Morpho-functional study of peritoneum in peritoneal dialysis patients

Silvio Volmer Bertoli, Laura Buzzi, Daniele Ciurlino, Massimo Maccario, Stefania Martino

Nephrology and Dialysis Unit, Policlinico MultiMedica, Sesto San Giovanni, Milan - Italy

ABSTRACT: *Background:* **Structure and function of the peritoneal membrane (PM) are impaired on peritoneal dialysis (PD). The aim of this study was to examine the relationship between dialytic parameters and histological and functional characteristics of the peritoneum of PD patients.**
Methods: **A peritoneal biopsy (PB) was performed on 31 PD patients during catheter removal due to malfunction or after drop-out from treatment. PB was performed at least 5 cm from the catheter insertion. For each patient PM transport was evaluated by the last peritoneal equilibration test (PET) before PB. Each daily glucose load was calculated. Tissue was formalin-embedded and stained for histological and immunohistochemical studies.**
Results: **(1) Duration of treatment was longer in patients with mesothelial impairment. (2) Patients showing submesothelial sclerosis (SS) and those with impairment of submesothelial basement membrane and subendothelial vascular membrane (SVM) were submitted to a larger daily glucose load. (3) SS exceeding 50 mm was more frequent among high transporters, who were exposed to larger daily glucose load compared to medium-high transporters. (4) Mesothelial loss correlated to SS and vascular alterations. (5) SS was related to vascular injuries but not to inflammatory infiltrate.**
Conclusions: **SS is not constant in PD patients and is not a prominent factor in treatment drop-out. Mesothelial impairment seems to be mainly related to duration of PD treatment. Glucose load seems to mainly damage the submesothelial layer.**

Key words: *Peritoneal dialysis, Peritoneal histology, Peritoneal membrane, Peritoneal biopsy*

INTRODUCTION

Derangement of peritoneal structure and function is the main factor of drop-out from peritoneal dialysis (PD) (1–3). The peritoneal membrane (PM) is composed of a layer of flat mesothelial cells, underlined by an electro-negative basement membrane and by loose connective tissue, composed of collagen I and III, elastic fibres and fundamental substance. Jaluronic acid and glucosaminoglicans, the main components of fundamental substance, make a network of pores and channels, involved in bi-directional transport of water and solutes between the peritoneal cavity and systemic circulation (4-7). The physical and chemical structure of peritoneum regulate water and solute transport: while convective permeability is mainly related to capillary endothelium, submesothelial interstitium interferes with diffusive permeability. Ultrafiltration depends 90% on small size pores and 10% on ultra-small pores of peritoneal capillary endothelial cells (8-12).
PD impairs the structure and function of PM (13). Histological changes of peritoneal mesothelium, interstitium and vessels have been described both in animals and in humans (14-17). Many factors seem to be involved in the pathogenesis of peritoneal damage: intrinsic peritoneal fragility; duration of treatment; low pH and hyperosmolarity of dialytic solutions (13-15, 18, 19); exposition to lactate, glucose, non-enzymatic glucose degradation and glycation products (13, 15, 21-26); chronic inflammatory state (27-29); oxidative stress (21); infections (30) and individual genetic predisposition (13, 15, 31). The relationship between these factors and PM morpho-functional impairment is still debated.
This study aimed to examine the relationship between epidemiological, clinical and dialytic parameters and peritoneum function and histology in PD patients submitted to peritoneal biopsy (PB).

Subjects and Methods

A PB was performed in 31 PD patients, 84% (n=26) on CAPD, 16% (n=5) on APD, after treatment drop-out (29 patients, 93.5%) or catheter removal due to malfunction (two patients, 6.5%). For each patient epidemiological, clinical, dialytic and histological parameters were collected.

Epidemiological characteristics

A PB was performed on 12 men (39%) and 19 women (61%), aged from 28-85 years (median age 65 years, mean age 65 ± 12 years). Mean body mass index (BMI) was 25.3 ± 5. In two patients (6.5%), aged 61 and 79 years, BMI was lower than 18 and they dropped out of treatment because of malnutrition after 62 and 45 months on PD. Cause of end-stage renal disease was polycystic kidney disease in five patients (16%); focal glomerulosclerosis (GSF) in three patients (10%); IgA nephropathy in two patients (6.5%); unknown in seven patients (23%); nephrolithiasis in one patient (3%). Nine patients (29%) had a history of past abdominal surgery. Two (6.5%) were diabetic and two had peripheral arteriopathy. Seven patients (22.5%) had used beta-blockers and seven patients (22.5%) had chronic HBV- or HCV-related hepatitis.

