Regulation of Angiopoietin-2 before and after Mechanical Circulatory Support Therapy

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None.

ABSTRACT

Gastrointestinal bleeding (GIB) during mechanical circulatory support (MCS) is a major unsolved comorbidity. Inadequate activation of angiopoietin-2-related systems is considered as a major cause of GIB. However, the regulation of angiopoietin-2 remains unknown. Consecutive 20 patients who received continuous-flow MCS therapy (MCS group) and 12 with advanced heart failure (HF; HF group) were prospectively enrolled and their angiopoetin-2 levels were compared. Angiopoietin-2 level had a moderate correlation with $\log_{10}$ B-type natriuretic peptide (BNP; $r = 0.39$, $p < 0.001$). The MCS group had significantly higher angiopoietin-2 level divided by $\log_{10}$ BNP compared with the HF group (2.80 ± 0.20 vs. 1.88 ± 0.17, $p < 0.001$). Angiopoetin-2 had a moderate correlation with central venous pressure and C-reactive protein during the MCS support ($r = 0.51$ and $r = 0.45$, respectively). Higher angiopoietin-2 level divided by $\log_{10}$ BNP (>4.3) was significantly associated with the occurrence of GIB with a hazard ratio of 296 (95% confidence interval 2.24–38620, $p = 0.0224$). Angiopoietin-2 was already elevated in the HF cohort and more elevated following MCS initiation. Among the MCS cohort, angiopoietin-2 was particularly elevated in patients with systemic congestion and inflammation and was associated with higher incidence of GIB.

Keywords: hemodynamics; angiogenesis; bleeding.

INTRODUCTION

Despite significant improvement in short-term survival among patients with advanced heart failure (HF) receiving mechanical circulatory support (MCS),[1] gastrointestinal bleeding remains one of the major unsolved device-related comorbidities.[2, 3] Particularly, the incidence of gastrointestinal bleeding during left ventricular assist device supports (LVADs) has recently been investigated.[4, 5] In addition to intensified anti-platelet and anti-coagulation therapy and acquired von Willebrand disease due to blood-device interaction,[6, 7] the development of arteriovenous malformation on the surface of gastrointestin[8, 9] due to inappropriate activation of inflammatory and angiogenesis systems including over-expression of angiopoietin-2 have been considered as major causes of gastrointestinal bleeding.[10, 11]

However, detailed mechanisms of over-expression of angiopoietin-2 during MCS remains unknown. Patel and colleagues showed that the hypervascularity of nasal mucosa, which was a surrogate of gastrointestinal bleeding from arteriovenous malformation during LVAD supports, was already observed in patients with advanced HF before LVAD implantation,[12] whereas the activity of angiopoietin-2 before and after MCS therapy remains unknown. The expression of angiopoietin-2 seems to vary in each patient with MCS therapy, whereas the cause of its variation also remains unknown.

In this study, (1) we compared the serum concentration of angiopoietin-2 between the HF cohort and those with MCS therapy and (2) investigated the association of angiopoietin-2 level with other clinical data to more clarify the mechanism of angiopoietin-2 regulation during MCS therapy.

This there is based on our published paper.[13]

METHODS

Patient selection:

Consecutive advanced HF patients with reduced ejection fraction who received continuous-flow MCS therapy (MCS group, N = 20) and those with continuous infusion of inotropes (HF group, N = 12) between Aug 2018 and Jun 2019 at our institute were prospectively enrolled in this study. Written informed consent was obtained from all participants before the enrollment. This study was approved by the local institutional review board beforehand.

Baseline characteristics:

Of all, 32 patients consisting of 12 HF patients and 20 patients with MCS were enrolled. Demographic characteristics are shown in Table 1. The mean age was 68.6 ± 15.2 years old and 21 (66%) were male. All HF patients were assigned to stage D HF with intravenous inotrope infusion. The devices of MCS group consisted of Impella, extracorporeal membrane oxygenation, Jarvik 2000, and HeartMate II. There were no significant differences in baseline characteristics between the MCS group and HF group.

Measurements of angiopoietin-2:

Serum level of angiopoietin-2 was measured at several timings in both group in addition to other conventional laboratory data including plasma level of B-type natriuretic peptide (BNP), by using Human Angiopoietin-2 Quantikine ELISA Kit.

Among the MCS group, blood samples were obtained at three days, seven

days, and every one month following the initiation of MCS therapy as well as at the time of bleedings as principals. Among the HF group, blood samples were obtained at three days and every two weeks following the admission. Other clinical data including the use of anti-platelet drugs were also obtained at each timing when blood samples were obtained.

Hemodynamic assessments during MCS therapy:

Hemodynamic data, including central venous pressure, pulmonary capillary wedge pressure, and Fick cardiac index were measured in patients with MCS using right heart catheterization when clinically indicated. All hemodynamic data were obtained at the same time with blood sampling.

Managements of bleeding during MCS therapy:

All MCS patients received guideline-directed medical therapy including antiplatelet therapy, oral proton pump inhibitor, and anticoagulation therapy with appropriate targets of activated partial thromboplastin time, activated whole blood clotting time, or international normalized ratio for each device, unless recurrent gastrointestinal bleeding occurred.

Bleeding was defined as any clinically documented bleeding from the gastrointestinal tract as indicated by the appearance of melena, hematochezia, hematemesis, or guaiac-positive stool that required any hemostatic treatments.

