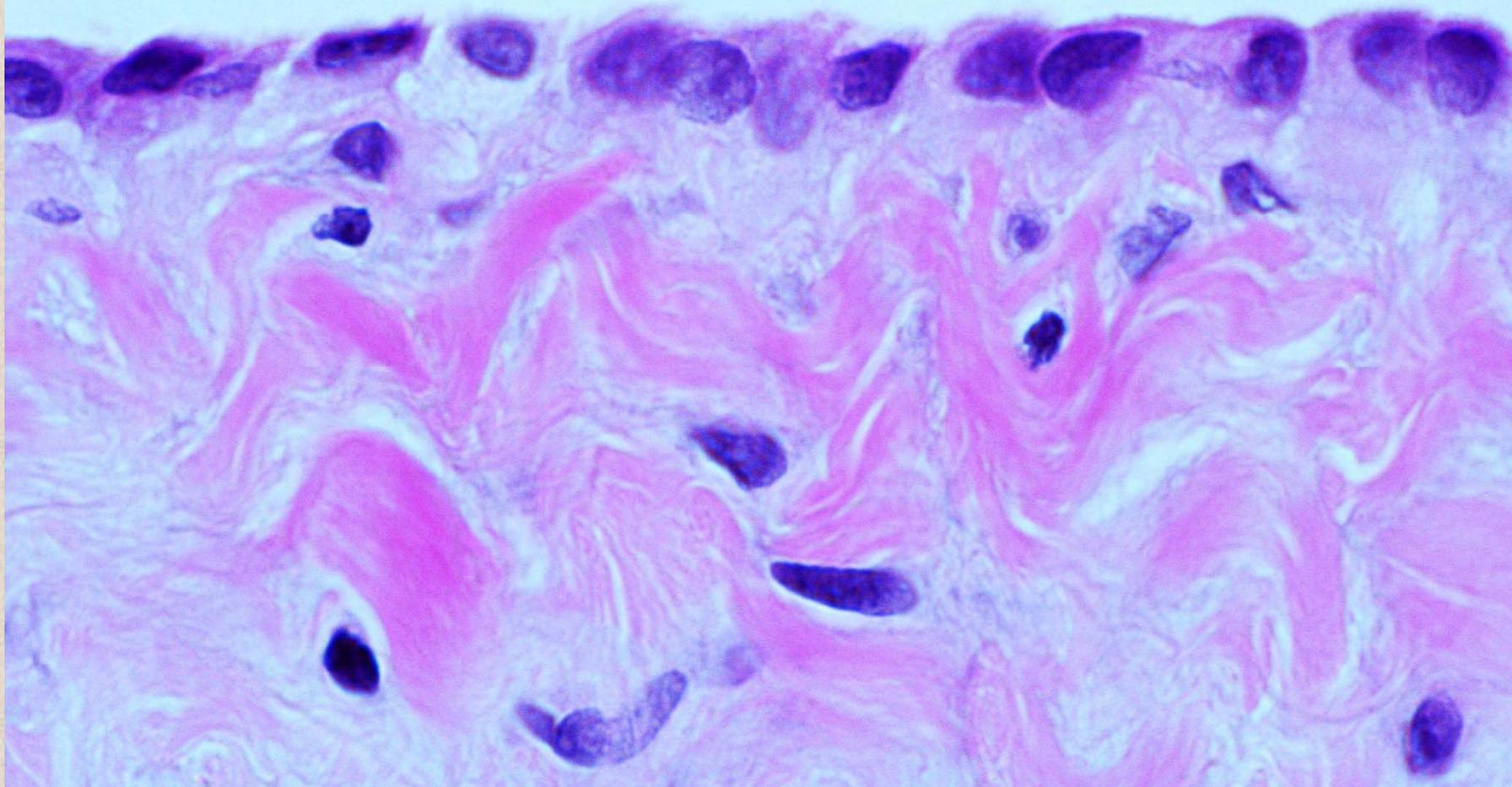


Análisis de biocompatibilidad a través de la biopsia peritoneal

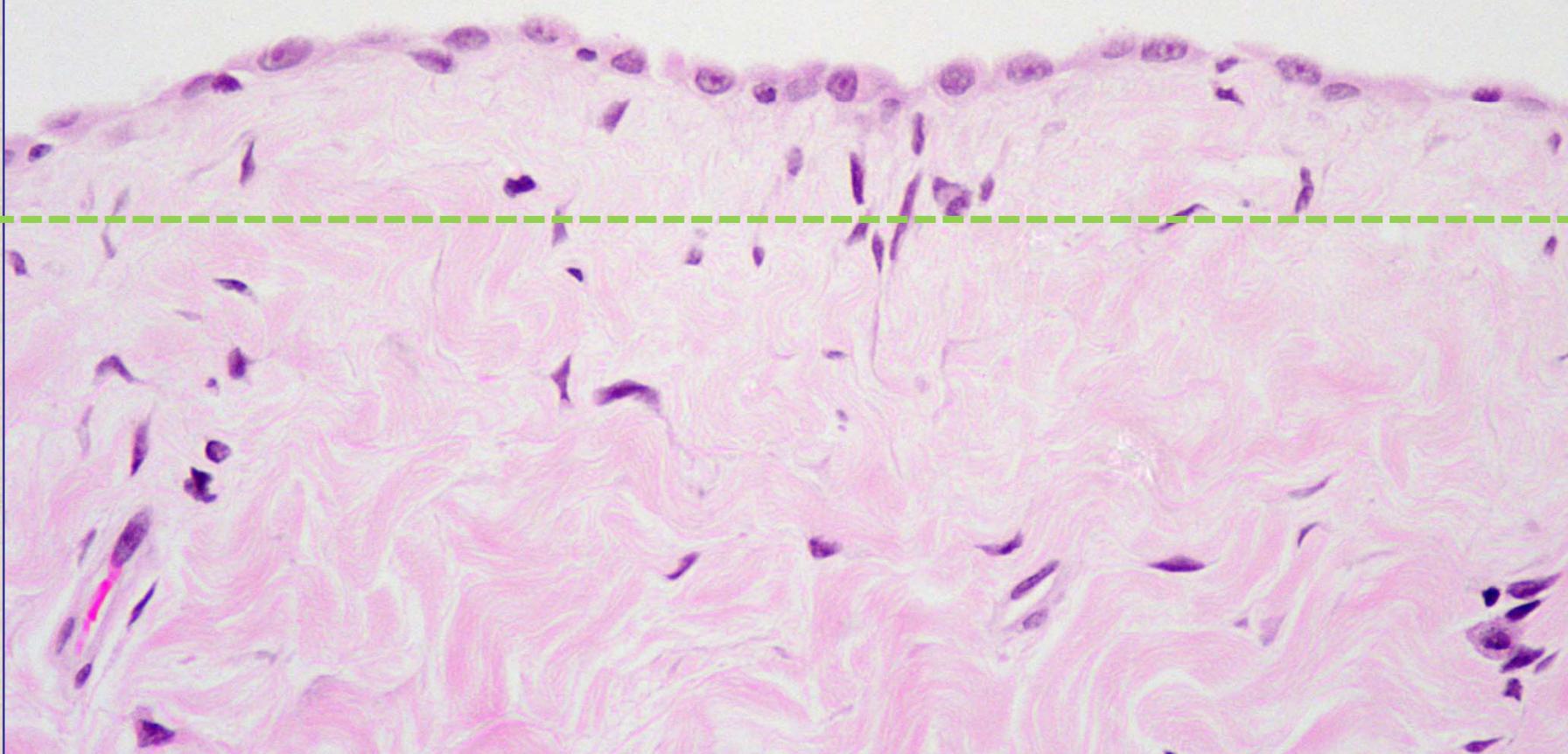
José A. Jiménez Heffernan
Hospital La Princesa, Madrid



