Received: 4 May 2021 | Revised: 21 September 2021 | Accepted: 14 October 2021

DOI: 10.1111/1744-9987.13747

Therapeutic Apheresis and Dialysis

WILEY

ORIGINAL ARTICLE

Ultrastructural observation of filtration membrane in cell-free and concentrated ascites reinfusion therapy for malignant ascites

Kenichi Koga[1] | **Takeshi Ishihara**[2] | **Yohei Doi**[1,3] | **Ryota Suzuki**[4] | **Masakazu Komatsu**[4] | **Kosei Abe**[5] | **Takahiro Tanaka**[5] | **Ryuji Iwaki**[6] | **Hiroyuki Hashi**[6] | **Akira Sugawara**[1]

[1]Department of Nephrology, Osaka Red Cross Hospital, Osaka, Japan

[2]Department of Clinical Engineering, Osaka Red Cross Hospital, Osaka, Japan

[3]Department of Nephrology, Osaka University Graduate School of Medicine, Suita, Japan

[4]Blood Purification Business Division, Asahi Kasei Medical Co., Ltd., Tokyo, Japan

[5]Development and Planning Department, Asahi Kasei Medical MT Corp., Oita, Japan

[6]Department of Palliative Care, Osaka Red Cross Hospital, Osaka, Japan

Correspondence
Kenichi Koga, Department of Nephrology, Osaka Red Cross Hospital, 5-30 Fudegasaki-cho, Tennoji-ku, Osaka 543-8555, Japan.
Email: kkogaosaka@gmail.com

Funding information
Asahi Kasei Medical Co., Ltd

Abstract

Introduction: Cell-free and concentrated ascites reinfusion therapy (CART) is used for the treatment of diuretic-resistant ascites. An increase in circuit pressure and clogging of the filtration membrane often occur in CART for malignant ascites.

Methods: To clarify the precise mechanism of filter clogging, we performed an ultrastructural observation study of the filtration membrane after the filtration of malignant ascites.

Results: The deposition on the filtration membrane was composed of blood cells, fibrin, or both. Cellular deposition was associated with a greater number of blood cells in the original ascites fluid. In contrast, fibrin deposition was associated with higher levels of interleukin-6, α1-antitrypsin, haptoglobin, and fibrinogen/fibrin degradation products.

Conclusion: Our results suggest that the specific pathophysiologies of malignancy (such as inflammation or coagulation/fibrinolysis) and characteristics of malignant ascites (highly concentrated and cell-rich) are associated with clogging of the filtration membrane during CART.

KEYWORDS

ascites, cancer, filtration, membranes, scanning electron microscopy

1 | INTRODUCTION

Massive ascites in patients with liver cirrhosis and malignant peritonitis is associated with appetite loss, dyspnea, and reduced quality of life. It is initially treated with diuretics; however, when diuretic resistance occurs, ascites drainage by abdominal paracentesis is performed [1]. However, repeated drainage often results in the loss of albumin and immunoglobulins [2, 3]. Hypoalbuminemia can lead to systemic swelling, intravascular dehydration, and pre-renal failure, and the loss of immunoglobulins is a risk factor for infection. Cell-free and concentrated ascites reinfusion therapy (CART) is used for the treatment of refractory ascites and avoids protein loss [3–6]. In CART, ascites is collected from a patient with refractory ascites and filtrated using a filtration filter to remove the cellular components including cancer cells and bacteria. Then, the ascites fluid is concentrated using a

concentration filter. The concentrated ascites is reinfused to the patient intravenously [2, 4]. Several beneficial effects of CART have been reported including the amelioration of diuretic resistance, reduction of abdominal tension, and improvement in quality of life [5]. CART was originally indicated for hepatic ascites in patients with cirrhosis, though the use of CART for malignant ascites has recently increased [2, 6]. However, increases in circuit pressure and cessation of the procedure often occur when CART is used for patients with malignant ascites, leading to protein loss [6, 7]. To achieve a more effective CART, a study regarding the precise mechanism of circuit pressure increase and circuit obstruction is necessary. To clarify this mechanism, we performed an ultrastructural observation study of the filtration membrane after the filtration of malignant ascites, which was scheduled to be discarded after the ascites drainage procedure.

2 | PATIENTS AND METHODS

2.1 | Ascites samples

This study was conducted between December 2015 and April 2018 at the Osaka Red Cross Hospital, Osaka, Japan. Ascites samples were obtained from patients with refractory malignant ascites who were scheduled for ascites drainage. Patients with refractory hepatic ascites were enrolled as a control group. Informed consent for the use of the samples for this study was obtained. Patients with concomitant infections, such as hepatitis B, hepatitis C, or human immunodeficiency virus, were excluded to avoid the risk of infection during the experimental procedures. Twenty-one consecutive patients with malignant ascites and four consecutive patients with hepatic ascites were registered for this study. Two patients with malignant ascites were excluded due to infection, and two patients were excluded because the procedures could not be accomplished due to obstruction of the header of the filtration cassette with a large clot. The final analysis included samples from 17 patients with malignant ascites and four patients with hepatic ascites. Heparin (500 IU/kg) was added to the original ascites fluid. This study protocol was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Osaka Red Cross Hospital (IRB number 672).

