

1 **Decreased Serum Levels of High Mobility Group Box 1 (HMGB-1) After Graft**

2 **Replacement or Stenting of Abdominal Aortic Aneurysm**

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15 Article word count: 1334 words

16 Article Category: Original article

17 Total number of tables and figures: 6

18

19 **Brief statement:** High mobility group box 1 (HMGB-1) has been associated with inflammation and  
20 atherosclerosis, which results in elevated HMGB-1 levels in patients with abdominal aortic aneurysms  
21 (AAA) because aneurysms contain a large amount of atherosclerotic tissue. There are no data regarding  
22 changes in HMGB-1 levels following surgical interventions for AAA. We investigated the serum HMGB-  
23 1 levels before and after either endovascular aortic repair (EVAR) or open aortic repair (OAR). The  
24 serum HMGB-1 was higher in patients with AAA than in controls. However, the elevated HMGB-1

25 levels normalized after graft replacement or stent placement for AAA. This is the first report describing  
26 changes in serum HMGB-1 after surgical treatment of AAA.

27 **Abstract**

28 **Objectives:** High-mobility group box 1 (HMGB-1) is a key substance mediating inflammation  
29 and development of atherosclerotic lesions (AL), including abdominal aortic aneurysms (AAA).  
30 Serum levels of HMGB-1 are increased in patients with AAA than in normal controls because  
31 the ALs in AAAs secrete HMGB-1. We therefore postulate that the serum HMGB-1 level should  
32 decrease after endovascular aortic repair (EVAR) or open aortic repair (OAR). However, there is  
33 no evidence of this in the literature. The purpose of this study was to investigate the changes in  
34 HMGB-1 levels after surgical intervention for AAA. We also aimed to determine if the HMGB-1  
35 levels varied between the two procedures.

36 **Design:** Prospective study.

37 **Materials and methods:** Serum HMGB-1 levels were determined in 24 patients with AAA and  
38 25 healthy controls. Twelve of the 24 AAA patients underwent EVAR while the other half  
39 underwent OAR. The relationship between HMGB-1 levels and presence of AAA or influence of  
40 operative methods on the serum HMGB-1 level were prospectively investigated.

41 **Results:** Serum HMGB-1 levels in AAA patients were significantly higher than in healthy  
42 controls ( $9.4 \pm 5.7$  vs.  $4.1 \pm 2.0$  ng/mL,  $P < 0.01$ ). The serum HMGB-1 levels in both the EVAR  
43 group and the OAR group were significantly decreased from baseline at both 3 months and 1  
44 year after surgery.

45 **Conclusions:** Removal or isolation of AL via surgical intervention significantly decreases serum  
46 HMGB-1 levels. The significant post-operative reduction in HMGB-1 levels suggests that  
47 important endocrinological changes occur after surgical treatment of AAA.

48 **Key Words:** Abdominal aortic aneurysm, Atherosclerosis, Covered stenting, High-mobility  
49 group box 1

50 **Introduction**

51 High-mobility group box 1 (HMGB-1) is a nonhistone DNA-binding protein consisting of  
52 215 amino acid residues organized into 3 domains that include 2 tandem HMGB domains (A box  
53 and B box) arranged in an L-shape configuration and a 30 amino acid long C-terminal tail.<sup>1, 2</sup>  
54 HMGB-1 functions as an intracellular regulator of gene transcription and promotes secretion of  
55 several inflammatory cytokines including interleukin (IL), tumor necrosis factor (TNF)- $\alpha$ ,  $\gamma$ -  
56 interferon, and macrophage inflammatory proteins-1 $\alpha$  and -1 $\beta$ .<sup>3,4,5,6</sup> HMGB-1 is therefore  
57 regarded as a key mediator of inflammation-related responses, including inflammation, tissue  
58 regeneration, cancer, infections, and development of atherosclerotic lesions (AL), including  
59 abdominal aortic aneurysms (AAA).<sup>5</sup>

