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Performance comparison of platelet function analyzers in cardiology patients: VerifyNow and Anysis-200 aspirin assays

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Abstract.
BACKGROUND: Analysis of responsiveness to antiplatelet therapy is crucial in the management of patients with cardiovascular diseases.
OBJECTIVE: This study aimed to evaluate a new platelet function analysis system (Anysis-200) and to compare it with VerifyNow (Accumetrics, San Diego, CA) in cardiology patients.
METHODS: Overall, 125 citrated blood samples were collected from 85 cardiology patients referred for platelet function testing. In Anysis-200, platelet function was measured as blood migration distance (MD) until clogging of flow passage, which is comparable to aspirin resistance units obtained using VerifyNow. The two devices were simultaneously used and compared.
RESULTS: The MDs before and after taking aspirin were 174 ± 51 and 247 ± 27 mm, respectively ($p < 0.0001$). Compared with VerifyNow (reference), the sensitivity and specificity of Anysis-200 was 91.5% and 75.5%, respectively (area under the curve, 0.829). Further, the true positive rate in patients newly taking aspirin was 85% for VerifyNow and 92.5% for Anysis-200, respectively. The Cohen's kappa coefficient between the two devices was 0.682, indicating a relatively high agreement.
CONCLUSIONS: Anysis-200, a novel system for assessing platelet aggregation, has accuracy and precision equivalent to that of, and significant agreement with, VerifyNow. Anysis-200 may be useful in screening patients with abnormal platelet reactivity and aspirin nonresponsiveness.

Keywords: Platelet function, aspirin, Anysis-200 analyzer, VerifyNow

1. Introduction

Platelets play an important role in primary hemostasis when blood vessels are damaged. *In vivo* hemostasis is accompanied by platelet activation, secretion, adhesion, aggregation, and coagulation. In healthy blood vessels, the endothelial glycocalyx determines vascular permeability, attenuates blood cell–vessel wall interactions, mediates shear stress sensing, enables balanced signaling, and fulfills a vasculoprotective role, but Inhibition of its function causes abnormally activated platelets in atherosclerosis. Beyond the well-known function in hemostasis, platelets are known to play an important role

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in atherosclerosis by promoting atherosclerotic plaque formation through P-selectin-dependent mechanisms and causing endothelial dysfunction [1]. With the increase in the incidence of cardiovascular diseases associated with platelet reactivity, antiplatelet drugs, such as aspirin and clopidogrel, are now being widely used as treatment and preventive agents. Dual anti-platelet therapy with aspirin and P2Y12 inhibitors has been widely used in patients at a high risk for cardiovascular disease, thus significantly reducing the mortality rate. However, balancing the ischemic and hemorrhagic risks in preoperative cardiac patients has been a controversial issue [2, 3]. Recent guidelines recommended cessation of aspirin several days before coronary artery bypass graft surgery, especially in patients at a high risk of bleeding as well as in stable patients, which has been shown to be effective in preventing death, myocardial infarction (MI), or stroke in patients with acute coronary syndrome [4–6]. Thereby, there has been an enormous need to test platelet function preoperatively and during routine checkup. In addition, a study of patients undergoing percutaneous transluminal coronary angioplasty showed that abnormal platelet function was the most important factor for subsequent development of restenosis after angioplasty [7]. As such, testing platelet functions has become increasingly important in the present era.

Owing to rapid developments in point-of-care (POC) technology, several types of devices are now available for assessing the antiplatelet therapeutic response and platelet function. The typical devices are Platelet Function Analyzer-200 (Siemens, Mississauga, Ontario, Canada), Multiplate Analyzer (Roche Diagnostics, Rotkreuz, Switzerland), and Plateletworks Kit (Helena Laboratories, Beaumont, TX, USA). For example, VerifyNow is a test method for measuring the platelet aggregation inhibitory action of drugs (aspirin and P2Y12 inhibitor) that can be quickly and easily performed regardless of the skill of the operator. Previous studies have described and compared these POC devices to demonstrate their usefulness in identifying patients with abnormal antiplatelet therapeutic response. However, a few recent studies reported that the measured results did not agree with the clinical outcomes [8, 9]. Furthermore, another study reported poor standardization and agreement between different tests (38.7%–62.8%) [10].

