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On aspirin treatment but not baseline thromboxane B2 levels predict adverse outcomes in patients with acute coronary syndromes

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The clinical benefits of acetylsalicylic acid (ASA) in the primary or secondary prevention of cardiovascular disease are attributed to its ability to inhibit the generation of platelet-derived thromboxane A₂, a potent vasoconstrictor and promoter of platelet aggregation [1]. Low doses of ASA irreversibly acetylate the platelet cyclooxygenase-1 (COX-1) pathway and are capable of blocking over 95% of subsequent thromboxane production for the entire life cycle of platelets [2]. Despite its wide use, ASA fails to prevent atherothrombotic events because 10–25% of patients with arterial thrombotic disease suffer a recurrent vascular event while on aspirin therapy [3]. The failure to prevent thrombotic events with therapeutic doses of ASA has been referred to as aspirin resistance [4]. The precise mechanism(s) for the inadequate or poor response to ASA therapy remain controversial, and may include genetic variations, increased platelet turnover, activation by additional

platelet pathway(s), alternative sources of thromboxane production, and/or drug bioavailability [5]. High thromboxane levels in cardiovascular patients on aspirin therapy have been associated with increased risk of adverse events, suggesting a causative role of thromboxane. The HOPE [6] and CHARISMA [7] studies demonstrated that elevated levels of the urinary thromboxane metabolite 11-dehydrothromboxane B₂ (11dhTx_{B2}) in ASA-treated patients predict future risk of stroke, myocardial infarction and cardiovascular death. However, the significance of baseline (aspirin-free) thromboxane B₂ levels for cardiovascular risk was not evaluated.

We studied 287 consecutive aspirin-free acute coronary syndrome (ACS) patients admitted to Kurashiki Central Hospital (Kurashiki, Japan) for percutaneous coronary intervention (PCI) from December 2007 to November 2010 to evaluate a possible association of urinary thromboxane B₂

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Table 1 Thromboxane B₂ levels before (baseline) and after ASA treatment in patients with ACS

Time	n	Urinary 11dhTx _{B2} (pg mg ⁻¹ creatinine)		P value** (vrs baseline)
		Mean ± SD	95% CI*	
Baseline	287	7082 ± 12 813	5598–8565	
7–14 days ASA	267	1402 ± 1178	1259–1544	< 0.0001
6 months ASA	242	1354 ± 886	1241–1466	< 0.0001
12 months ASA	132	1395 ± 947	1232–1558	< 0.0001

*95% confidence interval; **Wilcoxon/Kruskal-Wallis Rank Sum.

Table 2 Prevalence and relative risk for adverse events according to baseline and post-ASA 11dhTxB2 quartiles in patients with ACS

11dhTxB2 quartiles	Baseline*				Post-ASA**			
	<i>n</i>	% adverse events	Relative risk (95% CI) [#]	<i>P</i> value [^] (versus Q1)	<i>n</i>	% adverse events	Relative risk (95% CI) [#]	<i>P</i> value [^] (versus Q1)
Q1	72	19.4			66	9.1		
Q2	72	16.7	0.9 (0.829–1.127)	0.665	66	12.1	1.3 (0.489–3.631)	0.572
Q3	72	16.7	1.1 (0.580–2.345)	0.665	66	24.2	2.7 (1.112–6.391)	0.019
Q4	71	15.5	1.2 (0.612–2.574)	0.534	65	20.0	2.2 (0.890–5.436)	0.076
Q1 + 2 versus Q3 + 4	144 / 143	18.1 / 16.1	1.1 (0.673–1.871)	0.657	132 / 131	10.6 / 22.1	2.1 (1.156–3.766)	0.011

*Baseline quartiles: Q1 = 0–1826; Q2 = 1827–4235; Q3 = 4236–7171; Q4 = 7172–142 691 pg mg⁻¹. **Post-ASA (discharge) quartiles: Q1 = 0–772; Q2 = 773–1054; Q3 = 1055–1604; Q4 = 1605–12 796 pg mg⁻¹. [#]95% confidence interval. [^]Pearson's chi-square.

levels pre- and post-ASA ingestion with adverse events. Inclusion criteria included ST elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI) or early onset (within 24 h) invasive revascularization procedure. Upon enrollment and prior to PCI, a baseline (aspirin-free) urine sample was obtained, followed by a daily regimen of 100 mg of ASA. Urine samples from aspirin-treated patients were collected at hospital discharge (7–14 days) and upon 6 and 12 months follow-up. Adverse cardiovascular events (AEs) were recorded during a 12-month patient follow-up. Primary endpoints included stent thrombosis, Q wave myocardial infarction (QMI), non-Q wave myocardial infarction (NQMI) and death (cardiac and non-cardiac). Secondary endpoints included stroke, transient ischemic attack (TIA), target lesion revascularization of PCI or CABG, or other vascular event. Primary and secondary endpoints were used in the analysis of results. Urinary 11dhTxB2 was measured by ELISA and results were expressed as pg 11dhTxB2 mg⁻¹ creatinine.

