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奈良 信雄(編)

『疾患からまとめた病態生理 FIRST AID』

亀田総合病院リウマチ膠原病内科/東京大学・東京医科歯科大学・北里大学非常勤講師
岸本 暢将

日本の医学部にも精通するハワイ大学副医学部長Dr. Izutsuと日米の医学部教育についてディスカッションしたことがある. 日米の医学部教育の大きな違いは, 教育の目標が異なるということだそうである. 日本の医学部教育の目標は, ともすると国家試験合格におもに重点を置いてしまいがちな風潮があるが, 米国医学部教育の目標は, 卒業後ただちに現場で通用する研修医を育てるということが何よりも優先されるとのこと. 実際に, ハワイ大学内科新1年目研修医が研修初日の当直で, 新入院患者さんの指示をほとんどもれもなく書き終えていたのには指導する立場の上級研修医であった私も, 非常に驚かされた.

もう一点は塾通いの丸暗記教育に慣れている日本の学生に比べ, 米国医学部学生は4年制大学を卒業してから医学部に入学することもあり, 人間的に成熟した学生が多く, 理論概念を徹底的に理解するまで追及してから自分の知識とする傾向が強く, いわゆる丸暗記ではないのである.

そこで,『疾患からまとめた病態生理 FIRST AID』. 一つひとつの病気の診断・治療を述べた教科書は数多くあるが, ここではその病気の起こるメカニズム(病態生理)をコンパクトでかつ要点をしっかりまとめている. さらに, 病態生理を理解するために必要な正常生理についてもしっかり述べ, 診断のために重要で最小限必要な診察所見や検査所見, 治療についても解説してある. 臨床現場で活躍されている先生の"ワンポイント・アドバイス"も随所にちりばめられており, 時間のない研修医には貴重な一冊である. 臓器別にわけられた各章の最後には演習問題もあり, 病態生理を知り病気の理解を深めていただきたいポリクリ中の医学部高学年生にも, 読みやすく臨床の醍醐味を垣間見ることができる一冊である.

ひとつ内容をご紹介すると, 特発性と続発性の副腎不全を鑑別する時の重要な所見として, "アジソン病(特発性副腎不全)では色素沈着は起こるが続発性副腎不全や急性副腎不全では色素沈着は起きない"とある. これはアジソン病では副腎皮質2層目の束状層からコルチゾールが低下してフィードバックで下垂体からのACTHの分泌が増加する. このACTHはメラニン細胞を刺激し色素沈着を起こす. したがって, 下垂体からのACTH分泌が低下する続発性副腎不全では色素沈着は起きないといった具合である.

本書は, 時間のない研修医, 病態生理をしっかり勉強する必要がある医学生ばかりでなく, 回診で研修医をしっかり指導する上級医にも読んでいただきたい良書である. 本書を読むことによってぜひ一つひとつの知識に関して疑問をもち, 概念つまり病態生理を理解し解決していっていただきたい. そして, その知識を完全に自分のものとするために, 自分の得意なものがあれば同僚後輩に教えることを心がけていただきたい. 教えることが一番の知識蓄積になるそうである.

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Carotid Intima-Media Thickness and Risk of Cardiovascular Events in High-Risk Patients

Results of the Osaka Follow-Up Study for Carotid Atherosclerosis 2 (OSACA2 Study)

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Key Words
Intima-media thickness · Carotid atherosclerosis · Ultrasonography · Cardiovascular events · Stroke

Abstract
Background and Purpose: There is epidemiological evidence that increased carotid intima-media thickness (IMT) is a predictor of cardiovascular disease (CVD) events. However, the significance of carotid IMT in high-risk patients in whom risk factors are managed clinically has not been adequately investigated. The purpose of this study was to determine the usefulness of carotid IMT measurement in such patients. ***Methods:*** The study comprised 900 outpatients with cardiovascular risk factors or established atherosclerosis. Carotid IMT was calculated as the mean bilateral IMT of the common carotid artery, bifurcation, and internal carotid artery. Baseline vascular risk factors, medications, and history of CVD were recorded at the time of enrollment. The incidence of CVD events was determined prospectively. ***Results:*** During a mean follow-up period of 2.6 years, there were 64 CVD events. The relative risk (RR) of a CVD event increased with increased IMT. Association between CVD events and carotid IMT was significant after adjustment for risk factors and history of CVD, showing an increased risk per IMT tertile from the middle tertile (RR, 2.5; 95% confidence interval [CI]: 1.0–6.3) to the highest (RR, 3.6; 95% CI: 1.4–9.0). When patients with a history of CVD were excluded (n = 574), the predictive value of IMT was significant even after adjustment for risk factors (hazard ratio per 1 SD IMT increase was 1.57 [95% CI: 1.11–2.20]). ***Conclusions:*** Carotid IMT is an independent predictor of vascular events in high-risk patients in whom risk factors are managed clinically.

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Introduction

An increased carotid intima-media thickness (IMT) and increased plaque score, determined noninvasively by high-resolution ultrasound imaging, have proved to be associated with the presence of cardiovascular risk factors [1–3], cardiovascular disease (CVD), and atherosclerosis elsewhere in the arterial system [4–7]. Prospective follow-up studies in the population at large have shown a positive correlation between increased carotid IMT and the risk of myocardial infarction, stroke, and cardiovascular mortality, establishing carotid IMT as a surrogate marker of arteriosclerosis [8–12]. Whereas there is no doubt that measurement of carotid IMT is valuable for stratification of individuals at high risk of incident CVD [13], there is little information about the value of IMT measurement in clinical practice, particularly in relation to patients in whom risk factors are being controlled by modifications in lifestyle and by drug therapy [14, 15]. Thus we conducted a study to determine the predictive value of carotid IMT in terms of the occurrence of cardiovascular events in high-risk patients in whom risk factors were being managed clinically. We organized the Osaka Follow-Up Study for Carotid Atherosclerosis, Part 2 (OSACA2), as a multicenter study in which physicians control risk factors and administer drug therapy in high-risk patients for the purpose of primary and secondary prevention of cardiovascular events such as stroke and myocardial infarction.

Subjects and Methods

Patients

The OSACA2 Study was a nine-hospital, prospective follow-up study of CVD in high-risk patients aged ≥40 years. The protocol was approved by the institutional review board or ethics committee at each participating hospital, and written informed consent was provided by each patient. During the period, January 2001 through December 2002, 900 outpatients with more than one cardiovascular risk factor such as hypertension, diabetes mellitus, hyperlipidemia, or a history of smoking (current or former), or with established arteriosclerosis documented as a transient ischemic attack (TIA), stroke, coronary heart disease, or peripheral artery disease including aortic aneurysm, were enrolled in this cohort study. Patients' characteristics are shown in table 1. Patients were excluded from the study if they had experienced a clinical CVD event in the previous 3 months or had undergone carotid endartectomy or carotid artery stenting.

Measurement of IMT

To measure carotid IMT, ultrasonography of the left and right common carotid artery (CCA), carotid bifurcation, and internal carotid artery (ICA) was performed with a 3- to 11-MHz linear-array transducer. On a longitudinal two-dimensional ultrasound image of the carotid artery, the anterior (near) and posterior (far) walls of the carotid artery appear as two bright white lines separated by a hypoechogenic space. IMT was measured as the distance between the luminal-intimal interface and the medial-adventitial interface. This was done with the use of an electronic caliper on the frozen frame of a suitable longitudinal B-mode image in which the putative maximal IMT was shown in each segment: the CCA, bifurcation, and ICA. The severity of carotid atherosclerosis was evaluated by the mean max-IMT, i.e., the mean maximal wall thickness of the six segments (near and far walls of the left and right CCA, bifurcation, and ICA) shown in figure 1. All measurements were performed by stroke specialists (H.Y., H.H., T.I., Y.S., D.T., S.M., Ki.Ko., T.H., S.F., Y.N.) who were unaware of the patient data. To achieve consistency in imaging procedures and readings, the examiners held a meeting twice a year during the enrollment period. Intraobserver correlation between repeated IMT measurements in 36 patients was 0.98 ($p < 0.01$), with similar averages for the two sets of readings (1.45 ± 0.66 vs. 1.45 ± 0.62 mm, difference not significant). Interobserver correlation for 47 patients was 0.94 ($p < 0.01$), with similar IMT averages (1.27 ± 0.43 vs. 1.25 ± 0.43 mm, difference not significant).