2.2 | Procedure

This study focuses on the filtration process of ascites. The procedure consisted of three steps: first filtration, reverse wash, and second filtration to assess the mechanisms of clogging and the effects of reverse washes. These processes are different from the filtration process of regular CART in the clinical setting. During the filtration, ascites samples were processed simultaneously using two methods: internal and external, using hollow-fiber type ascitic filtration filters (AHF-MO, Asahi Kasei Medical, Tokyo, Japan) (Figure 1(A)). The ascites flows from the inside to the

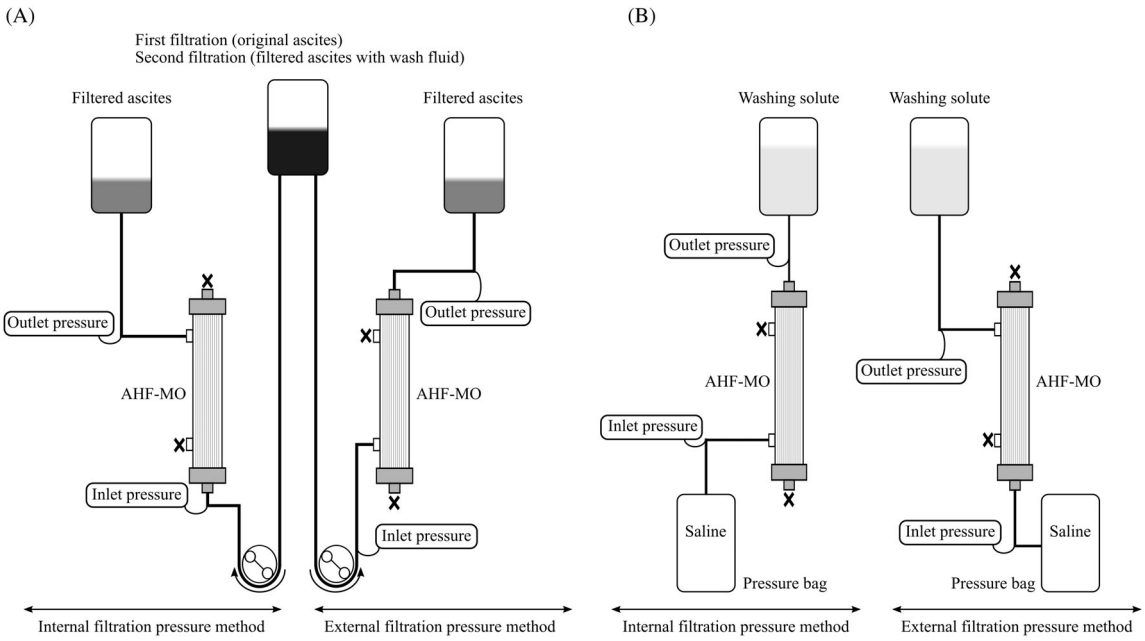


FIGURE 1 Circuit settings. (A) Two filtration methods are used to filter the original ascites samples at 50 mL/min. (B) After the first filtration, the filtration membrane is reverse washed using 500 mL of saline at 40 kPa. The filtered ascites is combined with 1000 mL of wash fluid and filtered again using the same circuit at the same treatment rate as the initial filtration. The circuit pressure was measured at the entry and exit sites of the filtration membrane, and the transmembrane pressure (TMP) was calculated

outside of the hollow fiber during the internal filtration method and from the outside to the inside of the hollow fiber during the external filtration method. The circuits of the internal and external filtration methods were connected to the same ascites bag, and the ascites fluid was simultaneously filtrated via both methods at a rate of 50 mL/min. The circuit pressures were continuously monitored at the entry and exit sites of each filtration filter, and the transmembrane pressures (TMPs) for each filter were calculated. When the TMP reached 500 mm Hg in either the internal or external method, that method was discontinued while the other method continued until the filtration was completed or discontinued due to increased TMP in that filter. This circuit was designed to allow for a direct comparison of the two filtration methods and reduce the volume of non-filtered ascites when clogging occurs in one filtration method. Membrane cleaning using a reverse wash during the filtration process, especially when the external filtration method is used, has been reported to prevent filter clogging and increase the volume of ascites that can be processed [4] and is commonly performed in the clinical setting. In this experimental model, a reverse wash of the filtration membrane was performed after the first filtration for each filtration method using 500 mL of saline at 40 kPa (Figure 1(B)). The filtered ascites were combined with 1000 mL of wash fluid, and filtered again during the second filtration to assess the efficacy of the reverse wash process under the same conditions that were used during the first filtration (Figure 1(A)). The protein recovery rate (%) was examined in samples showing a rise in the circuit pressure and was calculated as the proportion of protein in the concentrated ascites to the protein in the original ascites.

2.3 | Biochemical and proteome analyses

The total protein, albumin, interleukin-6 (IL-6), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), fibrinogen/fibrin degradation products (FDP), haptoglobin, α1-antitrypsin, and the number of cells in the original ascites samples were measured at the laboratory of SRL Co., Ltd. (Tokyo, Japan). Proteome analyses were performed to analyze the protein composition of the deposits on the filtration membrane. Protein solution samples were extracted from filtration membrane surfaces. Shotgun proteomics analyses were performed at the Chemical Evaluations and Research Institute (CERI), Japan.