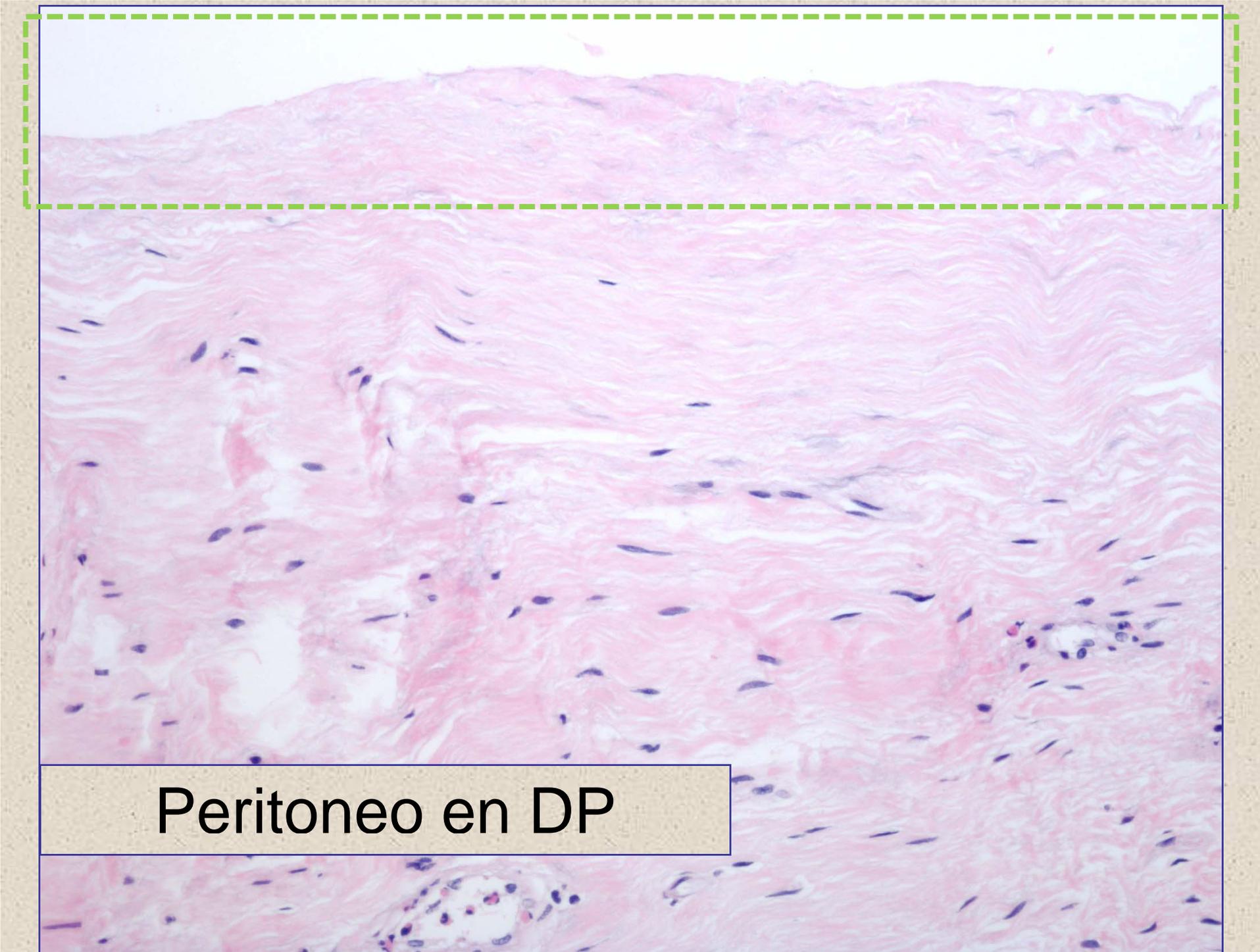
Las anomalías en la función peritoneal se relacionan con alteraciones estructurales de la membrana

Cambios relacionados con el uso de líquidos convencionales

- Pérdida de mesotelio
- Fibrosis submesotelial
- Vasculopatía hialinizante
- Angiogénesis

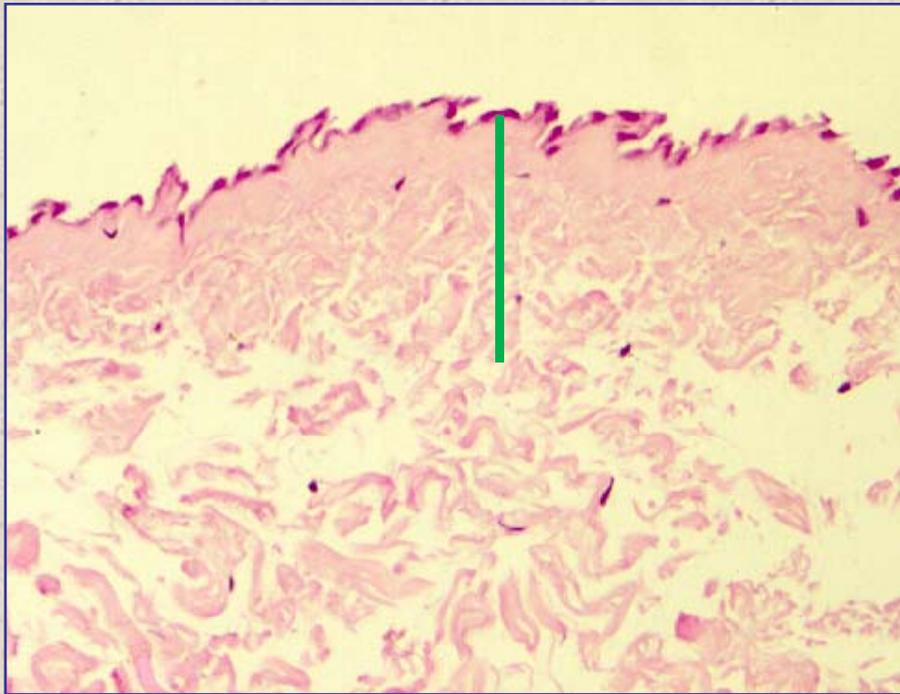


Peritoneo normal

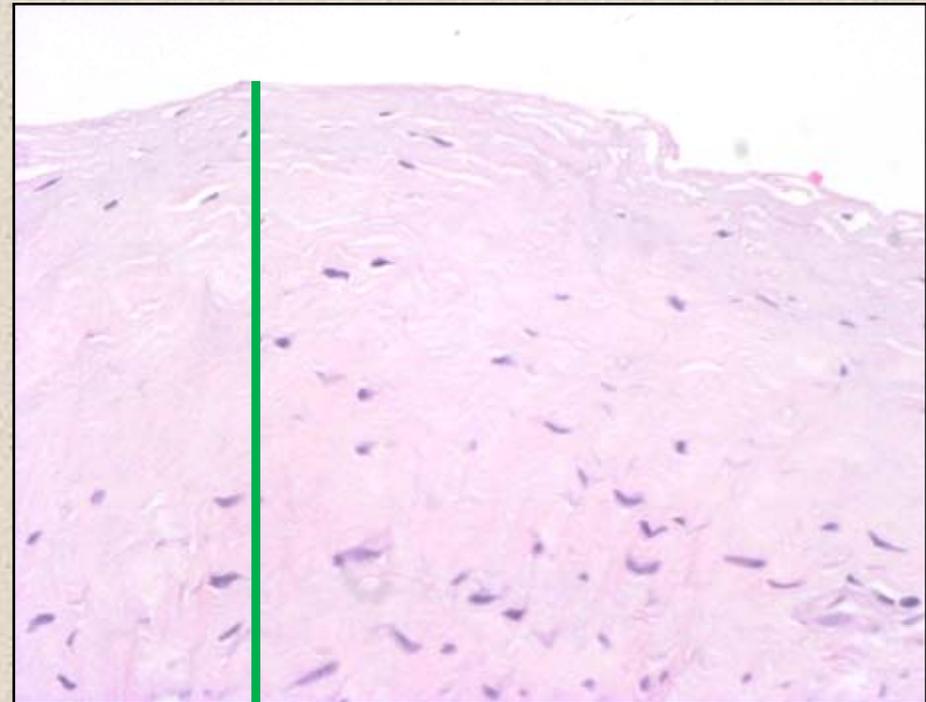


Peritoneo en DP

Fibrosis submesotelial (esclerosis)



Normal



DP

Vaculopatía Hialinizante



Reduplicación de la membrana basal endotelial

Biocompatibilidad: niveles de evidencia

- In vitro and ex vivo
- In vivo, con modelos animales
- **In vivo, con biopsias de pacientes en DP**

Estudios in vivo con modelos animales

- Garosi et al. PDI 1998 (conejo)
- Hekking et al. JASN 2001 (ratas)
- Mortier et al. KI 2004 (ratas)
- Musi et al. PDI 2004 (ratas)
- Zareie et al. PDI 2005 (ratas)
- Krediet et al. NDT 2008 (ratas)
- Van Westrhenen et al. PDI 2008 (ratas)
- De Graaff et al. PDI 2010 (ratas)

Soluciones BC evaluadas

- 
- PD solution with 1.1% aminoacids
 - Bicarbonate/lactate buffered solution (Physioneal)
 - Amino-acid based (Nutrineal)
 - Low GDP PD fluid (Gambrosol trio)
 - Sterile-filtered without GDP PD fluid
 - Glucose free lactate-buffered PD fluid
 - Pyruvate-buffered PD fluids
 - GLAD solution: aas, dextrose, glycerol

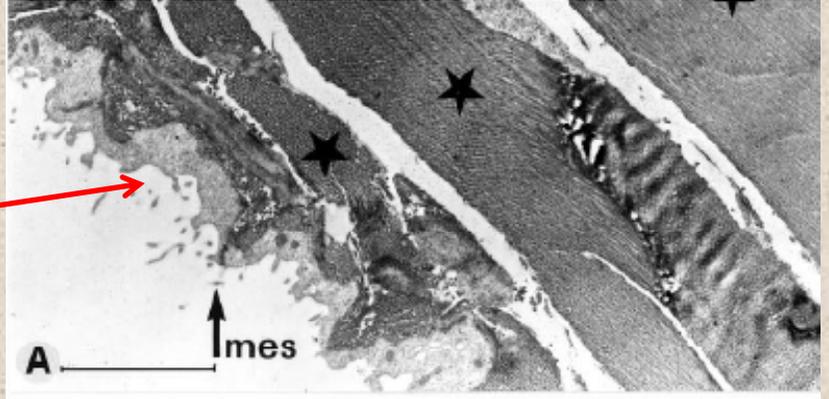
Cambios en modelos animales (BC vs BIC)

	Daño mesotelial	Fibrosis	Angiogénesis
Garosi et al	< BC	No diferencias	No diferencias
Hekking et al	< BC	Mínima diferencia (ns)	< BC
Mortier et al	< BC	< BC	< BC
Musi et al	< BC	No diferencias	No diferencias
Zareie et al	< BC	< BC	< BC*
Krediet et al	< BC	< BC	< BC
van Westrhenen et al	No evaluado	Variable	< BC
de Graaff et al	No evaluado	< BC	< BC

