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ORIGINAL ARTICLE

Usefulness of cell-free and concentrated ascites reinfusion therapy in the therapeutic management of advanced ovarian cancer patients with massive ascites

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Abstract

Background The management of refractory ascites in advanced ovarian cancer (AOC) is vital for patients with abdominal distention, respiratory distress, and anorexia due to massive ascites with cancer peritonitis. We analyzed the benefits of concentrated ascites reinfusion therapy (CART) in the management of AOC.

Methods We reviewed records of AOC patients who underwent CART between January 2011 and March 2017. We retrospectively analyzed patients' backgrounds and physiological changes, including body weight, abdominal girth, urine volume, blood component values, blood pressure, heart rate, and body temperature before and after CART. We investigated the clinicopathological significance of CART by measuring the mean number of ascites tumor cell (ATC) clusters before CART.

Results A retrospective analysis was performed on 29 cases of AOC with massive ascites involving 47 CART sessions. The patients' mean age was 56.6 ± 12.8 years, and the mean number of sessions was 1.7 ± 1.2. The mean volume of the processed ascites was 2,937 ± 820 mL, which was concentrated to 272 ± 84 mL containing 85.0 ± 33.2 g protein on average. Significant reductions in abdominal girth (− 5.30 ± 0.65 cm; $p < 0.0001$) and body weight (− 2.97 ± 0.26 kg; $p = 0.0011$), increased urine volume (+ 824.29 ± 145.21 mL; $p < 0.0001$), and improved serum albumin levels (+ 0.18 ± 0.34; $p < 0.0001$) were observed after CART. Analysis of variance revealed significant elevations in body temperature after CART in 11 patients with a small number of ATC clusters.

Conclusions CART is useful for the therapeutic management of AOC patients with refractory massive ascites. Elevations of body temperature after CART may be avoided by the investigation of patients' peritoneal cytology before CART.

Keywords Cell-free and concentrated ascites reinfusion therapy · Advanced ovarian cancer · Body temperature · Ascites tumor cells

Introduction

Management of refractory massive ascites in advanced ovarian cancer (AOC) patients is challenging during multimodality treatment [1]. The development of malignant ascites in patients undergoing treatment for AOC leads to symptoms, such as severe abdominal distention, fatigue, shortness of breath, nausea, vomiting, pain, appetite loss, and circulatory failure [2, 3]. The development of massive ascites affects the quality of life of patients, increasing symptom burden and the need for immediate hospitalization [1].

Normally, the capillary lymphatic vessels in the peritoneal cavity continuously absorb around 50–100 mL of fluid every hour; this allows for the easy passage of solutes between the peritoneum and the adjacent bowels. However, in cases of malignant ascites, an increased production of ascites is induced by the dissemination of tumors on the peritoneum, leading to increased leakiness of the tumor microvasculature and obstruction of lymphatic drainage, thereby preventing the reabsorption of peritoneal fluid and proteins in the abdominal cavity [1, 4]. As a result, fluid

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accumulation in the peritoneal cavity exceeds fluid reabsorption, leading to a high volume of ascitic fluid with a high protein content [5].

Various therapeutic options have been used in the management of the aforementioned symptoms [4]. The most frequently used treatments include paracentesis; however, this often causes electrolytic changes and cardiovascular effects. Paracentesis procedures vary; some research suggests using indwelling catheters for patients with ascites that builds up rapidly and others replacing intravenous fluids with albumin preparations; in addition, the speed of drainage also varies [6]. Gotlieb et al. demonstrated that the removal of a mean of 4.5 L of ascites over 30–90 min could be completely safe [7]. The use of diuretics is another therapeutic option [8]. However, this may lead to dehydration and venous thrombosis [2]. Furthermore, albumin administration is considered to be effective in preventing circulatory dysfunction due to large-volume paracentesis; however, the use of blood derivatives is associated with the presence of unknown microbes or viral infections as well as poor cost-effectiveness [9].

