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Association of atherogenic serum lipids and platelet activation with changes in arterial stiffness in patients with type 2 diabetes

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Abstract: Pulse wave velocity (PWV) is a potential marker for atherosclerosis severity and/or predictor of future atherosclerotic cardiovascular events. PWV is significantly correlated with carotid-intimal media thickness in patients with diabetes. However, its significance as a surrogate marker for the treatment of atherosclerotic cardiovascular risk in the management of type 2 diabetes has not been fully established. To elucidate the factors that determine the improvement or deterioration of PWV, we studied the association of clinical parameters, parameters for glucose metabolism, serum lipids including each lipoprotein fraction, serotonin as a marker for platelet activation, and change in PWV in 54 patients with type 2 diabetes. Systolic blood pressure and serum levels of non-high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol (LDL-C), and intermediate-density lipoprotein-cholesterol significantly decreased in the PWV-improved group after 2 months compared with those in the PWV-deteriorated group. The serotonin levels at baseline were significantly lower in the PWV-improved group than in the PWV-deteriorated group. The changes in systolic blood pressure and LDL-C levels were significantly and positively correlated with those in PWV. The serotonin levels at baseline were significantly and positively correlated with changes in PWV. Therefore, amelioration of blood pressure, serum lipid level, and platelet activation might be beneficially associated with PWV change. PWV-guided clinical practice for cardiovascular risk stratification could be useful in type 2 diabetes management.

***Keywords*:** atherosclerosis, diabetes, lipoprotein, pulse wave velocity, serotonin

Introduction

Type 2 diabetes and dyslipidemia are significant cardiovascular risk factors that should be managed (*1,2*). Therefore, it is important to regulate atherosclerotic risk factors and understand the progression of arteriosclerosis in patients with type 2 diabetes. Aortic pulse wave velocity (PWV) is an independent predictor of adverse cardiovascular events, including mortality (*3*). PWV reflects arterial stiffness and correlates with markers reflecting the severity of atherosclerosis, such as carotid intima-media thickness (IMT) (*4*). PWV is thought to be applicable as a marker for the severity of atherosclerosis and/or predictor of future atherosclerotic cardiovascular events (*4*). PWV has been significantly correlated with carotid IMT in patients with diabetes (*5,6*). Previous studies have reported that PWV can be beneficial in the clinical practice for cardiovascular risk stratification (*7,8*). However, its significance as a surrogate marker for the treatment of atherosclerotic cardiovascular risk in the management of type 2 diabetes has not been fully established.

Elevated of levels of atherogenic lipids, such as triglycerides (TG), low-density lipoprotein (LDL)-cholesterol (LDL-C), small dense LDL, and oxidized LDL as well as reduction of anti-atherogenic high-density lipoprotein (HDL)-cholesterol (HDL-C) level were observed in type 2 diabetes (*9*). Recently, attention has been focused on the important contribution of TG-rich lipoproteins, such as very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) to atherogenesis (*10*).

Serotonin is released from aggregating platelets and mediates platelet-induced vasoconstriction (*11*). In patients with diabetes, platelets are activated in endothelial injury promoted by atherosclerosis, and platelets are hyperreactive with intensified adhesion and aggregation (*12,13*). Activated platelets release serotonin stored in platelet-dense granules (*13*). It was previously reported that serotonin levels in platelet-poor plasma (PPP) increased in patients with coronary heart disease (CHD) (*14-16*). Platelet aggregatory response to serotonin is modulated by the disparate effects of lipoprotein fractions, corresponding to the recognized differences in the degree of atherogenicity of LDL and HDL (*11*). Amplification of serotonin-induced platelet

aggregation by LDL and its inhibition by HDL support the hypothesis that serotonin-mediated effects represent a mechanism clinically relevant to chronic progression of atherosclerosis (*11*).

To elucidate the factors that determine the improvement or deterioration of PWV, we studied the association of clinical parameters, parameters for glucose metabolism, serum lipids including each type of lipoprotein fraction, and serotonin level with the change in PWV.

