min differed significantly, in comparison with the standard 240 min dwell test categorization. D/Pcreatinine at 60 min overestimated small solute transport rate. Measurement of CA125 appearance rate gave added information on peritoneal membrane status and was consistently lower in the UFF group of patients.

Key words: peritoneal transport, PET, sodium sieving, CA125 appearance rate, ultrafiltration failure.

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Introduction

The characterisation of peritoneal membrane permeability is an important step for peritoneal dialysis prescription and adequacy tailoring. Nephrologists are now aware that it is utterly important to monitor not only membrane small solute transport, but also ultrafiltration, free-water transport and sodium removal. These parameters may impact on comorbidity and mortality [1]. Therefore it is necessary to use a method that will allow timely diagnosis and intervention on membrane function alterations. Relevant changes with different types of ultrafiltration failure may occur in long-term peritoneal dialysis patients [2].

The peritoneal equilibration test (PET) has widely been used to divide patients into transport categories [3]. The Committee on Ultrafiltration Failure of the International Society for Peritoneal Dialysis has proposed a PET with a 3.86% glucose solution for characterization of peritoneal membrane transport [4]. In comparison with the standard 2.27% PET, this modified test yields similar results of low molecular weight solute transport [5, 6] but has a higher sensitivity to detect changes in transcapillary water transport. It also allows studying sodium sieving by means of D/P Na⁺ which is an indirect expression of free water transport. However the PET 3.86% has not been widely accepted for routine clinical evaluation because of doubts about the interpretation of sodium sieving measurement: it is also influenced by diffusion of sodium, especially when the difference between plasma and dialysate concentrations is relatively large. Reference values using 3.86% glucose solutions during a standard peritoneal permeability analysis (SPA) have been established [7], but sophisticated formulas, hardly accessible to routine clinical practice, were used. Recently a formula for diffusion correction has been developed that can be applied on the dialysate sodium concentration at any time point of a PET 3.86% [8], including the end of the 4 hours dwell. The need for simple formulas of free-water transport evaluation deserved further studies [9, 10]: when compared with the more accurate, but investigational Smit *et al* [7] formulas, a fast –

fast PET with La Milia *et al*. [10] technique was found useful for estimation of transcellular water transport, with an acceptable error in everyday clinic. This however is limited to 60 minutes dwell determinations.

Added to peritoneal solute and fluid transport monitoring, the PET is also the ideal and standardized opportunity to longitudinally measure CA125 effluent levels, suggested to be a marker of mesothelial cell mass in stable patients [11]. Expectedly, functional changes will parallel structural alterations, possibly highlighted by this accessible marker, which has been investigated not only in biocompatibility studies [12, 13] but also in peritoneal sclerosis [14].

The aim of the present cross-sectional analysis of peritoneal equilibration tests was 4 fold: the establishment of reference values for 1) small solute transport parameters using a 3.86% glucose solution, 2) the sieving of sodium after one hour without and after correction for diffusion, 3) the CA125 appearance rate in effluent, and 4) to compare the results of the proposed fast-fast PET with a dwell time of one hour, with those obtained after 4 hour dwell, especially with regard to transport categories.

Patients and Methods

Patients

We cross-sectionally evaluated 69 prevalent patients of our PD Unit, in whom at least one PET with 2L, 3.86% glucose solution had been performed as a routine clinical evaluation. A dialysate sample was obtained after one hour and after drainage at 4 hours. The duration of PD ranged from 1.3 to 91.3 month, median 15.1 months.

All patients were treated with standard glucose and lactate -based dialysis solutions. None of the patients had peritonitis during the test or in the previous 6 weeks.

PETs with an ultrafiltered volume < 400 mL/4hrs were considered to represent acquired ultrafiltration failure (UFF), unless when present within the first year of PD [7]. UFF was detected in 6 patients, analyzed separately and compared with the stable group.

Measurements

Creatinine and glucose were measured with standard automatic analyser techniques. Creatinine Jaffé-compensated method (Cobas Integra) was used. Dialysate creatinine was corrected for the glucose concentrations according to local laboratory determinations. Sodium in dialysate and plasma was measured with indirect ion selective electrodes. Effluent CA 125 was determined with an electrochemiluminescence method on an automated analyzer (Elyesys 2010, Boehringer Mannheim, Indianapolis, IN, USA).

Calculations

Peritoneal transport status was determined using mean values and the standard deviation of D/P creatinine as described by Twardowski et al. [4]. The MTAC creatinine was calculated by the simplified Garred model [15, 16]. The MTAC creatinine was used to estimate the dialysate sodium concentration due to diffusion [8, 17]. This value was then subtracted from the measured sodium concentration in the dialysate. The later was used to calculate the D/P sodium [8]. The dip D/P sodium is the difference between the initial D/P sodium and the D/P sodium at 60 minutes. The expected D/P creatinine at one hour was calculated using the exponential relationship that is present for solute accumulation during a dwell, using D/P creatinine immediately after inflow (set at 0.1 in all patients) and the individual D/P creatinine after drainage. The appearance rate of CA 125 was calculated as the concentration of CA 125 present in the drained effluent divided by the duration of the dwell.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess significant deviation from normality. Except for time on treatment, all variables had a normal distribution. Results are expressed as mean $\pm$ standard deviation or as median and *inter-quartile* range; values of 95% confidence interval of the mean were calculated in the stable group. Statistically significant differences between groups were assessed using Mann-Whitney U test for continuous variables. Chi-Square test was used to compare categorical variables between groups. ANOVA was used to

compare continuous variables between transport groups. Relationship between variables was evaluated with Pearson correlation. Expected and measured D/Pcreatinine at 60 min dwell were compared by Bland and Altman analysis.

All analyses were performed using SPSS software for Windows, version 13.0.

Results

Eleven (17.5%) of the stable patients were classified as slow transporters (D/Pcreatinine < 0.63); 19 (30%) as slow-average (D/Pcreatinine 0.63-0.73); 22 (35%) as fast-average (D/Pcreatinine 0.73-0.83) and 11 (17.5%) as fast transporters (D/Pcreatinine > 0.83). Levels of mean and 95% CI of the mean for PET parameters are shown in Table 1.

PET 3.86%	Stable patients n= 63	
	Mean $\pm$ SD	95% CI
PET Drained volume (ml)	2803 $\pm$ 209	2751 - 2856
MTAC creatinine (mL/min)	9.6 $\pm$ 5	8.4 - 10.9
D/P creatinine	0.73 $\pm$ 0.10	0.70 - 0.76
D/D 0 Glucose	0.30 $\pm$ 0.07	0.28 - 0.31
D/PNa60 min	0.86 $\pm$ 0.03	0.86 - 0.87
Corrected D/PNa60 min	0.77 $\pm$ 0.05	0.75 - 0.78
Dip D/P Sodium	0.07 $\pm$ 0.03	0.06 - 0.08
Corrected Dip D/P sodium	0.17 $\pm$ 0.06	0.15 - 0.18
CA125 appearance rate (U/min)	150 $\pm$ 97	125 - 176

Table 1. PET 3.86% parameters including sodium sieving and CA125 appearance rate from a clinically stable population.

The effects of diffusion correction on D/P60 sodium and the dip according to transport group are shown in Table 2. The mean percentage increase of the sodium dip after correction was significantly higher in fast transporters than in the other transport categories ($P=0.04$). However both corrected and uncorrected values of sodium sieving significantly differed among the transport categories, presenting a similar distribution. Six long-term PD patients, on treatment for a median of 53 (range 30-58) months had acquired UFF. In comparison with stable patients, they had significantly higher median levels of D/P and MTAC creatinine and lower median levels of sodium dip and CA125 appearance rate (Table 3), falling clearly outside the 95% CI intervals given in Table 1.

Due to some expected overestimation of sodium diffusion because the simplified Garred method was used to calculate MTAC creatinine, which is higher than that of MTAC Na, calculations were also done in the UFF group

with the double value of the median MTAC creatinine (9 mL/min), but the obtained values of corrected dip Na (median 0.064, min 0.046, max 0.10) were similar (Table 3).

Table 2. Effects of diffusion correction on the D/P60 sodium and the sodium dip according to the transport group.

	<i>D/P60 sodium uncorrected</i>	<i>D/P60 sodium corrected</i>	<i>Sodium Dip uncorrected</i>	<i>Sodium Dip corrected</i>	<i>% Dip increase with correction</i>
Slow transporters	0.861	0.733	0.083	0.211	147
Slow-average transporters	0.859	0.753	0.085	0.192	151
Fast-average transporters	0.873	0.788	0.062	0.146	150
Fast transporters	0.896	0.823	0.044	0.118	271

Mean values, P <0.05 between transport groups, for all the variables

Table 3. Comparison between the stable patients (n=63) and the UFF patients (n=6): these had increased small solute transport combined with compromised free water transport and signs of a reduced mesothelial cell mass.

PET 3.86%	Stable patients		UFF Patients	
	median	IQ range	median	IQ Range
PET Drained volume (ml)	2800	2700 – 2950	2300*	2075 – 2462
MTAC creatinine (mL/min)	8.9	6.5 – 11.8	29.4*	18 – 38
D/P creatinine	0.73	0.66 – 0.81	0.95*	0.89 – 0.98
D/D 0 Glucose	0.29	0.25 – 0.33	0.18*	0.17 – 0.19
D/PNa60 min	0.86	0.85 – 0.88	0.91*	0.90 – 0.94
Corrected D/PNa60 min	0.77	0.73 – 0.81	0.87*	0.84 – 0.89
Sodium Dip	0.07	0.05 – 0.09	0.01*	0.007 – 0.03
Corrected sodium Dip	0.16	0.12 – 0.20	0.06*	0.05 – 0.08
CA125 appearance rate (U/min)	135	67 – 213	56**	24 – 76

* P<0.001 ** P=0.006

D/P creatinine 240 min					
	Slow	Slow Average	Fast Average	Fast	total
D/P creatinine 60 min					
Slow	6	3	0	0	9
Slow Average	2	11	9	2	24
Fast	2	4	10	4	20
Fast Average	1	1	3	5	10
total	11	19	22	11	63

Table 4 Comparison of transport categories based on D/P creatinine values at 60 min and 240 min dwell in 63 stable patients (Pearson Chi-Square P<0.0001)

Additional information on small solute transport was investigated, based on 60 min dwell PET parameters: D/Pcreatinine at 60 min correlated significantly with D/Pcreatinine at 240 min ($r=0.69$, $P<0.0001$) (Figure1). However, patient were differently categorized according to the two methods (Pearson Chi-Square $P<0.0001$) (Table 4). When investigating measured and expected values of D/Pcreatinine at one hour with a Bland and Altman analysis, we did not find randomly distributed data around the mean: a systematic error occurred with overestimation of D/Pcreatinine, especially marked the higher the D/Pcreatinine (Figure 2).

Figure 1. Correlation between standard D/P creatinine values at 240 min and values obtained at 60 min dwell (Pearson $r = 0.69$, $P < 0.0001$).

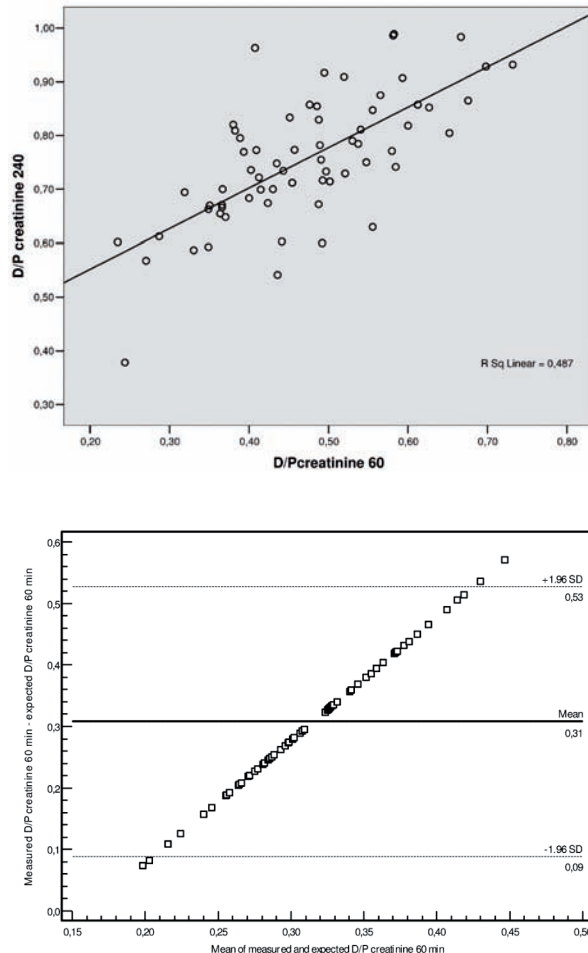


Figure 2. Bland and Altman analysis of measured and expected values for D/P creatinine: a systematic bias is evidenced with overestimation of small solute transport with measured D/P creatinine 60 min

Discussion

The present study has shown that a 3.86% glucose PET with a dialysate sampling after one hour provides results on peritoneal function that are similar to those obtained in the much more complicated SPA [7]. On the other hand, it avoids overestimation of small solute transport rate that might occur when categorization is based on D/P creatinine

values obtained in a Fast-Fast PET, after 60 min dwell only.

The PET allows calculation of the MTAC creatinine by the simplified Garred model, while in the SPA the Waniewski model is used. It has been shown previously that the MTAC creatinine is not significantly different between the simplified Garred method and the Waniewski method [16, 17]. The similarity between our results and those of Smit et al [7] suggests that it is also the case for the 3.86% glucose solution.

Simpler formulas for sodium sieving correction for diffusion could be applied to the PET 3.86%. This correction might be useful in case of a large vascular peritoneal surface, a situation where diffusion of sodium from circulation to the dialysate will be increased, blunting the D/P sodium dip and overestimating the impairment of transcellular free water transport. Different methods have been investigated to evaluate free water transport and adequately correct for sodium diffusion [5, 7-10, 18-20]. Reference values, using the standard peritoneal permeability analysis [7], have recently been published, but a volume marker is needed to allow fluid kinetic calculations and complicated equations were employed. Average MTAC Na was assumed to be similar to MTAC urate which is not measured during a PET and the MTAC creatinine was calculated with the Waniewski model, the best predictive model, but too sophisticated for routine clinical evaluation. Theoretically, simple modules for prediction of small solute transport across the peritoneal membrane should apply a correction factor F for the convective component, justified for a high ultrafiltration period [21]. However this modification has little or no significant effect on the calculation of MTAC during a dwell using 1.36% or 3.85 % [22]. In search for more accessible formulas, simplified methods of mass transfer area coefficients have previously been clinically validated [16, 17]. Although the simplified Garred model may be less correct than the Waniewski model, overestimating sodium sieving, an excellent correlation was found between Waniewski MTAC urate and Garred MTAC creatinine which allows the use

of this more easily applicable model in routine clinical sodium sieving estimation and correction for diffusion [17]. However our study showed that both corrected and uncorrected values of sodium sieving significantly differ among the transport categories, presenting similar distribution and were also both discriminative of UFF. Therefore for the simple clinical situation, correction for sodium sieving might be dispensable, since this methodology still only indirectly estimates free water transport. La Milia et al [10] developed a bedside formula for actual free water transport quantification, derived from a Fast-Fast PET with duration of only one hour. It was validated [9] as a clinically useful tool, but it is less precise than the Smit et al technique and a correction algorithm was still recommended.

Whichever the method to estimate or directly measure free water transport a distinction cannot be done between causes of its decrease. Functionally, UFF is in most cases characterized by an enhanced peritoneal mass transfer area coefficient for glucose combined with a largely unchanged peritoneal glucose osmotic conductance [23]. However, this may be decreased in cases of more severe acquired UFF in long-term PD patients [24]: aquaporin-1 dysfunction and/or interstitial fibrosis might be the causes of such late changes. It is interesting to note that if we make a correction for diffusion the sodium dip varies more consistently and significantly between the categories of small solute transport, as our data in table 2 suggest. It is possible that causes, other than increased mass transfer area coefficient for glucose, such as true aquaporin-1 dysfunction and a decrease of osmotic conductance to glucose due to interstitial fibrosis become more detectable after correction for diffusion, especially in fast transporters.

The La Milia group [10] also concluded that their method allowed standardized categorization of small solute transport. However reference values for D/P creatinine at one hour are still not available. It has also been reported that there is an overestimation of MTAC at 60 min, due to the contribution of convective transport and the fact that

permeability surface area during the first hour of dwell is significantly higher than during subsequent hours [25, 26]. In our study both D/P creatinine at 60 min and at 240 min were correlated, but patients transport categorization differed significantly with the two methods. Besides, Bland and Altman analysis of measured and expected values for D/P creatinine at 60 min dwell showed a systematic error with overestimation of small solute transport with measured D/P creatinine 60 min.

Our modified PET looks useful for routine clinical evaluation, because it allowed us to monitor small solute transport, using standardized categorization, and sodium sieving, as a discriminative estimate of free water transport. In our study the values for D/P creatinine, D/Do glucose and MTAC creatinine were similar to the reference values using the SPA. Only the dialysate/plasma ratio for Na⁺ showed a somewhat lower minimum value, therefore a more pronounced dip. As expected, this may have been caused by some overestimation of the diffusive component of sodium transport, because the MTAC creatinine was used for this purpose. In the present study an indirect ion-selective electrode methodology was used, thereby avoiding spurious estimations of D/P Na found using direct ion-selective electrode methods or flame photometry without correction for plasma water and a Donnan factor for Na measurement [27]; indirect ion-selective methodologies can be reliably used in the evaluation of D/P Na in everyday clinical practice [28].

Our methodology enabled us to identify one or more causes of ultrafiltration failure in all patients, a high MTAC creatinine being the most common one. This was combined with a low dip in the D/P Na⁺ pointing to reduced free water transport.

These patients had duration of peritoneal dialysis of more than 4 years. It supports the observation that impaired free water transport is especially found in long term PD patients [29]. Ultrafiltration failure due to a high lymphatic absorption rate cannot be identified with our methodology. However, no

effect of the duration of PD has been identified in cross-sectional analyses or during longitudinal follow-up [30].

Patients with ultrafiltration failure had lower effluent CA 125 appearance rates suggesting a reduced mesothelial cell mass [11], probably a general sign of extensive peritoneal membrane damage. Although this has been recently questioned [31] the finding of low levels of effluent CA125 in long-term PD, notably with UFF make it worthwhile to be routinely measured, in order to clarify its predictive value. It was not the purpose of this study to investigate the relationship between mesothelial cell mass and peritoneal transport: an indirect role has recently been suggested but this seems to vary in short and long term PD, possibly mediated earlier by production of vasoactive factors [32-34] or later through epithelial-mesenchymal transition [35]. This should be preferably investigated in longitudinal studies.

We conclude that the PET 3.86% with a simpler formula for diffusion corrected sodium sieving and measurement of the CA 125 appearance rate provides useful clinical information on the status of the peritoneal membrane and can identify causes of ultrafiltration failure in individual patients. Some expected overestimation of sodium sieving was clinically acceptable, but the uncorrected sodium dip was also informative. Small solute transport categorization based on D/P creatinine at 60 min differed significantly, in comparison with standard 240 min dwell test categorization. D/P creatinine measurement at 60 min dwell induced a systematic error with an overestimation of small solute rate, especially marked the higher the D/P creatinine.

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Evaluation of Effluent Markers Cancer Antigen 125, Vascular Endothelial Growth Factor, and Interleukin-6: Relationship with Peritoneal Transport

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Peritoneal hyperpermeability has been associated with increased levels of effluent vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6). Mesothelial cells can produce various vasoactive substances besides VEGF. A large mesothelial mass may possibly lead to high dialysate VEGF concentrations and may partly explain some cases of peritoneal hyperpermeability during a patient's early months on peritoneal dialysis (PD). Early peritoneal fast transport may therefore not necessarily be associated with systemic inflammation.

To investigate the relationship of effluent markers and peritoneal transport, we measured the appearance rates of cancer antigen 125 (CA125), VEGF, and IL-6 in 4-hour effluents from 69 peritoneal equilibration tests (PETs) using 3.86% glucose solution. At the same time, we measured serum VEGF and IL-6. Our analyses included an early group (EG), whose members had been on PD for 4.6 ± 3.3 months, and a later group (LG), whose members had been on PD for 30 ± 17 months.