Cell-free and concentrated ascites reinfusion therapy (CART) is an apheresis therapy for malignant ascites. A patient's own protein components (e.g., albumin and globulin) are recovered after the filtration of cell components and concentration from ascites, with cancer cells and bacteria removed using a filter [10]. The efficacy of CART was first demonstrated in 1976, in the treatment of massive ascites in patients with liver cirrhosis [11]. The removal of larger-volume ascites with minimal complications can lead to the long-term improvement of disease symptoms [12, 13]. Nevertheless, CART was not widely used in clinical settings for refractory ascites in cancer patients until 1981, when its use was approved for the treatment of refractory ascites in various diseases, including malignant ascites, under the National Health Insurance coverage in Japan. This could be because the safety and efficacy of CART were still inadequately tested for malignant ascites, with several reports demonstrating elevated body temperatures during reinfusion [10, 14–16]. In time, several publications demonstrated the efficacy and safety of CART for malignant ascites, with some Japanese retrospective studies demonstrating that patients with malignant ascites who received CART experienced a relief in their ascites-induced symptoms [10, 15, 17–21]. However, there is still a lack of adequate data on the usefulness of CART, particularly regarding the management of AOC. In addition, the various adverse events caused by CART have not been well validated in AOC patients. Therefore, in this study, we retrospectively assessed the usefulness and superiority of CART in the therapeutic management of AOC. CART is associated with various adverse events [19, 22], such as elevations in body temperature, which can be life-threatening to AOC patients who are neutropenic or susceptible to bacterial or viral infections. Hence, we performed further analysis to identify significant clinicopathological factors that may be associated with elevations in body temperature after CART.

Patients and methods

Patients and ethical issues

We retrospectively analyzed the clinical records of 29 AOC patients treated with CART at the University of Tokyo Hospital from January 2011 to March 2017. Patients were routinely evaluated before CART through blood examinations with tumor markers (CA125, CEA, CA19-9, etc.), chest and abdominal radiographs, trans-vaginal and trans-abdominal ultrasound examinations, and computed tomography (CT) for evidence of disease. Before treatment, all patients provided written informed consent in accordance with the ethical guidelines at the University of Tokyo Hospital for the CART procedure and for their records to be used in the clinical study.

Procedure

Standard CART was administered in accordance with the procedure reported by Ito et al. [18]. In brief, the ascites was drawn by paracentesis into a designated bag and processed at a rate of 50 mL/min with the CART system (first filter to remove cancer cells and microbes: AHF-MOW; second filter for concentration: AHF-UNH; Asahi Kasei Medical. Tokyo, Japan). A disposable indwelling needle was used for paracentesis to drain the ascites. Heparin (500 U per 1 L of ascites) was added to the ascites to prevent formation of fibrin. Intravenous hydrocortisone premedication (maximum of 200 mg) was routinely administered to patients prior to reinfusion to reduce the risk of body temperature elevation. The filtered and concentrated ascitic fluid was reinfused into the patients' veins at 100–150 mL/h (2 mL/min on average).

Peritoneal cytology

All ascites obtained by paracentesis from the patients before CART were examined by peritoneal cytology. As part of the routine protocol, to keep a constant number of cells in the field of view, cell components of 15 mL of ascites fluid were collected after centrifugal separation; a 20-μL aliquot of the suspension was then smeared on a glass slide. Conventional staining, comprising Papanicolaou, periodic acid Schiff, and May-Grunwald and Giemsa, was performed after dehydration. The peritoneal cytology was considered positive if atypical tumor cells were detected microscopically in the fluid. AOC usually presents as cellular specimens containing single cells or cohesive irregular cell clusters with pleomorphic nuclei and prominent nucleoli with condensed chromatin. The precise cellular composition of ascites tends to vary across patients; the fraction of ascites tumor cell (ATC) clusters is generally believed to be < 0.1% of harvested cells, with the remainder comprising only host cells [1]. Therefore, we categorized all cases into two groups by counting the number of ATC clusters per microscopic field at 40· magnification before CART: (1) many (> 10) clusters, and (2) few (≤ 10) clusters as suggested by Layfield et al. [23]. Representative figures of groups (1) and (2) are shown in Fig. 1. Using these categories examined by blinded cytopathologists, we analyzed the clinicopathological indices in association with the changes before and after CART.

