Evaluation of Platelet Indexes in Patients with Aortic Aneurysm

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Key Words

Platelet index · Mean platelet volume · Platelet-large cell ratio · Platelet distribution width · Aneurysm, aortic

Abstract

The purpose of this study was to investigate whether platelet indexes [platelet count, mean platelet volume (MPV), platelet-large cell ratio (P-LCR), and platelet distribution width (PDW)] could serve as diagnostic tools to evaluate the potential significance of platelet heterogeneity on thrombus formation. Blood samples were obtained from 54 patients with aortic aneurysm (AA; mean age 73 years; 40 males, 14 females), from 120 age-matched controls (AC; mean age 74 years; 61 males, 59 females), and from 38 young controls (YC; mean age 30 years; 20 males, 18 females). We measured the blood platelet indexes using an automated counter, as well as plasma D-dimer and thrombin-antithrombin III complex (TAT) using ELISA. The AA patients were divided into two groups, group A (platelet count more than $150 \times 10^9/l$, $n = 36$) and group B (platelet count below $150 \times 10^9/l$, $n = 18$). There was no difference in the platelet count between AC and YC. However, P-LCR was signifi-

cantly higher ($p = 0.0113$) in AC. MPV and PDW were also higher, but not significantly so. The platelet count was not different between group A and AC. MPV ($p = 0.0024$ and <0.0001 , respectively), P-LCR ($p < 0.0012$ and <0.0001 , respectively) and PDW ($p = 0.0006$ and 0.0005 , respectively) were significantly lower in group A than in group B and AC. The platelet indexes were significantly lower in the 54 AA patients than in the AC. There were significant inverse relationships between the platelet count and other indexes in the AC and 54 AA patients; however, no relationships were observed in group A, B and YC. The D-dimer and TAT levels were significantly higher in group B than in groups A and YC. In conclusion, these findings suggest that large platelets decrease rather than increase in patients with AA.

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Introduction

Circulating platelets are heterogeneous with respect to their size, density and reactivity. The elevated mean platelet volume (MPV) has been proposed by many authors as a risk factor for ischemic heart disease [1, 2]. In

the case of aortic aneurysm (AA), Balduini et al. [3] reported increased MPV and/or thrombocytopenia in patients with unruptured abdominal AA. It is generally accepted that large platelets are metabolically and enzymatically more active than small platelets [4, 5]. The degree of platelet activation may be assessed by parameters such as the platelet count, MPV and the percentage of large platelets. AA has been considered to arise as a direct consequence of atherosclerosis, in which platelet activation plays an important role in the prethrombotic events leading to thrombin formation. We compared the platelet indexes in age-matched controls (AC), in young controls (YC) and in patients with AA to evaluate the physiology of platelet heterogeneity on thrombus formation. Furthermore, we measured the D-dimer and thrombin-anti-thrombin III complex (TAT) in the patients and AC to estimate the platelet activation and the presence of hypercoagulability.

Materials and Methods

The subjects recruited were 54 patients with AA (aged 39–89 years, mean age 73 years; 40 males, 14 females) over a 12-year period from 1989 to 2000, including 23 abdominal AA, 20 thoracic AA and 11 thoracoabdominal aneurysms, as well as 120 AC from the Kure City Aged Conference (aged 63–86 years, mean age 73 years; 61 males, mean age 74 years; 59 females, mean age 72 years), and 38 YC (aged 18–40 years, mean age 30 years; 20 males, 18 females) from hospital workers. Patients with acute complications, such as rupture, infection, renal failure and those undergoing fibrinolytic therapy were excluded from this study. Persons who suffered from anemia, diabetes mellitus, thrombocytopenia (platelet count below $15 \times 10^9/l$) and those with a past history of ischemic heart disease or cerebral vascular disease were also excluded. The patients were divided into two groups and split at the lower limit of the normal range for platelet count, group A (platelet count more than $150 \times 10^9/l$; $n = 36$) and group B (platelet count below $150 \times 10^9/l$; $n = 18$), because the lower limit of the normal range for the platelet count in the Sysmex Corporation is $150 \times 10^9/l$.