In EG, dialysate-to-plasma creatinine at 4 hours (D/P_{Cr240}) correlated significantly with effluent CA125/min ($r = 0.51$, $p = 0.006$) and VEGF/min ($r = 0.57$, $p = 0.001$), but not with serum VEGF or IL-6. The values of CA125/min and VEGF/min also correlated ($r = 0.40$, $p = 0.034$). Fast transporters in EG had higher effluent CA125 ($p = 0.057$) and VEGF ($p = 0.0001$), but not serum or effluent IL-6. In LG, D/P_{Cr240} again correlated significantly with dialysate VEGF

($r = 0.51$, $p = 0.009$), but not with CA125. Fast transporters in LG tended to have higher levels of serum and effluent IL-6 and effluent VEGF.

We conclude that fast solute transport rates at the beginning of PD are associated with signs of a large mesothelial cell mass and not consistently associated with higher systemic IL-6. The VEGF produced by mesothelial cells can mediate early peritoneal hyperpermeability in some populations. Later, mesothelial mass is lost and is no longer related to increased intraperitoneal VEGF or IL-6.

Key words

Peritoneal transport, mesothelial cells, vascular endothelial growth factor, cancer antigen 125, interleukin-6

Introduction

Fast peritoneal solute transport rates at the beginning of peritoneal dialysis (PD) have been linked to systemic inflammation, with increased levels of plasma cytokines such as interleukin-6 (1). Severe associated comorbidity could explain the worse outcomes of these patients (2). However, baseline peritoneal hyperpermeability is not always associated with inflammation, and the dialysis population probably includes a heterogeneous group of patients (3,4). In some patients, a baseline profile of fast solute transport changes to an average category. In fact, longitudinal studies have often documented a centripetal progression in dialysate-to-plasma (D/P) creatinine (5,6).

Because mesothelial cells are able to constitutively produce vascular endothelial growth factor [VEGF (7)], a potent vasodilator and angiogenic factor, a larger mesothelial mass could therefore be assumed to promote higher levels of intraperitoneal VEGF. Those

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levels may induce recruitment of previously non-perfused capillaries, causing a functional increase in the effective capillary surface during the early months of PD. With longer time on PD, a reduction in mesothelial mass is observed (8); however, this reduction is paralleled by an anatomic increase in peritoneal vascular surface area (9). Cumulative exposition to glucose degradation products stimulates VEGF production not only by mesothelial cells, but also by capillary endothelial cells (10,11). Increased intraperitoneal levels of interleukins and growth factors might suggest ongoing chronic inflammation due to glucotoxicity (12).

The aim of the present study was therefore both to investigate the relationship between peritoneal solute transport and markers of inflammation (systemic and intraperitoneal IL-6) and to evaluate whether mesothelial mass and effluent VEGF are related to peritoneal hyperpermeability in the initial phase and the later stages of PD.

Patients and methods

We analyzed 69 peritoneal equilibration tests (PETs) performed in 58 patients with 3.86% or 4.25% PD solution. The average age of the patients was 50 years (range: 23 – 81 years). Median duration of PD was 13 months (range: 0.2 – 80 months). All patients were treated with commercially available glucose-based solutions.

We measured the appearance rates of interleukin-6 (IL-6), cancer antigen 125 (CA125), and VEGF in the effluent at 240 minutes of the PET. To measure VEGF in effluent and serum, we used a commercially available enzyme-linked immunosorbent assay [Quantikine (human VEGF): R&D Systems, Minneapolis, MN, U.S.A.] as previously described (13). To measure IL-6 in effluent and serum, we used a commercially available immunoenzymometric assay (Easia: Biosource Europe SA, Nivelles, Belgium). To measure CA125, we used an electrochemiluminescence method with a automated analyzer (Elecsys 2010: Boehringer Mannheim, Indianapolis, IN, U.S.A.).

The relationship between peritoneal transport and effluent markers was studied in two groups: an early group (EG) whose members had been on PD for a period ≤ 12 months ($n = 32$), and a later group (LG) whose members had been on PD for >12 months ($n = 37$).

Statistical analysis

We applied the Kolmogorov–Smirnov test for a normal distribution. Serum IL-6 and VEGF showed a non normal distribution, and logarithmic transformation was therefore performed before analysis. Variables with normal distribution are expressed as mean $\pm$ standard deviation, and asymmetrically distributed data are reported as medians and interquartile ranges. The Pearson correlation test was used to determine correlations between variables. The Mann–Whitney test was used to compare groups. Values of $p < 0.05$ were used to define statistical significance, and all statistical analyses were performed using the SPSS software program, version 11.5 (SPSS Inc., Chicago, IL, U.S.A.).

Results

The appearance rate of CA125 decreased with time on PD ($r = -0.30$, $p = 0.012$), as shown in Figure 1. No trend related to the duration of PD was observed for any of the other parameters investigated. Table I shows the results of the PET and the CA125, VEGF, and IL-6 analyses.

Further analysis of values for EG patients showed that D/P creatinine was correlated with the appearance rate of VEGF ($r = 0.57$, $p = 0.001$) and of CA125 ($r = 0.51$, $p = 0.006$; Figure 2). A correlation was also present between the appearance rate of CA125 and that of VEGF ($r = 0.40$, $p = 0.034$). No relationships were found between D/P creatinine, serum VEGF, the appearance rate of IL-6, and serum IL-6.

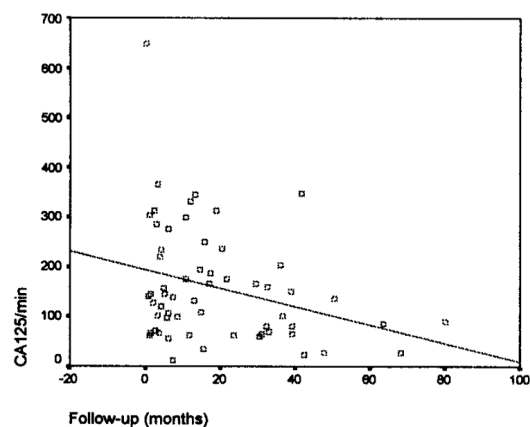


FIGURE 1 Relationship between the appearance rate of cancer antigen (CA125) and the duration of peritoneal dialysis ($p = 0.012$).

TABLE I Demographics of the patients in the early peritoneal dialysis group ($n=32$) and the later group ($n=37$), and their peritoneal transport characteristics, their dialysate appearance rates of cancer antigen 125 (CA125), vascular endothelial growth factor (VEGF), and interleukin-6 (IL-6), and their serum concentrations of VEGF and IL-6

	Groups ^a	
	Early	Later
Male/female (n)	12 / 20	17 / 20
Diabetic/nondiabetic (n)	7 / 25	10 / 27
Age (years)	49±15	51±16
Follow-up (months)	4.6±3.3	30±17
D/P creatinine	0.75±0.13	0.72±0.11
Ultrafiltration (mL/4 h)	803±229	817±286
Dialysate appearance rate (mean±SD)		
CA125 (U/min)	185±133	137±90
VEGF (pg/min)	260±106	255±196
IL-6 (pg/min)	690±520	1013±775
Serum concentration [median (interquartile range)]		
VEGF (pg/mL)	336 (218–558)	326 (230–483)
IL-6 (pg/mL)	6.3 (3.8–13.1)	15.7 (10.7–25.7)

^a No parameter was significantly different between the two groups, except for duration of follow-up.

D/P = dialysate-to-plasma concentration ratio; SD = standard deviation.

A comparison between EG fast transporters (D/P creatinine > 0.81) and other transport categories showed a tendency to higher CA125 values in the fast transporters and significantly higher VEGF appearance rates (Table II).

In the LG patients, D/P creatinine correlated only with the appearance rate of VEGF ($r = 0.38$, $p = 0.021$). A correlation between the appearance rates of CA125 and VEGF was not found in LG patients. Fast transport patients in LG showed a tendency to higher serum IL-6 concentrations ($p = 0.117$). The difference in the appearance rate of VEGF was of borderline significance ($p = 0.058$, Table II).

Discussion

The present study shows that relationships between peritoneal transport status and effluent and serum markers of inflammation and angiogenesis depend on the duration of PD. In the first year of PD, fast transporters were especially characterized by increased CA125 and VEGF appearance rates, but not by high serum or dialysate levels of IL-6. That result contrasts with the results obtained by Stenvinkel *et al.* (1), but accords with a recent study of nondiabetic PD patients investigated during the first 6 months of dialysis (14).

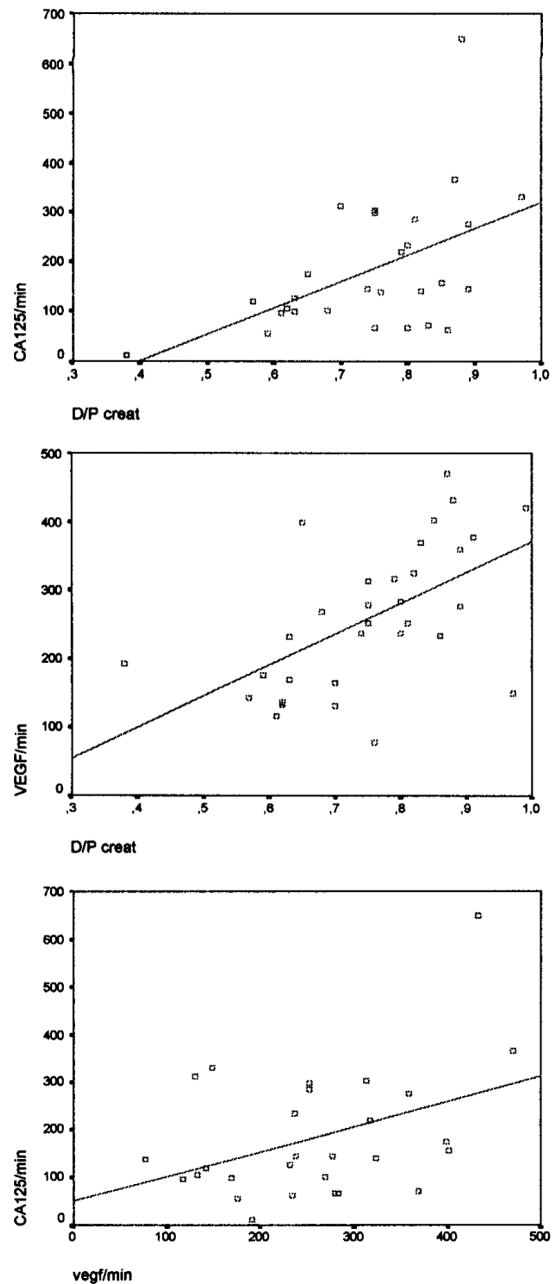


FIGURE 2 Correlations in the early-group patients between the dialysate-to-plasma concentration ratio (D/P) of creatinine and the appearance rate of cancer antigen 125 (CA125; $p = 0.006$), between D/P creatinine and the appearance rate of vascular endothelial growth factor (VEGF; $p = 0.001$), and between the appearance rates of VEGF and CA125 ($p = 0.034$).

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TABLE II Comparison between fast transporters (D/P creatinine > 0.81) and other transport categories by group

	Transport status			
	Early group		Later group	
	Fast (n=12)	Other (n=20)	Fast (n=6)	Other (n=31)
D/P creatinine	0.88±0.05	0.67±0.01	0.92±0.06	0.68±0.07
Ultrafiltration (mL/4 h)	683±157	875±239	600±301	859±268
Dialysate appearance rates				
CA125 (U/min)	249±176 ^a	149±90	185±144	129±77
VEGF (pg/min)	339±94 ^b	212±82	442±301 ^c	220±152
IL-6 (pg/min)	877±630	550±408	1017±436	877±617
Serum concentrations				
VEGF (pg/mL)	487±272	368±211	559±304	398±291
IL-6 (pg/mL)	10.48±12	13±23	33±23 ^a	17±12

^a $p = 0.10$.^b $p = 0.001$.^c $p = 0.06$.

The discrepancy underlines the need to interpret initial fast transporters as a heterogeneous population, not always inflamed and prone to a poor prognosis.

Our results suggest that the VEGF produced by mesothelial cells—such production having been established *in vitro* (7)—may be involved in the initial fast transport status. Our hypothesis is supported by the correlation between D/P creatinine and the appearance rate of VEGF, which has been reported previously (13), and by the correlations between CA125 and VEGF and between D/P creatinine and CA125.

The relationship between CA125 and peritoneal transport rates disappears during long-term PD (15), probably because mesothelial cell mass declines (16). Yet, solute hyperpermeability is still associated with higher dialysate appearance rates of VEGF in long-term PD patients (12), which suggests a non mesothelial site of production. In our longer-duration PD patients with fast transport status, we found some evidence of higher IL-6 levels, which suggests a role for ongoing intraperitoneal inflammation.

Conclusion

The presence of fast peritoneal transport status in the early stage of PD is often associated with high appearance rates of CA125 and VEGF, suggesting an indirect effect of mesothelial cell mass on solute transport rates—partly mediated by VEGF. In the later stages of PD, peritoneal angiogenesis and chronic inflammation are likely to be involved in peritoneal solute transport.

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Nephrol Dial Transplant (2006) 21: 763–769
doi:10.1093/ndt/gfi245
Advance Access publication 6 December 2005



Original Article

Peritoneal fast transport in incident peritoneal dialysis patients is not consistently associated with systemic inflammation

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Abstract

Background. The determinants of peritoneal fast transport status at the beginning of peritoneal dialysis (PD) are still under debate. The relationship between fast transport status and inflammation or co-morbidity, and its impact on patient survival are not fully elucidated. Our objective was to investigate if fast transport status in incident patients is associated with markers of inflammation and atherosclerosis, and its relationship to patient survival.

Methods. Seventy-three incident patients on PD performed a 3.86% peritoneal equilibrium test (PET) at 4.7 ± 2.7 months after starting PD. Doppler carotid wall intima-media thickness (IMT) and the presence of carotid plaque were used as markers of atherosclerosis. C-reactive protein (CRP) and serum interleukin-6 (IL-6) were evaluated as markers of systemic inflammation. Baseline plasma levels of albumin, homocysteine, lipoprotein (a) [Lp(a)] and other lipid parameters were measured. Body mass index and residual renal function (RRF) were calculated. Patients were classified with the Davies co-morbidity score.

Results. The dialysate–plasma creatinine ratio (D/P creatinine) was 0.75 ± 0.10 ; 26% were fast transporters (D/P ≥ 0.85). In comparison with other transport categories, these had similar age, body mass index and RRF, and did not present a higher co-morbidity score than non-fast transporters. IMT did not significantly differ between groups. By multiple regression analysis, baseline peritoneal small solute transport was not related to systemic inflammation biomarkers. Fast transporters did not present higher levels of CRP or serum IL-6. Plasma levels of lipids, Lp(a), calcium $\times$ phosphorus product and albumin also did not differ between groups. Similar results were obtained when patients were grouped according to mass transfer

area coefficient for creatinine. Patients with more than two co-morbidities had lower levels of plasma albumin (3.6 ± 0.58 vs 3.9 ± 0.9 g/dl, $P = 0.054$), significantly higher median levels of serum IL-6 (19.3 vs 9.2 pg/ml, $P = 0.003$) and wider IMT (0.90 ± 0.36 vs 0.65 ± 0.28 mm, $P = 0.017$). Multivariate analysis confirmed that baseline peritoneal transport was not a significant determinant of patient survival ($P = 0.848$), while the co-morbidity score remained significant (hazard ratio = 3.48, 95% confidence interval = 1.29–9.38, $P = 0.014$).

Conclusion. Initial fast transport was not associated with systemic inflammation and atherosclerosis. In a population with preserved RRF and absence of baseline serious co-morbidity, it was not predictive of worse prognosis. Other determinants of early peritoneal fast transport deserve investigation.

Keywords: inflammation; peritoneal transport; survival

Introduction

Hyperpermeability of the peritoneal membrane, defined as fast or high transport by the peritoneal equilibration test (PET [1]), can develop during long-term peritoneal dialysis (PD) [2,3], but may also be present from the start of dialysis [4]. Studies in the latter group of patients have reported an association with increased mortality [5,6], decreased technique survival [7] or a combination of both [8].

The association of a fast transport status with decreased technique survival can be explained because the resulting inadequate ultrafiltration usually leads to transfer from continuous PD to automated PD or haemodialysis when residual urine volume has decreased. However, the reason for the higher mortality reported in earlier studies has not been clarified.

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Pathogenic effects of a high peritoneal transport status have been documented [9], but poor outcome was not confirmed by all [10–12]. Some studies [13–15] reported an over-representation of a fast peritoneal transport status in patients with co-morbidity. It has been suggested that a chronic inflammatory status might determine baseline peritoneal transport [16]. As inflammation is associated with hyperaemia, it can be hypothesized that concomitant peritoneal hyperaemia leading to an increase in the effective vascular peritoneal surface area (the number of effectively perfused capillaries) might explain the relationship between co-morbidity and peritoneal transport status. Acute systemic inflammation is indeed associated with a temporary increase in the peritoneal solute transport rate in PD patients [17]. However, a causal relationship between inflammation and basal peritoneal transport is doubtful and was not supported in more recent investigations [18,19].

A large study [20] reported recently that a higher peritoneal transport status was not independently predicted by diabetes, other co-morbid diseases or residual renal function. The baseline dialysate–plasma creatinine ratio (D/P creatinine) also had no effect on patient or technique survival in a multicentre study of automated PD anuric patients [21].

Therefore, the diversity of peritoneal transport characteristics in different populations and controversial data concerning its determinants deserve more investigation. The validity of baseline peritoneal transport as an independent predictor of patient survival is questionable.

The aim of the present study was to investigate whether peritoneal hyperpermeability in incident PD patients was associated with inflammation, atherosclerosis or co-morbidity, and its impact on patient survival.

Patients and methods

Seventy-three incident PD patients (27 male, 46 female), aged 48 ± 16 years, were evaluated in a prospective observational study. Twenty (27%) patients were diabetic, 17 (23%) presented clinical ischaemic heart disease, five (6%) had a previous cerebrovascular event and nine (12%) had peripheral vascular disease. Patients were subjected to a basal PET with a 3.86% solution, at 4.7 ± 2.7 months after starting on PD. Mean D_4/P_4 creatinine was 0.75 ± 0.10 . Patients were categorized, according to Twardowski *et al.* [1], as high transporters ($D/P \geq \text{mean} \pm \text{SD}$, $n = 19$) and non-high transporters ($n = 54$). High and non-high transporters were then stratified using Davies's co-morbidity scores [22].

To characterize peritoneal small solute transport further, the mass transfer area coefficient (MTAC) for creatinine was calculated with the simplified Garred method, validated for clinical purposes [23–25]. Median MTAC creatinine was 9.3 ml/min [interquartile range (IQR) 6.6–13.1 ml/min]. Additional analysis was done to compare patients according to the level of MTAC creatinine (higher than *vs* lower than the median).

In search of markers of atherosclerosis, right and left carotid arteries were examined with a G.E. Logic 500 MD duplex scanner (7.5 MHz probe). All scannings were performed by the same trained sonographer. Intima-media thickness (IMT) was defined as the distance between the leading edge of the lumen–intima echo and the leading edge of the media–adventitia echo, measured 10 mm proximal to the common carotid artery bifurcation. A carotid plaque was reported if localized IMT > 1 mm and at least a 100% increase in thickness compared with adjacent wall segments was present.

Body mass index (BMI) and serum albumin were measured. Serum albumin was measured with the bromocresol green method. Residual renal function (RRF) was calculated from 24 h urine with the media of creatinine and urea clearances.

C-reactive protein (CRP) was used as an inflammatory marker. It was measured by nephelometry with a high sensitivity assay (Boehringer analyzer II; normal values: CRP < 0.5 mg/dl).

Serum interleukin-6 (IL-6) was additionally measured in 42 patients for whom samples were available. These patients were similar in terms of peritoneal permeability (D/P creatinine, MTAC creatinine), age, gender, RRF, plasma albumin and co-morbidity score compared with the remaining cohort ($n = 31$). A commercially available immunoenzymometric assay (IL-6 Easia; Biosource Europe SA, Nivelles, Belgium) was used.

Metabolic and humoral cardiovascular risk factors included: homocysteine, lipoprotein (a) [Lp(a)], other lipid parameters and calcium $\times$ phosphorus product ($\text{Ca} \times \text{P}$).

Total cholesterol, triglycerides and high-density lipoprotein cholesterol were assayed enzymatically by colorimetric methods (Olympus). Low-density lipoprotein cholesterol was calculated using the Friedwald's formula, and very low-density lipoprotein cholesterol was obtained by dividing the serum triglycerides level by 5. The plasma Lp(a) concentration was quantified by an immunoturbidimetric assay from Boehringer-Mannheim, using the automatic analyser Olympus AU 800 [Lp(a) normal values: < 30 mg/dl]. Total fasting homocysteine levels were determined by a fluorescence polarization immunoassay on an automated IMx analyzer from Abbott laboratories (Axis Biochemical's ASA, Oslo, Norway) (reference values of homocysteine ranged from 5 to $15 \mu\text{mol/l}$).