60 Elevated HMGB-1 expression has been detected in ALs in endothelial cells, vascular smooth  
61 muscle cells, and macrophages.<sup>7</sup> Increased HMGB-1 expression leads to progression of ALs and  
62 may result in development of AAAs. The ALs in AAAs then secrete more HMGB-1, further  
63 accelerating growth of the AAAs.<sup>8,9</sup> There are two surgical treatments for AAA: open aortic  
64 repair (OAR) and endovascular aortic repair (EVAR). In patients with AAA, serum HMGB-1 is  
65 increased compared to that in normal controls,<sup>8</sup> probably because ALs in AAAs secrete HMGB-  
66 1. Therefore, theoretically, the serum HMGB-1 level will decrease after OAR or EVAR because  
67 these procedures result in a large amount of the AL in the AAA being removed or isolated from  
68 the patient's blood circulation. However, there is no published evidence supporting this. The  
69 purpose of this study was to investigate the changes in HMGB-1 secretion after surgical  
70 intervention for AAA.

71

72 **Materials and Methods**

73 **Patients**

74 **Figure 1** shows the study design. We enrolled 49 subjects, consisting of 24 AAA patients and  
75 25 healthy volunteers. All 24 AAA patients underwent surgical interventions—12 underwent an  
76 OAR using a Dacron graft (the AL was removed from the blood circulation) and the other 12  
77 underwent EVAR (the AL was isolated from the blood circulation). There was no endoleak after  
78 EVAR. The serum HMGB-1 levels were measured at baseline in all participants and were not  
79 repeated in the control group. In the AAA group, serum HMGB-1 levels were repeated at 3  
80 months and 1 year after surgery. All patients and volunteers gave informed consent, and the  
81 study was approved by the Institutional Review Board at Okayama University Hospital  
82 (Okayama, Japan).

83

84 **Measurements**

85 Blood samples were collected in the conventional manner and centrifuged (3000 rpm, 10 min)  
86 to obtain serum, which was stored at  $-80^{\circ}\text{C}$ . The concentration of HMGB-1 in serum samples  
87 was determined using an enzyme-linked immunosorbent assay kit according to the  
88 manufacturer's protocol (Shino-Test, Sagamihara, Japan). We analyzed cell counts and  
89 biochemistry using standard methods established by the Department of the Central Clinical  
90 Laboratory, Okayama University Hospital.

91

92 **Statistical Analysis**

93 The characteristics of the AAA group and the control group were compared and the  
94 difference in serum HMGB-1 levels at baseline between the two groups was analyzed. Baseline  
95 serum HMGB-1 levels were compared against levels obtained at 3 months and 1 year post

96 surgery. Comparisons were also made between the EVAR and OAR groups to investigate the  
97 effect of the different surgical procedures on the serum levels of HMGB-1. All data were  
98 expressed as mean  $\pm$  SEM or SD. The Mann-Whitney U test was used to analyze differences  
99 between the quantitative data in the AAA and control groups. The chi-square test was used for  
100 analysis of the categorical variables. Time-course variation of serum HMGB-1 levels from their  
101 preoperative values to the values 1 year post intervention between the OAR and EVAR groups  
102 were tested using 2-way ANOVA. A probability value of  $< 0.05$  was considered to be  
103 statistically significant. Statistical analyses were performed with IBM SPSS software, version  
104 19.0.0 (SPSS Inc., Chicago, Illinois).

105

## 106 **Results**

### 107 **Baseline characteristics of all patients**

108 The baseline demographic and clinical characteristics of the AAA patients and controls are  
109 shown in **Table I**. The AAA group had significantly higher pulse wave velocity than the control  
110 group. Smoking, diabetes, and hyperlipidemia were more prevalent in the AAA group than in the  
111 control group.

112 **Table II** shows the laboratory data for the entire patient cohort. The AAA group had  
113 significantly higher levels of fibrinogen degradation products and D-dimer, and lower levels of  
114 hemoglobin, activated partial thromboplastin time, albumin, and high-density lipoprotein (HDL)  
115 cholesterol than the control group. Serum HMGB-1 levels were significantly higher in the AAA  
116 group than in the control group (**Figure 2**).