Statistical assessments:

Statistics were performed using JMP pro ver13.0 (SAS Institute Inc). Variables

with $p < 0.05$ were considered significant. Continuous data were described as mean ± standard deviation and compared between two groups using unpaired t-test or Mann-Whitney U test as appropriate. Categorical data were compared between two groups by Chi-square test or Fisher's exact test as appropriate. In this study, we performed three major analyses:

(1) Comparison in the angiopoietin-2 levels between the HF group samples and the MCS group samples among those without bleeding events: Associations between angiopoietin-2 and BNP were investigated by Pearson's correlation coefficient and compared between the two groups.

(2) Association between angiopoietin-2 and other clinical data among the MCS group: Correlations between angiopoietin-2 and other clinical data were assessed by Pearson's correlation coefficient among the MCS group.

(3) Association of angiopoietin-2 and bleeding events among the MCS group: The cutoff of angiopoietin-2 relative to BNP level for the mucosal bleeding events was calculated by the receiver operating characteristics analysis and the incidences of bleeding were compared between the two groups stratified by the cutoff. The impact of angiopoietin-2 on bleeding events was investigated by the logistic regression analysis by adjusting for the suspected confounders including age, mean blood pressure, prothrombin time with international normalized ratio, and use of anti-platelet. Changes in angiopoetin-2 levels between baseline and at time of bleedings were assessed by Wilcoxon signed-rank test.

RESULTS

(1) MCS vs. HF group sample comparison:

A total of 178 blood samples were collected from both groups. Eight samples from the HF group were obtained at the time of bleeding events and 15 samples were obtained at the timing of bleeding in the MCS group. We excluded these 23 samples from the inter-group comparison analysis between the HF group and the MCS group to exclude the effect of bleeding on any clinical data. Finally, 155 samples consisting of 87 from the HF group and 68 from the MCS group, which were collected without any episodes of bleeding, were analyzed.

Comparisons in the clinical parameters are shown in Table 2. BNP and creatinine were lower in the MCS group than the HF group ($p < 0.05$ for both). C-reactive protein was numerically lower in the MCS group ($p = 0.13$).

Of all 155 samples, angiopoietin-2 level had a moderate positive correlation with $\log_{10}$ BNP ($r = 0.39$, $p < 0.001$; Figure 1A). Furthermore, the MCS group had significantly higher angiopoietin-2 divided by $\log_{10}$ BNP compared with the HF group (2.8 ± 0.2 vs. 1.9 ± 0.2, $p < 0.001$; Figure 1B).

(2) Angiopoietin-2 regulation during MCS therapy:

We investigated the association of angiopoietin-2 level with other clinical variables among the MCS group samples (N = 68). Angipoietin-2 divided by $\log_{10}$ BNP had a moderate correlation with $\log_{10}$ BNP ($r = 0.442$), C-reactive protein (CRP) ($r = 0.448$), and creatinine ($r = 0.457$) ($p < 0.001$ for all; Table 3).

Association between angiopoetin-2 and hemodynamics are shown in Table 4 (N = 25). The hemodynamic data were collected on the same day of angiopietin-2 and

BNP measurement. Angiopoetin-2 had a moderate positive correlation with central venous pressure (CVP) ($r = 0.514$, $p = 0.009$) and mild negative correlation with mean arterial pressure ($r = -0.276$, $p = 0.027$).

The early-phase trends of BNP, angiopoietin-2, and angiopoietin-2 divided by $\log_{10}$ BNP in five MCS patients without any bleedings were shown (Appendix Figure 1). BNP levels decreased within a month after introduction of MCS ($p=0.012$). Angiopoietin-2 and angiopoietin-2 divided by $\log_{10}$BNP also had a tendency to decrease, though they did not reach statistical significance (angiopoietin-2; 8.2 ± 5.0 vs 4.0 ± 1.4, $p = 0.11$, angiopoietin-2 divided by $\log_{10}$BNP; 3.3 ± 2.0 vs 1.9 ± 0.7, $p = 0.21$) (Appendix Figure 1).

Twenty-one samples out of 31 ones showed a loss of large multimer of von Willebrand factor. There was no significant difference in the angiopoietin-2 divided by $\log_{10}$ BNP between those with or without loss of large multimer of von Willebrand factor (median 2.01 vs 3.42 ng/mL, $p = 0.2204$).

(3) Angiopoietin-2 and bleeding during MCS therapy:

A total of 83 samples including 15 collected at the time of mucosal bleedings during the MCS support were analyzed. The angiopoietin-2 divided by $\log_{10}$ BNP obtained at bleeding ($N = 15$) was higher than 68 samples without bleeding (5.4 ± 0.6 vs. 2.8 ± 0.3, $p <0.001$).

A cutoff of angiopoietin-2 divided by $\log_{10}$ BNP for the bleeding event was calculated as 4.3 with an area under the curve 0.73. The incidence of bleeding was significantly higher in the high angiopoietin-2 group (>4.3) than those with low angiopoietin-2 group (≤4.3) (55.6% vs. 6.0%, $p <0.001$; Figure 2).

Higher angiopoietin-2 level divided by $\log_{10}$ BNP (>4.3) was significantly associated with the bleeding event with an unadjusted hazard ratio 15 (95% confidence interval 4.28–60.0, $p < 0.0001$) and a hazard ratio 298 (95% confidence interval 2.24-39620, $p = 0.0224$) adjusted for age, mean blood pressure, prothrombin time with international normalized ratio, C-reactive protein, and the use of anti-platelet drugs.