Statistical analysis

Normality of data distribution was tested by the Kolmogorov–Smirnov test. Pearson's correlation was then used to assess the relationship between continuous variables with normal distribution.

Comparisons between high and non-high transporters for continuous variables were made with non-parametric techniques because of the different sizes of the compared groups. Group data are expressed as median (IQR), and differences between medians were analysed by Mann–Whitney *U*-test. Frequencies of categorical variables were compared by χ^2 -test or Fisher exact test.

A multiple regression analysis was done to examine the relationship between inflammation and baseline peritoneal transport. Statistical significance thresholds required for inclusion and exclusion at each stepwise run were set at

0.05 and 0.01, respectively. Baseline D/P creatinine, or MTAC in a subsequent analysis, was the dependent variable, and all possible predictors of baseline peritoneal transport were screened for inclusion as independent variables using univariate and multivariate general linear modelling.

Univariate survival analysis was carried out by the Kaplan–Meier method, using the log-rank test to compare survival between groups. Multivariate analysis was done using the Cox proportional hazard regression model to evaluate the independent influence of baseline peritoneal transport. Multivariate Cox regression analysis was performed using the method ‘enter’ and the results are reported as hazard ratios (HRs), with 95% confidence interval (95% CI) and *P*-value.

Statistical analyses were performed using the SPSS statistical program for Windows (version 10.0, SPSS Inc., Chicago, IL), and a *P*-value <0.05 was considered statistically significant.

Results

D₄/P₄ creatinine ranged from 0.54 to 0.96, with a mean level of 0.75 ± 0.10 . Demographic and laboratory data, and co-morbidity scores of the patients are presented in Table 1. The magnitude of RRF was not significantly different between high transporters and non-high transporters. No significant differences were found in co-morbidity in high transporters vs non-high transporters (10.5 vs 27.8%, *P* = 0.207) (Table 1).

In the subgroup of 42 patients from whom serum IL-6 was available, the median (IQR) level of this marker was 11.1 (4.6–17.8) pg/ml. Twenty-one patients had serum IL-6 levels higher than the median. Peritoneal solute transport did not differ significantly between the groups, categorized according to the median level of serum IL-6 (>the median vs ≤median level): MTAC 8.2 ± 4.4 vs 10.7 ± 6.3 ml/min, *P* = 0.16; D/P creatinine 0.73 ± 0.10 vs 0.76 ± 0.11 , *P* = 0.38, respectively.

Median levels of serum albumin, CRP and serum IL-6 were not significantly different in high and non-high transporters (Table 2). Plasma levels of lipids, including Lp(a), homocysteine, parathyroid hormone,

calcium × phosphorus product and IMT did not differ between groups. Similar results were obtained when patients were grouped according to MTAC for creatinine (analysis not shown).

Baseline peritoneal small solute transport (D₄/P₄ creatinine, MTAC creatinine) was not significantly correlated with markers of systemic inflammation, with either IL-6 (*P* = 0.273) or CRP (*P* = 0.804).

A multivariate analysis was performed to examine the relationship between inflammation and baseline peritoneal transport. Baseline D/P creatinine, or MTAC in a subsequent and similar analysis, was the dependent variable and all potential clinically relevant variables were examined, namely serum IL-6, CRP, plasma levels of lipids, including Lp(a), homocysteine, parathyroid hormone, calcium × phosphorus product and co-morbidity score. The presence of diabetes mellitus, clinical ischaemic heart disease, peripheral vascular disease and a previous cerebrovascular event were also tested.

Each variable was included one by one as independent variables (continuous or categorical). Except for homocysteine, none of the remaining variables was significantly related to D/P creatinine or MTAC. Even so, a multivariate analysis was performed, and all the variables were tested. Models with two, three, four, five and six variables were studied, in several combinations. We verified again that, except for homocysteine, none of the remaining variables was significantly related to D/P creatinine or MTAC.

Patients with more than two co-morbidities had lower levels of plasma albumin (3.6 ± 0.58 vs 3.9 ± 0.49 g/dl, *P* = 0.054), significantly higher median levels of serum IL-6 [19.3 (14–37.5) vs 9.2 (4–14.4) pg/ml, *P* = 0.003 and wider IMT (0.90 ± 0.36 vs 0.65 ± 0.28 mm, *P* = 0.017).

Baseline peritoneal fast transport did not show an impact on patient survival: fast transporters had 92, 77 and 77% of cumulative survival vs 98, 85 and 79% in non-fast transporters at 1, 2 and 3 years, respectively, *P* = 0.84 (Figure 1).

Table 2. Comparisons between high and non-high transporters for continuous parameters showed no significant differences

	High transporters (<i>n</i> = 19)	Non-high transporters (<i>n</i> = 54)
Age (years)	39 (30–61)	49 (37–64)
Gender (M/F)	4/15	23/31
Diabetes mellitus (Yes)	6 (31.6%)	14 (25.9%)
Interval (start PD to PET; months)	4 (2–7)	4.2 (2.5–6)
BMI (kg/m ²)	22.9 (21.7–25.3)	23.3 (21.2–26.1)
RRF (ml/min/1.73 m ²)	4.4 (3.0–7.9)	4.0 (3.0–5.0)
Co-morbidity (Davies score ≥2)	2 (10.5%)	15 (27.7%)

Values are given as median (interquartile range) for continuous variables and as *n* (%) for categorical variables.

P Statistically non-significant (*P* > 0.05) for all these variables.

	High transporters median (IQR)	Non-high transporters median (IQR)
Albumin (g/dl)	3.9 (3.4–4.3)	3.9 (3.6–4.2)
CRP (mg/dl)	0.24 (0.12–1.12)	0.52 (0.14–1.15)
IL-6 (pg/ml)	9.3 (7–17.5)	11.8 (4.2–18.6)
Ca × P	51.8 (42–56.3)	46.5 (38.6–54.3)
Parathyroid hormone (pg/ml)	204 (108–488)	224 (120–466)
Total cholesterol (mg/dl)	190 (165–234)	197 (164–239)
LDL cholesterol (mg/dl)	116 (104–147)	122 (92–151)
HDL cholesterol (mg/dl)	44 (35–51)	44 (38–80)
Triglycerides (mg/dl)	165 (129–220)	175 (126–197)
Lp(a) (mg/dl)	37 (11–65)	45.7 (16–104)
Homocysteine (μmol/l)	20 (16–27)	18.2 (14.3–22.7)
IMT (mm)	0.6 (0.50–0.67)	0.6 (0.5–0.8)

P Statistically non-significant (*P* > 0.05) for all these variables.

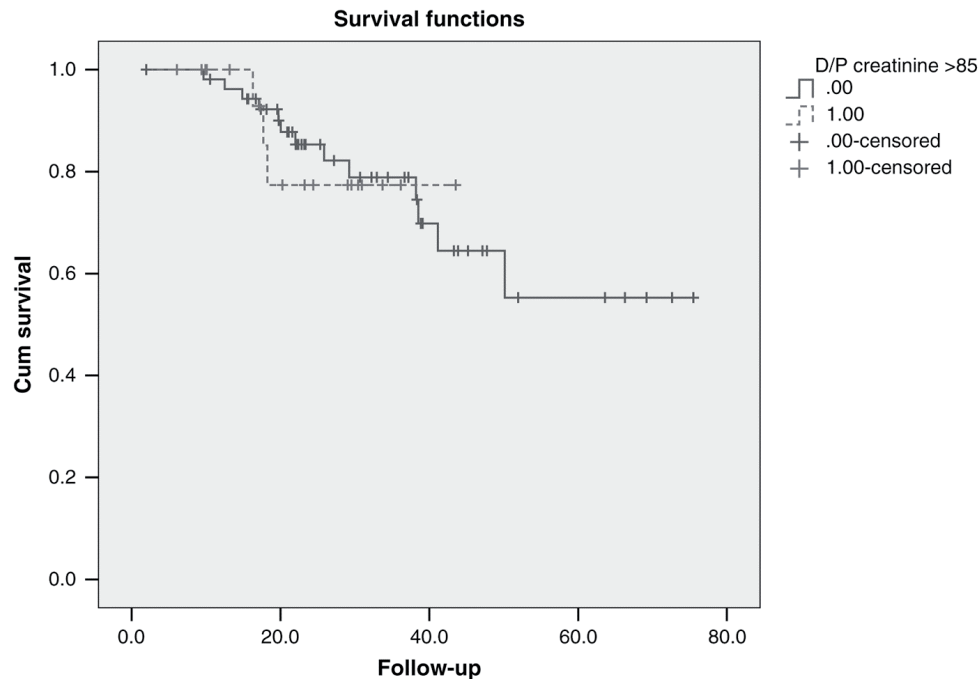


Fig. 1. Patient cumulative survival did not differ between baseline peritoneal transport groups [group 0 = non-high transporters, $n = 54$, 13 events; group 1 = high transporters (D/P creatinine >0.85), $n = 19$, three events, $P = 0.84$], follow-up given in months.

Patient survival was negatively influenced by co-morbidity score: patients with a co-morbidity score <2 had a cumulative survival of 98, 89 and 85 vs 93, 64 and 55% in patients with a co-morbidity score ≥ 2 , at 1, 2 and 3 years, $P = 0.006$ (Figure 2).

By Kaplan–Meier survival analysis, elevated levels of serum IL-6 were a significant predictor of mortality (Figure 3). Differences in survival between patients with elevated vs low serum IL-6 levels (higher vs lower median IL-6 levels) were compared using the log rank test. Eight patients died in the group with IL-6 levels higher than the median, while no fatal event occurred in the group with IL-6 equal to or below the median levels of IL-6 ($P = 0.0019$).

The Cox proportional hazards method was then used to evaluate the influence of baseline peritoneal hyperpermeability on patient survival of PD patients when combined with the co-morbidity score or markers of inflammation evaluated, namely CRP and IL-6. Because inclusion of covariates with high degrees of collinearity may inflate the variance of the model, inclusion of the co-morbidity score in the model was done separately from the inclusion of IL-6 and CRP. Including baseline peritoneal transport and co-morbidity score in the same model confirmed that baseline peritoneal transport (D_4/P_4 creatinine >0.85) was not a significant predictor of mortality in PD patients (HR = 1.13; 95% CI = 0.31–4.14; $P = 0.848$), while a co-morbidity score >2 remained a significant and independent marker of poor outcome (HR = 3.48;

95% CI = 1.29–9.38; $P = 0.014$) (Table 3). After excluding the morbidity score from the model and including IL-6, or CRP, with baseline peritoneal transport, neither of the two inflammation markers was predictive of mortality.

Similar conclusions were obtained when MTAC creatinine was used as a measure of peritoneal small solute transport.

Discussion

This study showed that baseline peritoneal fast transport was not associated with markers of systemic inflammation; neither was predictive of worse patient survival in incident PD patients. Our results are in accordance with recent investigations [19–21].

It has been speculated that peritoneal hyperpermeability in the beginning of PD might impact on patient mortality due to serious associated co-morbidity, such as underlying atherosclerosis [26] and chronic inflammatory status [16], or insufficient management of fluid load and humoral abnormalities [27]. However, controversial data came from relevant recent studies [20,21].

Concerning the relationship with inflammation, a single cross-sectional [28] study involving 40 patients documented a positive correlation between D/P creatinine and serum and effluent IL-6, a sensitive marker of inflammation. Recently, a multicentre study [29]

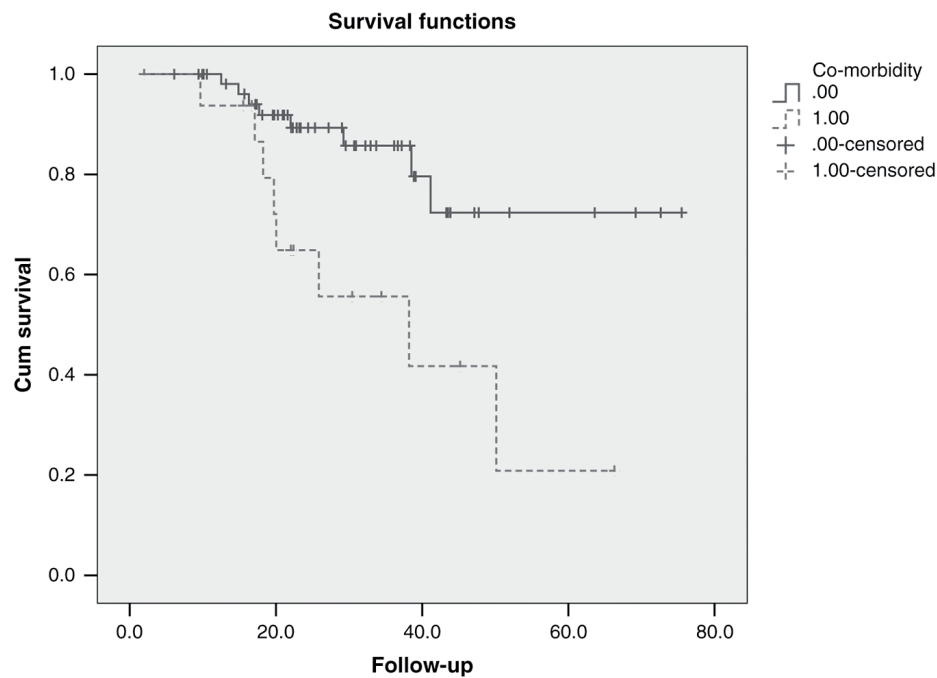


Fig. 2. Co-morbidity score impacted negatively on patient cumulative survival (group 0=co-morbidity score <2, $n=56$, eight events; group 1=co-morbidity score ≥ 2 , $n=17$, eight events, $P=0.006$) follow-up given in months.

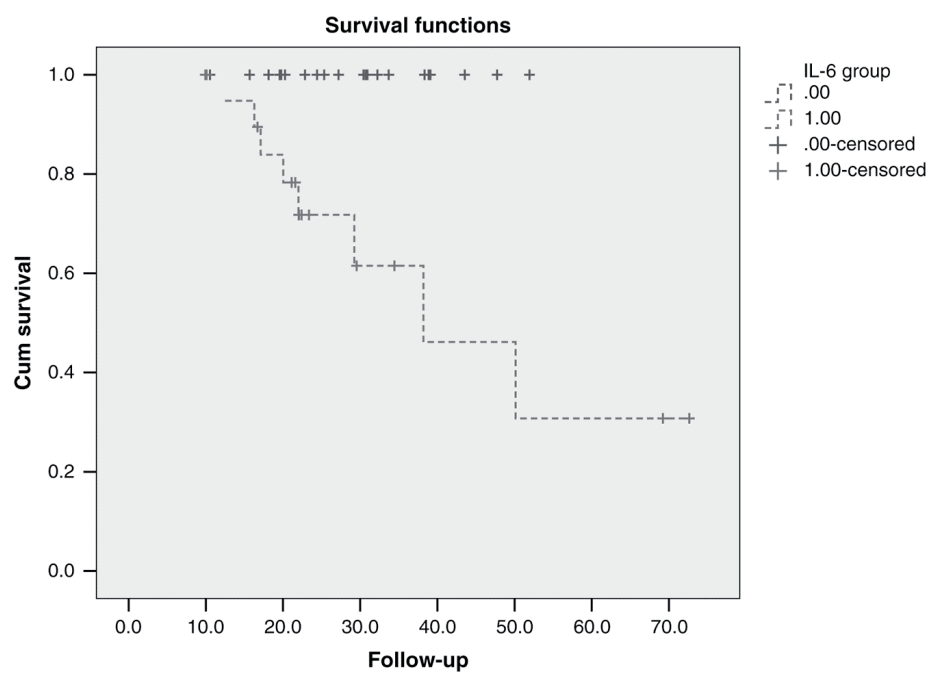


Fig. 3. Serum IL-6 impacted negatively on patient cumulative survival (group 0=IL-6 < median level, $n=21$, no events; group 1=IL-6 $\geq$ median level, $n=21$, eight events, $P=0.0019$) follow-up given in months.

Table 3. Cox proportional hazard regression model

Variable	B	SE	P	Risk	95% CI for Exp(B)	
					Lower	Upper
D ₄ /P ₄ creatinine >0.85	1.225	0.504	0.848	1.134	0.311	4.137
Co-morbidity score >2	1.575	2.518	0.014	3.476	1.289	9.376

Method: 'Enter'.

demonstrated an influence of certain genetic and clinical factors on the baseline peritoneal permeability: fast transporters had higher age, higher proportion of diabetic patients, and higher prevalence of high-grade co-morbidity and cardiovascular disease. Peritoneal fast transport in this population was associated with -174 GC and CC IL-6 polymorphism and higher levels of IL-6; this inflammation biomarker was only available in 56 patients from the studied cohort. This association, however, was not reproduced in other populations [18–20,30] certainly due to different epidemiological characteristics. It was reported recently that a fast peritoneal transport status in incident non-diabetic PD patients was not related to co-morbidity nor higher levels of serum IL-6 [19]. In our study, higher age, diabetes and co-morbidity were not over-represented in fast transporters, nor did they show higher lipid levels, higher IMT or lower albumin and BMI, surrogate markers of cardiovascular disease and malnutrition. In this population, markers of inflammation such as CRP and serum IL-6 were not increased in baseline fast transporters.

On the other hand, multivariate analysis demonstrated that, while baseline fast transport did not impact on patient survival, higher co-morbidity did. In agreement with our study, Passadakis *et al.* [10] also did not find differences comparing the survival curves of high transporters and patients of other transport types. Chung *et al.* [13] were able to document poorer patient survival only in patients who, besides a hyper-permeable peritoneum, had higher co-morbidity scores. The authors contended that the severity of the underlying co-morbidity was likely to influence the prognosis more strongly than PET categorization. Davies *et al.* [14] also failed to document peritoneal transport as an independent predictor of patient survival, but co-morbidity was clearly a determining factor.

A protective factor in our incident population could also have been preserved RRF. A strong association between low RRF and inflammation has been shown [31], both with an impact on outcomes.

Although the authors recognize the limitations of the present study due to the single-centre enrolled population, with a small number of patients with extensive co-morbidity, the results are supported by other studies. A multivariate analysis in a large study [20] revealed that higher peritoneal transport status was not independently predicted by diabetes, other co-morbid diseases or RRF. Another representative study reported a reduction of D/P creatinine at 1 year

after the start of PD [32], which also questions the causal relationship of systemic inflammation and baseline peritoneal transport.

All these studies suggest that other determinants for peritoneal transport may exist in non-inflamed patients: baseline hypermeability was shown to be associated with higher levels of vasoactive mediators produced by the mesothelium [18,19], certainly a more benign determinant of increased effective capillary surface at the beginning of PD.

It can be concluded that a fast peritoneal transport status in incident PD patients without an associated serious co-morbid condition cannot be explained by inflammation and does not impact negatively on patient survival. Other mechanisms of early peritoneal fast transport should be clarified, since patients with high peritoneal transport present as a heterogeneous group that is not always associated with worse outcomes.

Acknowledgements. The authors thank Professor R.T. Krediet for his invaluable scientific support. This study was granted from the Portuguese Nephrology Society, with the Roche Award 2003.

Conflict of interest statement. None declared.

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Received for publication: 6.6.05

Accepted in revised form: 5.10.05

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Characterization of peritoneal fast transport status: differences in free-water transport and the marker of systemic inflammation IL-6 in the early and late stage of peritoneal dialysis (PD)

Abstract

Fast transport status may be associated with transperitoneal water transport changes. Whether these are associated with systemic inflammation is currently not known. To investigate this, we studied 70 patients on PD for 16 ± 17 months. Markers of mesothelial cell mass (effluent CA125) and systemic inflammation (plasma IL-6) were measured after a PET 3.86%. Seventeen fast transporters from early stage group (≤ 24 months on PD, $n=64$) were compared with 9 fast transporters from late stage group (> 24 months on PD, $n=24$).

Late-stage fast transporters had significantly lower values of drained volume (2364 ± 397 mL vs. 2694 ± 196 mL, $P=0.025$), D/D_0 glucose (0.19 ± 0.01 vs. 0.25 ± 0.05 , $P=0.001$), free water peritoneal transport evaluated with corrected dipNa (0.10 ± 0.04 vs. 0.15 ± 0.04 , $P=0.016$) and CA125 appearance rate (101 ± 107 U/min vs. 198 ± 104 U/min, $P=0.019$) than baseline fast transporters; the longer the treatment the lower the dip ($r=-0.61$, $P=0.001$). Plasma IL-6 levels were higher in late-stage vs. baseline fast transporters (47.8 ± 43.5 , median 33.7 pg/ml, vs. 11.2 ± 9.6 , median 9 pg/ml, $P=0.052$).