Patients and Methods

A total of 54 patients with type 2 diabetes were recruited from the outpatient clinics in Kohnodai Hospital, National Center for Global Health and Medicine. We measured PWV, parameters for glucose metabolism, serum lipid profile, including each lipoprotein-cholesterol, and PPP serotonin at baseline and after 2 months. At 2 months, the treatments for diabetes, dyslipidemia, and hypertension were not changed intentionally. This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Medical Ethics Committee of the National Center for Global Health and Medicine (NCGM-G-000889). Written informed consent was obtained from each patient.

PWV was measured using a noninvasive vascular device with four pneumatic pressure cuffs (BP-203RPE, Omron Corp., Japan). Blood pressure levels and pulse waves were measured in the bilateral brachial and radial arteries after 5 min of rest on the bed. PWV was calculated as the transmission time and distance from the right or left arm to each ankle based on the body height. The mean value of the right and left PWV was used for analysis in this study.

Fasting plasma glucose, hemoglobin A1c (HbA1c), C-peptide, and small dense LDL levels were measured using commercial kits (HLC-723G and GA, Tosoh Corp., Japan; Sekisui Medical Co., Ltd., Japan; and Denka Seiken Co., Ltd., Japan, respectively).

The analysis method for lipoprotein profile using anion-exchange high-performance liquid chromatography (AEX-HPLC) has been previously reported (*17,18*). Briefly, major lipoprotein classes, HDL, LDL, IDL, and VLDL, in the plasma samples were separated using AEX-HPLC, and the cholesterol levels in the separated lipoprotein classes were measured using post-column reaction with a reagent containing cholesterol esterase and cholesterol oxidase. AEX-HPLC can be used as a substitute for ultracentrifugation. The PPP serotonin levels were measured using a previously reported method (*19*). In brief, PPP serotonin was separated in a column-switching system with two octadecyl-bonded silica columns. The separated serotonin was specifically converted into a fluorescent derivative with benzylamine and detected sensitively and quantitatively.

Data are presented as mean ± standard deviation. The Wilcoxon test was performed to compare the values at baseline and that after 2 months. The Mann-Whitney test and Fisher's exact probability test were performed to analyze the differences in data between the two groups. Correlations were estimated using the Spearman's rank test. Statistical significance was set at *P*-values of < 0.05.

Results

The clinical characteristics of patients with type 2 diabetes are summarized in Table 1. The mean body mass index and waist circumference were > 25 kg/m^2 and 0.9 m, respectively, suggesting that a relatively greater proportion of overweight and obese patients were included. Most patients were treated with insulin or oral anti-diabetic drugs. Approximately 60% and 70% of patients had used hypolipidemic and hypotensive drugs, respectively.

PWV and clinical and metabolic parameters at baseline and after 2 months of patients with type 2 diabetes are shown in Table 2. During the study period of 2 months, no intentional change in drugs was performed. No parameters, including PWV, showed significant changes. To determine the factors that improve or deteriorate PWV, we divided patients into PWV-improved (decreased) and PWV-deteriorated

Table 1.Clinical characteristics of studied patients with type 2 diabetes (*n* = 54)

Variables	Values
Age (years)	65.3 ± 11.1
Sex (male/female)	32/22
Smoker (smoker/non-smoker)	19/35
Duration for diabetes (years)	8.78 ± 9.72
Body mass index (kg/m^2)	25.6 ± 4.9
Waist circumference (m)	0.91 ± 0.12
Systolic blood pressure (mmHg)	141 ± 21
Diastolic blood pressure (mmHg)	80 ± 11
Pulse wave velocity (cm/sec)	1,774 ± 380
Anti-diabetic treatments (*n*)	
Insulin	10
Sulfonylurea	9
Glinides	5
Dipeptidyl peptidase-4 inhibitors	15
Thiazolidinedione	13
Metformin	31
a-glucosidase inhibitors	9
Hypolipidemic treatments (*n*)	
Statin	27
Fibrate	3
Ezetimibe	2
Anti-platelets treatments (*n*)	
Aspirin	5
Hypotensive treatments (*n*)	
Diuretics	1
Angiotensin converting enzyme inhibitors	2
Angiotensin II receptor blockers	19
b-blockers	2
Calcium antagonists	17

Table 2. Pulse wave velocity and metabolic parameters at baseline and after 2 months in patients with type 2 diabetes (*n* = 54)