After informed consent had been obtained, venous blood samples were taken from the patients and the controls in the morning into 7.5% edetic acid (EDTA) for the measurement of the platelet index, and into 3.2% trisodium citrate for D-dimer and TAT measurement. The platelet count, MPV, platelet-large cell ratio (P-LCR) and platelet distribution width (PDW) were calculated using a blood cell analyzer (E-4000; Sysmex Corporation, Kobe, Japan). P-LCR is the percentage of large platelets, defined as the platelet volume exceeding 12 fl. Plasma D-dimer and TAT were assayed by ELISA using assay kits (Dimer test EIA, AGEN, Australia; Enzygnost TAT, Behringwerke, Germany). The samples for platelet indexes were kept at room temperature and measured within 3 h; for D-dimer and TAT, aliquots of platelet poor plasma were stored in plastic tubes at -80°C until measurement. The values of the indexes were expressed as the mean $\pm$ standard deviation. Significances of individual differences were evaluated using the Scheffé test if ANOVA was significant. Statistical differences between the two groups were assessed using the Mann-Whitney U test. Correlations between the variables were determined by calculating Spearman's rank correlation coefficients. 'Group A', 'group B' and AC were compared with respect to the platelet indexes. Statview 5 for Macintosh was used for all statistics. Differences were considered significant at $p < 0.05$.

Results

Platelet Indexes in AC and YC

The mean values for platelet count of AC and YC were not different ($236.1 \pm 52.9 \times 10^9/l$ and $223.9 \pm 57.9 \times 10^9/l$, respectively). MPV and PDW were larger in AC than in YC, but the difference was not significant (10.42 ± 0.79 vs. 10.27 ± 0.48 fl and 11.90 ± 1.45 vs. 11.52 ± 0.83 fl, respectively). However, P-LCR was significantly higher ($p = 0.0113$) in AC ($27.48 \pm 6.50\%$) than in YC ($24.25 \pm 5.53\%$). The platelet indexes were not different between males and females in AC.

Table 1. Mean platelet indexes in patients with AA and AC

	Median age ^a years	Patient number ^b	PLT $\times 10^9/l$	MPV fl	P-LCR %	PDW fl
AC	72 (61.5–82.5)	120 (59/61)	236.1 ± 52.9	10.42 ± 0.79	27.48 ± 6.50	11.9 ± 1.45
Group A	74 (64.5–83.5)	36 (6/30)	247.1 ± 71.5	9.61 ± 0.80	21.43 ± 6.22	10.6 ± 1.39
Group B	75 (67.5–82.5)	18 (5/13)	122.0 ± 25.4	10.49 ± 1.07	28.94 ± 8.19	12.4 ± 2.06
Total AA	75 (66.7–83.3)	54 (11/43)	202.2 ± 83.5	10.00 ± 1.01	24.59 ± 7.90	11.40 ± 1.83

^a Interquartile range is given in parentheses.

^b Female/male ratio is given in parentheses.

Platelet Indexes in AC and AA Patients

The mean platelet index values of AC were $236.1 \pm 52.9 \times 10^9/l$ for platelet count, 10.42 ± 0.79 fl for MPV, $27.48 \pm 6.50\%$ for P-LCR, and 11.90 ± 1.45 fl for PDW. The mean values of 54 AA patients were $202.2 \pm 83.5 \times 10^9/l$ for platelet count, 10.00 ± 1.01 fl for MPV, $24.59 \pm 7.90\%$ for P-LCR and 11.40 ± 1.83 fl for PDW. The mean values of group A were $247.1 \pm 71.5 \times 10^9/l$ for platelet count, 9.61 ± 0.80 fl for MPV, $21.43 \pm 6.22\%$ for P-LCR and 10.68 ± 1.39 fl for PDW. The mean values of group B were $122.0 \pm 25.4 \times 10^9/l$ for platelet count, 10.49 ± 1.07 fl for MPV, $28.94 \pm 8.19\%$ for P-LCR and 12.47 ± 2.06 fl for PDW (table 1). The platelet count in group B was significantly lower ($p < 0.0001$) than that in group A and in AC. MPV ($p = 0.0024$ and <0.0001 , respectively), P-LCR ($p = 0.0012$ and <0.0001 , respectively) and PDW ($p = 0.0006$ and 0.0005 , respectively) in group A were significantly lower than those in group B and AC. The platelet indexes in the 54 AA patients were significantly lower than those in AC ($p = 0.0005$, 0.0008 , 0.0031 , 0.0093 , respectively). There were no differences between group B and AC in MPV, P-LCR and PDW (table 2).

Correlations among Platelet Indexes

There were significantly inverse relationships between the platelet count and other indexes in the 54 AA patients (MPV: $\rho = -0.271$, $p = 0.0486$; P-LCR: $\rho = -0.287$, $p =$

0.0367 ; PDW: $\rho = -0.355$, $p = 0.0098$), and in AC (MPV: $\rho = -0.294$, $p = 0.0013$; P-LCR: $\rho = -0.298$, $p = 0.0012$; PDW: $\rho = -0.341$, $p = 0.0002$); however, no significant relationships were observed in group A, B and YC, as shown in table 3. There were strong relationships among MPV, P-LCR and PDW in the four groups.