2.4 | Ultrastructural observation

The filtration membrane cartridges were observed via scanning electron microscopy (SEM, S-3000N; Hitachi Limited, Tokyo, Japan) at nine locations, as shown in Figure 2(A). The existence of deposition was defined as positive if significant deposition was observed at more than two out of nine observation points. Deposit formation was defined as significant

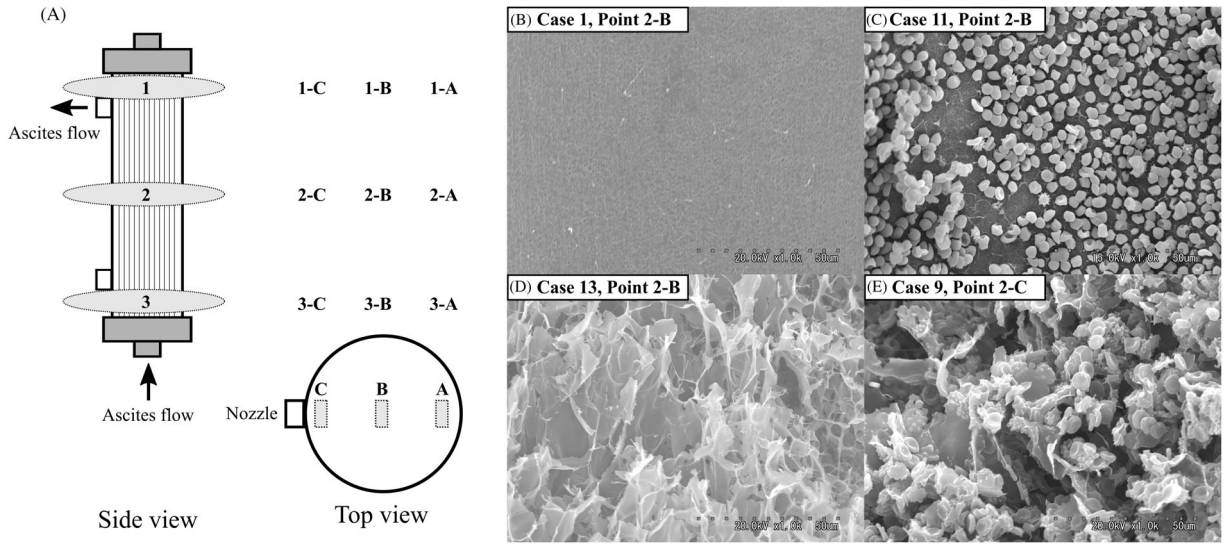


FIGURE 2 Ultrastructural observation of the filtration membrane cartridge. (A) The locations of the nine observation points on the filtration membrane cartridge are shown. (B) Representative images of the filtration membrane cartridge used to filter ascites are shown. The filtration membrane cartridge used to filter ascites from a patient with liver cirrhosis (case 1) showed no deposition on the filter membrane. The filtration membrane cartridges from a patient with (C) papillary renal cell carcinoma (case 11), (D) uterine cancer (case 13), and (E) ovarian cancer (case 9) were found to have deposits of cells, fibrin, and cells and fibrin, respectively (original magnification 1000×)

when deposition materials covered more than half of the field of view captured using SEM at an original magnification of 1000×. Each captured field was 89 × 128 μm.

2.5 | Statistical analysis

Data were analyzed using JMP Software (JMP version 11, SAS Institute, Cary, USA), and statistical significance was set at $p < 0.05$. Continuous data are expressed as medians and interquartile ranges (IQRs) and categorical data are expressed as numbers and percentages. Continuous data were analyzed using Wilcoxon's signed-rank test, and categorical data were analyzed using Fisher's exact test.

3 | RESULTS

3.1 | Patient characteristics

The patient characteristics are shown in Table 1 and the Supplementary Table 1. The primary cancer types were ovarian (35.3%), gastrointestinal (29.4%), pancreatic (5.9%), bile duct (5.9%), lung (5.9%), bladder (5.9%), papillary renal cell carcinoma (5.9%), and uterine (5.9%).

3.2 | Characteristics of original ascites samples

The characteristics of the original ascites samples are presented in Table 1. The mean weight of the original ascites

