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Original article

Serum interleukin-18 levels as a predictor for patients with genetic dysfunction of cytochrome P450 2C19 in dual antiplatelet therapy with clopidogrel

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ABSTRACT

Background: $P2Y_{12}$ reaction unit (PRU) is an index of platelet activity upon treatment with clopidogrel. In spite of suitable $P2Y_{12}$ reactions in dual antiplatelet therapy (DAPT) with clopidogrel after percutaneous coronary intervention (PCI), cardiovascular events actually occur in some patients, possibly due to a genetic dysfunction of cytochrome P450 2C19 (CYP2C19), which is a major metabolic enzyme of clopidogrel. As testing the CYP2C19 phenotypes to predict such patients may lack general versatility in daily clinical practice, the aim of this study was to examine whether measuring the blood levels of some cytokines in patients showing desirable PRUs in DAPT with clopidogrel could be a substitute for testing the CYP2C19 phenotypes.

Methods: We analyzed relationships among PRU, serum levels of 51 cytokines, and CYP2C19 phenotypes in 22 patients receiving DAPT with aspirin and clopidogrel after PCI.

Results: Seventeen, 18, and 19 of 22 patients indicated PRU $\leq$ 208, PRU $\leq$ 230, and PRU $\leq$ 262, respectively. Approximately 60% of the patients had a genetically metabolic dysfunction of CYP2C19, and the serum levels of interleukin-18 were independently increased in those patients ($p = 0.024$ in patients with PRU $\leq$ 208, $p = 0.021$ with PRU $\leq$ 230, and $p = 0.020$ with PRU $\leq$ 262). The area under the curves in plot receiver operating characteristics curves for the serum levels of interleukin-18 were 0.94, 0.96, and 0.90 in the non-extensive metabolizer patients with PRU $\leq$ 208, PRU $\leq$ 230, and PRU $\leq$ 262, respectively.

Conclusions: The serum levels of interleukin-18 may be a predictor to diagnose patients who receive undesirable DAPT with clopidogrel, possibly due to the genetic dysfunction of CYP2C19 in spite of suitable $P2Y_{12}$ reactions after PCI.

Introduction

Dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ inhibitor is recommended to prevent major adverse cardiovascular events including stent thrombosis in patients after percutaneous coronary intervention (PCI) [1]. $P2Y_{12}$ reaction unit (PRU), which is an index of platelet activity in the presence of a $P2Y_{12}$ inhibitor, such as clopidogrel, prasugrel, and ticagrelor, is occasionally measured to assess the effect of DAPT with the $P2Y_{12}$ inhibitor. Studies in Caucasians have reported that stent thrombosis and myocardial infarction after PCI occurred more frequently in patients with PRU > 208 [2,3] and PRU > 230 [4] than in those with PRU $\leq$ 208 and PRU $\leq$ 230 in DAPT with aspirin and clopidogrel, respectively. Another study in the Japanese population has reported that cardiovascular death, non-fatal myocardial infarction, and stroke after PCI occurred more frequently in patients with PRU > 262 than in patients with

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PRU ≤ 262 in DAPT with aspirin and clopidogrel or prasugrel [5]. These reports suggest that it might be necessary to change DAPT with clopidogrel to an alternative DAPT with another antiplatelet agent in patients with PRU > 208 after PCI. Meanwhile, these studies have also reported that considerable major adverse cardiovascular events in DAPT with clopidogrel after PCI, such as stent thrombosis for 0.3–0.8% of the patients, myocardial infarction for 0.9–4.0% of the patients [2–4], and composite events for 1.4–5.0% of the patients [4,5], actually occurred in the patients despite indicating first PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262. These unexpected cardiovascular events in the patients might be due to transient incidences of undesirable DAPT with clopidogrel. The undesirable DAPT might be attributed to transient increases in PRU from a desirable baseline.

Clopidogrel is a pro-drug that is principally metabolized by an enzyme, cytochrome P450 (CYP) 2C19. Its active metabolite binds to the $P2Y_{12}$ receptor as an adenosine diphosphate receptor on platelets for inhibiting platelet aggregation. The polymorphism of CYP2C19 involves the antiplatelet effect of clopidogrel [6], and the CYP2C19 phenotypes are classified based on the rate of the drug metabolizing activity into three groups: extensive metabolizer (EM), intermediate metabolizer (IM), and poor metabolizer (PM) [7]. Non-EM that is composed of the IM and PM indicates metabolic dysfunction for clopidogrel and the prevalence of the non-EM has been reported to be higher in the Japanese population than in Western populations [8,9]. In patients receiving DAPT with clopidogrel after PCI, a higher incidence of cardiovascular events has occurred in patients with CYP2C19 gene variation than in patients without the variation [7,10,11]. Nagashima et al. have reported that PRU of the non-EM patients was higher than PRU of the EM patients in not only an early but also a late phase of acute coronary syndrome [12]. Taken together, it is considered that genetic metabolic dysfunction for clopidogrel fails to inhibit platelet aggregation sufficiently and takes the patients to a higher incidence of cardiovascular events with increased PRU. Moreover, there is a possibility that a transient augmentation of the genetic metabolic dysfunction for clopidogrel has some reasons that it unexpectedly increases PRU in patients who have ever indicated desirable PRU in DAPT with clopidogrel. Accordingly, both measuring PRU and testing CYP2C19 phenotypes may be desirable for patients receiving DAPT with clopidogrel after PCI to estimate whether the DAPT is appropriate to prevent cardiovascular events in the future. However, these examinations lack general versatility due to cost, implying the necessity of affordable examinations to diagnose undesirable DAPT with clopidogrel.

Previous epidemiological studies have reported the relationships between inflammatory biomarkers, such as C-reactive protein and pro-inflammatory cytokines, and cardiovascular diseases [13–15]. Although Frye et al. have reported an inverse relationship between both tumor necrosis factor-α and interleukin-6 plasma concentrations and the activity of CYP2C19 in patients with congestive heart failure [16], there are no reports on any relationships among the blood levels of cytokines, phenotypes of CYP2C19, and PRU in patients receiving DAPT with clopidogrel after PCI. Therefore, in this study, we examined those relationships in the blood levels of 51 cytokines and evaluated whether measuring the blood levels of some cytokines could contribute to an easy diagnosis of undesirable CYP2C19 phenotypes in patients showing desirable PRUs.

