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Platelet Aggregation is not Altered in Men with Aortic Aneurysms

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Abbreviations
Abdominal aortic dilation (AAD)
Adenosine diphosphate (ADP)
Arachidonic acid (AA)
Ascending thoracic aortic dilation (ATAD)
Interquartile range (IQR)
Protease-activated receptor 4 (PAR-4)
Thrombin receptor-activating peptide (TRAP)

Introduction

Platelets play an important role in arterial diseases that extends beyond their traditional function in hemostasis and thrombosis(1). The development and progression of ascending thoracic aortic dilations (ATAD) and abdominal aortic dilations (AAD) have been associated with increased platelet activation response(1, 2). Consequently, platelets have been suggested as potential medical targets to slow expansion rates and reduce risk of ruptures.

This study aimed to evaluate if increased in-vitro platelet aggregation and hyperresponsiveness of platelets were associated with the presence of ATAD and AAD, and to estimate potential correlations between platelet activation response and ascending and abdominal aortic diameters, respectively.

Methods

In the population-based DANCAVAS trial (Danish Cardiovascular Multicenter Screening Trial), 45,000 men aged 65-74 years were randomized 1:2 to undergo screening or to participate passively as controls(3). At screening, participants underwent medical history interviews, electrocardiogram-gated non-contrast computed tomography scans, blood-pressure readings on upper and lower extremities, and blood samplings. Blood samplings subsequently used for platelet studies were obtained using 3.2% (105 mM) trisodium citrate. The DANCAVAS trial was approved by the Regional Scientific Ethical Committee of Southern Denmark (S-20140028, 2014).

In this study, 566 consecutively enrolled men (September 2016 to January 2017) with platelet counts >$100x10^9$/L and no use of platelet inhibitors had 96-well light transmission platelet aggregometry performed, as previously described(4). Platelets were stimulated with the following agonists (concentrations per well): thrombin receptor-activating peptide (TRAP)(2.5, 5.0, and 10.0 μM), adenosine diphosphate (ADP)(0.55, 1.10, and 6.40 μM), collagen type 1 (0.2, 0.4, and 6.4

μg/mL), arachidonic acid (0.03, 0.06, and 0.50 mM), and protease-activated receptor (PAR)-4 agonist (AYPGKF-NH_2, 12.5, 25.0, and 100.0 μM), respectively. Platelet aggregation was estimated after 2 and 10 minutes and reported as percentages relative to optical densities (595 nm) in platelet-rich or platelet-poor plasma.

In the primary analyses, ATAD was defined as ≥40 mm and AAD as ≥25 mm in diameter, respectively(5). The ascending and abdominal aortic diameters were measured anterior-posteriorly at the first circular level above the sinotubular junction and just above the bifurcation, respectively(3, 5). In sensitivity analyses, the thresholds for ascending and abdominal aortic aneurysms were increased to ≥45 mm and ≥30 mm, respectively. Hyperresponsiveness of platelets was defined as ≥50% platelet aggregation at low or medium agonist concentration levels(4).

Comparison between groups were tested with the Mann Whitney U-test and the chi-squared test, as appropriate. Univariate correlation between ascending and abdominal aortic diameters and platelet aggregation were estimated using Spearman's rho, respectively. In multivariate linear regression analyses, correlations between the aortic diameters and logarithmic-transformed platelet aggregations were adjusted for age, smoking, hypertension, and diabetes. Bonferroni corrected p-values <0.003 were considered statistically significant. All analyses were performed with STATA/IC 16.1 (StataCorp LLC, College Station, Texas).

Results

Of the 566 included men, the median age was 69 years (interquartile range (IQR): 67-71) and platelet count was median 229x10^9/L (IQR: 204-265). The mean ascending and abdominal aortic diameters were 37.3 ±3.9 mm and 20.3 ±4.2 mm, respectively. Prevalence of ATAD and AAD were 24.4% (n=138) and 8.8% (n=50), respectively. Overall, platelet aggregation increased with all agonists dose-dependently.

No differences were observed for platelet aggregation between the agonists at any concentration level for neither ATAD nor AAD compared to controls (Table 1), respectively. Increasing the thresholds for ascending (n=17) and abdominal (n=18) aortic aneurysms made no essential differences.

No differences in frequencies of platelet hyperresponsiveness were observed for any agonists between cases of ATAD (frequency range: 53.0%-85.6%) and controls (frequency range: 47.5%-85.8%)(*P*-values: 0.29-0.95) or AAD (frequency range: 44.2%-87.7%) and controls (frequency range: 49.2%-85.6%)(*P*-values: 0.40-0.78), respectively. Frequencies of platelet hyperresponsiveness in the sensitivity analyses were equally non-significantly different between aneurysmal and none-aneurysmal men.