Angiopoietin-2 divided by $\log_{10}$ BNP significantly increased at the time of bleeding compared with baseline (hemodynamically stable without bleeding) among five MCS patients ($p=0.004$; Appendix Figure 2).

DISCUSSION

In this study, we compared the level of angiopoietin-2 between the HF cohort and the MCS cohort and investigated the association of angiopoietin-2 with other clinical data as well as the bleeding events. The main findings are as follows: (1) Angiopoietin-2 had a positive moderate correlation with BNP in the overall cohort. Angiopoietin-2 relative to BNP was higher in the MCS group over the HF cohort; (2) Among the MCS cohort, angiopoietin-2 was associated with systemic congestion and inflammation; (3) Elevated angiopoietin-2 level was associated with the occurrences of gastrointestinal bleedings.

Angiopoietin-2 in HF cohort:

Inflammatory and angiogenesis systems, particularly the activation of angiopoietin-2 are receiving great concerns as a dominant cause of non-surgical bleeding from arteriovenous malformation on the gastrointestin during continuous-flow

MCS therapy.[10, 11] However, scarcity has been reported about the systems before MCS therapy.

Patel and colleagues demonstrated that hypervascularity of the nasal mucosa, which was associated with gastrointestinal bleeding from arteriovenous malformation in the MCS patients, has already developed in the HF cohort at a similar prevalence to the MCS cohort, although their hypervascularity was less severe than the MCS cohort.[12] They suspected that the HF itself would be a trigger of the development of hypervascularity, although the detailed mechanism remained unknown.

In this study, angiopoietin-2 was elevated both in the HF cohort and the MCS cohort, which would support their results and hypothesis. Angiopoietin-2 had a correlation with the severity of HF indicated by the BNP level. This finding is compatible with other previous studies.[14, 15]

Furthermore, the rate of angiopoietin-2 relative to BNP was significantly higher in the MCS cohort than the HF cohort. The detailed mechanism remains unknown, but the finding might be explained by the two-hit therapy: (1) HF itself stimulates inflammatory and angiogenesis signal cascade as it progresses.[16, 17] (2) device-related hematological instability further stimulates the system and inappropriately increase angiopoietin-2 level.[11] Anti-platelet and anti-coagulation therapies, as well as acquired von Willebrand disease, would also contribute to the vulnerability of arteriovenous malformation.[18, 19]

Regulation of angiopoietin-2 during MCS therapy:

Nevertheless, the levels of angiopoietin-2 vary in each MCS patients and not all MCS patients have mucosal bleedings. Among hemodynamic parameters, higher

central venous pressure and serum creatinine level, which indicate renal dysfunction probably due to systemic congestion were associated with increased angiopoietin-2. Furthermore, one of the major indexes of inflammation, C-reactive protein, had also correlation with angiopoietin-2. There are several studies investigating the clinical association between right ventricular failure and gastrointestinal bleeding during MCS therapy.[2, 5] Our findings would support and explain their previous findings, i.e., right ventricular failure, which often develops following the MCS implantation at early or late phase, might stimulate inflammatory and angiogenesis systems that trigger the development of arteriovenous malformation and mucosal bleedings.[20, 21]

Interestingly, pulmonary artery pulsatility index and central venous pressure divided by pulmonary capillary wedge pressure, both of which represent right ventricular function, had no significant association with angiopoietin-2. Right ventricular failure, indicated by elevated central venous pressure, might stimulate angiopoietin-2 secretion, whereas right ventricular dysfunction itself might not necessarily cause systemic congestion and successive angiopoietin-2 secretion. More detailed investigation using device type, arterial pulsatility, and echocardiographic and laboratory parameters are warranted to clarify the mechanism of angiopoietin-2 regulation.

Angiopoietin-2 and bleeding during MCS therapy:

It is hypothesized that angiopoietin-2-associating inflammatory and angiogenesis cascade has an important role in the development of arteriovenous malformation on the wall of gastrointestine and gastrointestinal bleeding together with other factors including tumor necrotic factor alpha.[11, 22] Clinically, Tabit and

colleagues showed that the elevated angiopoietin-2 (>12.3 ng/mL) was associated with gastrointestinal bleeding within future 3 months.[10] We demonstrated in this study that angiopoietin-2 was elevated just at time of bleeding. Angiopoietin-2 levels might change dynamically depending on the patients' hemodynamic, inflammatory, and hematological conditions as shown in Appendix Figure 2. Nevertheless, we cannot conclude that the elevated angiopoetin-2 level is a sole cause of bleeding, which should be multifactorial. Therefore, we performed multivariate Cox proportional hazard ratio regression analyses instead of univariate analysis.

Furthermore, we cannot exclude the impact of other un-investigated factors including device arterial pulsatility, device rotational speed, and the severity of acquired von Willebrand disease on the occurrence of bleeding.

We performed receiver characteristic operating analysis to calculate the threshold of angiopoetin-2 level to predict the occurrence of bleeding: cutoff level of angiopoietin-2 divided by $\log_{10}$ BNP for the bleeding event was calculated as 4.3 with an area under the curve 0.73.