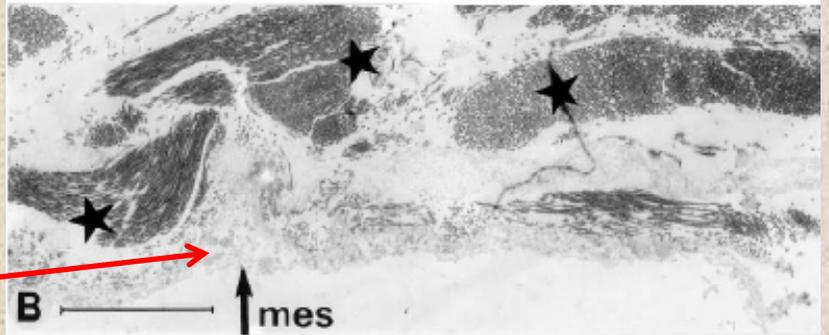
Los líquidos BC inducen menos daño mesotelial

Hekking et al. JASN 2001

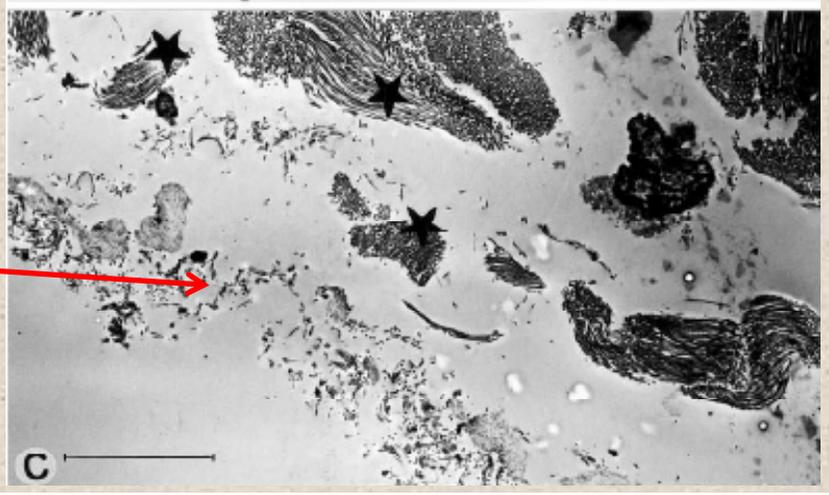
Célula mesotelial normal
(microvellosidades)



Célula mesotelial
modificada (presente
pero con pérdida de
microvillosidades)

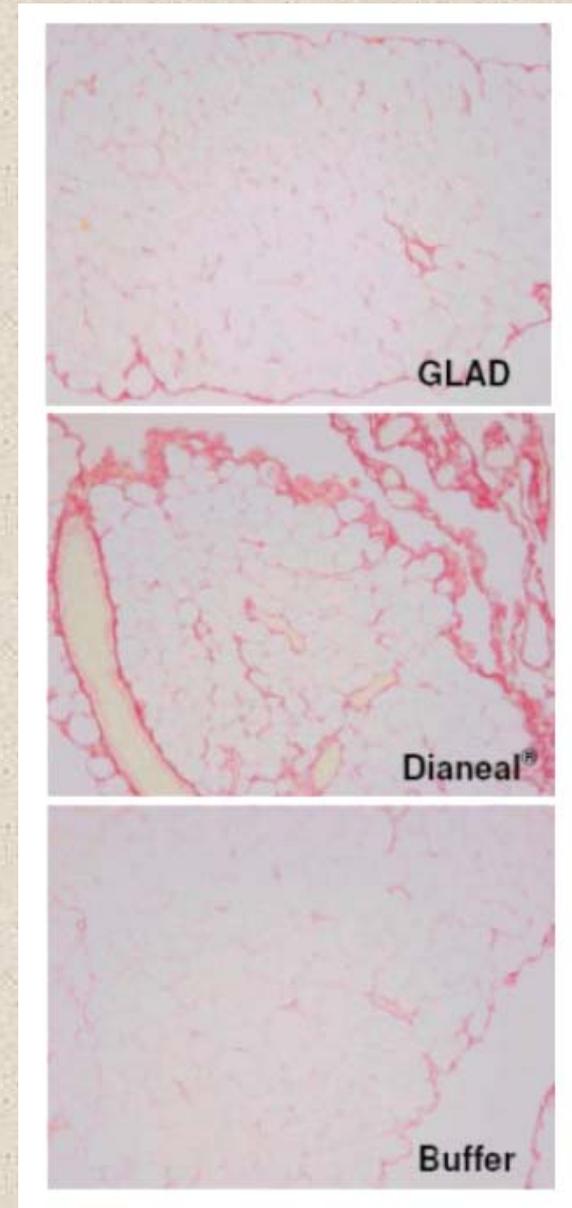
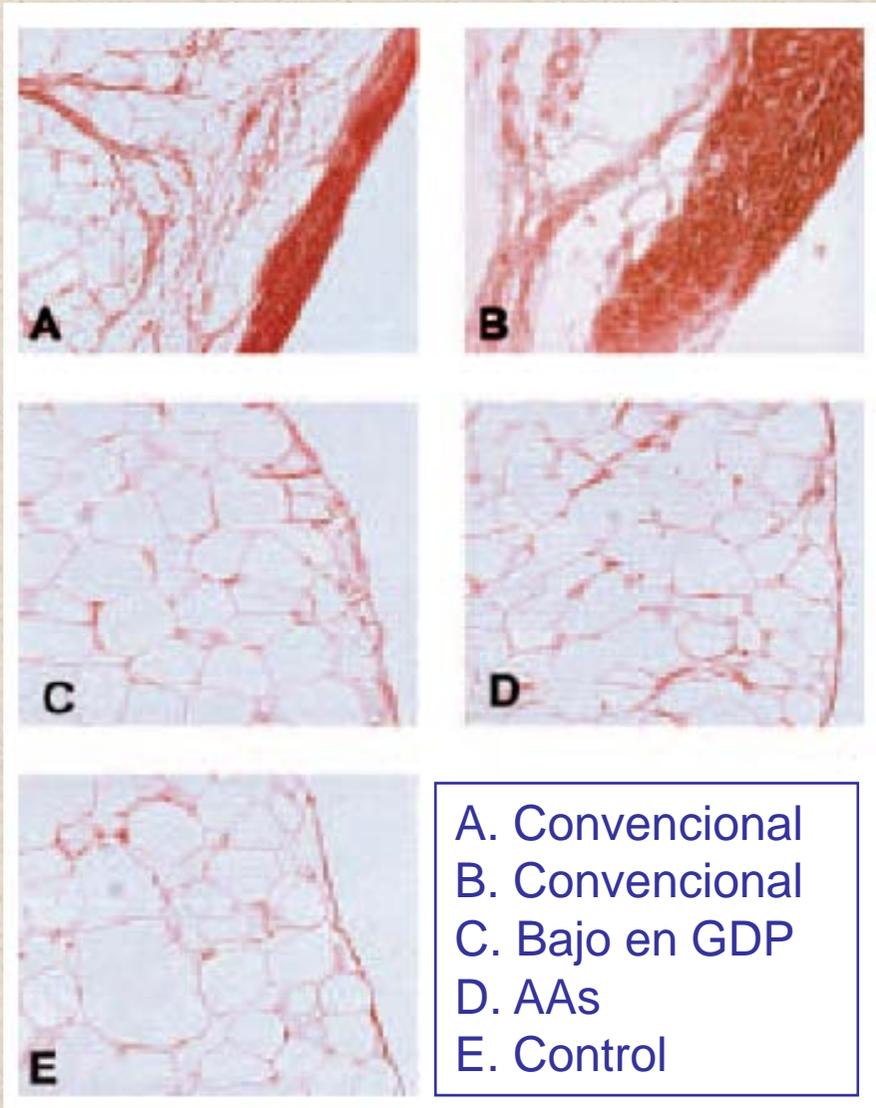


Células mesoteliales
necróticas



Los líquidos BC inducen menos fibrosis

Mortier et al, KI 2004; De Graaf et al PDI 2010



Histoquímica: Sirius red (collagen)

En relación a la fibrosis los resultados son variables

- *Garosi et al: no diferencias*
- *Musi et al: no diferencias*
- *Hekking et al: diferencias no significativas estadísticamente*
- *Van Westrhenen et al: fibrosis variable según zonas*

Las soluciones BC inducen menor angiogenesis

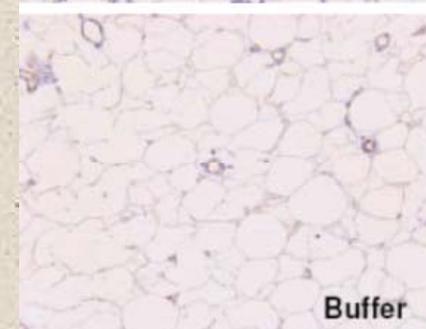
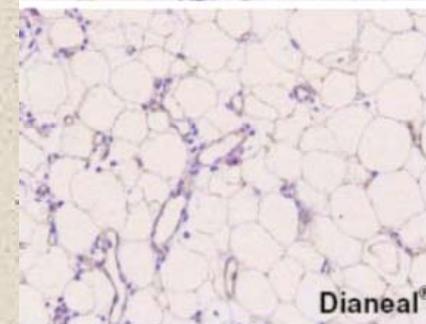
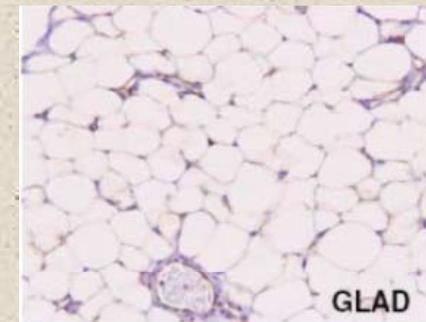
Mortier et al KI, 2004

