

Angiopoietin Balance in Septic Shock Patients With Acute Lung Injury: Effect of Direct Hemoperfusion With Polymyxin B-Immobilized Fiber

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Abstract: Acute lung injury (ALI) in sepsis is characterized by an increase in microvascular permeability, resulting in pulmonary edema. Several studies have suggested that angiopoietin-1 and -2 play a contributory role in the pathogenesis of ALI. Polymyxin B-immobilized fiber column hemoperfusion is effective for sepsis-induced ALI. We investigated the angiopoietin levels before and after direct hemoperfusion with polymyxin B-immobilized fiber column (PMX) therapy. Enzyme-linked immunoassay was used to measure the serum angiopoietin-1 and -2 levels in 25 patients with septic shock treated with PMX. Eleven of the 25 patients were diagnosed with ALI. There was a significant positive correlation between the angiopoietin-1 level and the PaO₂/FiO₂ ratio, but there was a significant inverse correlation between the angiopoietin-2 level and the PaO₂/FiO₂ ratio. The mean angiopoietin-1 level before

PMX therapy in the ALI group was significantly lower and the mean angiopoietin-2 level was significantly higher than in the non-ALI group. The mean angiopoietin-1 level of the ALI patients in response to PMX therapy was increased during PMX therapy, but that of the non-ALI patients with newly occurring ALI showed a decreased angiopoietin-1 level. On the other hand, the mean angiopoietin-2 level of the responders was decreased during PMX therapy, but that of patients with newly occurring ALI showed an increased angiopoietin-2 level. This result suggested that each angiopoietin-1 and -2 level may play a role in the pathogenesis of ALI and that PMX therapy ameliorates the angiopoietin balance in patients with ALI in sepsis. **Key Words:** Acute lung injury, Angiopoietin-1, Angiopoietin-2, Direct hemoperfusion with polymyxin B-immobilized fiber column, PaO₂/FiO₂ ratio, Sepsis.

Sepsis is characterized as a systemic inflammatory response to a microbial pathogen. Although mortality associated with sepsis has declined in the past 20 years (1), it remains a common and deadly condition. Individuals with sepsis who develop shock and multi-organ dysfunction are at greatest risk of death (2). Capillary permeability, a tightly regulated feature of microcirculation in all organ beds, is fundamentally

altered in sepsis, resulting in net extravasation of fluid out of the vascular space and into tissues. Dramatic manifestations of this phenomenon are acute lung injury (ALI) and septic shock. ALI is characterized by activation of the pulmonary endothelium, disruption of the endothelial and alveolar epithelial barriers, and an increase in microvascular permeability, resulting in pulmonary edema (3).

Several angiogenic factors, such as vascular endothelial growth factor (VEGF) and angiopoietins, may be associated with capillary permeability. Angiopoietin-1 is a positive regulator of blood vessel development, remodeling, and maturation (4). This protein is associated with the maintenance of vascular integrity and supports recruitment of endothelial

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cells and perivascular cells (5). Angiotensin-2 is a competitive inhibitor of angiotensin-1 (6). It may also play a pro-angiogenic role by mediating the destabilizing interaction between endothelial and perivascular cells (7). Several recent studies have suggested that angiotensin-1 and -2 play a contributory role in the pathogenesis of ALI (8,9). Treating blood vessels with blood from patients with ALI or with only angiotensin-2 made these blood vessels leaky *in vitro*, but reversal of the leakiness by subsequent treatment with angiotensin-1 was shown (8). Moreover, injecting angiotensin-2 into the blood of healthy mice caused ALI-like symptoms *in vivo*, but these symptoms can be reversed with angiotensin-1 (8). Angiotensin-1 reduces pulmonary inflammation and permeability (9). It was reported that the ratio of the concentration of angiotensin-2 relative to angiotensin-1 may be a prognostic biomarker of endothelial activation in ALI patients and may be useful for risk stratification of ALI patients (10). Angiotensin-2 interferes with angiotensin-1, resulting in pulmonary inflammation and increased permeability (8–10).

For the treatment of septic shock, direct hemoperfusion with a polymyxin B-immobilized fiber column (PMX) that potentially adsorbs plasma endotoxins has been widely used and has resulted in both decreased plasma endotoxin levels and the improvement of hemodynamic instability (11). Several investigations demonstrated that PMX therapy is effective against ALI induced by direct or indirect pulmonary injury (12,13). To verify the efficacy of PMX therapy for ALI and the relation between ALI and the angiotensin balance, we investigated the angiotensin levels in ALI patients treated with PMX.