Materials and methods

Subjects

This study was conducted as a part of a clinical study named "CONVERT 2" [17], which was a multicenter, randomized, open-label, parallel-group comparison study, in accordance with the Declaration of Helsinki, ethical guidelines for clinical research, ethical guidelines for human genome/gene analysis research and followed the ICH-GCP guidelines. Full details of inclusion and exclusion criteria for subjects in this study were published previously [17]. All subjects received DAPT with aspirin (81 or 100 mg/day) and clopidogrel (75 mg/day). This study was approved by the Committees on the Ethics Review Board of the Kurume University School of Medicine.

Measurement of PRU and test of CYP2C19 phenotypes

The PRU value was measured using the VerifyNow-$P2Y_{12}$ assay system (Instrumentation Laboratory, Bedford, MA, USA) [18]. The assay is a rapid platelet-function cartridge-based assay designed to measure directly the effects of drugs on the $P2Y_{12}$ receptor. We obtained blood samples from an antecubital vein using a 21-gauge needle. A part of the blood samples was drawn into two 1.8-mL blood collection tubes containing 0.2 mL buffered 3.2% sodium citrate solution and PRU was measured within 2 h as suggested by the manufacturers. Meanwhile, a part of the blood samples was drawn into an EDTA-2Na tube for testing CYP2C19 phenotypes by a real-time polymerase chain reaction method in an external laboratory (SRL Co., Tokyo, Japan).

Measurement of serum cytokines and high-sensitivity C-reactive protein

The serum of blood samples was stored at -80 °C. The concentrations of 50 types of cytokines in the samples were measured with a magnetic bead-based multiplex assay kit (Bio-Plex Pro™ Human Cytokine Standard Group I 27-Plex, Group II 21-Plex, and TGF-β 3-Plex, Bio-Rad Laboratories, Hercules, CA, USA). The 51 cytokines were as follows: interleukin (IL)-1β, IL-1 receptor α (IL-1rα), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic fibroblast growth factor (b-FGF), eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), MIP-1β, platelet-derived growth factor-BB (PDGF-BB), regulated upon activation normal T-cell expressed and secreted (RANTES), tumor necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF), cutaneous T-cell-attracting chemokine (CTACK), growth-regulated alpha (GRO-α), hepatocyte growth factor (HGF), IFN-α2, IL-1α, IL-2Rα, IL-3, IL-12 (p40), IL-16, IL-18, leukemia inhibitory factor (LIF), MCP-3, macrophage colony-stimulating factor (M-CSF), macrophage migration inhibitory factor (MIF), macrophage-induced gene (MIG), β-nerve growth factor (β-NGF), stem cell factor (SCF), stem cell growth factor-β (SCGF-β), stromal cell-derived factor-1α (SDF-1α), TNF-β, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), transforming growth factor-β1 (TGF-β1), TGF-β2, and TGF-β3. In addition, we measured the concentration of erythropoietin (EPO) with an enzyme-linked immunosorbent assay kit (abcam, Boston, MA, USA). All assays were performed according to the manufacturer's instructions [19]. High-sensitivity C-reactive protein (hs-CRP) was measured by nephelometory in an external laboratory (SRL Co.).

Statistical analysis

Continuous variables are presented as the mean ± SD. Statistical comparisons of baseline characteristics and serum cytokine concentrations between the EM and the non-EM were performed using unpaired two-sample Student's *t*-test, Wilcoxon rank sum

Table 1
Baseline characteristics of 22 patients.

Characteristics	
Age, years	71.0 ± 12.0
Height, cm	159.0 ± 7.3
Weight, kg	61.8 ± 12.2
Body mass index, kg/m^2	24.3 ± 4.1
Male, n (%)	14 (63.6)
Female, n (%)	8 (36.4)
Current smoking, n (%)	9 (40.9)
Hypertension, n (%)	19 (86.4)
Dyslipidemia, n (%)	18 (81.8)
Diabetes mellitus, n (%)	17 (77.3)
CYP2C19 genotype	
Extensive metabolizer, n (%)	7 (31.8)
Intermediate metabolizer, n (%)	13 (59.1)
Poor metabolizer, n (%)	2 (9.1)
Diagnosis of CAD before PCI	
Effort angina	5 (22.7)
Unstable angina	4 (18.2)
Acute myocardial infarction, n (%)	7 (31.8)
Silent myocardial infarction, n (%)	6 (27.3)
Duration of DAPT, years	5.9 ± 2.8

CAD, coronary artery disease; DAPT, dual antiplatelet therapy; PCI, percutaneous coronary intervention.
Parts of data are described by mean ± standard deviation.

test, and Fisher's exact test. Statistically significant differences in the baseline characteristics were used as confounding factors for logistic regression analyses. The point-biserial correlation coefficient between a continuous variable and a dichotomous variable was calculated by applying the Pearson correlation coefficient. A multiple stepwise regression analysis was performed to identify independently-associated serum cytokines for the diagnosis of CYP2C19 phenotypes in each patient group with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262. For the identified cytokines, a logistic regression analysis was performed to estimate the association between serum cytokine concentrations and CYP2C19 phenotypes, calculate odds ratios, and plot receiver operating characteristics (ROC) curves. Odds ratios were calculated with 95% confidence interval (CI). Statistical significance was assumed at a value of $p < 0.05$. Data were analyzed using JMP Pro 13.0 (SAS Institute, Cary, NC, USA).

Results

Twenty-two patients participated in this study. Table 1 shows the patients' characteristics. The average duration of DAPT was 5.9 ± 2.8 years. The percentages of patients who were EM and non-EM were 31.8% and 68.2% of the participants, respectively. Seventeen (77.3%) of the 22 patients, 18 (81.8%) of the 22, and 19 (86.4%) of the 22 indicated PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262, respectively (Fig. 1A, B, and C). All patients with PRU > 208, PRU > 230, and PRU > 262 were non-EM; we were unable to compare the serum concentrations of 51 cytokines between the EM patients and the non-EM patients. In the 17 patients with PRU ≤ 208, 7 (42.2%) and 10 (58.8%) patients were EM and non-EM, respectively (Fig. 1A). In the 18 patients with PRU ≤ 230, 7 (38.9%) and 11 (61.1%) were EM and non-EM, respectively (Fig. 1B). In the 19 patients with PRU ≤ 262, 7 (36.8%) and 12 (63.2%) were EM and non-EM, respectively (Fig. 1C). In the baseline characteristics of the EM and the non-EM patients with PRU ≤ 208, patients with PRU ≤ 230, and patients with PRU ≤ 262 (Online Table 1), there was a significant difference in sex between the EM and the non-EM in patients with PRU ≤ 230 ($p = 0.047$), suggesting that sex was a confounding factor for the following analysis in patients with PRU ≤ 230.

