GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 18, Number 9, 2014

Pp. 599–604
DOI: 10.1089/gtmb.2014.0077

Genetic Polymorphisms of Platelet Receptors in Patients with Acute Myocardial Infarction and Resistance to Antiplatelet Therapy

Jana Ulehlova,[1] Ludek Slavik,[1] Jana Kucerova,[1] Vera Krcova,[1] Jan Vaclavik,[2] and Karel Indrak[1]

Methods: The studied group comprises 124 patients with acute myocardial infarction on dual antiplatelet therapy with acetylsalicylic acid (ASA) and thienopyridines. Antiplatelet therapy was monitored by platelet-rich plasma light transmittance aggregometry (LTA) using the APACT 4004 analyzer (Helena Laboratories) and by whole blood impedance aggregometry (multiple electrode aggregometry [MEA]) using the Multiplate analyzer (Dynabyte). Platelet aggregation was detected after stimulation with arachidonic acid for detection of aspirin resistance and with adenosine diphosphate (ADP) and prostaglandin E_1 for detection of thienopyridine resistance. To determine the frequencies of P2Y12 (i-744T > C; rs2046934), P2Y12 (34C > T; rs6785930), COX-1 (-842A > G; rs10306114), GPVI (13254T > C; rs1613662), and GPIbA (5T > C; rs2243093) polymorphisms, DNA of patients with AIM was tested by real-time-polymerase chain reaction and melting curve analysis using the LightCycler 480 analyzer (Roche Diagnostics). *Results:* The cut-off points used for patients with effective ASA therapy are 25% of aggregated platelets and 220 area under the curve (AUC)/min if LTA or MEA, respectively. The cut-off points used for effective thienopyridine therapy are 45% of aggregated platelets or 298 AUC/min, respectively. Both LTA and MEA found that aspirin and thienopyridine therapies failed in 14.51% and 25.8%, respectively. The data were statistically processed using the SPSS version 15 software (SPSS, Inc.). Associations between receptor mutation status and response to therapy were assessed with Fisher's exact test. The significance level was set at 0.05. *Conclusion:* The aim of our work was to use the two functional laboratory methods described earlier to assess both aspirin and thienopyridine resistance and to determine the contribution of genetic polymorphisms of platelet receptors to resistance to antiplatelet therapy in AIM. Fisher's exact test showed a significant statistical correlation between platelet function tests suitable for monitoring ASA resistance, that is, LTA and MEA, and mutation status of COX1_A1 (-A842G). Fisher's exact test showed no statistically significant correlations between platelet function tests suitable for monitoring ASA resistance, that is, LTA and MEA, and mutation status of GP1bA (-5T > C) and GP6 (T13254C). Fisher's exact test showed no statistically significant correlation between mutational statuses of the receptors P2RY12 (i-T744C), P2RY12 (C34T), GP1bA (-5T > C), or GP6 (T13254C) and response to antiplatelet therapy with 75 mg of clopidogrel.

Introduction

Acute myocardial infarction (AMI) remains one of the leading causes of morbidity and mortality in developed countries, including the Czech Republic. AMI is the most severe form of ischemic heart disease (IHD). Most cardiovascular diseases, with an exception of congenital disorders, tend to develop in adulthood. Myocardial infarction is acute focal ischemic necrosis of a different extent in the myocardium due to sudden closure or progressive extreme narrowing of a coronary artery supplying blood to a particular region. In more than 95% of cases, the cause is coronary atherosclerosis with intimal rupture and thrombosis at the plaque site. Recently, there has been increasing information on the causes and pathogenetic mechanisms of very early manifestations of IHD in the form of AMI at a young age, in men aged 18–45 years and women aged 18–55 years. The incidence of AMI in young patients is eight to nine times lower than in older adults, accounting for only 2–6% of all AMI cases. In numerous aspects, this group of younger

[1]Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital, Olomouc, Czech Republic.
[2]Department of Internal Medicine I–Cardiology, University Hospital, Olomouc, Czech Republic.

patients is different from those who suffered their first AMI later than the age of 60. Previous studies reported that hospital mortality of younger patients with AMI ranged from 2.9% to 5%, as compared with ~20% in older AMI sufferers.