PATIENTS AND METHODS

Subjects

Every weekday during a one-year period, all patients admitted during the preceding 24 h to the medical intensive care unit at Mito Saiseikai General Hospital, Ibaraki, Japan, were screened for study eligibility. Twenty-five patients were diagnosed with sepsis and septic shock according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee (14). Eleven of the 25 patients were diagnosed with ALI according to the criteria of the American-European Consensus Conference (15). PMX treatment was initiated if the patients exhibited sustained shock despite fluid resuscitation and vasopressor drugs. All patient records were reviewed to obtain demographic data, and these data, as well as details of

the initial clinical presentation at the time PMX was started and the Acute Physiology And Chronic Health Evaluation version II (APACHE-II) score (16), were used for the quantitative assessment of each patient.

PMX with continuous hemodiafiltration

PMX was achieved with continuous renal replacement therapy (CRRT). A dual-lumen catheter (Arrow International, Reading, PA, USA) was inserted into a central vein. Blood flow was kept at 60 to 100 mL/min. A PMX column immobilizing polymyxin B (Toraymyxin PMX -20R; Toray Industries, Tokyo, Japan) was used for direct hemoperfusion, and a polysulfone hollow-fiber hemofilter (Hemofeel SH-0.8; Toray Industries) was used for CRRT. Because the hemodynamics were unstable, continuous hemodiafiltration (CHDF) was chosen as the CRRT method. PMX with CHDF carried out for 24 h was considered one session of therapy. Therapy was discontinued when adverse events appeared or when it was judged that PMX would be difficult to continue. Otherwise, PMX was carried out twice on each patient. Anticoagulation of the circuit was achieved with nafamostat mesilate (Torii Pharmaceuticals, Tokyo, Japan) at 30 to 35 mg/h to maintain an activated coagulation time of 150 to 200 s.

The study protocol was accepted by the ethics committee of our institution, and written informed consent was obtained from all patients or their immediate family members. This study also conformed to the provisions of the Declaration of Helsinki of 1995.

Blood sampling and assay of angiotensins

Serial blood samples were collected in plasma separator tubes before and after PMX therapy. The samples were then separated at 1000 g for 15 min and stored at -80°C for analysis.

The samples were measured by commercially available enzyme-linked immunoassay kits (Quantikine; R & D Systems, Minneapolis, MN, USA) in duplicate. In brief, 96-well microplates were first coated with either of two anti-angiotensins. Samples from the controls were diluted 50-fold (in angiotensin-1) or 5-fold (in angiotensin-2), and the samples from septic patients were diluted 5-fold (in angiotensin-1) or 20-fold (in angiotensin-2). Then, 50 µL of diluted sample was added to each well. The plates were first incubated for 2 h at room temperature and then washed, and each of two horseradish peroxidase-conjugated polyclonal anti-angiotensin antibodies was added at 200 µL/well. The plates were then incubated for 2 h at room temperature and washed, and chromogen (tetramethylbenzidine) and

TABLE 1. Characteristics of patients at the start of PMX therapy

	Total patients	With ALI	Without ALI	P-value
Number of patients	25	11	14	
Age (years)	68.5 ± 17.8	71.4 ± 12.3	66.2 ± 21.4	0.805
Gender (Male : Female)	16:6	9:2	11:3	0.840
Mean arterial pressure (mm Hg)	62.0 ± 13.2	57.8 ± 10.9	65.3 ± 14.2	0.132
APACHE II	26.1 ± 8.70	29.9 ± 7.56	23.1 ± 8.67	0.080
Treatment time of DHP-PMX (h)	32.3 ± 19.0	34.7 ± 19.6	30.4 ± 19.0	0.366
Site of infection				0.085
Intra-abdominal	18/25	6/11	12/14	
Others	7/25	5/11	2/14	
Arterial pH	7.343 ± 0.129	7.330 ± 0.135	7.353 ± 0.128	0.661
White blood cell count (/μL)	15 540 ± 10 328	17 464 ± 10 953	14 029 ± 9 950	0.477
Hemoglobin (g/dL)	11.20 ± 2.50	11.00 ± 2.27	11.44 ± 2.79	0.763
Platelet count (×10 ⁴ /μL)	16.1 ± 11.5	9.18 ± 3.92	21.6 ± 12.7	0.047
Serum creatinine levels (mg/dL)	2.70 ± 2.55	3.19 ± 2.11	2.30 ± 2.87	0.106
Serum CRP levels (mg/dL)	21.7 ± 10.6	24.6 ± 10.8	19.4 ± 10.3	0.298
Plasma endotoxin (pg/mL)	21.35 ± 54.17	11.0 ± 21.9	30.5 ± 72.4	0.413
Positive for blood culture	12/25	5/11	7/14	0.821
PaO ₂ /FiO ₂ ratio	225.7 ± 101.8	170.8 ± 59.1	268.8 ± 109.0	0.018