Future perspective:

Given already elevated angiopoietin-2 level in the HF cohort, we might risk stratifying HF patients for the post-MCS bleedings before the surgery. Following the MCS implantation, aggressive medications including β-blocker and diuretics such as arginine vasopressin type-2 antagonist or adjustment of device setting with hemodynamic ramp tests might improve systemic congestion and reduce angiopoietin-2 level.[23] Unsaturated fatty acid omega-3,[24] digoxin,[25] direct angiopoietin-2 blocker, or any other anti-inflammatory agents might also be useful to reduce

angiopoietin-2 activity and prevent mucosal bleeding, although not yet demonstrated in any prospective randomized control trials. Given our two-hit hypothesis, i.e., the progression of HF and the increased share stress caused by the interaction between the device and patients' blood, an additional third group of patients with elevated BNP level plus shear stress due to a valvular pathology including aortic stenosis might strengthen our hypothesis and clarify the mechanism of angiopoetin-2 regulation.

Study limitations:

This study is a proof of concept and was conducted in a small sample size cohort. Any similarity cannot be concluded even though several comparisons showed statistical non-significance. We used multiple blood samples from the same patient, and such procedures would have a selection bias. Given the difference in the interval of blood sampling, we cannot simply compare the trends of angiopoetin-2 levels between the HF cohort and the MCS cohort. We excluded blood samples obtained at time of bleeding in the comparison study between the HF group and the MCS group given the significant association between angiopoietin-2 and bleeding, which might also have a selection bias. We could not assess the impact of device type and device settings including rotational speed on the level of angiopoietin-2 given small sample size, which would be a future concern. Although we adjusted for several factors to investigate the impact of angiopoietin-2 on the occurrence of gastrointestinal bleeding, there might be other uninvestigated variables including pulsatility, device rotational speed, and the severity of acquired von Willebrand disease on the occurrence of bleeding.

We did not assess the correlation of loss of high molecular monomer of von Willebrand factor and the level of angiopoietin-2. We do not have complete longitudinal

data, which would more clarify the association between angiopoietin-2 and MCS. We did not find bleeding origins for all cases, and bleedings might not come from arteriovenous malformation in all cases. We did not show the cytokine markers including tumor necrosis factor and vascular endothelial growth factor associating with systemic inflammation and angiogenesis.

CONCLUSION

Angiopoietin-2 was already elevated in the HF cohort, particularly those with elevated BNP levels. The angiopoietin-2 level relative to BNP was higher in the MCS cohort than the HF cohort. Among the MCS cohort, angiopoietin-2 was particularly elevated in patients with systemic congestion and inflammation and was associated with higher incidence of gastrointestinal bleedings. Novel strategies to approach elevated angiopoietin-2 to reduce the risk of gastrointestinal bleedings would be the next concern.

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None.

DISCLOSURE

None.

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FIGURE LEGENDS

Figure 1: Correlation between angiopoietin-2 and B-type natriuretic peptide (A) and comparison in the angiopoietin-2 divided by logarithm of B-type natriuretic peptide between MCS group and HF group (B)

MCS, mechanical circulatory support; HF, heart failure. *p <0.05 by Pearson's correlation coefficient. †p <0.05 by Mann-Whitney U test.

Figure 2: Incidences of bleeding stratified by the cutoff of angiopoietin-2 divided by logarithm of B-type natriuretic peptide among the MCS group

*p <0.05 by Fisher's exact test.

Appendix Figure 1: Trends of BNP (A), angiopoietin-2 (B), and angiopoietin-2 divided by $\log_{10}$ BNP (C) levels in five MCS patients at one week after and one month after initiation of MCS who did not experience any bleedings

Ang-2, angiopoietin-2; BNP, B-type natriuretic peptide; MCS, mechanical circulatory support.

Appendix Figure 2: Trends of BNP (A), angiopoietin-2 (B), and angiopoietin-2 divided by $\log_{10}$ BNP (C) levels at baseline and at the time of bleeding in five MCS patients who experienced bleedings

*p <0.05 by Wilcoxon singed-rank test.

Ang-2, angiopoietin-2; BNP, B-type natriuretic peptide; MCS, mechanical circulatory support.

Table 1. Baseline characteristics

	Total patients (N = 32)	MCS group patients (N = 20)	HF group patients (N = 12)	p-value
Age (years)	68.6 ± 15.2	66.7±17.8	71.8±10.5	0.37
Male sex	21 (66%)	15 (75%)	6 (50%)	0.25
Ischemic etiology	18 (56%)	13 (75%)	5 (42%)	0.28
Left ventricular ejection fraction (%)	27.8 ± 10.3	26.9±10.2	29.4±10.8	0.50
Atrial fibrillation	14 (44%)	8 (40%)	6 (50%)	0.72
History of stroke	9 (28%)	7 (35%)	2 (17%)	0.42
Anti-platelet therapy	20 (63%)	15 (75%)	5 (42%)	0.13

Variables were compared by unpaired t-test or Fisher’s exact test.

Table 2. Comparison in laboratory parameters among those without bleeding events

	Total samples (N = 155)	MCS group samples (N = 68)	HF group samples (N = 87)	p-value
Log_{10} BNP (pg/mL)	2.50 ± 0.46	2.27 ± 0.38	2.67 ± 0.44	<0.001*
Albumin (mg/dL)	3.40 ± 0.63	3.80 ± 0.47	3.10 ± 0.56	<0.001*
Creatinine (mg/dL)	1.07 ± 0.56	0.84 ± 0.28	1.25 ± 0.66	0.007*
Total bilirubin (mg/dL)	1.00 ± 1.31	1.00 ± 0.49	1.01 ± 1.70	0.97
Lactate dehydrogenase (U/mL)	439.2 ± 395.6	584.8 ± 312.6	325.4 ± 417.4	<0.001*
C-reactive protein (mg/dL)	2.27 ± 4.20	1.68 ± 3.58	2.74 ± 4.60	0.13
PT-INR	1.66 ± 0.73	1.99 ± 0.60	1.33 ± 0.70	<0.001*

MCS, mechanical circulatory support; HF, heart failure; BNP, B-type natriuretic peptide; PT-INR, prothrombin time with international normalized ratio.

