A Synergistic Effect of Albumin and Fibrinogen on Immunoglobulin-Induced Red Blood Cell Aggregation

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Running head: Immunoglobulin-Induced Red Blood Cell Aggregation

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Abstract

Therapeutic administration of immunoglobulins (Ig) has the potential to precipitate thrombotic events. This phenomenon may be explained by red blood cell (RBC) aggregation, which can be potentiated by Ig. The contribution of plasma albumin and fibrinogen to Ig-induced RBC aggregation is unclear. We examined RBC aggregation in three settings: (1) patients receiving therapeutic infusions of Ig; (2) plasma supplemented in vitro with Ig; and (3) RBC suspensions in standard buffer with varying concentrations of albumin, Ig and fibrinogen. Ig infusion augmented aggregation of RBCs from patients with normal or high plasma levels of albumin, but decreased aggregation in those with lower plasma albumin concentrations. In vitro, RBC aggregation was significantly increased only when all three components, fibrinogen, albumin and Ig, were present at or above normal concentrations in the suspension, but was unaffected when any one of the components was absent from the suspension. Our results suggest a three-way interaction among fibrinogen, Ig and albumin, which synergistically induce RBC aggregation in plasma. Understanding these interactions may help predict clinically important phenomena related to RBC aggregation, such as thrombotic complications of Ig infusion.

Keywords: hemorheology, thrombosis, adverse effects
Introduction

Immunoglobulin (Ig) preparations are routinely administered to patients suffering from a variety of autoimmune disorders, immune deficiencies and the Guillan-Barre syndrome. Clinical data implicates therapeutically administered intravenous Ig in the precipitation of life-threatening thrombotic events [7, 9, 10, 13, 21, 25, 28, 29, 31, 35]. Ig preparations have been shown to induce red blood cell (RBC) aggregation, which may contribute to the increased blood viscosity associated with their use [11, 23, 37]. RBC aggregation affects blood viscosity most markedly in areas of low shear stress (<4 dyn/cm²), such as arterial bifurcations. The same sites are also prone to atherosclerosis and thrombosis [18]. Increased RBC aggregation and the resulting elevated blood viscosity promote low-flow downstream from atherosclerotic lesions, a condition which favors thrombogenesis [12]. Regional low-flow resulting from RBC aggregation can therefore set the stage for vascular thrombosis, especially in persons with underlying vascular atherosclerotic disease. Elucidating the mechanism for Ig-induced RBC aggregation could help identify those patients at risk for thrombotic complications.

Aggregation of RBCs is governed by opposing forces: on the one hand the repulsive electrical force between negatively charged cells and the shear force exerted by blood flow, which together disaggregate RBCs, and on the other hand the cohesive force induced by the presence of various plasma proteins which promote the formation of rouleaux structures and larger aggregates [8, 20, 26, 30]. The equilibrium between these forces determines the extent of RBC aggregation, which in turn is the major
determinant of blood viscosity at low shear rate [2], and thus a key element in hemorheology.

The relative roles of plasma proteins in RBC aggregation is not clear, as disparate findings have been reported. It is believed that fibrinogen, a 340 kD fibrous hexamer, is the most potent aggregator of RBCs in plasma [26]. Some studies [11, 37] have shown that Ig induces RBC aggregation while others found no effect [16]. Data on albumin are even more conflicting. While it is clear that albumin does not directly aggregate RBCs, inconsistent results have been obtained regarding its effect when combined with other plasma proteins. Two studies found an inverse relationship between plasma albumin and RBC aggregation in diabetics [14, 38]. Some investigations described enhancement of fibrinogen-induced RBC aggregation by albumin [17, 22], but one study showed inhibition [14]. Ig-induced RBC aggregation has been inhibited by albumin in one study [17], but enhanced by albumin in another [34]. Reinhart et al found that albumin increased the erythrocyte sedimentation rate when added to fibrinogen and Ig, but inhibited it when added to either alone [24]. However, the sedimentation rate is an imperfect correlate of RBC aggregation, as it is affected by the shape of RBC aggregates, RBC aggregates are not visualized directly, and aggregation is measured under static, no-flow conditions [36].