TABLE 1 Characteristics of patients and original ascites samples

	Hepatic ascites	Malignant ascites	*p*-Value
Number of patients	4	17	
Age (years), mean (SD)	56.5 (53.8–57.8)	71 (66.0–76.5)	<0.05
Sex (male), No. (%)	4 (100)	3 (17.7)	<0.01
Types of cancer, No. (%)			
Ovary	-	6 (35.3)	
Gastrointestinal	-	5 (29.4)	
Pancreas	-	1 (5.9)	
Gallbladder and bile duct	-	1 (5.9)	
Lung	-	1 (5.9)	
Bladder	-	1 (5.9)	
Uterine	-	1 (5.9)	
Ascitic volume (mL)	3489 (2813–3542)	2488 (1425–3671)	N.S.
Bloody ascites, No. (%)	3 (75.0)	10 (58.8)	N.S.
Total protein (g/dL)	1.65 (1.0–2.9)	3.6 (2.8–4.5)	<0.05
Albumin (g/dL)	0.75 (0.6–1.6)	1.8 (1.2–2.2)	0.054
IL-6 (pg/mL)	1895 (1125–7442)	4910 (1980–15 800)	0.15
IgG (mg/dL)	436 (258–528)	718 (527–1140)	<0.05
IgA (mg/dL)	77 (54–119)	122 (68–181)	N.S.
IgM (mg/dL)	16 (15–18)	26 (18–41)	<0.05
FDP (μg/mL)	392 (190–1203)	890 (448–1880)	0.20
Haptoglobin (mg/dL)	15 (10–69)	61 (40–109)	0.13
α1-antitrypsin (mg/dL)	41 (31–146)	158 (96–227)	<0.05
WBC (10^2/μL)	1.3 (0.9–2.4)	1.0 (0.5–3.3)	N.S.
RBC (10^4/μL)	1 (0.3–1.8)	1 (0–2.0)	N.S.
Plt (10^2/μL)	0.2 (0.1–0.4)	0.3 (0.2–0.4)	N.S.

Note: Continuous variables are presented as medians and interquartile ranges and categorical variables are presented as number (%).

Abbreviations: FDP, fibrinogen/fibrin degradation products; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-6, interleukin-6; N.S., not significant; Plt, platelets; RBC, red blood cells; WBC, white blood cells.

samples was 3489 g (IQR: 2813–3542 g) in the hepatic ascites group and 2488 g (IQR: 1425–3671 g) in the malignant ascites group. The mean weight was not significantly different between the groups. Then, 3 samples in the hepatic ascites group (75%) and 11 samples in the malignant ascites group (58.8%) were classified as bloody ascites. The concentration of total protein in the malignant ascites group was higher than that in the hepatic ascites group ($p < 0.05$). The malignant ascites group showed a tendency of higher albumin level than the hepatic ascites group ($p = 0.054$). The levels of IgG, IgM, and α1-antitrypsin were significantly higher in the group with malignant ascites. There were no significant differences in the number of white blood cells (WBCs), red blood cells (RBCs), or platelets between the two groups.

3.3 | Circuit pressure measurements

With the exception of case 11 (ascites from a patient with papillary renal cell carcinoma), the TMP did not increase during the first or second filtration procedures in either method (Figure 3(A–C)). An increase in TMP occurred during the first and second filtration procedures for case 11 (Figure 3(D–F)) and was observed earlier when the external method was used compared to when the internal method was used. The protein recovery rates in case 11 were higher when the internal method was used compared to when the external method was used: total protein (internal: 51.2%; external: 36.6%), albumin (internal: 52.2%; external: 34.8%), and IgG (internal: 47.3%; external: 30.9%). There were no significant differences in the recovery rates of total protein, albumin, or immunoglobulins between the two methods, except in case 11.

3.4 | SEM analysis

No residual material was observed on the filtration membrane in the hepatic ascites group (Figure 2(B)). In the malignant ascites group, 10 samples had deposition on the filtration membrane. Three cases (17.6%) had cellular

