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Endotoxin Activity Reflects an Increase in Body Temperature in Cirrhotic Patients With Ascites Undergoing Cell-free and Concentrated Ascites Reinfusion Therapy

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Abstract. *Background: Ascites commonly complicates cirrhosis and is refractory to the vasopressin-2 antagonist tolvaptan and fluid restriction in approximately 60% of patients. We aimed to identify risk factors associated with adverse events following cell-free and concentrated ascites reinfusion therapy (CART) in patients with cirrhosis and ascites. Patients and Methods: We evaluated the efficacy and tolerability to the CART system in 18 patients with decompensated liver cirrhosis and ascites. We determined serum endotoxin activity using endotoxin activity (EA) assays and serum and ascitic fluid concentrations of interleukin 6 (IL6) and tumor necrosis factor-α (TNFα) before and after the CART procedures. Results: Body weight and waist circumference significantly decreased after CART (both p<0.001). Body temperature (BT) increased significantly at an average rate of 1.1˚C during CART (p<0.001). The change in BT was correlated with EA and not interleukin IL6 or TNFα. The rise in BT was positively correlated with serum EA levels at baseline. The increase in BT was significantly higher in the group with high EA (≥0.37) than in the low EAA group (<0.37) (p=0.02). TNFα and serum IL6 levels in ascites were significantly increased during CART (both p<0.001). However, no significant differences in the EA, serum TNFα or IL6 levels were found in ascitic fluid before and after the CART procedures. Conclusion: Although this discovery warrants further study, EA assay can indicate an increase in BT during effective CART in patients with cirrhosis and ascites.*

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Liver cirrhosis, the end stage of liver disease, causes clinical manifestations including ascites, variceal hemorrhage, hepatic encephalopathy, or jaundice in the decompensated stage. Refractory ascites, one of the most serious complications in patients with cirrhosis and ascites, has a prevalence of 5-10% in 5 years (1) and a post diagnosis survival rate of 50% at 6-12 months (2, 3). Most patients with decompensated cirrhosis who develop large volumes of ascitic fluid notice abdominal distention, which may markedly impair the patients' quality of life (4). Evidence-based clinical practice guidelines for liver cirrhosis have shown that diuretic resistance or poor diuretic response is an indication for large abdominal paracentesis or cell-free and concentrated ascites reinfusion therapy (CART) (5, 6), with fever, high blood pressure, headache, and shaking with chills having been reported as CART-related adverse events (7). Most patients experience high fever during the reinfusion of ascitic fluid (8), with evidence implicating endotoxin or inflammatory cytokines as a cause (9). Nonetheless, premedication immediately before reinfusion of ascites has been shown to prevent fever. Thus, identifying risk factors predicting fever during CART is critical.

Patients and Methods

Patients. The present study enrolled 145 patients with decompensated cirrhosis who received treatment at the Department of Gastroenterology, Nara Medical University Hospital between September 2005, and May 2019. Among them, 18 patients with

cirrhosis who underwent CART were included. Potential complications, including intraperitoneal hemorrhage from perforation of the inferior epigastric artery, abdominal wall hematoma, bowel perforation, persistent leakage of fluid, infection at the entry site, or peritonitis, were not observed in this study. Body temperature (BT) and proinflammatory cytokine levels were measured before and immediately after reinfusion. We determined the proinflammatory cytokine levels in original and processed ascitic fluid. Laboratory data were also examined several days prior to or on the day of CART and 1 day after the procedure.

Written informed consent was obtained from all patients enrolled prior to the collection of blood and ascitic fluid samples.