The present study was undertaken to examine the combined effects of albumin and fibrinogen on Ig-induced RBC aggregation. First, we examined the effect of Ig administered in vivo on RBC aggregation, as a function of plasma albumin and fibrinogen concentrations. The combined effects of albumin and fibrinogen on Ig-induced RBC aggregation were further investigated in vitro, using either human
plasma or a suspension containing controlled concentrations of Ig, fibrinogen and albumin as a medium for RBC aggregation.
Materials and Methods

All patients and healthy (control) subjects gave their informed consent for participation, and the study protocol was reviewed and authorized by the institutional review board at the Sourasky Tel Aviv Medical Center.

1. Aggregation of RBCs from patients receiving intravenous Ig therapy

To study the effect of intravenous Ig administered in vivo, we recruited patients scheduled for Ig treatment from the Tel Aviv Medical Center hematology and rheumatology clinics. All patients filled a clinical questionnaire. Venous blood was drawn for determination of RBC aggregation immediately before the infusion of Ig (Omr-IgG-am, Omrix biopharmaceuticals, Rehovot, Israel) was started, and again at its termination. Plasma fibrinogen concentration was determined by the method of Clauss [5] using a thrombin reagent (Dade Behring, Newark, DE) according to the manufacturers instructions. Plasma albumin was measured by the Bayer Advia® 1650 system (Bayer Diagnostics, Tarrytown, NY). Plasma Ig was measured by nephelometry using the BN II system (Dade Behring).

Preparation of RBC suspension for determination of RBC aggregability

Samples of venous blood were drawn from the ante-cubital vein and collected into EDTA containing Vacutainers. The RBCs were isolated by centrifugation (2000 rpm for 10 minutes), washed with phosphate buffered saline (PBS) pH 7.4, and resuspended at the desired hematocrit in either autologous plasma or PBS supplemented with predetermined concentrations of human fibrinogen (F4883, Sigma St. Louis, MO), human serum albumin (A3782, Sigma), and Ig, as described below.
Determination of RBC aggregation

All aggregation measurements were conducted immediately following venipuncture. RBC aggregability was studied using a cell-flow properties analyzer as previously described [3]. Briefly, RBC suspension was prepared at 5% hematocrit. The suspension was then introduced into a cell-flow properties analyzer, consisting of a narrow-gap (30 micrometer) flow-chamber, connected to a pump exerting laminar flow and a pressure transducer that monitored shear stress during the experiment. The RBC dynamic organization (aggregation/disaggregation) in the flow-chamber was directly visualized and recorded through a microscope connected to a charge-coupled device (CCD) video camera, which transmitted the RBC images to a computer. Images were then analyzed by image analysis software [a modified version of SigmaScan Pro (SPSS, Chicago, Il)], which computes the average aggregate size (number of RBCs per aggregate) by dividing total aggregate volume by the volume of a single RBC.

RBC aggregation was monitored in the flow-chamber under increasing shear stress, and characterized by the area under the curve (AUC) of average aggregate size plotted as a function of the shear stress exerted (AUC_{AAS}). The wall shear stress taken for this calculation ranged from 0.15 to 4.00 dyn/cm^{2}, at which normal RBCs are singly dispersed. The AUC expresses both the extent of RBC aggregation and its dependence on shear stress, thus reflecting the strength of the intercellular interactions. We previously found this index to faithfully represent clinically relevant aggregation in various disease states [1]. The change in AUC after Ig infusion was expressed as the relative increment in RBC aggregation, calculated as:

\[
100 \times \frac{(AUC \text{ after Ig}) - (AUC \text{ before Ig})}{(AUC \text{ before Ig})}
\]
2. **RBC aggregation after the addition of Ig to plasma in vitro**

In order to study the effect of Ig on RBC aggregation over a wide range of plasma fibrinogen concentrations, we drew blood samples from healthy volunteers and hospitalized individuals with acute coronary syndromes. The latter group is known to have elevated levels of plasma fibrinogen [1].

RBC aggregation was examined in autologous plasma at baseline. Aggregation studies were then repeated after adding Ig in vitro to achieve a final concentration of 25 mg/ml, approximately the concentration resulting in vivo from the administration of Ig at a dose of 0.5 grams per kilogram body weight.