GM-CSF, IFN-α2, IL-2, IL-5, IL-12 (p40), IL-12 (p70), IL-15, LIF, and TNF-β were not detected in the serum of the 22 patients. IL-10, MCP-3, and VEGF were detected in the serum of a small number of the 22 patients. In the 17 patients with PRU ≤ 208, the serum concentrations of INF-γ, IL-17, IL-18, and M-CSF were significantly greater in the non-EM patients than in the EM patients (Table 2). In the 18 patients with PRU ≤ 230, a sex-adjusted logistic regression analysis showed that the serum concentrations of IL-1α and IL-18 were significantly greater in the

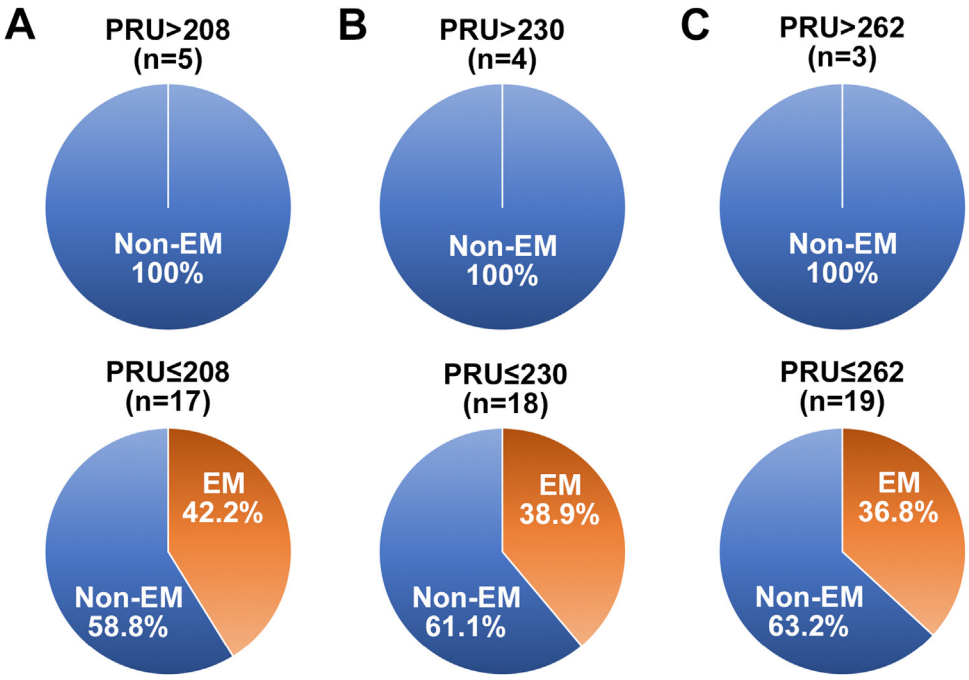

Fig. 1. Percentages of the EM and non-EM in patients with PRU > 208, PRU ≤ 208 (A), PRU > 230, PRU ≤ 230 (B), PRU > 262, and PRU ≤ 262 (C) in DAPT with clopidogrel. EM, extensive metabolizers for cytochrome p450 2C19; PRU, $P2Y_{12}$ reaction unit; DAPT, dual anti-platelet therapy.

Table 2
Serum cytokines' levels in two types of CYP2C19 metabolizers.