	Dianeal® (N = 10)	Physioneal® (N = 10)	Nutrineal® (N = 9)	Earle's Balanced Salt Solution (EBSS) (N = 10)
Blood vessels number/mm ²	174 ± 17 ^a	97 ± 9	102 ± 2	95 ± 5

De Graaff et al PDI 2010

Quantification of the Amount of Fibrosis in the Omentum and the Number of Blood Vessels

	GLAD (n=11)	Dianeal (n=7)	Buffer (n=13)
Submesothelial area	1±1	2±1 ^a	1±0
Intersegmental area	2±1	3±0 ^b	2±0
Perivascular area	1±1	2±1 ^b	1±0
Over-all fibrotic score	4±1	7±2 ^b	4±1
Vessels per microscopic field (n)	20±9	35±8 ^b	17±5



Resumen de los estudios in vivo en modelos animales

- Los líquidos BC producen menos daño mesotelial (hallazgo universal)
- Tendencia a una menor fibrosis
- Tendencia a menor angiogénesis
- La vasculopatía hialinizante no resulta evaluable

Estudios in vivo de BC en humanos

- *Ayuzama et al.* Tokio PDI 2012
(11 pacientes)
- *Kawanishi et al.* Tokio PDI 2013
(12 pacientes)
- *Del Peso et al.* Madrid PDI 2014
(23 pacientes)

PERITONEAL MORPHOLOGY AFTER LONG-TERM PERITONEAL DIALYSIS WITH BIOCOMPATIBLE FLUID: RECENT CLINICAL PRACTICE IN JAPAN

Nobuhiro Ayuzawa,¹ Yoshitaka Ishibashi,² Yutaka Takazawa,³ Haruki Kume,⁴ and Toshiro Fujita¹

- 11 pacientes al menos tres años en DP
- 7 con tto. mixto DP/HD
- Lactato-buffered, pH neutro, solución baja en GDP
- No grupo control
- Evalúan fibrosis y vasculopatía pero no integridad mesotelial

- Menor fibrosis (APT: 180 μm , 96-1424)
- Menor vasculopatía
- Al no existir control comparan con los valores de normales de otros estudios (*Williams et al*, *Honda et al*)
- No mención al mesotelio

Good news but limited because of low number of patients, hybrid treatment and no control group

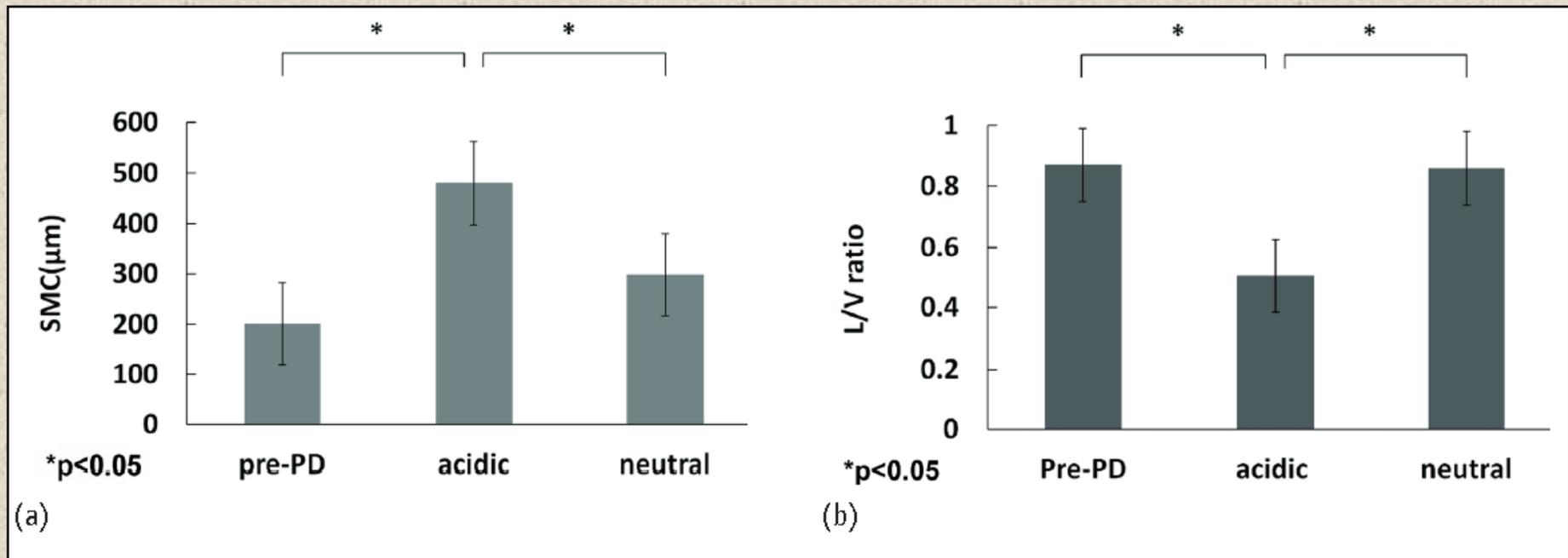
ORIGINAL ARTICLES

NEUTRAL SOLUTION LOW IN GLUCOSE DEGRADATION PRODUCTS IS ASSOCIATED WITH LESS PERITONEAL FIBROSIS AND VASCULAR SCLEROSIS IN PATIENTS RECEIVING PERITONEAL DIALYSIS

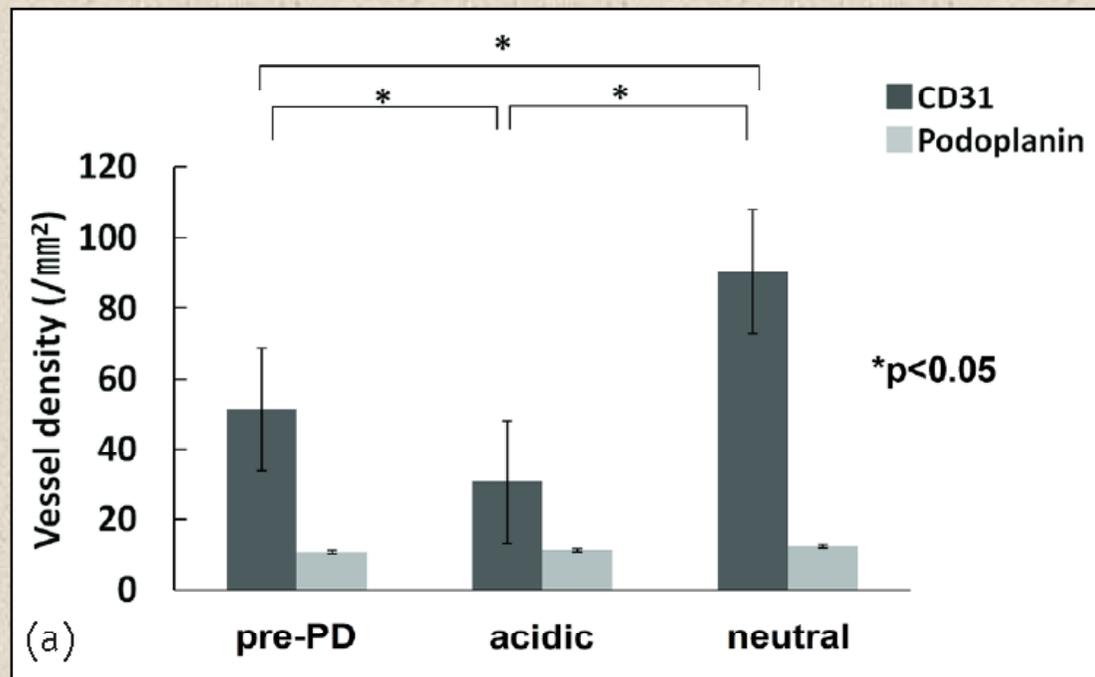
Kunio Kawanishi,¹ Kazuho Honda,² Misao Tsukada,¹ Hideaki Oda,² and Kosaku Nitta¹

- 1) Grupo de soluciones ácidas (Dianeal): 12 pacientes
- 2) Grupo de soluciones neutras: 12 pacientes
- 3) Grupo control