Recently, the Anysis-200 analyzer (Rheo Meditech Inc., Seoul, Korea), a POC device for platelet function testing, has been developed [11–13]. A study showed that Anysis-200 is useful for screening cardiac patients with abnormal platelet function, showing moderate fair agreement with Platelet Function Analyzer-200 [14]. Anysis-200 is an automated laboratory-on-a-chip microfluidic system that uses the migration distance (MD) of blood through a microchannel to analyze platelet adhesion and aggregation. The MD, which is comparable with the aspirin resistance units (ARUs) obtained using VerifyNow, is automatically analyzed based on the images obtained by a camera. The present study aimed to evaluate the feasibility of the Anysis-200 system in screening cardiac patients with abnormal platelet functions and to compare it with VerifyNow, one of the most widely used antiplatelet responsiveness tests.

2. Materials and methods

2.1. Patient samples

In total, 125 whole blood samples were collected at Yonsei University Gangnam Severance Hospital between October 2018 and April 2019. The exclusion criteria for this study were as follows: platelet count $<100 \times 10^9$/L, hematocrit <35% and >60%, abnormal value of either prothrombin time or activated partial thromboplastin time within the previous 1 month, pregnancy, and use of anticoagulation agents. The samples were divided into two groups: aspirin treated ($n = 75$) and controls ($n = 50$). The study protocol was approved by the Institutional Review Board of Yonsei University

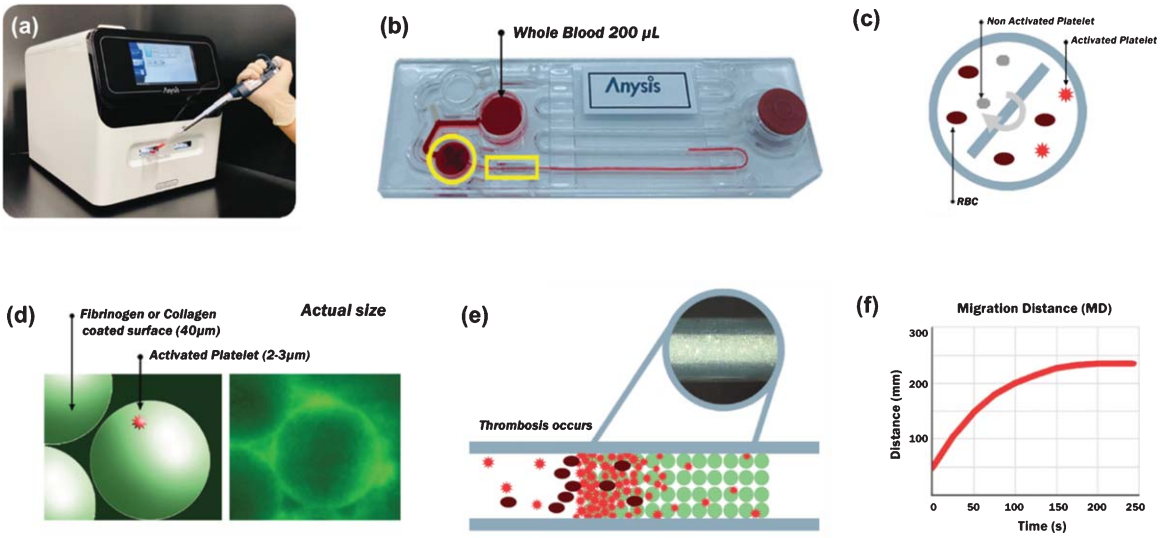

Fig. 1. Anysis-200 platelet function analyzer. (a) Anysis-200 instrument. (b) Anysis aspirin microfluidic chip, which consists of a sample loading chamber, a sample chamber (yellow circle) with a stirrer containing arachidonic acid, and a microbead-packed tube and a running tube. (c) The stirrer mixes arachidonic acid with blood in the sample chamber. (d, e) Fibrinogen-coated microbeads. (f) Migration distance of blood samples with respect to time.