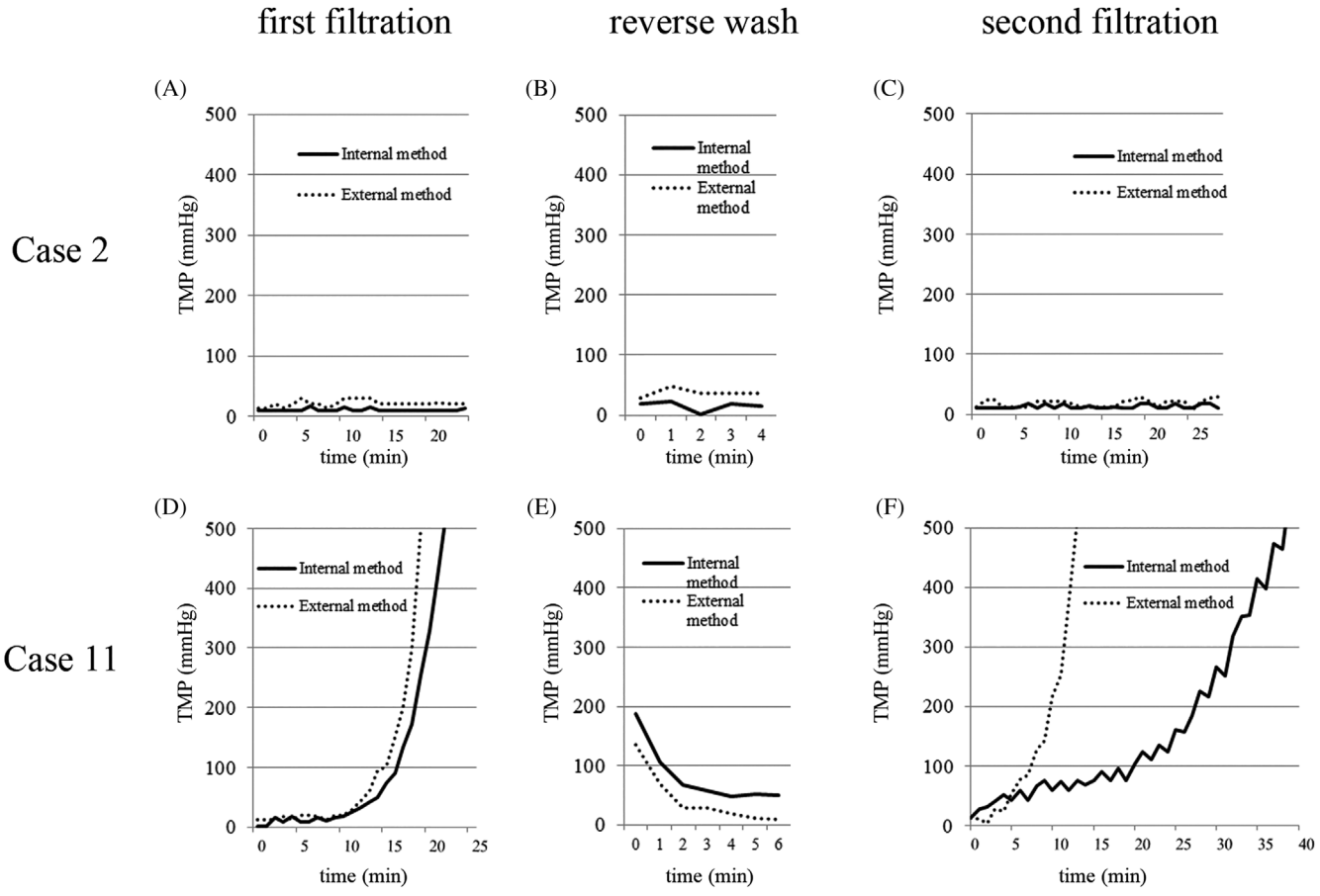


FIGURE 3 Circuit pressures in ascites from a patient with liver cirrhosis and ascites from a patient with papillary renal cell carcinoma. The transmembrane pressure (TMP) of the filtration membrane in during the filtration of ascites from a patient with liver cirrhosis (case 2) is shown in panels (A–C) and that of the ascites from a patient with papillary renal cell carcinoma (case 11) is shown in panels (D–F) Case 2 is representative of the hepatic ascites cases included in this study. Panels (A) and (D) represent the first filtration, (B) and (E) represent the reverse wash, and (C) and (F) represent the second filtration. An increased pressure was observed during filtration in case 11

deposition that was predominately RBCs that had a unique discoid morphology of 7–8 μm in diameter (Figure 2(C)). Six cases had fibrous deposition (35.2%) (Figure 2(D)). These fibrous materials were presumed to consist mostly of fibrin as fibrin is known to be abundant in malignant ascites [8] and intrinsically forms clots, especially on the surface of artificial materials such as a hemodialyzer [9]. One case (5.9%) had both types of deposition on the filter membrane (Figure 2(E)). Case 11, the only case in which the circuit pressure increased, showed cellular deposition.

3.5 | Protein composition of ascites samples

The ascites samples that resulted in cellular deposition on the filtration membrane cartridges showed the tendency of a higher prevalence of bloody ascites and a higher concentration of total protein than the samples that did not have cellular deposition on the filtration membrane cartridges (Table 2). The cellular deposition group had a significantly larger number of WBCs, RBCs, platelets, and IgA than the samples that did not result in cellular deposition. The original ascites of case 11 had the highest number of RBCs (61×10^4/mL) and high concentrations of total protein (4.2 g/dL) and albumin (2.3 g/dL). The ascites samples that resulted in fibrin deposition on the filtration membrane cartridges had significantly higher concentrations of FDP, IL-6, haptoglobin, and α1-antitrypsin than the ascites samples that did not result in fibrin deposition (Table 3). The fibrin deposition group also showed the tendency of a higher concentration of total protein.

3.6 | Proteome analyses

Proteome analyses were performed using samples from two patients with hepatic ascites (cases 1 and 2) and 10 patients with malignant ascites (cases 5–14). The results of the proteome analyses are shown in Figure 4. The protein of the ascites samples consisted of globulin, albumin, glycoproteins, fibrinogen, apolipoproteins, hemoglobin subunits, and complement-related proteins. Although bloody ascites samples tended to have a higher composition of hemoglobin subunits, there was no specific composition pattern associated with membrane deposition or increases in pressure. The samples from a patient with liver cirrhosis (case 2) and a patient with papillary renal cell carcinoma (case 11) that were classified as bloody ascites had high concentrations of hemoglobin subunits (Figure 5). The number of RBCs in case 11 was approximately 60 times that in case 2 (case 11: 610 000/μL; case 2:10 000/μL).