3. **RBC aggregation under controlled concentrations of fibrinogen, Ig and albumin**

In order to define more precisely the contribution of the major plasma proteins to RBC aggregation, we studied the aggregation of RBCs from healthy volunteers under a range of controlled concentrations of fibrinogen, Ig and albumin. To establish dose-response, RBC aggregation was examined at concentrations of fibrinogen ranging from 0 to 1000 mg/dl (increments of 200 mg/dl), in a suspension containing 0 or 25 mg/ml Ig and 0 or 4.5gr/dl albumin; and at concentrations of albumin ranging from 0 to 5.5 g/dl (increments of 1 g/dl), in a suspension containing 0 or 25 mg/ml Ig and 250 or 500 mg/dl fibrinogen.

**Statistical analysis**

Differences in RBC aggregation (AUC$_{AAS}$) and plasma protein concentrations before and after Ig administration were calculated with the student’s t-test. Correlations
between continuous variables were analyzed with Pearson’s bivariate correlation. All values are expressed as mean ± SEM. All statistical tests were two-sided. P values were considered significant when less than 0.05. Statistical calculations were performed with the SPSS software package.
Results

1. Effect of Ig administered in vivo on RBC aggregation

Thirteen patients (ten males, three females), received intravenous Ig treatment for a total of 18 sessions (five patients were sampled twice). The indication for Ig treatment was chronic lymphocytic leukemia in 4 patients, non-Hodgkin’s lymphoma in 1, Hodgkin’s disease in 1, multiple myeloma in 4, autoimmune vasculitis in 2 and common variable immune deficiency in 1. Ig was given at a mean dose of 0.4 gr/kg (range 0.3-0.6 gr/kg). Results were analyzed per treatment session rather than per patient.

Baseline values of plasma proteins are shown in Table 1. After Ig treatment, plasma globulin increased from 3.5 ± 0.5 gr/dl to 4.2 ± 0.5 gr/dl (P < 0.001), plasma fibrinogen decreased from 325 ± 26 mg/dl to 290 ± 19 mg/dl (P < 0.01), and plasma albumin decreased from 3.8 ± 0.1 gr/dl to 3.4 ± 0.1 gr/dl (P < 0.001).

Overall, RBC aggregation was not significantly altered following the administration of Ig. The relative change in AUC following Ig infusion ranged from an increase of 78% to a decrease of 43%. The results, depicted in Figure 1, show that the response to Ig differed among patients with low plasma albumin (≤ 3.5 gr dl) and those with normal to high plasma albumin (> 3.5 gr dl). Prior to Ig infusion, AUC was higher in the low albumin group compared to the normal-high albumin group. However, Ig infusion decreased AUC_{AAS} by 12.29 ± 10.14% in the low albumin group, and increased it by 23.18 ± 9.95% in the normal-high albumin group (P = 0.02) (Figure 1). In the group as a whole, RBC aggregation did not correlate with plasma fibrinogen, either before or after Ig infusion. Baseline AUC_{AAS} before Ig infusion did not correlate
with plasma fibrinogen in either of the albumin groups. However, after Ig infusion, in patients with plasma albumin above 3.5 gr/dl, AUC$_{AAS}$ correlated positively with plasma fibrinogen ($r = 0.74$, $P = 0.006$). In contrast, in the low albumin group there was no correlation between plasma fibrinogen and AUC$_{AAS}$ following Ig infusion ($r = -0.55$, $P = 0.2$). The relative increment in AUC$_{AAS}$ following Ig infusion correlated with plasma fibrinogen in the normal-high albumin group ($r = 0.59$, $P = 0.04$) but not in the low albumin group ($r = 0.04$, $P = 0.9$).

2. Effect of Ig on RBC aggregation in vitro

Plasma was collected from 15 patients hospitalized with an acute coronary syndrome, and four healthy individuals. Concentrations of plasma proteins are shown in Table 1. Baseline RBC aggregation in human plasma correlated positively with plasma globulin ($r = 0.58$, $P = 0.009$) and plasma fibrinogen ($r = 0.47$, $P = 0.04$), and negatively with plasma albumin concentrations ($r = -0.54$, $P = 0.01$). As with patients receiving Ig in vivo, addition of Ig to human plasma in vitro was not associated with an overall change in mean AUC$_{AAS}$. However, the effect of Ig on RBC aggregation was dependent on the concentrations of fibrinogen and albumin in plasma; the relative increment in RBC aggregation correlated with plasma fibrinogen levels ($r = 0.64$, $P = 0.003$). A marked increase in RBC aggregation was noted in plasma samples with fibrinogen concentrations of above 450 mg/dl (Figure 2). The plasma sample with the highest fibrinogen concentration (600 mg/dl) exhibited a 29-fold increase in AUC$_{AAS}$ following the addition of Ig.