Gangnam Severance Hospital, and written informed consent was obtained from all patients before study enrolment.

Blood samples were obtained using 21-G needles and collected into 3.2% sodium-citrate tubes (0.109 mol/L buffered sodium citrate; BD Vacutainer Systems, Franklin Lakes, NJ, USA) in both the VerifyNow and Anysis-200 assays. After blood collection, the tubes were gently inverted (five to six times). During the transportation, special care was taken to avoid platelet agitation. Thereafter, the blood samples were stored at room temperature for 30 min before the test. All tests were performed within 90 min after blood collection.

2.2. Anysis-200 platelet function analyzer

Anysis-200 is a recently developed platelet function analyzer with a disposable microfluidic system (Fig. 1). The microfluidic chip has three components: 1) sample chamber with a rotating stirrer, 2) tube packed with fibrinogen-coated microbeads, and 3) running tube. In the sample chamber, a specific reagent is coated and lyophilized. When vacuum pressure is applied to the test chip, the blood sample is aspirated into the sample chamber. With a rotating stirrer, agonists can be easily mixed with the blood sample for 30 s to activate platelets. In the aspirin assay, arachidonic acid is used as an agonist. The rotation is gently generated to avoid any shear-induced activation of platelets. After the mixing process, the blood sample is passed through the bead packing tube and then through the running tube. Activated platelets may adhere to the fibrinogen-coated surface, aggregate with each other, and eventually block the flow passages in the microbead-packed tube. By using a charge coupled device camera, the MD of the blood sample is monitored and analyzed with respect to time. The MD measured in the Anysis-200 assay is comparable to the ARU obtained in the VerifyNow assay. The unit of MD is millimeters. Abnormal MD results are defined as >204 mm, according to the manufacturer's instructions. Any values >204 mm is considered abnormal or indicative of aspirin-inhibited platelet function.

The bead-packed tube has a length of 2 mm and a diameter of 450 μm. The diameter of the microbeads is 40 ± 3 μm, and the effective diameter of the minimum pore is approximately 10 μm. Any blood

cells can freely pass through the pores formed in the microbead-packed tube. However, adhesion and aggregation of platelets to the beads rapidly reduces the effective diameter of the pores, which eventually blocks all pores. The required volume of a blood sample to block the bead-packed tube within a couple of minutes is <250 μL. For each test, a whole blood sample is pipetted into a sample loading chamber and the MD is automatically measured within 4 min. Anysis-200 is a microfluidic flow system that mimics the *in vivo* hemodynamic environment of the vasculature, with an upstream activation and downstream aggregation of platelets [11–13]. In fact, the downstream response of platelets after upstream activation by either agonists or shear stress has been increasingly recognized for its clinical significance in thrombotic analysis [15, 16].

2.3. VerifyNow

The VerifyNow system, a POC device for measuring platelet aggregation, is a turbidimetric-based optical detection system. The system consists of an instrument and disposable assay chip with four chambers. Each chamber includes a magnetic steel ball and various reagents (such as fibrinogen-coated beads), a platelet agonist (such as arachidonic acid), peptide, bovine serum albumin, buffer, and a stabilizer. These materials are clustered in pellet form in each chamber. The sample from the patient is citrated whole blood, which is automatically dispensed from the blood collection tube into the assay chip by the instrument (i.e., no blood handling by an operator. The magnetic ball is used for mixing the reagent pellet with the blood sample. Fibrinogen-coated microparticles tend to aggregate with activated platelets via glycoprotein IIb/IIIa receptors. The VerifyNow aspirin assay results are reported in ARUs. Abnormal ARUs are defined as <550, according to the manufacturer's instructions. Any values <550 are considered abnormal or indicative of aspirin-inhibited platelet function.