No correlations between aortic diameters and platelet aggregation at lowest agonist levels were observed for the ascending aorta (Spearman's rho range: 0.06-0.11, *P*-values: 0.09-0.28) and the abdominal aorta (Spearman's rho range: -0.18-0.00, *P*-values: 0.07-0.94), respectively. Adjusting for previously mentioned confounders made no noteworthy changes.

Discussion

In this large study based upon well-characterized men from the general Danish population with detailed phenotyped platelet information, we did not find higher platelet aggregation or increased frequencies of hyperresponsiveness in men with dilated and aneurysmal ascending and abdominal aortas. Additionally, no correlations between platelet activation response and increasing ascending and abdominal aortic diameters were observed.

Previous studies suggesting potential associations between aortic dilations and elevated platelet activation responses either failed to examine platelet function, had limited sample-sizes with numerous unadjusted tests, or only observed significant associations in Marfan patients(1, 2).

The presented study was limited by only relying on data from men, and few cases of large aortic aneurysms were included. Also, non-classical hemostatic functions were not tested, i.e., functions of significance for hemostasis during inflammation and angiogenesis.

In conclusion, this study suggests that other medical agents than platelet inhibitors should be prioritized in the hunt for effective medical treatment of the dilated ascending and abdominal aorta.

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Table 1: Platelet aggregation in 65–74-year-old men not receiving platelet inhibitors with and without ascending and abdominal aortic dilations

	ATAD (N = 138)	**No ATAD (N = 428)**	***P***	**AAD (N = 50)**	**No AAD (N = 516)**	***P***
Agonist (concentration)	Platelet aggregation [% (IQR)]			Platelet aggregation [% (IQR)]		
TRAP (10.0 µM)	71.7 (60.4-77.2)	69.8 (59.8-75.7)	0.23	71.1 (62.6-74.9)	70.3 (59.1-76.3)	0.97
TRAP (5.0 µM)	73.8 (59.1-81.3)	71.9 (57.5-80.3)	0.53	73.8 (61.7-79.6)	72.4 (57.4-81.0)	0.77
TRAP (2.5 µM)	57.6 (40.4-72.2)	54.9 (34.9-70.8)	0.31	56.8 (36.7-68.2)	55.6 (36.1-72.2)	0.52
ADP (6.4 µM)	71.2 (63.8-78.4)	72.3 (64.8-77.1)	0.78	71.6 (66.1-78.2)	72.1 (64.7-77.5)	0.87
ADP (1.1 µM)	69.3 (49.4-80.3)	66.7 (48.1-76.7)	0.25	65.3 (44.7-77.2)	67.4 (49.3-77.2)	0.67
ADP (0.55 µM)	54.9 (40.3-71.0)	51.5 (33.7-69.6)	0.19	50.1 (36.8-71.6)	53.1 (34.4-70.0)	0.94
Collagen (6.4 µg/mL)	66.0 (49.8-75.0)	67.6 (50.6-76.2)	0.51	70.1 (45.2-75.8)	67.2 (51.5-76.1)	0.80
Collagen (0.4 µg/mL)	42.5 (29.1-64.4)	38.9 (26.8-64.0)	0.41	41.8 (27.7-51.7)	40.3 (27.1-65.4)	0.67
Collagen (0.2 µg/mL)	43.6 (26.4-71.4)	39.1 (23.0-61.1)	0.17	45.7 (27.5-58.4)	39.1 (23.0-65.6)	0.72
AA (0.5 mM)	77.6 (66.8-84.9)	77.2 (67.3-82.6)	0.48	76.1 (66.5-82.8)	77.5 (67.3-83.4)	0.34
AA (0.06 mM)	46.7 (27.8-72.1)	43.1 (25.3-73.1)	0.35	46.8 (28.1-71.0)	44.2 (25.6-73.3)	0.72

AA (0.03 mM)	37.2 (21.5-71.3)	36.8 (19.2-65.7)	0.16	36.3 (22.5-61.0)	37.2 (19.3-67.3)	0.93
PAR-4 (100.0 µM)	79.2 (73.2-85.2)	80.8 (70.7-85.7)	0.68	79.0 (70.0-84.2)	80.7 (71.6-85.7)	0.96
PAR-4 (25.0 µM)	66.2 (41.0-83.0)	65.1 (42.3-82.3)	0.99	62.4 (37.5-81.2)	65.6 (42.4-82.6)	0.44
PAR-4 (12.5.0 µM)	37.8 (21.1-68.0)	33.7 (17.5-55.8)	0.05	37.5 (20.6-51.1)	34.1 (17.9-60.0)	0.85

Values are presented as %-platelet aggregation reported as median (25-75% interquartile ranges). P-values test for differences between dilated cases and none-dilated controls.

Arachidonic acid (AA), Abdominal aortic dilation (AAD), Adenosine diphosphate (ADP), Protease-activated receptor 4 (PAR-4), Ascending thoracic aortic dilation (ATAD), Thrombin receptor-activating peptide (TRAP)