117

### 118 **Time-course variations in HMGB-1 after vascular surgery**

119 All patients in the AAA group survived surgery and were well 1-year post intervention with  
120 no major complications. Serum HMGB-1 levels were significantly decreased after surgical  
121 intervention (**Figure 3**). Both OAR and EVAR significantly decreased serum HMGB-1 levels at  
122 3 months and 1 year after the surgery (**Figure 4-A,B**). In addition, 2-way ANOVA analysis  
123 showed that there were no significant differences in the degree of reduction in serum HMGB-1  
124 levels between the OAR group and the EVAR group 1 year after intervention (**Figure 4-C**).

125

## 126 **Discussion**

127 This study showed significantly increased serum HMGB-1 levels in AAA patients than in  
128 controls. These results are consistent with previous reports that HMGB-1 expression is enhanced  
129 in all layers of the aortic wall, including atheromatous lesions in AAA patients,<sup>3</sup> and that plasma  
130 HMGB-1 levels are increased in AAA patients.<sup>8</sup> The results of this study confirmed the  
131 relationship between increased serum HMGB-1 levels and the presence of AAA.

132 This study is the first to demonstrate a significant reduction in serum HMGB-1 levels in AAA  
133 patients after surgical intervention with similar reductions seen after either OAR or EVAR. The  
134 postulated reason for this phenomenon is that a large amount of HMGB-1 secreting AL was  
135 removed or isolated from the blood circulation by graft replacement or covered-stent placement.  
136 A previous study showed that HMGB-1 was highly expressed in inflammatory cells in the  
137 adventitia, media, and atherosclerotic plaques. HMGB-1 was also expressed in smooth muscle  
138 cells and endothelial cells in AAA tissue.<sup>3</sup> In this study, there is a possibility that the remaining  
139 aortic wall may continue to secrete HMGB1 after EVAR or after graft replacement and the  
140 secreted HMGB1 will enter the circulation via the remaining vasa vasorum. However, the  
141 decrease of HMGB1 after AAA surgery was significant. In addition, the only difference between

142 pre- and post-operative conditions in the patients was removal or isolation of the atherosclerotic  
143 plaque. Furthermore, there were no cases of endoleak after EVAR in this study. These results  
144 suggest that the main cause of HMGB1 increase in patients with AAA was secretion from the  
145 atherosclerotic plaque in AAA, not from the aortic wall itself.

146 The long-term outcome of decreased HMGB-1 could not be determined from this study. Our  
147 observations indicate that the eventual post-operative reduction in serum HMGB-1 is not related  
148 to adverse outcomes in terms of immediate survival or incidence of major post-surgical  
149 complications. The decreased HMGB-1 levels following surgical treatment of AAA clearly  
150 demonstrate that these interventions trigger an important endocrinological change, which  
151 suggests surgical intervention is likely to have a significant impact in terms of long-term  
152 outcomes. In a study using a transgenic mouse model, reduction of inflammatory cytokines,  
153 including HMGB-1, was shown to reduce the development of atherosclerotic changes.<sup>10</sup>  
154 Therefore, decreased HMGB-1 levels after surgery for AAA may have beneficial effects for  
155 long-term vascular outcomes. Further study is necessary to determine if this is truly the case.

156

### 157 **Study Limitations**

158 This study was limited by the relatively small number of patients.

159

### 160 **Conclusion**

161 The baseline serum HMGB-1 levels were significantly increased in the AAA group compared  
162 to those in the control group. The HMGB-1 levels in AAA patients significantly decreased after  
163 OAR or EVAR. Removal or isolation of large AL may suppress progression of atherosclerotic  
164 disease due to the decreased secretion of HMBG-1 after intervention. Further studies are required



165 to determine whether decreased HMBG-1 levels truly improve outcomes of atherosclerotic  
166 vascular diseases.

167

### 168 **Conflict of Interest Statement**

169 The authors report no conflicts of interest. This research did not receive any specific grant  
170 from funding agencies in the public, commercial, or not-for-profit sector.