*p <0.05 by Mann-Whitney U test or Fisher's exact test as appropriate.

Table 3. Correlation between angiopoietin-2/log_{10} BNP and laboratory data among the MCS group samples

N = 68	R-value	p-value
Log_{10} BNP (pg/mL)	0.442	<0.001*
Hemoglobin (g/dL)	-0.251	0.0223*
Creatinine (mg/dL)	0.457	<0.001*
Total bilirubin (mg/dL)	0.144	0.19
Lactate dehydrogenase (U/mL)	0.085	0.44
C-reactive protein (mg/dL)	0.448	<0.001*

BNP, B-type natriuretic peptide; MCS, mechanical circulatory support. *p <0.05 by Pearson's correlation coefficient.

Table 4. Correlation between angiopoietin-2/log$_{10}$ BNP and hemodynamic data among the MCS group samples

N = 25	**R-value**	**p-value**
Mean arterial pressure (mmHg)	-0.276	0.027*
Pulmonary capillary wedge pressure (mmHg)	0.318	0.40
Cardiac index (L/min/m^2)	-0.214	0.41
Central venous pressure (mmHg)	0.514	0.009*
Pulmonary artery pulsatility index	-0.281	0.23
Central venous pressure/pulmonary capillary wedge pressure	-0.009	0.97

MCS, mechanical circulatory support. *p <0.05 by Pearson's correlation coefficient.

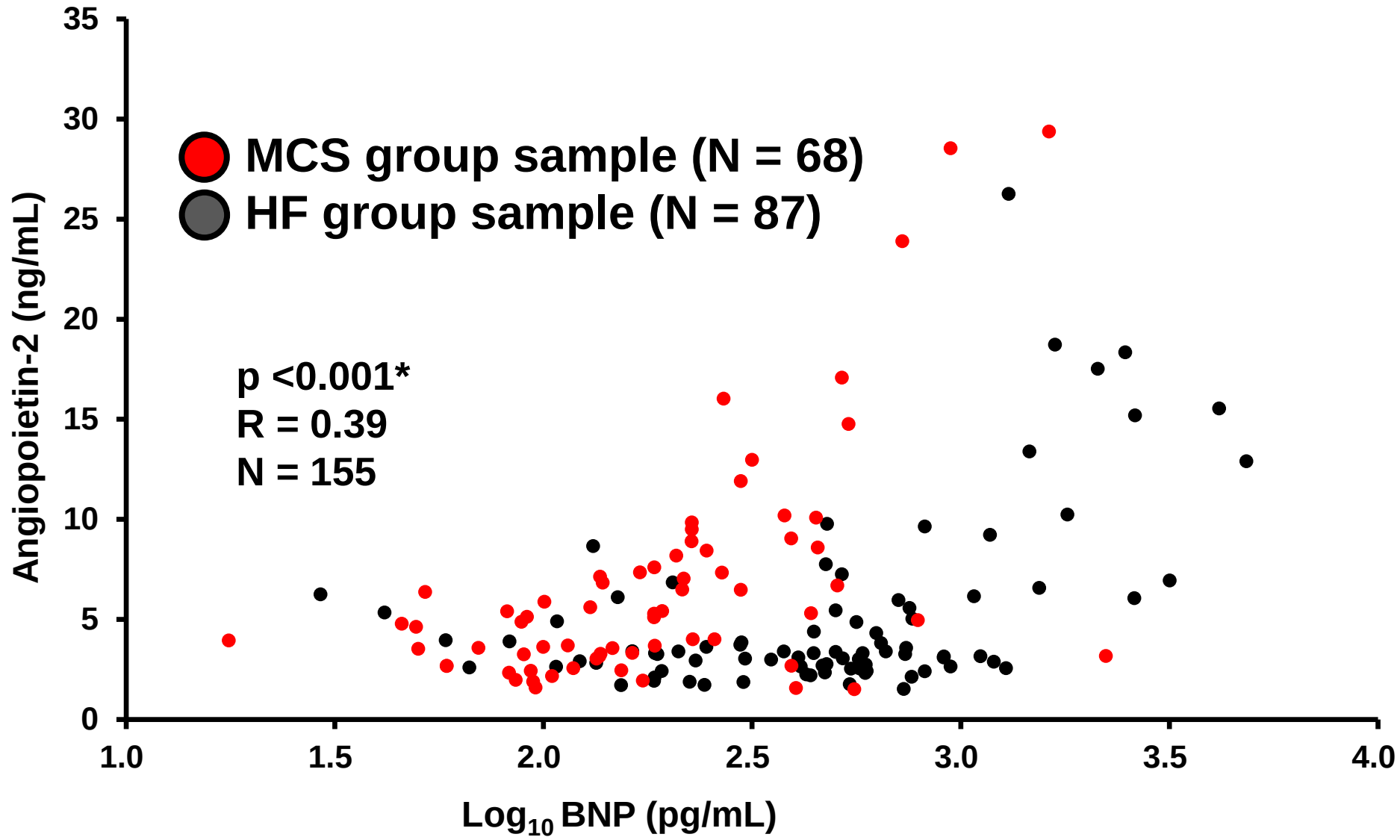

MCS group sample (N = 68)
HF group sample (N = 87)
p <0.001*
R = 0.39
N = 155
Angiopoietin-2 (ng/mL)
Log10 BNP (pg/mL)
0
5
10
15
20
25
30
35
1.0
1.5
2.0
2.5
3.0
3.5
4.0