Variables	Baseline	after 2 months	*P* value
Pulse wave velocity (cm/sec)	1,774 ± 380	1,777 ± 382	0.6421
Clinical parameters			
Body mass index (kg/m^2)	25.6 ± 4.9	25.5 ± 4.8	0.728
Waist circumference (m)	0.91 ± 0.12	0.91 ± 0.11	0.797
Systolic blood pressure (mmHg)	141 ± 21	139 ± 19	0.420
Diastolic blood pressure (mmHg)	80 ± 11	79 ± 10	0.115
Parameters for glucose metabolism			
Fasting plasma glucose (mg/dL)	135 ± 28	134 ± 26	0.436
Hemoglobin A1c (%)	7.2 ± 0.9	7.2 ± 0.8	0.781
Fasting serum C-peptide (ng/mL)	1.73 ± 0.7	1.92 ± 1.0	0.280
Serum lipids			
Triglyceride (mg/dL)	114 ± 47	112 ± 50	0.390
Non-HDL-C (mg/dL)	128 ± 26	123 ± 26	0.091
LDL-C (mg/dL)	116.3 ± 26.0	113 ± 25.3	0.240
IDL-C (mg/dL)	6.6 ± 2.1	6.7 ± 2.6	0.607
VLDL-C (mg/dL)	12.1 ± 7.3	11.1 ± 7.0	0.155
HDL-C (mg/dL)	58.3 ± 16.0	58.1 ± 15.3	0.904
Small-dense LDL (mg/dL)	27 ± 11	27 ± 12	0.801
Oxidized LDL (U/mL)	92 ± 28	95 ± 24	0.740
Serotonin levels			
PPP serotonin (nmol/L)	20.9 ± 19.6	22.7 ± 17.2	0.196

Abbreviations: Non-HDL-C, non-high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; IDL-C, intermediate-density lipoprotein-cholesterol; VLDL-C, very-low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; PPP, platelet-poor plasma.

Table 3. Differences in clinical characteristics, parameters for glucose metabolism, serum lipids and serotonin between PWV-improved group and PWV-deteriorated group

Variables	PWV-improved group (*n* = 25)			PWV-deteriorated group (*n* = 28)			Comparison between 2 groups	
	Baseline	after 2 months	*P* values *vs*. baseline	Baseline	after 2 months	*P* values *vs*. baseline	*P* values at baseline	*P* values after 2 months
Clinical parameters								
Body mass index (kg/m^2)	26.1 ± 5.4	26.1 ± 5.1	0.379	25.2 ± 4.5	24.9 ± 4.5	0.223	0.521	0.368
Waist circumference (m)	0.92 ± 0.13	0.92 ± 0.13	0.346	0.91 ± 0.1	0.90 ± 0.1	0.288	0.726	0.368
Systolic blood pressure (mmHg)	140 ± 21	135 ± 17	0.044	141 ± 21	143 ± 20	0.235	0.470	0.076
Diastolic blood pressure (mmHg)	79 ± 10	77 ± 8	0.053	81 ± 12	80 ± 11	0.392	0.762	0.184
Treatments								
Calcium antagonists (*n*)	12	12	NA	5	5	NA	0.020	0.020
Parameters for glucose metabolism								
Fasting blood glucose (mg/dL)	127 ± 23	133 ± 23	0.029	141 ± 30	135 ± 29	0.197	0.149	0.817
Hemoglobin A1c (%)	7.1 ± 0.8	7.1 ± 0.7	0.354	7.3 ± 1.0	7.2 ± 0.8	0.489	0.438	0.464
Fasting serum C-peptide (ng/mL)	1.9 ± 0.8	2.1 ± 1.2	0.173	1.6 ± 0.6	1.7 ± 0.9	0.308	0.250	0.107
Serum lipids								
Triglyceride (mg/dL)	112 ± 34	115 ± 56	0.495	116 ± 56	108 ± 47	0.127	0.728	0.669
Non-HDL-C (mg/dL)	140 ± 26	129 ± 28	0.014	117 ± 21	118 ± 22	0.400	0.001	0.086
LDL-C (mg/dL)	128.6 ± 26.6	118.2 ± 28.0	0.016	104.9 ± 20.5	107.4 ± 22.5	0.233	0.001	0.139
IDL-C (mg/dL)	6.6 ± 1.4	6.1 ± 1.7	0.010	6.6 ± 2.5	6.9 ± 2.9	0.239	0.510	0.392
VLDL-C (mg/dL)	11.1 ± 4.3	11.3 ± 7.3	0.404	12.8 ± 9.3	10.8 ± 7.0	0.055	0.880	0.715
HDL-C (mg/dL)	58.8 ± 14.9	55.8 ± 13.7	0.069	58.8 ± 17.4	60.3 ± 16.7	0.052	0.748	0.285
Small-dense LDL (mg/dL)	31.5 ± 10.7	30.6 ± 13.0	0.295	23.1 ± 9.1	25.0 ± 10.3	0.135	0.001	0.024
Oxidized LDL (U/mL)	97 ± 27	99 ± 24	0.371	88 ± 28	91 ± 18	0.193	0.240	0.247
Serotonin levels								
PPP serotonin (nmol/L)	14.7 ± 9.3	19.0 ± 10.5	0.038	26.2 ± 24.3	23.6 ± 19.2	0.477	0.032	0.466