The key component of secondary prevention of myocardial infarction is dual antiplatelet therapy with acetylsalicylic acid (ASA) and thienopyridine derivatives. However, this therapy may fail in 5–30% of patients due to their resistance to antiplatelet drugs (Gurbel and Tandry, 2007; Cuisset *et al.*, 2009). This phenomenon was first described in administration of ASA and subsequently in thienopyridines. The causes of resistance are multifactorial, and their laboratory assessment is based on several possible ways of detection (Ševčíková, 2006). In recent years, novel laboratory methods have been developed to be used for monitoring failing antiplatelet therapy. The most frequent approach for assessing platelet function is aggregation testing. Nowadays, the so-called golden standard is platelet aggregation testing in platelet-rich plasma (PRP) using light transmission aggregometry (Cattaneo, 2007; Cattaneo *et al.*, 2007). A novel option for determining residual platelet aggregation is the use of multiple electrode aggregometry (MEA) in whole blood (Mueller *et al.*, 2007; Seyfert *et al.*, 2007). It measures electrical impedance between two electrodes in hirudin-treated whole blood. Recorded are changes in impedance resulting from a build-up of platelet aggregates on two independent electrodes.

Another method, only used to determine aspirin resistance, is detection of 11-dehydrotromboxane B_2 (in serum or urine). Compared with the aggregation methods described earlier, however, this test is more time consuming and its results may be influenced by the presence of this metabolite from other than platelet sources. Thienopyridine resistance may also be tested by flow cytometry with a vasodilator-stimulated phosphoprotein phosphorylation assay (Blais *et al.*, 2009).

The risk for atherothrombogenesis is currently considered associated with gene polymorphisms of glycoprotein receptors on the platelet surface or their enzymes. These are known polymorphisms of the receptor for fibrinogen–integrin GP III (HPA-1), receptor for adenosine diphosphate (ADP) P2Y12 (H1/H2 haplotypes as well as 34C > T), receptor for thrombin PAR-1 (IVS -14A > T), cyclooxygenase 1 enzyme (-842A > G), receptor for collagen GP Ia/IIa (807C > T), and also the GP VI gene (13254T > C) associated with not only higher incidence rates of coronary events but also venous thrombosis (Lepäntalo *et al.*, 2006; Linnemann *et al.*, 2008). No definite decision on the clinical significance of individual platelet receptor gene polymorphisms has been made as yet. In the P2Y12 ADP receptor, four polymorphisms are known (i-139C > T, i-744T > C, i-ins801A, and 52G > T); these are in linkage disequilibrium and form haplotypes referred to as H1 and H2. The H2 allele has been suggested as a potential cause for an increased risk of atherothrombogenesis. Of particular interest were results of the GENDER study showing that after percutaneous coronary angioplasty and stent insertion, patients carrying the common H1 had lower rates of restenosis (Rudež *et al.*, 2008). A polymorphism of the enzyme cyclooxygenase 1 is considered as influencing platelet response to ASA therapy.

In addition to the earlier genetic causes, platelet aggregation is affected by numerous other factors. These include insufficient bioavailability (noncompliance, inadequate dose, insufficient absorption, or interference with other drugs), increased platelet function (incomplete TXA_2 production, increased platelet turnover), or interaction of platelets with other blood cells. A rather varied group of acquired factors such as smoking, hypercholesterolemia, stress, and increased sympathetic activity also has an impact on platelet aggregation. Another limitation is the platelet count. For most methods for determining platelet aggregation, the threshold count is 100×10^9/L.

Materials and Methods

Patients

The group comprised 124 patients (89 men and 35 women) who underwent antiplatelet tests at least 7 days after the diagnosis of AMI and initiation of therapy with daily doses of 100 mg of ASA and 75 mg of clopidogrel. The median and mean ages were 48 and 51, respectively (range 19–83 years).