Figure 1A

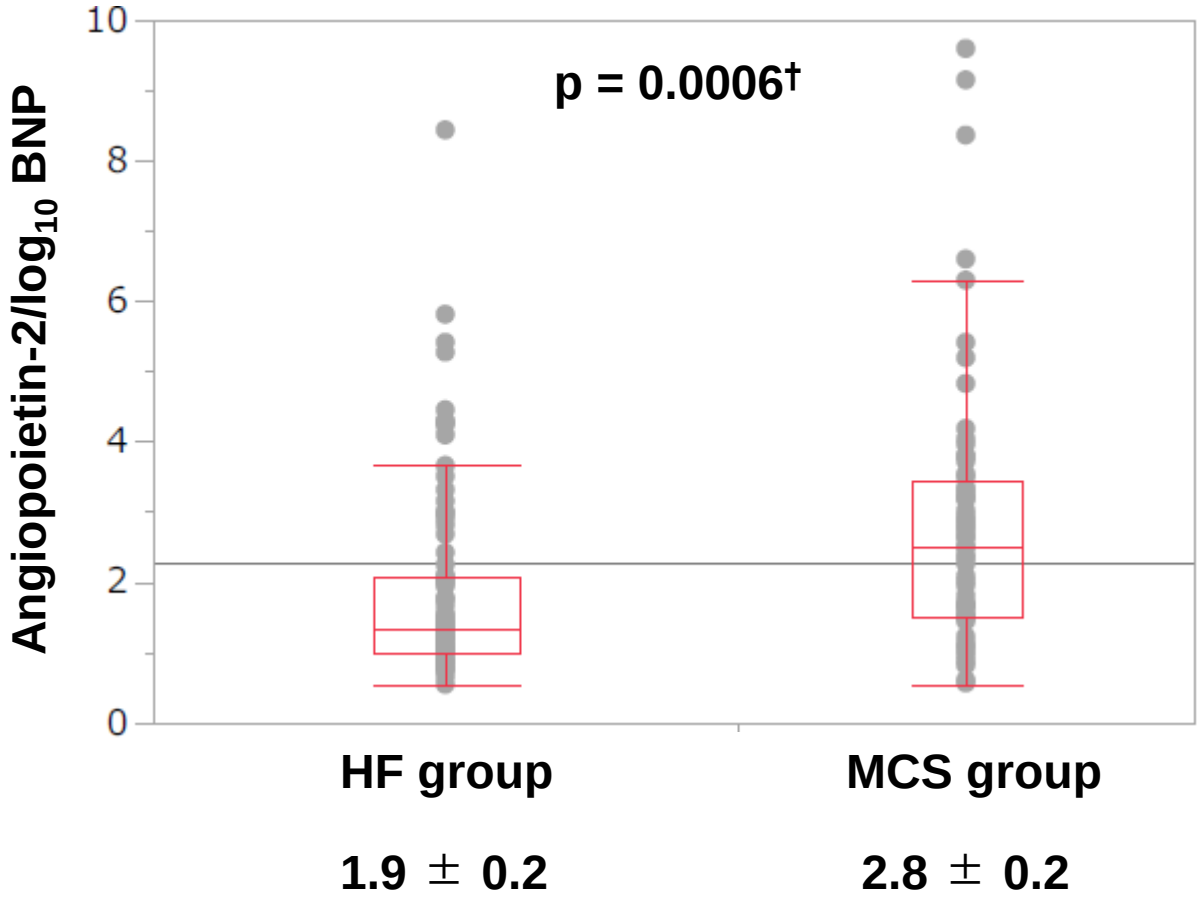

Angiopoietin-2/log10 BNP
10
8
6
4
2
0
p = 0.0006†
HF group
MCS group
1.9 ± 0.2
2.8 ± 0.2