D-Dimer and TAT in the Patients and the AC

D-dimer levels of group B, A and AC were $1,732.3 \pm 2,447.2$, 679.3 ± 518.2 and 111.8 ± 48.3 ng/ml ($n = 91$), respectively, being significantly higher in group B than in group A and AC ($p = 0.0003$ and <0.0001 , respectively). The TAT levels of group B, A and AC were 26.7 ± 19.0 , 13.5 ± 10.3 , and 3.79 ± 6.28 ng/ml ($n = 88$), respectively, being significantly higher in group B than in group A and AC ($p = 0.0001$ and <0.0001 , respectively).

Discussion

In the present study, P-LCR in AC was significantly higher than that in YC. MPV, P-LCR and PDW were significantly lower in group A than in group B and AC. There were no differences between group B and AC with respect to MPV, P-LCR and PDW. The platelet indexes were significantly lower in the 54 AA patients than in AC. Platelet count was significantly inversely correlated with MPV, P-LCR and PDW in AC and in the 54 AA patients. However, in YC these correlations were not significant. In group A and B, such correlations were not observed. Such findings had not been reported before.

Balduini et al. [3] reported increased MPV in unruptured AA (10.8 ± 1.0 fl) using NE-8000 (Sysmex Corporation), which was different to our results. However, our results showed that in 87 patients with angina pectoris who underwent coronary angiography, MPV was almost the same as that in the AA patients of Balduini et al. and in our AC (data not shown). Increased MPV was reported

Table 2. Significances of individual differences between the patient groups and AC

	PLT	MPV	P-LCR	PDW
Group A vs. group B	<0.0001	0.0024	0.0012	0.0006
Group A vs. AC	NS	<0.0001	<0.0001	0.0005
Group B vs. AC	<0.0001	NS	NS	NS
Total AA vs. AC	0.0005	0.0008	0.0031	0.0093

Table 3. Correlation coefficient between the platelet count and other platelet indexes in patients with AA and the controls

	n	MPV		P-LCR		PDW	
		ρ	p	ρ	p	ρ	p
AC	120	-0.295	0.0013	-0.298	0.0012	-0.341	0.0002
YC	38	-0.122	0.495	-0.137	0.446	-0.235	0.191
Group A	36	-0.022	0.895	0.027	0.875	-0.011	0.510
Group B	18	0.044	0.856	0.042	0.861	-0.038	0.876
Total AA	54	-0.271	0.0486	-0.287	0.0368	-0.355	0.0098

in patients with angina pectoris [4]. Thus, it is suggested that smaller platelets than in the controls or in angina pectoris exist in the circulation of AA patients.

Larger platelets have been thought to be young platelets because it had been suggested previously that platelets decrease in size as they age in the circulation [5]. However, there are controversial reports about platelet aging and platelet size [6]. The significance of platelet heterogeneity under various pathophysiological conditions is not yet fully understood.

There is a significant inverse relationship between MPV and platelet count in healthy volunteers [7]. It has been considered that platelet production is regulated to maintain a constant functional platelet mass, giving a constant hemostatic potential [8]. We showed the same relationships in AC and in the 54 AA patients in the present study. During stimulated thrombopoiesis, there was an increase both in the platelet count and in platelet volume. Platelets produced under such conditions, so-called 'stress' platelets, show an increase in the MPV compared with the normal platelets [9]. Large platelets are metabolically and enzymatically more active than small platelets, as assessed by *ex vivo* aggregometry [10, 11]. Irrespective of certain remaining controversies regarding the origin of platelet size heterogeneity, there is agreement that stress platelets are larger than steady-state platelets and have greater functional capacity than smaller platelets. A similar situation is seen in acute coronary syndromes [1]. However, it was also reported that MPV does not increase in stable coronary artery disease [12].