Variable	Dialysate type		<i>p</i> Value
	Acidic	Neutral	
Patients (<i>n</i>)	12	12	
Mean age (years)	46.1±5.3	43.0±5.3	NS
Sex (men/women)	7/5	9/3	NS
Primary disease of renal failure			
CGN	8	9	NS
Diabetes mellitus	1	1	
Others	3	2	
PD duration (months)	57.0±5.97	51.9±5.9	NS
Use of high glucose solution [<i>n</i> (%)] ^a	8 (66.7)	7 (58.3)	NS
Ultrafiltration failure [<i>n</i> (%)] ^b	8 (66.7)	3 (25)	0.0128
Mean RRF (mL/day)			
First 6–12 months of PD	512.5±95.2	580±95.1	NS
Last 6–12 months of PD	52.5±42.7	214.1±42.7	0.0139
Ultrafiltration (mL/day)			
First 6–12 months of PD	787.5±96.1	691.7±96.1	NS
Last 6–12 months of PD	224.2±77.6	578.4±77.5	0.0039
PET D/P Cr			
First 6–12 months of PD	0.61±0.03	0.58±0.03	NS
L/LA/HA/H	2/5/5/0	3/6/2/1	
Last 6–12 months of PD	0.76±0.03	0.62±0.03	0.0042
L/LA/HA/H	0/3/5/4	1/7/3/1	
Use of RAAS inhibitor	6 (50)	8 (66.7)	NS
Use of statin	3 (25.0)	4 (33.3)	NS
Use of vitamin D	7(58.3)	7 (58.3)	NS



- El grupo BC tenía menos fibrosis (482 vs 281 vs 201 μm)
- El grupo BC tenía menos vasculopatía (lumen/vessel ratio: 0.5 vs 0.86 vs 0.87)
- No evalúan la integridad mesotelial



Resultado inesperado:

La vascularización (angiogénesis) era mayor en el grupo BC (sin correlación con permeabilidad peritoneal)

Resumen de los dos estudios con biopsias de pacientes en DP

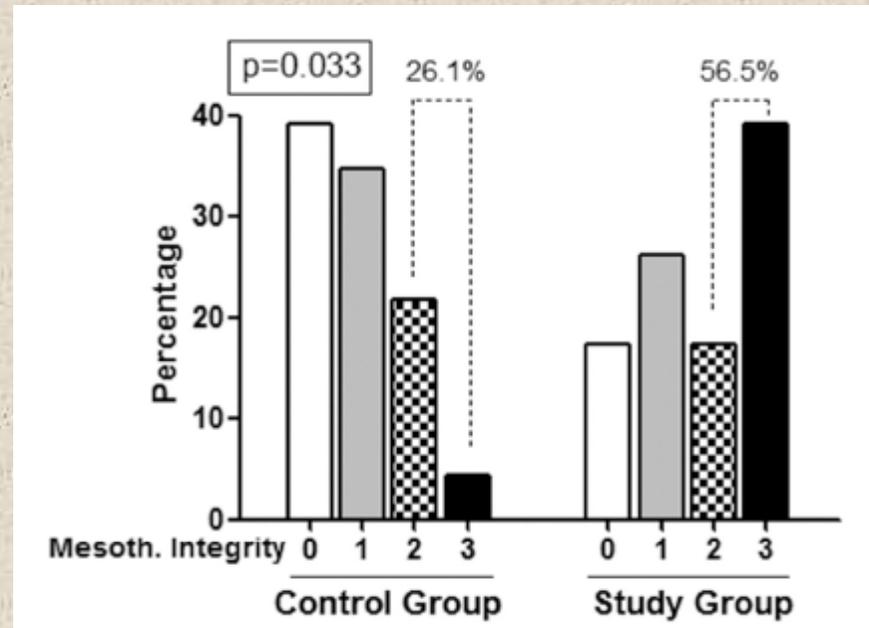
- Los líquidos BC parecen producir menor fibrosis
- Inducen menos vasculopatía
- Resultados no esperados en cuanto a vascularización
- No se analizó la integridad mesotelial

Del Peso et al. Grupo de
estudios peritoneales de Madrid
(Rafael Selgas)

“Biocompatible dialysis solutions
preserve peritoneal mesothelial
cell and vessel wall integrity. A
case-control study on human
biopsies”

BIOCOMPATIBLE DIALYSIS SOLUTIONS PRESERVE PERITONEAL MESOTHELIAL CELL AND VESSEL WALL INTEGRITY. A CASE-CONTROL STUDY ON HUMAN BIOPSIES

Gloria del Peso,¹ José Antonio Jiménez-Heffernan,² Rafael Selgas,¹ César Remón,³ Marta Ossorio,¹
Antonio Fernández-Perpén,⁴ José Antonio Sánchez-Tomero,⁴ Antonio Cirugeda,⁵
Erika de Sousa,¹ Pilar Sandoval,⁶ Raquel Díaz,¹ Manuel López-Cabrera,⁶
and María Auxiliadora Bajo¹



Pacientes

- Grupo BC (n=23): 7 Physioneal (Baxter), 5 Balance, 6 Bicavera y 5 GambrosolTrio (Fresenius)
- Grupo control BiC (n=23): 20 Dianeal (Baxter) y 3 Stay-safe (Fresenius)
- Mismo tiempo en DP (24.2 ± 18 y 22.7 ± 16 meses)

Table 1. Patient characteristics at the time of peritoneal biopsy

	CONTROL GROUP (n=23)	STUDY GROUP (n=23)	P
Age (years)	43.8±14	51.5±14	ns
Male gender	12 (52%)	15 (65%)	ns
Time on PD (months)	24.2±18	22.7±16	ns
Automated PD	21 (91%)	10 (43.5%)	0.001
Accumulated glucose load (Kilograms)	139.2±151	101.08±110	ns
Diabetes	2 (8.7%)	3 (13%)	ns
Peritonitis antecedent	5 (21.7%)	11 (48%)	ns
Mean number of peritonitis	1.6±1.3	2.09±1.44	ns
Accumulated days of peritonitis	2.8±2	5.2±4	ns
Urea-MTC (ml/min)	21.5±7	23.5±4	ns
Cr-MTC (ml/min)	8.9±4	9.3±2.5	ns
UF capacity (ml/4h)	849±257	666±261	0.041
Residual renal function (ml/min)	3.22±3.46	5.08±3.5	ns

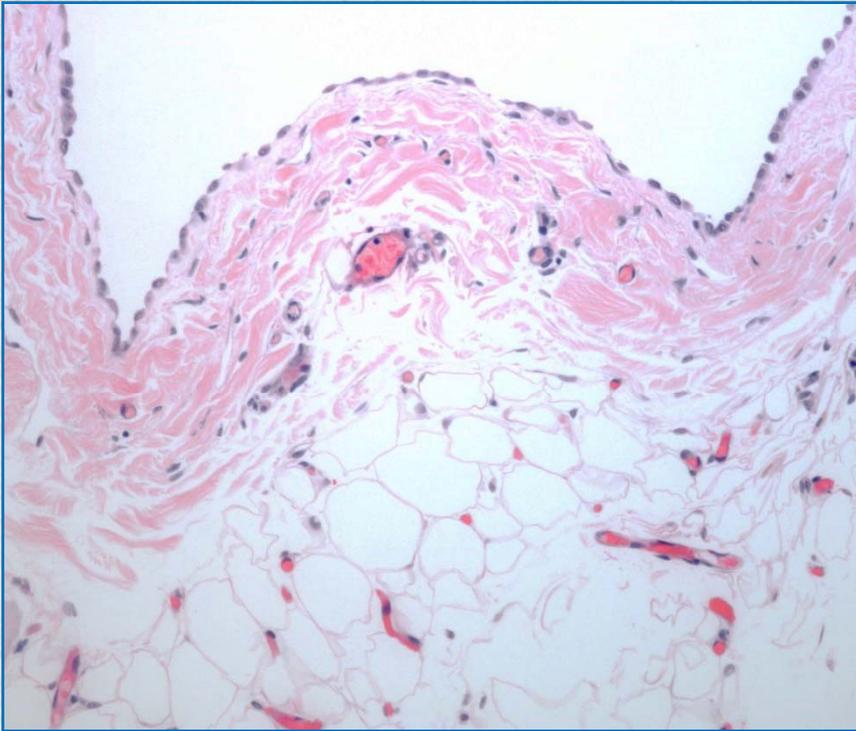
MTC= mass transfer coefficient. UF= ultrafiltration

Parámetros morfológicos evaluados

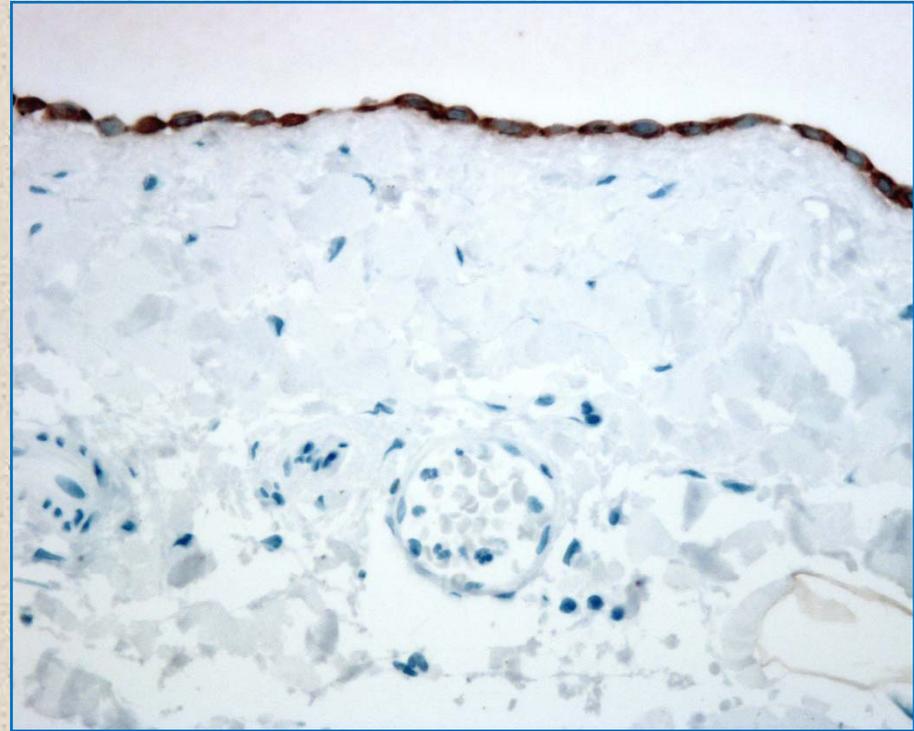
- Integridad mesotelial
- Espesor submesotelial (fibrosis)
- Vasculopatía hialinizante
- In vivo EMT (fibroblastos queratina+)