Furthermore, the effect of Ig on RBC aggregation in the presence of a high fibrinogen concentration was strongly dependent on the albumin concentration. When
plasma fibrinogen was less than 450 mg/dl, there was an inverse correlation between albumin levels and AUC_{AAS} (r = -0.69, P = 0.004, figure 3A). However, in the four plasma samples in which fibrinogen concentration exceeded 450 mg/dl, AUC_{AAS} correlated linearly with the plasma albumin concentration (r = 0.99, P = 0.006, Figure 3B).

3. RBC aggregation under controlled concentrations of fibrinogen, Ig and albumin

The response of RBC aggregation to increasing concentrations of albumin (0 to 5.5 gr/dl) was examined in four sets of suspensions, produced by the following combinations: 1) Low or high fibrinogen concentrations (250 and 500 mg/dl, respectively); and 2) Presence or absence of Ig at a concentration of 25 mg/ml. In a suspension containing Ig (25 mg/ml) and a high fibrinogen concentration, AUC_{AAS} correlated positively with albumin concentration (r = 0.94, P = 0.01) (Figure 4). However, in the other three suspensions, there was no effect of increasing albumin concentration on RBC aggregation. At albumin concentrations of 0 to 3.5 gr/dl, AUC_{AAS} did not differ significantly among the four suspensions. At albumin concentrations of 4.5 and 5.5 gr/dl, AUC_{AAS} in the suspension containing a high fibrinogen concentration supplemented with Ig was increased two–fold over the other three suspensions (P < 0.001) (Figure 4).

We also assessed the contribution of fibrinogen to RBC aggregation in four sets of suspensions, produced by the following combinations: 1) Presence or absence of a physiologic concentration of albumin (4.5 gr/dl); and 2) Presence or absence of Ig at a concentration of 25 mg/ml.
RBC aggregation (Log AUC\textsubscript{AAS}) correlated with fibrinogen concentration in a suspension containing Ig 25 mg/ml and albumin 4.5 gr/dl (r = 0.86, P = 0.02). In contrast, fibrinogen concentration did not affect AUC\textsubscript{AAS} in albumin-free suspensions (Figure 5). At fibrinogen concentrations above 400 mg/dl, the absolute value of AUC\textsubscript{AAS} was significantly greater in a suspension containing both Ig and albumin compared to the other three suspensions (P < 0.01) (Figure 5).
Discussion

Our results suggest a three-way interaction among the three main plasma proteins involved in RBC aggregation. The observations in vivo imply that infusion of Ig increases RBC aggregation in the presence of an elevated plasma fibrinogen concentration and a normal to high plasma albumin concentration. Both these conditions are required for Ig to augment RBC aggregation, and their effects are synergistic. These findings are supported by the results of the in vitro studies; RBC aggregation in autologous plasma was enhanced following the addition of Ig if the plasma fibrinogen concentration exceeded 450 mg/dl; at this fibrinogen concentration, RBC aggregation correlated linearly with the plasma albumin concentration. However, at a lower plasma fibrinogen concentration, we found no enhancement of RBC aggregation by Ig, and AUC_{AAS} correlated negatively with the plasma albumin concentration. These interactions were further delineated at controlled concentrations of fibrinogen, albumin and Ig. RBC aggregation was significantly greater in suspensions containing Ig, albumin and fibrinogen compared to suspensions deficient in any one of these macromolecules. The presence of albumin (4.5 gr/dl) and Ig (25 mg/ml) in the RBC suspension facilitated enhancement of RBC aggregation in response to increasing fibrinogen concentrations. Likewise, a fibrinogen concentration of 500 mg/dl and an Ig concentration of 25 mg/dl facilitated the enhancement of RBC aggregation in response to increasing albumin concentrations; in an RBC suspension free of either fibrinogen or Ig, there was no correlation between albumin and RBC aggregation. Taken together, these results show that enhanced RBC aggregation occurs only when all three plasma proteins are present at or above threshold concentrations, indicating that RBC aggregation is dependent on a synergistic interaction between Ig, albumin and fibrinogen.
The effect of albumin on RBC aggregation merits special consideration. Previous studies varied widely in their findings concerning the role of albumin in RBC aggregation [14, 17, 22, 34, 38]. These discrepancies may result from failure to take into account the interactions among various plasma proteins. Our findings in vivo and in vitro confirm a negative correlation between plasma albumin concentration and RBC aggregation, as reported by others [14, 38]. However, when studied at high concentrations of both fibrinogen and Ig, albumin takes on an important role as an inducer of RBC aggregation.