Sample collection

Blood samples were collected using the VACUETTE® collection tubes and needles (Greiner Bio-One). For both light transmittance aggregometry (LTA) aggregation tests and DNA isolation, the VACUETTE collection tubes contained sodium citrate buffer solution at a concentration of 0.109 M (3.2%). For impedance aggregometry, tubes from the same manufacturer and the same sampling technique were used, with the anticoagulant hirudin (15 IU/mL), a direct inhibitor of thrombin. Immediately after collection, each sample was carefully mixed and transported to the laboratory as quickly as possible. All samples were processed within 2 h of collection.

For all analyses, samples were collected by the same healthcare team using the same collection technique described earlier.

Light transmission aggregometry

Platelet aggregation was measured in PRP (Linnemann *et al.*, 2008) using a turbidimetric method with the APACT 4004 analyzer (LABiTec). This was followed by centrifugation at 150 or 2000 *g* for 10 min at room temperature to obtain PRP or platelet-poor plasma, respectively (Cattaneo, 2007). The resulting platelet count for analysis was adjusted to 250×10^{12}/L (range $221–282 \times 10^{12}$/L).

As a specific inducer for aspirin resistance assessment (Cuisset *et al.*, 2009; Abaci and Kilickesmez, 2013), arachidonic acid at a concentration of 1 μM was used (Helena Biosciences). To determine an insufficient therapeutic effect with arachidonic acid-induced LTA (acetyl salicid acid induced aggregation (ASPI) test), the cut-off point was set at 25% (Slavik *et al.*, 2011; Úlehlová *et al.*, 2011).

For detection of thienopyridine resistance (Gurbel and Tandry, 2007), stimulation of aggregation was induced by ADP with prostaglandin E_1 (PGE_1) at a concentration of 10 μM. The final PGE_1 concentration was 9.4 nM. To determine an insufficient therapeutic effect with ADP + PGE_1-induced LTA (adenosin phosphate induced aggregation with prostaglandin E1 (ADPHS) test), the cut-off point was set at 45% (Slavik *et al.*, 2011; Úlehlová *et al.*, 2011).

Multiple electrode aggregometry

Platelet aggregation was measured in whole unclotted blood using an impedance method with the Multiplate analyzer (Dynabyte). The reaction was started by diluting

hirudin whole blood with saline (0.9% NaCl) at a ratio of 1:2. The fluids were mixed in a measuring cuvette for 3 min at a constant temperature of 37°C (Mueller *et al.*, 2007; Seyfert *et al.*, 2007). To determine aspirin resistance, arachidonic acid as an inducer was added at a final concentration of 15 mM. To determine thienopyridine resistance, a specific combination of ADP with PGE_1 at a concentration of 10 μM as an inducer was used. The final PGE_1 concentration was 9.4 nM. Residual platelet aggregation was monitored in the form of increasing impedance and expressed in aggregation units per period of time; that is, area under the curve (AUC) per minute, with cut-off points of 200 AUC/min and 298 AUC/min for the ASPI and ADPHS tests, respectively (Slavik *et al.*, 2011; Úlehlová *et al.*, 2011).

Genotyping

DNA isolation was performed using peripheral blood leukocytes with the Puregene kit. In the obtained DNA samples, the following polymorphisms were detected: H1/H2 haplotypes of the ADP platelet receptor gene P2Y12, determined by assessing i-744T > C (rs2046934) and 34C > T (rs6785930) polymorphisms in the same gene. Further, -842A > G (rs10306114) polymorphism of the cyclooxygenase gene, GPVI 13254T > C (rs1613662) polymorphism of the glycoprotein VI gene, and 5T > C (rs2243093) polymorphism of the glycoprotein IbA gene were assessed. This was performed by real-time polymerase chain reaction (PCR) with fluorescent probes and melting curve analysis based on a reduction in fluorescence, as fluorescent probes are released from PCR products. The method is beneficial in that it allows detection directly in a PCR tube, that is, immediately after amplification without having to open the reaction tube, reducing a need for manipulation with the sample and thus the risk of contamination. The analysis itself only lasts for a few minutes, thus being time efficient. The procedure was performed using the LightCycler 480 analyzer and LightCycler 480 Genotyping Master® kits (Roche Diagnostics) according to the manufacturer's instructions.