Integridad mesotelial

Análisis semicuantitativo (grado 3, normal; grado 0 (ausencia total). Tal y como describe *Plum et al* (KI 2001)

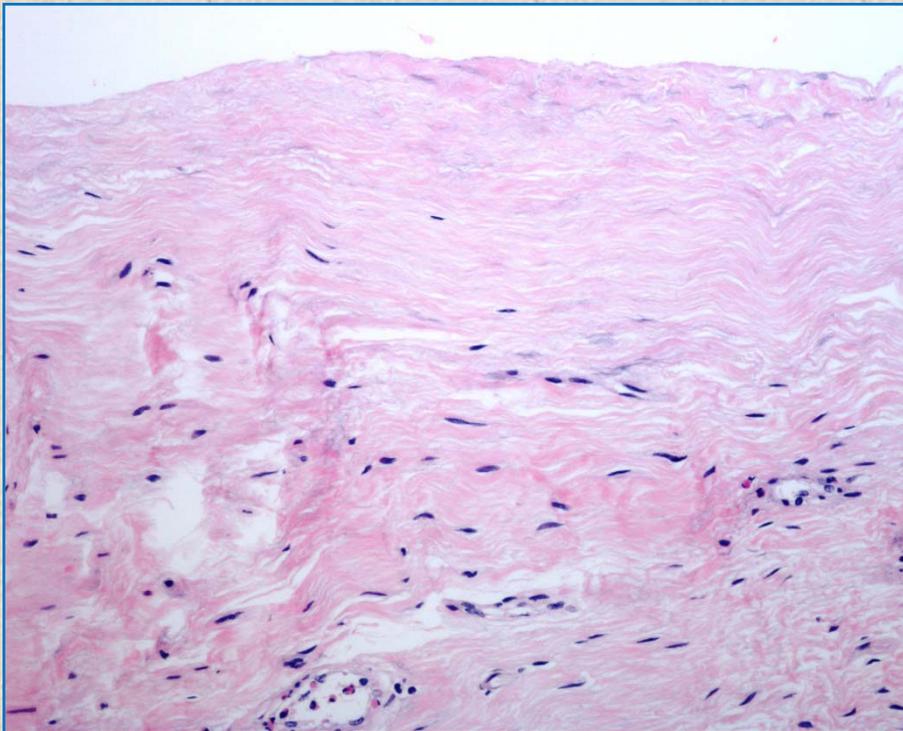


Grade 3

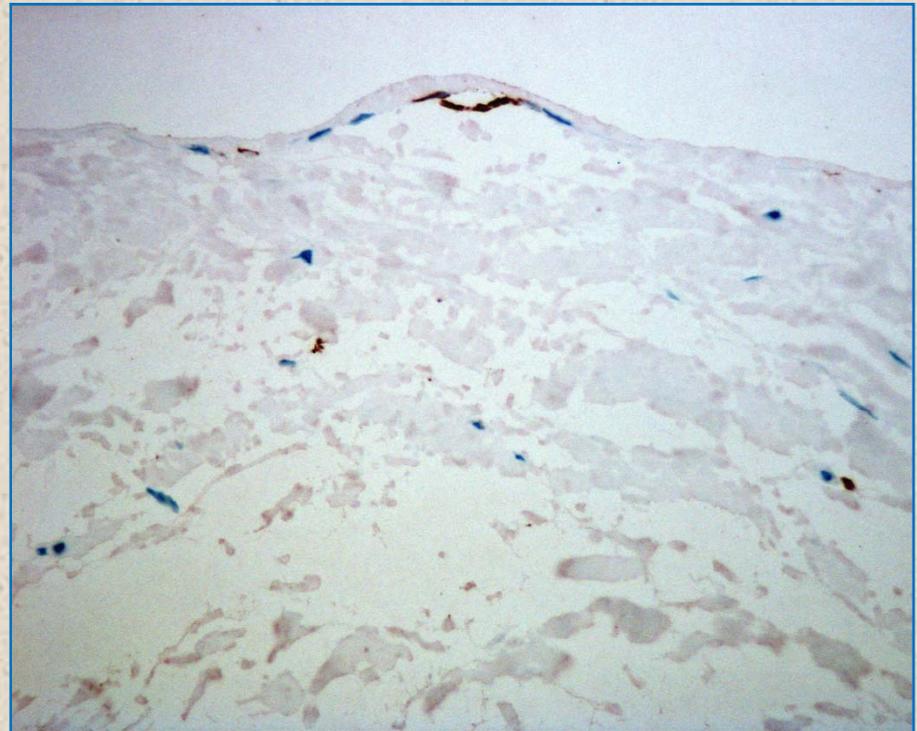


Grade 3

- Mesothelial cell integrity. It was measured using a semiquantitative scale (grade 3, normal cell density; grade 0, complete denudation) as described by Plum et al (11).