CART procedure. The standard CART procedure comprises three steps: i) Drainage of the ascites into a designated bag *via* abdominal paracentesis; ii) removal of malignant cells and bacteria *via* filtration, and removal of excess fluid and electrolytes using concentration method; and iii) reinfusion of the filtered and processed ascites (10). Ascitic fluids were processed at the rate of 50-100 ml/min using the CART system. A plasmapheresis device (KPS-8800Ce; Asahi Kasei Kuraray Medical, Tokyo, Japan) and circuit (KMT-8601; Kawasumi Laboratories Co., Tokyo, Japan) were used to process the ascites. The concentration method was an internal pressure type system. Ascites obtained from patients were filtered through AHF-MO ascites filtration filters (Asahi Kasei Medical Co., Ltd). The filtered ascites were subsequently concentrated using AHF-UP ascitic concentration filters (Asahi Kasei Medical Co., Ltd). We confirmed that endotoxins were not detected in the collected ascites as CART has no influence on the ability to remove endotoxins (11). Furthermore, the total white blood cell count in ascites was examined before performing the CART procedures. Patients with spontaneous bacterial peritonitis were excluded. The absolute polymorphonuclear cell count was calculated by multiplying the total number of white blood cells by the percentage of polymorphonuclear cells. Infusion of processed ascitic fluid was commenced at the rate of 1.0 ml/min and maintained at 1.0-5.0 ml/min.

Measurement of endotoxin activity (EA). Whole-blood EA was assessed using the commercially available Endotoxin Activity Assay (EAA) kit (Spectral Diagnostics, Toronto, Canada), as described elsewhere (12). In brief, EAA measurements were based on the principle that endotoxin binds to anti-endotoxin antibodies and is delivered to neutrophils by complement receptors. In the presence of β-glucan and luminol, these neutrophils encounter a respiratory burst with the emission of light. A chemiluminometer was used to quantify the light produced, with its intensity being proportional to the amount of endotoxin present in the sample (13). EA was expressed in relative units derived from the integral of the basal and stimulated chemiluminescent response (on a scale from 0 to 1). Accordingly, an EA level of 0.4 is approximately equivalent to a lipopolysaccharide (LPS) concentration of 25-50 pg/ml, with healthy controls supposedly having EA levels <0.2 (14). EA levels were measured before paracentesis and after reinfusion.

Proinflammatory cytokine levels. Serum and ascitic fluid levels of proinflammatory cytokines tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL6) were measured using commercially available enzyme-linked immunosorbent assay kits (human TNF-alpha Quantikine ELISA Kit and hIL-6 QKit) from R&D Systems (Minneapolis, MN, USA) according to the manufacturers' instructions. The concentrations of these cytokines were determined by comparing the absorbance of samples to standard curves. The assay range for TNFα and IL6 was 15.6-1,000 pg/ml and 31.2-2,000 pg/ml, respectively.

Table I. *Baseline characteristics before concentrated ascites reinfusion therapy procedures in patients with liver cirrhosis (n=18).*

Parameter		Value
Age, years	Mean±SD	69.3±14.7
Body mass index, kg/m^2	Mean±SD	24.0±5.6
Sex, n	Male	5
	Female	13
Etiology, n	HCV	6
	Alcohol	7
	PBC	3
	NASH	2
Child–Pugh score, n	8	2
	9	4
	10	4
	11	6
	12	1
	13	1
Child–Pugh grade, n	B	5
	C	13
Hepatocellular carcinoma, n	Yes	8
	No	10
Poral thrombosis	Yes	3
	No	15
Amount of drained ascitic fluid, ml	Mean±SD	5,847.2±2,245.9
Amount of processed ascites, ml	Mean±SD	340.6±191.6
Reinfusion speed, ml/min	Mean±SD	2.4±1.1

HCV: Hepatitis virus C; PBC: primary biliary cholangitis; NAFLD: non-alcoholic fatty liver disease.

Statistical analysis. Data were expressed as median±standard deviation. All statistical analyses were performed using the GraphPad Prism version 8 software (GraphPad Software, La Jolla, CA, USA) (15). The paired *t*-test was performed for comparisons of two paired samples with normally distributed data (body weight, systolic blood pressure, waist circumference, hemoglobin, white blood cell count, platelet, total protein, albumin, blood urea nitrogen, creatinine, glomerular filtration rate, antithrombin III, and BT). The Wilcoxon signed-rank test was performed for comparisons of two paired samples with non-normally distributed data (urine volume, aspartate aminotransferase, alanine aminotransferase, total bilirubin, ammonia, C-reactive protein, fibrin degradation products, fibrinogen, prothrombin time, EA, ascites, and serum TNFα and IL6). Correlations among continuous variables were evaluated using Spearman's correlation coefficient. A result with a two-tailed value of $p<0.05$ was considered statistically significant.