Risk Factors

Information pertaining to medical history of cerebrovascular, coronary, and peripheral artery disease, current medications, and smoking habits was obtained from patients' clinical records at the time of enrollment. Patients were categorized as having CVD if they had a history of coronary heart disease (myocardial infarction, angina, a history of coronary artery bypass surgery or coronary artery angioplasticity), cerebrovascular disease (stroke and TIA), aortic aneurysm, or peripheral vascular disease. Fasting blood glucose, serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (TG) levels were determined. Hypertension was defined as casual blood pressure ≥140/90 mm Hg or current use of antihypertensive agents. Diabetes mellitus was defined as fasting blood glucose ≥7.0 mmol/l, a glycosylated hemoglobin A1c (HbA1c) concentration ≥5.8%, or the use of glucose-lowering agents. Dyslipidemia was defined as fasting total serum cholesterol ≥5.7 mmol/l, TG ≥1.7 mmol/l, HDL cholesterol <1.1 mmol/l, or the use of cholesterol-lowering agents. Smoking status was categorically evaluated based on self-reports, with smoking defined as a history of smoking ≥10 cigarettes/day ≥1 year.

Ascertainment of Incident CVD

Patients were followed up to determine the incidence of CVD events by July 31, 2004, in each hospital. Clinical end points were assessed by investigators blinded to the ultrasound measurements. Follow-up was terminated when patients withdrew from the study because of death (n = 25) or for personal reasons (n = 94). However, the follow-up time for each patient was included in the analysis.

A cerebrovascular event was confirmed by clinical signs of an acute-onset neurological deficit of presumed vascular origin. A TIA was defined as focal symptoms lasting <24 h. Ischemic and hemorrhagic stroke were confirmed by computed tomography scanning or magnetic resonance imaging. Subtypes of cerebral

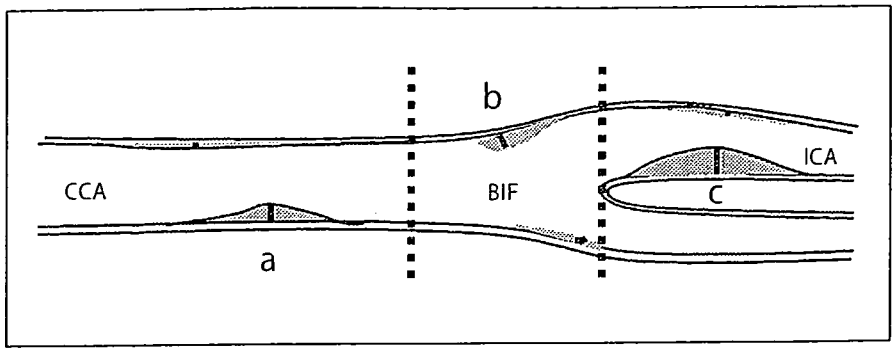

Fig. 1. Evaluation of carotid IMT. The carotid artery was divided into three parts, each 15 mm in length, beginning at the flow divider (CCA = Common carotid artery; BIF = carotid bifurcation; ICA = internal carotid artery). IMT was determined for each part as the maximal IMT of the near and far wall. Carotid IMT was taken as the mean of six measurements, i.e., bilaterally from the CCA, BIF, and ICA. Carotid IMT = (a + b + c + contralateral sum)/6 (mm).

Table 1. Characteristics of the 900 study patients at enrollment

	Total	CVD event (+)	CVD event (–)
Number	900	64	836
Age, years	65.3±9.5	67.1±9.4	65.2±9.5
Men sex, %	56.8	78.1*	55.4
Body mass index, kg/m^2	23.3±3.1	22.7±3.0	23.3±3.1
Hypertension, %	75.8	82.8	75.4
Blood pressure, mm Hg			
Systolic	136.1±17.8	136.8±19.4	136.1±17.6
Diastolic	78.2±11.3	77.6±10.9	78.3±11.4
Dyslipidemia, %	56.8	48.4	57.5
Total cholesterol, mmol/l	5.4±0.9	5.0±0.7	5.4±0.9
Triglyceride, mmol/l	1.6±0.8	1.4±0.6	1.6±0.8
HDL cholesterol, mmol/l	1.5±0.5	1.4±0.3	1.5±0.4
Diabetes mellitus, %	28.7	34.3	28.3
Fasting blood glucose, mmol/l	6.0±1.9	6.2±1.9	6.0±1.9
Smoking, %	21.9	28.1	21.4
Atrial fibrillation, %	3.9	3.5	3.9
Medical treatment			
Antiplatelet drugs use, %	334 (37)	45 (70)*	289 (35)
ACEI or ARB use, %	261 (29)	23 (36)	238 (28)
Statin use, %	267 (30)	22(34)	246 (29)
History of CVD, %	326 (36)	46 (72)*	280 (33)
Carotid IMT, mm	1.14±0.46	1.38±0.47*	1.12±0.46

Values are mean ± SD except where otherwise indicated; values in parentheses are median. ACEI = Angiotensin-converting enzyme inhibitors; ARB = angiotensin II type-1 receptor blocker; CVD = cardiovascular disease; IMT = intima-media thickness.

* $p < 0.05$ compared with a CVD event (–) group.

infarction were also determined. A coronary heart disease event was defined as myocardial infarction and hospitalization for unstable angina, coronary artery bypass surgery, or coronary artery angioplasty. A peripheral artery disease event was defined as hospitalization for therapeutic intervention, revascularization, or a surgical procedure.

Statistical Analysis

Statistical analyses were performed with SPSS 9.0J for Windows (SPSS Japan Inc., Tokyo, Japan). Patients were divided into groups according to tertile IMT values. One-way analysis of variance (ANOVA) and post-hoc analysis (Bonferroni's multiple comparison tests) were applied for comparisons of normally distributed data (risk factors) between IMT tertiles. χ^2 analysis was applied to differences in cardiovascular risk factors between groups. Cumulative event-free survival was calculated by the Kaplan-Meier method, and differences were analyzed by log-rank test. To estimate the relative risk per tertile of a new CVD event, Cox regression for the multivariate analysis was applied with adjustment for cardiovascular risk factors. When not otherwise specified, data are presented as mean ± SD. A two-tailed p value of <0.05 was considered statistically significant.

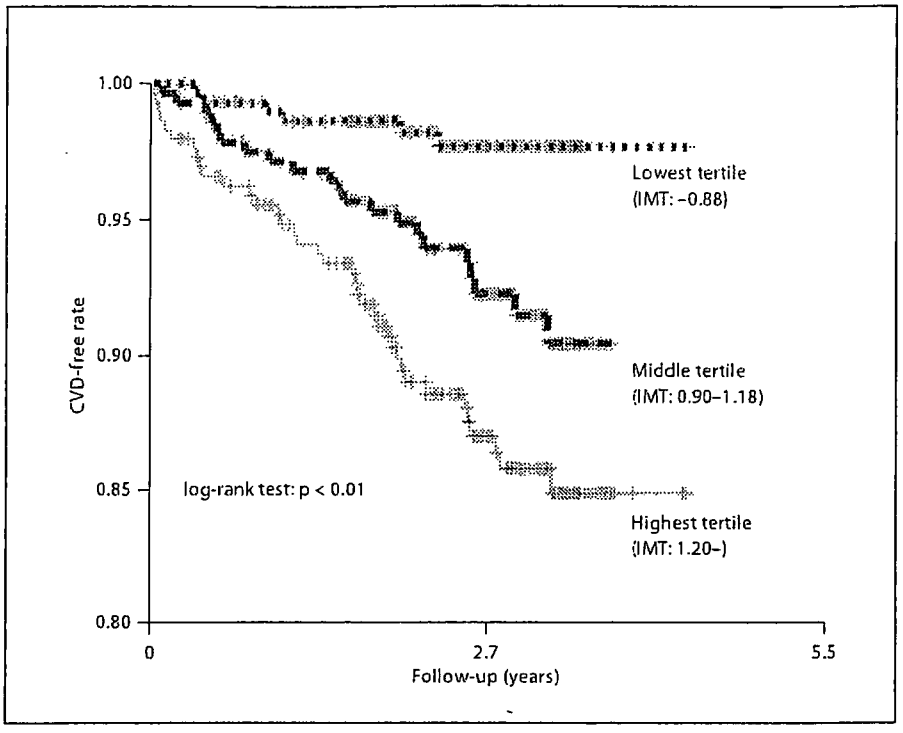

Fig. 2. Cumulative event-free survival for the cardiovascular events in the lowest, middle, and highest third of carotid IMT in all 900 patients.