Ultrafiltration capacity failure (UCF) was detected in 4 baseline and 4 late-stage fast transporters. The later had comparable drained volume (2125 ± 290 mL vs. 2325 ± 50 mL, $P=0.2$) but abolished sodium sieving (0.08 ± 0.02 vs. 0.16 ± 0.03 , $P=0.029$) and further lower CA125 U/min (40 ± 19 U/min vs. 123 ± 74 U/min, $P=0.043$).

Acquired fast transport with UCF was associated to higher serum IL-6 (62 ± 53 pg/ml vs. 13.8 ± 12.7 pg/ml, $P=0.043$). We conclude that increased small solute transport is not

associated to free-water transport abnormalities in early stage PD. Diminished free-water transport is coexistent in long-term fast transport as a cause of UCF. In this later stage, fast transport is associated with increased marker of systemic inflammation, IL-6. Corrected sodium dip correlated negatively with time on dialysis in fast transporters

Keywords: Peritoneal transport; Peritoneal equilibration test; Interleukin-6

Introduction

A fast peritoneal solute transport status may be present as a baseline characteristic in recently started peritoneal dialysis patients, or it may be acquired during long-term PD treatment. In both situations ultrafiltration failure (UFF) may occur due to a rapid dissipation of the osmotic gradient, caused by absorption of glucose [1]. This phenomenon is likely caused by the presence or development of a large effective peritoneal capillary surface area. Such enlargement can be functional (more perfused microvessels) or anatomic (more microvessels). The former is likely to be present in some patients in the beginning of peritoneal dialysis [2] and during acute infectious peritonitis [3]. The presence of an inherent fast transport status is often a temporary phenomenon [4] and has been associated with co-morbidity [5], inflammation [6] and cancer antigen 125 (CA 125), a marker of mesothelial cell mass [7]. In a recent study by one group no single clinical determinant of an inherent fast transport status could be identified [8].

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An anatomic increase in the peritoneal vascular surface area due to neoangiogenesis is a phenomenon that may develop during peritoneal dialysis [9]. The vascular abnormalities are accompanied by fibrous changes [10]. These morphological changes lead to the development of a fast peritoneal transport status with UFF [11, 12]. The development of the above abnormalities has been associated with the peritoneal exposure to high amounts of glucose and/or glucose degradation products [13]. The UFF in the acquired fast transporters is not only due to a rapid disappearance of the osmotic gradient, but a reduction in the amount and contribution of free water transport is also likely [14, 15].

The aim of the present study was to compare peritoneal fluid and solute transport, CA-125 and interleukin-6 (IL-6) in 4 patient groups: (1) fast transport patients with a PD duration of ≤ 2 yrs, (2) non-fast transport patients with a PD duration ≤ 2 yrs, (3) fast transport patients with a PD duration of PD > 2 yrs, and (4) non-fast transport patients with a duration of PD > 2 yrs.

Patients and methods

We analysed 88 PET from 70 patients (aged 50 ± 16 years, on PD for 16 ± 17 months).

All patients were standard treated with glucose and lactate-based dialysis solutions. None of the patients had peritonitis during the test or in the previous 6 weeks.

All investigations were done with a peritoneal equilibration test (PET) using a 3.86% glucose lactate buffered dialysis solutions.

PETs with an ultrafiltered volume < 400 mL/4hrs were considered to represent acquired ultrafiltration failure (UFF), unless when present within the first year of PD. These UFF tests were analyzed separately.

Measurements

Creatinine and glucose were measured with standard automatic analyser techniques. Creatinine Jaffé-compensated method was used (Cobas Integra) Dialysate creatinine was corrected for the high glucose concentrations according to local laboratory determinations. Sodium in dialysate and plasma was measured with indirect ion selective electrodes.

Effluent CA125 and plasma IL-6 were measured as markers of mesothelial cell mass and systemic inflammation, respectively.

We measured the dialysate appearance rate of interleukin 6 (IL-6) and cancer antigen 125 (CA125) in the effluent at 240 min of the PET. To measure IL-6 in effluent and serum, we used a commercially available immuno-enzymometric assay (IL-6 Easia: Biosource Europe SA, Nivelles, Belgium). Effluent CA 125 was measured with an electrochemiluminescence method with an automated analyser (Elecsys 2010: Boehringer Mannheim, Indianapolis, IN, U.S.A.).

Calculations

Peritoneal transport status was determined using mean values and the standard deviation of D/P creatinine. The MTAC creatinine was calculated by the simplified Garred model [5]. The MTAC creatinine was used to estimate the dialysate sodium concentration due to diffusion. This value was then subtracted from the measured sodium concentration in the dialysate. The later was used to calculate the D/P sodium. The dip D/P sodium is the difference between the initial D/P sodium and the D/P sodium at 60 minutes.

Appearance rates of effluent markers were calculated as the amount of these present in the drained effluent divided by the duration of the dwell.

Statistical analysis

Fast transporters (D/P creatinine > 0.81) in the Baseline group (first 24 months of treatment (n=64) were compared with fast transporters in Late group (after 24 months of PD, n=24). In those patients with more than one PET only one test was included for analysis in each time period.

Stable patients were compared with patients with ultrafiltration capacity failure (UCF) defined as: ultrafiltration < 400 mL at PET test.

Variables are expressed as mean $\pm$ standard deviation and median for asymmetrically distributed variable.

Comparisons between groups were performed with T-test, and Mann-Whitney test as

appropriate. Values of $p < 0.05$ were used to define statistical significance

Statistical analysis was performed with SPSS software, version 11.5.

Results

Comparisons between fast and non-fast transporters in early and late PD patients and a comparison between early and late patients with a fast transport status are summarized in table 1. In early PD group, fast transporters did not show increased levels of serum IL6, in comparison with non-fast transporters.

Late fast transporters had significantly lower values of drained volume, D/D_0 glucose, free

water peritoneal transport evaluated with corrected dipNa and CA125 appearance rate than baseline fast transporters. Plasma IL-6 levels were higher in late vs. early fast transporters.

Aquaporin dysfunction expressed by corrected sodium dip was correlated with time on dialysis in fast transporters: the longer the treatment the lower the dip (Figure 1). Such correlation was not found in non-fast transporters. Comparing baseline fast transporters with UCF and late fast transporters with acquired UCF, comparable drained volume (2125 ± 290 mL vs. 2325 ± 50 mL, $P=0.2$) but abolished sodium sieving (0.08 ± 0.02 vs. 0.16 ± 0.03 , $P=0.029$) and further lower CA125 U/min (40 ± 19 U/min vs. 123 ± 74 U/min, $P=0.043$) was seen (Table 2).

	PD ≤ 2 years		PD > 2 years	
	fast transport n=17	non-fast transport n=47	fast transport n=9	non-fast transport n=15
Drained volume (mL)	2694 $\pm$ 196	2836 $\pm$ 202 ^a	2364 $\pm$ 397*	2805 $\pm$ 197 ^b
MTAC creatinine (mL/min)	16.9 $\pm$ 4.3	7.6 $\pm$ 2.6 ^c	23.4 $\pm$ 10.9	9.1 $\pm$ 2.8 ^c
D/P creatinine	0.87 $\pm$ 0.04	0.69 $\pm$ 0.08 ^c	0.87 $\pm$ 0.04	0.69 $\pm$ 0.08 ^c
D/Do glucose	0.25 $\pm$ 0.05	0.31 $\pm$ 0.07 ^b	0.19 $\pm$ 0.01**	0.31 $\pm$ 0.05 ^c
Corrected dip Na	0.15 $\pm$ 0.04	0.22 $\pm$ 0.06 ^c	0.10 $\pm$ 0.04**	0.21 $\pm$ 0.05 ^c
Corrected D/P Na60	0.78 $\pm$ 0.05	0.71 $\pm$ 0.06 ^c	0.72 $\pm$ 0.06**	0.83 $\pm$ 0.05 ^c
CA 125 appearance rate (U/min)	198 $\pm$ 104	132 $\pm$ 87 ^a	118 $\pm$ 117*	142 $\pm$ 88
Serum IL-6(pg/mL)	11.2 $\pm$ 9.6	21.1 $\pm$ 42.6	47.8 $\pm$ 43*	12.9 $\pm$ 11.1
			median 33.7	median 13.1

^a $p \leq 0.05$ ^b $p \leq 0.01$ ^c $p \leq 0.001$ compared to fast transportes, both early and late

* $p \leq 0.05$ • $p \leq 0.01$ compared to early fast transporters

Table 1. Comparisons between fast and non-fast transport status in early and late PD patients, and a comparison between early and late patients with a fast transport status

	Baseline UCF	Late UCF
	n=4	n=4
Drained volume mL	2325 $\pm$ 50	2125 $\pm$ 290 ^c
MTAC creatinina	12.6 $\pm$ 5.2	27.6 $\pm$ 13.2
D/P creatinina	0.81 $\pm$ 0.08	0.93 $\pm$ 0.06
D/Do glucose	0.24 $\pm$ 0.03	0.19 $\pm$ 0.01
Corrected Dip Na	0.16 $\pm$ 0.03	0.08 $\pm$ 0.02 ^a
Corrected D/PNa60	0.75 $\pm$ 0.05	0.85 $\pm$ 0.03 ^b
Ca125 U/min	123 $\pm$ 74	40 $\pm$ 19 ^b
Serum IL-6pg/ml	13.8 $\pm$ 12.7	62 $\pm$ 53 ^b
	median 10	median 52

Table 2. Comparison of PET parameters and serum IL-6 between baseline and late UCF cases (^a $p=0.02$, ^b $p=0.04$, ^c $p=0.13$).

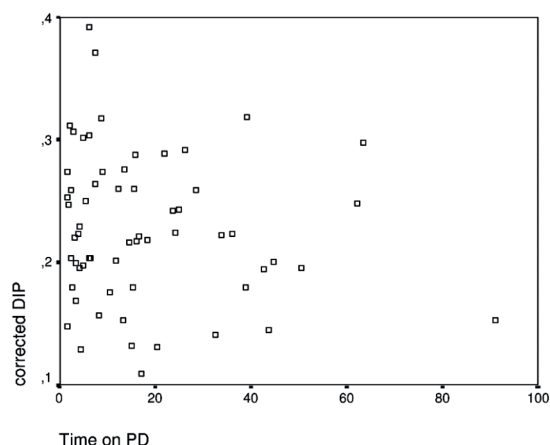
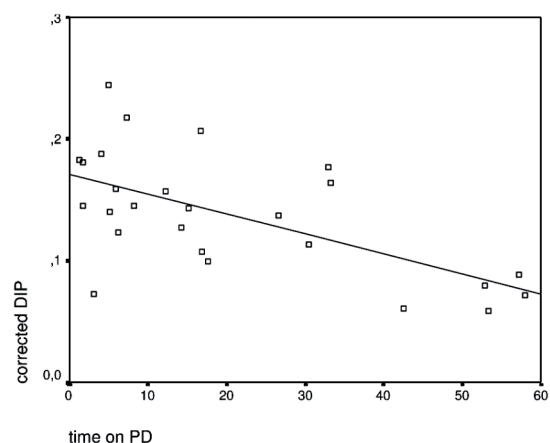


Fig.1 Relationship between transcellular water transport evaluated by the corrected Na Dip and time (months) on peritoneal dialysis (PD), in non-fast transporters ($r = -0.11$, $P = \text{NS}$), upper panel; and in fast transporters group ($r = -0.61$, $P = 0.001$), lower panel.



Discussion

Baseline and long-term peritoneal fast transport status are heterogeneous conditions with debatable determinants [7, 16, 17, 18], but compromised peritoneal fluid removal is present in both stages.

This study confirms that incident fast transporters do not show higher levels of systemic IL-6, a reliable marker of systemic inflammation. They also do not show free water transport abnormalities in the first years on PD.

Levels of corrected Dip were lower in baseline fast transporters than in the other categories of transport but still within normal values. At this early stage we can only speculate if uremia per se, involving oxidative processes and protein glycation, can induce some

transcellular abnormalities even before peritoneal dialysis induction. This however was not supported by the recent study in a population of incident diabetics who showed no differences in fluid or solute transport in comparison to non-diabetic patients [19].

Lower levels of corrected dip were found, in this study, with time on PD: noteworthy, for similar drained volume, significant differences in sodium sieving were found in baseline and acquired UCF patients. In long-term PD patients free-water transport was compromised and almost abolished in the subgroup with ultrafiltration capacity failure.

This profile goes along with an increase of systemic markers of inflammation. Late fast transporters had indeed higher levels of systemic IL-6. It is not known if there is a causal relationship between inflammation and water channel dysfunction. Others have reported a relationship between inflammation and volume overload [20].

Oxidative metabolism that accompanies intraperitoneal inflammation has also been pointed as cause of aquaporin dysfunction. It is known that NO-mediated increase in effective peritoneal surface area is followed by dissipation of the osmotic gradient—a mechanism accounting for loss of UF in acute acquired fast transport. But NO might also initiate angiogenesis or modification of aquaporin 1 function through structural modifications [21, 22]. Investigation is needed to clarify the mechanisms of aquaporin dysfunction to search for treatment options. Specific pharmacological regulation of AQP1 with corticosteroids has been documented in a rat model suggested by an increase of sodium sieving and net ultrafiltration contrasting with a lack of effect on the osmotic gradient and small solute transport [23].

This study shed some light to the time course of fluid transport processes during PD treatment. In accordance with previous studies it underlines that baseline fast peritoneal transport was not determined by systemic inflammation. On the other hand, abnormalities of free water transport are absent in baseline fast transporters but develop later with long-term PD. At this stage it is associated to higher levels of inflammation markers.

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Evaluation of Peritoneal Transport and Membrane Status in Peritoneal Dialysis: Focus on Incident Fast Transporters

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Key Words

Peritoneal transport · Mesothelial cells · Vascular endothelial growth factor · CA-125 · Interleukin-6

Abstract

Background/Aim: The determinants of baseline fast solute transport are still unclear. We prospectively investigated the relationship of peritoneal solute transport with markers of inflammation, angiogenesis, and membrane status, with a focus on fast transporters. **Methods:** Seventy-one incident peritoneal dialysis patients were assessed with baseline and annual peritoneal equilibration tests, using a 3.86% glucose dialysis solution. Residual renal function and markers of inflammation, including systemic and intraperitoneal interleukin-6 (IL-6), effluent cancer antigen 125 (CA-125), and vascular endothelial growth factor (VEGF) appearance rates (ARs), were investigated. The time course of the dialysate-to-plasma ratio of creatinine (D/P creatinine ratio) and its relationship with the biomarkers were investigated by a mixed linear model. **Results:** Incident fast/fast average transporters had a similar age, diabetes prevalence, and serum and effluent IL-6 levels, but significantly higher levels of CA-125 and VEGF ARs than the slow/slow average group; the D/P creatinine

ratio was not correlated with systemic IL-6, but was correlated with effluent CA-125 AR ($r = 0.45$, $p < 0.0001$) and VEGF AR ($r = 0.52$, $p < 0.0001$). The D/P creatinine ratio decreased with a U-shaped profile ($p = 0.02$). Intraperitoneal IL-6 was the significant and positive determinant of the time course of the D/P creatinine ratio ($p < 0.0001$). Effluent CA-125 decreased with time on peritoneal dialysis ($p = 0.013$). **Conclusions:** Baseline peritoneal fast transport was not associated with systemic inflammation, but was related to peritoneal locally produced substances able to mediate transitory hyperpermeability. The D/P creatinine ratio changed during the follow-up period with a U-shaped profile. This was associated with effluent IL-6 and partly with VEGF. CA-125 decreased throughout the follow-up period.

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This work was presented in part at the 24th Annual Conference on Peritoneal Dialysis [abstract in Perit Dial Int 2004;24(suppl 1):11] and at the 1st Joint ISPD/EUROPD Congress [abstract in Perit Dial Int 2004;24(suppl 2):8].

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Introduction

The determinants of peritoneal fast solute transport status in peritoneal dialysis (PD) patients at the beginning of PD are not fully elucidated [1–5].

From a physiological point of view, a fast transport status is mainly dependent on the effective peritoneal capillary surface area [6]. This may be enlarged due to vasoactive agents inducing vasodilation and recruitment of previously nonperfused vessels or, instead, due to an increase in the number of capillaries (neoangiogenesis). The association of peritoneal fast transport with hypoalbuminemia, even before the start of PD, supports the link between inflammation or comorbidity and peritoneal transport [7]. High levels of plasma interleukin-6 (IL-6), a marker of systemic inflammation and a strong predictor of patient survival, have been reported in fast transporters [5]. This would help to explain the poor outcomes reported in these patients [8, 9]. However, these studies usually focused on prevalent PD fast transporters with scarce knowledge on the characteristics and determinants of this status in early-stage PD. Some investigators [10–12] were also unable to document the association between peritoneal transport and systemic inflammation consistently, while other studies [13–18] did not indicate baseline peritoneal transport status as an independent predictor of mortality. Therefore, a causal link between systemic inflammation and baseline peritoneal transport remains controversial.

Fast transporters are a heterogeneous group with different causes of membrane hyperpermeability. We hypothesize that the short-term peritoneal transport profile may have determinants different from those that induce long-term changes. An initial fast transport status may be related mainly to intraperitoneal processes, such as the reactivity of a larger mesothelial cell mass, able to produce increased levels of intraperitoneal vasoactive substances, among which the vascular endothelial growth factor (VEGF) [19–22], without detrimental impact on patient survival [11].

Long-term PD, however, may induce intraperitoneal chronic inflammation, causing structural and functional alterations of the peritoneal membrane. The Peritoneal Biopsy Study Group [23] revealed important morphological results, but only cross-sectional data of the PD patient membrane structure were obtained, without a dynamic relationship to the membrane function. Since repeated peritoneal biopsies in patients are not feasible, the search for markers of peritoneal pathological processes, more accessible and informative of earlier abnor-

malities, is clinically justified. Therefore, the relationship between peritoneal solute transport, as measured by dialysate-to-plasma ratio of creatinine (D/P creatinine ratio), and systemic and effluent markers of inflammation, angiogenesis, and membrane status was prospectively studied, with a focus on the incident fast solute transporters.

Patients and Methods

Peritoneal equilibration tests (PET), using a 3.86% glucose dialysis solution, are performed on an annual basis in our PD population. A cohort of 71 incident patients, in whom at least one PET was done, was included. The patients were 48 ± 16 years old and were followed for a median time of 18 (range 6.8–53) months after start on PD. Twenty patients (28%) were diabetic. All patients were treated with continuous ambulatory PD, using conventional commercially available glucose-based solutions. All patients underwent a PET 4 ± 2 months after the initiation of PD. One hundred and forty-seven PET evaluations were obtained: one in 71 patients, two in 42 patients, three in 19 patients, four in 12 patients, and five in 3 patients. The 3.86% glucose PET was performed according to the recommendations of the International Society for Peritoneal Dialysis. None of the patients had peritonitis or acute exit site or tunnel infections at the time of the PET or during the previous 6 weeks. Informed consent was obtained from all patients who participated in the study.

Baseline epidemiological data and serum albumin and C-reactive protein levels were registered. The residual renal function was calculated from 24-hour urine collection data, using Adequest® software (Baxter, Deerfield, Ill., USA). As a marker of inflammation, serum and effluent IL-6 levels were determined. Effluent cancer antigen 125 (CA-125) and VEGF appearance rates (ARs) at the end of the PET were also measured to investigate whether these parameters are related to the peritoneal transport status. The patients were grouped for analysis according to the cutoff level of the mean baseline D/P creatinine ratio (0.77): fast/fast average (F/FA) and slow/slow average (S/SA) transport groups.

Creatinine was measured with a Cobas Integra automatic analyzer (Roche Diagnostics, Basel, Switzerland), using the creatinine Jaffé-compensated method, with correction for high dialysate glucose concentrations according to the local laboratory. VEGF was measured in effluent and serum, with a commercially available enzyme-linked immunosorbent assay (Quantikine; R & D Systems, Minneapolis, Minn., USA). To measure IL-6 in effluent and serum, we used a commercially available immunoenzymometric assay (IL-6 Easia; BioSource Europe, Nivelles, Belgium). Effluent CA-125 was measured with an electrochemiluminescence method using an automated analyzer (Elecys 2010; Boehringer Mannheim, Indianapolis, Ind., USA). The AR values were calculated as the concentrations in effluent divided by the dwell time.