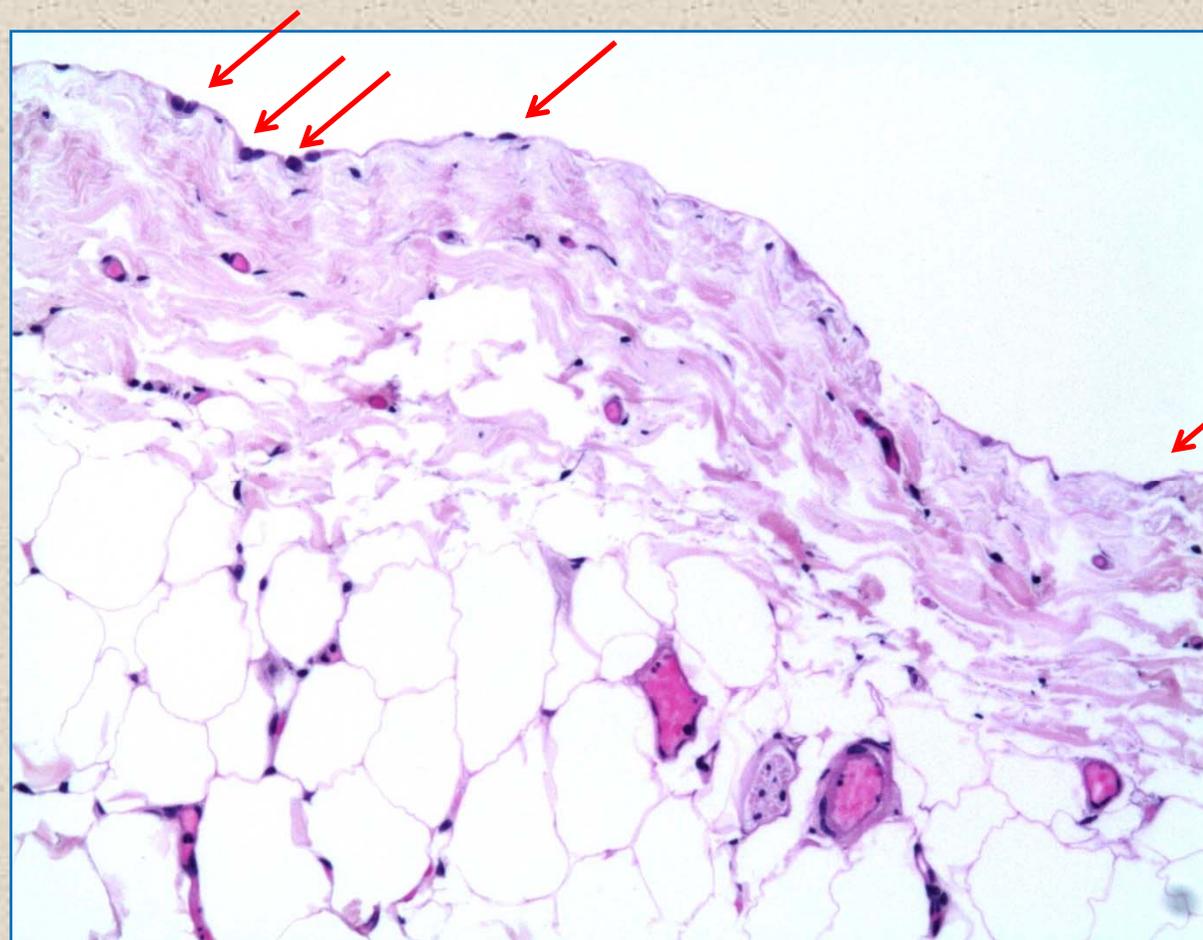


Grade 0

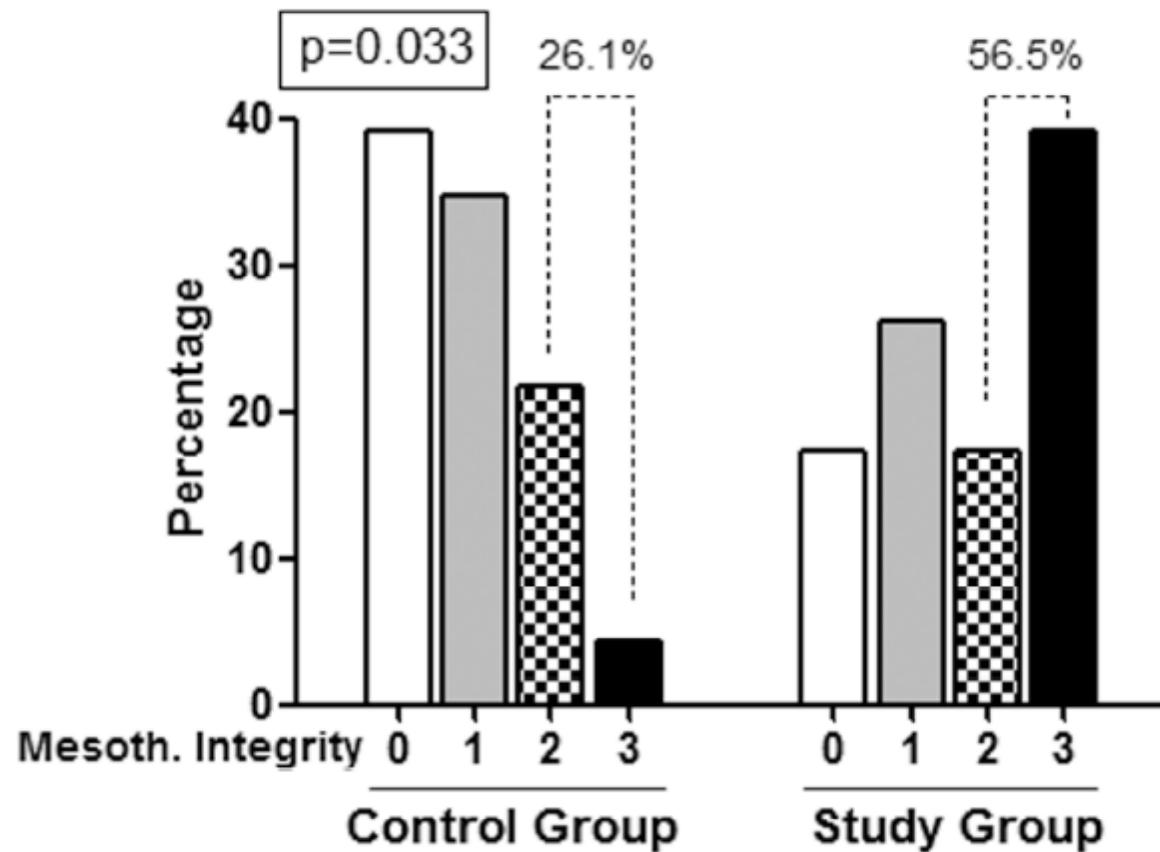


Grade 0

- Mesothelial cell integrity. It was measured using a semiquantitative scale (grade 3, normal cell density; grade 0, complete denudation) as described by Plum et al (11).



Grade 1



Patients in SG showed significantly greater mesothelial cell preservation score than those in CG (mean values 1.78 ± 1.16 vs. 0.91 ± 0.9 , $p=0.007$). 56.5% of SG patients showed total or partial preservation of mesothelial cells (score 2-3), in contrast with 26.1% of patients in CG ($p=0.036$) (**Figure 2**).

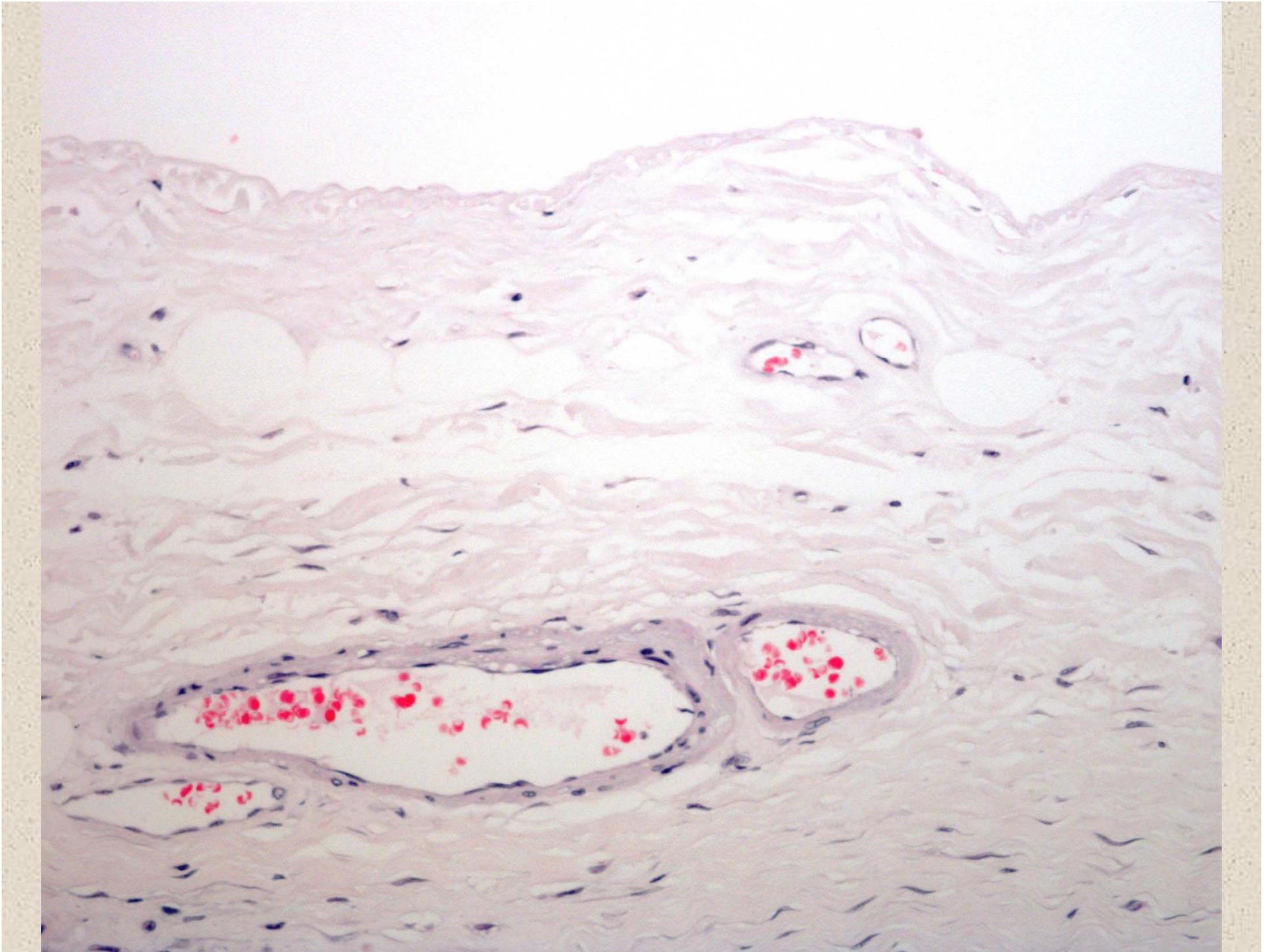
Espesor submesotelial (fibrosis)

- Era menor en el grupo BC pero sin alcanzar significación estadística (47.8% en BC vs 69.6% en control BiC, ns)
- Los valores extremos de fibrosis (Grado 3, $>350 \mu\text{m}$) eran menos frecuentes en el grupo BC, pero de nuevo sin significación (8.7% vs 17.4%, ns)
- Cuando se excluyen aquellos pacientes con episodios previos de peritonitis se observó menor fibrosis en el grupo BC (41.7% vs 77.8%, $p=0.04$)

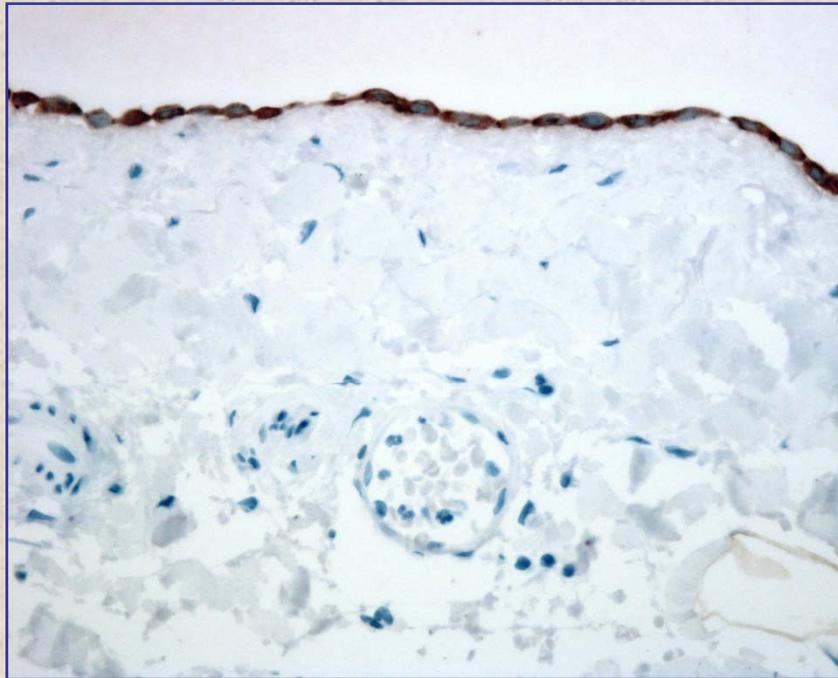
Vasculopatía hialinizante

- Hyalinizing vasculopathy (HV). It was measured using the four grade system described by *Honda* et al (12): grade 0, no abnormalities; grade 1, mild thickening without stenosis of the lumen; grade 2, moderate thickening with partial luminal stenosis; and grade 3, intense thickening with marked stenosis and luminal distortion or complete occlusion.