The mean age of the ACS patients was 68.9 ± 11.9 years. Age did not influence baseline 11dhTxB2 levels ($r = 0.060$, $P = 0.310$), but female patients had significantly higher mean baseline 11dhTxB2 (7675 ± 8180 pg mg⁻¹) compared with male patients (6949 ± 14 076 pg mg⁻¹, $P = 0.0171$). The mean baseline aspirin-free 11dhTxB2 (7082 pg mg⁻¹) for this cohort of ACS patients was two to three times higher than that of various healthy populations we have previously studied (range 2450–3337 pg mg⁻¹). ASA significantly suppressed (81%, $P > 0.0001$) baseline 11dhTxB2 levels to 1402 ± 1178 pg mg⁻¹ at discharge and beyond (Table 1). The overall rate of AEs was 17.1%. The rate of AEs according to baseline aspirin-free 11dhTxB2 decreased slightly from 19.4% in quartile 1 to 15.5% in quartile 4 (Table 2). In contrast, the rate of AEs in aspirin treatment quartiles increased from 9.1% in quartile 1 to 24.2% in quartile 3 and 20% in quartile 4. The relative risk for AEs of quartile 3 was 2.7 (1.112–6.391, $P = 0.019$). When upper quartiles (3 and 4) were compared with lower quartiles (1 and 2), the relative risk was 2.1 (1.156–3.766, $P = 0.011$). The correlation between AEs and discharge 11dhTxB2 was not observed at 6- or 12-month measurements.

High baseline 11dhTxB2 levels were associated with poor ASA response (data not shown), a finding consistent with a possible underlying platelet hyperactivity that may contribute to the development of atherothrombosis. To determine whether higher doses of ASA reduce the rate of poor responders and improve outcomes will require additional studies. However, baseline aspirin-free 11dhTxB2 levels did not predict 1-year AEs. The degree to which platelet COX-1 or COX-2 pathways contribute to high baseline 11dhTxB2 levels in ACS requires further investigation. In spite of a significant inhibition (81%) of urinary 11dhTxB2 generation by ASA, 29% of ACS patients were classified as poor ASA responders. This rate was over three times the rate of poor responders we have seen in previous studies of healthy volunteers (7.0–8.5%), and two times that of type 2 diabetic patients (14.8%). Thus, high levels of 11dhTxB2 after ASA ingestion are likely to represent extra-platelet (i.e. monocyte/macrophage-derived) COX-2 production of thromboxane. The increased relative risk (2.7) for AEs associated with high post-ASA 11dhTxB2 levels (upper quartiles) suggests that COX-2 production of thromboxane may be a factor associated with a cardiovascular inflammatory process. Healthy subjects with poor aspirin response manifest an incomplete inhibition of COX-1 in a proinflammatory milieu with enhanced oxidative stress [8]. Aspirin-insensitive thromboxane generation has been associated with oxidative stress in diabetes. Amongst several biomarkers tested, only baseline urinary 8-isoPGF2 α discriminated between normal and poor thromboxane responders, suggesting that oxidative stress may maintain platelet function irrespective of COX-1 inhibition and/or increased systemic generation of thromboxane from non-platelet sources [9]. Thromboxane alone may not be directly implicated in atherothrombosis. Nonetheless, our results confirm previous reports that post-ASA urinary 11dhTxB2 may be useful in predicting adverse outcomes in ACS patients [6,7].

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Disclosure of Conflict of Interests

LRL is an employee of Corgenix. KG has received consulting fees from Corgenix.

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Utility of multiplex ligation-dependent probe amplification (MLPA) for hemophilia mutation screening

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Hemophilia A (HA) and B (HB) are estimated to affect 1 in 5000 male births in the United States each year.[1] Inheritance of mutations in the factor (F)VIII (*F8*) gene or FIX (*F9*) gene causes these bleeding disorders. Identification of mutations causing a patient's hemophilia can lead to a better understanding of risk of complications [2], as well as aid in carrier detection in family members [3]. Mutation screening for HA has involved testing for inversions of introns 1 and 22 of *F8*, as approximately 45% of severe HA patients carry an inversion as their causative mutation [2], and sequencing of the coding regions of *F8* to identify point mutations, deletions or splice-site mutations. Similarly, mutation screening for HB has involved sequencing of the coding regions of *F9*. However, a subset of

patients presenting with hemophilia do not have a detectable mutation with these methods [4]. Duplication of part of *F8* or *F9*, for example, may not be detected. Also, female family members heterozygous for a large *F8* or *F9* deletion may not be identified as carriers using these methods, as dosage of the genes is not determined. Recently, Multiplex Ligation-Dependent Probe Amplification (MLPA®) (MRC Holland, Amsterdam, the Netherlands) has been successfully used to identify large deletions and duplications within *F8* and *F9* [5–7]. This assay quantitatively compares copy numbers of a set of DNA sequences in a patient sample to those in a control sample to screen for the presence of deletions or duplications [8]. The assessment of how this or similar technologies will fit into currently used mutation screening protocols should be critically evaluated.

We describe here our experience using MLPA® in combination with inversion testing and DNA sequencing for identification of mutations in a large series of hemophilia patients. Additionally, we present our analyzes of how this duplication/deletion testing fits into mutation screening algorithms and highlight the importance of careful assay validation and interpretation.

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