Results

Baseline characteristics of the 900 study patients are given in table 1. The mean age of the patients was 65.3 years, and 56.8% were men. The mean follow-up period was 2.57 years (median 2.74 years; range 0.03–4.36 years). 36% of the patients (n = 326) had a history of at least one CVD event. Of those 326 patients, 243, 78, 21 and 15 patients had at least one history of cerebrovascular disease, coronary artery disease, peripheral artery disease and aortic aneurysm, respectively. The subjects were outpatients, 75.8, 56.8, and 28.7% had hypertension, dyslipidemia, and diabetes mellitus, respectively, and 81.3% received at least antiplatelet drugs, antihypertensive drugs or statins. However, blood pressure, blood glucose, and serum lipid levels were fairly well controlled by lifestyle modifications and drug therapy. There were 37 new cerebrovascular events, 23 new cases of coronary artery disease, 2 new aortic aneurysms, and 2 new cases of peripheral artery occlusive disease. Of the 37 cerebrovascular events, 23 (62%) were cerebral infarctions, and 7 (19%) were cerebral hemorrhages. Characterization of the risk in both CVD event (+) (n = 64) and CVD event (–) (n = 836) groups was shown in table 1. Frequency of male sex, anti-platelet drugs use and history of CVD were significantly higher in CVD event (+) group than that in CVD event (–) group. Carotid IMT in CVD event (+) group was also significantly more than that in CVD event (–) group. Cumulative event-free survival is shown by carotid IMT tertiles in figure 2. Cox regression analysis showed IMT to be significantly associated with risk of a CVD event after adjustment for age and sex ($p < 0.01$ by the test for trend), with patients in the highest tertile having a risk that was 5.5 times greater than that of patients in the lowest tertile (table 2). The associated risk remained significant ($p < 0.01$ by the test for trend), although slightly reduced in magnitude, after adjustment for risk factors and a history of CVD (table 2). Cox regression analysis with IMT used as a continuous variable also revealed strong association between IMT and CVD events. The age- and sex-adjusted relative risk associated with a change of 1 SD in the IMT was 1.35 (95% confidence interval [CI]: 1.11–1.62) and remained significant after adjustment for risk factors (hazard ratio [HR]: 1.34, 95% CI: 1.10–1.63). Patient characteristics are shown per tertile group in table 3. Although male sex, hypertension, diabetes mellitus, and CVD history were most prevalent in the highest tertile, body mass index, blood pressure, and plasma lipid and glucose level was similar between groups because of clinical management of risk factors.

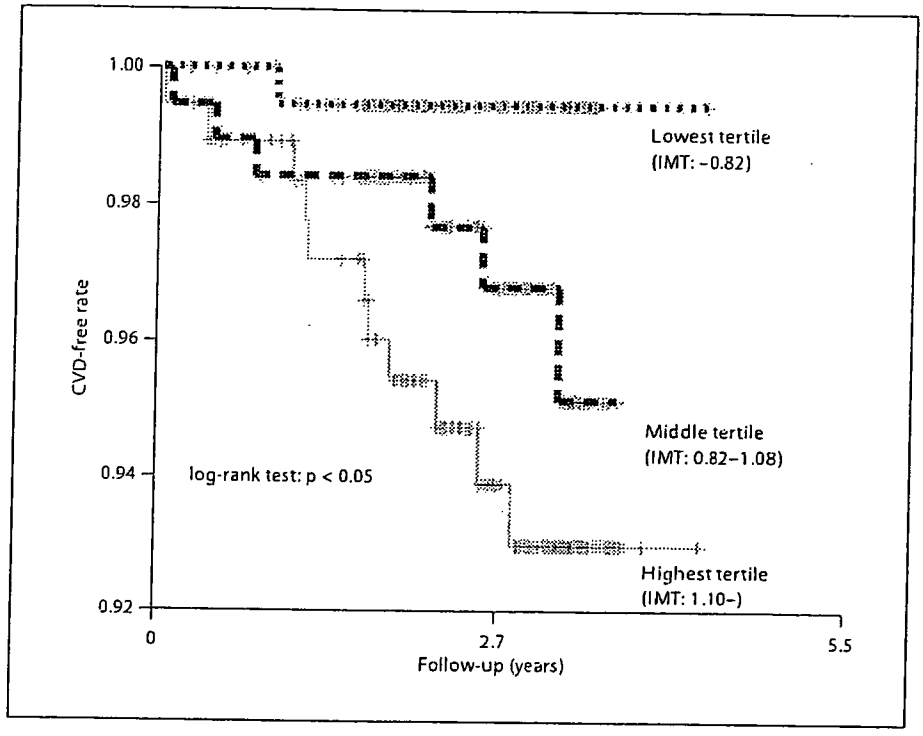

Fig. 3. Cumulative event-free survival for the cardiovascular events in the lowest, middle, and highest third of carotid IMT in 574 patients without a history of CVD.

Table 2. Relative risk of cardiovascular events as a function of the carotid IMT expressed as tertiles and as a continuous variable

IMT, mm	Events/risk	Adjusted for		
		age and sex	age, sex, risk factors	age, sex, risk factors and CVD history
All 900 patients				
Lowest, <0.90	6/311	1.00	1.00	1.00
Middle, 0.90–1.18	21/289	3.4 (1.3–8.4)	3.3 (1.3–8.1)	2.5 (1.0–6.3)
Highest, >1.18	37/300	5.5 (2.2–13.7)	5.1 (2.0–12.7)	3.6 (1.4–9.0)
Per 1 SD increase		1.35 (1.11–1.62)	1.34 (1.10–1.63)	1.18 (0.96–1.45)
574 patients without CVD history				
Lowest, <0.83	1/194	1.00	1.00	
Middle, 0.83–1.10	6/193	6.5 (0.8–55.4)	6.4 (0.7–55.2)	
Highest, >1.10	11/187	12.1 (1.4–100.7)	13.4 (1.6–115.4)	
Per 1 SD increase		1.65 (1.12–2.33)	1.57 (1.11–2.20)	

CVD = Cardiovascular disease, IMT = intima-media thickness. The risk factors were: presence of hypertension, dyslipidemia, diabetes mellitus, and smoking.

Cumulative event-free survival is shown per IMT tertile for the 574 patients without a history of CVD in figure 3. There were 18 new CVD events. In this group, increased IMT was significantly associated with the risk of a CVD event after adjustments for age, sex ($p < 0.01$ by the test for trend), and risk factors ($p < 0.02$ by the test for trend) (table 2). The relative risk associated with a change of 1 SD in IMT was 1.57 (95% CI: 1.11–2.20) after adjustment for risk factors.