		PRU ≤ 208 (n = 17)		
		EM (n = 7)	Non-EM (n = 10)	P-Value
CTACK	($\times 10^2$ pg/mL)	7.9 ± 2.3	9.2 ± 2.9	0.344
Eotaxin	($\times 10$ pg/mL)	7.3 ± 4.1	9.5 ± 2.3	0.174
EPO	(mIU/mL)	6.7 ± 4.7	5.6 ± 3.3	0.581
G-CSF	($\times 10^2$ pg/mL)	1.2 ± 0.7	1.6 ± 0.4	0.150
GM-CSF		N.D	N.D	
HGF	($\times 10^2$ pg/mL)	2.6 ± 0.9	2.7 ± 1.3	0.891
IFN-α2		N.D	N.D	
IFN-γ	(pg/mL)	3.1 ± 0.7	4.4 ± 1.3	**0.030**
IL-1α	(pg/mL)	5.5 ± 6.3	3.9 ± 2.4	0.624
IL-1Ra	($\times 10^2$ pg/mL)	1.1 ± 0.4	1.6 ± 0.8	0.218
IL-2		N.D	N.D	
IL-2Ra	($\times 10$ pg/mL)	3.2 ± 1.2	4.8 ± 1.8	0.055
IL-3	($\times 10^{-1}$ mIU/mL)	1.0 ± 0.3	1.3 ± 0.3	0.110
IL-5		N.D	N.D	
IL-7	($\times 10$ pg/mL)	1.2 ± 0.7	1.5 ± 0.4	0.206
IL-8	(pg/mL)	5.9 ± 1.5	7.6 ± 3.4	0.230
IL-9	($\times 10$ pg/mL)	4.1 ± 1.0	4.9 ± 0.3	**0.125**
IL-12 (p40)		N.D	N.D	
IL-12 (p70)		N.D	N.D	
IL-13	(pg/mL)	0.8 ± 0.5	0.9 ± 0.4	0.714
IL-15		N.D	N.D	
IL-16	($\times 10$ pg/mL)	3.0 ± 0.9	3.7 ± 1.6	0.358
IL-17	($\times 10$ pg/mL)	1.0 ± 0.4	1.3 ± 0.2	**0.044**
IL-18	($\times 10$ pg/mL)	2.5 ± 1.2	5.3 ± 1.5	**0.001**
IP-10	($\times 10^2$ pg/mL)	7.8 ± 4.6	8.1 ± 7.2	0.907
LIF		N.D	N.D	
MCP-1	($\times 10$ pg/mL)	2.5 ± 1.2	2.5 ± 1.6	0.963
M-CSF	($\times 10$ pg/mL)	1.0 ± 0.3	1.7 ± 0.6	0.012
MIF	($\times 10^2$ pg/mL)	5.0 ± 1.8	5.7 ± 2.4	0.514
MIG	($\times 10^2$ pg/mL)	6.5 ± 8.6	5.4 ± 6.1	0.772
MIP-1α	(pg/mL)	2.5 ± 0.7	2.4 ± 1.1	0.796
MIP-1β	($\times 10$ pg/mL)	3.8 ± 1.0	4.0 ± 0.9	0.620
PDGF-BB	($\times 10^3$ pg/mL)	1.8 ± 0.8	2.8 ± 1.4	0.115
RANTES	($\times 10^3$ pg/mL)	5.5 ± 1.7	6.0 ± 1.9	0.594
SCF	($\times 10$ pg/mL)	9.5 ± 4.5	9.4 ± 3.1	0.951
SCGF-β	($\times 10^5$ pg/mL)	1.1 ± 0.4	1.3 ± 0.4	0.412
SDF-1α	($\times 10^2$ pg/mL)	2.8 ± 0.5	3.1 ± 0.9	0.436
TGF-β1	($\times 10^4$ pg/mL)	3.9 ± 1.8	4.8 ± 1.2	0.204
TGF-β2	($\times 10^2$ pg/mL)	3.3 ± 0.3	3.5 ± 0.5	0.330
TGF-β3	($\times 10$ pg/mL)	4.7 ± 1.0	5.4 ± 0.4	0.111
TNF-α	($\times 10$ pg/mL)	1.1 ± 0.2	1.3 ± 0.3	0.163
TNF-β		N.D	N.D	
TRAIL	($\times 10$ pg/mL)	2.0 ± 0.6	2.6 ± 0.8	0.105
Log hs-CRP	(mg/dL)	2.9 ± 0.7	2.9 ± 0.4	0.807
		EM (n = 5)	**Non-EM (n = 10)**	
FGF basic	($\times 10$ pg/mL)	0.9 ± 0.3	1.1 ± 0.5	0.437
		EM (n = 5)	**Non-EM (n = 8)**	
IL-1β	(pg/mL)	0.6 ± 0.6	0.5 ± 0.3	0.782
		EM (n = 4)	**Non-EM (n = 10)**	
IL-4	(pg/mL)	0.6 ± 0.5	0.8 ± 0.5	0.598
		EM (n = 4)	**Non-EM (n = 9)**	
GROα	($\times 10$ pg/mL)	6.0 ± 2.9	10.3 ± 4.9	0.145
		EM (n = 6)	**Non-EM (n = 5)**	
IL-6	(pg/mL)	0.2 ± 0.1	0.6 ± 0.3	0.098
		EM (n = 1)	**Non-EM (n = 6)**	
MCP-3	($\times 10^{-1}$ pg/mL)	4.7	1.9 ± 1.1	
		EM (n = 2)	**Non-EM (n = 4)**	
VEGF	($\times 10^2$ pg/mL)	2.8 ± 3.8	2.3 ± 3.7	0.873
		EM (n = 2)	**Non-EM (n = 3)**	
IL-10	(pg/mL)	1.5 ± 1.9	1.9 ± 1.4	0.847

	PRU ≤ 230 (n = 18)			PRU ≤ 262 (n = 19)		
	EM (n = 7)	Non-EM (n = 11)	P-value	EM (n = 7)	Non-EM (n = 12)	P-value
CTACK	7.9 ± 2.3	9.3 ± 2.7	0.538	7.9 ± 2.3	9.2 ± 2.6	0.303
Eotaxin	7.3 ± 4.1	9.2 ± 2.4	0.946	7.3 ± 4.1	9.5 ± 2.7	0.151
EPO	6.7 ± 4.7	5.7 ± 3.2	0.478	6.7 ± 4.7	6.6 ± 4.4	0.981
G-CSF	1.2 ± 0.7	1.5 ± 0.4	0.782	1.2 ± 0.7	1.6 ± 0.4	0.159
GM-CSF	N.D	N.D		N.D	N.D	
HGF	2.6 ± 0.9	2.6 ± 1.2	0.949	2.6 ± 0.9	2.7 ± 1.2	0.863
IFN-α2	N.D	N.D		N.D	N.D	
IFN-γ	3.1 ± 0.7	4.3 ± 1.3	0.454	3.1 ± 0.7	4.2 ± 1.3	0.056
IL-1α	5.5 ± 6.3	3.9 ± 2.3	**0.046**	5.5 ± 6.3	3.8 ± 2.2	0.552
IL-1Ra	1.1 ± 0.4	1.6 ± 0.8	0.392	1.1 ± 0.4	1.5 ± 0.8	0.299
IL-2	N.D	N.D		N.D	N.D	

Table2 (*Continued*)