Results

Baseline characteristics of the patients with cirrhosis. The baseline characteristics of the 18 patients with cirrhosis enrolled are summarized in Table I.

Table II. *Clinical indices before and 1 day after concentrated ascites reinfusion therapy.*

Parameter	Before	After	*p*-Value
Body weight, kg	65.59±17.20	59.93±15.32	**<0.001**
Systolic blood pressure, mmHg	112.8±13.8	107.56±14.53	0.02
Urine volume, ml/day	898.6±498.8	1,016.3±674.4	0.31
Waist circumference, cm	96.4±13.6	88.3±13.7	**<0.001**
Hemoglobin, g/dl	10.19±1.94	9.61±1.84	**<0.001**
White blood cell, U/l	4,472.2±1,267.1	4,194.1±1,291.8	0.11
Platelet, mg/dl	9.49±2.69	8.38±2.21	0.01
Total protein, g/dl	5.94±0.62	5.92±0.72	0.86
Albumin, g/dl	2.54±0.27	2.56±0.31	0.69
Aspartate aminotransferase, IU/l	45.83±18.94	45.94±24.41	0.12
Alanine aminotransferase, IU/l	25.17±12.21	25.00±14.67	0.12
Total bilirubin, mg/dl	1.74±1.13	1.78±1.16	0.51
Ammonia, μg/dl	54.02±29.76	55.82±29.01	0.93
Urea nitrogen, mg/dl	34.28±19.06	34.67±18.49	0.71
Creatinine, mg/dl	1.36±0.97	1.67±1.03	0.54
Glomerular filtration rate, ml/min/1.73 m^2	50.36±22.80	40.24±20.73	0.08
Antithrombin III, %	56.5±14.08	54.82±12.43	0.25
C-Reactive protein, mg/dl	0.82±0.12	0.95±0.34	0.14
Fibrin degradation products, μg/ml	17.50±10.00	31.12±18.62	**<0.001**
Fibrinogen, g/l	229.00±75.98	210±72.2	**0.008**
Prothrombin time, %	62.11±15.70	60.7±16.77	**<0.001**

All data are presented as the mean±standard deviation. Statistically significant *p*-values are shown in bold.

Changes in clinical indices before and after CART. Body weight, Systolic blood pressure, waist circumference, platelet count, hemoglobin, fibrin degradation products, and prothrombin time were significantly decreased 1 day after the CART procedure ($p<0.001$, $p=0.02$, $p<0.001$, $p=0.01$, $p<0.001$, $p=0.008$, and $p<0.001$, respectively) (Table II). Although significant changes were observed in the systolic blood pressure 1 h after reinfusion, the reduction in blood pressure was small, indicating that the reinfusion process only had minor effects on hemodynamics (data not shown). Fibrin degradation product levels were significantly higher after than that before CART ($p<0.001$). However, no significant changes in albumin nor C-reactive protein levels were observed 1 day after the CART procedure ($p=0.69$ and $p=0.14$, respectively).

BT and EA measurements before and after CART. BT increased significantly by 1.1±0.7˚C on average after CART ($p<0.001$) (Figure 1A). No significant differences in baseline EA were found between before and immediately after CART procedures ($p=0.87$) (Figure 1B).

Serum and ascitic fluid cytokine levels before and immediately after CART. TNFα levels were significantly increased in processed ascitic fluid compared to original ascitic fluid ($p<0.001$) (Figure 2A). No significant differences between serum TNFα levels were found before and after CART procedures ($p=0.40$) (Figure 2B). No significant differences in IL6 levels were found between processed and original ascitic fluid ($p=0.63$), whereas serum IL6 levels were significantly greater after completion of reinfusion than before reinfusion ($p<0.001$) (Figure 3).