Table 3. Baseline risk factors in carotid IMT tertiles

	IMT tertile			p
	lowest ≤0.88 mm	middle 0.90–1.18 mm	highest ≥1.20 mm	
Age, years	61.1±9.7	65.8±8.8	69.2±8.0	<0.01
Men sex, %	43.4	58.1	70.0	<0.01
Body mass index, kg/m^2	23.7±3.3	23.3±2.9	22.9±3.0	<0.01
Hypertension, %	70.7	73.0	84.0	<0.01
Blood pressure, mm Hg				
Systolic	134.5±17.2	135.5±17.7	138.5±18.1	0.015
Diastolic	79.9±11.0	78.5±11.1	76.4±11.6	<0.01
Dyslipidemia, %	58.8	54.7	57.0	N.S.
Total cholesterol, mmol/l	5.6±1.0	5.4±0.8	5.2±0.9	<0.01
Triglyceride, mmol/l	1.6±0.9	1.6±0.8	1.5±0.8	N.S.
HDL cholesterol, mmol/l	1.6±0.5	1.5±0.5	1.5±0.5	<0.01
Diabetes mellitus, %	24.4	27.7	34.3	<0.05
Fasting blood glucose, mmol/l	5.9±2.1	6.0±1.7	6.2±1.9	N.S.
Current/past smoking, %	20.9	19.7	25.0	N.S.
History of CVD, %	20.6	39.4	49.3	<0.01

Discussion

Results of this study showed the value of carotid IMT as a predictive factor for a CVD event in high-risk patients to be similar to that found in epidemiological studies of the general population. The adjusted odds ratio for a CVD event at a mean IMT increase of 1 SD (0.46 mm) was 1.18 (0.96–1.45) in the total 900 patients and 1.57 (1.11–2.20) in the 574 patients without a history of CVD (table 2). These data confirm the findings of previous large-scale population-based studies. The Cardiovascular Health Study showed that a CCA IMT increase of a 1 SD and ICA IMT increase resulted in an age, sex and other risk factor-adjusted HR for CVD event were 1.27 (1.17–1.38) and 1.30 (1.20–1.41), respectively. The Rotterdam study showed that a CCA IMT increase of 1 SD resulted in an age, sex and risk factor-adjusted HR of 1.34 (1.08–1.67) for stroke and 1.25 (0.98–1.58) for myocardial infarction. Our results led us to conclude that IMT measurement is clinically useful for stratification of patients at high risk for a CVD event.

Because we and others have shown the significance of carotid IMT as a predictive factor for CVD, it is important to determine how carotid IMT measurement can be used in clinical care of high-risk patients with cardiovascular risk factors. It remains undetermined whether atherosclerosis risk factors such as hypertension and hyperlipidemia should be more strictly controlled in patients of the highest IMT tertile than in those of the lowest tertile. Management of blood pressure varies according to the presence of diabetes or renal disease and age. The recommended lipid level also varies according to the presence of CVD history and diabetes mellitus. Physicians make efforts to control risk factors in patients. As shown in table 3, the levels of these variables are similar between IMT tertile groups, although the frequency of hypertension and diabetes mellitus differ significantly between these groups. To further strengthen the clinical value of carotid IMT measurement, hospital- or clinic-based cohort studies evaluating the management of risk factors together with measurement of carotid IMT in large groups of high-risk patients during longer follow-up periods are needed.

In clinical practice, carotid IMT measurement has been believed to be a surrogate marker [13], although del Sol et al. [16] reported that adding IMT to a risk function did not result in a substantial increase in the predictive value. Treatment with statin or angiotensin-converting enzyme inhibitor may reverse or retard an increase in carotid IMT [17, 18]. Management of traditional risk factors alone may not be adequate to prevent atherosclerosis progression and CVD events. Over the past decades, we have acknowledged the prominent role of inflammation in atherosclerosis and its complications such as myocardial infarction and most strokes [19]. The high-sensitivity CRP (hs-CRP) level predicts the risk of CVD in a variety

of clinical settings [20]. Our previous study also showed that hs-CRP is an independent predictor of the rate of carotid atherosclerosis progression [21]. Measurement of inflammatory markers in combination with carotid IMT measurement might be useful in predicting a new CVD event.

Conclusions

We have shown that carotid IMT independently predicts vascular events in high-risk patients. Carotid IMT measurement is of value for clinical stratification of patients at high risk of vascular events. Clinical trials are needed to determine how carotid IMT measurement should be included in the clinical management of risk factors in high-risk patients.

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Appendix

The participating hospitals and researchers are listed in order of the number of eligible patients entered in the study. *Osaka University Graduate School of Medicine, Suita*: K. Kitagawa, H. Hougaku, H. Yamagami, K. Kondo, E. Omura, T. Hoshi, S. Furukado, Y. Abe, M. Sakaguchi, Y. Nagai, M. Matsumoto, M. Hori. *National Osaka Hospital, Osaka:* H. Hashimoto, M. Tagaya, H. Niki, M. Fujiwara, H. Etani. *Hoshigaoka-Kouseinenkin Hospital, Hirakata:* T. Itoh, S. Sugiura, N. Ohyama, Y. Terasaki, R. Fukunaga. *National Osaka-minami Hospital, Kawachinagano:* D. Takahashi, S. Yamamoto, K. Takasawa, M. Nukada, M. Watanabe. *Osaka Seamen's Hospital, Osaka:* S. Murata, Y. Terayama. *Kobe Ekisaikai Hospital, Kobe*: Y. Shimizu, S. Furukawa, D. Uematsu, T. Yoshikawa. *Bobath Memorial Hospital, Osaka:* Y. Seike. *Osaka Rousai Hospital, Sakai:* Y. Okazaki, T. Araki. *All Nippon Airway Healthcare Center, Ikeda:* H. Maeda.

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CLINICAL RESEARCH

Determinants of thrombin generation, fibrinolytic activity, and endothelial dysfunction in dual-antiplatelet therapy: involvement of factors other than platelet aggregability in Virchow's triad

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Aims The aim of the study was to assess mechanisms and clinical backgrounds in order to determine residual platelet aggregability in dual-antiplatelet therapy and to ascertain whether platelet aggregability is involved in systemic thrombogenicity.

Methods and results A cross-sectional study was conducted in 85 consecutive patients who underwent dual-antiplatelet therapy (aspirin and thienopyridine/cilostazol) after percutaneous coronary intervention (PCI). Although serum thromboxane B_2 and dephosphorylation of vasodilator-stimulated phosphoprotein were significantly abolished, the platelet aggregation tests showed inter-individual differences that could be partly explained by plasma glucose levels. Platelet aggregability was not related to other factors involved in thrombogenicity. Thrombin generation assessed by soluble fibrin was independently associated with total cholesterol ($\beta = 0.349$, $P < 0.001$), brain natriuretic peptide ($\beta = 0.222$, $P = 0.018$), and ankle-brachial index ($\beta = -0.330$, $P = 0.001$). Plasminogen activator inhibitor-1 was associated with the apnea–hypopnea index ($\beta = 0.300$, $P = 0.006$). E-selectin was correlated with diabetes mellitus ($\beta = 0.279$, $P = 0.008$) and body mass index ($\beta = 0.323$, $P = 0.002$).

Conclusion Although dual-antiplatelet therapy effectively inhibited its pharmacological targets, thrombin generation, inhibition of fibrinolytic activity, and endothelial dysfunction were determined by other clinical backgrounds. Our data suggested that some patients remain at risk of thrombotic complications after PCI and that these may benefit from anticoagulant treatment despite adequate dual-antiplatelet therapy.

Keywords Percutaneous coronary intervention • Aspirin • Thienopyridine • Antiplatelet drug resistance • Thrombin generation

Introduction

Platelet aggregation plays a central role in the development of thrombotic complications after percutaneous coronary intervention (PCI).[1–3] The role of aspirin in secondary prevention of ischaemic cardiovascular diseases is universally accepted. Furthermore, dual-antiplatelet therapy of aspirin combined with thienopyridine (and/or cilostazol) including clopidogrel or ticlopidine is

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the gold standard for preventing major cardiovascular events in patients undergoing PCI, especially since the beginning of the balloon-expandable stent era.[4–6] In contrast, nearly 20% of patients continue to have further cardiovascular events after PCI, despite the superior protection conferred by dual-antiplatelet therapy, as shown in a number of clinical trials.[7]

The mechanism by which antiplatelet therapy fails in certain patients after PCI, in part, thought to be attributed to the fact that some individuals have impaired antiplatelet responses, is referred to as 'aspirin resistance' or 'clopidogrel resistance'.[8–10] There is evidence that not all patients respond comparably to antiplatelet drugs, as evaluated by non-specific laboratory test such as aggregometry, and hence the concept of drug 'resistance' has arisen.[11–14] However, recent evidence suggest that when the definition of resistance is limited to situations in which the drugs fail to hit their pharmacological targets, resistance against antiplatelet drug appears to be rare.[15–18] Many published studies of antiplatelet resistance have been carried out using nonspecific platelet aggregation tests, which merely identify patients on antiplatelet therapy with high residual platelet activation.[7,18] Despite this drawback, identification of patients with high residual platelet reactivity may be useful for predicting individuals risks of atherothrombotic events.[7–10,13,17]

The results of clinical trials on the use of anticoagulant agents and the involvement of fibrin fibrils and inflammatory cells in the formation of occlusive thrombi suggest that not only platelets but also the coagulation cascade, fibrinolytic system, inflammation, and endothelial dysfunction may orchestrate *in vivo* thrombus formation, thereby leading to clinical treatment failure under dual-antiplatelet therapy.[19–21] Indeed, the clinical outcomes of patients undergoing PCI were reported to be associated with the levels of D-dimer, plasminogen activator inhibitor-1 (PAI-1), E-selectin, and markers for thrombin generation.[22–25] However, there is no sufficient data that correlate heightened platelet reactivity during dual-antiplatelet therapy with other markers for coagulation, fibrinolysis, and endothelial dysfunction. The aims of the present study were to assess the various clinical backgrounds associated with high residual platelet aggregability under dual-antiplatelet therapy and to clarify any association with thrombin generation, fibrinolytic activity, and endothelial dysfunction that might lead to clinical failure against antiplatelet therapy.