Data collection

We collected the following data: patient age, body weight, abdominal girth, Eastern Cooperative Oncology Group (ECOG) performance status (PS) [24], primary diagnosis, number of CART procedures, volume of ascites, protein concentration and the amounts both in the original ascites and filtrated and concentrated ascites. Before and after CART, blood pressure, heart rate, body temperature, body weight, abdominal girth, daily urine volume, and peritoneal cytology and blood examination values were recorded. Patients' body temperatures and blood pressures were measured before and 1 day after reinfusion. All data were retrospectively collected from medical records. All adverse events associated with CART were recorded in accordance with MedDRA/J version 18.0 [25]. Particularly, according to the guideline by the Japan Society of Transfusion Medicine and Cell Therapy, a high fever was defined as a 1 °C elevation in the body temperature from the pre-CART level at ≥38 °C.

Statistical analyses

Standard statistical analyses were utilized. To assess the usefulness of CART in AOC patients, paired *t* tests were used for the comparison of two groups (pre-CART and post-CART). *P* values lower than 0.05 were considered statistically significant. The clinicopathological factors associated with the ATC cluster categories were also examined by a Mann–Whitney *U* test, while the factors influencing high body temperature (1 °C elevation in the body temperature) were analyzed by Fisher's exact test. All statistical analyses were performed using JMP®-Pro software version 14 (SAS Institute Inc.)

Results

The baseline characteristics of the 29 AOC patients who received CART are summarized in Table 1. CART was performed a total of 47 times during treatment for the 29 cases, with an average of 1.7 ± 1.2 (1–6) sessions between January 2011 and March 2017. The mean age was 56.6 years (range 34–80 years). All patients had a significant amount of ascites beyond the pelvic cavity with severe abdominal distention. Twenty-eight patients (96.5%) had an ECOG PS of 2 or 3; only one patient (3.5%) had an ECOG PS of 1. Regarding the histological findings, 12 cases showed evidence of serous carcinoma (41.4%), 7 cases showed clear cell carcinoma (24.1%), and 8 cases (27.6%) were not further characterized by ascites cytology but diagnosed as adenocarcinoma arising from the ovary. The objective of CART was the palliation of severe abdominal distention in our hospital. The pre-CART clinical statuses were diverse and varied across patients but were predominantly divided into 14 cases of therapy (13