Correlation between changes in BT and EA and cytokines at baseline. Baseline EA values were significantly positively correlated with BT (R=0.57, $p=0.01$; Figure 4A). A total of 18 patients were grouped into two categories using an EA cut-off of 0.37. The high EA group (≥0.37) included eight patients and the low EA group included 10 patients. The increase in BT was significantly greater for the high EA group than for the low EA group ($p=0.02$) (Figure 4B). BT changes were not significantly correlated with serum levels of IL6 (R=–0.18, $p=0.48$) nor TNFα (R=–0.00008, $p=1.0$) (Figure 5).

Discussion

CART is now widely available for patients with malignant ascites. However, only a few reports are available on the safety and efficacy of CART in patients with cirrhosis. The present study therefore sought to investigate factors that elicit changes in BT during effective CART. Overall, our findings showed that EA, a marker of the presence of endotoxin in the blood, based on bacterial translocation from the gut, may predict the rise in BT among patients with decompensated cirrhosis and ascites during CART. Notably, the high EA group (≥0.37) at baseline had a significantly

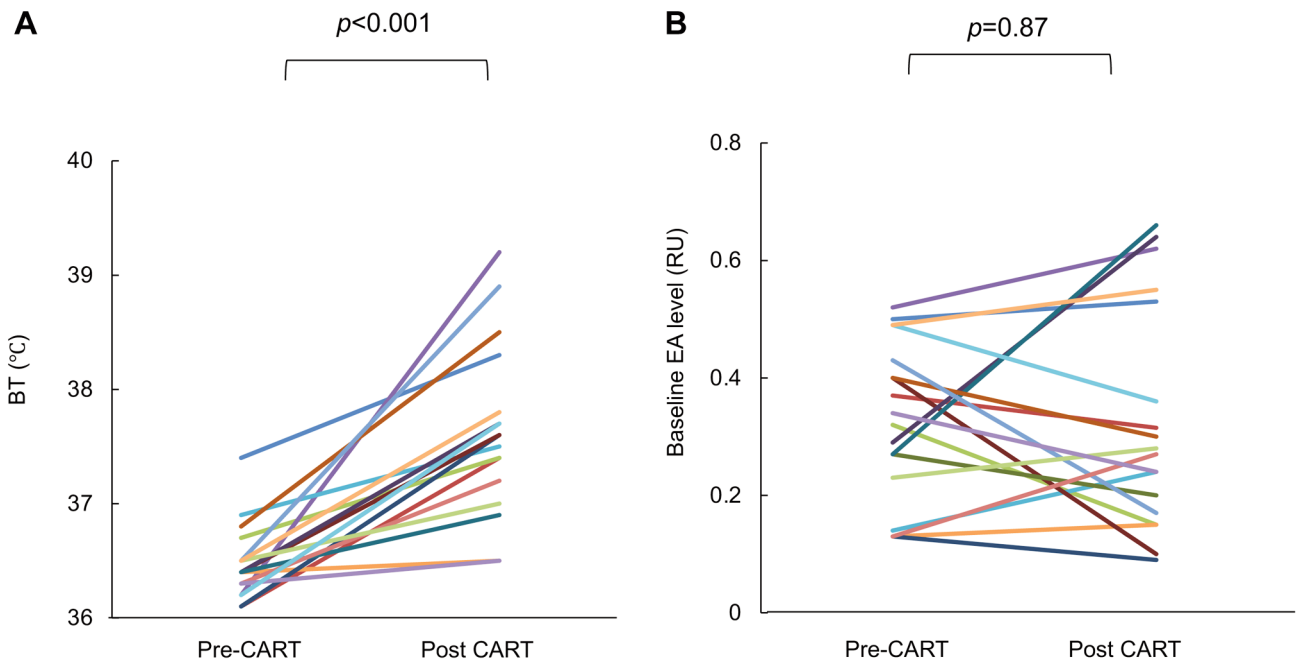

Figure 1. *Body temperature (A) and endotoxin activity (EA) levels (B) before and after concentrated ascites reinfusion therapy (CART). RU: Relative units.*