Methods

Patients and study protocol

The institutional review board at the Jichi Medical University approved the study protocols, and written informed consent was obtained from all participants. We enrolled consecutive hospitalized patients from July 2006 to April 2007 who were treated by PCI because of symptomatic coronary artery disease, including unstable angina, and non-ST-elevation or ST-elevation myocardial infarction. We estimated the sample size required using a general formula for the correlation coefficient.[26] We set $\alpha = 0.05$, $\beta = 0.20$, and expected a correlation coefficient, $r = 0.30–0.35$. Using the formula, at least 62–85 participants would be required for the study. All patients had taken dual-antiplatelet therapy, consisting of 100 mg/day of aspirin and 200 mg/day of ticlopidine, 75 mg/day of clopidogrel, or 200 mg/day of cilostazol (*Table 1*). The exclusion criteria were as follows: acute coronary syndrome within 10 days; New York Heart Association Class III or IV heart failure; ingestion of other drugs affecting platelet function or coagulation; platelet counts of $<10\times10^7$ or $>40\times10^7$ ml^{-1}; myeloproliferative disorders; autoimmune diseases; malignant diseases; and atrial fibrillation. Compliance with antiplatelet drugs was determined by nursing staff during hospitalization. After normalization of cardiac enzymes (just before discharge), patients underwent blood sampling, ambulatory blood pressure monitoring (ABPM; TM-2425; A&D Co., Inc., Tokyo, Japan), ankle-brachial index (ABI) monitoring (FORM/ABI; Colin Co. Ltd., Ehime, Japan), and cardiorespiratory monitoring (Somte; Compumedics, Melbourne, Australia).

Table 1 Characteristics of the study population

Variables	Total subjects ($n = 85$)
Age (years)	60.0 ± 13.1
Men, *n* (%)	70 (82)
Body mass index (kg/m^2)	24.3 ± 3.3
Current smoker, *n* (%)	50 (59)
Family history of coronary artery disease, *n* (%)	26 (31)
Hypertension, *n* (%)	59 (69)
Diabetes mellitus, *n* (%)	36 (42)
Dyslipidemia, *n* (%)	75 (88)
Prior myocardial infarction, *n* (%)	10 (12)
Presenting symptoms, *n* (%)	
Unstable angina	63 (26)
Myocardial infarction	22 (74)
Coronary artery disease, *n* (%)	
One-vessel disease	41 (48)
Two-vessel disease	26 (31)
Three-vessel disease	18 (21)
Concomitant medications	
Antiplatelet agents, *n* (%)	
Aspirin	85 (100)
Ticlopidine	72 (85)
Clopidogrel	3 (4)
Cilostazol	10 (12)
Antihypertensive medication, *n* (%)	
Beta blocker	51 (60)
Angiotensin-converting enzyme inhibitor	39 (46)
Angiotensin II receptor blocker	32 (38)
Calcium channel blocker	17 (20)
Diuretic	14 (16)
Nitrate, *n* (%)	5 (6)
Statin, *n* (%)	66 (78)
Proton pump inhibitor, *n* (%)	1 (1)
Non-steroidal anti-inflammatory drug, *n* (%)	0 (0)

Data for continuous variables are expressed as the mean ± SD. BMI, body mass index; CAD, coronary artery disease; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; PPI, proton pump inhibitor; NSAID, non-steroidal anti-inflammatory drug.

To assess the effects of antiplatelet therapy, 20 healthy individuals who were not taking any antiplatelet drugs were enrolled as controls.

Platelet aggregation

A fasting venous sample was carefully collected via a 21-gauge needle into a syringe containing 1/10 volume of sodium citrate between 07:30 and 08:00 h. Platelet-rich plasma (PRP) was obtained by centrifuging whole blood at 200 g for 12 min. The time from blood collection to measurement was standardized to 1 h. The aggregation response was measured based on the light scattering intensities obtained with a PA-200 Platelet Aggregation Analyzer (Kowa Co. Ltd., Tokyo, Japan).[27] This device is particularly sensitive for detecting the sizes of small platelet aggregates.[27,28] Platelet aggregation was performed without any agonists, or with collagen (Hormon-Chemie, Munich, Germany), ADP (MC Medical Co., Tokyo, Japan), and thrombin receptor-activating peptide (TRAP; Invitrogen Co., Carlsbad, CA, USA), a specific agonist for protease-activating receptor-1. Spontaneous small platelet aggregation was defined by small aggregate formation by stirring without agonist.

Phosphorylation of vasodilator-stimulated phosphoprotein in platelets

Phosphorylation of vasodilator-stimulated phosphoprotein (VASP) is regulated by the cAMP level, which is thus believed to be a marker of $P2Y_{12}$ receptor reactivity.[29] To determine the VASP phosphorylation state of whole blood, we used a standardized flow cytometric assay (PLT VASP/P2Y12; Biocytex, Marseille, France) with some modifications. We found that the commercially available VASP phosphorylation assay appeared to contain an extremely high concentration of ADP. In our protocol, cAMP elevation by 1 μM PGI_2 increased the VASP phosphorylation level by stimulation of adenylate cyclase. When simultaneously stimulated with 2 μM ADP, the signaling from G_i activation mediated via $P2Y_{12}$ reduced the phosphorylation of VASP induced by PGI_2. However, when the $P2Y_{12}$ receptor was successfully inhibited by active metabolites of thienopyridines or phosphodiesterase that was inhibited by cilostazol, ADP was unable to reduce PGI_2-induced VASP phosphorylation. The phosphorylation of VASP was quantified by flow cytometry according to the manufacturer's instructions. The reduction of VASP phosphorylation induced by ADP was expressed as the % of PGI_2; the mean fluorescence intensity of PGI_2 plus ADP was devided by that of PGI_2.

Laboratory testing, ambulatory blood pressure monitoring, ankle-brachial index, and cardiorespiratory monitoring

Methods are described in detail in the supplementary materials. The intraassay and interassay coefficients of laboratory tests were all <10%. The data obtained from patients are shown in *Table 2*.

Statistical analysis

All statistical analyses were performed with SPSS version 11 software (SPSS, Inc., Chicago, IL, USA). The Mann–Whitney *U*-test was used to compare measurements of platelet activation between patients and healthy volunteers. The associations between the individual parameters were calculated using Spearman's correlation method. To identify independent factors, we used a step-wise multivariable linear regression analysis in which a *P*-value of 0.05 or less in a simple regression analysis was used as the criterion for entry into the model. We validated independent explanatory variables by Mann–Whitney *U*-test after categorization into two groups. All reported

Table 2 Physiological and biochemical characteristics of the study population

Biochemical markers	
White blood cells ($\times 1000\ mm^{-3}$)	7.1 ± 1.8
Haemoglobin (g/dL)	13.7 ± 1.8
Platelets ($\times 1000\ mm^{-3}$)	306.7 ± 84.0
Fasting glucose (mg/dL)	116.2 ± 46.7
Total cholesterol (mg/dL)	167.9 ± 35.6
Triglycerides (mg/dL)	130.9 ± 52.6
High-density lipoprotein cholesterol (mg/dL)	41.3 ± 11.8
Low-density lipoprotein cholesterol (mg/dL)	100.3 ± 29.5
Adrenalin (pg/mL)	31.1 ± 21.9
hsCRP (mg/L)	5.69 ± 7.60
Brain natriuretic peptide (pg/mL)	145.7 ± 174.7
PAI-1 (ng/mL)	56.6 ± 20.2
E-selectin (ng/mL)	20.4 ± 10.1
D-dimer (μg/mL)	1.8 ± 2.5
Soluble fibrin (μg/mL)	4.3 ± 7.5
Physiological markers	
24-h SBP (mmHg)	120.0 ± 14.5
24-h DBP (mmHg)	72.6 ± 9.1
24-h HR (b.p.m.)	68.2 ± 11.1
AHI ≥ 5/h, *n* (%)	75 (88)
AHI ≥ 15/h, *n* (%)	50 (59)
ABI	1.08 ± 0.123

Data for continuous variables are expressed as the mean ± SD. HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; BNP, brain natriuretic peptide; PAI-1, plasminogen activator inhibitor-1; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; AHI, apnea–hypopnea index; ABI, ankle-brachial index.