### 171 **Acknowledgements**

172 We gratefully acknowledge the work of past and present members in the Division of  
173 Cardiovascular Surgery and Pharmaceutical Science.

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232 **Table I. Baseline demographic and clinical characteristics of all patients**

	Control (n = 25)	AAA (n = 24)	p value
Male % (n)	56.0 (14)	73.6 (18)	0.28
Age (years)	69.8 ± 5.3	71.8 ± 6.7	0.2
BMI	24.1 ± 3.8	23.2 ± 4.0	0.15
Systolic blood pressure (mmHg)	122.4 ± 16.7	130.1 ± 19.7	0.13
Diastolic blood pressure (mmHg)	75.5 ± 9.1	73.7 ± 11.6	0.87
ABI (right)	1.18 ± 0.11	1.08 ± 0.13	0.67
ABI (left)	1.15 ± 0.13	1.05 ± 0.18	0.62
PWV (right) (cm/s)	1547 ± 366	2089 ± 481	< 0.01
PWV (left) (cm/s)	1540 ± 370	2022 ± 488	0.02
Smoker % (n)	8.0 (2)	24.0 (10)	0.01
Medication			
Hypertension % (n)	48.0 (12)	58.5 (14)	0.18
Diabetes % (n)	0	29.2 (7) <sup>1</sup>	0.01
Hyperlipidemia % (n)	24.0 (6)	62.5 (15)	< 0.01

233 Data are shown as mean ± SD (or percentage and number). AAA; abdominal aortic aneurysm, ABI; ankle  
 234 branch index, BMI; body mass index, PWV; pulse wave velocity.

235

236 **Table II. Baseline laboratory based parameters of all subjects**

Laboratory values	control (n = 25)	AAA (n = 24)	p value
WBC × 10 <sup>4</sup> (/μL)	6.2 ± 2.7	5.9 ± 1.6	0.70
Hb (mg/dl)	13.7 ± 2.0	12.6 ± 1.7	0.03
Hct (%)	40.0 ± 5.1	37.8 ± 4.6	0.08
Plt × 10 <sup>4</sup> (/μL)	212 ± 46	224 ± 86	0.45
APTT (sec)	113 ± 18	102 ± 26	0.048
Fibrinogen (mg/dl)	396 ± 127	487 ± 204	0.16
FDP (μg/ml)	4.8 ± 1.8	16.7 ± 14.3	0.03
D-dimer (ng/ml)	1.5 ± 1.0	8.3 ± 7.0	0.03
TP (g/dL)	7.1 ± 0.5	7.1 ± 0.5	0.87
Albumin (g/dL)	4.2 ± 0.3	3.9 ± 0.5	0.01
Creatinine (mg/dL)	1.3 ± 1.2	1.0 ± 1.2	0.94
T-chol (mg/dL)	194 ± 27	192 ± 38	0.78
HDL-chol (mg/dL)	67.8 ± 15.1	49.5 ± 10.8	< 0.01
HbA1c (%)	5.8 ± 0.4	5.9 ± 0.5	0.47
BNP (pg/mL)	31.6 ± 57.5	54.5 ± 62.8	0.29
CRP (mg/dL)	0.5 ± 0.9	2.2 ± 4.8	0.14
HMGB-1 (ng/ml)	4.1 ± 2.0	9.4 ± 5.7	< 0.01

237 Data are shown as mean ± SD. AAA; abdominal aortic aneurysm, APTT; activated partial thromboplastin  
 238 time, BNP; brain natriuretic peptide, CRP; C-reactive protein, FDP; fibrinogen degradation products, Hb;  
 239 hemoglobin, Hct; hematocrit, HDL-chol; high-density lipoprotein-cholesterol, HMGB-1, high-mobility  
 240 group box 1, Plt; platelet, T-chol; total cholesterol, TP; total protein, WBC; white blood cell.

241 **Figure legends**

242 **Figure 1. Tree diagram of patients enrolled in this study**

243 AAA; abdominal aortic aneurysm, EVAR; endovascular aortic repair, HMGB-1; high-mobility  
244 group box 1, OAR; open aortic repair

245

246 **Figure 2. Serum HMGB-1 levels before intervention**

247 The serum HMGB-1 level was higher at baseline in the AAA group than in the control group. All  
248 data are expressed as mean  $\pm$  SEM. \*P < 0.01, AAA vs. control group.