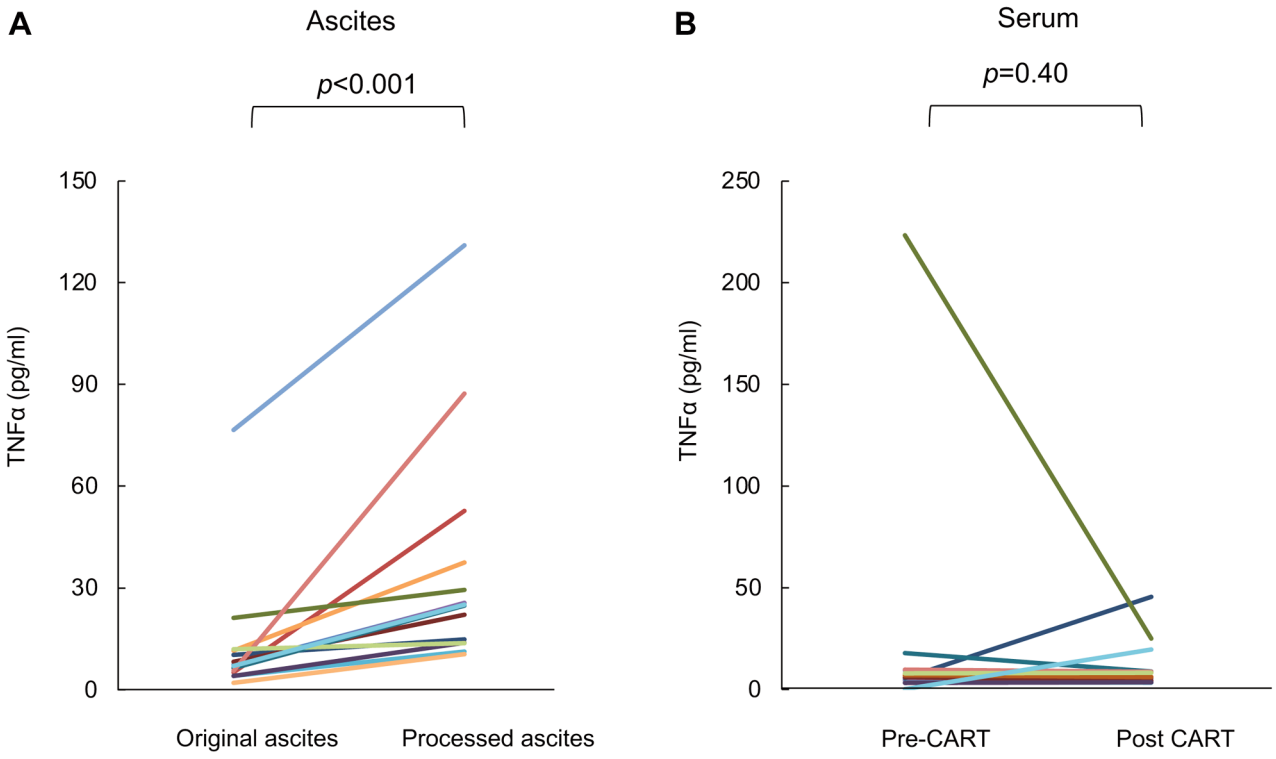

Figure 2. *Serum and ascitic fluid levels of tumor necrosis factor-alpha (TNFα) before and after concentrated ascites reinfusion therapy (CART).*