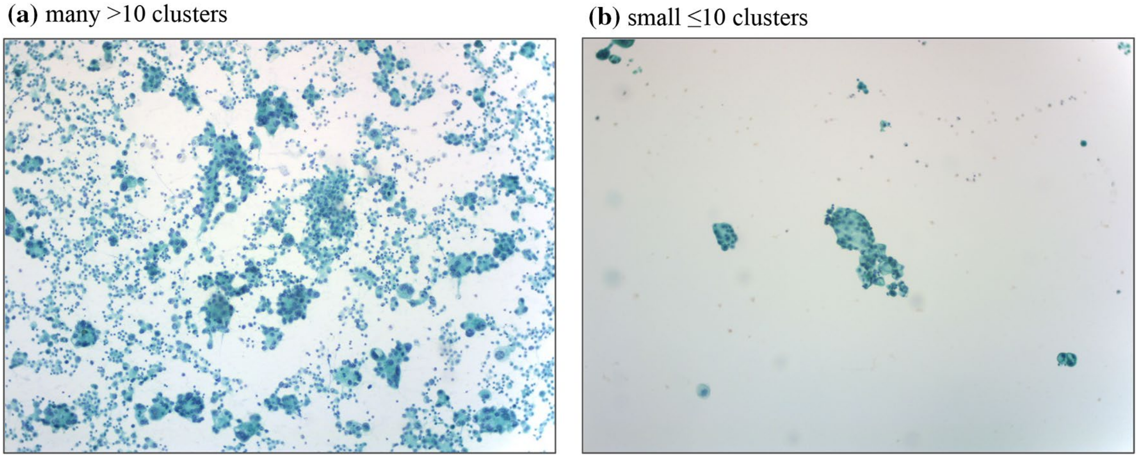

Fig. 1 Atypical cells in the peritoneal cytology. We categorized all cases into two groups by counting the number of ATC clusters per microscopic field at 40· magnification before CART: **a** many (> 10) clusters, and **b** few (≤ 10) clusters

Table 1 Characteristics of the patients

Characteristic	*n*
Total patients	29
Total CART procedures	47
Age (years), mean ± SD (range)	56.6 ± 12.8 (34–80)
ECOG performance status[a]	
0	0 (0.0%)
1	1 (3.5%)
2	13 (44.8%)
3	15 (51.7%)
4	0 (0.0%)
Patient diagnosis (tissue type)	
Clear cell carcinoma	7 (24.1%)
Serous carcinoma	12 (41.4%)
Mucinous carcinoma	1 (3.4%)
Endometrioid carcinoma	1 (3.4%)
Adenocarcinoma	8 (27.6%)
Pre-CART clinical status	
Pretreatment	3 (10.3%)
Chemotherapy	13 (44.8%)
Radiation therapy	1 (3.4%)
Palliative care	12 (41.4%)
Post-CART progress (March 2018)	
Non-recurrence	2 (6.9%)
Chemotherapy	2 (6.9%)
Death	21 (72.4%)
Palliative care	4 (13.8%)

CART concentrated ascites reinfusion therapy, *SD* standard deviation, *ECOG* Eastern Cooperative Oncology Group

[a]Median (range) 3 (1–3); mean 2.5

Table 2 Outcomes of CART procedures

Outcomes	Mean ± SD (range)
Total, *n*	47
Per patient	1.7 ± 1.2 (1–6)
Ascitic volume (mL)	
Pre-CART	2,937 ± 820 (1,500-5,500)
Post-CART	272 ± 84 (100–500)
Concentration ratio[a]	9.6 ± 2.7 (2.9–20.0)
Total protein concentration (g/dL)	
Pre-CART	5.3 ± 1.2 (1.4–7.8)
Post-CART	30.8 ± 5.9 (17.0–42.0)
Reinfused amount of protein (g)	85.0 ± 33.2 (18.4–168)
Retrieval rate (%)	58.2 ± 23.3 (17.5-142.9)
Processing time (min)	102.3 ± 25.5 (59–160)

CART concentrated ascites reinfusion therapy, *SD* standard deviation

[a]Concentration ratio refers to the ratio of the amount of original ascites to the amount of processed ascites

chemotherapy cases and 1 radiation therapy case) and 20 cases of palliative care.

As shown in Table 2, the mean volume of the processed ascites in a single session was 2,937 ± 820 mL (range 1500–5500 mL), which was concentrated to 272 ± 84 mL containing 30.5 ± 5.9 g/dL (range 17–42 g/dL) of protein. The mean amount of reinfused protein was 85.0 ± 33.2 g (retrieval rate 58.2 ± 23.3%; range 17.5–142.9%), and the processing time for filtration and concentration was 102.3 ± 25.5 min (range 59–160 min).