	PRU ≤ 230 (n = 18)			PRU ≤ 262 (n = 19)		
	EM (n = 7)	Non-EM (n = 11)	P-value	EM (n = 7)	Non-EM (n = 12)	P-value
IL-2Ra	3.2 ± 0.6	4.6 ± 0.4	0.714	3.2 ± 0.6	4.6 ± 0.4	0.066
IL-3	1.0 ± 0.3	1.3 ± 0.3	0.667	1.0 ± 0.3	1.2 ± 0.3	0.103
IL-5	N.D	N.D		N.D	N.D	
IL-7	1.2 ± 0.7	1.5 ± 0.3	0.219	1.2 ± 0.7	1.5 ± 0.3	0.091
IL-8	5.9 ± 1.5	7.2 ± 3.5	0.249	5.9 ± 1.5	7.0 ± 3.5	0.444
IL-9	4.1 ± 1.0	4.9 ± 1.0	0.267	4.1 ± 1.0	4.9 ± 0.3	0.107
IL-12 (p40)	N.D	N.D		N.D	N.D	
IL-12 (p70)	N.D	N.D		N.D	N.D	
IL-13	0.8 ± 0.5	0.9 ± 0.4	0.667	0.8 ± 0.5	0.9 ± 0.4	0.754
IL-15	N.D	N.D		N.D	N.D	
IL-16	3.0 ± 0.9	3.8 ± 1.6	0.334	3.0 ± 0.9	3.7 ± 1.6	0.612
IL-17	1.0 ± 0.4	1.2 ± 0.2	0.520	1.0 ± 0.4	1.3 ± 0.2	**0.049**
IL-18	2.5 ± 1.2	5.2 ± 1.5	**0.003**	2.5 ± 1.2	4.9 ± 1.7	**0.004**
IP-10	7.8 ± 4.6	7.6 ± 7.0	0.822	7.8 ± 4.6	8.1 ± 7.0	0.903
LIF	N.D	N.D		N.D	N.D	
MCP-1	2.5 ± 1.2	2.4 ± 1.6	0.620	2.5 ± 1.2	2.6 ± 1.5	0.943
M-CSF	1.0 ± 0.2	1.6 ± 0.1	0.351	1.0 ± 0.2	1.6 ± 0.6	**0.012**
MIF	5.0 ± 1.8	5.9 ± 2.4	0.325	5.0 ± 1.8	5.7 ± 2.4	0.528
MIG	6.5 ± 8.6	5.1 ± 5.9	0.591	6.5 ± 8.6	5.0 ± 5.7	0.666
MIP-1α	2.5 ± 0.7	2.3 ± 1.0	0.463	2.5 ± 0.7	2.2 ± 1.1	0.550
MIP-1β	3.8 ± 1.0	4.0 ± 0.8	0.984	3.8 ± 1.0	3.9 ± 0.9	0.796
PDGF-BB	1.8 ± 0.8	2.7 ± 1.4	0.665	1.8 ± 0.8	2.6 ± 1.5	0.240
RANTES	5.5 ± 1.7	6.2 ± 1.8	0.947	5.5 ± 1.7	6.0 ± 1.8	0.576
SCF	9.5 ± 4.5	9.3 ± 2.9	0.921	9.5 ± 4.5	9.2 ± 2.8	0.875
SCGF-β	1.1 ± 0.4	1.3 ± 0.4	0.999	1.1 ± 0.4	1.3 ± 0.4	0.337
SDF-1α	2.8 ± 0.5	3.1 ± 0.9	0.171	2.8 ± 0.5	3.1 ± 0.9	0.474
TGF-β1	3.9 ± 1.8	4.8 ± 1.2	0.801	3.9 ± 1.8	4.6 ± 1.2	0.283
TGF-β2	3.3 ± 0.3	3.5 ± 0.5	0.822	3.3 ± 0.3	3.4 ± 0.5	0.375
TGF-β3	4.7 ± 1.0	5.4 ± 0.4	0.182	4.7 ± 1.0	5.3 ± 0.4	0.163
TNF-α	1.1 ± 0.2	1.3 ± 0.3	0.512	1.1 ± 0.1	1.2 ± 0.1	0.280
TNF-β	N.D	N.D		N.D	N.D	
TRAIL	2.0 ± 0.6	2.5 ± 0.8	0.051	2.0 ± 0.6	2.5 ± 0.8	0.140
Log hs-CRP	2.9 ± 0.7	2.8 ± 0.4	0.518	2.9 ± 0.7	2.9 ± 0.3	0.790
	EM (n = 5)	**Non-EM (n = 11)**		**EM (n = 5)**	**Non-EM (n = 12)**	
FGF basic	0.9 ± 0.3	1.1 ± 0.4	0.490	0.9 ± 0.3	1.1 ± 0.4	0.357
	EM (n = 5)	**Non-EM (n = 9)**		**EM (n = 5)**	**Non-EM (n = 10)**	
IL-1β	0.6 ± 0.6	0.5 ± 0.3	0.541	0.6 ± 0.6	0.4 ± 0.3	0.524
	EM (n = 4)	**Non-EM (n = 11)**		**EM (n = 4)**	**Non-EM (n = 12)**	
IL-4	0.6 ± 0.5	0.7 ± 0.5	0.791	0.6 ± 0.5	0.8 ± 0.5	0.598
	EM (n = 4)	**Non-EM (n = 10)**		**EM (n = 4)**	**Non-EM (n = 10)**	
GROα	6.0 ± 2.9	9.9 ± 4.8	0.162	6.0 ± 2.9	9.9 ± 4.8	0.160
	EM (n = 6)	**Non-EM (n = 6)**		**EM (n = 6)**	**Non-EM (n = 6)**	
IL-6	0.2 ± 0.1	0.6 ± 0.3	0.417	0.2 ± 0.1	0.6 ± 0.3	**0.016**
	EM (n = 1)	**Non-EM (n = 7)**		**EM (n = 1)**	**Non-EM (n = 7)**	
MCP-3	4.7	1.7 ± 1.1	0.437	4.7	1.7 ± 1.1	
	EM (n = 2)	**Non-EM (n = 4)**		**EM (n = 2)**	**Non-EM (n = 4)**	
VEGF	2.8 ± 3.8	2.3 ± 3.7	0.871	2.8 ± 3.8	2.3 ± 3.7	0.873
	EM (n = 2)	**Non-EM (n = 3)**		**EM (n = 2)**	**Non-EM (n = 4)**	
IL-10	1.5 ± 1.9	1.8 ± 1.4	0.062	1.5 ± 1.9	1.7 ± 1.2	0.836

CYP2C19, cytochrome P450 2C19; PRU, $P2Y_{12}$ reaction unit; EM, extensive metabolizers for cytochrome p450 2C19; CTACK, cutaneous T-cell-attracting chemokine; EPO, erythropoietin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGF, hepatocyte growth factor; IFN, interferon; IL. interleukin; LIF, leukemia inhibitory factor; MCP, monocyte chemoattractant protein; M-CSF, macrophage colony-stimulating factor; MIF, macrophage migration inhibitory factor; MIG, macrophage-induced gene; MIP, macrophage inflammatory protein; PDGF-BB, platelet-derived growth factor-BB; RANTES, regulated upon activation normal T-cell expressed and secreted; SCF, stem cell factor; SCGF, stem cell growth factor; SDF, stromal cell-derived factor; TGF, transforming growth factor; TNF, tumor necrosis factor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; hs-CRP, high-sensitivity C-reactive protein; FGF, fibroblast growth factor; GROα, growth-regulated alpha; VEGF, vascular endothelial growth factor. Data are described by mean ± standard deviation. *p*-values in patients with PRU ≤ 230 are adjusted for sex. *p*-values in bold are significant at $p < 0.05$.