TABLE 2 Composition of original ascites samples among samples that resulted in cellular deposits and those that did not

	No cellular deposition	Cellular deposition	*p*-Value
Number of samples (%)	14 (82.3)	3 (17.6)	
Ascitic volume (mL)	2530 (1479–3564)	1720 (1235–4039)	N.S.
Bloody ascites, No. (%)	7 (50)	3 (100)	0.11
Total protein (g/dL)	3.5 (2.5–4.0)	4.3 (4.1–4.9)	0.10
Albumin (g/dL)	1.7 (1.1–2.1)	2.3 (1.4–2.7)	N.S.
IL-6 (pg/mL)	5620 (3193–16 650)	1690 (716–15 200)	N.S.
IgG (mg/dL)	699 (509–1139)	718 (653–1825)	N.S.
IgA (mg/dL)	108 (54–152)	234 (134–273)	<0.05
IgM (mg/dL)	28 (17–42)	26 (21–34)	N.S.
FDP (μg/mL)	909 (376–1823)	725 (548–2000)	N.S.
Haptoglobin (mg/dL)	60 (32–122)	68 (61–98)	N.S.
α1-antitrypsin (mg/dL)	149 (97–237)	158 (60–218)	N.S.
WBC (10^2/μL)	0.8 (0.4–2.1)	7.8 (2.5–8.5)	<0.05
RBC (10^4/μL)	1.0 (0–1.0)	14.0 (2.0–61.0)	<0.05
Plt (10^2/μL)	0.25 (0.1–0.3)	0.6 (0.4–0.6)	<0.05

Note: Continuous variables are presented as medians and interquartile ranges and categorical variables are presented as number (%).

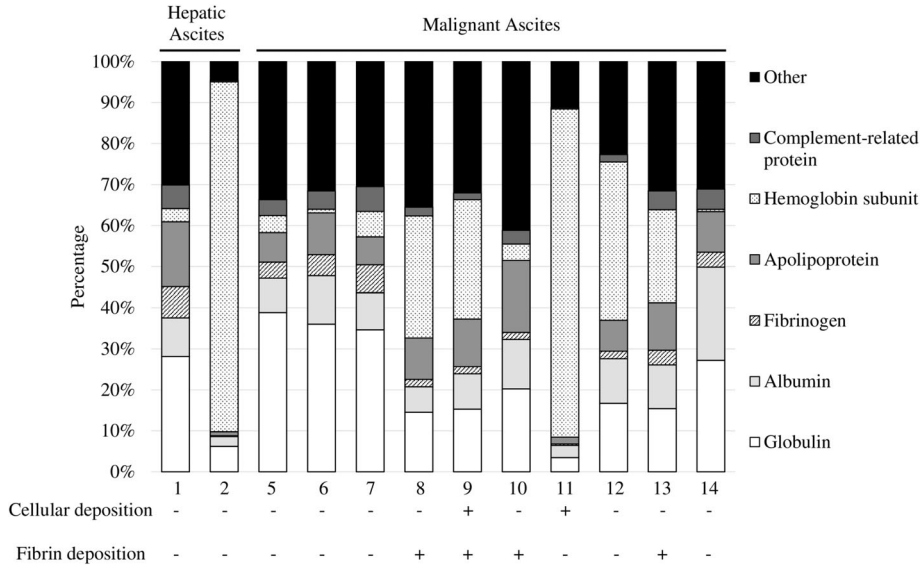
Abbreviations: FDP, fibrinogen/fibrin degradation products; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-6, interleukin-6; N.S., not significant; Plt, platelets; RBC, red blood cells; WBC, white blood cells.

TABLE 3 Composition of original ascites samples among samples that resulted in fibrin deposits and those that did not

	No fibrin deposition	Fibrin deposition	*p*-Value
Number of patients (%)	11 (67.4)	6 (35.2)	
Ascitic volume (mL)	3250 (1316–4039)	1932 (1458–2900)	N.S.
Bloody ascites, No. (%)	7 (63.6)	3 (50)	N.S.
Total protein (g/dL)	3.3 (2.2–4.1)	4.3 (3.4–5.3)	0.11
Albumin (g/dL)	1.4 (1.1–2.1)	2 0.1 (1.5–2.7)	0.16
IL-6 (pg/mL)	3500 (1640–4910)	15 800 (12 083–18 300)	<0.01
IgG (mg/dL)	653 (504–1143)	732 (630–1293)	N.S.
IgA (mg/dL)	122 (50–144)	147 (85.8–207)	N.S.
IgM (mg/dL)	26.0 (17–38)	27.5 (20–48)	N.S.
FDP (μg/mL)	548 (369–890)	2005 (945–2410)	<0.005
Haptoglobin (mg/dL)	55 (10–68)	109 (82–155)	<0.05
α1-antitrypsin (mg/dL)	105 (54–168)	239 (204–254)	<0.005
WBC (10^2/μL)	1.1 (0.5–3.1)	0.5 (0.0–8.8)	0.16
RBC (10^4/μL)	0.3 (0.2–0.5)	0.2 (0.1–0.4)	N.S.
Plt (10^2/μL)	0.3 (0.2–0.5)	0.3 (0.1–0.4)	N.S.

Note: Continuous variables are presented as medians and interquartile ranges and categorical variables are presented as number (%).
Abbreviations: FDP, fibrinogen/fibrin degradation products; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-6, interleukin-6; N.S., not significant; Plt, platelets; RBC, red blood cells; WBC, white blood cells.