Thrombus formation is responsible not only for the acute manifestation of atherosclerosis, but also for the continuous progression of atheroma [13]. Increased larger platelets in AC of the present study might possibly be a result of age-related atherosclerosis, possibly entailing chronic platelet consumption at the site of the atherosclerotic plaque. Thrombus formation is generally strong in patients with AA, as reflected by elevated D-dimer and TAT [14, 15], especially in group B. The increase in platelet size was accompanied by a decrease in platelet count, and the life span of 'stress' platelets will be remarkably shortened and thus, as a compensatory response, thrombopoiesis may be strongly accelerated leading to the formation of larger platelets. Although this phenomenon was present in AA patients, the size was significantly smaller than that in AC in this study. In the group A patients, although accelerated thrombopoiesis could maintain the platelet count within the normal range, the larger platelets will decrease in number due to consumption. However, in the group B patients, the excess platelet consumption

might possibly be due to chronic disseminated intravascular coagulation, as reflected in the remarkably elevated the D-dimer and TAT, ultimately entailing the fact that platelet count could not be maintained within the normal range. This is based on the assumption that platelets of all sizes would equally be prone to consumption. This is the reason for decreased platelet count and the normal platelet size.

The limitations of this study are its retrospective nature, the limited number of patients and the lack of detailed and documented clinical findings (such as aneurysmal size and complications) which could, therefore, not be correlated with the platelet parameters. However, we were able to estimate the aneurysmal size and volume by measuring D-dimer and TAT [15].

In conclusion, the platelet indexes decreased rather than increased when the platelet count was within the normal range in the group A patients, and were within the normal range when the platelet count decreased in the group B patients. This study is, to our knowledge, the first to study platelet heterogeneity of AA.

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References

- 1 Endler G, Klimesch A, Sunder-Plassmann H, Schillinger M, Exner M, Mannhalter C, Jordanova N, Christ G, Thalhammer R, Huber K, Sunder-Plassmann R: Mean platelet volume is an independent risk factor for myocardial infarction but not for coronary artery disease. *Br J Haematol* 2002;117:399–404.
- 2 Mathur A, Robinson MS, Cotton J, Martin JF, Erusalimsky JD: Platelet reactivity in acute coronary syndromes: evidence for differences in platelet behaviour between unstable angina and myocardial infarction. *Thromb Haemost* 2001;85:989–994.
- 3 Balduini CL, Salvini M, Montani N, Noris P, Sepedini P, Belletti S, Gamba G: Activation of the hemostatic process in patients with unruptured aortic aneurysm before and in the first week after surgical repair. *Haematologica* 1997;82:581–583.
- 4 Pizzulli L, Yang A, Martin JF, Luderitz B: Changes in platelet size and count in unstable angina compared to stable angina or non-cardiac chest pain. *Eur Heart J* 1998;19:80–84.
- 5 Thompson CB, Love DG, Quinn PG, Valeri CR: Platelet size does not correlate with platelet age. *Blood* 1983;62:487–494.
- 6 Levin J, Bessman JD: The inverse relationship between platelet volume and platelet number. *J Lab Clin Med* 1983;101:295–307.
- 7 Thompson CB, Jakubowski JA: The pathophysiology and clinical relevance of platelet heterogeneity. *Blood* 1998;72:1–8.
- 8 Tong M, Seth P, Penington DG: Proplatelets and stress platelets. *Blood* 1987;59:522–528.
- 9 Karparkin S, Strick N: Heterogeneity of human platelets. V. Differences in glycolytic and related enzymes with possible relation to platelet age. *J Clin Invest* 1972;51:1235–1243.
- 10 Corash L, Tau H, Gralnick HR: Heterogeneity of human whole blood platelet subpopulations. I. Relationship between buoyant density, cell volume, and ultrastructure. *Blood* 1977;49:71–87.
- 11 van der Loo B, Martin JF: A role for changes in platelet production in the cause of acute coronary syndromes. *Arterioscler Thromb Vasc Biol* 1999;19:672–679.
- 12 Haflbmayer WM, Haushofer A, Radek J, Schon R, Deutsch M, Fischer M: Platelet size, fibrinogen and lipoprotein (a) in coronary heart disease. *Coron Artery Dis* 1995;6:397–402.
- 13 Nachman RL: Thrombosis and atherogenesis: molecular connections. *Blood* 1992;79:1897–1906.
- 14 Ihara A, Kawamoto T, Matsumoto K, Kawamoto J, Katayama A, Yoshitatsu M, Izutani H, Ihara K: Relationship between hemostatic markers and circulating biochemical markers of collagen metabolism in patients with aortic aneurysm. *Pathophysiol Haemost Thromb* 2003;33:221–224.
- 15 Nomura F, Ihara A, Yoshitatsu A, Tamura K, Katayama A, Ihara K: Relationship between coagulation cascade, cytokine, adhesion molecule and aortic aneurysm. *Eur J Cardiothorac Surg* 2003;23:1034–1039.