

Review

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Recommendations for pathological diagnosis on biopsy samples from peritoneal dialysis patients

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Abstract: Peritoneal dialysis (PD) has been established as an essential renal replacement therapy for patients with end stage renal disease during the past half century. Histological evaluation of the peritoneal membrane has contributed to the pathophysiological understanding of PD-related peritoneal injury such as peritonitis, fibrosis, and encapsulating peritoneal sclerosis (EPS). Hyalinizing peritoneal sclerosis (HPS), also known as simple sclerosis, is observed in almost all of PD patients. HPS is morphologically characterized by fibrosis of the submesothelial interstitium and hyalinizing vascular wall, particularly of the post-capillary venule (PCV). Two histological factors, the thickness of submesothelial compact zone (SMC) and the lumen/vessel ratio (L/V) at the PCV, have been used for the quantitative evaluation of HPS. The measuring system on SMC thickness and L/V ratio is easy and useful for evaluating the severity of HPS. On the other hand, EPS is characterized by unique encapsulation of the intestines by an “encapsulating membrane”. This newly formed membranous structure covers the visceral peritoneum of the intestines, which contains fibrin deposition, angiogenesis, and proliferation of fibroblast-like cells and other inflammatory cells. This review will cover the common understandings of PD-related peritoneal alterations and provide a basic platform for clinical applications and future studies in this field.

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Introduction

Since peritoneal dialysis (PD) therapy began at the end of the 1970s, many technological advances have been made and contributed to the long-term use of this therapy. Extended PD treatment gave rise to several serious problems that interfere with the continuation of this therapy. One major problem is a peritoneal deterioration or “simple sclerosis”, characterized by interstitial fibrosis and hyalinizing vasculopathy with functional loss of ultrafiltration (UF) capacity [1–6]. In this review, “hyalinizing peritoneal sclerosis (HPS)” is proposed for the first time in the literature instead of “simple sclerosis” to emphasize their pathological features. Another major issue is encapsulating peritoneal sclerosis (EPS), which rarely occurs, but can be life-threatening in some long-term PD patients [7–14]. In these circumstances, histological evaluation of the peritoneal membrane has become necessary to determine the extent of the damage induced by PD. In addition, increasing reports of EPS allow us to establish criteria for the diagnosis and anticipation of this complication. The purposes of peritoneal biopsy in PD patients are (1) assessment for cause and state of infectious peritonitis; (2) evaluation of peritoneal damage by PD therapy (evaluation of HPS); and (3) diagnosis and prediction of EPS. To make an appropriate diagnosis from peritoneal biopsy samples, we need to establish a consensus on the terminology for the disease entities and pathological lesions, and on the methods for qualitative and quantitative histological evaluation of each lesion. The Japanese Society for Peritoneal Dialysis established a working committee on peritoneal pathology in 2002, and has been discussing this issue in collaboration with a group from the University of Wales. Thereafter Honda et al. reported their quantitative histological evaluation for HPS associated with PD therapy; finding that severe HPS significantly correlated with long-term PD [15]. Several studies have applied this

histological evaluation, however, peritoneal biopsy and diagnosis for PD patients is not applied for routine PD therapy. Here, we present a joint proposal of peritoneal biopsy standards to facilitate appropriate evaluation and diagnosis of peritoneal disease in PD patients.

Procedures of peritoneal biopsy and sample processing

Parietal peritoneum biopsy from PD catheter insertion site

Peritoneal biopsy should be considered at the time of PD catheter insertion or PD discontinuation (protocol biopsy). Briefly, the parietal peritoneum is exposed and suture loop is inserted through the part of peritoneum to be sampled. By using the suture to lift the peritoneum, an ellipse (approximately 1–2 cm in size and up to 5 mm in depth) that includes the knot at one end can be excised. The site attached to the PD catheter should not be taken to avoid sampling errors as the area usually includes mechanical alterations caused by the catheter itself or suturing. The sample is placed in phosphate buffered saline, immediately pinned onto a silicone board with the mesothelial surface uppermost, extended to the same size as *in situ*, and fixed with 20 % buffered formalin. After overnight fixation at room temperature, the peritoneum is cut perpendicularly into vertical sections. The samples are routinely processed for light microscopy and embedded in paraffin. The 4 µm sections are cut and stained with hematoxylin & eosin (H&E) and Masson's trichrome stain. To identify vessels or lymph vessels, cluster of differentiation (CD) 31 (or CD34) and podoplanin staining is added to the procedure [16, 17]. Podoplanin staining is also useful to identify mesothelial cells, or podoplanin positive fibroblasts or “fibroblast-like cells (FLC)” [18, 19]. Preparation of frozen specimens and electron microscope specimens is recommended when precise investigations are required for special cases or for research purposes. Care must be taken when processing to avoid mechanical alteration, for example, the loss of mesothelial cells, or the distortion of SMC.