non-EM patients than in the EM patients (Table 2). In the 19 patients with PRU ≤ 262, the serum concentrations of IL-6, IL-17, IL-18, and M-CSF were significantly greater in the non-EM patients than in the EM patients (Table 2). The serum levels of IL-18, but not IL-1α, IL-6, IL-17, INF-γ, or M-CSF, were significantly greater in the male patients than in the female patients (48.3 ± 15.8 vs. 24.1 ± 16.0, $p = 0.010$), suggesting that sex was a confounding factor for the following multiple stepwise regression analysis. The point-biserial correlation coefficient of the confounding factor was 0.57 for the serum level of IL-18 ($p = 0.013$). A sex-adjusted multiple stepwise regression analysis for several cytokines that were adopted from each population by the above univariate analysis picked out only IL-18 as a significant and independent cytokine that was increased in the non-EM patients with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262. Moreover, in a sex-adjusted logistic regression analysis, the serum levels of IL-18 were significantly correlated with the presence of the non-EM patients with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262 (Table 3). The area under the curves in ROC curves for the serum levels of IL-18 were 0.94 in the non-EM patients with PRU ≤ 208 (Fig. 2A), 0.96 in the non-EM patients with PRU ≤ 230 (Fig. 2B), and 0.90 in the non-EM patients with PRU ≤ 262 (Fig. 2C), respectively.

Table 3
Serum cytokines as predictors of non-EM patients with PRU ≤ 208, PRU ≤ 230, or PRU ≤ 262 in DAPT with clopidogrel.

		Odds ratio	95%CI	*p*-value
PRU ≤ 208	IL-18	1.154	1.019–1.307	0.024
PRU ≤ 230	IL-18	1.147	1.006–1.308	0.041
PRU ≤ 262	IL-18	1.118	1.018–1.229	0.020

EM, extensive metabolizers for cytochrome p450 2C19; PRU, $P2Y_{12}$ reaction unit; DAPT, dual antiplatelet therapy; CI, confidence interval; IL, interleukin.

Discussion

This study provided some new findings as follows: (1) approximately 60% of patients with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262 on long-term (average: 5.9 ± 2.8 years) DAPT with aspirin and clopidogrel after PCI were non-EM, (2) the serum levels of IL-18 independently increased in the non-EM patients with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262, and (3) although each sample size was small, the area under the curves in the ROC curves for the serum levels of IL-18 to diagnose the non-EM were higher than or equal to 0.9 in the non-EM patients with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262.

Approximately 68% of patients who participated in this study were non-EM, corresponding to the CYP2C19 phenotype distribution in previous studies of Japanese patients with coronary artery disease [8,11]. PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262 were reported as suitable indices of PRU to prevent cardiovascular events after PCI [2–5]. In this study, approximately 60% of patients with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262 surprisingly were non-EM, suggesting that a greater-than-expected number of patients in this study might be at a high risk of future cardiovascular events after PCI and it might be fortunate that no stent thrombosis or myocardial infarction after PCI occurred in those patients for a long period until they participated in this study. However, this result also corresponded to previous reports [8,11]. Given previous reports that a higher incidence of cardiovascular events in DAPT with clopidogrel after PCI occurred in patients with genetic dysfunction of CYP2C19 [7,10,11], cardiovascular events after PCI may occur in the non-EM patients even if they have ever indicated PRU ≤ 208, PRU ≤ 230, or PRU ≤ 262 at a certain point in DAPT with clopidogrel [2–5]. Nevertheless, in order to prevent considerable cardiovascular events after PCI in patients with PRU ≤ 208, PRU ≤ 230, or PRU ≤ 262 in DAPT with clopidogrel [2–5], it may be desirable to know both PRU and the CYP2C19 phenotype of the patients and thereby require an appropriate change to another $P2Y_{12}$ inhibitor from clopidogrel [20].

However, there is a problem that those examinations (i.e. measuring PRU and testing CYP2C19 phenotype) lack general versatility and convenience in clinical situations due to ethical and cost issues. In this study, we found that the serum levels of IL-18 independently increased in patients who were non-EM of CYP2C19 phenotypes but indicated PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262 in a long term DAPT with clopidogrel. Although this result may help medical facilities where PRU measurement is performed in daily medical practice, the problem has not been resolved yet. Further studies to find serum cytokines defined as biomarkers for the non-EM showing any PRUs are surely necessary for other medical facilities where PRU measurement is difficult in daily medical practice.

The pro-inflammatory cytokine IL-18 was identified as an INF-γ inducing factor in Kupffer cells and macrophages [21]. Mallat et al. have reported that macrophage- and smooth muscle cell-produced IL-18 expressions increase in unstable human atherosclerotic plaques and the increase of IL-18 expression is responsible for strokes [22]. In mice, an intraperitoneal administration of IL-18 has been reported to increase atherosclerotic lesion size [23]. These results have suggested the important role of IL-18 in atherogenesis. Blankenberg et al. have reported in prospective studies for stable coronary artery disease patients [24] and healthy middle-aged men [25] that IL-18 levels in the peripheral blood are independent predictors of cardiovascular events. In a meta-analysis of 29 prospective studies, a 1-SD higher baseline level for IL-18 has been associated with 13% higher risk of non-fatal myocardial infarction or coronary heart disease death [15]. Thus, IL-18 circulating in peripheral blood is assumed to be involved in the progression of cardiovascular disease.