2.4. Statistical analysis

Normally distributed data are expressed as mean ± standard deviation (SD). The sensitivity and specificity were analyzed considering the results from VerifyNow as true positive and negative values. The pairwise agreement between the two platelet function assays was assessed using Cohen's kappa coefficient, and the results were interpreted as follows: ≤0, no agreement; 0.01–0.20, none to slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–1.00, almost perfect agreement [17]. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using MedCalc version 12.1.4 software (MedCalc software, Mariakerke, Belgium).

3. Results

3.1. Descriptive characteristics and hematologic parameters

A total of 42 male and 43 female patients, aged 49.8 ± 19.62 years (mean ± 1SD), were included (Table 1). Among them, 32, 38, and 13 patients were diagnosed with hypertension, dyslipidemia, and diabetes, respectively. Among the hypertensive patients, 31 were diagnosed with dyslipidemia and 13 were diagnosed with diabetes. The clinical characteristics of the patients are shown in Table 1. The platelet count was $247 \pm 57 \times 10^9$/L (mean ± 1SD).

Table 1
Baseline patient characteristics

Variable	Overall (N = 85)
Age (years), mean ± SD	49.8 ± 19.62
Male sex, *n* (%)	42 (49.4)
Risk factors	
Hypertension, *n* (%)	32 (37.6)
Diabetic mellitus, *n* (%)	13 (15.3)
Dyslipidemia, *n* (%)	38 (44.7)
Current smoking, *n* (%)	4 (4.7)
BMI (kg/m^2)	24.01 ± 3.17
Medication	
Statin, *n* (%)	34 (40)
Calcium channel blocker, *n* (%)	22 (25.9)
Beta-blocker, *n* (%)	21 (24.7)
ACEI, *n* (%)	1 (1.2)
ARB, *n* (%)	26 (30.6)
Nitrate, *n* (%)	12 (14.1)
Diuretics, *n* (%)	9 (10.6)
Platelet count (×10^9/L)	246.56 ± 57.34

Continuous data are shown as mean ± 1SD. Dichotomous data are shown as *n* (%). Abbreviations: BMI, body mass index; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; SD, standard deviation.

Table 2
Comparison of migration distances (a) between normal controls and patients using aspirin and (b) between before and after using aspirin

	Groups	Migration distance (mm) in Anysis-200	
		Mean ± SD	*p*-Value
(a)	Normal controls (*n* = 50)	171.7 ± 47.5	<0.0001
	Patients using aspirin (*n* = 35)	236.5 ± 34.2	
(b)	Before using aspirin (*n* = 40)	175.0 ± 51.4	<0.0001
	After using aspirin (*n* = 40)	247.3 ± 27.1	

Abbreviation: SD, standard deviation.

3.2. Comparative measurements between the two platelet function assays

Anysis-200 measured the MDs in normal controls who did not use aspirin and in patients who used aspirin (Table 2). The mean value in the normal control group was 171.7 ± 47.5 mm, whereas that in patients using aspirin was 236.5 ± 34.2 mm. Additionally, 40 patients who did not initially use aspirin eventually used aspirin, and the values before and after using aspirin were compared in these patients (Table 2b). The mean value before using aspirin was 175.0 mm, which significantly increased to 247.3 mm after using aspirin.

Of the 40 patients who did not initially use aspirin, those with VerifyNow ARUs < 550 and Anysis-200 MD > 204 mm were examined for changes after aspirin use (Table 3). There were 4 (10%) and 34 (85%) patients with measured VerifyNow ARUs of <550 before and after using aspirin, respectively

Table 3
Measurement comparison between before and after using aspirin with (a) VerifyNow and (b) Anysis-200

	Variable	Before using aspirin, *n* (%)	After using aspirin, *n* (%)	*p*-Value
(a) VerifyNow	ARUs ≥ 550	36 (90%)	6 (15%)	<0.0001
	ARUs<550	4 (10%)	34 (85%)	<0.0001
(b) Anysis-200	MD ≤ 204 mm	34 (75%)	3 (7.5%)	<0.0001
	MD>204 mm	7 (25%)	37 (92.5%)	<0.0001

Abbreviations: ARUs, aspirin resistance units; MD, migration distance.