Alternative sampling strategy during surgery with or without laparoscope

Peritoneal biopsies are recommended at the time of correction of a malpositioned catheter, and any kind of

abdominal surgeries including those for malignant disease, hernia fixation, cholecystectomy, etc. to check pathological status associated with concurrent PD therapy. Although laparoscopic surgery is not required for removing a PD catheter, the images gained could be useful with regards to any underlying malignant disease [20–22]. There are few studies about macroscopic findings on PD patients, however, a scoring system including taking into account peritoneal appearance, hypervascular changes, adhesions and encapsulation may be useful [23, 24]. Visceral peritoneum biopsy could be considered in some cases, but it also presents a risk of perforation. Further studies need to be done, however, as laparoscope-guided biopsies might be an option in high-risk cases of EPS. Surgical enterolysis and visceral peritoneum biopsies will be performed simultaneously in severe EPS cases.

Terminology concerning PD peritoneal pathology

Parietal peritoneal architecture

Normal parietal peritoneum is consisted of mesothelial cells (surface lining), the submesothelial interstitial layer and underlying peritoneal adipose tissue or peritoneal fascia (Figure 1A schema and a). The peritoneal fascia is composed of dense connective tissue that appears as a compact assemblage of collagen bundles. Sometimes, the peritoneal fascia is adjacent to the submesothelial interstitial layer without the intercalation of peritoneal adipose tissue. The vascular network of parietal peritoneum is mainly located between the submesothelial interstitial layer and peritoneal adipose tissue and is composed of small artery, arteriole, capillary, post-capillary venule and veins (Figure 1A-b), occasionally with lymphatic vessels. Of note is that the vascular population varies dependent on age and mesothelial location; blood and lymph vessel density in parietal peritoneum is higher in infant age [17], lymphatic vessels are more abundant in the visceral peritoneum than the parietal [19, 25].

The definition of the special terms used in this field of peritoneal pathology is described below:

Submesothelial compact zone (SMC)

The term “submesothelial compact zone (SMC)” was proposed by Williams et al. [6], naming the widened

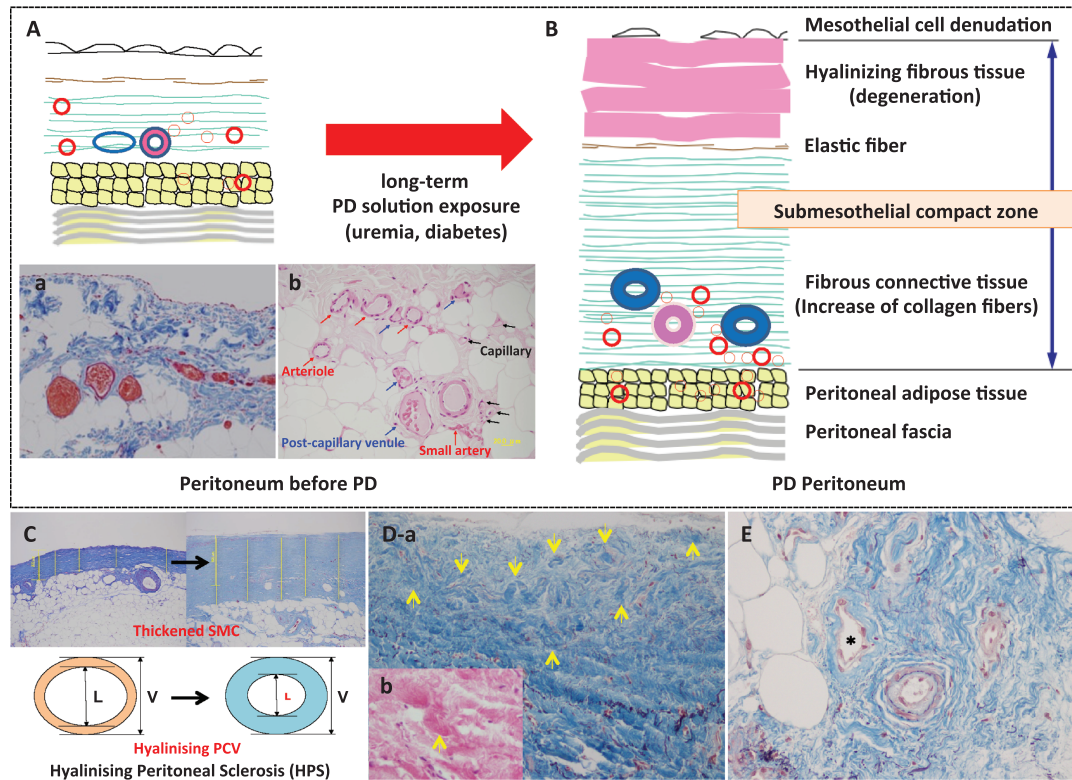


Figure 1: Parietal peritoneum before peritoneal dialysis and hyalinizing peritoneal sclerosis (HPS).