FIGURE 4 Proteome analyses. The results of the proteome analyses in two samples from the hepatic ascites group (cases 1 and 2) and 10 samples from the malignant ascites group (cases 5–14) are shown



4 | DISCUSSION

This is the first study to use SEM to analyze the deposits on the filtration membranes cartridges used during CART for malignant ascites. This study revealed that membranes used to filter malignant ascites had deposits composed of blood cells, fibrin, or both.

The blood–membrane interaction in hemodialysis has been investigated to improve the biocompatibility of the hemodialysis membrane, and SEM has been used to reveal blood-cell adhesion and fibrin formation on the surface of the hemodialysis membrane [9]. To the best of our knowledge, no study has investigated deposit formation on the filtration membrane in CART. The filtration of ascites samples from patients with hepatic ascites did not result in any deposition on the membrane. On the other hand, the ascites samples from patients with malignant ascites had higher concentrations of total protein,

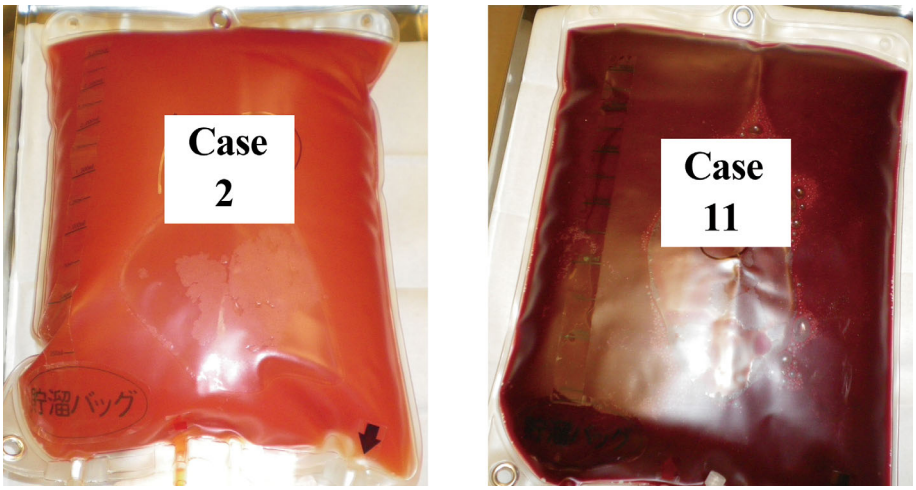


FIGURE 5 Bloody ascites. The bloody ascites samples from a patient with liver cirrhosis (case 2) and a patient with papillary renal cell carcinoma (case 11) are shown. The bloody ascites was more apparent in case 11, which had a higher concentration of red blood cells

IgG, IgM, and α1-antitrypsin. Based on these results, we hypothesized that the deposition on filtration membrane cartridges is associated with the protein composition in malignant ascites.

We compared the composition of the original ascites among samples that resulted in deposits on the filtration membrane with those that did not. The original ascites samples that resulted in cellular deposition on the filtration membranes had a higher number of WBCs, RBCs, and platelets and a higher concentration of total protein than samples that did not result in cellular depositions. Case 11, the only case in which the circuit pressure increased, exhibited cellular deposition, and the original ascites of case 11 had the highest number of RBCs. This result indicates that a high concentration of blood cells is associated with deposit formation and increases in circuit pressure. A comparison of the internal and external methods in case 11 revealed a lower protein recovery rate in the external method. This may be attributed to the premature termination of the CART procedure and the reduced volume of processed ascites when the external method was used.

The deposition of fibrin on the filtration membrane may be due to other mechanisms. Malignant ascites samples that resulted in fibrin deposition had higher levels of IL-6, α1-antitrypsin, haptoglobin, and FDP than those that did not result in fibrin deposition, and the number of blood cells did not differ between the two groups. IL-6, α1-antitrypsin, and haptoglobin are pro-inflammatory proteins [7, 10], and FDP is a marker of coagulation and fibrinolysis. Generally, inflammation and coagulation or fibrinolysis are enhanced in patients with malignancy [11]. These pathological conditions may be associated with the fibrin deposition on the filtration membrane. Our results are consistent with those of a previous study that reported that markers of inflammation and coagulation in malignant ascites are associated with an increase in circuit pressure during CART [7].

Proteome analyses were performed using residual materials on the filtration membrane to investigate the composition of the deposits on the filtration membrane. We did not find specific patterns in ascites samples that resulted in membrane deposition or an increase in pressure. The tendency of higher levels of hemoglobin subunits was observed in samples classified as bloody ascites. Cases 2 and 11 were both bloody ascites and both had a high proportion of hemoglobin subunits on proteome analysis; however, the filtration of the ascites in case 2 did not result in a pressure increase or cellular membrane deposition, as occurred in case 11. Case 11 had the highest number of RBCs and high total protein levels, suggesting that the number of RBCs or the density of proteins in the ascites are more important factors to predict deposit formation than the protein composition. As shown in Tables 2 and 3, the tendency of higher total protein levels was observed in both the fibrin and cellular-deposition groups, suggesting that protein density is essential in deposit formation. This is consistent with previous studies that reported the association of higher total protein levels with increased circuit pressures and low protein recovery rates [6, 7].