Causes of drop-out from treatment

The drop-out rate because of infections was 39%, which included peritonitis (10 patients, 32.5%) and tunnel infections (two patients, 6.5%). The second cause of drop-out (seven patients, 22.5%) was clearance failure. Loss of ultrafiltration and malnutrition caused PD failure in two patients (6.5%). Other causes of drop-out were transplantation, bowel occlusion, infarction and hemoperitoneum, and dementia.

Dialytic parameters

Duration of treatment ranged from 11 to 150 months, with a median of 45 months and a mean of 53 ± 34 months. Mean daily dialysate volume was 9.2 ± 3 litres. Mean daily glucose load (calculated for the last month of PD treatment) was 132 ± 67 g. Six patients (19%) used polyglucose solution and 15 patients (48%) used iodopovidone and PVC. All other patients used hypochlorite and Clear-FlexR. Incidence of peritonitis was 0.68 ± 0.58 episodes/yr. Infections were caused by gram positive (52%), gram negative (35%) and fungi (3%). In 19% of infections the pathogenic germ was not isolated.

Peritoneal functional parameters

Peritoneal functional parameters were derived from the last peritoneal equilibration test (PET) preceding the PB (performed 1-3 months before the biopsy). According to these parameters, patients were classified as high (n=16), medium-high (n=10) and medium-low (n=2) transporters; recent data were not available for three patients.

Histological parameters

A PB was performed by the excision of the parietal tissue sample at a distance of 5 cm from catheter insertion. Each specimen was formalin-embedded and stained with hematoxylin and eosin, trichrome and van Gieson. For immunohistochemical study, specific antibodies for CD8, CD4, macrophages, myofibroblasts (actin) and fibroblasts (vimentin) were employed. For each patient, characteristics were reported of mesothelium, submesothelial basement membrane (SBM), submesothelial sclerosis (SS), vessels, subendothelial vascular membrane (SVM), chronic inflammation and fibrin deposition. Thickness of SS was quantified as > or < 50 µm, the cut-off point commonly used to define simple peritoneal sclerosis (32-35). Histological patterns observed are shown in Figures 1-4.

Statistical analysis

Results are presented as mean ± standard deviation (SD). A Student's t-test and a Fisher test were performed.

Results

Table I shows histological patterns observed at light microscopy.

Infections represented the major cause of early drop-out from PD: mean and median duration of treatment were 38 ± 27 and 31 months for patients who dropped out because of infections, and 63 ± 35 and 56 months for other patients (p=0.03). No statistical difference in PM histology was observed between the two groups.

Patients with a damaged mesothelium had longer treatment (57 ± 34 months vs. 23 ± 7 months; p=0.0002). After 34 months, all patients showed mesothelial damage, mesothelium loss being the most frequent finding.

Patients with SS and those with alterations of the SBM and SVM had been submitted to a larger daily glucose load (Fig. 5: submesothelial thickening: 158 ± 49 g/day vs. 97 ± 38 g/day, p=0.023; SBM: 150 ± 67 g/day vs. 100 ± 28 g/day, p=0.02; SVM: 141 ± 67 g/day vs. 101 ± 28 g/day, p=0.037). All patients who had been exposed to a daily glucose load >145 g showed diabetic alterations of SVM.

APD patients were a small group in comparison to

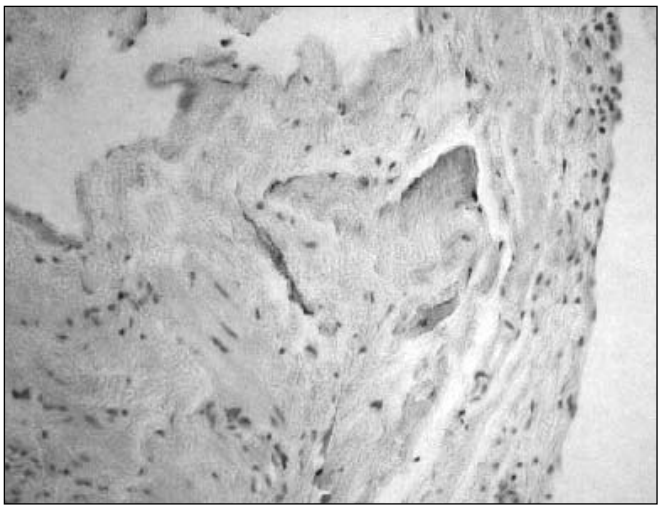

Fig. 1 - Normal morphology of parietal peritoneum: a single layer of flat mesothelial cells coating a poorly vascularized loose connective tissue. Hematoxylin and Eosin 100 x.