249

250 **Figure 3. Time course variance of changes in HMGB-1 levels after surgical intervention for**

251 **AAA**

252 Following intervention, the AAA patients showed decreased serum HMGB-1 levels on follow up  
253 at 3 months and 1 year compared to baseline. All data are expressed as mean  $\pm$  SEM. \*P < 0.01,  
254 3 months or 1 year vs. baseline.

255

256 **Figure 4. Changes in serum HMGB-1 levels after EVAR or OAR**

257 The serum HMGB-1 levels decreased after surgery in both EVAR and OAR groups at 3 months  
258 and 1 year follow-up compared with baseline (A, B). No significant differences in post-surgical  
259 HMGB-1 levels were observed between the two groups (C). \*P < 0.05, baseline vs. 3 months,  
260 \*\*P < 0.01, baseline vs. 1 year. EVAR; endovascular aortic repair, OAR; open aortic repair.

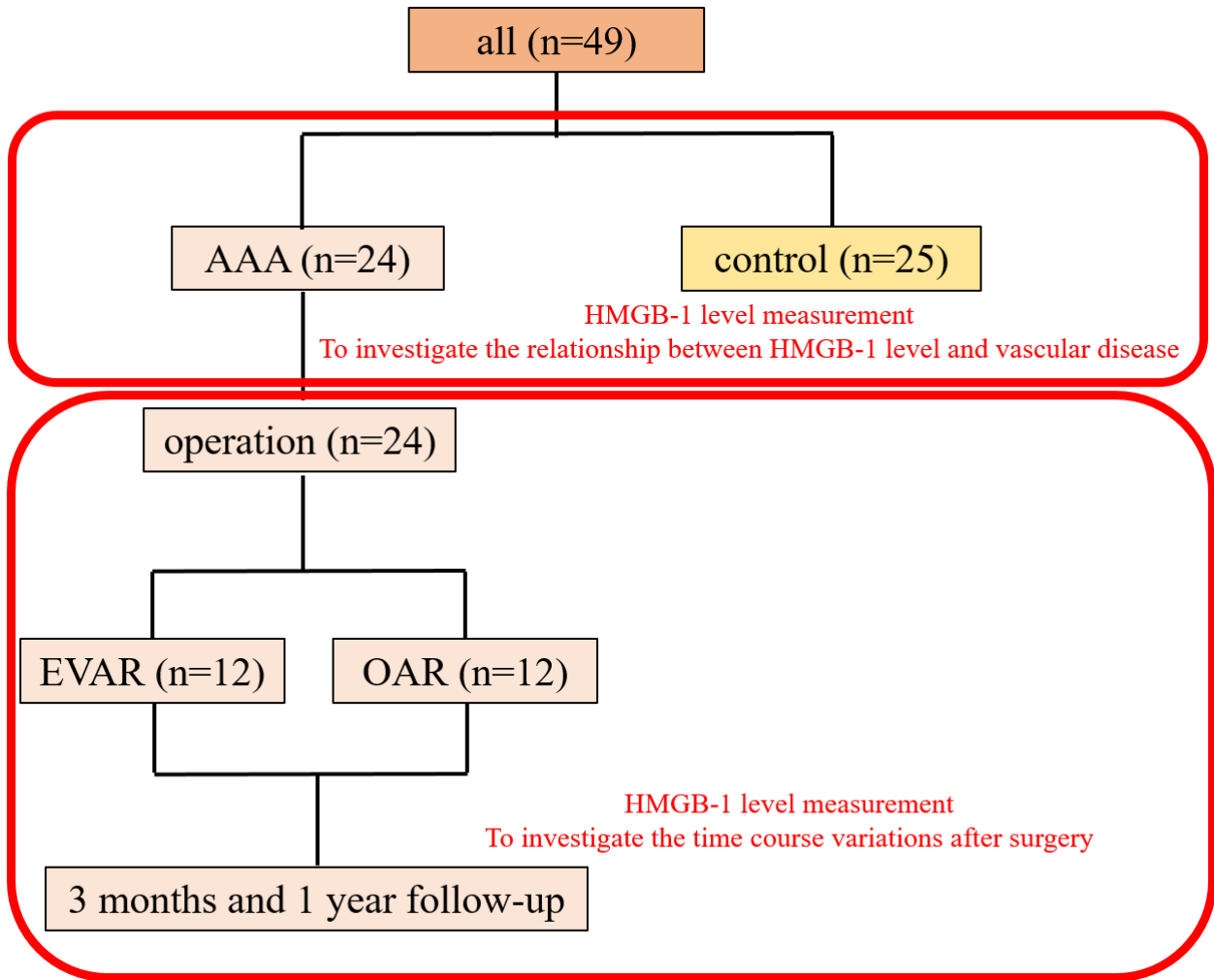
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Figure 1. Tree diagram of patients enrolled in this study