Aggregation of RBCs in a suspension increases with increasing molecular size of macromolecules in the suspension [4]. Therefore, it is reasonable to speculate that synergistic enhancement of RBC aggregation by plasma proteins is a result of the formation of multi-protein complexes. Being an anionic protein, it is likely that albumin by itself directly disaggregates negatively charged RBCs. Albumin may however interact with other plasma proteins, with the effect of enhancing their ability to aggregate RBCs. Fibrinogen and Ig are cationic proteins, and thus can bind to albumin. It is possible to construct a plausible model to explain the synergistic effects of all three plasma proteins, based on the following assumptions: 1) albumin can bind to both fibrinogen and Ig simultaneously, using two separate binding sites; 2) fibrinogen and Ig interact with albumin using binding sites which are distinct from the RBC membrane binding sites of these molecules. If these assumptions hold true, then sufficient concentrations of these plasma proteins could result in the formation of large, positively charged complexes of fibrinogen, Ig and albumin, which efficiently bridge RBCs and promote the formation of large aggregates.
Several observations support this model. Albumin-IgG complexes are present in normal human plasma [27]. Such complexes probably increase in number after the administration of Ig. Albumin-fibrinogen complexes can be produced experimentally, and these complexes have been shown to interact with platelets [32]. Both Ig and fibrinogen have been extensively shown to interact with RBCs. Ig infused into healthy subjects coats RBCs without causing hemolysis [19]. Fibrinogen binding to RBC membranes has recently been shown to be inhibited by the peptide Arg-Gly-Asp-Ser, suggesting both specific and non-specific fibrinogen binding to RBCs [15]. Thus, both fibrinogen and Ig can each bind to albumin and RBC membranes, and could theoretically form an intercellular matrix which stabilizes RBC aggregates. The significant decrease in plasma fibrinogen and albumin following intravenous Ig administration, reported also by Madl et al [16], is consistent with the formation of Ig-albumin-fibrinogen complexes, resulting in reduced levels of free albumin and fibrinogen.

The level of plasma albumin, a so-called “negative acute phase protein”, decreases in acutely ill patients, and albumin levels are inversely related to the risk of death. A recent meta-analysis has shown that the time-honored practice of administering albumin to critically ill patients does not reduce mortality, and may in fact increase it [6]. In view of our findings, it is tempting to hypothesize that reduction in plasma albumin levels in acutely ill patients is an adaptive response, which reduces the viscosity of blood containing high concentrations of RBC-aggregating acute phase proteins such as fibrinogen and Ig. Increased RBC aggregation may reduce oxygen
delivery to tissues by increasing the thickness of the marginal cell-free layer [33], thus reducing the already compromised tissue oxygenation in critical patients.

In clinical practice, fibrinogen and albumin concentrations tend to vary reciprocally, and it is uncommon to find elevated levels of both in the same patient. Madl et al studied patients with sepsis who received Ig and found no change in RBC aggregation [16]. These patients probably had high concentrations of fibrinogen and low concentrations of albumin, as is typical of acutely ill patients.

Induction of RBC aggregation by therapeutically administered Ig may have important clinical consequences. Thrombotic complications of Ig treatment are increasingly reported, and include myocardial infarction [9, 10, 25], ischemic stroke [7, 28, 31] and retinal vein occlusion [21]. Prophylactic administration of Ig to bone marrow transplant recipients was associated with increased mortality in two large registries. Death was attributed to venoocclusive disease of the liver, which could be explained by increased blood viscosity resulting from RBC aggregation [13, 35]. Immune globulin is currently contraindicated in children with cyanotic heart disease, who have an elevated hematocrit and blood viscosity, as it was found to promote cyanotic episodes and poor surgical outcomes [29]. Knowing what constellation of plasma protein concentrations places a patient at an increased risk of these complications may facilitate the selection of candidates for Ig treatment.