(A) Schema for parietal peritoneum before starting peritoneal dialysis (PD); mesothelial cells line on the peritoneum surface. Submesothelial interstitial layer and peritoneal adipose tissue or peritoneal fascia are underlying. (a) Biopsy sample from a chronic kidney disease patient at the beginning of peritoneal dialysis. (b) Peritoneum contains various types of blood vessels classified by the diameter and structure, i. e., small artery, arteriole, post-capillary venule (PCV), and capillary. Submesothelial compact zone (SMC) is defined between mesothelial cell layer surface and the upper border of peritoneal adipose tissue or peritoneal fascia (double arrow). (B) Schema for long-term exposure to PD solution causes thickened SMC with degeneration of the collagen, and hyalinizing vasculopathy with or without narrowing or obstruction of the lumen. (C) Pathological evaluations for SMC thickness and lumen per vessel ratio of PCV. (D-a) Biopsy samples from a long-term PD patient showed thickened SMC that contains homogeneous hyaline change (yellow arrow), (D-b) which is recognized as pink and glassy substance in H&E stain. (E) Hyalinizing vasculopathy is observed mainly in the PCV (*). [A-a, C, D-a, E, Masson's Trichrome stain; A-b, D-b, H&E stain].

submesothelial layer with fibrous connective tissue either with or without degeneration of collagen fibers. Width of the submesothelial compact zone was defined as a distance from the mesothelial surface to the upper border of peritoneal adipose tissue or peritoneal fascia. The width of submesothelial compact zone is regarded as the thickness of parietal peritoneum.

Post-capillary venule (PCV)

Post-capillary venules (PCVs) are described as venous-sided blood vessels, connecting capillaries to small veins. The diameter of PCVs ranges approximately from 20 to 100 μm in diameter. The PCV has a thin wall structure containing a few slender smooth muscle cells within the wall, and

usually co-localizes with arterioles (Figure 1A-b). It is noteworthy that hyalinizing vasculopathies in the PD peritoneum primarily affect PCVs [6, 15].

Definition of the pathological lesions

Peritoneal fibrosis

The pathological condition which is manifested by increased thickness of submesothelial compact zone replaced by interstitial fibrous material is generally called as peritoneal fibrosis (PF), regardless of its etiology; infection, chemical or physical irritation, inflammation, malignancy, metabolic disorders (diabetes and uremia), surgical damage and PD treatment.

Hyalinizing peritoneal sclerosis (HPS)

HPS, also known as “simple sclerosis”, is a pathological condition characterized by thickened SMC and hyalinizing vasculopathy (Figure 1B schema). It is predominantly encountered in PD patients but can be observed in some uremic patients before PD treatment [6, 15]. HPS is proposed for the first time instead of “simple sclerosis” to emphasize their different pathological features from PF. Because the thickened SMC in HPS can be morphologically differentiated from PF by the loss of fibrous bundles of collagen fibers and alteration to homogeneous hyaline substances (Figure 1C upper and D). Hyaline in the peritoneal interstitium or vascular wall is recognized as glassy and pink substances without inflammation in H&E or Periodic acid–Schiff (PAS) stains [6]. Otherwise, picrosirius red is useful for measuring collagen volume fraction (collagen density). Moreover the extra cellular matrix (ECM) in the interstitium such as proteoglycan is also changed during PD therapy; decorin, which is one of inhibitor of TGF- β , is decreased (pro-fibrosis way), and versican, which is a key factor in the inflammatory process, is increasing (pro-inflammatory way) in the long term PD peritoneum [26]. The cause of hyalinizing PF may be multifactorial, incorporating inflammation, ischemia, uremia, physical and chemical irritation and the deposition of advanced glycation end products (AGEs) related to bio-incompatible factors within PD solutions [27, 28], i. e. high glucose, glucose degradation product (GDP), acidic pH, lactate.

Hyalinizing vasculopathy is defined as hyalinization of vascular walls with or without narrowing or obstruction of the lumen (Figure 1C lower and E). It primarily affects PCVs and capillaries [6, 15]. Larger vessels; such as small arteries, arterioles and veins can be involved in more advanced cases. Electron microscopy reveals degenerative changes of vascular smooth muscle cells and lamination of vascular basement membrane in the peritoneal walls in cases of hyalinizing vasculopathy [4]. The cause of hyalinizing vasculopathy is also undetermined. PD-related factors as AGE deposition in the vascular wall is considered a primary candidate, with deposition leading to degeneration of collagen fiber components of the vessel wall [29]. Uremic toxins like β_2 -microglobulin (β_2 -MG) may have a role on the alteration, because β_2 -MG deposition is observed dominantly in the perivascular areas, and overlapped with AGE deposition [30].

Encapsulating peritoneal sclerosis

EPS is pathologically defined by the encapsulation of intestines or entire abdominal organs with cocoon like

thin white encapsulating membranes. It is often accompanied by edema of intestinal walls, ascites and adhesions to the abdominal wall (parietal peritoneum) in advanced cases. The encapsulating membrane is a newly-formed fibrous membranous structure that covers the visceral surface of the intestines and other abdominal organs (Figure 2A, B). Histologically, the encapsulating membrane is composed of fibrin deposits and is accompanied by swelling and the proliferation of peritoneal fibroblasts, inflammatory cell infiltration, angiogenesis and fibrosis (Figure 2C) [31]. The components of the encapsulating membrane are variable depending on the stage of its formation; fibrin is a main component in earlier stages (Figure 2D), whereas collagen fibers are dominant in later stages along with increased fibroblast or FLC proliferation [18, 19]. Similar histological findings can be observed in the parietal peritoneum such as the greater omentum (Figure 2E). Hyalinizing vasculopathy is significantly observed underneath the EPS membrane (Figure 2E). Severe blood and lymph angiogenesis is also found in some EPS cases (Figure 2F–H). These histological features can be modified by therapeutic interventions, such as steroids, anti-inflammatory agents or surgical treatment.