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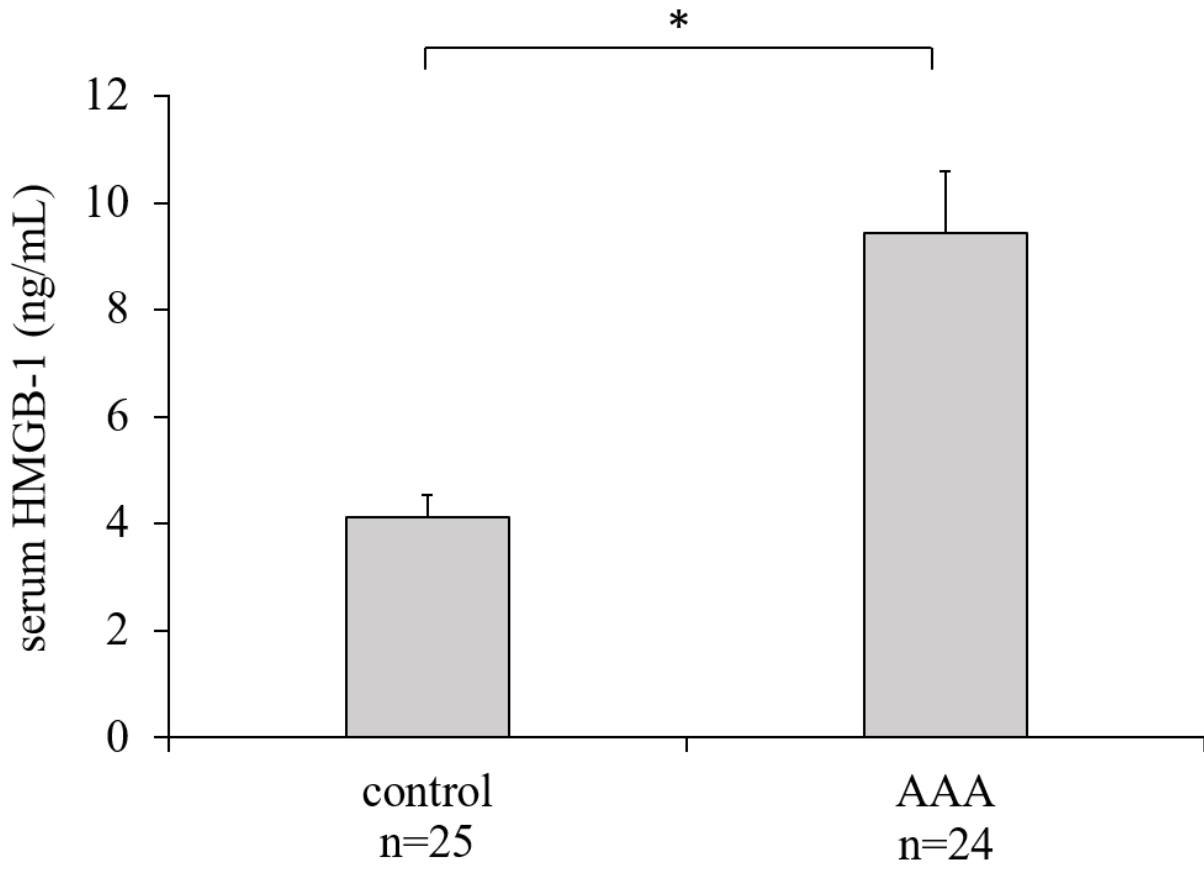
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274 [Figure 2. Serum HMGB-1 levels before intervention](#)