P-values are two-sided; a *P*-value of less than 0.05 was considered to be statistically significant.

Results

Patients

Of the 94 patients recruited, two were not included because of advanced gastric cancer or spastic angina, and three did not take dual-antiplatelet drugs at the time blood was collected. An additional four patients were excluded from the analysis because of incomplete blood collection or failure of polysomnography or ABPM. Thus, 85 patients were finally included in the analysis (*Table 1*).

Dual-antiplatelet therapy effectively inhibits its pharmacological targets

To precisely assess the effects of aspirin, we measured serum thromboxane B_2 (TxB_2) concentration, which reflects platelet-COX-dependent TxA_2 production. As has been described,[15,17] the serum TxB_2 concentration was uniformly abolished in all patients compared with control patients (*Figure 1A*). We also simultaneously evaluated VASP dephosphorylation after ADP stimulation, which reflects Gi-dependent cAMP reduction. As shown

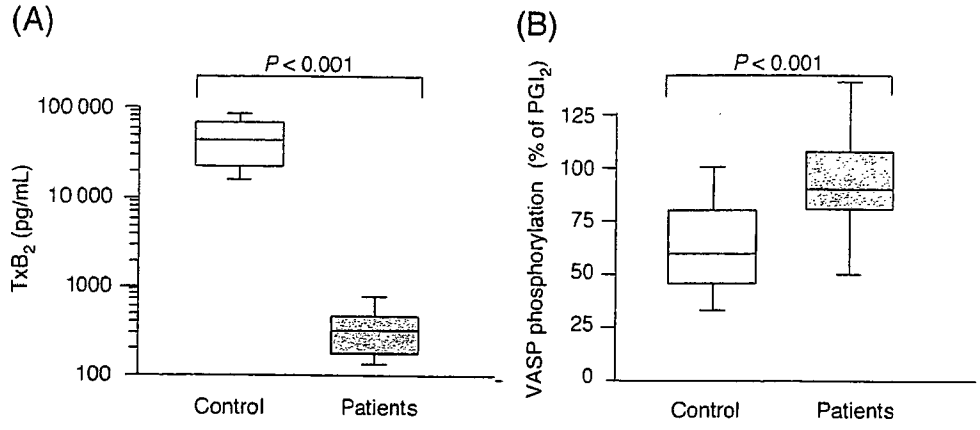

Figure 1 Serum thromboxane B_2 (TxB_2) concentration and vasodilator-stimulated phosphoprotein index in patients taking dual-antiplatelet therapy. (*A*) The serum concentration of TxB_2 was measured by EIA. (*B*) The vasodilator-stimulated phosphoprotein phosphorylation was assessed by flow cytometry. ADP-induced vasodilator-stimulated phosphoprotein dephosphorylation was expressed as % of PGI_2. Data are expressed as box-and-whisker plots.

in *Figure 1B*, cAMP reduction by ADP was effectively inhibited by dual-antiplatelet therapy. These data suggested that dual-antiplatelet therapy efficiently inhibits its pharmacological targets in patients undergoing PCI.

Inter-individual differences in platelet reactivity under dual-antiplatelet therapy

Next, we examined the effects of dual-antiplatelet therapy on platelet aggregation patterns using an aggregometry method that simultaneously measures both light transmission and light scattering. Although platelet aggregation assessed by light transmission was significantly decreased in the patients, the results of platelet aggregation tests induced by different agonists showed some inter-individual differences compared with serum TxB_2 and VASP phosphorylation (*Figure 2A*). We compared the changes of VASP phosphorylation and all platelet aggregations in the cilostazol group ($n = 10$) with those in the thienopyridine group ($n = 75$). We did not find any significant differences in platelet activation status, suggesting that drug differences could not explain the heterogeneity of platelet aggregation. Use of a laser-light scattering method to quantitatively evaluate the aggregate sizes and numbers revealed that the number of small aggregates increased after stimulation with all agonists, except for the lower concentration of ADP (*Figure 2B*). The inhibition of medium and large aggregates was clearer for low-dose agonist stimulation (data not shown), indicating that the platelet reactivity generating large platelet aggregates from small aggregates after agonist stimulation was highly concentration-dependent. Furthermore, the degrees of platelet aggregation induced by different agonists within a given subject significantly correlated with each other (*Table 3*). The number of small platelet aggregates spontaneously formed without agonist stimulation was significantly correlated with the collagen-induced platelet aggregation assessed by light transmission ($R = 0.398$, $P < 0.001$). We also found that small aggregate formation induced by a lower dose of agonist (1 μg/mL of collagen or 2 μM ADP) strongly correlated with light transmission induced by all higher concentrations of agonist ($R = 0.563$–0.815, $P < 0.001$). These data suggested that platelet aggregability under dual-antiplatelet therapy may be determined by differences in the thresholds of each patient's platelets, rather than by differences in antiplatelet drug efficacies.

As activated platelets offer the scaffold of a coagulation cascade in arterial thrombus formation, we supposed that residual platelet activation under dual-antiplatelet therapy may be involved in a systemic thrombin generation. To determine whether *in vitro* platelet aggregation is related to blood thrombogenicity, we compared the results of platelet aggregation tests with the plasma levels of SF (a marker for thrombin generation), D-dimer (a marker for fibrinolysis), PAI-1 (an inhibitor of fibrinolysis), and E-selectin (a marker for endothelial dysfunction). None of these variables was associated with the results of platelet aggregation (*Table 3*). Next, we attempted to determine factors influencing platelet aggregability by comparing the clinical backgrounds and other laboratory tests. Interestingly, we found that only the fasting glucose level was significantly correlated with the number of spontaneously formed small platelet aggregates and collagen-induced platelet aggregates ($R = 0.498$, $P < 0.001$ and $R = 0.243$, $P = 0.025$, respectively), regardless of the presence of diabetes mellitus (*Table 4*). Although many drugs including angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and statin can influence platelet activation and blood coagulation, the use of these drugs did not affect the results of platelet aggregation tests, or the levels of PAI-1, D-dimer, SF, or E-selectin (data not shown).