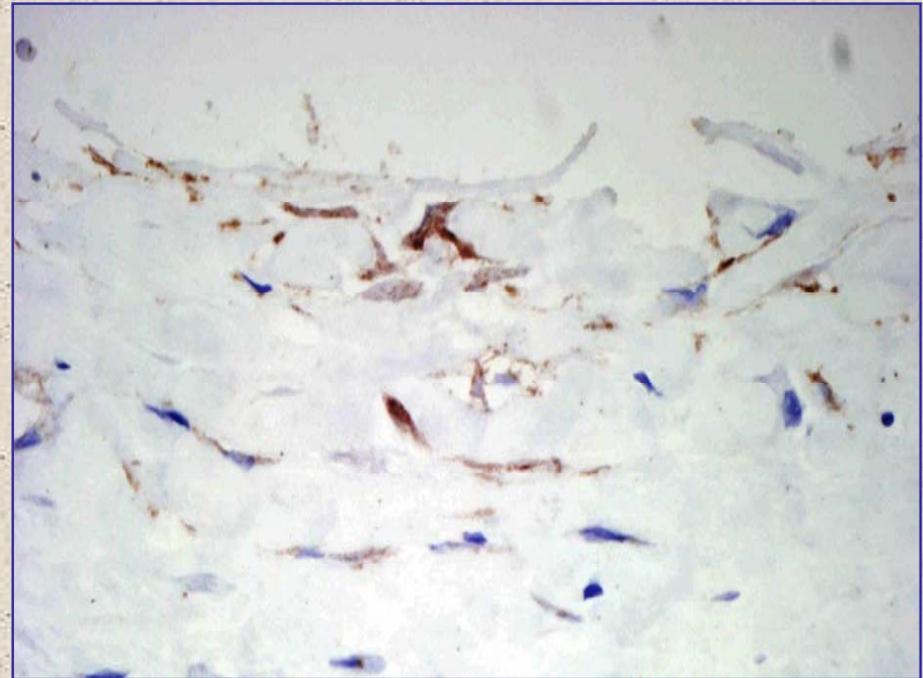
- **La prevalencia de HV** era menor en el grupo BC: 4.3% vs 30.4% ($p=0.02$).
- **La intensidad lesional** fue menor en el grupo BC: 0.09 ± 0.41 vs 0.48 ± 0.8 ($p=0.05$).



Transición epitelio-mesenchimal (keratin+ fibroblastic cells)



Distribución normal de queratinas en células mesoteliales



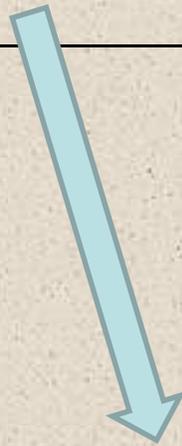
EMT: expresión de queratinas en fibroblastos localizados en submesotelio

Epithelial-to-Mesenchymal transition (Submesothelial cytokeratin staining)

EMT was detected only in 10 patients (22%), with a lower prevalence in SG group, but it did not reach statistical significance probably due to the low number of patients (13%, SG vs. 30.4%, CG, $p=0.15$). Interestingly, when EMT was present, mesothelial layer integrity (scores 2-3) never appeared (for both

La existencia de EMT se correlacionó con una menor preservación del mesotelio

	MC damage	Fibrosis	Angiogenesis/ Vasculopathy
Ayuzama et al	No evaluado	< BC	No evaluado/ < BC
Kawanishi et al	No evaluado	< BC	Inesperado/ < BC
Del Peso et al	< BC	Tendencia a < (ns)	No evaluado/ < BC



**Importancia de la INTEGRIDAD
MESOTELIAL**

The initiation of peritoneal chronic inflammation and repair is at least, partly due to mesothelial cell injury. Hekking et al 2001

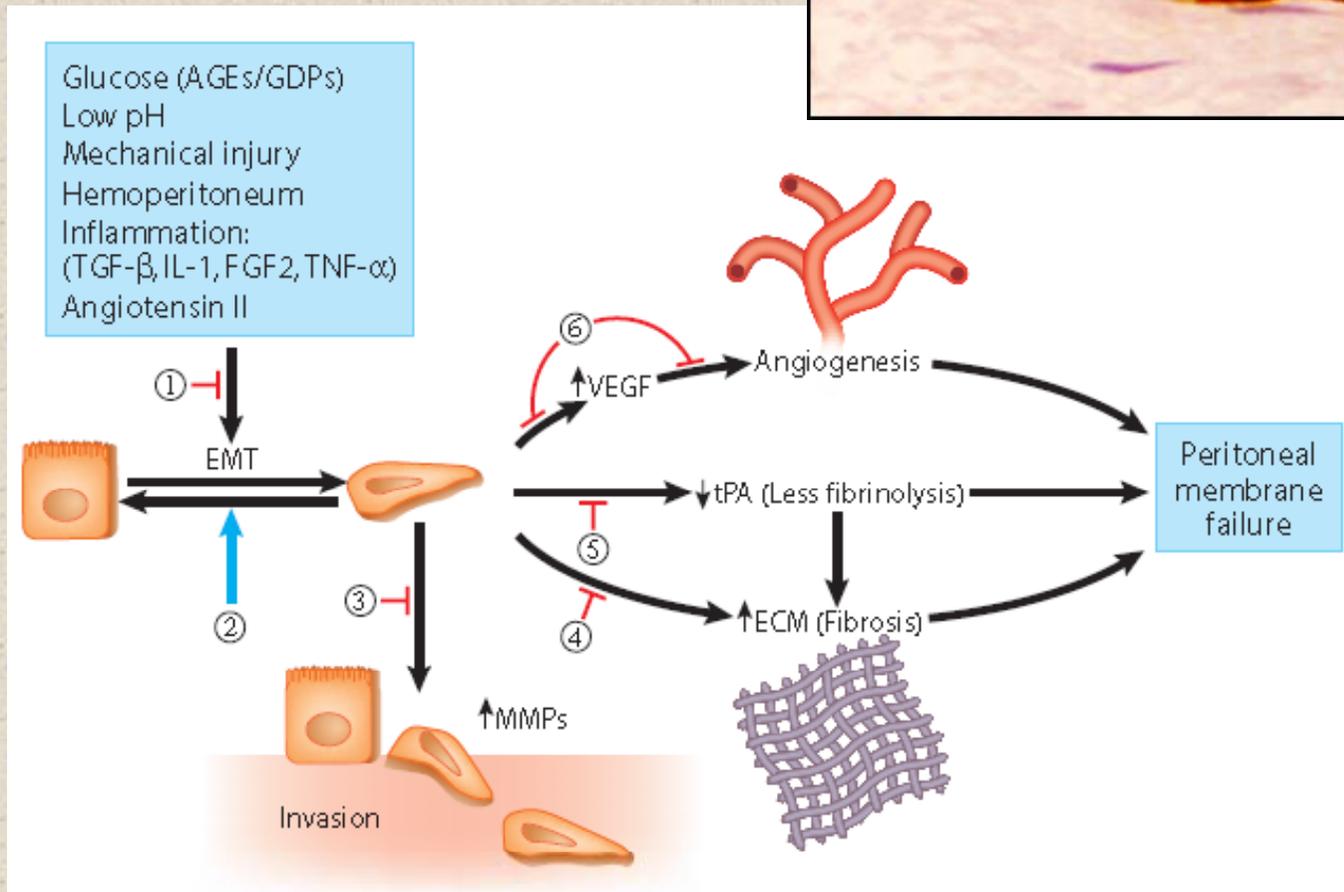
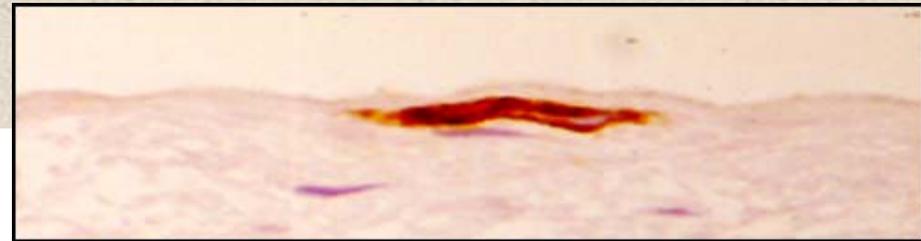


Figure 3. Therapeutic strategies for peritoneal membrane failure based on EMT of MC.

En conclusión, los líquidos BC:

- Asocian una mayor integridad mesotelial (in vitro, ex vivo e in vivo)
- Muestra menor tendencia al desarrollo de fibrosis (no son cambios dramáticos)
- Inducen menor vasculopatía hialinizante

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- Manuel López Cabrera y Rafael Selgas