This study has several limitations. First, a circuit pressure increase was observed in only one case. The original ascites samples were divided and filtered using two methods, resulting in a small volume of the original ascites. The small volume of samples may have affected the results. To clarify the association between the pressure increase and cellular membrane deposition, a larger number of cases and a larger volume of original ascites are needed. In addition, due to the small sample size, the characteristics of the two types of deposition were analyzed separately and the interaction effect between the two types of deposition could not be assessed. The efficacy of reverse washes may also not be evaluated as most cases in this study did not include membrane clogging. Second, this study did not determine the association

between fibrin deposition and an increase in circuit pressure as pressure increases were not observed in cases with fibrin deposition. Yamada et al. recently reported that ascitic FDP levels were elevated in samples that resulted in increased circuit pressures, suggesting that increases in circuit pressure may be associated with the activation of the coagulation fibrinolysis system [7]. In our study, heparin was added to the original samples, which may have prevented the increase in circuit pressure during filtration. It has been reported that heparin is added to the ascites fluid in 26.6% of the CART sessions to prevent fibrin clot formation, and heparin use is associated with a higher total protein recovery rate [6]. Although the previous report did not demonstrate fibrin formation on the filtration membrane in the case of increased circuit pressure, it is possible that fibrin formation on the membrane is an important factor associated with increases in circuit pressure and lower protein recovery rates. Third, in the experimental model, the proteins on the surface and inside of the membrane that were too small to be observed via SEM could not be fully evaluated. The proteome analysis showed a variety of proteins, suggesting the existence of smaller molecules. These molecules may play a role in the clogging of the filter membrane.

5 | CONCLUSION

This is the first report to use SEM to analyze deposit formations on the filtration membrane of CART for malignant ascites samples. The membranes were found to have deposits of blood cells, fibrin, or both. Cellular deposition was associated with a larger number of blood cells in the ascites, and fibrin deposition was associated with markers related to the pathophysiology of malignancy, such as inflammation and coagulation/fibrinolysis markers. More studies are needed to develop a more effective CART system based on these results.

ACKNOWLEDGMENT

This study was supported by Asahi Kasei Medical Co., Ltd.

CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

ORCID

Kenichi Koga https://orcid.org/0000-0002-3758-6805

REFERENCES

1. Kozaki K, IInuma M, Takagi T, IInuma M, Takagi T, Fukuda T, et al. Cell-free and concentrated ascites reinfusion therapy for decompensated liver cirrhosis. Ther Apher Dial. 2016;20(4):376–82.
2. Ito T, Hanafusa N. CART: cell-free and concentrated ascites reinfusion therapy against malignancy-related ascites. Transfus Apher Sci. 2017;56(5):703–7.
3. Yamada Y, Inui K, Hara Y, Fuji K, Sonoda K, Hashimoto K, et al. Verification of serum albumin elevating effect of cell-free and concentrated ascites reinfusion therapy for ascites patients: a retrospective controlled cohort study. Sci Rep. 2019;9(1):10195.
4. Japanese CART Study Group, Matsusaki K, Ohta K, Yoshizawa A, Gyoda Y. Novel cell-free and concentrated ascites reinfusion therapy (KM-CART) for refractory ascites associated with cancerous peritonitis: its effect and future perspectives. Int J Clin Oncol. 2011;16(4):395–400.
5. Ito T, Hanafusa N, Fukui M, Yamamoto H, Watanabe Y, Noiri E, et al. Single center experience of cell-free and concentrated ascites reinfusion therapy in malignancy related ascites. Ther Apher Dial. 2014;18(1):87–92.
6. Hanafusa N, Isoai A, Ishihara T, Inoue T, Ishitani K, Utsugisawa T, et al. Safety and efficacy of cell-free and concentrated ascites reinfusion therapy (CART) in refractory ascites: post-marketing surveillance results. PLoS One. 2017;12(5):e0177303.
7. Yamada S, Hasegawa M, Nii N, Kato M, Ohashi A, Suzuki R, et al. Comparison between the internal and external pressure filtration method of cell-free and concentrated ascites reinfusion therapy using the same cancerous ascites. Ther Apher Dial. 2019;23(3):237–41.
8. Shahid S, Iman A, Matti U, Rachid K, Assaf A, Eveno C, et al. Fibrin deposit on the peritoneal surface serves as a niche for cancer expansion in carcinomatosis patients. Neoplasia. 2019;21(11):1091–101.
9. Hofbauer R, Moser D, Frass M, Oberbauer R, Kaye AD, Wagner O, et al. Effect of anticoagulation on blood membrane interactions during hemodialysis. Kidney Int. 1999;56(4):1578–83.
10. Pang WW, Abdul-Rahman PS, Wan-Ibrahim WI, Hashim OH. Can the acute-phase reactant proteins be used as cancer biomarkers? Int J Biol Markers. 2010;25(1):1–11.
11. Falanga A, Marchetti M, Vignoli A. Coagulation and cancer: biological and clinical aspects. J Thromb Haemost. 2013;11(2):223–33.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Koga K, Ishihara T, Doi Y, Suzuki R, Komatsu M, Abe K, et al. Ultrastructural observation of filtration membrane in cell-free and concentrated ascites reinfusion therapy for malignant ascites. Ther Apher Dial. 2022;26:649–57. https://doi.org/10.1111/1744-9987.13747