Statistics

The Kolmogorov-Smirnov test was applied to test for a normal distribution. Variables without a normal distribution were logarithmically transformed before analysis with parametric tests.

Table 1. Comparison between baseline transport groups among 71 incident patients: S/SA versus F/FA

	S/SA patients (n = 30)	F/FA patients (n = 41)	p (t test)
Follow-up period, months	5.2 ± 3.4	4.7 ± 2.5	0.53
Age, years	48.4 ± 13.4	49.1 ± 16.6	0.85
Residual renal function, ml/min	3.9 ± 3.7	5.2 ± 3.6	0.14
Urine volume, ml	895 ± 735	1,213 ± 831	0.083
Serum albumin, g/dl	3.8 ± 0.46	3.8 ± 0.49	0.68
C-reactive protein, mg/dl	0.61 (0.18–1.48)	0.51 (0.20–1.13)	0.84
D/P creatinine ratio	0.66 ± 0.076	0.85 ± 0.068	<0.0001
Drained volume, ml	2,846 ± 245	2,717 ± 199.8	0.017
Effluent			
CA-125, U/min	98.38 (67.5–135.9)	151.6 (97.5–232.9)	0.011
VEGF, pg/min	203 (140.6–271.3)	277.0 (204.1–389.95)	0.016
IL-6, pg/min	647.7 (298.5–1,347.5)	636.2 (533.8–1,191.3)	0.13
Serum			
VEGF, pg/ml	443.7 (187.9–652.1)	481.7 (298.2–635.9)	0.27
IL-6, pg/ml	10.1 (2.9–14.1)	9.4 (5–27.7)	0.19
Variables with not a normal distribution given as median (interquartile range).			

Variables with a normal distribution are expressed as mean values $\pm$ SD, and asymmetrically distributed data are given as median and interquartile range. The Pearson correlation test was used to determine correlations between variables. Comparisons between baseline transport groups were performed with the unpaired t test. Changes over time were determined with the paired t test and by applying a mixed linear model approach. $p < 0.05$ was considered statistically significant, and all statistical analyses were performed using SPSS version 12.0.1. for Windows (SPSS, Chicago, Ill., USA).

Results

The baseline analysis showed that the F/FA category had similar age, diabetes prevalence, and C-reactive protein and serum albumin concentrations. Residual renal function and urine volume were not significantly different, but tended to be greater in the F/FA patients, as shown in table 1. Serum and effluent IL-6 levels were not significantly different between the groups, but the F/FA patients had higher levels of intraperitoneal CA-125 and VEGF than the S/SA group (table 1). No relationships were found between D/P creatinine ratio and serum IL-6 concentration. The peritoneal solute transport correlated with both effluent CA-125 ARs ($r = 0.45$, $p < 0.0001$) and VEGF ARs ($r = 0.52$, $p < 0.0001$; fig. 1). A correlation between systemic and intraperitoneal concentrations of the markers was not found.

The D/P creatinine ratio decreased from 0.86 ± 0.7 at baseline to 0.77 ± 0.12 in the 2nd year in the F/FA group ($p = 0.007$), and the drained volume increased from $2,689 \pm 220$ ml/4 h at baseline to $2,789 \pm 238$ ml/4 h ($p = 0.003$). Such changes were not found in the S/SA patients.

The time course of the D/P creatinine ratio was investigated using a mixed linear model, where the D/P creatinine ratio was the dependent variable. The following cofactors were entered: age, residual renal function, urine volume, serum and effluent IL-6 and VEGF, and effluent CA-125. The estimated mean values over time of D/P creatinine ratio and of the effluent ARs of the biomarkers during the follow-up period of the patients are given in figure 2. The D/P creatinine ratio changed significantly over time and showed a U-shaped profile. A similar although statistically not significant profile was found for effluent VEGF. Effluent CA-125 decreased significantly with the duration of PD ($p = 0.03$).

Univariate analysis showed that the time course of the D/P creatinine ratio was positively associated with both effluent VEGF and effluent IL-6 (table 2). The association with IL-6 was marginally stronger than with VEGF. The estimate for effluent IL-6 was 0.0016 ($p < 0.0001$); this means that in a patient with an IL-6 effluent level which is 1 pg/min higher than in another patient, its D/P creatinine ratio would be 0.0016 times higher. The addition of effluent VEGF to the D/P creatinine-IL-6 model in multivariate analysis (table 2) did not improve the

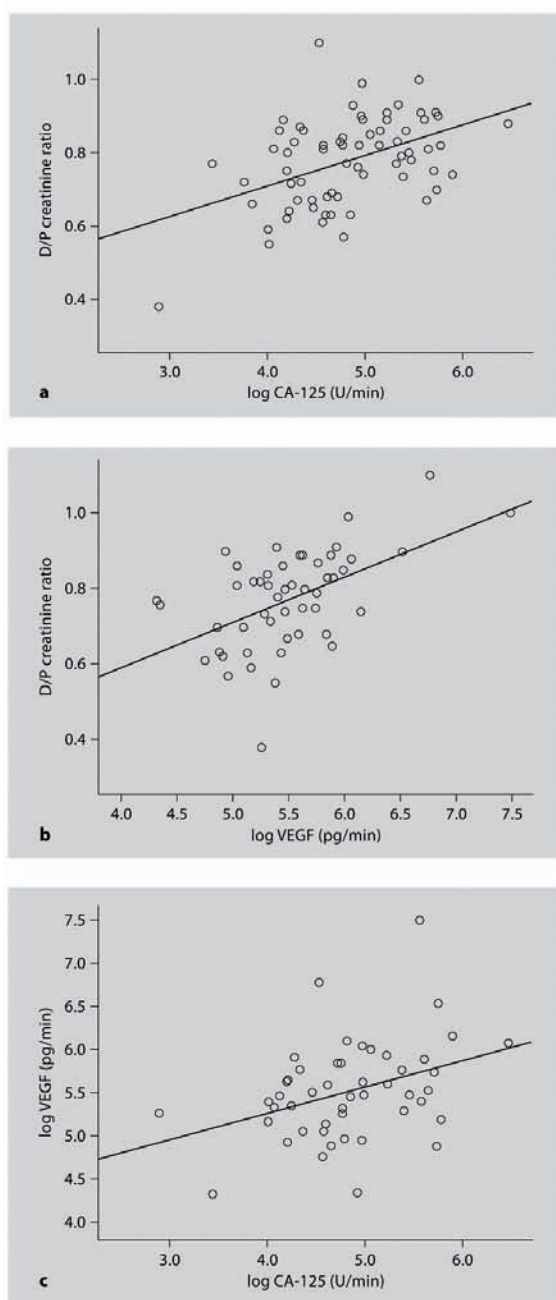


Fig. 1. Baseline analysis of the first PET in incident patients ($n = 71$). Relationships between ARs of effluent CA-125 (**a**) ($r = 0.45$, $p < 0.0001$) and VEGF (**b**) ($r = 0.52$, $p < 0.0001$) and between these two markers (**c**) ($r = 0.32$, $p = 0.02$).

Table 2. Relationship of effluent VEGF and IL-6 AR values with the time course of the D/P creatinine ratio

Parameter	Mean estimate $\times 10^{-4}$	95% CI	p
<i>Univariate</i>			
VEGF model			
Effluent VEGF, pg/min	22	12–32.4	<0.0001
IL-6 model			
Effluent IL-6, pg/min	0.16	0.09–0.23	<0.0001
<i>Multivariate</i>			
VEGF-IL6 model			
Effluent VEGF, pg/min	0.82	0.82–2.46	0.3
Effluent IL-6, pg/min	0.12	0.04–0.23	0.04

Dependent variable: D/P creatinine ratio.

model, but it reduced the effect and the significance of effluent IL-6.

As expected, the residual renal function decreased significantly with time on PD ($p = 0.007$), but no association was statistically significant between residual renal function or urine volume and D/P creatinine ratio.

The time course of effluent CA-125 was positively related to effluent VEGF (estimate 0.509, $p = 0.002$) and negatively related to effluent IL-6 (estimate -4.40 , $p = 0.06$). Analysis of the time courses of effluent VEGF and IL-6 showed a significant relationship (estimate 13.43, $p < 0.0001$), suggesting a link between inflammation and angiogenic processes.

Discussion

Patients starting PD have a large interindividual variability in peritoneal transport rates. We have shown that epidemiological variables account for very little of such profiles, a conclusion also made by other groups [24–26]. Patients also vary in alterations that may occur during follow-up. Our study supports the contention that a baseline fast transport rate does not share the same determinants as a later acquired fast transport status and might represent in some populations a transitory benign short-term condition, not related to systemic inflammation. We hypothesized that a fast transport rate is influenced by locally produced substances that can be measured in effluent and may reflect some aspects of the peritoneal membrane status.

Effluent CA-125 is likely to be dependent on mesothelial cell mass or turnover [19]. CA-125 is usually measured in solution biocompatibility studies, and it was also recommended as a tool for longitudinal membrane status evaluation. Although Breborowicz et al. [27] reported limitations of CA-125 as an index of peritoneal mesothelial cell mass, they demonstrated a reduction of CA-125 secretion and expression with chronic exposure to glucose solutions. VEGF is spontaneously produced by cultured human mesothelial cells [21], but also by a variety of other cells, like vascular smooth muscle cells [28]. The proinflammatory cytokine IL-6 is also secreted constitutively by cultured human mesothelial cells, but especially after stimulation with other cytokines [29]. Similar to VEGF, IL-6 can also be produced by other cells, like macrophages, neutrophils, and endothelial cells [30]. Its effluent concentration, however, has been considered a marker of local peritoneal inflammation [5, 31].

The present study shows that the baseline peritoneal solute transport status, as judged by the D/P creatinine ratio, was related to effluent CA-125 and VEGF, while for IL-6 there was no statistical significance. Also no differences were found for serum concentrations of VEGF or IL-6. These results are in accordance with those of a study previously performed by our group in Portugal in a smaller number of patients [22] and also with those of a study from The Netherlands performed in nondiabetic PD patients without any peritonitis episode, examined during the first 6 months of PD [12]. The findings are partly dissimilar to those from a study done in Sweden [5] and from one performed in Brazil [31]. The study from Sweden [5] reported a highly significant increase in the D/P creatinine ratio already during the 1st year of PD, associated with increases in plasma and effluent IL-6. The extremely high D/P creatinine ratio after 1 year is not in accordance with results reported in other recent prospective studies [32] and suggests that some of the patients may have suffered from severe inflammation. This is supported by findings that plasma IL-6 increased during follow-up, but the D/P IL-6 ratio did not.

In the study from Brazil [31], the results for effluent VEGF were similar to ours, but differences were found

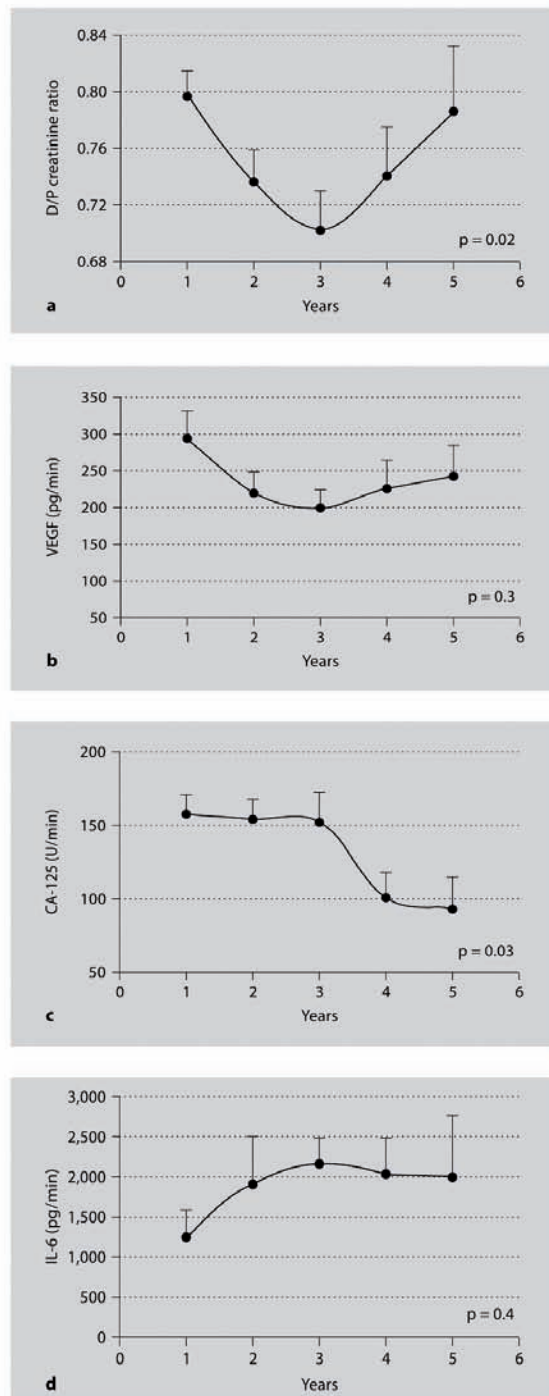


Fig. 2. Mixed linear model estimated marginal mean values of variables by time in incident patients: D/P creatinine ratio (a), VEGF ARs (b), CA-125 ARs (c), and IL-6 ARs (d). Analyses were done with 71 patients, annual PET evaluation, and 147 PETs, including 71 PET 1, 42 PET 2, 19 PET 3, 12 PET 4, and 3 PET 5.

for dialysate and plasma IL-6. These divergent results might be explained by the cross-sectional design of the study reported by Pecoits-Filho et al. [31]. The duration of PD ranged from 3 to 56 months, and the patients were older and had a high prevalence of diabetes mellitus. Involvement of IL-6 in baseline peritoneal transport characteristics has also been suggested in a study on the IL-6 -174G/C polymorphism [33]. In this study the GC and CC genotypes were more often associated with fast solute transport rates than the GG genotype. The latter was associated with lower plasma and dialysate IL-6 values than other genotypes. However, possible relationships of effluent IL-6 concentration with peritoneal transport in individual patients were not reported. Of note, the study on IL-6 polymorphisms was done in a population of fast transport patients that was older and had more cardiovascular disease and lower plasma albumin concentrations than the population of the present study. As we also previously reported [11], the baseline fast transport status, as measured by D/P creatinine ratio, was not an independent risk factor for patient survival in our population, but comorbidity and increased serum levels of IL-6 were.

The relationships between D/P creatinine ratio, CA-125, and VEGF at baseline in our study are in accordance with previous findings [12, 22]. They suggest that the peritoneal solute transport during the 1st year of PD is influenced by mesothelial cell mass and that VEGF produced by mesothelial cells may be the link between the two. The results of our study and those of the ones discussed above suggest that the determinants of baseline fast transport rate are to a large extent dependent on the population examined: associations with a reactive mesothelial cell mass, as expressed by effluent CA-125 and VEGF, can be expected in a population with a low risk of death; associations with systemic IL-6 are likely to be found in a population of elderly patients with higher comorbidity.

The D/P creatinine ratio showed some decrease during the longitudinal follow-up period, followed by an increase. This short-term decrease of the D/P creatinine ratio is apparently at odds with the reported increase of the D/P creatinine ratio with time on PD. But earlier studies included patients beginning dialysis treatment in the 1980s, and the peritonitis rate was high at this time, possibly conditioning the results, but this could not be reproduced in a recent investigation [32]. However, we also documented an increase of the D/P creatinine ratio with longer treatment time. On the other hand, early membrane changes are scarcely explored, but this U-

shaped curve of the D/P creatinine ratio was also present in other more recent longitudinal studies on peritoneal transport [25, 34]. We could speculate that this transitory short-term fast transport status mimics the reported not detrimental increase of D/P creatinine ratio and effluent CA-125 with short-term use of more biocompatible solutions. While much is already known about long-term acquired membrane changes, there is less knowledge about short-term adaptive membrane changes. This profile also indicates that many patients presenting a fast transport rate at the start of PD can expect to benefit from the modality without a poor prognosis.

In contrast to peritoneal transport, effluent CA-125 showed a significant decrease with increasing duration of PD. It confirms previous results in another PD patient population [35]. The lack of significant changes during the time course of effluent VEGF and IL-6 may be due to the variety of cells that can synthesize these factors, although a type 2 error cannot be excluded due to the smaller number of late-term data in our study.

The linear mixed model showed a significant relationship between the time course of D/P creatinine ratio and IL-6, partly influenced by VEGF. This finding suggests that local peritoneal inflammation and neoangiogenesis are both involved in the enlargement of the vascular peritoneal surface area that may develop during long-term PD. It is likely that the contribution of mesothelial cells to the effluent concentrations of IL-6 and VEGF decreases with the duration of PD, but that the effects of other cells become more important. For instance, it has been shown recently [36] that mesothelial cells transformed to myofibroblasts produced more VEGF than epithelial-like mesothelial cells. However, our clinical study cannot distinguish between the sources of effluent IL-6 and VEGF. Yet, the borderline negative association between the time courses of CA-125 and IL-6 supports the contention that the main source of production of mediators may change during PD.

Although none of our results definitively prove causality, the significant associations are clinically relevant, because they clarify the time-dependent determinants of peritoneal transport as measured by the D/P creatinine ratio. Stronger statistical significance is hardly achievable in clinical studies investigating biological variables. We were able to highlight short-term membrane changes, not usually explored, but the main limitation of our study is the small number of late-term evaluations.

It can be concluded that effluent determinants of baseline peritoneal transport status are influenced by the mix of the population studied and that a baseline fast trans-

port rate is not necessarily detrimental. Effluent CA-125, representing the mesothelial cell mass, is related to the baseline peritoneal solute transport possibly mediated by VEGF in a low-risk population without peritonitis. During longitudinal follow-up, the solute transport shows a U-shaped profile, and the CA-125 level decreases. Effluent IL-6, probably produced by nonmesothelial cells, becomes more important with longer treatment duration. This suggests low-grade peritoneal inflammation, either induced by exposure to PD solutions, recurrent peritonitis, or systemic inflammation. Whether these changes are influenced by the use of the so-called biocompatible PD solutions is currently not known.

Acknowledgements

This work was supported by grants-in-aid for scientific research from the Ministry of Health of Portugal and received the Roche Award 2005 from the Portuguese Nephrology Society. The authors thank Maria Emília Moreira Fernandes Pinto Silva, Silvia Maria Reis Santos, and Maria Julia Reis for their valuable laboratory and biotechnical assistance. We also acknowledge the support from our dedicated PD nurse team.

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Peritoneal Rest May Successfully Recover Ultrafiltration in Patients Who Develop Peritoneal Hyperpermeability with Time on Continuous Ambulatory Peritoneal Dialysis

Anabela Rodrigues, Antonio Cabrita, Pedro Maia, Serafim Guimarães

Temporary transfer to hemodialysis, as a peritoneal rest, may be a rescue therapy to recover ultrafiltration (UF) in patients who develop peritoneal hyperpermeability as a complication of continuous ambulatory peritoneal dialysis (CAPD). However, peritoneal sclerosis has been reported after peritoneal pause.

Since the beginning of our CAPD program in 1985, 12°elective peritoneal pauses have been performed in 11°patients who developed type°I ultrafiltration failure (D/P₂₄₀ creatinine: 0.88 ± 0.09) after 42 ± 14 °months on CAPD. Eight patients recovered UF and remained on CAPD with standard solutions for 10 ± 9 °months more (minimum: 5°months; maximum: 29°months). Only 3 of those patients were later switched to hemodialysis because of recurring UF failure. One patient remains on CAPD (62°months of follow-up). Four patients failed to respond and were permanently transferred to hemodialysis, without signs of developing encapsulating peritoneal sclerosis. The failed pauses were performed later after the detection of UF failure than were the successful ones (483 ± 574 °days vs. 54 ± 52 °days).

In our study, 8 of 12 peritoneal pauses (66.6%) successfully treated type°I UF failure and prolonged CAPD retention. If a pause is initiated soon after diagnosis of UF failure, results may improve further. We urge prospective studies to better determine the best and timely therapeutic approach in patients with loss of ultrafiltration.

Key words

Peritoneal rest, ultrafiltration failure, peritoneal transport

Introduction

Cumulative long-term exposure of the peritoneal membrane to glucose solutions causes neoangiogenesis, interstitial fibrosis, and mesothelial loss (1,2). Functionally, those changes usually result in hyperpermeability with ultrafiltration loss. In rare cases, the patient also may develop a more serious and irreversible condition with peritoneal sclerosis.