Determinants of thrombin generation, fibrinolytic activity, and endothelial dysfunction

Finally, we examined the clinical characteristics that determine thrombin generation, fibrinolytic activity, and endothelial dysfunction. SF was significantly correlated with total cholesterol, BNP, ABI, and the number of coronary vessels affected *Table 4*. By

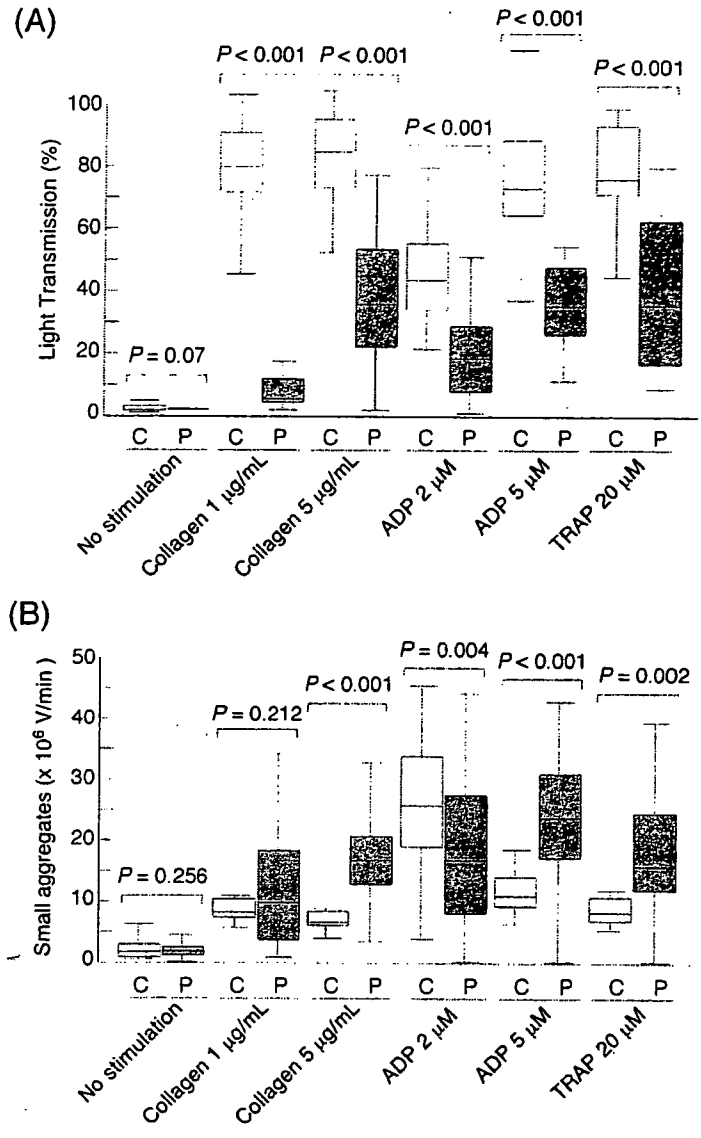

Figure 2 Platelet aggregation patterns in patients taking dual-antiplatelet therapy. Platelets in platelet-rich plasma obtained from control subjects (*C*) or patients taking dual-antiplatelet therapy (P) were stimulated with the indicated agonists for 5 min. (*A*) Changes in the maximum light transmission were monitored using conventional methods. (*B*) Light scattering intensities that represent small aggregate formation were measured simultaneously. Data are expressed as box-and-whisker plots.

multivariable regression analysis including these significant covariates, total cholesterol, BNP, and ABI remained independently correlated with the SF level (*Table 5*). BNP was also an independent predictor of the D-dimer level in a multivariable regression analysis (*Table 5*). On the other hand, PAI-1 was significantly correlated with body mass index (BMI) and AHI *Table 4*. By multivariable analysis, only AHI remained independently correlated with the PAI-1 level (*Table 5*). E-selectin was significantly associated with age, BMI, diabetes mellitus, 24 h DBP, and AHI (*Table 4*). By multivariable regression analysis, BMI and diabetes mellitus remained independently correlated with the E-selectin level (*Table 5*). The significance of these explanatory variables was confirmed by Mann–Whitney *U*-test after categorization into two groups (see Supplementary material online, Figure S1). These results suggested that total thrombogenicity under antiplatelet therapy may be orchestrated by a variety of patient backgrounds that affect platelet reactivity, thrombin generation, fibrinolysis, and endothelial dysfunction.

Discussion

Activated platelets are critically involved in thrombotic complications after PCI and in acute coronary syndrome.[8–10] The issue of resistance to antiplatelet agents has been emphasized in the literature, leading to growing concern about the efficacy of antiplatelet therapy and about possible unfavorable clinical outcomes.[10–12] However, the term 'resistance' is frequently misleading when it refers to individuals who develop cardiovascular events despite

Table 3 Spearman's correlation coefficients among platelet aggregation (light transmission), PAI-1, D-dimer, SF, and E-selectin

	Collagen (1 μg/mL)	Collagen (5 μg/mL)	ADP (2 μg/ml)	ADP (5 μg/mL)	TRAP	PAI-1	D-dimer	Soluble fibrin
Collagen (1 μg/mL)	–	–	–	–	–	–	–	–
Collagen (5 μg/mL)	0.788 ($P < 0.001$)	–	–	–	–	–	–	–
ADP (2 μg/mL)	0.635 ($P < 0.001$)	0.853 ($P < 0.001$)	–	–	–	–	–	–
ADP (5 μg/mL)	0.608 ($P < 0.001$)	0.868 ($P < 0.001$)	0.931 ($P < 0.001$)	–	–	–	–	–
TRAP	0.417 ($P < 0.001$)	0.716 ($P < 0.001$)	0.646 ($P < 0.001$)	0.688 ($P < 0.001$)	–	–	–	–
PAI-1	−0.142 ($P = 0.194$)	−0.121 ($P = 0.271$)	−0.163 ($P = 0.137$)	−0.177 ($P = 0.105$)	−0.016 ($P = 0.884$)	–	–	–
D-dimer	0.050 ($P = 0.648$)	−0.007 ($P = 0.947$)	−0.007 ($P = 0.948$)	−0.047 ($P = 0.669$)	−0.177 ($P = 0.104$)	−0.169 ($P = 0.122$)	–	–
Soluble fibrin	0.100 ($P = 0.364$)	−0.046 ($P = 0.675$)	−0.022 ($P = 0.840$)	−0.021 ($P = 0.852$)	−0.170 ($P = 0.121$)	−0.061 ($P = 0.580$)	0.190 ($P = 0.082$)	–
E-selectin	−0.087 ($P = 0.427$)	−0.062 ($P = 0.572$)	−0.073 ($P = 0.506$)	−0.083 ($P = 0.049$)	0.031 ($P = 0.781$)	0.232 ($P = 0.032$)	0.042 ($P = 0.701$)	−0.105 ($P = 0.340$)

TRAP, thrombin receptor-activating peptide; PAI-1, plasminogen activator inhibitor-1.

antiplatelet therapy.[10–12] More accurately, we should properly distinguish patients who develop cardiovascular events despite antiplatelet therapy as 'treatment failure'.[30] From the viewpoint of Virchow's triad, arterial thrombosis may occur through complex interactions of a variety of components, including platelet activation, coagulation/fibrinolytic activity, endothelial dysfunction, and blood flow.[31,32]

On the basis of the results of our study, true antiplatelet drug resistance as defined by a specific test appears rare. This observation is consistent with recent studies, reporting that aspirin resistance other than non-compliance appears to be exceptional.[15–18,33] Although studies that used specific tests to measure the pharmacological effects of thienopyridines showed a wide variability in the responses to these drugs,[12] VASP dephosphorylation was significantly inhibited by dual-antiplatelet therapy, and was not associated with ADP-induced platelet aggregation (data not shown). This discrepancy may be because of differences in the concentrations of ADP used; the commercially available VASP phosphorylation kit appears to use a high concentration of ADP (see Materials and Methods). As well, it is possible that pharmacokinetic differences related to race exist in the metabolism of thienopyridine antiplatelet drugs.

Although antiplatelet resistance has been defined by *in vitro* platelet function, there appears a widespread misunderstanding that *in vitro* platelet function directly represents inhibition of a drug target.[30] Here, we found that platelet aggregation elicited by different agonists were significantly correlated with each other and associated with small aggregate formation without or with lower agonist stimulation. These data suggest that the platelet aggregability under dual-antiplatelet therapy may be determined by differences in the thresholds of each patient's platelets, rather than by differences in antiplatelet drug efficacies. Our finding is supported by recent reports that a 150 mg maintenance dose of clopidogrel is associated with enhanced antiplatelet effects compared with a 75 mg dose, although suboptimal responses were still present in 60% of patients.[34] Furthermore, Michelson *et al.*[35] reported that pre-existing variability in platelet responses to ADP accounts for clopidogrel resistance assessed by aggregometory.