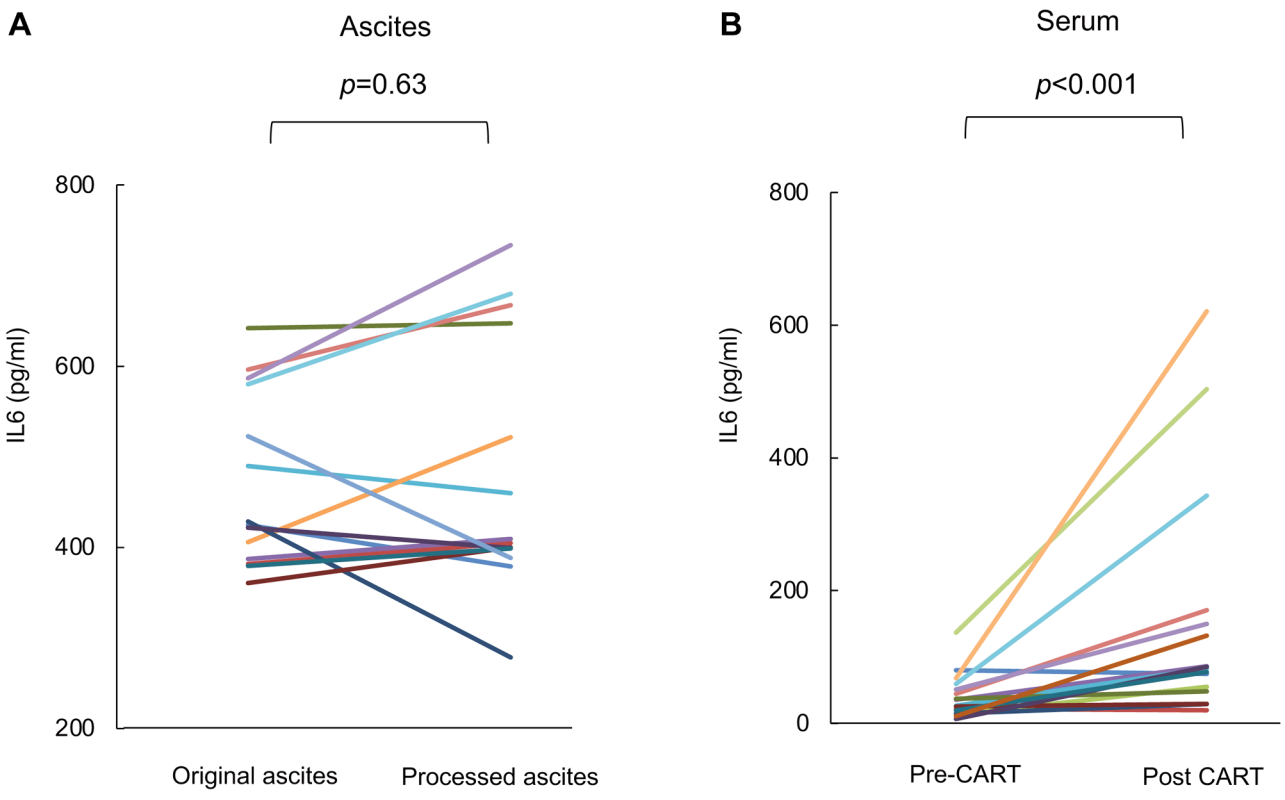

Figure 3. *Serum and ascitic fluid levels of interleukin-6 (IL6) before and after concentrated ascites reinfusion therapy (CART).*