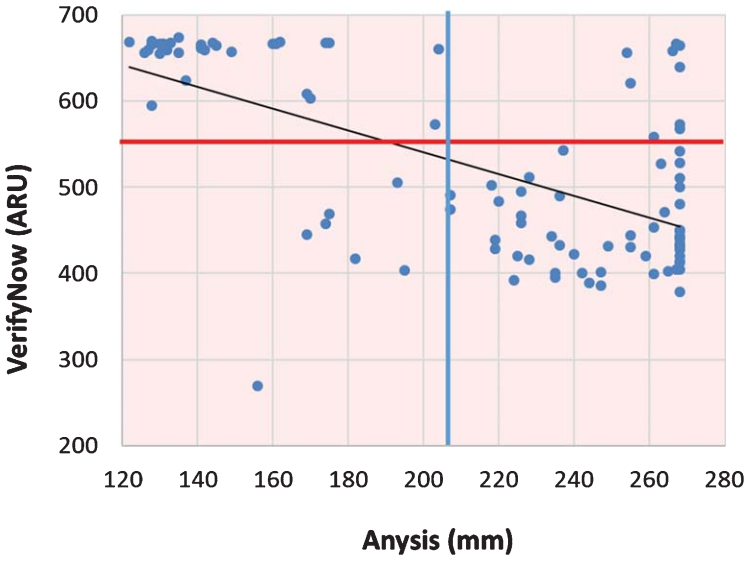

Fig. 2. Scatter plot comparing Anysis-200 and VerifyNow. Abbreviation: ARUs, aspirin resistance units.

(Table 3a). There were 10 (25%) and 37 (92.5%) patients with measured Anysis-200 MD > 204 mm before and after using aspirin, respectively (Table 3b).

In seven repeated measurements in the Anysis-200 assay, the intraclass correlation coefficient (ICC) was 0.960 (95% confidence interval [CI], 0.948–0.970), confirming the reliability of the method in yielding highly reproducible results. The agreement rate between Anysis-200 and VerifyNow was 0.682 (95% CI, 0.551–0.812; Cohen's kappa coefficient), which is the second highest level among the agreement comparisons.

The sensitivity and specificity of Anysis-200 was 91.5% (95% CI, 85.1%–98.0%) and 75.5% (95% CI, 63.9%–87.1%), respectively (Fig. 2). The correlation between Anysis-200 and VerifyNow was moderate (Pearson correlation coefficient $r = -0.615$, $p < 0.0001$). With the provided cutoff value, Anysis-200 results were further analyzed using the receiver operating characteristic curve in comparison with those of VerifyNow (Fig. 3). The area under the curve for Anysis-200 was 0.829, which indicated that Anysis-200 had nearly the same performance as VerifyNow.

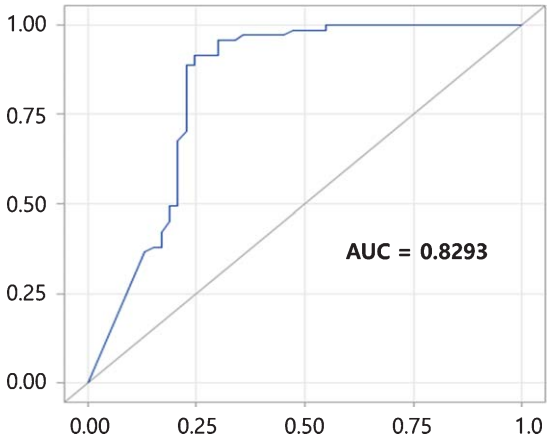

Fig. 3. Receiver operating characteristic curve comparing Anysis-200 and VerifyNow. Abbreviation: AUC, area under the curve.