How to evaluate the extent of HPS

The adequacy of specimens for histological evaluation is determined at first in terms of size, site and orientation of the specimen. The specimen should have enough material sampled and be orientated such that the sample contains all layers of peritoneum (mesothelial, submesothelial and adipose tissue) and blood vessels, especially PCV of 25–50 μ m in external diameter.

How to evaluate hyalinizing PF (peritoneal thickening)

The extent of hyalinizing PF is determined by the thickness of the submesothelial compact zone. Five portions are randomly selected for measurement of submesothelial thickness in a well-oriented area of the peritoneum where all peritoneal layers are embedded properly and vertically. The portion where the peritoneum looks severely fibrotic due to tangential embedding or miscellaneous inflammatory reactions should be excluded from measurement. The thickness of the submesothelial compact zone is measured using microscopic lens or with an image analyzer. The average of the five values measured at different portions of the submesothelial compact zone represents the peritoneal thickness (Figure 1C upper).

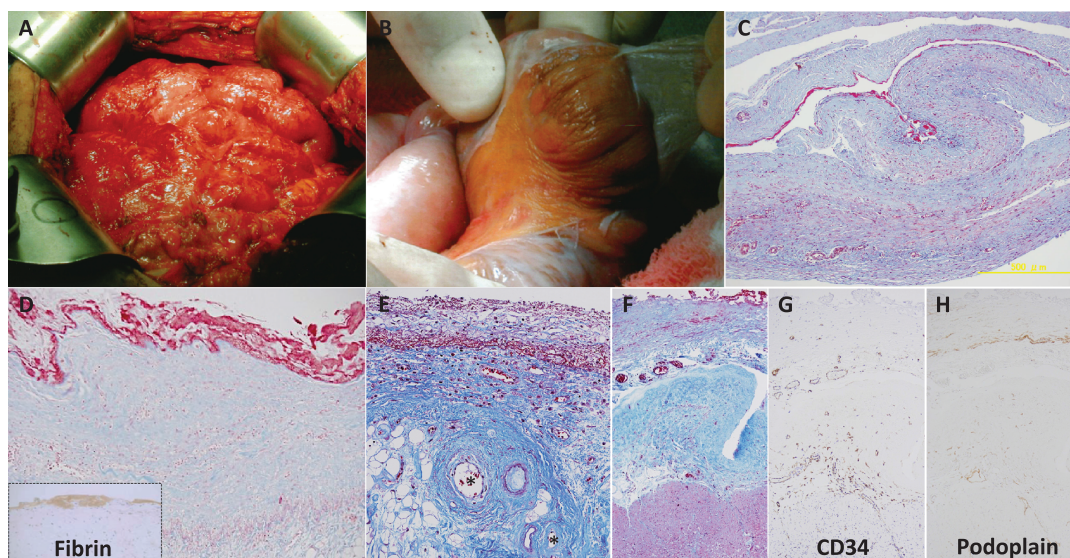


Figure 2: Pathological features of encapsulating peritoneal sclerosis (EPS).

(A, B) The encapsulating membrane is a newly formed fibrous membranous structure that covers the visceral surface of the intestines and other abdominal organs. (C) Histologically, the encapsulating membrane is composed of organized deposits of fibrin, along with enlargement and proliferation of peritoneal fibroblasts, inflammatory cell infiltration, angiogenesis and fibrosis. (D) Active fibrin formation was detected on the surface of EPS membrane. (E) Similar pathological components are also revealed in visceral mesothelium such as the greater omentum, and hyalinizing vasculopathy (*) underneath the EPS membrane. (F–H) The EPS case showed hyper-density of blood and lymph vessels in the visceral peritoneum (small intestine). [(C–F) Masson's Trichrome stain; dot square of (D) Fibrin stain; (G) CD34 stain; (H) Podoplanin stain].

The grading of the peritoneal thickening is difficult to establish because the variation of peritoneal thickness is enormously wide-ranged. Therefore, the average thickness of SMC is simply described for the quantification of PF in each sample. As a reference, the peritoneal thicknesses of normal, pre-PD and PD patients with different PD durations have been shown by Honda et al. [15].

How to evaluate the hyalinizing vasculopathy

The extent of hyalinizing vasculopathy is determined by the presence of hyalinosis, and the severity of luminal narrowing and luminal obstruction in PCVs. To evaluate the severity of luminal narrowing, a ratio of luminal diameter to vessel external diameter (L/V) is determined, which represents the extent of luminal patency (Figure 1C lower). PCVs with a diameter between 25 μ m and 50 μ m is selected for measurement, as the L/V ratio is influenced by the size of the blood vessel examined. The most severely affected vessel of each specimen is chosen for the measurement.