Data on the vital conditions before and after CART (pre-CART and post-CART) are shown in Table 3. A significant reduction in body weight and abdominal girth, and a significant improvement in urine volume and serum albumin level were observed. In contrast, after CART, no significant changes were observed in the levels of serum total protein, serum creatinine, or C-reactive protein. Increases in the white blood cell (WBC) count were observed in this study, as was a significant reduction in the platelet count; however, these changes were ranked as less than Common Terminology Criteria for Adverse Events grade 1 in all patients who had no clinical complications. Importantly, patients' body temperatures were significantly elevated after CART (36.8 ± 0.4 °C pre-CART vs. 37.2 ± 0.6 °C post-CART; $p < 0.0001$). No other clinically important changes in vital signs including blood pressure were observed.

Next, we investigated the clinicopathological factors associated with body temperature changes after CART, including age (< 60 years vs. ≥60 years), PS (< 3 vs. ≥3), pre-CART clinical status (chemotherapy vs. palliative care), histologic type (clear cell, mucinous vs. endometrioid, serous), ascites cytology (number of ATC clusters: small vs. large), pre-CART WBC counts (< 9000/μL vs. ≥ 9000/μL), pre-CART neutrophil/lymphocyte (N/L) ratio (< 4 vs. ≥4), and processing time for filtration and concentration (< 100 min vs. ≥ 100 min). The peak body temperature was recorded on each post-infusion day. As shown in Table 4, the univariate analysis revealed that the presence of a small number of ATC clusters (≤ 10 clusters per microscopic field at 40· magnification of ascites) in the ascites cytology was significantly correlated to a high body temperature after CART ($p = 0.0446$). Notably, the variation in the small number of ATC clusters with a high body temperature were also visualized in Fig. 2, suggesting a significant difference in correlation. In addition, the high body temperature (1 °C elevation in the body temperature) was significant for the presence of a small number of ATC clusters (Table 5).

Discussion

AOC is associated with the highest mortality rate of all gynecological cancers, worldwide [26]. Because the disease is asymptomatic, early detection is difficult; at the time of

Table 3 Changes of clinical condition between pre- and post-CART

	Pre-CART	Post-CART	Δ	*P* value
PS (*n* = 47)	2.5 ± 0.6 (1–3)	2.5 ± 0.6 (1–3)	0	–
Body weight (kg) (*n* = 39)	54.7 ± 10.3 (37.3–86.7)	51.7 ± 9.6 (35.1–82.3)	− 2.97 ± 0.26	< 0.0001
Abdominal girth (cm) (*n* = 29)	91.3 ± 9.4 (78.0–116.0)	86.0 ± 9.8 (68.0–113.5)	− 5.30 ± 0.65	< 0.0001
Urine volume (ml/day) (*n* = 29)	567.0 ± 406.9 (150–2080)	1391.2 ± 708.6 (175–2640)	824.29 ± 145.21	< 0.0001
Body temperature (℃) (*n* = 47)	36.8 ± 0.4 (36.0–37.7)	37.2 ± 0.6 (35.9–38.7)	0.38 ± 0.08	< 0.0001
Systolic blood pressure (mmHg) (*n* = 47)	109.4 ± 13.7 (86–148)	112.6 ± 17.6 (66–180)	3.11 ± 2.41	0.2054
Diastolic blood pressure (mmHg) (*n* = 47)	65.7 ± 11.2 (48–98)	63.8 ± 12.4 (41–98)	− 1.87 ± 1.81	0.3064
Heart rate (/min) (*n* = 47)	94.1 ± 15.9 (64–125)	92.0 ± 18.1 (50–128)	− 2.15 ± 1.63	0.1944
Serum total protein (g/dL) (*n* = 34)	5.32 ± 0.79 (3.4–6.3)	5.59 ± 0.70 (3.7–6.8)	0.26 ± 0.13	0.0504
Serum albumin (g/dL) (*n* = 34)	2.40 ± 0.42 (1–3.2)	2.58 ± 0.49 (1.3–3.5)	0.18 ± 0.34	< 0.0001
WBC ($\times 10^3$/μL) (*n* = 35)	7.08 ± 3.70 (2.3–17.4)	7.68 ± 3.58 (2.7–20.1)	0.60 ± 0.28	0.0385
N/L ratio (*n* = 43)	9.98 ± 13.91 (1.0–66.4)	11.39 ± 15.09 (0.3–74.6)	1.41 ± 0.93	0.1382
Hct (%) (*n* = 35)	30.6 ± 5.1 (22.1–40.3)	28.7 ± 5.5 (19.4–39.3)	− 1.90 ± 0.53	0.0011
Platelet ($\times 10^4$/μL) (*n* = 35)	42.4 ± 20.9 (5.8–83.9)	36.9 ± 17.9 (5.5–74.3)	− 5.52 ± 1.49	0.0007
BUN (mg/dL) (*n* = 38)	21.1 ± 9.4 (6.6–47.5)	18.3 ± 7.7 (6.2–37.9)	− 2.76 ± 0.69	0.0003
Cre (mg/dL) (*n* = 38)	0.97 ± 1.48 (0.31–9.67)	0.70 ± 0.31 (0.32–1.94)	− 2.79 ± 0.24	0.2537
e-GFR (mL/min/1.73 m^2) (*n* = 36)	72.04 ± 28.87 (21.6–187.5)	79.49 ± 28.45 (24.6–181.1)	7.45 ± 1.93	0.0005
CRP (mg/dL) (*n* = 41)	8.36 ± 6.82 (0.66–27.74)	8.05 ± 5.96 (0.67–26.30)	− 0.31 ± 0.51	0.5459