The method is based on amplifying a polymorphism-containing segment of patient DNA. This amplification is observed in real time. In addition to normal components, the PCR mixture contains two fluorescent probes. One is directly at the site of polymorphism; the other is close to the end of the first probe. The adjacent ends of the probes are labeled with different fluorescent dyes. After excitation of one fluorophore, resonance energy transfer to the other fluorophore occurs and the emitted light is detected. After amplification, the temperature is lowered to a level at which both probes anneal, producing fluorescence. On gradual heating, the probe specific for a polymorphism is displaced and fluorescence decreases. If DNA contains a sequence different from the probe sequence, the probe is not fully complementary, being displaced at a lower temperature as compared with full complementarity. This may be measured as different fluorescence peaks on the temperature axis. If the patient has two alleles with other than standard variations, fluorescence is decreased, for instance, only at a lower temperature. In case of both wild-type alleles, the decrease is at a higher temperature. A heterozygote has two peaks at different temperatures. The sequences of primers and probes used in the study are shown in Table 1.

Results

The pilot study assessed 124 patients with AMI taking daily doses of 100 mg of ASA and 75 mg of clopidogrel for 7 days. Both LTA and MEA found that aspirin and thienopyridine therapies failed in 14.51% and 25.8%, respectively. An important factor in the failure of antiplatelet therapy could have been a genetic predisposition, namely platelet receptor polymorphisms.

Table 1. Names and Sequences of Primers and Probes Used in the Study

Gene name	*SNP*	*SNP identif.*	*Oligonucleotides*
P2RY12	i-T744C	rs2046934	5′ATTTATCTAAATATCTTTTACACgAA 5′AAATAAAATATAggTTATTACCACA 5′AAAAgATTACAAACgTCATTTCAA—FL 5′LC640-TTCCCAAgATgTAgATgCCATATAgCA—PH
P2RY12	C34T	rs6785930	5′AAgTTACACACAgAgATAACAgC 5′gAAgATCAgAAATgACTgTgTTC 5′CgCAgAggTgAgATTgTCg—FL 5′LC640-CggCTTgCATTTCTTgTTggTTACCTAgAg—PH
COX1_A1	-A842G	rs10306114	5′CCTTCCgATAACTgAgAACCT 5′TTTCTAgCCCTCAgTATTCTCAT 5′CAATgAgggAATgCACACAAATCTCCTgg—FL 5′LC640-gCAgTgCCCAgCATgTAg—PH
GP1bA	-5T > C	rs2243093	5′gCAgggggATCCACTCAA 5′ggTTgTgTCTTTCggCAgg 5′CCACAggCCCTCATgCCTC—FL 5′CCTCCTCTTgCTgCTCCTgCTgCC—PH
GP6	T13254C	rs1613662	5′CAAATCTgTgAAAgAACCAACT 5′gATTTCCCAggAACCTCTgT 5′gCACCAgAATggACCCTgCAgAACCT—FL 5′LC640-CCTgCTACCgAggAAggTgg—PH

SNP, single nucleotide polymorphism.

Table 2. Rates of Individual Polymorphisms in the Studied Group of 124 Patients with Acute Myocardial Infarction and Association with Aspirin Resistance

Polymorphism	Type of mutation	Entire group n	Entire group %	Resistant n	Resistant %
COX1_A1; -A842G (rs10306114)	Heterozygote	9	7.26	5	27.78
	Homozygote	0	0.00	0	0.00
	Wild type	115	92.74	13	72.22
GP1bA; -5T>C (rs2243093)	Heterozygote	26	20.97	5	27.78
	Homozygote	4	3.23	0	0.00
	Wild type	94	75.80	13	72.22
GP6; T13254C (rs1613662)	Heterozygote	16	12.90	4	22.20
	Homozygote	0	0.00	0	0.00
	Wild type	108	87.10	14	77.80

Tables 2 and 3 show the rates of polymorphisms COX1_A1, -A842G (rs10306114); P2RY12, C34T (rs6785930); P2RY12, i-T744C (rs2046934); GPVI 13254T>C (rs1613662); and GPIbA 5T>C (rs2243093) in the group.