It remains unclear why the serum levels of IL-18 in patients receiving a desirable DAPT with clopidogrel (e.g. DAPT with PRU ≤ 208, PRU ≤ 230, or PRU ≤ 262) independently increase in non-EM of CYP2C19 phenotypes. It has been reported that the serum levels of IL-18 is elevated in patients with type 2 diabetes [26]. The percentage of patients with diabetes mellitus in this study was 77.3%, suggesting that the high prevalence of diabetes mellitus might be associated with the mechanism. However, there was no difference in the prevalence of diabetes mellitus between the EM patients and the non-EM patients (Online Table 1). It has been reported that IL-18 promoter −137 G/C polymorphism influences the increase in the serum levels of IL-18 in patients with in-stent restenosis after PCI [27]. Another study has reported an association between IL-18 + 183 A/G polymorphism and the blood level of IL-18 on the risk of clinical events in patients with stable coronary artery disease [28]. Although we did not test IL-18 polymorphism in this study, some patients with CYP2C19 polymorphism might share IL-18 polymorphism, and the shared

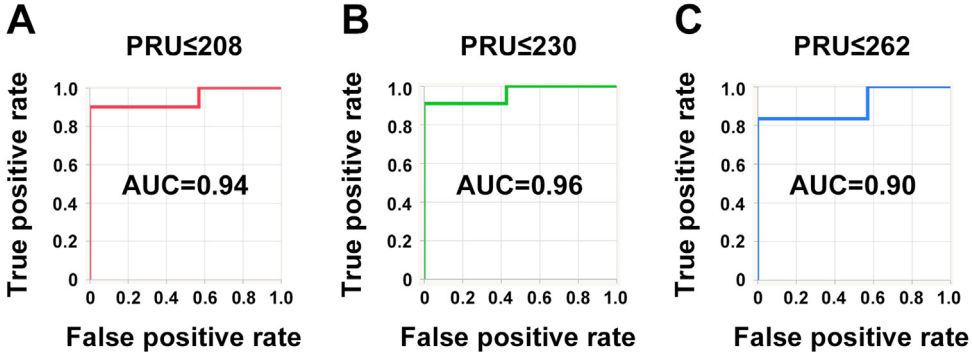

Fig. 2. Receiver operating characteristics curves of the serum levels of interleukin-18 for predicting the non-EM in patients with PRU ≤ 208 (A), PRU ≤ 230 (B), and PRU ≤ 262 (C) in dual antiplatelet therapy with clopidogrel. Red, green, and blue lines indicate the curves.
EM, extensive metabolizers for cytochrome p450 2C19; PRU, $P2Y_{12}$ reaction unit; AUC, area under the curve.

status might be regulating the platelet activity (i.e. PRU) in DAPT with clopidogrel and the serum levels of IL-18. Although there was a significant difference in sex between the EM and the non-EM in patients with PRU ≤ 230 in a comparison of several demographic characteristics between the two groups in patients with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262 (Online Supplementary Table 1), racial characteristics were not compared in this study, which was conducted only in Japanese patients. In order to test the hypotheses above based on our speculation, it will be desirable to study the relationship among IL-18 polymorphism, CYP2C19 polymorphism, atherosclerotic disease, and platelet activity in a multiracial population. East Asian patients are known to show a similar or even a lower rate of ischemic events after PCI than Caucasian patients, despite a higher level of platelet reactivity in DAPT and a higher frequency of the CYP2C19 loss-of-function alleles. Meanwhile, East Asian patients are also known to be at a greater risk of bleeding than Caucasian patients [29]. These notable differences in the risk profiles for thrombotic and bleeding events between East Asian and Caucasian patients suggest that clinical trial results in Western countries and American and European guidelines for antiplatelet therapy after PCI should be advisedly applied to clinical practice for East Asian patients. This study may additionally suggest that a more tailored approach to appropriate antiplatelet therapy after PCI should be considered according to racial risks in the profiles for not only thrombotic and bleeding events [30], but also polymorphism of CYP2C19 and serum cytokines, possibly including IL-18.

This study had several limitations. First, the study population was small, resulting that each analysis for the EM, IM, and PM in patients with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262 in DAPT with clopidogrel lacked. Second, it was unclear whether switching clopidogrel to another antiplatelet agent, such as prasugrel or ticagrelor, in the non-EM patients with PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262 followed by initial DAPT with clopidogrel after PCI could reduce the serum levels of IL-18 in the non-EM patients. Third, even though the aim of this study was to find the serum biomarker for the non-EM patients showing desirable PRU in DAPT with clopidogrel, the serum levels of 51 cytokines were not compared between the EM and the non-EM in patients showing any PRUs, suggesting that this study is not useful to medical facilities where PRU measurement is difficult in daily medical practice.

Conclusion

In the non-EM patients receiving DAPT with clopidogrel, cardiovascular events after PCI may occur in the patients despite indicating PRU ≤ 208, PRU ≤ 230, and PRU ≤ 262, which have been reported as indices of desirable DAPT to prevent the cardiovascular events. Although the present study had a small sample size and selection bias for sampling, the serum levels of IL-18 independently increase in such patients, implying a biomarker to diagnose such patients easily, as compared to a set biomarker with PRU and the CYP2C19 phenotype of the patients.

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Conflict of interest

Ken-ichiro Sasaki has received a personal fee from Daiichi Sankyo Co. Yoshihiro Fukumoto has received personal fees from Daiichi Sankyo Co., Ltd. and Bayer Yakuhin, Ltd. and research funds from Daiichi Sankyo Co., Ltd., Bayer Yakuhin, Ltd., and Sanofi K.K. Takafumi Ueno has received personal fees from Daiichi Sankyo Co., Ltd., Bayer Yakuhin, Ltd., and Sanofi K.K and a research fund from Sanofi K.K. Atsushi Harada is an employee of Daiichi Sankyo Co., Ltd. Yuji Hirakawa is an employee of Daiichi Sankyo Co., Ltd. Other authors have nothing to disclose regarding the current study.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jjcc.2020.06.008.