CART concentrated ascites reinfusion therapy, *PS* performance status, *WBC* white blood cell, *N/L* neutrophil/lymphocyte ratio, *Hct* hematocrit, *BUN* blood urea nitrogen, *Cre* creatinine, *e-GFR* estimated glomerular filtration rate, *CRP* C-reactive protein

diagnosis, the tumor has often metastasized (International Federation of Gynecology and Obstetrics stages III and IV) [27]. Even with optimal debulking surgery followed by aggressive front-line chemotherapy, AOC cannot be cured in the majority of cases. This is due to the development of chemoresistant disease, which results in recurrence within 16–22 months and a 5-year survival rate lower than 27% [28]. More than one-third of ovarian cancer patients present with malignant ascites at diagnosis. Moreover, the development of ascites is a fundamental part of having a chemoresistant condition [27]. An anti-vascular endothelial growth factor (VEGF) therapeutic agent—bevacizumab—has been recently introduced in the chemotherapy for AOC patients. Because VEGF is a very major factor in the alteration of vascular permeability, targeting VEGF has the potential to suspend ascites production [29]. However, it may take anywhere between a few weeks and some months to observe an ultimate response in the reduction of massive ascites. Thus, the management of large-volume malignant ascites can be a major problem, and many patients are subjected to frequent paracentesis to temporarily relieve symptoms [1, 7, 30].

Although it may be necessary to investigate a larger number of cases to be able to draw a definite conclusion on the effectiveness of CART in the management of AOC, our findings provide useful data on CART for AOC management. Our data regarding the mean concentration ratio and the recovery rate were similar to those in previous studies of CART for patients with malignant ascites [18]. The effect of protein supplementation by CART may be underestimated by plasma osmotic pressure because reinfusion of albumin dilutes the blood with influx of interstitial fluid into vascular spaces [19]. However, our data with a significant improvement in serum albumin levels and urine volume and a significant decrease in hematocrit level indicate that CART caused fluid inflows due to elevated plasma colloid osmotic pressure. The safety of CART was reflected in the limited negative effects on hemodynamics. Moreover, although our data show a slight decrease in the platelet count, we did not see any hemorrhagic diathesis after CART.