The data were statistically processed using the SPSS version 15 software (SPSS, Inc.). Associations between receptor mutation status and response to therapy were assessed with Fisher's exact test. The significance level was set at 0.05. Fisher's exact test showed a statistically significant correlation between platelet function tests suitable for monitoring ASA resistance, that is, LTA and MEA, and mutation status of COX1_A1;-A842G (rs10306114) ($p=0.003$). Fisher's exact test did not confirm statistically significant correlations between platelet function tests suitable for monitoring ASA resistance, that is, LTA and MEA, and mutation status of GP1bA; -5T>C (rs2243093) ($p=0.755$) or mutation status of GP6; T13254C (rs1613662) ($p=0.249$). Further, Fisher's exact test did not confirm statistically significant correlations between platelet function tests suitable for monitoring thienopyridine resistance, that is, LTA and MEA, and mutation status of P2RY12; i-T744C (rs2046934) ($p=0.424$); mutation status of P2RY12; C34T (rs6785930) ($p=0.129$ vs. $p=0.061$); mutation status of GP1bA; -5T>C (rs2243093) ($p=0.488$ vs. $p=0.812$); or mutation status of GP6; T13254C (rs1613662) ($p=1.000$).

Table 3. Rates of Individual Polymorphisms in the Studied Group of 124 Patients with Acute Myocardial Infarction and Association with Thienopyridine Resistance

Polymorphism	Type of mutation	Entire group n	Entire group %	Resistant n	Resistant %
P2RY12; i-T744C (rs2046934)	Heterozygote	22	17.74	7	27.78
	Homozygote	0	0	0	0.00
	Wild type	102	82.26	25	72.22
P2RY12; C34T (rs6785930)	Heterozygote	54	43.55	16	50.00
	Homozygote	15	12.10	5	15.63
	Wild type	55	44.35	11	34.37
GP1bA; -5T>C (rs2243093)	Heterozygote	26	20.97	6	18.70
	Homozygote	4	3.23	2	6.30
	Wild type	94	75.80	24	75.00
GP6; T13254C (rs1613662)	Heterozygote	16	12.90	4	22.20
	Homozygote	0	0.00	0	0.00
	Wild type	108	87.10	14	77.80

Table 4. Endogenous Causes of Acetylsalicylic Acid Antiplatelet Therapy Failure

Cellular	*Genetic*
Pathways not blocked by ASA (aggregation induced by erythrocytes, thrombin, collagen, adrenalin, ADP, cytokines)	Polymorphisms of COX-1 (alters the active site and inhibits acetylation by aspirin), COX-2, TxA2 synthase
Sensitivity of platelets to collagen and ADP	ADP polymorphisms of GP Ia/IIa, Ib/V/IX, IIb/IIIa receptors
COX-2 overexpression (rapid regeneration of platelets)	Polymorphisms of receptors for collagen GP VI, vWf GP Ia
Regenerated COX-1 (Mo, Ma, endothelial cells)	Polymorphism of f XIII (Val34Leu) leading to inhibition of factor XIII activation in ASA therapy
Resolvins	
8-iso-PGF production	

ADP, adenosine diphosphate; ASA, acetylsalicylic acid.

Discussion

Monitoring of the effectiveness of antiplatelet therapy appears to be a key factor in the assessment of secondary prevention of myocardial infarction, especially in young patients and with the emergence of novel therapeutic options.

High treatment failure rates, 14.51% in ASA therapy and 25.8% in thienopyridine therapy, are suggestive of the severity of the problem. Determining the causes of therapeutic failure is one of the key factors for rationalization and personalization of antiplatelet therapy as well as for further stratification of the management, particularly with regard to prevention of recurrent atherothrombotic events. In recent years, numerous endogenous (Table 4) and exogenous (Tables 5 and 6) causes of antiplatelet therapy failure have been identified.

Rates of individual endogenous and exogenous factor influencing treatment failure are varied, depending on the particular antiplatelet drug and its metabolism and with regard to the patient's genetic predisposition.