ALI, acute lung injury; APACHE II, Acute Physiology and Chronic Health Evaluation version II; CRP, C-reactive protein; DHP-PMX, direct hemoperfusion with polymyxin B-immobilized fiber column.

hydrogen peroxide were added to each well. The final incubation plates were incubated for 30 min at room temperature, and 200 μL of 2 N sulfuric acid solutions was added to each well. The plates were immediately read on a microplate reader (Sunrise Remote; Tecan Japan, Kanagawa, Japan) set at a 450 nm wavelength and at 540 nm for wavelength correction. The inter- and intra-assay variations were less than 10%.

Statistical analysis

Variables were expressed as mean ± standard deviation or as numbers with percentages of the total. Statistically significant differences in the mean values between the two subject groups were evaluated by the Mann-Whitney *U*-test. The mean values that had time-dependent changes were analyzed by the Wilcoxon signed-rank test. The analysis of variance (ANOVA) was used to assess differences among three or more subject groups, and post-hoc comparisons were made using the Bonferroni/Dunn test. We compared categorical data between the two groups using the Chi-squared test with Yates' continuity correction and Fisher's exact test. Correlation analysis was performed using Pearson's correlation method, and linear regression analysis was carried out to determine whether or not the angiopoietins were related to the arterial blood's oxygen partial pressure (PaO₂) to the fraction of inspired air consisting of the oxygen (FiO₂) ratio. Statistical significance was defined as a *P*-value of less than 0.05. All statistical analyses were performed on a computer using the StatView program, version 5.0J (SAS Institute, Cary, NC, USA) for Windows.

RESULTS

Twenty-five Japanese patients (16 males and 9 females) with septic shock were included in the study. The patients' characteristics and laboratory data are shown in Table 1. At the initiation of PMX therapy, 11 patients fulfilled the criteria for ALI (the ALI group), but 14 patients did not (the non-ALI group). Four patients continuously had ALI during PMX therapy (Group A), but 7 of the other 11 ALI patients were improved by the end of PMX therapy (Group B). By the end of PMX therapy, 3 of the 14 patients without ALI had deteriorated (Group C), but no recurrence of ALI was observed in the remaining 11 patients during PMX therapy (Group D). Characteristics and laboratory data of these 4 groups are shown in Table 2. There was a significant positive correlation between the angiopoietin-1 level and the PaO₂/FiO₂ ratio ($r = 0.427$, $P < 0.001$, Fig. 1A). On the other hand, there was a significant inverse correlation between the angiopoietin-2 level and the PaO₂/FiO₂ ratio ($r = 0.302$, $P = 0.003$, Fig. 1B).

The mean PaO₂/FiO₂ ratio of the ALI group was significantly higher than that of the non-ALI group at the initiation of PMX therapy, but there was no significant difference between the ALI group and non-ALI group at the end of PMX therapy. Among the four groups, the mean PaO₂/FiO₂ ratios of Groups A and C were decreased, but the mean PaO₂/FiO₂ ratio of Group B was significantly increased during PMX therapy ($P = 0.018$, Fig. 2).