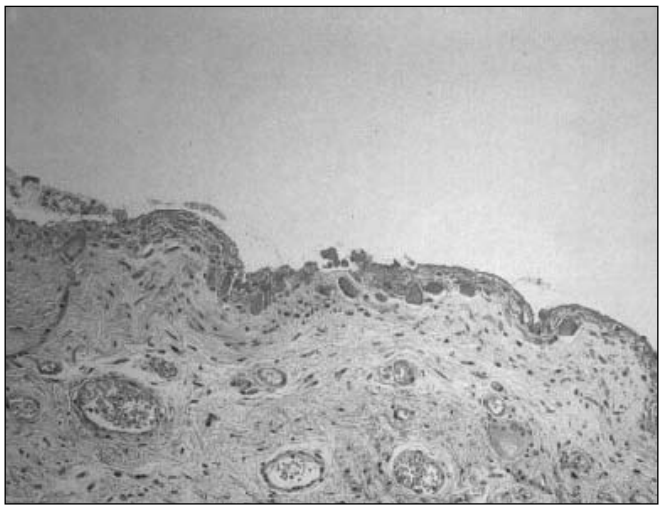

Fig. 2 - Morphological changes of parietal peritoneum: mesothelial thickening with cellular swelling and activation; submesothelial layer with neoangiogenesis and increased collagen deposition. Hematoxylin and Eosin 100 x.

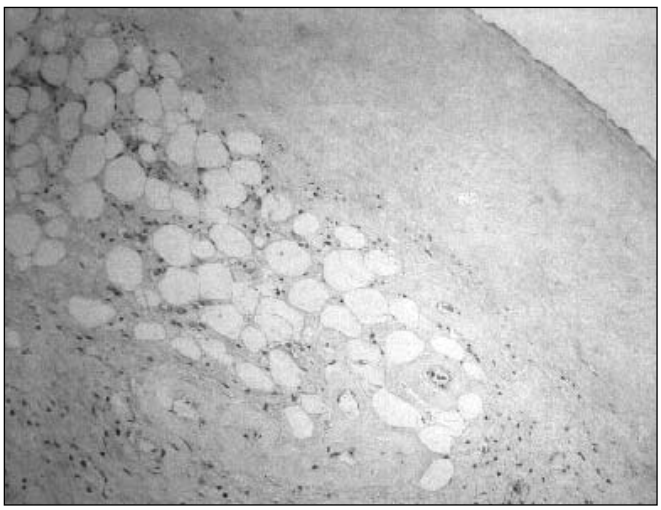

Fig. 3 - Disappearance of mesothelial layer. Submesothelial collagen thickening >50 µm. Neoangiogenesis with vascular sclerosis. Fibrous tissue infiltrating the adipose layer. Hematoxylin and Eosin 100 x.

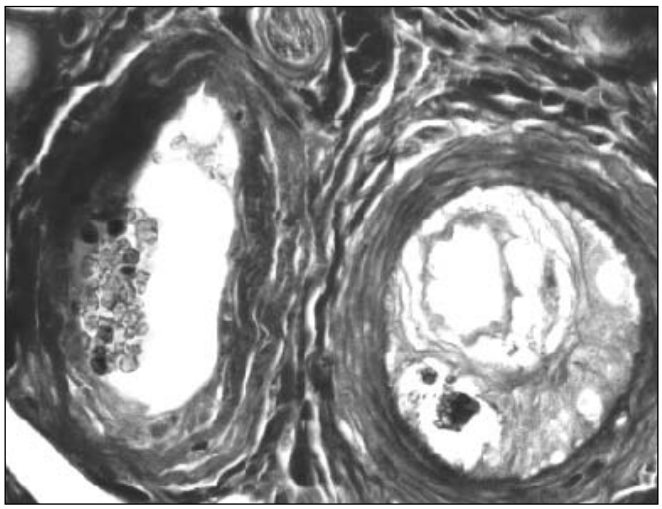

Fig. 4 - Neoangiogenesis with vascular sclerosis and partial occlusion of the lumen. Thickening of the *intima,* focal calcification and deposition of collagen on the *media*. Mallory 400 x.