However, the patients' body temperatures increased to 0.38 ± 0.08 °C after CART despite the use of intravenous injections of hydrocortisone premedication to prevent allergic reactions, such as acute inflammation. Although the use of non-steroidal anti-inflammatory drugs can successfully ameliorate elevations in body temperature, it may mask urgent physical changes in AOC patients receiving CART; therefore, it is not routinely prescribed during CART. Some inflammatory cytokines, including interleukin (IL)-6, may be responsive factors in the elevation of body temperatures after CART [16, 19, 31], but it is not realistic to measure the cytokine levels in ascites before every CART procedure. In the case of peritoneal carcinomatosis, elevations in the WBC count can be assumed to reflect the presence of peritonitis. Also, it was recently demonstrated that blood cell counts are risk factors for refractory paracentesis [5], indicating a causal relationship between the quantity of cell components

Table 4 Clinical factors influencing changes in body temperature after CART

	n (%)	°C	*P* value
Age (years) ($n=29$)[a]			
<60	17 (58.6%)	0.34 ± 0.16	0.6728
≥60	12 (41.4%)	0.33 ± 0.19	
PS ($n=29$)[a]			
<3	14 (48.3%)	0.40 ± 0.18	0.2733
≥3	15 (51.7%)	0.28 ± 0.17	
Pre-CART status ($n=25$)[a]			
Chemotherapy	13 (52.0%)	0.47 ± 0.14	0.1993
Palliative care	12 (48.0%)	0.21 ± 0.15	
Histologic type ($n=21$)[a]			
Clear cell and mucinous	8 (38.1%)	0.45 ± 0.27	0.8843
Endometrioid and serous	13 (61.9%)	0.34 ± 0.21	
Ascites cytology (the number of ATC clusters) ($n=27$)[a]			
Small	11 (41.7%)	0.71 ± 0.19	0.0446
Large	16 (59.3%)	0.09 ± 0.16	
Pre-CART WBC (/μL) ($n=44$)			
<9000	31 (70.5%)	0.45 ± 067	0.0615
≥9000	13 (29.5%)	0.09 ± 0.31	
Pre-CART N/L ratio ($n=43$)			
<4	16 (37.2%)	0.44 ± 0.70	0.7338
≥4	27 (61.8%)	0.30 ± 0.56	
Processing time (min) ($n=47$)			
<100	21 (44.7%)	0.29 ± 0.66	0.7639
≥100	26 (33.3%)	0.37 ± 0.55	

CART concentrated ascites reinfusion therapy, *PS* performance status, *ATC* ascites tumor cells, *WBC* white blood cell, *N/L* neutrophil/lymphocyte ratio

[a]Data from before the first CART session

Table 5 The impact of clinical factors correlated with high body temperature after CART

	Odds ratio	95% CI	*P* value
Age (years) ($n=29$)[a]			
<60			
≥60	2.50	0.35–17.94	0.6221
PS ($n=29$)[a]			
<3			
≥3	0.56	0.08–4.01	0.6513
Pre-CART status ($n=25$)[a]			
Chemotherapy			
Palliative care	0.30	0.03–3.41	0.5930
Histologic type ($n=21$)[a]			
Clear cell and mucinous			
Endometrioid and serous	0.90	0.12–7.03	1.0000
Ascites cytology (the number of ATC clusters) ($n=27$)[a]			
Small			
Large	0	-	0.0057
Pre-CART WBC (/μL) ($n=44$)			
<9000			
≥9000	0	-	0.0820
Pre-CART N/L ratio ($n=43$)			
<4			
≥4	0.52	0.11–2.46	0.4433
Processing time (min) ($n=47$)			
<100			
≥100	0.77	0.17–3.55	1.0000

CART concentrated ascites reinfusion therapy, *CI* confidence interval, *PS* performance status, *ATC* ascites tumor cells, *WBC* white blood cell, *N/L* neutrophil/lymphocyte ratio