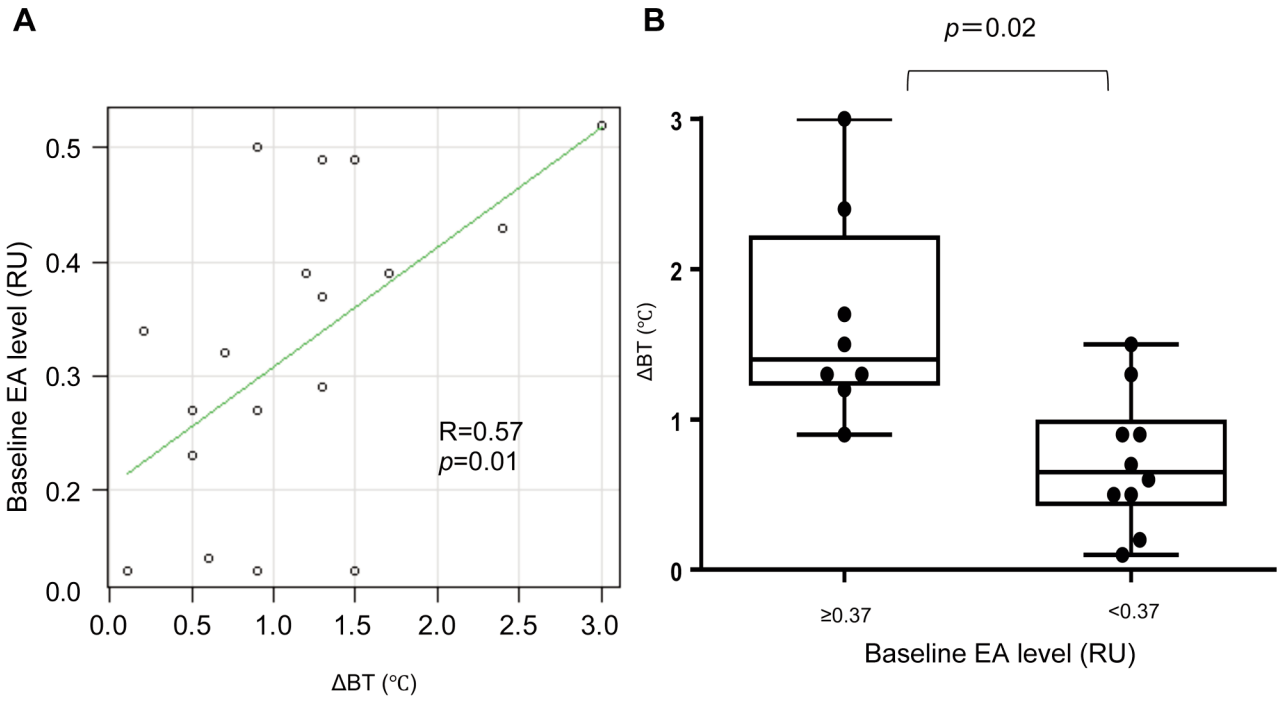

Figure 4. *Correlation of changes in body temperature (BT) and endotoxin activity (EA) levels.*