We previously showed that an unknown factor, other than COX-1, determines inter-individual differences in platelet aggregation in aspirin-treated patients.[17] In this study, only fasting glucose level was significantly correlated with platelet aggregability, regardless of diabetes mellitus. Acute hyperglycemia during oral glucose tolerance tests was correlated with the number of small platelet aggregates.[28] Angiolillo *et al.*[34] reported that patients with hyperglycemia exhibit increased platelet reactivity, despite dual-antiplatelet therapy, that continues to persist even after administration of a higher maintenance dose of clopidogrel. These findings indicate the importance of suppressing transient hyperglycemia by tight glucose control to prevent thrombotic complications after PCI. Indeed, elevated plasma glucose, with or without a diabetic status, was reportedly an independent predictor of outcomes in acute coronary syndrome patients.[36,37]

Treatment failure under antiplatelet drug therapy may be influenced by many factors. The coagulation cascade and its regulation are important contributors to clinical events after PCI.[19–21]

Table 4 Spearman's correlation coefficients between patient characteristics and thrombogenetic factors in patients taking dual-antiplatelet therapy

	Platelet aggregation[a]	PAI-1	E-selectin	D-dimer	Soluble fibrin
Patient characteristics					
Age (years)	0.038 ($P = 0.729$)	−0.051 ($P = 0.645$)	−0.241 ($P = 0.026$)*	0.167 ($P = 0.127$)	0.079 ($P = 0.472$)
BMI (kg/m^2)	−0.057 ($P = 0.804$)	0.234 ($P = 0.032$)*	0.310 ($P = 0.004$)**	−0.254 ($P = 0.020$)*	−0.163 ($P = 0.139$)
Hypertension	0.042 ($P = 0.452$)	0.181 ($P = 0.132$)	−0.166 ($P = 0.128$)	−0.016 ($P = 0.396$)	0.029 ($P = 0.794$)
Diabetes mellitus	0.063 ($P = 0.570$)	−0.150 ($P = 0.172$)	0.253 ($P = 0.019$)*	−0.009 ($P = 0.943$)	0.145 ($P = 0.186$)
Prior myocardial infarction	−0.106 ($P = 0.334$)	0.058 ($P = 0.598$)	0.131 ($P = 0.131$)	−0.273 ($P = 0.011$)*	−0.046 ($P = 0.579$)
Number of vessel diseases	0.089 ($P = 0.420$)	−0.006 ($P = 0.953$)	0.011 ($P = 0.918$)	−0.185 ($P = 0.073$)	0.248 ($P = 0.022$)*
Biochemical markers					
Fasting glucose	0.243 ($P = 0.025$)*	−0.119 ($P = 0.277$)	0.205 ($P = 0.059$)	0.090 ($P = 0.411$)	−0.097 ($P = 0.378$)
Total cholesterol	−0.010 ($P = 0.928$)	−0.125 ($P = 0.256$)	−0.154 ($P = 0.158$)	0.095 ($P = 0.387$)	0.426 ($P = < 0.001$)***
BNP	0.146 ($P = 0.191$)	−0.071 ($P = 0.527$)	−0.177 ($P = 0.111$)	0.411 ($P = < 0.001$)***	0.296 ($P = 0.005$)**
Adrenalin	0.099 ($P = 0.367$)	0.169 ($P = 0.122$)	0.022 ($P = 0.843$)	0.002 ($P = 0.969$)	0.148 ($P = 0.177$)
hsCRP	0.009 ($P = 0.932$)	0.115 ($P = 0.295$)	0.132 ($P = 0.230$)	0.064 ($P = 0.581$)	0.049 ($P = 0.655$)
Physiological markers					
AHI	0.048 ($P = 0.671$)	0.304 ($P = 0.005$)**	0.269 ($P = 0.015$)*	0.082 ($P = 0.465$)	0.111 ($P = 0.320$)
ABI	−0.078 ($P = 0.480$)	−0.010 ($P = 0.920$)	−0.111 ($P = 0.836$)	−0.009 ($P = 0.933$)	−0.452 ($P = < 0.001$)***
24-h SBP	−0.128 ($P = 0.494$)	0.109 ($P = 0.333$)	0.135 ($P = 0.231$)	−0.056 ($P = 0.620$)	0.032 ($P = 0.779$)
24-h DBP	−0.188 ($P = 0.255$)	0.148 ($P = 0.186$)	0.292 ($P = 0.008$)**	−0.042 ($P = 0.707$)	−0.120 ($P = 0.285$)

[a]Light transmission assessed by 1 μg/mL of collagen.

BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; BNP, brain natriuretic peptide; PAI-1, plasminogen activator inhibitor-1; SBP, systolic blood pressure; DBP, diastolic blood pressure; AHI, apnea–hypopnea index; ABI, ankle-brachial index.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

Table 5 Multivariate analyses for determination of thrombogenetic factors in patients taking dual-antiplatelet therapy

	(R^2; P)	Variables	β	β (95%CI)	P
PAI-I	(0.09, 0.006)	BMI	0.167	–	0.147
		AHI	0.300	0.402 (0.116–0.687)	0.006
E-selectin	(0.203, <0.001)	Age	−0.127	–	0.236
		BMI	0.323	0.983 (0.365–1.601)	0.002
		Diabetes mellitus	0.279	5.736 (1.566–9.906)	0.008
		AHI	0.126	–	0.253
		24-h DBP	0.169	–	0.136
D-dimer	(0.126, 0.001)	BMI	−0.064	–	0.564
		Prior MI	−0.075	–	0.484
		BNP	0.356	1.928 (0.793–3.063)	0.001
SF	(0.366, <0.001)	Number of VD	0.085	–	0.372
		Total cholesterol	0.349	0.075 (0.035–0.113)	<0.001
		BNP	0.222	3.681 (0.651–6.711)	0.018
		ABI	−0.330	−17.953 (−28.203–7.704)	0.001

β, standardized coefficient; CI, confidence interval; PAI-1, plasminogen activator inhibitor-1; AHI, apnea–hypopnea index; BMI, body mass index; BNP, brain natriuretic peptide; DBP, diastolic blood pressure; ABI, ankle-brachial index; VD, vessel diseases.

Activated platelets provide phosphatidylserine exposure on their surface that provokes the coagulation cascade, thereby amplifying thrombin generation.[38,39] However, residual platelet activation was not correlated with systemic thrombin generation assessed by plasma SF and resultant fibrinolytic activation assessed by the D-dimer level. The major determinant of thrombin generation was found to be independently associated with total cholesterol, BNP, and ABI, suggesting that thrombin generation in PCI subjects under dual-antiplatelet therapy is mainly determined by the degree of impaired cardiac function and/or arteriosclerosis. Plasma PAI-1 was also associated with the presence of sleep apnea syndrome. Although circulating platelets account for increases in plasma PAI-1 and release it following activation,[40] platelet aggregability was not associated with PAI-1. Taken together, these data suggested that many factors may be involved in systemic thrombogenicity, independent of platelet aggregability.

Our data suggested that some patients may benefit from the addition of anticoagulant treatment after PCI. The American College of Cardiology/American Heart Association guidelines recommend anticoagulant therapy in patients with an acute ST-elevation myocardial infarction with extensive regional wall motion abnormalities. However, the routine use of anticoagulant drugs without thienopyridine should be avoided in patients who have undergone PCI because treatment with aspirin and ticlopidine results in a lower rate of stent thrombosis as compared with a combination of aspirin plus warfarin.[41] No trial has closely evaluated the safety and efficacy of anticoagulant therapy in combination with dual-antiplatelet therapy in patients undergoing PCI. Large-scale trials are thus needed to confirm any recommendations. Our study should be interpreted in light of its limitations; for ethical reasons we could not obtain proper control patients who had not taken any antiplatelet drug after PCI. This was because dual-antiplatelet therapy is the gold standard to reduce clinical events in patients who have undergone PCI.

In conclusion, the current study has demonstrated that dual-antiplatelet therapy effectively inhibited its pharmacological targets, although we found inter-individual variability in platelet aggregation, which was at least partly explained by hyperglycemia. On the other hand, thrombin generation, inhibition of fibrinolytic activity, and endothelial dysfunction were not determined by platelet aggregability, but by other aspects of the patients' backgrounds, such as obesity, sleep apnea, diabetes mellitus, cardiac dysfunction, and/or atherosclerotic burden. Our findings indicated that some patients remain at risk of subsequent thrombotic complications after PCI despite adequate dual-antiplatelet therapy. Large-scale prospective studies are required to determine which markers are associated with the risk of further cardiovascular events after PCI and to examine interventions such as tight plasma glucose control, anticoagulation, and continuous positive air way pressure therapy.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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