When ultrafiltration failure occurs, patients usually are changed to automated peritoneal dialysis or permanently transferred to hemodialysis. Such a change has a negative effect in technique survival and financial expense, not to mention considerable psychological and social costs.

Strategies to prevent ultrafiltration failure are therefore mandatory (3). Structural abnormalities and transport hyperpermeability may sometimes be reversible if a timely intervention is proposed. That intervention may be either to stop hypertonic glucose solutions and to use alternatives such as icodextrin, amino acids, and neutral pH solutions, or to transfer temporarily to hemodialysis to assure a peritoneal rest.

This important clinical problem has been investigated with rat models that proved that peritoneal resting improves ultrafiltration by decreasing peritoneal thickening and hyperpermeability to glucose (4) and by restoring the surface layer and normal peritoneal transport (5).

De Alvaro *et al* (6) published a relevant clinical experience showing that peritoneal resting is beneficial in hyperpermeability and ultrafiltration failure. That study has been followed up only by anecdotal reports (7—12). Some investigators are particularly concerned that peritoneal rest may accelerate the progression to peritoneal sclerosis (13). To clarify those concerns, we analyzed our center's experience with

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peritoneal rest in patients who developed persistent ultrafiltration failure.

Patients and methods

Since the beginning of our continuous ambulatory peritoneal dialysis (CAPD) program in 1985, 12° peritoneal pauses have been performed in 11° patients (5° women, 6° men; 1° diabetic patient; 3° patients on CAPD as first treatment modality; previous time on hemodialysis: 47 ± 67 months).

After 42 ± 14 months on CAPD, 11° patients developed type°I ultrafiltration failure (D/P_{240} creatinine: 0.88 ± 0.09). In each case, a peritoneal pause (minimum: 30° days) was electively performed. Ultrafiltration failure was defined as a long-lasting decrease in ultrafiltration with an increased need for hypertonic solution, confirmed by a PET test. Temporary ultrafiltration failure associated with recent peritonitis or catheter-related problems was excluded.

Results

Ultrafiltration was recovered in 8° patients: it increased from 897 ± 294 mL in 24° hours, achieved with $2.54\% \pm 0.67\%$ glucose solution before peritoneal resting, to 1370 ± 452 mL in 24° hours, achieved with $1.79\% \pm 0.30\%$ glucose solution after therapy. In PET analysis, D/P_{240} creatinine changed from 0.88 ± 0.09 to 0.81 ± 0.13 .

The patients remained on CAPD with standard solutions for 10 ± 9 months more (minimum: 5° months; maximum: 29° months). Only 3 of the patients were later switched to hemodialysis because of recurring ultrafiltration failure after the pause (at 12° months, 22° months, and 29° months respectively). The other causes of drop-out included a fatal cardiovascular event and three peritonitis episodes. One patient remains on CAPD (62° months follow-up).

Four patients failed to respond to the pause and were permanently transferred to hemodialysis, without signs of developing peritoneal encapsulating sclerosis. The failed pauses were performed later after the detection of ultrafiltration failure than were the successful ones (483 ± 574 days vs. 54 ± 52 days). The reasons for the delay in implementing the strategy were either that the patients had no vascular access or refused earlier transfer to hemodialysis. Three of the patients had severe ultrafiltration failure, and even APD was supporting them inadequately when the pause was tried. One of them was efficiently switched

to CAPD with icodextrin and maintains the same schedule 12° months after peritoneal resting.

Therefore, 8 of 12 peritoneal pauses (66.6%) successfully treated type°I ultrafiltration failure and prolonged CAPD retention. The data suggest that if the pause is initiated soon after diagnosis, results may improve further.

Discussion

The present study strongly emphasizes that peritoneal rest is a useful rescue strategy to recover ultrafiltration in patients with type°I ultrafiltration failure. More than one half of the patients were safely maintained on peritoneal dialysis after a peritoneal rest, returning to their baseline prescriptions.

It must be noted that the patients were not changed to the alternative solutions only recently more accessible in clinical practice. Strong investigational evidence exists that solutions that are more biocompatible may further reduce development of peritoneal lesions (14–16). Therefore, we believe that timely intervention with a peritoneal rest, followed by maintenance in CAPD with alternative solutions, may be able to achieve longer retention on peritoneal dialysis.

Because we all aim for safe strategies (keeping in mind the menace of peritoneal sclerosis), the peritoneal rest should be performed as soon as the changing profile of peritoneal permeability is documented. Longitudinal measurement in effluent of the appearance rate of cancer antigen 125, a marker of mesothelial mass, may be added information to help monitor membrane status (17,18). None of the patients in our study developed peritoneal sclerosis, although some of them had a delayed peritoneal rest.

We urge prospective studies to better determine predictive markers of ultrafiltration failure so that a timely therapeutic approach is viable. Peritoneal rest may be a successful therapy for ultrafiltration loss.

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SHORT REPORTS

Sclerosing Encapsulating Peritonitis: A Case Successfully Treated with Immunosuppression

Sclerosing encapsulating peritonitis (SEP) associated with peritoneal dialysis (PD) has been reported frequently since its initial description by Gandhi in 1980 (1). The etiology remains elusive: peritonitis might be the most important risk factor, but acetate-based dialysis solutions, chlorhexidine as a disinfectant, and β -blocking drugs have also been implicated as causative agents.

Sclerosing encapsulating peritonitis, a particular presentation of sclerosing peritonitis (SP) should be distinguished from simple peritoneal sclerosis (SS). The latter is almost always present, in variable degrees, in PD patients for more than a few months and leads to a deterioration of membrane permeability. Bioincompatibility of solutions and time on PD are its presumed etiological factors. Usually, SEP presents as small bowel obstruction, malnutrition, sometimes with blood-stained dialysate, and is characterized by cocooning of the small bowel by a very thick fibrous sheath, often with sparse calcified plates lining the peritoneum.

The mean SP incidence in recently published studies ranged between 3.5 (2) and 4.2/1000 patients/year (3). Sclerosing encapsulating peritonitis is a severe complication, although rare, with a mortality rate exceeding 60%, due to sepsis and malnutrition. The treatment is still debated: surgical management is difficult and frequently leads to fatal bowel perforation. Immunosuppressive agents have been associated with some success, supporting the hypothesis that SEP could be immunologically mediated.

We report a clinical case of a continuous ambulatory peritoneal dialysis (CAPD) patient, with clinical and radiological signs of SEP, successfully treated with prednisone and azathioprine, parenteral nutrition, and transfer to hemodialysis (HD). She recovered from the intestinal obstruction and remains asymptomatic 18 months later.

CLINICAL REPORT

The patient is a female Caucasian, born in November of 1975, with juvenile nephronophthisis. She began

HD in May 1984. In March 1985 she had her first cadaveric kidney transplant (CKT), with immediate function but it failed 4 months later because of non-compliance. A definitive vascular access was not possible and she was transferred to CAPD with a lactate-based solution. She had two peritonitis episodes by unknown agents before her second CKT in October 1986. This graft had excellent function, but was complicated with ureteral necrosis, which was surgically resolved, and one corticosteroid-sensitive acute rejection. She recovered and her Tenckhoff catheter was removed 5 months later. In June 1993 a late rejection, again due to noncompliance, subsided. A second Tenckhoff catheter was introduced and she returned to CAPD, with some residual renal function, in November 1994 using four bags per day of a lactate-based solution, glucose 1.36%, calcium 1.25 mmol/L Baxter system.

In February 1995 she had a new *Staphylococcus epidermidis* uncomplicated peritonitis. She had a third CKT in May 1995, complicated by immediate vascular thrombosis and graft infarction; she remained on CAPD. In July 1995, she had another uncomplicated *S. epidermidis* peritonitis. Following this incident, she needed one 3.86% glucose dialysis per day to maintain adequate ultrafiltration (UF). She became hypersensitized, with a panel-reactive antigen of 84%. In January 1996, a peritoneal equilibration test (Twardowski method) showed a low-average transport with a 4-hour dialysate-to-plasma ratio (D/P) creatinine of 0.54, similar to that of 1 year before (0.58).

During 1996, there were three new peritonitis episodes (one *Streptococcus viridans* and two *S. epidermidis*) which promptly resolved. In December 1996, acute rejection episodes on stopping steroids led to transplantectomy (second graft). Hemoperitoneum without peritonitis persisted for 3 days. Since then, she has needed one 2.27% and one 3.86% bag per day. On 6 February 1997, she was admitted with a new peritonitis episode, which was treated with vancomycin and ceftazidime. A methicillin-sensitive *S. epidermidis* was identified and treatment was changed to cefazolin. Severe abdominal pain and fever persisted, and the peritoneal catheter was substituted 6 days later. On 18 February 1997, with persisting fever and abdominal complaints, *Candida parapsilosis* was detected in the culture of the catheter tip and in a new sample of peritoneal effluent. Abdomi-

nal ultrasonography (US) showed no collections and was interpreted as normal. The patient was treated with intraperitoneal fluconazole for 2 days, then changed to intravenous amphotericin B. She was transferred to HD, with intradialytic nutritional supplementation. The Tenckhoff catheter stayed in place for 5 more days, maintaining peritoneal washings. Her fever disappeared but her abdominal pain increased severely, relieved only by opioids, and obstructive symptoms, nausea, vomiting, and malnutrition with an 8-kg weight loss developed. A new US was interpreted as normal.

On 10 March 1997, an abdominal computed tomography (CT) showed intestinal adhesions and the bowel abnormally restricted to the central part of the abdomen, peritoneal thickening, and loculation of the abdominal cavity (Figure 1). The typical cocoon image emerged and allowed the diagnosis of SEP. Total parenteral nutrition, epidural analgesia, and immunosuppression with azathioprine 50 mg/day plus prednisone 100 mg/day were prescribed. Surgical management was discussed but not done owing to the unsatisfactory results previously reported. Three days later complaints diminished, epidural analgesia could be stopped and 10 days later she started progressive oral nutrition with intradialytic parenteral supplementation for 1 month more. A follow-up CT scan performed on 18 March 1997 showed some improvement. Azathioprine was stopped at 2.5 months of treatment and steroids were continued for 2 months more with progressive tapering without relapse of pain or occlusion. She remains on HD using a central catheter. The patient recovered her lost weight and had a serum albumin of 3.7 g/dL. A last CT performed in August 1997 showed normal distribution of the bowel; the "cocoon" had disappeared (Figure 2).

DISCUSSION

With the increasing number of PD patients and the increasing survival on PD, SP becomes an important problem. An Australian study (3) showed a near exponential increase in SP incidence with time on PD: from 1.9% for patients on dialysis less than 2 years, to 6.4%, 10.8%, and 19.4% for more than 5, 6, and 8 years, respectively, suggesting the duration of the exposure of the peritoneum to PD to be a major risk factor. However, some cases occur early in the course of PD.

Peritoneal sclerosis has two distinct forms: the SS form – thought mainly dependent on the bio-incompatibility of the solutions – with a sclerotic submesothelial layer not exceeding 40 μ , and without significant inflammatory infiltrate (4); and the SP form where there is a dramatic progression of the sclerosis after an inflammatory insult, such as peritonitis. The

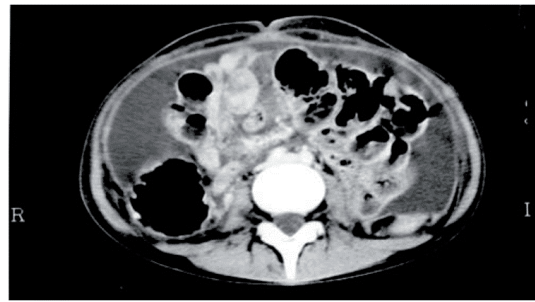


Figure 1 – Abdominal computed tomography image at time of diagnosis of sclerosing encapsulating peritonitis, showing adherent bowel loops, with posterior tethering and luminal narrowing, peritoneal thickening, and loculated fluid.

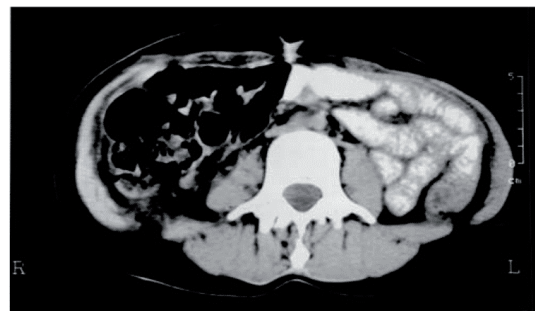


Figure 2 – Abdominal computed tomography image 5 months after Figure 1, showing normal distribution of the bowel loops, filling of all the cavity, with luminal patency.

thickness of the sclerotic tissue reaches much higher values (1000 – 4000 μ) than in SS, and a marked chronic inflammatory infiltrate is invariably present (4). Although bioincompatibility is a risk factor, a definitive etiology for SEP is still unknown. Peritonitis is the most commonly invoked, but not obligatory, pathogenic factor. The underlying process of SP may be immunological. A particular form of SP, SEP (encapsulating form), is characterized by bowel obstruction due to the extensive fibrosis covering and enclosing the gut like a rigid bag. The case we described presented the clinical and radiological features of SEP. The image of a cocoon bowel observed on CT, based on a suggestive clinical presentation, was diagnostic (2,3) and we considered peritoneal biopsy too risky.

This patient was on PD for 42 months and had an incidence of peritonitis of 2.6 episodes/year, much higher than our mean incidence of 0.7 episodes/pt/year. The impact of peritonitis on mesothelial cells, and on their intrinsic fibrinolytic activity are well known. After this damage, the multipotential stem cells of the subserosa may differentiate as mesothelial cells, causing a new epitheliation of the perito-

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neum or, to the contrary, leading to fibronogenesis and peritoneal sclerosis. This process of regeneration versus fibrosis is affected not only by the number and duration of peritonitis episodes, but also by their characteristics: persisting peritonitis (2), late peritonitis of a previously damaged peritoneum (2), and the severity of the last peritonitis (2). More aggressive agents such as *S. aureus*, *Pseudomonas*, and fungi are more likely to damage the peritoneum. The increased fibrinous exudate due to increased coagulability and decreased fibrinolysis justifies the decision of some authors, including these authors, to maintain the catheter in place for 48 hours, if possible, to maintain peritoneal washing. The fact that two of three SP cases present after transfer from PD suggests that PD helps to remove fibrin accretion (3).

Peritonitis is only one of many risk factors for SP (3). The peritonitis rate has decreased in the past 10 years and, on the contrary, SP has progressively increased: from 1.5/1000 in the past decade, to 4.2/1000 (3) in the first half of the present decade. In many cases, a previous peritonitis episode could not be documented, which suggests other factors are implicated in SP genesis.

The number of abdominal surgeries, related or not to the catheter, might be another risk factor (2,5). The use of acetate-based PD solution in the early 1980s was undoubtedly related with SP process. Chlorhexidine and β -blocking agents have also been responsible for a considerable number of SP cases. Peritoneal exposure to glucose, hypertonicity, low pH, plasticizers, glucose degradation products (GDP) by heat sterilization, and even trauma from the tip of the catheter (5), have all been implicated as risk factors (2,3). Some antimicrobial agents administered intraperitoneally are likely to negatively affect re-epithelization of the peritoneum. Our patient received fluconazole intraperitoneally for 3 days, as proposed by peritonitis treatment recommendations, although there is little experience with this route of administration. We do not know if it contributed to the SP; however, small bowel obstructive symptoms appeared before its use.

Some authors consider UF failure as an alerting sign for PS, and suggest systematic screening for SP in patients on PD for more than 4 - 5 years who present loss of UF (5). Peritoneal effluent CA125 may also be a sign. Our patient presented UF deterioration.

Imaging of the abdominal cavity became progressively more important during the present decade for the diagnosis of SEP. Abdominal x ray is of low utility (5). Ultrasonography can show peritoneal thickening and a preperitoneal membrane, forming the typical image of trilayer membrane or "sandwich-like" membrane, and a fixed and dilated bowel (5). The CT is the most useful method for this diagnosis (2,3,5). The characteristic image of a cocoon, with the small bowel

restricted to the central part of the abdomen due to the fibrotic sheath covering and closing the loops, and the resulting contracted mesentery, peritoneal thickening, calcifications, and the septa leading to fluid loculation are the features often seen on CT (2,3,5), and were present in our patient. The colonic transit study, although now simplified, takes 2 or 3 days to show an increased colonic transit time (5).

Therapeutic strategy is a difficult decision. One invariable attitude is to stop PD and oral nutrition and transfer the patient to total parenteral nutrition and HD. The cases diagnosed after stopping PD suggest a possible causal relationship between this stop and SP expression, and it may be advisable to maintain the catheter and lavage if infection can be ruled out. Surgical adhesiolysis is invasive and difficult to perform: to find a cleavage plane for the lysis of the adhesions and remove the fibrotic sheath is difficult and sometimes impossible work, especially if fibrosis overpasses the superficial layer. Occasional intestinal perforation may be fatal.

Immunosuppression is another option of therapeutic strategy, as an alternative to surgery or sometimes trying to facilitate the ensuing surgery. The improvement of small bowel obstruction in some SP patients after transplantation encouraged this strategy. Some authors only have SP survivors among those who were treated with immunosuppressive drugs, mostly renal transplanted patients (6). Immunosuppressive regimens were variable between reports, but all of them had prednisone and another drug, usually azathioprine. This supported our decision to use these two drugs, but the ideal prescription is not known; some have advocated intraperitoneal prednisone alone. When we started the immunosuppression, the fungal peritonitis seemed fully treated, with microbiological tests repeatedly negative, but we maintained amphotericin for 14 days.

From the experience acquired with this case, we believe that the therapeutic strategy adopted, which included prednisone and azathioprine, was decisive for the favorable outcome.

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VI. General Discussion

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General Discussion

Peritoneal dialysis (PD) has already progressed through important stages [1], and, in the late years, basic research with cell cultures and animal models allowed increased knowledge on the molecular pathways of membrane structural and functional alterations under chronic exposition to PD solutions.

However, clinical studies are ultimately needed to validate the results of these laboratory investigations and evaluate the effect of the therapy on hard clinical PD outcomes such as small solute transport and fluid removal.

Fast transporters are a clinical challenge because adequate fluid management and avoidance of excessive glucose exposure are particularly difficult in these patients, with potential compromise of technical maintenance on PD and, in some reports, also of patient survival. ADEMEX and other studies refuted this association, but a recent meta analysis [2], including twenty observational studies, demonstrated a mortality relative risk of 1.15 for every 0.1 increase in the D/Pcreatinine. However, studies usually focused on prevalent and late term PD fast transporters with scarce knowledge on the characteristics and determinants of this status in early stage PD. Besides clinical results might depend more on the strategies of fluid management [3] not usually detailed in publications.

We developed an observational study to prospectively evaluate baseline characteristics of fast transporters in our PD population and the impact of this status on patient survival. We investigated the determinants of the time course of D/P creatinine during follow-up. Hopefully this will help to clarify more the natural history of “in patient” peritoneal membrane and transport alterations.

Assessment of peritoneal membrane in clinical practice

We have elected the ISPD recommended protocol with PET 3.86% [4] to evaluate small solute transport rate and water transport. We avoided complex formulas [5] and applied a method applicable to the PET [6] to estimate free water transport with correction for sodium diffusion. Some authors state that a theoretically justified correction for convective transport would be advisable to estimate MTAC creatinine when using a dialysate with a high glucose concentration [7]. However this modification has little or no significant effect on the calculation of MTAC during a dwell using 1.36% or 3.85% [8;9].

Measurement of D/P Na at 60 min dwell also allows to detect cases of ultrafiltration failure (UFF) associated with impaired free water transport. Functionally, UFF is in most cases characterized by an enhanced peritoneal mass transfer area coefficient for glucose combined

with a largely unchanged peritoneal glucose osmotic conductance [10]. However, this may be decreased in cases of more severe acquired UFF in long-term PD patients [11]: aquaporin-1 dysfunction and/or interstitial fibrosis might be the causes of such late changes.

Recently La Milia and co-workers [12], proposed the fast-fast PET with only 60 min dwell determinations, which allows quantification of actual free water removal, while also categorizing small solute transport based on D/P creatinine at 60 min. But an overestimation of MTAC at 60 min may occur due to the contribution of convective transport and the fact that PS (permeability. surface area) during the first hour of dwell is significantly higher than during subsequent hours [13-15].

Facing these doubts, a recent review [16] suggested that, either the use of maximum dip of D/P sodium or the exact amount of free water transported through the water channels, by the method of la Milia, are accurate in following our patients, while the use of the mini-PET as a tool for small solute characterization should still be explored.