CAPD patients, and they showed more changes of mesothelial (p=0.022), SBM (p<0.001) and submesothelial layer (p=0.001).

Table II represents the relationship between histological and clinical parameters.

High transporters had been exposed to a larger daily glucose load (154 ± 82 g/day vs. 108 ± 25 g/day, p=0.047), although SS >50 µm was not more frequent in high transporters than medium-high transporters (p=ns).

Absence of mesothelial layer was related to SS and vascular injuries (p=0.045 and p=0.05); the presence of SS was related to vascular alterations (p=0.001). Presence of superficial fibrin was related to the absence of

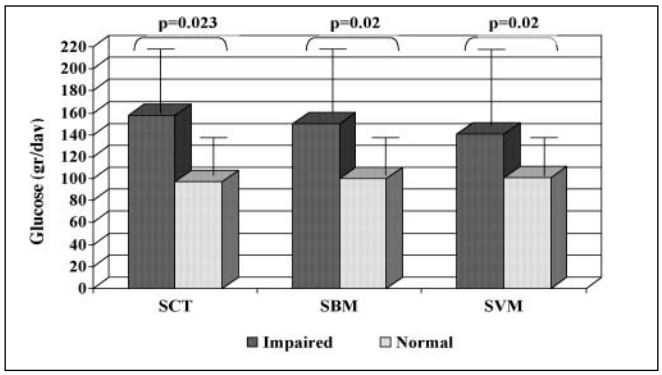

Fig. 5 - Histological changes according to daily glucose load. SCT: submesothelial connective tissue; SBM: submesothelial basement membrane; SVM: subendothelial vascular membrane.

TABLE I - PERITONEAL HISTOLOGICAL PATTERNS BY LIGHT MICROSCOPY

Peritoneal constituents	Histology	Samples
Mesothelium	Normal	17%
	Loss	46%
	Degeneration	15%
	Regeneration	6%
	Cellular activation	6%
	Not assessable	10%
Submesothelial membrane	Normal	39%
	Loss	43%
	Fragmentation	3%
	Reduplication	6%
	Thickening	6%
	Not assessable	3%
Submesothelial sclerosis	Absent	36%
	< 50 µm	22%
	> 50 µm	39%
	Not assessable	3%
Blood vessels	Normal	39%
	SVM reduplication	45%
	Neoangiogenesis	45%
	Sclerosis	48%
Chronic inflammation	Present	23%
	CD4	13%
	CD8	3%
	Macrophages	16%
Fibrin	Absent	48%
	Present	52%
Myofibroblasts	Absent	71%
	Present	29%
Fibroblasts	Absent	55%
	Present	45%

the mesothelial layer and the presence of vasculopathy (p=0.004 and p=0.047).
There was no association between findings of SS and inflammatory cells and the development of peritoneal functional impairment.

DISCUSSION

Our study confirmed that infections – in particular peritonitis – are the major cause of early PD drop-out (median duration of treatment: 31 vs. 56 months). In contrast to previous reports, we did not observe more peritoneal structural changes in patients who dropped out due to infections as compared to other patients. This finding could be because a PB was performed not during infection but after healing. This observation also suggests that infections can accelerate peritoneal wear: compared to patients who dropped out for other causes, PM changes were as severe in patients who dropped out because of infections with a shorter treatment period.
Duration of treatment seems to be involved in mesothelial impairment: after 34 months of PD, mesothelial damage was observed in all patients, mesothelium absence being the most frequent finding. Patients with normal mesothelium showed the following: normal submesothelial basal membrane, collagen band (when present) <50 µm, absence of vascular sclerosis, normal SVM, absence of fibrin and absence of inflammatory infiltrate. We demonstrated the strict correlation between the absence of mesothelial layer and the presence of SS and vascular injuries (Tab. II), suggesting that an intact mesothelial layer preserves the integrity of submesothelium and the loss of the mesothelial layer plays a fundamental role in