Table 5. Exogenous Causes of Acetylsalicylic Acid Antiplatelet Therapy Failure

Malabsorption
Drug interactions (nonsteroidal anti-inflammatory drugs)
Smoking
Physical strain, postural reactions, stress
Age, gender
Increased platelet turnover
Hyperlipidemia, hyperglycemia
Nonthrombotic vascular occlusion (arteritis, embolism)
Hypercoagulable state in acute coronary syndromes
Dose dependence

Table 6. Causes of Clopidogrel Resistance

Drug interactions with CYP3A4
BMI, diabetes, insulin resistance, stress
Interindividual and intraindividual variability in CYP 45 activity
Accelerated platelet turnover
CYP 450 gene polymorphisms
Platelet receptor gene polymorphisms
Noncompliance, underdose

The situation is relatively well documented in ASA, with the COX-1 gene polymorphism increasing the risk of premature myocardial infarction. The COX-1 allele is an independent predictor for sCD40L levels in the acute phase of premature AMI as well as 1 year after the event. The prevalence of the COX-1 polymorphism in 15% of patients with coronary artery disease is more than 7.26% in the presented group of patients, suggesting that this genetic change does not pose a risk. However, the situation changes dramatically if the COX-1 mutation is assessed in the group of patients with inadequate treatment response, with the prevalence being 27.78%.

From this point of view, it is apparent including the assessment of residual platelet aggregation is important in the determination of genetic changes in platelet receptors.

The situation is more complicated in clopidogrel, the metabolism of which is rather complex and in the final phase, only 15% of the prodrug is converted to an active metabolite. Intestinal absorption of the prodrug is limited by the P-glycoprotein efflux pump encoded by the ABCB1 gene. Most of the effective drug is metabolized to inactive metabolites by omnipresent esterases. The formation of active metabolites is influenced by numerous single nucleotide polymorphisms (SNPs) in CYP3A5, P2RY12, or ITGB3. Individual frequencies of variant polymorphisms of platelet receptors associated with higher platelet reactivity and resistance to clopidogrel therapy were reported in a relatively high percentage of patients with coronary artery disease (Antoniades *et al.*, 2006).

The prevalence rates of clinically significant genetic polymorphisms of receptors P2RY12; i-T744C (rs2046934) and P2RY12; C34T (rs6785930) are 17.74% and 12.10%, respectively. When compared with the healthy population, these are lower in both P2Y12 (i-T744C), with 27.40% of heterozygotes, and P2Y12 (C34T), with 2.63% of homozygotes.

However, when assessing patients with inadequate treatment response, the rates of both genetic changes are higher than in the healthy population, being 27.78% in P2RY12; i-T744C (rs2046934) and 15.63% in P2RY12; C34T (rs6785930). The effect of antiplatelet therapy failure is apparent.

Variable effectiveness of antiplatelet drugs with regard to the complicated process of their action and many factors influencing their effect presents a complex problem for providing secondary prevention of IHD (Cuisset *et al.*, 2009; Abaci and Kilickesmez, 2013).

Inconsistent findings in studies on the significance of genetic changes in platelet glycoproteins result from differences in sample sizes, proportions of ethnic groups, patient and control selection, endpoints of individual studies, and extreme variability of environmental factors interacting with genetic factors in various ways (Kvasnička *et al.*, 2008).

Conclusion

Determining frequencies of variant polymorphisms of platelet receptor genes could provide information about the proportion of patients in whom response to antiplatelet therapy cannot be expected, as the polymorphisms alter the binding site of platelet receptors and, thus, these receptors are not inhibited by active metabolites of antiaggregants. One of the objectives of this study was to obtain data on the frequency of selected polymorphisms associated with increased platelet activity and aggregability and resistance to ASA or clopidogrel therapy in patients with AMI. Fisher's exact test showed a statistically significant correlation between platelet function tests suitable for monitoring ASA resistance, that is, LTA and MEA, and mutation status of COX1_A1 (-A842G). Fisher's exact test showed no statistically significant correlations between platelet function tests suitable for monitoring ASA resistance, that is, LTA and MEA, and mutation status of GP1bA (-5T > C) and GP6 (T13254C). Fisher's exact test showed no statistically significant correlation between mutational statuses of the receptors P2RY12 (i-T744C), P2RY12 (C34T), GP1bA (-5T > C), or GP6 (T13254C) and response to antiplatelet therapy with 75 mg of clopidogrel.