The mean angiopoietin-1 level before PMX therapy in the ALI group (2.98 ± 3.31 ng/mL) was

TABLE 2. Characteristics of patients at the start of PMX therapy

	Group A	Group B	Group C	Group D
Number of patients	4	7	3	11
Age (years)	73.5 ± 5.45	70.1 ± 15.3	59.0 ± 24.2	68.2 ± 21.4
Gender (Male : Female)	3:1	6:1	0:3	7:4
Mean arterial pressure (mm Hg)	59.3 ± 9.38	56.9 ± 12.3	63.1 ± 17.9	65.9 ± 14.0
APACHE II	30.5 ± 9.00	29.6 ± 7.37	26.0 ± 6.56	22.3 ± 9.26
Treatment time of DHP-PMX (h)	26.0 ± 25.4	39.7 ± 15.5	19.7 ± 20.6	33.3 ± 18.4
Site of infection				
Intra-abdominal	0/4	6/7	2/3	10/11
Others	4/4	1/7	1/3	1/11
Arterial pH	7.302 ± 0.065	7.345 ± 0.165	7.222 ± 0.156	7.389 ± 0.100
White blood cell count (μL)	12 050 ± 12 251	20 557 ± 9 706	21 667 ± 7 129	11 945 ± 9 812
Hemoglobin concentration (g/dL)	10.13 ± 2.38	11.50 ± 2.22	10.10 ± 2.75	11.80 ± 2.82
Platelet count (×10 ⁴ /μL)	10.8 ± 3.50	8.27 ± 4.10	14.9 ± 3.44	23.5 ± 13.7
Serum creatinine levels (mg/dL)	3.74 ± 2.50	2.88 ± 1.99	1.40 ± 0.73	2.55 ± 3.21
Serum CRP levels (mg/dL)	24.0 ± 10.1	25.0 ± 11.9	22.8 ± 4.00	18.4 ± 11.4
Positive for blood culture	0/4	5/7	2/3	5/11

ALI, acute lung injury; APACHE II, Acute Physiology And Chronic Health Evaluation version II; CRP, C-reactive protein; DHP-PMX, direct hemoperfusion with polymyxin B-immobilized fiber column.

significantly lower than that in the non-ALI group (10.14 ± 9.75 ng/mL, $P = 0.019$). However, after PMX therapy, there was no significant difference between the mean angiotensin-1 level in the ALI group, 3.82 ± 3.57 ng/mL, and that in the non-ALI patient group, 7.81 ± 10.47 ng/mL ($P = 0.411$). The mean angiotensin-1 level of Group C was decreased and that of Group A was maintained at the lower level, but that of Group B was increased during PMX therapy (Fig. 3).

The mean angiotensin-2 level before PMX therapy in the ALI group (48.26 ± 30.35 ng/mL) was significantly higher than that in the non-ALI group, 21.49 ± 12.54 ng/mL, ($P = 0.049$). However, after

PMX therapy, there was no significant difference in the mean angiotensin-2 level between the ALI group (33.01 ± 24.39 ng/mL) and non-ALI group, 27.26 ± 28.16 ng/mL ($P = 0.477$). The mean angiotensin-2 level of Group C was increased and that of Group D was maintained at the lower level, but the level of Group B was decreased during PMX therapy (Fig. 4).

DISCUSSION AND CONCLUSIONS

In a previous study, the circulating angiotensin-1 level was not different between septic patients and controls, but the angiotensin-2 level in septic