Abbreviations: Non-HDL-C, non-high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; IDL-C, intermediate-density lipoprotein-cholesterol; VLDL-C, very-low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; PPP, platelet-poor plasma; NA, not applicable.

(increased) groups and analyzed the differences between both groups. Table 3 shows the differences in clinical parameters, parameters for glucose metabolism, serum lipid levels, and PPP serotonin levels between the PWV-improved and PWV-deteriorated groups.

Systolic blood pressure significantly decreased in the

PWV-improved group after 2 months. A significantly greater number of patients had used calcium antagonists at baseline and after 2 months in the PWV-improved group than in the PWV-deteriorated group. Fasting blood glucose levels significantly increased in the PWV-improved group. Serum levels of non-high-density lipoprotein-cholesterol (non-HDL-C), LDL-C, and IDL-cholesterol (IDL-C) significantly decreased after 2 months in the PWV-improved group. Serum non-HDL-C, LDL-C, and small-dense LDL levels at baseline and small-dense LDL levels after 2 months were significantly higher in the PWV-improved group than in the PWV-deteriorated group. PPP serotonin levels significantly increased in the PWV-improved group. However, PPP serotonin levels at baseline were significantly lower in the PWV-improved group than in the PWV-deteriorated group.

We analyzed the correlation of parameters that showed a significant change with change in PWV. The correlation of changes in systolic blood pressure and fasting blood glucose, non-HDL-C, LDL-C, IDL-C, and PPP serotonin levels with changes in PWV is shown in Figure 1. Changes in the systolic blood pressure and LDL-C levels were significantly and positively correlated with changes in PWV. However, changes in the fasting blood glucose, non-HDL-C, IDL-C, and PPP serotonin levels were not significantly correlated with changes in PWV.

The parameters for clinical characteristics, glucose metabolism, and serum lipids were not significantly correlated with PPP serotonin levels at baseline or after 2 months. Furthermore, changes in any parameters did not correlate with that in PPP serotonin levels. The correlations of PPP serotonin levels with PWV at baseline or after 2 months and with the change in PWV are shown in Figure 2. PPP serotonin levels at baseline were significantly and positively correlated with changes in PWV.

Discussion

Since 2001, PWV measurement has been applied for the risk stratification of patients with atherosclerotic cardiovascular disease and/or its risk factors in Japan (*4*). Several cross-sectional studies have demonstrated a significant correlation between PWV and known risk factors for cardiovascular diseases. The treatment of cardiovascular risk factors and lifestyle modifications have been shown to improve PWV (*4*).

Several meta-analyses have demonstrated that PWV reduces by antihypertensive treatments (*20-23*). A significant reduction in systolic blood pressure in the PWV-improved group and a significant and positive correlation between changes in systolic blood pressure and PWV suggest that a reduction in systolic blood pressure is beneficially associated with PWV.