Grading of hyalinizing vasculopathy is determined by the following criteria: Grade 0, no hyalinizing vasculopathy; Grade 1, hyalinizing vasculopathy without narrowing [$L/V > 0.6$]; Grade 2, hyalinizing vasculopathy with mild luminal narrowing [$0.3 < L/V < 0.6$]; Grade 3,

hyalinizing vasculopathy with severe luminal narrowing or distortion [$L/V < 0.3$]; Grade 4, hyalinizing vasculopathy with luminal obliteration [$L/V = 0$]. Basically, the grading of hyalinizing vasculopathy is performed using the criteria of Williams et al. by qualitative observation [6] but modified using the L/V ratio instead of the thickness of vascular hyalinosis [15]. In general, the grading represents the overall grading of HPS.

How to evaluate EPS

There are a limited number of studies focusing on the pathological features of EPS in comparison to HPS, as EPS occurs very rarely [32–36]. Tawada et al. compared non-EPS and EPS cases to identify predictive factors of EPS by analyzing the pathological findings in peritoneal biopsies [37]. It is hard to normalize the studies, as their criteria were different, i.e. sampling methods (parietal and/or visceral), participant backgrounds (genetic factor, diabetes, and infectious peritonitis, PD solutions and therapeutic strategy. Although specific pathological parameters have not been established yet, HPS (thickened SMC and hyalinizing PCVs), inflammation, angiogenesis, increased proliferation of fibroblasts or fibroblast-like cells, fibrin deposition, increased size of fibroblasts, mesothelial denudation, and calcification (and ossification

in some cases) were all reported to be features of EPS cases [32–36]. Otherwise, decreased cellularity and positive iron or podoplanin staining were more prevalent in EPS peritoneum [36]. However, it is still difficult to differentiate EPS from HPS even through a well-organized scoring system by the European EPS study group [36]. Here we recommend the following histological parameters to be evaluated in the pathological report for PD peritoneum biopsy samples for future studies to establish more reliable diagnostic markers or scoring system (Table 1).

Interpretation of PD-related peritoneal pathology

Is HPS a precursor lesion of EPS?

HPS is diagnosed by thickened SMC, and the decreased ratio of L/V by hyalinizing alteration found in most PD

therapy patients. Typically the average of SMC thickness increases, and the L/V ratio at PCV decreases according to PD duration (4–8 years) [6, 15]. On the other hand, the pathological definition of EPS is based on the presence of a newly formed encapsulating fibrin matrix, along with the increased size and proliferation of peritoneal fibroblasts (or fibroblast-like cells, which co-expresses podoplanin and α -smooth muscle actin), inflammatory cell infiltration, vascular and lymph-angiogenesis and fibrosis [10, 18, 32–36]. There is no definitive marker, including immunohistochemistry, for EPS cases so far, but some immuno-stains are useful for detection (Figure 2). The main lesion of EPS occurs in the visceral peritoneum, typically alongside severe forms of HPS. However, not all EPS patients show severe HPS lesions. Moreover, EPS can occur in non-PD patients such as autoimmune disease, malignancies and associated chemotherapy, infection, ascites, surgery, β -blocker administration [3]. Because of this, EPS and HPS are believed to be different disease entities, and additional initiating factors are considered to be required for the onset of

Table 1: Proposal for pathological diagnosis of HPS and EPS in PD peritoneum.

Histological parameters	Evaluation	Additional techniques	References
SMC thickness (fibrosis) ^a	Average of length or area		[15–17, 32–37]
L/V ratio at PCV (vasculopathy)	Semiquantitative grade		[15, 16, 35, 37]
Inflammation ^a			[32, 34, 36]
i) Inflammatory cell infiltration	Semiquantitative grade		
ii) Dominant cell type: neutrophilic, monocyte, etc.		CD3, CD68, etc	
Vascular and lymph angiogenesis (density and distribution) ^a	Semiquantitative grade	CD31 and Podoplanin	[8, 9, 16, 17, 19, 35–55]
Mesothelial cell denudation ^b	0 or 1	Podoplanin	[32, 34, 36]
Fibroblast swelling or fibroblast-like cell proliferation	0 or 1	Podoplanin and α -SMA	[18, 19, 36, 37]
Fibrin exudation	0 or 1	Fibrin	[32, 34, 36, 37]
Encapsulating membrane (new membrane)	0 or 1		[34, 37]
Decreased cellularity	0 or 1		[36]
Optional study			
i) Advanced glycation end products (AGEs)	semiquantitative grade	AGE	[16, 27–30, 37]
ii) Hyalinosis (SMC and vascular wall)	0 or 1	PAS	[6]
iii) ECM change	semiquantitative grade	Proteoglycan, Picrosirius red	[26, 37, 38]
iv) Calcification	0 or 1	Von Kossa	[33, 36]
v) Ossifications	0 or 1		[33, 36]
vi) Fe deposits	0 or 1	Fe	[36]
vii) Ultrastructure alteration in vessels, pericyte, FLC, etc.	semiquantitative grade	TEM	[2, 4, 19]

Peritoneal biopsies are recommended at the time of PD catheter insertion and catheter removal (protocol biopsies), or any kind of abdominal surgery.