In our study, we indeed found that D/P creatinine at one hour overestimates peritoneal transport rate in CAPD. However, D/P creatinine at 60 min dwell may be useful to guide automated peritoneal dialysis (APD) prescription, when short cycles, with individualized dwell times, must be prescribed.

The time-course of peritoneal membrane characteristics

D/P creatinine change with time on peritoneal dialysis has been a matter of debate. Some authors reported a sustained increase of D/P creatinine already from the first year of PD [17], while others found no clinically significant changes up to three or four years under treatment [18;19], particularly in those patients without serious peritonitis. Also not all long-term PD patients develop a fast transport status.

These variable and inconsistent results underline the extreme interpatient variability of peritoneal transport and its determinants.

Other issue that merits discussion is about when the first PET should be performed, after the beginning of PD. There is evidence of increasing peritoneal transport rate in the first 2-4 weeks [20], therefore it has been recommended that the first PET should be preferably done after the 2-3 months under treatment [21]. We followed this strategy, but our investigation highlighted that changes still proceed during the first and second year. However these might vary according to the characteristics of the populations and the insults related with the therapy.

Our studies are in accordance with a recent investigation [22] which concluded that the peritoneal transport parameters tended toward normalization during the first year on PD, mainly with a decrease of small solute transport and an increase of ultrafiltration capacity. This

evolution was independent of age, gender, diabetes and higher exposure to glucose in PD solutions. Our reported U-shaped curve of D/P creatinine (chapter IV.III), with the lowest values in the second year of PD, is similar to the results of an other study from The Netherlands [23]. We hypothesized that an indirect role of mesothelial cells could exist, as they are important sources of vasoactive mediators such as vascular endothelial growth factor (VEGF), a potent angiogenic factor [24-26]. Local intraperitoneal processes mediated by mesothelial cells could be determinants of fast transport status at PD start [26], indirectly influencing baseline peritoneal effective capillary surface through the recruitment and vasodilatation of peritoneal vessels, therefore causing a state of temporary functional hyperpermeability. Our investigation supports this hypothesis [25]. This might explain that not all fast transporters will progress with worse outcomes [27] and some will tend to normalize D/P creatinine in short-time PD.

Some temporary, not clinically relevant increase of D/P creatinine and decrease of ultrafiltration, associated with the increase of effluent CA125 has also been documented with the use of alternative more biocompatible solutions [28], presumably meaning a more healthy and “reactive” peritoneal membrane.

Our results are similar to more recent studies reporting preserved peritoneal membrane function in the first years of PD treatment, but differ from earlier studies on longitudinal transport evaluation, which showed a more dramatic and earlier detrimental increase of D/P creatinine. However, those earlier studies included patients treated in the eighties, with higher peritonitis rate and glucose exposure which might justify such discrepancy.

To correlate functional and morphological peritoneal membrane changes in our patients, repeated biopsies are not feasible. Therefore in vivo markers of peritoneal pathological processes, more accessible and informative of earlier abnormalities would be useful. In fact, the Peritoneal Biopsy Registry [32;33] allowed important investigation of vascular and fibrotic changes but only cross-sectional results of PD patient membrane could be obtained without dynamic functional-structural correlation.

A number of effluent markers have been previously investigated [34] in vitro, but the rate of production in vitro is 10-30 times higher and, on the other hand, the uremic milieu and dialysis solutions may modulate this secretion.

We elected effluent CA125, IL-6 and VEGF to be monitored longitudinally.

Our investigation supports the contention that among many investigated cytokines and growth factors, cancer antigen-125 (CA125) seems to be most useful [35;36]. Both in vitro and in vivo studies [37;38] support a role for CA125 as an informative marker of membrane status, related to mesothelial cell mass. Exposition to more biocompatible solutions was

associated with mesothelial repair and increase of CA125 [39]. Although some authors suggested limitations of CA125 as an index of peritoneal mesothelial cell mass [40] they demonstrated a reduction of CA125 secretion and expression with chronic exposition to glucose solutions. Indeed, we have demonstrated a consistent decrease of CA125 appearance rate with time on dialysis(chapter IV.III) paralleling functional estimates such as decreased sodium sieving (chapter IV.II), and increased D/P creatinine. Its prognostic significance is also supported by the fact that lower levels are found in patients with peritoneal sclerosis. However the natural history of CA125 and its informative role is still questioned because its regulation is not known yet nor its biological significance [41].

We found a significant association of effluent CA125 appearance rate with D/P creatinine at the beginning of peritoneal dialysis. The relationship of CA125 with peritoneal transport, as measured by D/P creatinine, has been controversial. It was first assumed that no association existed between mesothelial cell_CA125_ and peritoneal transport, supporting the concept of the mesothelial layer as a passive structure, mainly involved in host defence without influence on solute and fluid transport. Previous contradictory publications are due to great interindividual variation on the magnitude of intraperitoneal CA125, in cross sectional studies including patients at different stages of PD.

Within other potential useful markers [34] we also investigated effluent VEGF and IL-6, but did not measure TGF- β since its tissue concentration would be more informative than effluent levels. Both effluent VEGF and IL6 were associated with fast peritoneal transport.

VEGF was first associated with fast transport rate in a study by Zweers and co-workers [42], and more recent molecular investigation confirmed its role in the intermingled process of peritoneal neoangiogenesis and fibrogenesis [43]. Our results suggest that although different cells might be the source of VEGF, it is related with D/P creatinine: in the early PD period it is associated with a functional (temporary) hyperpermeability, while in late term PD it probably induces anatomical neoangiogenesis.

Considering IL-6, we found that it was not associated with baseline peritoneal fast transport but it seemed determinant, as an intraperitoneal inflammation marker, of the time-course increase of D/P creatinine, under PD treatment. This biomarker has been earlier associated with peritoneal membrane hyperpermeability [44]. It was also cross-sectionally associated with peritoneal fast transport as measured by D/P creatinine but this was documented in inflamed patients [45;46]. Diffusion from the circulation of increased systemic levels of IL-6 could not be excluded in those studies.

One can argue that IL-6 has both pro and anti-inflammatory effects but clinically it is mainly considered as an inflammation marker. It is produced by numerous types of immune cells such as mesothelial, monocytes, lymphocytes and fibroblasts in response to stimuli such

as TNF- α , IL-1 β , endotoxins, and oxidative stress. Our studies are also unable to detect the measured IL-6 cell source. Yet, the borderline negative association between the time-courses of CA125 and IL-6 supports the contention that the main source of production of mediators may change during PD.

We have not measured IL-6 receptor. It is known that IL-6 acts in target cells through the complex IL6- IL6 receptor. Soluble forms of IL-6 receptor modulate the effects of IL-6 and the complex IL6-IL6 receptor is considered an important signalling pathway involved in the transition between the early and the late phase of inflammatory [47] response. It would be interesting to explore the IL-6 system longitudinally. However apart from a single study that have measured it in PD patients [46], IL-6 itself has been widely assumed as a reliable marker of inflammation [48;49].

Types of fast peritoneal transport status and their determinants

A fast transport status may be present either from the beginning of PD (“inherent”) or be acquired during treatment. Different causes are presumed to induce such different situations.

Inconsistent results have been previously presented in the literature about the determinants of peritoneal baseline fast transport and its outcomes. Some aspects may contribute to such conflict.

First, scarce studies focused on the early PD period and the timing of the first PET varies widely.

Secondly, the case-mix of the studied population also varies between studies: clinical variables such age, prevalence and type of diabetes, comorbidity or residual renal function might influence the results, as we underlined in chapter IV.III. In our investigation, baseline fast transport status, as measured by D/P creatinine, was not an independent risk factor for patient survival; comorbidity and increased serum levels of IL-6 were. Our population showed a lower prevalence of aged and diabetic patients, in comparison with other studies, which could have explained that we did not found the association of baseline peritoneal fast transport with systemic inflammation nor with lower patient survival (chapter IV.I). But the study alerts for the fact that patients presenting small solute fast transport rate in the beginning of PD can be successfully managed if adequate individualized PD prescription is offered, often improving ultrafiltration with later normalization of D/P creatinine as we (chapter IV.III) and others reported [21;22].

The causative link between inflammation and baseline fast transport was also refuted in our studies. In fact, the previously reported association between systemic inflammation, as measured by IL-6, and D/P creatinine, documented in cross-sectional analysis of prevalent

patients, could be explained by the fluid overload, known to induce higher levels of inflammatory cytokines in plasma [29;30].

Interestingly, some authors [31] have argued that D/P creatinine, used in routine clinical assessment of peritoneal transport actually does not differentiate between small solute fast transport rate (dependent on an anatomical “normal” peritoneal capillary surface) and hyperpermeability (vasodilated and leaky capillaries associated with inflammation). In addition to the effective surface area available for diffusion, equivalent to the D/P creatinine or MTAC, the flow through the large pores, an estimated (though not measured) variable in the Peritoneal Dialysis Capacity Test (PDC test, Gambro software) could be an additional information to diagnose a fast transport status with actual hyperpermeability, associated with inflammation. Large pore flow was indeed related with higher comorbidity in a recent study [18].

Importantly our studies support the contention that a baseline fast transport rate does not share the same determinants as later acquired fast transport status, and might represent in some populations, a transitory benign short-term condition, not related with systemic inflammation.

We highlighted that baseline fast transport status was associated with higher levels of effluent CA125 and VEGF suggesting a role of the mesothelium as a source of vasoactive factors. The results support that VEGF, at that stage, is a determinant of a temporary functional fast transport rate, possibly due to recruitment of previously non-perfused vessels, in contact with the PD solutions (chapter III.II and chapter IV.III). VEGF is also related to the increase of D/P creatinine concordant with the documented neoangiogenesis occurring in late-term PD. Other peritoneal cells such as endothelial cells and mesothelial derived fibroblasts become more potent sources of VEGF, up regulating fibrogenic processes. In this intraperitoneal process, inflammation, as measured by effluent IL-6, shown to be significantly associated with the time course increase of D/P creatinine (chapter IV.III). This fits with the clinical knowledge of the cumulative inflammatory insults derived from peritonitis and solutions.

The significant associations that we found between mesothelial cell mass, VEGF, IL-6 and D/P creatinine, in the different stages of PD, are clinically relevant because they clarify the time –dependent determinants of peritoneal transport.

We therefore support that the classical categorization of fast transport status as inherent and acquired, should still include in the inherent group, two subgroups: 1) associated with a higher mesothelial cell mass, a favourable and transitory situation, and 2) associated with comorbidity, with obviously worse prognosis. Lower ultrafiltration in the situation of a

transitory baseline hyperpermeability can usually be supported by residual renal function or icodextrin in the long dwell. Comorbidity usually is of more complex approach.

On the other hand, acquired fast transport status, probably depends on insults occurring during therapy such as peritonitis rate and bioincompatibility of the solutions, causing intraperitoneal inflammation. It is usually associated also with more severe ultrafiltration failure with compromise of sodium sieving as an estimate of aquaporin dysfunction and/or loss of glucose osmotic conductance due to interstitial fibrosis.

Peritoneal rest

Reversibility of membrane changes were documented with peritoneal rest (chapter V.I) although published reports on this strategy have been scarce. The main objective of this strategy is to avoid encapsulating peritoneal sclerosis, which is a rare although serious and life threatening PD complication [50]. Fast transport state is a risk factor and an early marker of encapsulating peritoneal sclerosis [51;52].

Apart from some pharmacological attempts to avoid peritoneal sclerosis and/or encapsulating peritonitis, peritoneal rest demonstrate that peritoneal lesions may be reversible after a peritoneal rest, at least if it is offered soon after membrane functional changes are documented. Clinical experience with peritoneal rest showed that this strategy may successfully recover ultrafiltration failure associated with acquired fast transport during PD [53;54]. Temporary transfer to hemodialysis, for at least 28 days, is able to give time for peritoneal remesothelialization and reduction of D/P creatinine in some patients [55]. Interruption of PD may determine progression of fibrin and sclerosis but periodical lavages with intraperitoneal heparin is an adjuvant therapy to avoid such complication [56-58].

New animal model studies [59] and cultured human peritoneal mesothelial cell model [60] reproducing this ability of membrane recover after peritoneal rest brought novelty to this topic, now reemerging not only as a valuable therapeutic strategy but also as opportunity to investigate on pharmacological weapons against functional and structural peritoneal membrane changes.

We reported the benefit of immunosuppressive therapy in a case of peritoneal encapsulating peritonitis (chapter V.II). Combination of prednisolone with azathioprine was effective. Tamoxifen, which interferes with TGF β 1, has also been used (10 mg-20 mg daily), with apparent success [61]. It could be a useful strategy mainly in earlier phases of the peritonitis sclerosing syndrome [62;63], although benefits in advanced stages of encapsulating peritonitis were also reported recently [64]. It is not known if renin-angiotensin system (RAS)

inhibitors and anti fibrotic agents or phosphatidylcholine, can translate its reported protective benefits from animal models [65] to clinical cases.

Future investigations

Genetic determinants of inherent and acquired peritoneal membrane fast transport status

In the search for baseline peritoneal fast transport determinants also genetic factors were presumed to explain why some patients are intrinsically fast transporters at the beginning of PD or develop this status with time on PD.

Vascular endothelial growth factor polymorphisms have been associated with susceptibility to diabetic microvascular complications [66] and retinopathy [67]. In peritoneal dialysis it was found that there was no relation between VEGF genotype and baseline peritoneal transport group but AA genotype of VEGF promoter at -2578 position was associated with progressive increase in peritoneal transport [68;69]. It was also suggested that systemic and local peritoneal VEGF production may be differentially regulated. An other recent study on genetic polymorphisms pointed to a possible pathologic involvement of receptor for advanced glycation end products (RAGE) for development of encapsulating peritoneal sclerosis in Japanese CAPD patients [70].

That genetic and clinical factors influence the baseline permeability of the peritoneal membrane has also been supported in a recent investigation [71]: univariate and multivariate analyses identified comorbidity, serum albumin, and the -174G/C polymorphism of IL-6 as independent predictors of small solute transport. The -174G/C polymorphism of IL-6 was associated with significantly higher IL-6 mRNA levels in the peritoneal membrane and higher plasma and dialysate IL-6 concentrations, suggesting a dominant effect of the C allele.

Therefore we have begun a complementary prospective study on our patients, determining VEGF and IL-6 polymorphisms to analyse its relationship with peritoneal transport profile.

Alternative PD solutions: expectation on hard clinical outcomes

Considerable efforts were developed to understand the effect of dialysis solutions on the peritoneal membrane. In vitro and ex vivo models of investigation have been used to further document the alterations of mesothelial and fibroblast cells exposed to glucose, glucose degradation products and other components of dialysis solutions. However the doubt still remains that these test systems do not mimic the in vivo equilibration of dialysis solutions

and may overestimate the clinical relevance of some in vitro effects [72]. Besides they lack the natural humoral mediators and cellular components resident in the peritoneal cavity.

Animal models also present limitations [73]: consensus still is needed to elect a standardized and valid long-term exposure animal model to more accurately reproduce the in vivo human membrane alterations [72]. Uremia and other comorbid conditions such as diabetes, for instance, have not been simulated in the present animal models. Also the degree of exposition with 10-30 week period of exposition to 3.86% glucose may overestimate the normal pathophysiology of peritoneal membrane. The human situation is certainly more complex and still unclear.

However alternative solutions indeed promise less angiogenic, inflammatory and fibrogenic peritoneal membrane changes, presumably avoiding acquired fast transport status and peritoneal sclerosis. Cost issues limit their wider use. But hard clinical end points from the use of these alternative solutions will need to be investigated, although long-term PD is necessary to definitively prove patient and technique survival advantages. Investigation on the effect of alternative solutions on peritonitis rate and residual renal function are needed.

Investigations on the new roles of aquaporin-1 and on the causes of its dysfunction

In our investigation we could see a decrease of sodium sieving with time on PD, as an expression of free water transport compromise, but specific causes of water channel dysfunction are still not defined. Interestingly, corticosteroids were reported to induce aquaporin-1 expression and function in peritoneal dialysis patients with UFF [74]. This is a clue for therapeutical investigation.

Very recently, aquaporin-1 has been reported to be involved in cell migration and angiogenesis [75]. Less aquaporin-1 expression or dysfunction is functionally associated with ultrafiltration failure but can possibly be structurally associated with mesothelialization and decreased membrane repair capability, often seen in long-term UFF patients.

Animal models would make possible to further address this new topic while manipulating aquaporin-1 expression and function will be a future step.

Investigation on anti-fibrotic agents to prevent and treat peritoneal sclerosis

Some preliminary results from basic science are promising [76]. In the field of peritoneal dialysis it has been demonstrated [77] that human peritoneal mesothelial cells constitutively express a renin-angiotensin system (RAS): angiotensin II (Ang II) produced by these cells

mediates high glucose-induced up-regulation of TGF- β 1 and fibronectin expression. Both losartan and captopril inhibited high glucose-induced up-regulation of TGF- β 1 and fibronectin expression in peritoneal mesothelial cells in a dose-dependent manner. Relevance of this RAS on cultured human peritoneal mesothelial cells was also supported by other investigators [78].

Other anti-fibrotic agents have already been also investigated: the hepatocyte growth factor (HGF), known as an anti-fibrotic and anti-TGF- β 1 agent shown to be an effective agent in the regeneration of peritoneal membrane damaged by high glucose solution [79;80].

Epithelial-mesenchymal transition was also actively reversed into the opposite direction, into mesenchymal-epithelial transition by treatment with bone morphogenic protein-7 (BMP-7) [81].

Neutralizing monoclonal anti-RAGE antibodies also prevented the up-regulation of TGF- β , epithelial-to-mesenchymal transition of mesothelial cells and fibrosis in uraemia [82].

Ex vivo analysis of effluent derived human peritoneal mesothelial cells allows important investigation [83] on potential therapeutical agents for peritoneal membrane sclerosis. Rapamicine, an immunosuppressor with anti fibrosis and anti_VEGF effects showed only partial benefit [84].

Therefore, further studies concerning prevention, early detection and pharmacological treatment of encapsulating peritoneal sclerosis are needed.

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I - Introduction

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Sumário e Conclusões

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Summary and conclusions

Peritoneal dialysis (PD) offers efficient fluid and solute removal, while better preserving residual renal function, in comparison with standard hemodialysis. This translates in a patient survival advantage for the majority of chronic renal patients treated by PD as a first renal replacement therapy. Worse results are obtained in patients with more baseline comorbidity which challenge therapy protocols and obliges to individualized prescription. In these patients specific care should be given to avoid cardiovascular complications, obesity and excessive cumulative exposition to GDPs. Peritoneal transport evaluation is a key factor in the management of PD patients. It makes it possible to detect the presence and development of a fast transport status. The present studies focused on this heterogeneous group of patients, presenting with a fast peritoneal transport rate. In some of them this was associated with worse outcomes, but the determinants of this condition were not fully elucidated.

First we have given the epidemiological context (**chapter II**) for the present investigation. We showed that standard and updated therapy according to the international best practice guidelines can be applied while similar clinical outcomes are achieved, in comparison with international PD programmes. This is the first international report on long-term PD experience in Portugal. It appeared an effective modality of renal replacement therapy, reflected by the growing patient preference in our PD programme. However median PD retention was limited to 36 months. After access related infections, inadequate ultrafiltration came second as a cause of drop-out. It was present in 26% of the patients transferred to hemodialysis. This crude number included both incident patients with ultrafiltration capacity failure already in the beginning of PD, and patients with acquired ultrafiltration failure, many of them without peritoneal small solute rate categorization.

For routine baseline characterization and monitoring of peritoneal transport status, we than systematically performed a PET 3.86% with 60 min D/P sodium and 4hours effluent CA125 appearance rate measurements. We noticed that the method did not allow differentiation between abolition of sodium sieving due to free water transport abnormality or sodium sieving blunting due to fast early sodium diffusion in patients with a larger effective capillary surface area.

Correcting the sodium sieving for sodium diffusion was done with a formula applicable to the PET. We investigated the benefits and limitations of its use with 60 min dwell PET data (**chapter III.I**). We found that the 3.86% (4 h) PET with an additional dialysate sampling after one hour provides results on peritoneal function that are similar to those obtained with more complicated function tests. Some expected overestimation of sodium sieving was clinically acceptable but uncorrected sodium dip was also informative. D/P creatinine at 60 min dwell

overestimated peritoneal small solute rate. This observation adds to the debate in the international PD community, about which clinical tool is the best to evaluate peritoneal fluid and solute transport.