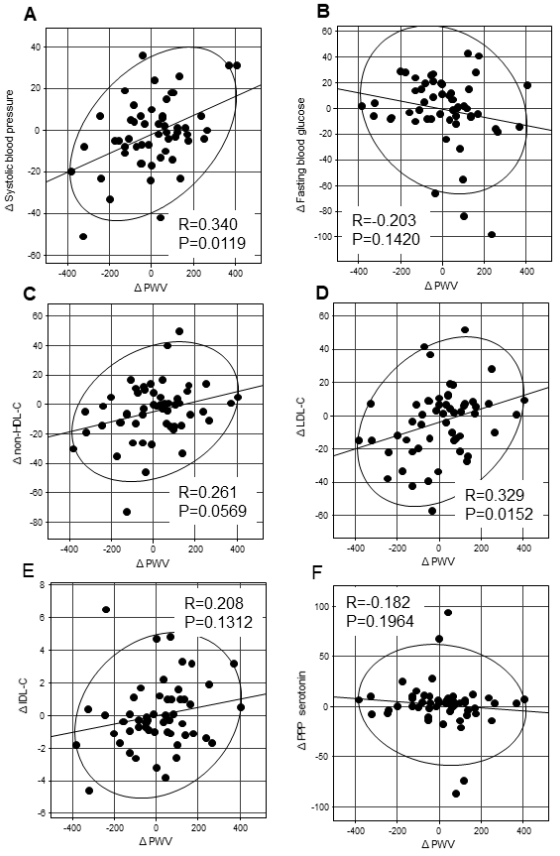

Figure 1. Correlation of changes in the systolic blood pressure (A) and fasting blood glucose (B), non-high-density lipoprotein-cholesterol (non-HDL-C) (C), low-density lipoprotein-cholesterol (LDL-C) (D), intermediate-density lipoprotein-cholesterol (IDL-C) (E), and platelet-poor plasma (PPP) serotonin (F) levels with the change in pulse wave velocity (PWV). R indicates the correlation coefficient.

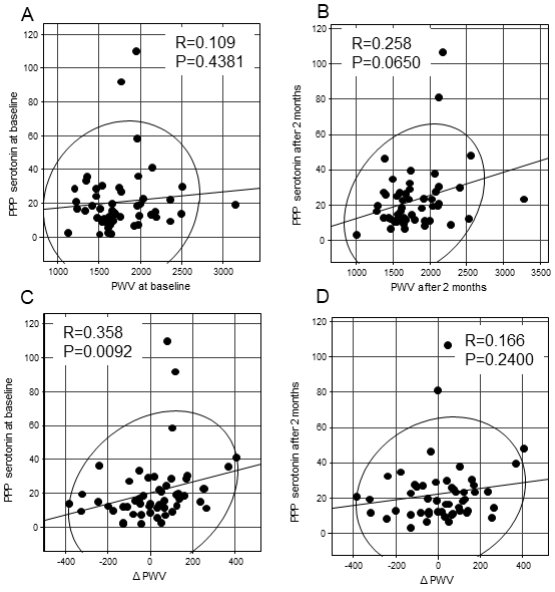

Figure 2. Correlations of the platelet-poor plasma (PPP) serotonin levels with pulse wave velocity (PWV) at baseline (A) and after 2 months (B) and with the change in PWV at baseline (C) and after 2 months (D). R indicates the correlation coefficient.

In a previous study with 4938 healthy adults, the sample was categorized into four groups using the 75 g oral glucose tolerance test (*24*). PWV increased in the following order: normal glucose tolerance, impaired fasting glucose, impaired glucose tolerance, and newly diagnosed diabetes (*24*). Another study showed that PWV increased in the order of normal subjects, patients with impaired fasting glucose, and patients with diabetes and was positively correlated with fasting blood glucose and HbA1c levels (*25*). Although fasting blood glucose levels significantly increased in the PWV-improved group, fasting blood glucose levels at baseline or after 2 months in the PWV-improved group were lower than those in the PWV-deteriorated group. Furthermore, no significant correlation between changes in fasting blood glucose levels and PWV was observed. In our patients, fasting blood glucose levels may not be associated with the determination of PWV.