Figure 1B

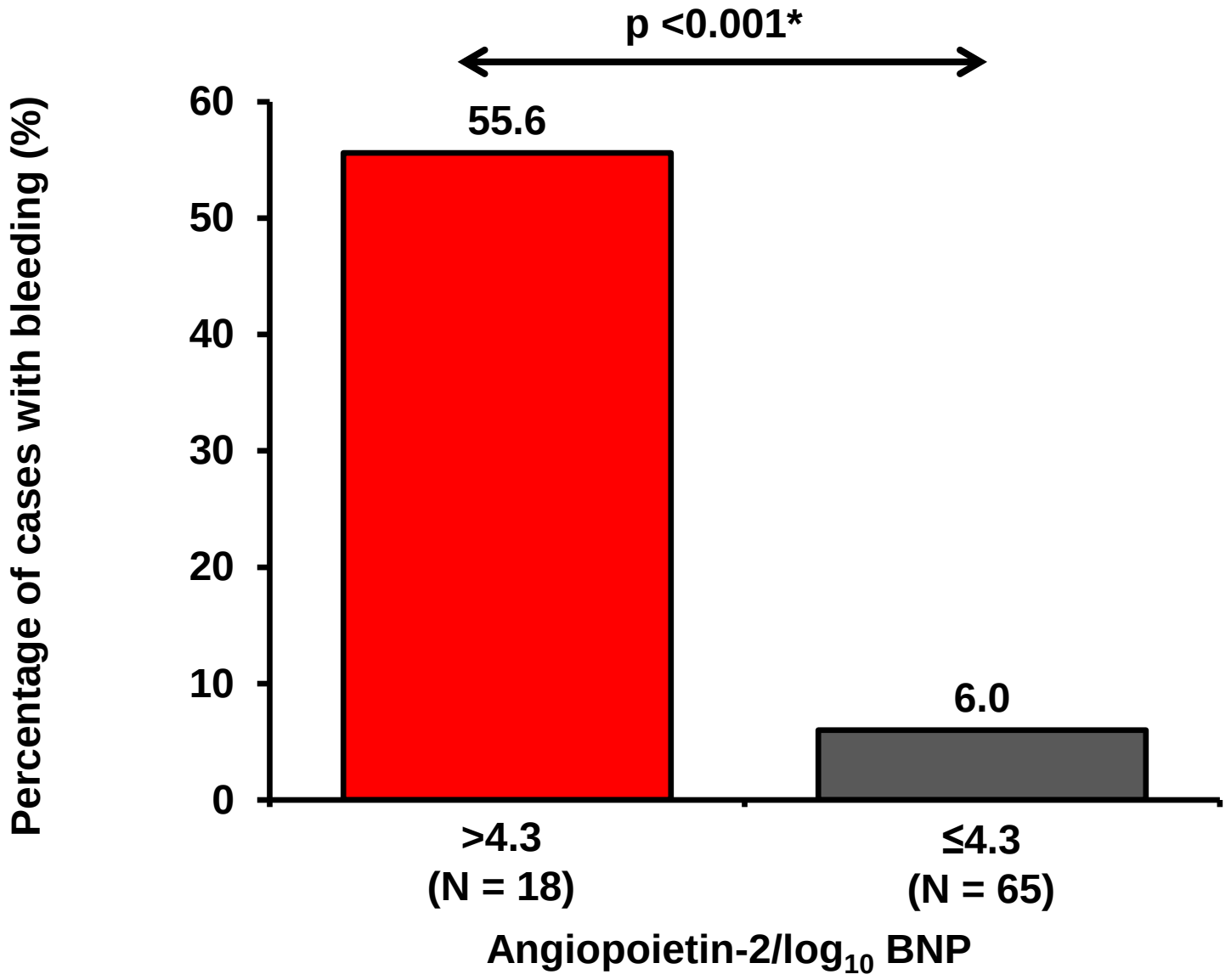
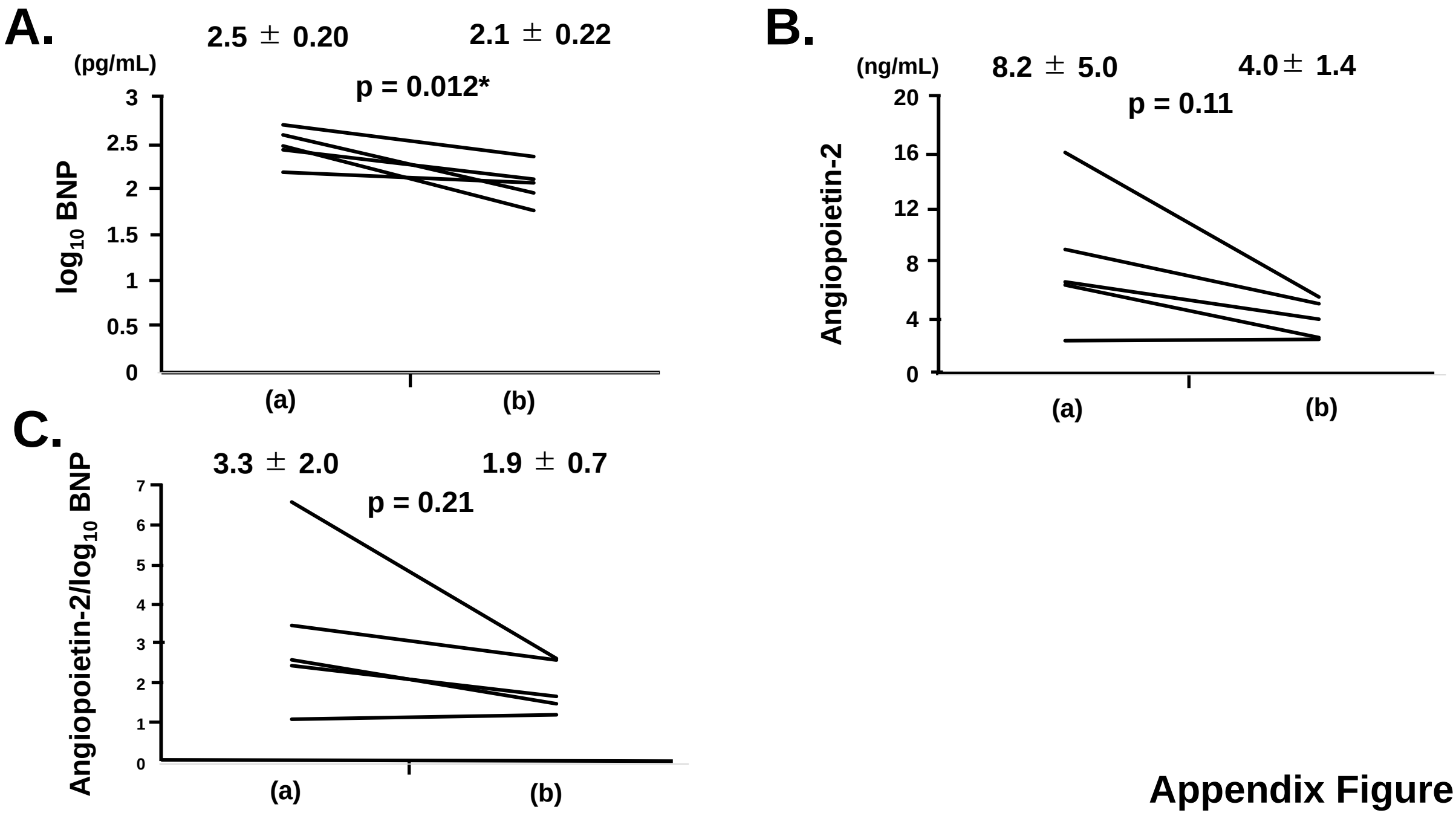
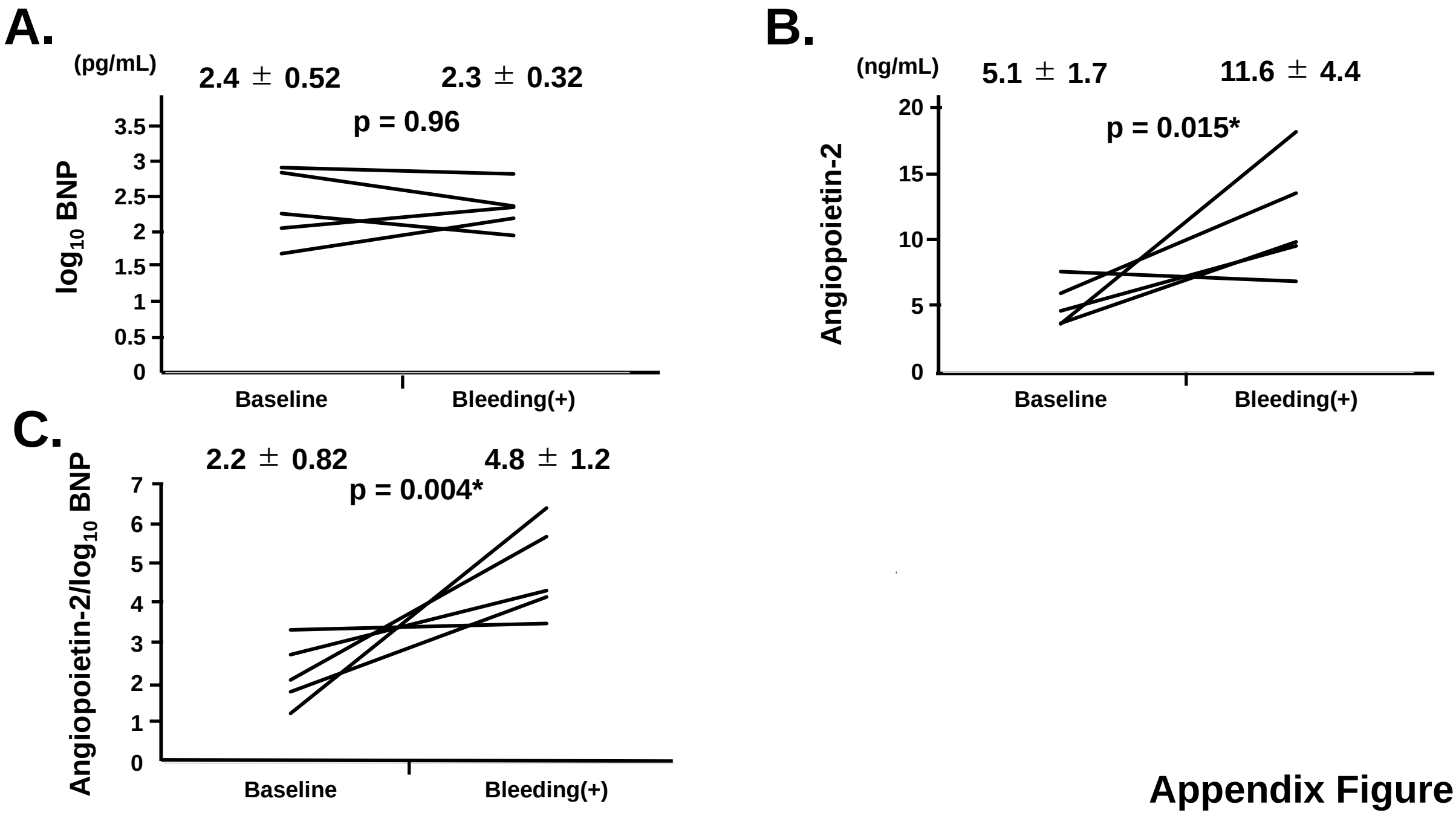

p <0.001*
55.6
6.0
60
50
40
30
20
10
0
Percentage of cases with bleeding (%)
>4.3
(N = 18)
≤4.3
(N = 65)
Angiopoietin-2/log10 BNP

Figure 2



Appendix Figure 1



Appendix Figure 2