[a]Data from before the first CART session

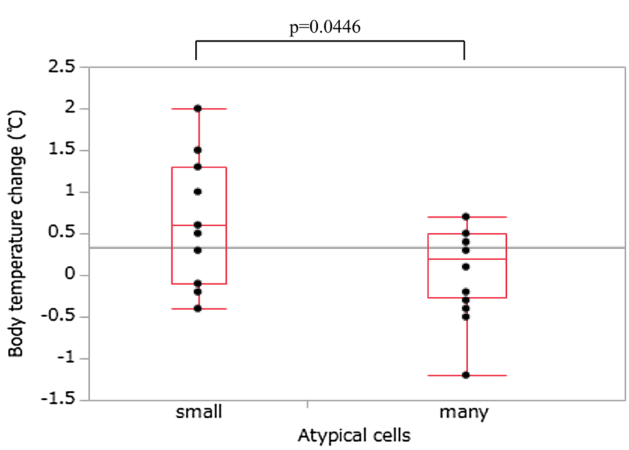

Fig. 2 Comparison of changes in body temperature due to differences in the number of atypical cells in peritoneal cytology. The presence of a small number of ATC clusters significantly correlates with the elevation in body temperature

such as the neutrophil/lymphocyte (N/L) ratio and a patient's status [32]. To our knowledge, ours is the first study to provide a comprehensive evaluation of body temperature elevation in AOC patients after CART, despite modifications in intravenous administration using hydrocortisone and reductions in the filtration flow rate to avoid the risk of high body temperatures during the procedure [16, 19].

At first, we considered that WBC counts, and the N/L ratio may affect elevations in body temperature. We also focused on inflammatory cytokines such as IL-6, which were released from activated WBCs in association with the processing step for filtration and concentration. However, unexpectedly, as shown in Table 4, the WBCs in the ascites were not significantly correlated with elevations in body temperature; this could represent patients' fragile status and reflect a delicate balance in patients' status. Next, we focused on the peritoneal cytology results before CART; this can provide valuable information on ovarian surface involvement and peritoneal dissemination of AOC. The presence of a positive peritoneal cytology is a predictor of poor survival in AOC patients receiving interval debulking surgery during initial treatment [33]; therefore, the positive peritoneal cytology may reflect the peritoneal carcinomatosis status. Therefore, we hypothesized that the number of ATC clusters was correlated to the levels of cytokines and chemokines. It is because cytokines such as IL-1β, tumor necrosis factor α, and IL-6 mediate the acute phase response to peritonitis in response to the tumor microenvironment [22]. Additionally, in general, IL-1β and IL-6 are produced by monocytes and endothelial cells. IL-6 is also produced in T cells and fibroblasts in the cancer microenvironment [29]. High levels of cytokines in ascites may be from the interaction of ATC clusters with cellular components, including blood cells, and soluble proteins. We believe that when large numbers of ATC clusters are visible in the ascites, massive ascites is induced by the lymphatic drainage obstruction due to a large number of ATC clusters and dissemination in the peritoneum. In contrast, when massive ascites is caused by the inflammatory cytokines synthesized in the peritoneal cavity with a small number of ATC clusters, an immune-active tumor microenvironment that induces leakiness of tumor microvasculature exists in the peritoneal cavity. The presence of a small number of ATC clusters may therefore be significantly correlated with high levels of inflammatory cytokines. Future studies should investigate which cytokines are responsible for body temperature elevation by measuring the levels of inflammatory cytokines in ascites.

Our study has several limitations. First, this is a retrospective, observational, single-center design that included a small sample size. Additionally, patient backgrounds may be varied in terms of disease status, and no cytokine measurements were available from the study data. However, this is the first study to demonstrate the safety and efficacy of CART for AOC treatment, investigate the factors inducing body temperature elevations due to CART, and reveal the possible relationship between the number of ATC clusters and high body temperature after CART.

In conclusion, our findings confirm the effectiveness of CART in the management of massive malignant ascites in AOC. Thus, it may be a good practice to investigate patients' peritoneal cytology and count the number of ATC clusters before CART initiation. Further cohort studies should be conducted to evaluate the usefulness of CART and how adverse events in AOC treatment can be avoided.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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