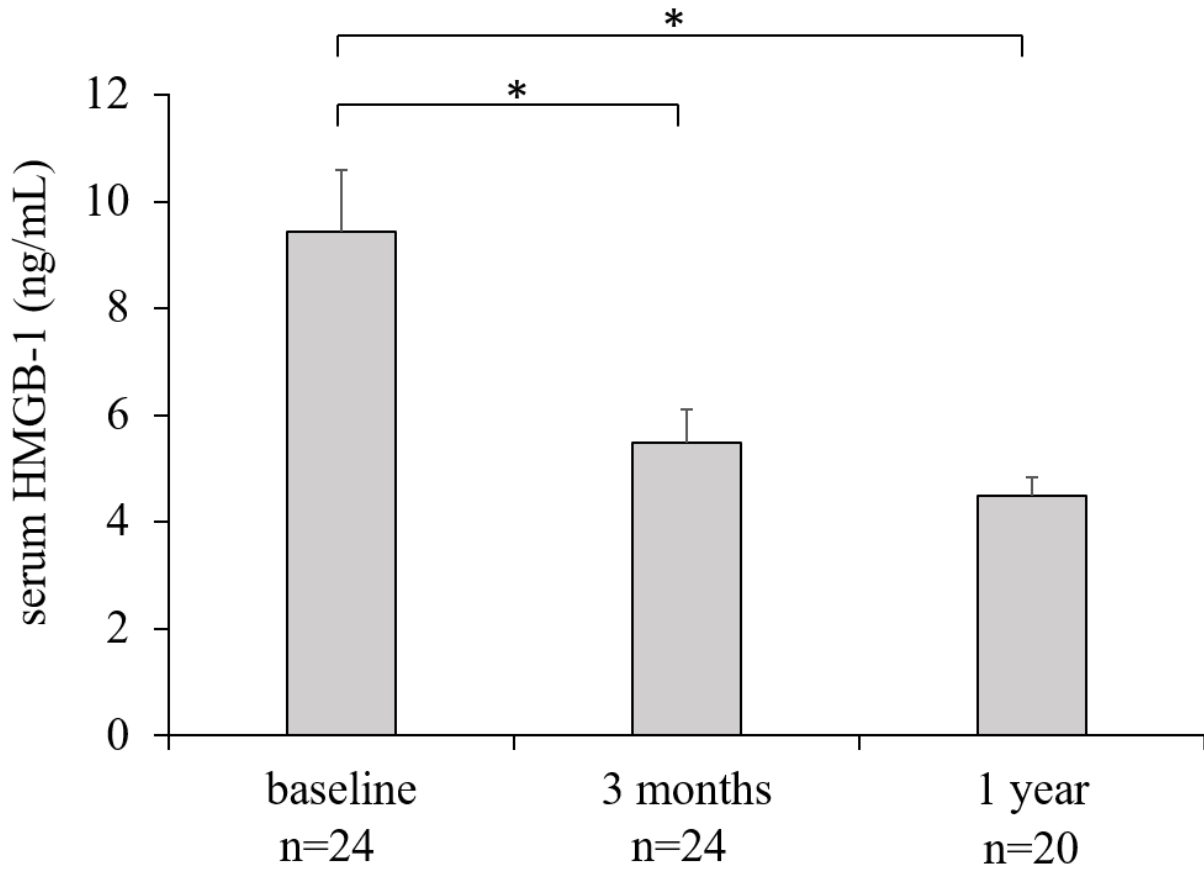


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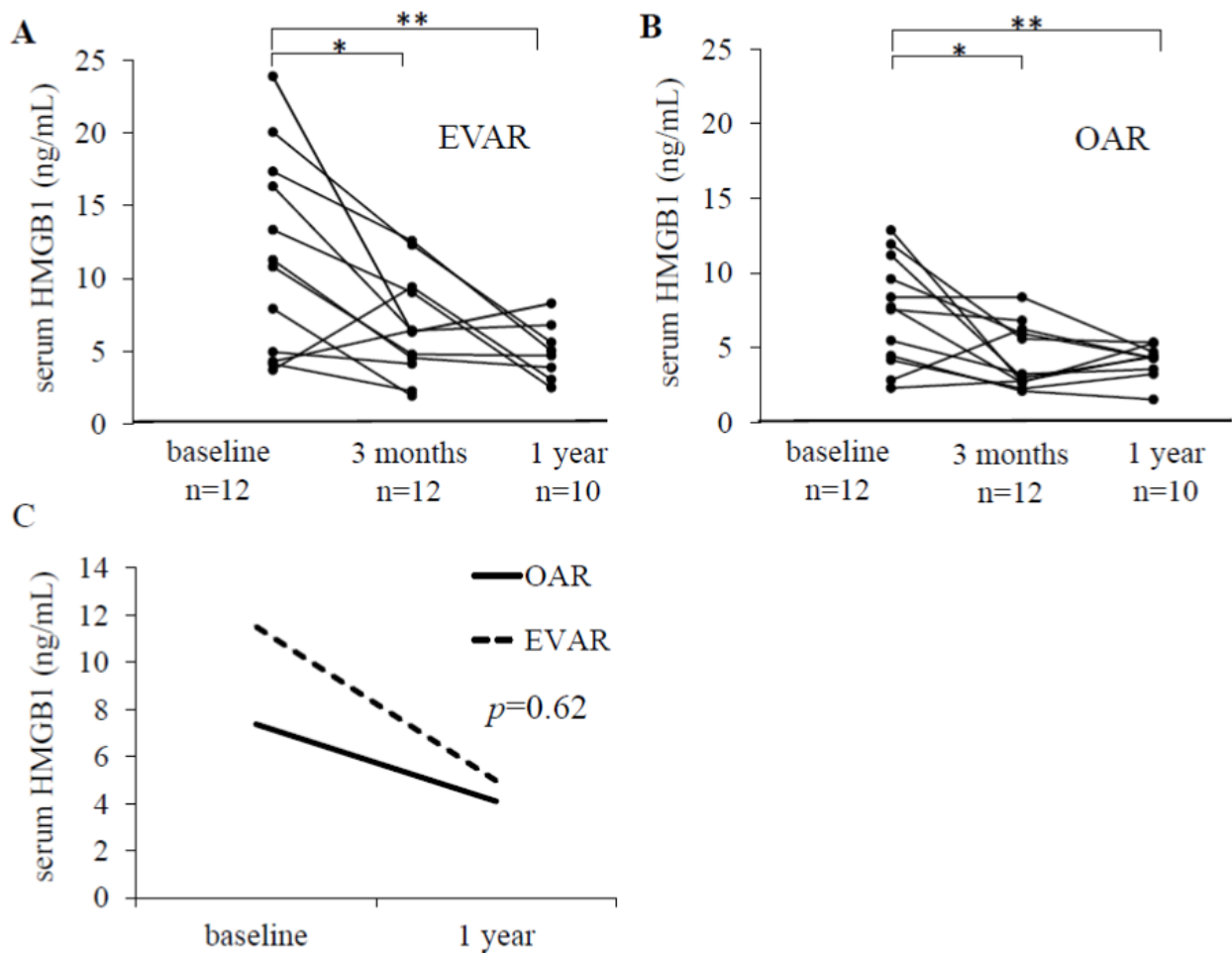
[Figure 3. Time course variance of changes in HMGB-1 levels after surgical intervention for AAA](#)



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297 [Figure 4. Changes in serum HMGB-1 levels after EVAR or OAR](#)



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