^aImaging software is used for more precise quantification. Detachment of mesothelial cells can occur as an artifact during biopsy procedures and histological sample processing. It is often difficult to decide whether it is derived from a pathological or artificial event. HPS, hyalinizing peritoneal sclerosis; EPS, encapsulating peritoneal sclerosis; PD, peritoneal dialysis; SMC, submesothelial compact zone; L/V, luminal diameter to vessel external diameter; PCV, post-capillary venule; α -SMA, α -smooth muscle actin; AGEs, advanced glycation end products; PAS, Periodic acid–Schiff; TEM, transmission electron microscopy.

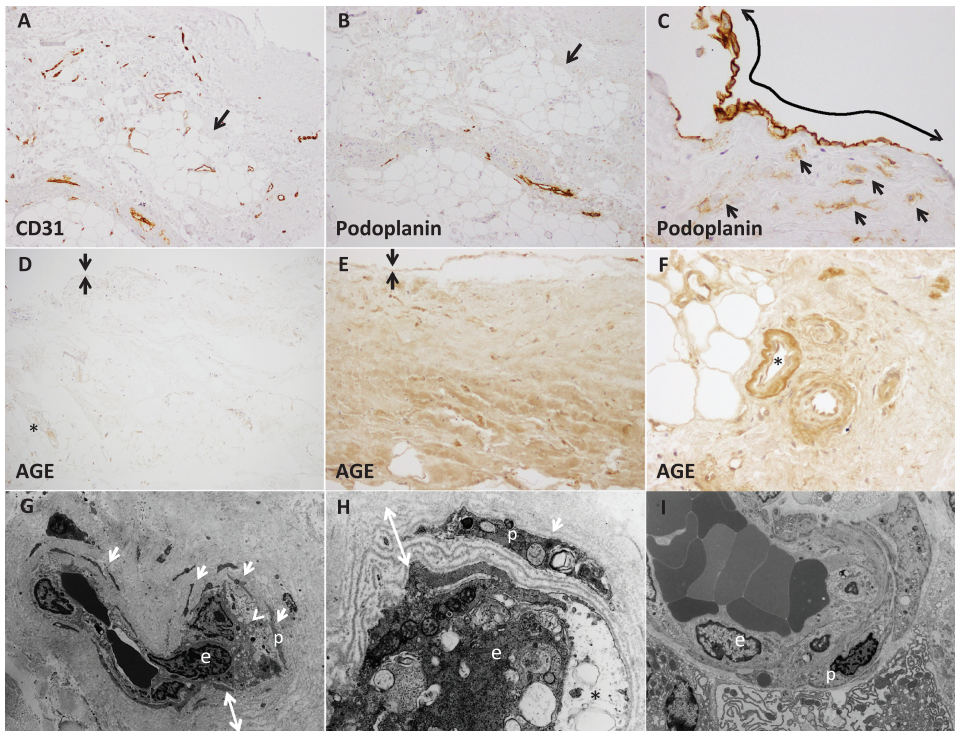


Figure 3: Useful tools for evaluation of PD parietal peritoneum.

(A) CD31 staining is used for evaluating blood vessel density. (B) Podoplanin staining showed lymph vessels in the serial section (arrow showed same lesion). (C) Mesothelial cells (double arrow) and fibroblast like cells (arrow) are positive for podoplanin staining. (D, F) Advanced glycation end product (AGE) staining revealed that AGE deposition are more intensive in the long term PD case (E, F) than in a patient before starting PD therapy (D), particularly in the interstitium and vessel walls (black arrows showed mesothelial cell layer, * showed post-capillary venule). (G, H) Transmission electron microscope (TEM) images of parietal peritoneum samples from a PD patient who dominantly used conventional PD solution, and (I) from “biocompatible” neutral PD solution. (G, H) TEM shows severe alterations with lamellar-structure on the basal lamina (double white arrow), and atrophied pericytes (p) (white arrow), on the other hands, the endothelial cell structures (e) are relatively well preserved. Cell vacuolation (v-shaped arrow) and detachment (*) were observed in the conventional PD solution case. (I) The alterations were ameliorated in the patient using more “biocompatible” PD solutions. [The images were taken from parietal peritoneum samples of non-EPS cases. (A) CD31 stain; (B, C) Podoplanin stain; (D-F) CML-AGE stain; (G-H) TEM images, $\times 2,000$, $\times 12,000$, $\times 2,500$, respectively].

EPS (2-hit theory) [38, 39]. The second hit has not yet been defined, but it is believed to be a pro-inflammatory stimulation such as infectious peritonitis, endotoxins, chemical agents, uremic toxins [40] or an endogenous receptor for advanced glycation end products (RAGE-mediated oxidative stimuli) [41] as well as genetic characteristics of RAGE [42, 43]. This concept has been supported by the fact that EPS occurs after discontinuation of PD in most cases [11], suggesting acute withdrawal of PD might be removing some protective effects of PD solution lavage against EPS in spite of the risk carried by PD itself [44, 45]. Renal transplantation might also increase the risk of EPS progression [46–49]. The pro-fibrosis effect of immunosuppressive medication in transplant patients such as calcineurin inhibitor, which induces transforming growth factor- β (TGF- β) [50], could be associated with EPS development in kidney recipients in addition to

acute PD withdrawal. However, a recent study revealed that there was no significant morphological difference between post-transplant and non-transplant EPS cases, except that the time required for EPS development was shorter in post-transplant cases [51].