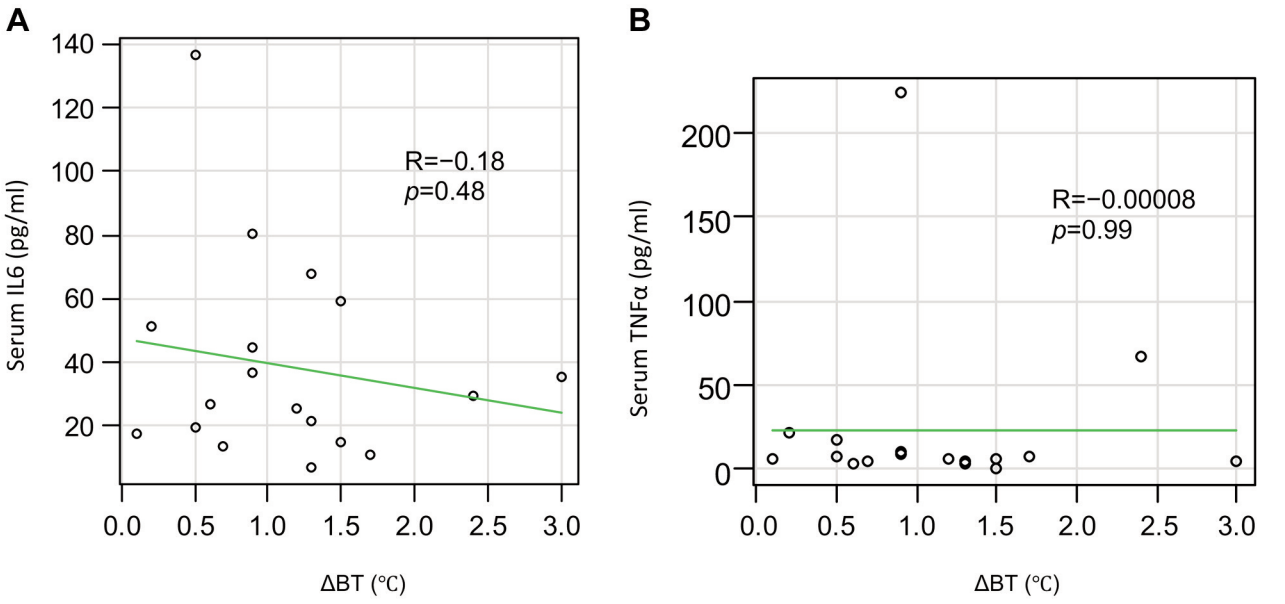

Figure 5. *Correlation of changes in body temperature (BT) with serum interleukin-6 (IL6) levels and tumor necrosis factor-alpha (TNFα) levels.*

greater BT after CART than the low EA group (<0.37). As far as we are aware, this is the first study to show that baseline EA levels increased with BT during CART in patients with cirrhosis and ascites. Randomized controlled clinical trials have shown that LPS administration induced a significant increase in BT (16, 17). Endotoxins are considered pyrogens given that they trigger the innate immune system and produce fever when bacterial translocation occurs in humans (18). However, it is highly unlikely that exogenous endotoxins cause fever by acting directly on the hypothalamic thermoregulatory center considering that countless fever-producing microbial products exist with different 3-dimensional structures (19). Nevertheless, changes in BT were correlated with endotoxin perhaps due to the gradual rise in BT after the time from pathogen exposure (20). There was no significant impact of the rise in BT on the EA levels in patients with cirrhosis. As the baseline serum EA levels are already high in patients with cirrhosis, a rise in BT may not have a significant additional impact on the serum EA levels (21). These findings reconfirm the potential role of serum EA levels in patients with cirrhosis and ascites during the CART procedure, suggesting this may be a potential new biomarker for predicting the most frequent CART-related adverse event.

IL6 is a cytokine that regulates systemic inflammation and fever response to pathogens, such as LPS (22). IL6 infusion inhibits hepatic albumin synthesis and promotes cachexia (23). Our findings showed that serum IL6 increased after completing reinfusion. However, serum albumin levels were not reduced after CART in cirrhosis patients with hypoalbuminemia and ascites. These differences in the role of IL6 on albumin production remain unclear. However, one possible explanation might be the differences in the underlying liver disease between studies. All 18 patients in our study developed cirrhosis, whereas the study by Ueda *et al*. enrolled 22 patients with hepatocellular carcinoma (24). Furthermore, fever normalization was observed in patients treated with anti-IL6 therapeutics despite IL6 response (25). Moreover, excessive production of TNFα after stimulation by LPS may result in fever and inflammation (26). However, no significant correlations were observed between changes in BT and serum levels of either IL6 or TNFα, indicating that endotoxin, and not inflammatory cytokines, contributed to the increase in BT among patients with cirrhosis who underwent CART in this study.

Nevertheless, this study has some limitations worth noting. Firstly, the sample size was extremely small given the need for patients to be admitted for CART. Secondly, a control group in which only abdominal paracentesis was conducted was not established. Moreover, the possibility of selection bias cannot be ruled out. Given the retrospective observational nature of this study, other factors could not be considered. Larger prospective studies are hence warranted to obtain higher level evidence on the use of biomarkers for fever triage.

In conclusion, baseline EA levels were able to represent a rise in BT among patients with cirrhosis and ascites during effective CART. The presence of endotoxins causes adverse events during CART procedures. Further investigations are

needed to determine whether anti-endotoxin drugs, including probiotics, prebiotics, and antibiotics, such as rifaximin, might inhibit the onset of CART-related adverse events, the most common of which is fever.

Conflicts of Interest

None declared.

Authors' Contributions

Conceptualization, HY; investigation, YT, NY, NN, KK, and HT; data curation, YF, YS, NS, K. Kaji, HK, and KM; resources and investigation, TA and MA; visualization and supervision, MK; formal analysis, TI; writing–original draft preparation, TN; writing–review and editing, TN. All Authors read and approved the final article.

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