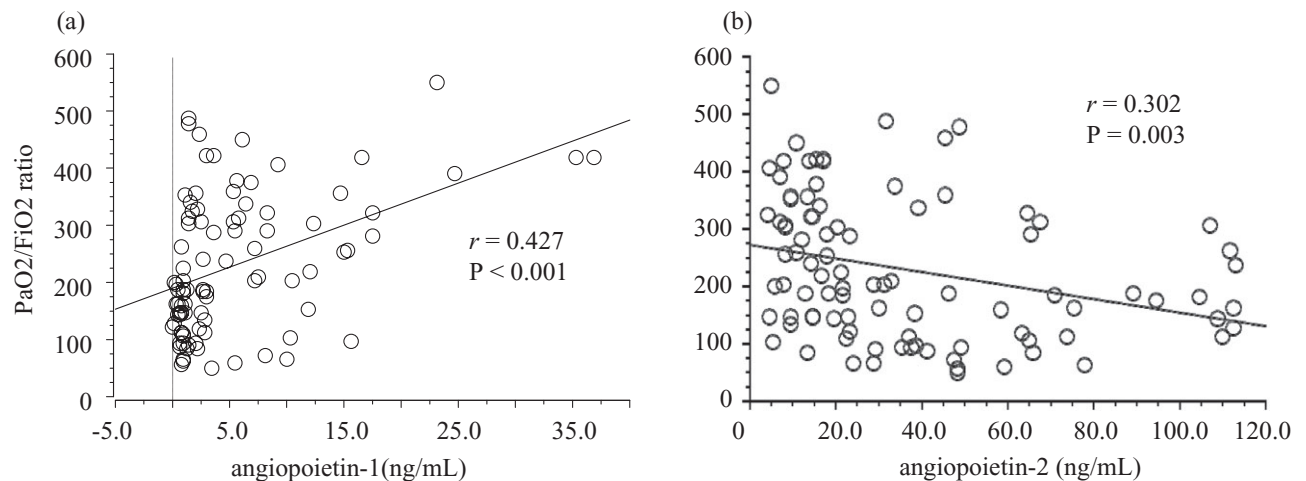


FIG. 1. Relation between angiotensin levels and the ratio of partial pressure of oxygen in arterial blood (PaO_2) to fraction of inspired oxygen (FiO_2) ($\text{PaO}_2/\text{FiO}_2$). Figures show the relation between the $\text{PaO}_2/\text{FiO}_2$ ratio and angiotensin-1 (A) or -2 (B). There was a significant correlation between the angiotensin-1 level and the $\text{PaO}_2/\text{FiO}_2$ ratio ($r = 0.427$, $P < 0.001$). The angiotensin-2 level was inversely correlated to the $\text{PaO}_2/\text{FiO}_2$ ratio ($r = 0.302$, $P = 0.003$).

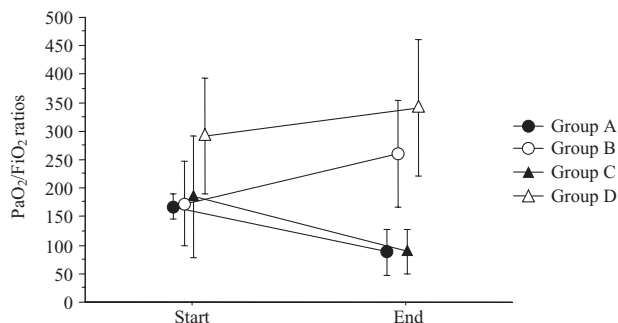


FIG. 2. Changes in the ratio of partial pressure of oxygen in arterial blood (PaO₂) to fraction of inspired oxygen (FiO₂) (PaO₂/FiO₂) during polymyxin B-immobilized fiber column (PMX) therapy. Group A is the patients who continuously had acute lung injury (ALI) during PMX therapy (closed circle), Group B the ALI patients who improved during PMX therapy (open circle), Group C the patients without ALI who deteriorated during PMX therapy (closed triangle), and Group D the patients in which ALI was never observed during the follow-up periods (open triangle). The mean PaO₂/FiO₂ ratios of Groups A and C were decreased, but the mean PaO₂/FiO₂ ratio of Group B was significantly increased during PMX therapy (*P* = 0.018).

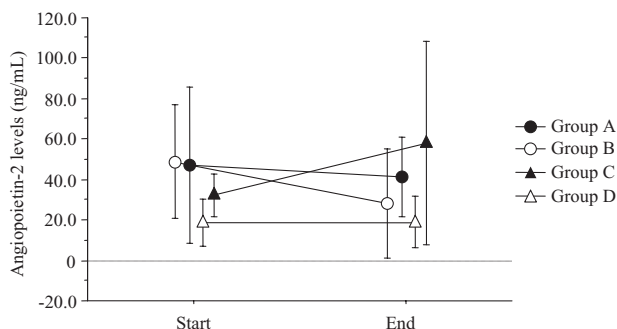


FIG. 4. Changes in the angiotensin-2 level during polymyxin B-immobilized fiber column (PMX) therapy. Group A is the patients who continuously had acute lung injury (ALI) during PMX therapy (closed circle), Group B the ALI patients who improved during PMX therapy (open circle), Group C the patients without ALI who deteriorated during PMX therapy (closed triangle), and Group D the patients in which ALI was never observed during the follow-up periods (open triangle). The mean angiotensin-2 level of Group C was increased and that of Group D maintained at the lower level, but that level of Group B was decreased during PMX therapy.

patients was significantly higher (8). Moreover, the angiotensin-2 level in ALI patients (PaO₂/FiO₂ < 200) was significantly higher than that in the group of individuals with better oxygenation (PaO₂/FiO₂ > 200). In the present study, there was a significant positive correlation between the angiotensin-1 level and the PaO₂/FiO₂ ratio. On the other hand, there was a significant inverse correlation between the angiotensin-2 level and the PaO₂/FiO₂ ratio. These results strongly suggest that angiotensin plays a role in the pathogenesis of ALI in septic patients. If angiotensin-2 is confirmed as a relevant biomarker