Available data from clinical studies have so far provided controversial results with regard to the impact of platelet receptor polymorphisms on the effectiveness of antiplatelet drugs. However, experimental models and results from some studies point to the fact that these genetic variants may, under certain circumstances, be important risk factors in some groups of patients.

Acknowledgment

This study was supported by grant project LF-2014-001 and IGA NT 14394.

Author Disclosure Statement

No competing financial interests exist.

References

Abaci O, Kilickesmez KO (2013) Aspirin resistance: where are we now? Anadolu Kardiol Derg 13:370–373.

Antoniades C, Tousoulis D, Vasiliadou C, *et al.* (2006) Genetic polymorphisms of platelet glycoprotein Ia and the risk for premature myocardial infarction: effects on the release of sCD40L during the acute phase of premature myocardial infarction. J Am Coll Cardiol 47:1959–1966.

Blais N, Pharad C, Lordkipanidze M, *et al.* (2009) Response to aspirin in healthy individuals. Cross-comparison of light transmission aggregometry, VerifyNow system, platelet count drop, thromboelastography (TEG) and urinary 11-dehydrothromboxane B_2. Thromb Haemost 102:404–411.

Cattaneo M, Lecchi A, Zighetti ML, *et al.* (2007) Platelet aggregation studies: autologous platelet poor plasma inhibits platelet aggregation when added to platelet rich plasma to normalize platelet count. Haematologica 92:694–697.

Cattaneo M (2007) Resistance to platelet drugs: molecular mechanisms and laboratory detection. J Thromb Haemost 5:230–237.

Cuisset T, Frere C, Quilici J, *et al.* (2009) Aspirin noncompliance is the major cause of ''aspirin resistance'' in patients undergoing coronary stenting. Am Heart J 157:889–893.

Gurbel PA, Tandry US (2007) Clopidogrel resistance? Thromb Res 120:311–321.

Kvasnička J, Hajkova J, Bobčikova P, *et al.* (2008) Polymorfizmy krevních destiček a možnosti monitorace účinku protidestičkové léčby. Interv Akut Kardiol 7:215–218.

Lepäntalo A, Mikkelsson J, Reséndiz JC, *et al.* (2006) Polymorphisms of COX-1 and GPVI associate with the antiplatelet effect of aspirin in coronary artery disease patients. Thromb Haemost 95:253–259.

Linnemann B, Schwonberg J, Mani H, *et al.* (2008) Standardization of light transmittance aggregometry for monitoring antiplatelet therapy: an adjustment for platelet count is not necessary. J Thromb Haemost 6:677–683.

Mueller T, Dieplinger B, Poelz W, *et al.* (2007) Utility of whole blood impedance aggregometry for the assessment of clopidogrel action using the novel Multiplate analyzer—comparison with two flow cytometric methods. Thromb Res 121:249–258.

Rudež G, Pons D, Leebeek F, *et al.* (2008) Platelet receptor P2RY12 haplotypes predict restenosis after percutaneous coronary interventions. Hum Mutat 29:375–380.

Ševčíková H (2006) Rezistence na protidestičkovou terapii. Interv Akut Kardiol 5:256–258.

Seyfert UT, Haubelt H, Vogt A, *et al.* (2007) Variables influencing Multiplate(TM) whole blood impedance platelet aggregometry and turbidimetric platelet aggregation in healthy individuals. Platelets 18:199–206.

Slavik L, Úlehlová J, Krcova V, *et al.* (2011) Detection of clopidogrel resistance by MEA and LTA. Transfuze a Hematologie Dnes 17:92–96.

Úlehlová J, Slavík L, Krčová V, *et al.* (2011) The assessment of aspirin resistance by using light transmission and multiple electrode aggregometry. Int J Lab Hematol 33:305–309.

Address correspondence to:
Ludek Slavik, PhD
Department of Hemato-Oncology
Faculty of Medicine and Dentistry
Palacky University Olomouc and University Hospital
Olomouc 77520
Czech Republic

E-mail: ludek.slavik@fnol.cz