In search for the determinants of baseline fast transporters we cross-sectionally investigated the relationship between effluent markers and peritoneal transport (**chapter III.II**). We concluded that fast solute transport rate at the beginning of PD is associated with signs of a large mesothelial cell mass and not with higher systemic IL-6. The results also suggested that VEGF, known to be produced by mesothelial cells could mediate this early phase peritoneal hyperpermeability. Refuting the presumed link of peritoneal fast transport and systemic inflammation, these results pointed to more benign determinants of baseline fast transport small solute rate. These data were validated by our investigation on the outcomes of incident fast transporters (**chapter IV.I**): we documented that baseline fast transport was not an independent factor of worse patient survival since it was also not consistently associated with systemic inflammation and atherosclerosis.

This clinical profile differs with acquired membrane peritoneal changes. Characterization of the fast transport status in the early and late stage of peritoneal dialysis (**chapter IV.II**) showed that acquired fast transport is associated with increased levels of the inflammation marker IL-6 and abolition of sodium sieving. Free water transport, expressed as sodium sieving, correlated with time on PD: the longer the treatment the lower the dip.

To further clarify the determinants of peritoneal transport we prospectively studied a cohort of incident patients (**chapter IV.III**). Our longitudinal study was able to document that: CA125, as an index of mesothelial cell mass, significantly decreases with time on PD. It is related with peritoneal transport but, in different ways according to the stage of PD. Higher levels in the beginning of PD are associated with intrinsic fast peritoneal transport, the results suggesting that this association is mediated by VEGF. In late term PD we were able to show that ultrafiltration failure is associated with lower levels of CA125 but VEGF tend to increase in accordance with the recent discovery that the mesothelial cell phenotype is lost while transdifferentiation occurs and mesothelial derived fibroblasts become important sources of VEGF. This promotes the development of an acquired fast transport status. A U shaped curve for D/P creatinine was found. The intraperitoneal inflammation marker IL-6 was significantly associated with the time course of D/P creatinine supporting the role of cumulative exposition to PD related infections and biocompatibility as promoter of membrane changes.

As a rescue therapy, the institution of a peritoneal pause improved UF failure associated with a fast transport status and prolonged CAPD retention (**chapter V.I**). This occurred in 67% of the cases but better results could be expected if the peritoneal rest was offered as soon as membrane changes are documented. It underlines the potential

reversibility of the membrane lesion process when instituted in an early phase and should be a matter for future investigation.

The development of a fast transport status is a risk factor for the life-threatening encapsulating peritoneal sclerosis in some patients. We add our report of a case successfully treated with immunosuppressive therapy (**chapter V.II**). This underlines the severe inflammatory status that can be present in this severe acquired PD complication.

Sumário e Conclusões

A Diálise Peritoneal (DP) permite a remoção eficaz de solutos e fluidos, com preservação mais duradoura da função renal residual, em comparação com a modalidade de hemodiálise. Apresenta também vantagem na sobrevivência cumulativa de um vasto número de doentes tratados por DP como primeira forma de tratamento substitutivo renal crónico. No entanto os resultados clínicos não são tão favoráveis em doentes com maior comorbilidade ao início do tratamento, um desafio à individualização do esquema terapêutico para evitar complicações cardiovasculares, obesidade e excessiva exposição a produtos de degradação da glicose. A avaliação das características de transporte peritoneal e a detecção do estado de transporte rápido é um factor chave no manuseamento dos doentes em DP. A presente investigação pretendeu caracterizar este grupo heterogéneo de doentes, transportadores rápidos, situação também associada a piores resultados clínicos, mas cujos determinantes não estão ainda definidos.

Reportamos pela primeira vez a experiência de um programa de diálise peritoneal continua ambulatoria crónica em Portugal, no decurso de vinte anos de actividade (capítulo II). Mostramos que são seguidas orientações internacionais de pratica clínica com obtenção de resultados similares aos reportados por outros grupos europeus e americanos. Verificamos um aumento da prescrição de DP por opção do doente, no nosso programa. No entanto, o tempo médio de retenção na modalidade de DP limitou-se a 36 meses. A falência de ultrafiltração foi causa de transferência para hemodiálise em 26 % dos nossos doentes, o que incluiu doentes com transporte rápido basal, transporte rápido adquirido no decurso do tratamento e em muitos doentes, não categorizado.

Para monitorização clínica do estado de transporte da membrana peritoneal elegemos o Teste de Equilíbrio Peritoneal (TEP) com solução 3,86%, incluindo a razão dializado/plasma (D/P) do sódio medido aos 60 minutos de permanência e da taxa de aparecimento do marcador CA125 no efluente, no final do teste, às 4 horas de permanência. Corrigimos o *seiving* do sódio para a difusão com uma formula aplicável ao TEP. Obtivemos resultados sobreponíveis aos que são reportados com métodos mais sofisticados, como o Standard Peritoneal Analysis (SPA) (capítulo III.I). Foi clinicamente irrelevante a esperada sobrestimação do *seiving* do sódio com a correcção efectuada. O *seiving* de sódio não corrigido foi também discriminativo, na avaliação do transporte de agua livre em função da categoria de transporte de pequenos solutos e na falência de ultrafiltração. O D/P da creatinina aos 60 minutos sobrestima a taxa de transporte peritoneal de pequenos solutos. Este resultado vem somar-se à actual controvérsia sobre qual o melhor método de monitorização do transporte peritoneal de fluidos e solutos nos nossos doentes.

Para avaliar os factores que condicionam o estado de transporte rápido no início de DP, fizemos um estudo transversal sobre a relação de biomarcadores de massa mesotelial peritoneal, inflamação e angiogénese e o D/P creatinina. Concluímos que o estado de transportador rápido inicial está associado a sinais de maior massa mesotelial peritoneal (CA125) e valores mais elevados no efluente do mediador vasoactivo vascular endotelial growth factor (VEGF) produzido por essas células, não tendo sido evidenciada relação do D/P basal e os valores sistémicos de interleuquina-6 (IL-6) (capítulo III.II). Estes resultados refutam a associação do estado de transporte peritoneal rápido no início de DP com inflamação sistémica e sugerem uma etiologia mais benigna para esta condição basal. A investigação dos resultados clínicos deste grupo de doentes (capítulo IV.I) corrobora esta hipótese: o estado de transporte rápido basal não foi factor independente de pior sobrevivência e não se associou a inflamação ou aterosclerose, mas a comorbilidade e valores mais elevados de IL-6 foram-no.

Este perfil clínico é diferente no estado de transporte peritoneal adquirido, estando este associado a valores mais elevados do marcador de inflamação sistémica IL-6 e a abolição do seiving do sódio (capítulo IV.II). O transporte de água livre estimado pelo seiving corrigido de sódio relacionou-se com o tempo em DP: quanto mais tempo de duração de tratamento menor o dip do sódio.

Para investigar prospectivamente a relação em função do tempo em DP, dos marcadores de massa mesotelial, inflamação e angiogénese, e o transporte peritoneal avaliado pelo D/P creatinina, estudamos uma coorte de doentes incidentes (capítulo IV.III). O nosso estudo permitiu documentar que: 1) o CA125, indicador de massa de células mesoteliais, diminui com o tempo em diálise, 2) relaciona-se com o transporte peritoneal mas de forma diferente consoante o estadio em DP – valores mais elevados de CA125 no início do tratamento associam-se ao estado de transporte rápido “intrínseco”, sendo os resultados sugestivos de que esta relação é mediada pelo VEGF. Numa fase mais tardia de DP, a falência de ultrafiltração com transporte rápido adquirido associa-se a valores mais baixos de CA125, mas os valores de VEGF aumentam tendencialmente, de acordo com o conhecimento recente de que as células mesoteliais que sofreram transdiferenciação epitelial-mesenquimal são ainda importantes fontes de VEGF.

O estudo longitudinal revelou alterações significativas do D/P creatinina com um perfil em U. Os valores de IL-6 intraperitoneal associaram-se significativamente com a evolução de D/P creatinina, o que suporta que o tratamento dialítico, implicando exposição cumulativa a soluções bioincompatíveis e insultos infecciosos, induz inflamação intraperitoneal e desenvolvimento de transporte rápido adquirido.

Como terapêutica de salvamento nesta complicação, uma pausa peritoneal mostrou ser capaz de induzir recuperação da capacidade de ultrafiltração e permitir prolongar a manutenção de DP (capítulo V.I). Este benefício foi obtido em 67% dos casos, mas a investigação sugere que melhores resultados seriam obtidos se a pausa peritoneal for efectuada precocemente após detecção do estado de transporte rápido adquirido. Esta experiência clínica aponta para a potencial reversibilidade das lesões da membrana peritoneal sob medidas precocemente instituídas, o que merece investigação futura.

O desenvolvimento do estado de transporte rápido é um factor de risco para a ocorrência de peritonite esclerosante encapsulante. Reportamos a eficácia terapêutica do tratamento com imunossuppressores usado em tal situação (capítulo V.II) o que sublinha o estado inflamatório severo associado a essa complicação em DP.

Sommaire et conclusions

La dialyse péritonéale (DP) permet une soustraction efficace de l'eau et solutés tout en permettant une meilleure préservation de la fonction rénale résiduelle par rapport à l'hémodialyse standard. Ceci se traduit par une meilleure survie des insuffisants rénaux chroniques qui débutent l'épuration extrarénale par dialyse péritonéale. Des résultats moins bons sont soulignés chez les patients, avec des pathologies qui mettent en échec les protocoles thérapeutiques standards et nécessitent une prescription individualisée : des traitements spécifiques ont pour but d'éviter les complications cardiovasculaires, l'obésité et l'exposition excessive aux produits de dégradations du glucose des solutions de dialyse. L'évaluation des caractéristiques de transport du péritoine est un aspect fondamental de la gestion des patients en DP, également pour détecter les transporteurs rapides. Le présent travail s'est concentré sur ce groupe hétérogène de malades qui présentent une hyperperméabilité en début de traitement par DP, associée à de plus mauvais résultats dont les causes déterminantes n'ont pas été entièrement élucidées.

Nous avons d'abord décrit le contexte épidémiologique (chapitre II) de la recherche actuelle. Nous avons montré que le traitement est appliqué selon les recommandations internationales avec des résultats cliniques semblables aux programmes internationaux de DP. Cela représentait le premier rapport international sur une expérience à long terme de DP au Portugal. La DP s'avérait une modalité efficace de traitement de l'insuffisance rénale terminale, reflété par la prévalence croissante des patients dans notre programme de DP. La durée médiane de traitement dans cette modalité a été limitée à 35.5 mois. Après les infections liées aux accès, ultrafiltration insuffisante vient en second lieu comme cause de transfert de 25,5 % des patients en hémodialyse. Ce nombre brut a inclus les patients incidents présentant une perte d'ultrafiltration dès le début et ceux qui ont eu une perte d'ultrafiltration ultérieure, un nombre important sans évaluation des caractéristiques de transports péritonéaux.

Pour l'évaluation basale et la surveillance du statut de transport péritonéal, nous avons systématiquement exécuté un test d'équilibration péritonéale (TEP) avec une solution à 3.86%, et nous avons évalué le rapport D/P du sodium (60 min) et mesuré le taux de Ca²⁺ (240 min) dans l'effluent.

Nous avons noté que cette technique n'a pas permis de différencier si la disparition du coefficient de tamisage du sodium était liée à une anomalie du transport de l'eau ou à une surface capillaire efficace plus élevée.

La correction du coefficient de tamisage pour la diffusion de sodium a été faite avec une formule applicable au TEP. Nous avons étudié les avantages et les limitations de son

utilisation avec les résultats obtenus au TEP 60 min (chapitre III.I). Nous avons constaté que le TEP 3.86% avec un prélèvement additionnel de dialysat après une heure fournit les résultats sur la fonction péritonéale qui sont semblables à ceux obtenus avec la plus compliquée SPA. Une certaine surestimation prévue du *coefficient de tamisage* du sodium était cliniquement acceptable mais les résultats non corrigés du D/P du sodium (60 min) étaient également instructifs. Le taux de transfert péritonéal était surestimé avec le D/P créatinine (60 min) : c'est une observation à ajouter aux discussions de la communauté néphrologique internationale lorsque le débat porte sur le choix du meilleur outil clinique pour évaluer le transport péritonéal.

Dans la recherche des causes déterminantes du statut de transport rapide en début de DP et plus tard au cours du traitement nous avons étudié le rapport entre les marqueurs de l'effluent et le transport péritonéal (chapitre III.II). Nous avons conclu que le transport rapide de solutés au début de la DP est associé aux signes d'une grande masse de cellules mésotéliales sans élévation d'IL-6 systémiques. L'étude a également suggéré que le VEGF, connu pour être produit par les cellules mésotéliales pourrait induire une hyper perméabilité péritonéale transitoire au début de la DP, réfutant le lien présumé du transport rapide péritonéal et de l'inflammation systémique. Ces résultats suggèrent des causes plus bénignes déterminantes du taux rapide de transport péritonéal basal. Ces données ont été validées par notre recherche sur les transporteurs rapides incidents (chapitre IV.I) : nous avons documenté que le transport rapide basal n'était pas un facteur indépendant d'une plus mauvaise survie du patient puisqu'il n'a pas été également associé à l'inflammation ni à l'athérosclérose systémiques.

Ce profil clinique diffère lors des changements péritonéaux acquis de la membrane. La caractérisation du statut de transporteur rapide en début de DP ou acquis pendant le traitement de la dialyse péritonéale (chapitre IV.II) a prouvé que le transport rapide acquis est associé aux plus hauts niveaux du marqueur d'inflammation IL-6 et de l'abolition du *tamisage* du sodium. Le transport libre de l'eau, exprimé comme le *tamisage* du sodium, est corrélé avec le temps en DP: le plus long le traitement, le plus inférieure est la valeur du *tamisage* du sodium.

Afin de clarifier davantage les causes déterminantes du transport péritonéal nous avons étudié une cohorte de patients incidents (chapitre IV.III). Notre étude longitudinale pouvait documenter cela : le CA125, comme index de la masse des cellules mésothéliales, diminue de manière significative avec le temps en DP ; on le relie avec le transport péritonéal mais de différentes manières selon l'étape de la DP ; des niveaux plus élevés en début de DP sont associés à un transport péritonéal rapide intrinsèque, les résultats suggérant que cette

association est induite par le VEGF. Plus tard en DP nous pouvons prouver que la perte d'ultrafiltration est associée à des niveaux plus bas de CA125 mais le VEGF tend à augmenter, selon la découverte récente que le phénotype mésothélial des cellules est perdu tandis que la trans-différentiation se produit et que les fibroblastes dérivés des cellules mésothéliales deviennent des sources importantes de VEGF en favorisant également le transport rapide acquis. Une courbe en U pour le D/P créatinine a été trouvée. Le marqueur d'inflammation intra péritonéal IL-6 a été significativement associé avec le D/P créatinine au cours du temps ; cela souligne le rôle des expositions cumulées aux infections et à la bio-incompatibilité des liquides de DP comme instigateur des changements de membrane.

Comme solution thérapeutique, en cas de perte d'ultrafiltration associée à des transports rapides acquis, la pause péritonéale s'est avérée efficace en permettant un maintien plus prolongé en DP (chapitre V.I). Le succès de cette pause a été observé dans 66.6% des cas. Cependant de meilleurs résultats seraient probables si le repos péritonéal était institué dès que des changements de membrane sont documentés. Cette stratégie témoigne la réversibilité potentielle du processus de lésion de membrane et ouvre la voie à de futures recherches. La présence d'un état de transporteur rapide est un facteur de risque de péritonite sclérosante mettant en jeu le pronostic vital : nous ajoutons notre rapport d'un cas traité avec succès par thérapie immunosuppressive (chapitre V.II), qui souligne l'état inflammatoire grave dans une telle complication acquise en DP.

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These words are public.

Others will rest intimate. Do not fit here.

Receive my grateful smile, all which believed in me and
helped me to accomplish this happy pursuit.

Estas palavras são públicas.

Outras serão íntimas. Não cabem aqui.

*Um sorriso grato a todos os que acreditaram e me
ajudaram a fazer um percurso feliz.*

Obrigada, queridas enfermeiras com quem trabalho tão de perto, na nossa Unidade de DP_ companheiras no entusiasmo, nas angústias, no empenho. Conteí sempre convosco e senti a confiança recíproca. Obrigada a cada uma de vós, sentidamente.

Thank you, dear Professor Doctor Krediet, for your continuous support, confidence, and fruitful discussions, without which I would have been unable to accomplish this task. You have shared my thoughts, doubts, risks and enthusiasms. You have shown your commitment. My gratitude can hardly be expressed in words.

Obrigada Dr. António Cabrita, pelo voto de confiança. Permitiu-me o trabalho e os afectos, com os doentes que eu gosto de tratar. Semeou desafios. Colheu de mim vontade, compromisso, neste trajecto de já longos anos de trabalho cúmplice.

Obrigada Professor Doutor Castro e Melo por ter aceite ajudar-me neste projecto. Dei os primeiros passos na investigação com a sua colaboração, no Serviço de Imunologia, muitos anos antes. Não o esqueci.

Querida amiga Isabel Fonseca, obrigada pelo teu companheirismo e generosidade. A estatística foi só pretexto para a nossa comunhão.

Obrigada Margarida Martins pela tua colaboração preciosa, imprescindível, rigorosa, no laboratório e fora dele. E pela tua amizade.

Obrigada querida Luísa Lobato, pelas tuas orientações sábias, de quem já trilhou o mesmo caminho, este percurso de perseverança e humildade.

Ao Dr. António Sarmento estou grata pelas palavras, pelo humor, pela confiança (e o CA125 é um ótimo pretexto de conversa, porque não?).

Obrigada Dr. Guilherme Rocha. Recebo os doentes que me referencia. Gosto de conversar consigo. Falamos mais da vida do que de nefrologia.

Obrigada aos meus colegas, a todos os que tendo feito ou fazendo parte do Serviço de Nefrologia do HGSA, partilharam comigo o esforço e os sonhos. O meu percurso é também uma construção, feita de amizades, confrontos, partilhas, de que todos vós fizestes parte.

Obrigada querida Laurinda Teixeira pela boa disposição, o estímulo, a dádiva. E pelo registo paciente das análises dos nossos doentes.

Agradeço também o apreço que as secretárias, enfermeiras e auxiliares do nosso Serviço tantas vezes me manifestaram, em gestos e palavras. Acompanharam a minha vivência hospitalar. Fazem parte dela.

Agradeço ao Dr. Carvalho dos Santos, Dr. Mário João Santos, Dr. José Carlos Oliveira, Dra. Fernanda Bravo, Emília Pinto e a todos os médicos e técnicos que de algum modo, com trabalho ou cumplicidade amiga, no Serviço de Química Clínica, acompanharam os meus trabalhos de investigação.

Obrigada Dr. Carlos Nogueira, a orientação cirúrgica oportuna dos meus doentes, é mais um testemunho de trabalho de equipa e compromisso. Além da amizade.

Obrigada Professor Doutor João Cabeda, pela sua disponibilidade para projectos futuros na área da Biologia Molecular. A sua orientação irá ser imprescindível nos meus próximos passos de investigação.

Obrigada querida Patrícia Branco pela tua imensa simpatia e confiança. A distância geográfica não impediu que partilhasses comigo o entusiasmo. A estatística. Os nossos doentes. A angústia às vezes. Conta comigo também.

Uma palavra aos meus colegas, que acreditam na Diálise Peritoneal e a prescrevem. Somos poucos. Obrigada pelo vosso reconhecimento, pela forma como se dispõem a trabalhar juntos para que mais doentes possam beneficiar da modalidade.

Agradeço ao Professor Doutor Manuel Pestana a confiança que tem manifestado no meu trajecto profissional.

Agradeço ao Dr. Serafim Guimarães, longe e tão perto, ter-me incentivado a trilhar o caminho da investigação e o doutoramento, um percurso de crescimento.

Thank You Doctor Machteld Zweers, for your suggestions on laboratory measurements, your references were important to my work.

I am grateful to Doctor Johanna Korevaar. You have kindly received me in Amsterdam. I have learned with you, and your advice in the statistical investigation was determinant.

Thank you, Annemieke Coester, for your warm reception and help during my stay in Amsterdam. I wish you will succeed with your thesis.

Thank you, Doctor Dick Struijk, for your supportive words, whenever we met. When we feel insecure, those words are precious.

I am also thankful to Dr. Christian Verger for his friendship and his help correcting my written French.

"É o mar cumpria-se"
outra vez
sempre que nós quisermos
Ar. del. Rodrigues