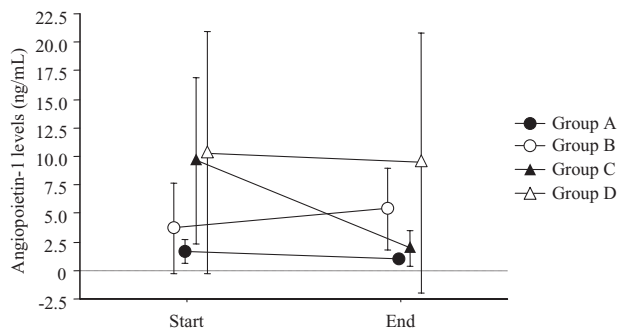


FIG. 3. Changes in the angiotensin-1 level during polymyxin B-immobilized fiber column (PMX) therapy. Group A is the patients who continuously had acute lung injury (ALI) during PMX therapy (closed circle), Group B the ALI patients who improved during PMX therapy (open circle), Group C the patients without ALI who deteriorated during PMX therapy (closed triangle), and Group D the patients in which ALI was never observed during the follow-up periods (open triangle). The mean angiotensin-1 level of Group C was decreased and that of Group A was maintained at a lower level, but that level of Group B was increased during PMX therapy.

of ALI, then clinicians may be able to more easily determine the prognosis and stratify patients, especially in clinical trials to treat ALI. It was reported that PMX therapy is effective against ALI induced by direct or indirect pulmonary injury (12). In another retrospective study of 38 ALI patients treated with PMX (13), the survival rate was 55% at 30 days after PMX therapy, and PMX therapy significantly improved the PaO₂/FiO₂ ratio, heart rate and systolic blood pressure in the survivors compared to the non-survivors. In the present study, 63.6% (7/11) of septic patients with ALI were improved by PMX therapy, and the PaO₂/FiO₂ ratios were also significantly elevated during PMX therapy in those patients. However, 21.4% (3/14) of septic patients without ALI at the initiation of PMX therapy had been complicated with ALI in spite of PMX therapy. The PaO₂/FiO₂ ratios were also decreased during PMX therapy, but there was no significant difference among these ratios because of the small number of subjects. Although it is difficult to conduct prospective randomized controlled trials for septic shock because of the urgency of this disease, these trials should be investigated to confirm the efficacy of PMX therapy for ALI.

Previous reports suggested that many mediators, such as high mobility group box-1 protein (17), oxidative stress (17,18), interleukin-8, plasminogen activator inhibitor-1, and neutrophil elastase (19), play a role in the pathogenesis of ALI. These reports also demonstrated that PMX therapy may ameliorate increased levels of those mediators in patients with ALI (17–19). We previously demonstrated that the

mean angiopoietin-2 level was significantly higher and the mean level of angiopoietin-1 was significantly lower before PMX therapy than after, and that the angiopoietin-2 levels are inversely correlated with the mean blood pressure (20). In the present study, the mean angiopoietin-1 level before PMX therapy in the ALI group was significantly lower and the mean angiopoietin-2 level before PMX therapy in the ALI group was significantly higher than in the non-ALI group. However, after PMX therapy there was no significant difference in the mean angiopoietin-1 and -2 levels between the ALI and non-ALI groups. The mean angiopoietin-1 level of the ALI patients with response to treatment was increased during PMX therapy, but that of the non-ALI patients with a new occurrence of ALI showed a decreased angiopoietin-1 level. On the other hand, the mean angiopoietin-2 level of the responders was decreased during PMX therapy, but that of patients with newly occurring ALI showed an increased angiopoietin-2 level. This result suggests that each angiopoietin-1 and -2 level may play a role in the pathogenesis of ALI, and that PMX therapy ameliorates the angiopoietin balance in patients with ALI in sepsis. However, there was no significant difference in both levels of angiopoietins before and after the PMX therapy in the ALI patients who responded to treatment because of the small sample size. Therefore, further study is needed to identify the association between the efficacy of PMX therapy for ALI and the angiopoietin balance.

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Conflicts of Interest: None declared.

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