TABLE II - RELATIONSHIP BETWEEN ANY HISTOLOGICAL AND CLINICAL PARAMETERS (FISHER EXACT TEST)

Submesothelial sclerosis (<50µm 7pts, >50µm 11pts)	vs.	Peritoneal transport (H 12 pts, MH 6 pts)	p=NS
Mesothelial layer (normal 3 pts, absent 9 pts)	vs.	Submesothelial sclerosis (>50 µm 7 pts, absent 5 pts)	p=0.045
Submesothelial sclerosis (present 19 pts, absent 12 pts)	vs.	Vasculopathy (present 19 pts, absent 12 pts)	p=0.001
Superficial fibrin (present 13 pts, absent 16 pts)	vs.	Vasculopathy (present 24 pts, absent 5 pts)	p=0.047

peritoneal impairment (36-40). The correlation between submesothelial fibrosis and peritoneal vasculopathy that we found (Tab. II) can depend on increased production of growth factors by deranged mesothelial cells, and also on a relative ischemia secondary to vasculopathy that exacerbates development of fibrosis, as recently suggested by Williams (41). Based on our results, submesothelial fibrosis seems to be also related to the daily glucose load that is involved in SBM and SVM damage. Focal or diffuse duplication and thickness of SVM was found in patients submitted to a glucose load >145 g/day. Such damage is similar to vascular changes commonly observed in diabetic patients: chronic hyperglicemia impairs arterial micro-circulation, causes an intracellular sorbitol filling (which increases intracellular osmolarity and impairs cellular metabolism) and enhances non-enzymatic glycation of plasma and tissue proteins (42-44)). In a recent *in vitro* study Mandl-Weber found that non-enzymatic glycation products like glycated albumin up-regulate VEGF expression by cultured HPMCs, promoting peritoneal vasculopathy (40). A Japanese study of 15 CAPD patients showed that advanced glycosylation end-products (AGEs) deposit mainly on the vascular peritoneal wall and the amount of deposited AGEs is related to the duration of CAPD and to peritoneum permeability (24). We did not examine the presence and distribution of AGEs and their receptors (RAGE); in addition, from our data a correlation between peritoneal permeability and vascular impairment can be excluded, as 26 of 31 patients were high or medium-high transporters and only two were medium-low transporters. No differences in treatment duration and causes of drop-out between high and medium-high transporters were observed, and could be because high transporters were submitted to a larger daily glucose load. In addition, they presented more frequently a thickness of submesothelial fibrosis >50 µm (p=0.03) and tended to have more vascular alterations compared to medium-high transporters (p=N.S.). All the high transporters had an impaired submesothelial layer. Glucose load seems to be involved in submesothelial impairment acting both directly through glycation end-products and indirectly through derangement of mesothelial metabolism (21). Nevertheless, SS and peritoneal thickening are not a constant event in PD patients, as also observed by Williams (41).

We used the method of PB and collection validated by Di Paolo in several histologic reports (6-7, 32-34). We choose not to measure the exact thickness of each biopsy specimen, but just to quantify SS thickness, when present, as < or >50 µm. This cut-off was proposed by Di Paolo to define simple peritoneal sclerosis (32-34). Peritoneal fibrosis was observed in approximately two-thirds of our patients; in 39% thickness was >50 µm; we did not notice an evolution of sclerosing encapsulating peritonitis. However, SS and peritoneal thickening do not seem to be strong determinants in treatment drop-out.

In conclusion, our study aimed to routinely apply PB in clinical practice to improve PD treatment. Considering the relationship between epidemiological, clinical, dialytic and histological parameters, duration of PD seems to be mainly involved in mesothelial impairment. Loss of mesothelial integrity favors the development of SS and vasculopathy and they are related. Daily glucose load seems to be involved mainly in submesothelial impairment, favoring fibrosis and the alteration of the mesothelial basement membrane and the SVM. Nevertheless, peritoneal fibrosis is not a constant event in PD patients and is not a crucial factor *per se* in PD technique failure.

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Address for correspondence:
Silvio V. Bertoli, M.D.
Unità Operativa di Nefrologia e Dialisi
Policlinico MultiMedica
Via Milanese, 300
20099 Sesto San Giovanni (MI), Italy
silvio.bertoli@multimedica.it

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