References

[1] Lugo LM, Ferreiro JL. Dual antiplatelet therapy after coronary stent implantation: individualizing the optimal duration. J Cardiol 2018;72:94–104.
[2] Stone GW, Witzenbichler B, Weisz G, Rinaldi MJ, Neumann FJ, Metzger DC, et al. Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES): a prospective multicentre registry study. Lancet 2013;382:614–23.
[3] Stuckey TD, Kirtane AJ, Brodie BR, Witzenbichler B, Litherland C, Weisz G, et al. Impact of aspirin and clopidogrel hyporesponsiveness in patients treated with drug-eluting stents: 2-year results of a prospective, multicenter registry study. JACC Cardiovasc Interv 2017;10:1607–17.
[4] Price MJ, Berger PB, Teirstein PS, Tanguay JF, Angiolillo DJ, Spriggs D, et al. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. JAMA 2011;305:1097–105.
[5] Nakamura M, Isshiki T, Kimura T, Ogawa H, Yokoi H, Nanto S, et al. Optimal cutoff value of $P2Y_{12}$ reaction units to prevent major adverse cardiovascular events in the acute periprocedural period: post-hoc analysis of the randomized PRASFIT-ACS study. Int J Cardiol 2015;182:541–8.
[6] Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood 2006;108:2244–7.
[7] Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med 2009;360:354–62.
[8] Yamamoto K, Hokimoto S, Chitose T, Morita K, Ono T, Kaikita K, et al. Impact of CYP2C19 polymorphism on residual platelet reactivity in patients with coronary heart disease during antiplatelet therapy. J Cardiol 2011;57:194–201.
[9] Myrand SP, Sekiguchi K, Man MZ, Lin X, Tzeng RY, Teng CH, et al. Pharmacokinetics/genotype associations for major cytochrome P450 enzymes in native and first- and third-generation Japanese populations: comparison with Korean, Chinese, and Caucasian populations. Clin Pharmacol Ther 2008;84:347–61.
[10] Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, et al. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med 2009;360:363–75.
[11] Ogawa H, Isshiki T, Kimura T, Yokoi H, Nanto S, Takayama M, et al. Effects of CYP2C19 allelic variants on inhibition of platelet aggregation and major adverse cardiovascular events in Japanese patients with acute coronary syndrome: the PRASFIT-ACS study. J Cardiol 2016;68:29–36.
[12] Nagashima Z, Tsukahara K, Morita S, Endo T, Sugano T, Hibi K, et al. Platelet reactivity in the early and late phases of acute coronary syndromes according to cytochrome P450 2C19 phenotypes. J Cardiol 2013;62:158–64.
[13] Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
[14] Stoll G, Bendszus M. Inflammation and atherosclerosis: novel insights into plaque formation and destabilization. Stroke 2006;37:1923–32.
[15] Kaptoge S, Seshasai SR, Gao P, Freitag DF, Butterworth AS, Borglykke A, et al. Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. Eur Heart J 2014;35:578–89.
[16] Frye RF, Schneider VM, Frye CS, Feldman AM. Plasma levels of TNF-alpha and IL-6 are inversely related to cytochrome P450-dependent drug metabolism in patients with congestive heart failure. J Card Fail 2002;8:315–9.
[17] Shimamatsu J, Sasaki KI, Katsuki Y, Kawasaki T, Murasato Y, Ajisaka H, et al. Prasugrel effectively reduces the platelet reactivity units in patients with

genetically metabolic dysfunction of cytochrome P450 2C19 who are treated with long-term dual antiplatelet therapy after undergoing drug-eluting stent implantation. Heart Vessels 2020;35:312–22.
[18] Ueno T, Koiwaya H, Sasaki KI, Katsuki Y, Katsuda Y, Murasato Y, et al. Changes in $P2Y_{12}$ reaction units after switching treatments from prasugrel to clopidogrel in Japanese patients with acute coronary syndrome followed by elective coronary stenting. Cardiovasc Interv Ther 2017;32:341–50.
[19] Sasaki KI, Ishizaki Y, Sasaki M, Nakayoshi T, Ohtsuka M, Matsuse H, et al. Hybrid training system-induced myokine secretion in healthy men. Cytokine 2018;111:178–81.
[20] Kim C, Shin DH, Hong SJ, Ahn CM, Kim JS, Kim BK, et al. One-year clinical outcomes of ticagrelor compared with clopidogrel after percutaneous coronary intervention in patients with acute myocardial infarction: From Korean Health Insurance Review and Assessment Data. J Cardiol 2019;73:191–7.
[21] Novick D, Kim S, Kaplanski G, Dinarello CA. Interleukin-18, more than a Th1 cytokine. Semin Immunol 2013;25:439–48.
[22] Mallat Z, Corbaz A, Scoazec A, Besnard S, Lesèche G, Chvatchko Y, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. Circulation 2001;104:1598–603.
[23] Whitman SC, Ravisankar P, Daugherty A. Interleukin-18 enhances atherosclerosis in apolipoprotein E(−/−) mice through release of interferon-gamma. Circ Res 2002;90:E34–8.
[24] Blankenberg S, Tiret L, Bickel C, Peetz D, Cambien F, Meyer J, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. Circulation 2002;106:24–30.
[25] Blankenberg S, Luc G, Ducimetière P, Arveiler D, Ferrières J, Amouyel P, et al. Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). Circulation 2003;108:2453–9.
[26] Zaharieva E, Kamenov Z, Velikova T, Tsakova A, El-Darawish Y, et al. Interleukin-18 serum level is elevated in type 2 diabetes and latent autoimmune diabetes. Endocr Connect 2018;7:179–85.
[27] Liu W, Liu Y, Jiang H, Ding X, Zhu R, Li B, et al. Plasma levels of interleukin 18, interleukin 10, and matrix metalloproteinase-9 and -137G/C polymorphism of interleukin 18 are associated with incidence of in-stent restenosis after percutaneous coronary intervention. Inflammation 2013;36:1129–35.
[28] Opstad TB, Pettersen AÅ, Arnesen H, Seljeflot I. The co-existence of the IL-18 +183 A/G and MMP-9-1562 C/T polymorphisms is associated with clinical events in coronary artery disease patients. PLoS One 2013;8e74498.
[29] Saito Y, Kobayashi Y, Tanabe K, Ikari Y. Antithrombotic therapy after percutaneous coronary intervention from the Japanese perspective. Cardiovasc Interv Ther 2020;35:19–29.
[30] Mangieri A, Gallo F, Sticchi A, Khokhar AA, Laricchia A, Giannini F, et al. Dual antiplatelet therapy in coronary artery disease: from the past to the future prospective. Cardiovasc Interv Ther 2020;35:117–29.