Atherogenic dyslipidemia comprises a triad of increased blood small dense LDL, decreased HDL, and TG levels (*26*). As a typical feature of type 2 diabetes, atherogenic dyslipidemia has emerged as an important coronary risk factor. The Framingham Heart Study (2,693 men, 3,101 women) has shown that non-HDL-C level is a stronger predictor of CHD risk than LDL-C (*27*). An abundance of small dense LDL increases CHD risk by three-fold (*28*). The metabolic milieu associated with small dense LDL includes insulin resistance, increased IDL, increased susceptibility to oxidative damage, impaired reverse cholesterol transport, and increased postprandial lipemia (*28*). A significant decrease in non-HDL-C, LDL-C, and IDL-C levels was observed in the PWV-improved group compared with in the PWV-deteriorated group. This decrease might be beneficially associated with PWV. In our study, low-density LDL levels in the PWV-improved group at baseline or after 2 months were significantly higher than those in the PWV-deteriorated group. However, small dense LDL decreased in the PWV-improved group and increased in the PWV-deteriorated group, thereby diminishing the negative impact of high small dense LDL levels on PWV in the PWV-improved group. Otherwise, a significant reduction in non-HDL-C, LDL-C, and IDL-C levels might have diminished the negative impact of high small dense LDL levels on PWV in the PWV-improved group.

Accelerated atherosclerosis and increased risk of thrombotic vascular events in patients with diabetes may result from dyslipidemia, endothelial dysfunction, platelet hyperreactivity, impaired fibrinolytic balance, and abnormal blood flow. The importance of platelets in the atherothrombotic process has led to the investigation for the use of antiplatelet agents to reduce cardiovascular risks. A meta-analysis conducted by the Antiplatelet Trialists' Collaboration demonstrated that aspirin reduced the risk of ischemic vascular events as a secondary prevention strategy in numerous high-risk groups, including patients with diabetes (*29*). Activated platelets release serotonin stored in platelet-dense granules (*13*). In the present study, significantly lower PPP serotonin levels were observed in the PWV-improved group than in the PWV-deteriorated group, suggesting a significant contribution of serotonin to atherogenesis. Furthermore, the PPP serotonin levels at baseline were significantly and positively correlated with changes in PWV. The PPP serotonin levels have been reported to be increased in patients with CHD (*14-16*), supporting our results.

Our study showed that a significantly greater number of patients had used calcium antagonists at baseline and after 2 months in the PWV-improved group compared with that in the PWV-deteriorated group. Calcium antagonists have been reported to inhibit the amplifying effect of LDL on serotonin-induced platelet aggregation (*11*). In our study, the PPP serotonin levels in patients receiving calcium antagonists (n = 16, 15.7 ± 13.2 nmol/L) were lower than those in patients who were not receiving calcium antagonists (n = 36, 23.2 ± 21.6 nmol/L) (P = 0.057). Furthermore, PWV decreased in patients using calcium antagonists (n = 17, −57 ± 176 cm/s) but increased in patients who had not used calcium antagonists (n = 37, 37 ± 149 cm/s) (P = 0.053). This suggested a possible beneficial effect of calcium antagonists on PWV. A meta-analysis showed that calcium antagonists significantly reduced PWV compared with placebo in long-term trials (*20*). However, calcium antagonists did not show superiority in improving PWV compared with other types of antihypertensive drugs. Further studies are needed to elucidate the influence of antihypertensive drugs on PWV.

The Steno-2 study demonstrated that intensified multifactorial intervention with tight glucose regulation and the use of renin-angiotensin system blockers, aspirin, and lipid-lowering agents had sustained beneficial effects with respect to vascular complications and on mortality rates from any cause, including cardiovascular causes (*30*). While no parameters improved in the PWV-deteriorated group, four parameters were significantly improved in the PWV-improved group, suggesting that the improvement of multiple coronary risk factors led to an improvement in PWV.

This study has some limitations. First, the number of participants was small. Second, since this was an observational study, the causal relationship of the results could not be explained clearly. To elucidate the influence of metabolic parameters on PWV, a randomized controlled trial using interventions is recommended in the future.

In conclusion, a reduction in blood pressure; a decrease in the levels of atherogenic lipids, including non-HDL-C, LDL-C, and IDL-C; and lower levels of platelet activation might be beneficially associated with PWV. PWV-guided clinical practice for cardiovascular

risk stratification could be useful in the management of type 2 diabetes.

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