A more recent approach to differentiate non-EPS from progressive EPS cases by analyzing peritoneal biopsy showed that a lower L/V ratio of PCVs and fibrin deposition could be predicting factors of EPS, suggesting vascular endothelial damage cause fibrin leakage and promotion of EPS [37]. Notably the authors also confirmed new membrane formation not only in progressive EPS cases but also in non-EPS cases [37] as in a previous study [34]. These findings are compatible with clinical suggestions that the new membrane could exist even in non-EPS cases and may be visualized laparoscopically [24]. Also a lower level of CA125 and

higher levels of the inflammatory cytokine Interleukin-6 (IL-6) in PD effluent have been reported as potential diagnostic markers for EPS [52]. Dynamic MRI scanning could also be useful to detect early stages of EPS [53]. Further studies are needed to confirm these suggestions, however, peritoneal biopsy is essential for diagnosis, particularly in high-risk cases.

Pathological findings of PD associated infectious peritonitis

Peritonitis is a common complication in PD, and important risk factor (2nd hit) in the development of EPS [44], particularly peritoneal infections by *Pseudomonas* spp., and fungi [8, 9, 54, 55]. Representative pathological findings of severe peritonitis are fibrin exudation associated with strong inflammation in the acute phase [37], which causes mesothelial cell injury and interstitial fibrosis [56]. Such fibrin exudation and subsequent fibrosis are similarly found in EPS cases as described above. The inflammation and endothelial injury caused by peritonitis or other factors are believed to cause high vascular permeability and fibrin exudation based on PD related vasculopathy in progressive EPS cases. Repetitive peritonitis also causes angiogenesis, with vessels expanding beyond the three distinct vascular layers found in normal peritoneum [17, 57]. IL-6 is thought to be a mediator of increased solute transport related to infection, seen in an IL-6 KO peritonitis model [58], and supported by clinical findings of elevated level of IL-6 in PD effluent [59]. Recent clinical studies are compatible with those findings; recurrent peritonitis may cause increased permeability and decreased UF in time dependent manner after episode of peritonitis [60, 61]. The activated peritoneal macrophages are involved via producing fibrosis chemokine (C-C motif) ligand 18 (CCL18) [62, 63], and high level of CCL15 in PD effluent was detected together with elevated IL-6 and monocyte chemoattractant protein-1 (MCP-1) [64]. Otherwise inflammation derived fibrosis formation are produced by tumour necrosis factor-like weak inducer of apoptosis (TWEAK)/Fn14 [65] and T helper 17 cell/IL-17 [66] (for review [67]).

Which is the main pathophysiological factor to evaluate peritoneal function in conventional PD era?

The peritoneal equilibration test (PET) with keratinize concentration in the dialysate is globally used to estimate solute clearance and ultrafiltration (UF) capacity

(peritoneal function) in PD patients. In general, a greater rate of membrane solute transport (high or fast PET) tends to show enhanced clearance of small solutes, and poor UF [68]. PET test has already been established in the conventional PD (higher GDP, acidic pH, lactate buffered) era as a standard tool for the management including prescription and discontinuation of PD. As far as conventional PD era, it is well known that increased solute transport and decreased UF in accordance to PD duration is a risk for patient prognosis [69, 70]. However, the view is changing due to increased use of automated peritoneal dialysis (APD) [71–73], icodextrin [74–76], and “biocompatible PD solution”, which enables lower GDP and neutral pH conditions, but still contains glucose and lactate/lactate/bicarbonate [77, 78]. These options can produce better clinical outcomes even for high-fast transporter. Histologically, prolonged exposure to conventional PD solutions causes PF, vasculopathy (HPS) as described above. Vascular proliferation (angiogenesis) coincidences with vasculopathy have been reported in PD patients with severe loss of UF capacity or with PD-related surgery [5, 6]. On the other hand, vascular density does not increase at least in uncomplicated PD patients, while severe vasculopathy (decreased L/V ratio) are found according to long-term PD durations [79–81]. One study with baseline biopsy samples showed that baseline peritoneal permeability was associated with density of CD68-positive macrophages, Interleukin (IL)-6 positive cells, CD31 positive and pathologische anatomie leiden endothelium (PAL-E) antibody positive (vesicle-associated protein-1, PV-1 positive) blood vessels [82]. High level of vascular endothelial growth factor (VEGF) in peritoneum and blood induced via AGE-RAGE signaling should be associated with angiogenesis and decreased UF in PD patients [83, 84]. Numerous studies have revealed signaling pathways of angiogenesis and fibrosis in the context of epithelial mesenchymal transition (EMT) (mesothelial cell to myofibroblast) associated with PD-derived peritoneal injury (for review [67, 85]). However, the primary origin of myofibroblast in PD peritoneum is argued by recent insight [86].