In conclusion, albumin, fibrinogen and Ig synergistically induce RBC aggregation. Therefore, patients with high plasma concentrations of both albumin and fibrinogen are susceptible to enhanced RBC aggregation after Ig infusion, which may
place them at risk for thrombotic complications. Our results should aid in constructing a model of RBC aggregation by plasma proteins, which takes into account interactions among the proteins themselves as well as between proteins and RBCs.
Acknowledgements:

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References


Figure 1. Relative Increment in RBC aggregation following therapeutic administration of Ig, in patients with low and normal values of plasma albumin.

- Alb ≤ 3.5 gr/dl
- Alb > 3.5 gr/dl

P = 0.02
**Figure 2.** RBC aggregation in Ig-supplemented plasma as a function of fibrinogen concentration.
Figure 3. RBC aggregation in plasma before and after Ig-supplementation, as a function of fibrinogen and albumin concentrations.
**Figure 4.** RBC aggregation as a function of albumin concentration in a suspension with controlled concentrations of Ig and fibrinogen.
Figure 5. RBC aggregation as a function of fibrinogen concentration in a suspension with controlled concentrations of Ig and albumin.
Legends for Figures

Figure 1.
Relative increment in RBC aggregation, expressed as the area under the curve for average aggregate size (AUC$\text{AAS}$), of RBCs from patients receiving intravenous Ig therapy. Relative increment was positive among patients with plasma albumin concentrations above 3.5 gr/dl and negative in patients with plasma albumin below 3.5 gr/dl ($P = 0.02$).

Figure 2.
RBC aggregation (AUC$\text{AAS}$), as a function of serum fibrinogen concentration, in plasma supplemented with Ig. Markedly elevated AUC$\text{AAS}$ is noted at fibrinogen concentrations above 450 mg/dl.

Figure 3.
RBC aggregation in plasma (AUC$\text{AAS}$), as a function of plasma albumin concentration; A. Without Ig supplementation, no correlation is evident between AUC$\text{AAS}$ and plasma albumin. B. With Ig supplementation, AUC$\text{AAS}$ correlates negatively with plasma albumin at plasma fibrinogen concentration below 450 mg/dl ($r = -0.69, P = 0.004$), but increases linearly with increasing plasma albumin concentration at plasma fibrinogen above 450 mg/dl ($r = 0.99, P = 0.006$).

Figure 4.
RBC aggregation (AUC$\text{AAS}$) as a function of albumin concentration in four sets of suspensions with controlled concentrations of fibrinogen and Ig. AUC$\text{AAS}$ correlates
with albumin concentration only when both Ig (25 mg/ml) and high fibrinogen concentration (500 mg/dl) are present in the medium, but not in the absence of either.

Figure 5.

RBC aggregation (AUC_{AAS}) as a function of fibrinogen concentration in four sets of suspensions with controlled concentrations of albumin and Ig. Log AUC_{AAS} correlates with fibrinogen concentration in a suspension containing Ig (25 mg/ml) and albumin (4.5 gr/dl), but not in an albumin-free suspension. AUC_{AAS} is significantly greater at a fibrinogen concentration above 400 mg/dl in a suspension containing Ig and albumin compared to the other three suspensions.
Table 1. Baseline Concentrations of Plasma Proteins in the Various Patient Groups Studied.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Fibrinogen (mg/dl)</th>
<th>Ig (gr/dl)</th>
<th>Albumin (gr/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients receiving Intravenous Ig</td>
<td>13</td>
<td>325.8 ± 26.5</td>
<td>3.5 ± 0.5</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Patients with ischemic heart disease</td>
<td>15</td>
<td>380.6 ± 31</td>
<td>2.7 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Control subjects</td>
<td>4</td>
<td>268.9 ± 28.4 *</td>
<td>2.7 ± 0.1</td>
<td>5.2 ± 0.1 †</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

* Value smaller than in patients with ischemic heart disease (P = 0.02).

† Value greater than in patients with ischemic heart disease (P = 0.001) and in patients receiving intravenous Ig (P < 0.001).