Otherwise, the dialysate level of connective tissue growth factor (CTGF), which inhibits bone morphogenetic protein-7 (BMP-7) and activates transforming growth factor (TGF)- β (pro-fibrosis factor), was shown be correlated to high-fast PET [87]. Furthermore CTGF mRNA level in the peritoneum from PD patients with UF failure were also higher, and was correlated with SMC thickness [87]. Aquaporin (AQP)-1 null mice study showed that AQP-1 expressed in peritoneal endothelium plays an essential role in water permeability and UF

during PD [88]. However, recent analysis with biopsy samples from EPS patients showed that AQP-1 expression did not change between EPS and long-term PD or uremic peritoneum, instead of significant upregulated VEGF and endothelial nitric oxide synthase (eNOS) with increasing vascular density in EPS cases [89]. In this study, SMC thickness and collagen volume fraction showed significant correlation with UF failure and sodium sieving, but not with small solute transport, and these pathological parameters were significantly severe in EPS than in other groups [89]. This meaningful study showed that UF capacity and sodium sieving could be used as a predicting risk factor for EPS, however, SMC thickness and collagen in EPS cases should have been measured from existing peritoneum (HPS lesion) excluding newly formed membrane of EPS (the study included EPS membrane in the pathological analysis), because the EPS membrane does not reflect functional test histories before the lesion is formed. Otherwise, higher levels of lymphangiogenesis mediator vascular endothelial growth factor-C (VEGF-C) is detected from biopsy samples (expressed in mesothelium cells and macrophages), dialysate effluents, and mesothelial cells in vitro (TGF- β dose dependent manner) particularly in patients with UF failure, suggesting lymphangiogenesis associated with TGF- β signal could be related to UF failure [90].

Can biocompatible PD solution (lower GDP and neutral pH) ameliorate PD-related alterations?

Conventional PD solutions are bioincompatible due to their high glucose, GDP, lactate, acidic pH and high osmolality. The pathophysiological impacts of this on PD patients have been confirmed in several animal and human studies, including pathophysiological findings described above. One of the main factors is AGE-RAGE signaling, and AGE accumulation in the peritoneal interstitium and vessel walls [27–29] (Figure 3D–F) with ultrastructural alterations [2–4] (Figure 3G, H). At least four studies have revealed that milder HPS lesions are found in biopsy samples from PD patients using biocompatible PD solutions when compared to conventional solution [16, 91–93]. One multi-center study showed no significant difference in SMC thickness between conventional ($n=80$) and biocompatible ($n=61$) solutions groups, but did show significantly less vasculopathy in the biocompatible group [92]. Furthermore, we are now seeing milder ultrastructural alterations of endothelial cells,

pericytes, and collagen fibers, which could be attributed to the use of biocompatible PD solutions (Figure 3G–I, unpublished data). These ultrastructural findings might be linked not only to former pathological studies [16, 91–93] but also to clinical findings following the use of biocompatible solutions, for example, preserved UF capacity despite increasing small solute transport [94, 95]. However, the impact on peritoneal membrane transport function has not been quantified by meta-analysis [96–98]. Last but not least, biocompatible PD solution is not fully biocompatible, and still needs to be improved to counter unsolved problems such as infection, PD membrane preservation, and patient survival (for review [57]). One prospective observational study, “Neutral solution, Extraneal use, and current PD outcome in Japan (NEXT-PD)” [99] showed that multidisciplinary approaches including the use of biocompatible PD solution, reduction of high glucose solutions, introduction of an incremental prescription style [100], combination therapy (PD and hemodialysis) [101], and planned PD discontinuation for long-term cases with membrane damage [101] contributed to a decreased incidence of EPS [99, 102]. One systematic pathological analysis on healthy peritoneum including a wide range of ages (0.1–60 years old) showed that age dependent vascular and lymph blood vessel densities form three distinct layers together with nerve fibers, related to SMC thickness without inflammation, pro-fibrosis and mesothelial cell translocation [17]. Another systematic review of peritoneal vascular anatomy and function also highlights that the peritoneal vascular plexus and vascularization is under the influence of neurological factors as well as other physical, chemical, and hormonal factors [103]. Furthermore, recent image analysis tools and methodologies enable us to overcome semiquantitative evaluation, i. e. vascular network analysis, collagen tissue quantification, computer-assisted quantification of immuno-stains [104–106]. These studies suggest important areas for future study in order to better understand the pathophysiological impact of biocompatible PD solutions.

Summary

HPS and EPS are two peritoneal alterations associated with PD therapy. PD associated peritonitis is a common complication and one of the most considerable risk factors (2nd hit) for EPS in PD patients. Peritoneal biopsies can produce important information for PD management, including pathological diagnosis of HPS, EPS, and

peritonitis, evaluation of membrane function, and planning of PD withdrawal, however, it is not currently performed routinely in PD patients. The pathophysiological impact of currently available “biocompatible” PD solutions could be different from conventional solutions. A multi-disciplinary approach should be used for all patients where possible to reduce the complications of PD therapy. Peritoneal biopsy and laparoscopic findings in collaboration with basic and clinical research has an important part to play in understanding the pathophysiology of PD in more detail, and will lead to improved safety and more stable PD therapy for end stage of renal disease patients.

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