4. Discussion

Platelets are disk-shaped cells in the bloodstream that are specifically involved in the formation of blood clots, which, in turn, play an important role in MI, stroke, and peripheral vascular disease. Typically, platelets can detect a disruption in the lining of blood vessels and responds by forming aggregates to stop bleeding. At the site of vessel wall damage, platelets are rapidly engaged in sequential functional responses that lead to their activation, including adhesion, spreading, shape change, aggregation, release reaction, procoagulant surface exposure, and clot retraction. The rapid progression of these different processes causes activated platelets to form a hemostatic plug that occludes the site of injury to prevent blood loss [18]. However, adverse clotting frequently results in serious adverse events such as MI or stroke, which are the major causes of morbidity and mortality in Western countries [19, 20]. Platelets may adhere and aggregate within atherosclerotic lesions, forming occluding arterial thrombi that can result in thromboembolic diseases. Moreover, platelets play a key role in mediating stent thrombosis, which is the major cause of ischemic events in the immediate period after percutaneous coronary intervention (PCI). Several studies have demonstrated the effectiveness of dual or triple antiplatelet therapy with aspirin, clopidogrel, and glycoprotein IIb/IIIa inhibitors in patients with acute coronary syndrome and those undergoing coronary stent implantation. Therefore, antiplatelet therapy, which is started at the time of PCI and continued for at least 30 days, is the cornerstone of antithrombotic therapy after PCI. However, despite optimal antiplatelet therapy, patients often develop cardiovascular events, including stent thrombosis. The individual response to dual antiplatelet therapy is not uniform, and consistent findings across multiple investigations support the association between a lower degree of platelet inhibition, high on-treatment platelet reactivity, and occurrence of atherothrombotic events [21–23]. Especially, several studies have shown that high residual platelet reactivity during antiplatelet treatment is predictive of major cardiovascular events in patients undergoing PCI [24–26]. Therefore, monitoring antiplatelet therapy is becoming increasingly important in the identification of hypo- or hyper-responding patients at a risk of both thrombosis and hemorrhage.

The clinical application of platelet function tests has improved in the last two decades, and these tests have been increasingly used in monitoring antiplatelet treatment in patients with cardiovascular disease who are at a risk of arterial disease [27]. The VerifyNow system is a platelet function-waived POC test consisting of a device that assesses whole blood platelet aggregation through turbidimetric-based optical detection and by using a system cartridge containing fibrinogen-coated beads and platelet

agonists [28]. Owing to convenience of operation, the method has been widely employed to monitor antiplatelet therapies in clinical environments outside of specialized facilities such as the emergency cardiac operating room [29]. However, the major drawbacks of VerifyNow include the high cost per test and the disagreement of results with clinical outcomes.

The newly developed platelet function analyzer Anysis-200 has been shown to overcome the above drawbacks of VerifyNow through technological innovations. Anysis-200 is one of the most user-friendly POC platelet function tests that can provide rapid and precise results at the patient's bedside. Considering the Cohen's kappa coefficient ($\kappa = 0.682$) and Pearson correlation coefficient ($r = -0.615$), Anysis-200 has a substantial agreement with VerifyNow. Furthermore, in the comparison analysis of before and after aspirin use in patients who did not initially take aspirin, the concordance rate of VerifyNow and Anysis-200 was 85% and 92%, respectively. This result confirms that both devices are reliable for testing aspirin reactivity. The ICC of the results of seven repeated tests in Anysis-200 assay was 0.96, indicating the high reliability of the device. Thus, Anysis-200 assay, which is a novel method for assessing platelet aggregation, has equivalent accuracy and precision, and moderate agreement, to VerifyNow. This new platelet function test can significantly improve prognosis and survival by identifying patients who are not responding to aspirin in order to prevent thrombosis and allow replacing aspirin with other effective treatments.

5. Limitations

Abnormal ARUs were defined as values of <550 according to the manufacturer's instructions. However, the cutoff values for distinguishing responders from nonresponders in the VerifyNow assay are based on results of studies with a relatively small sample size. Therefore, these thresholds should be used with utmost caution. Nevertheless, in this study, the sensitivity and specificity of Anysis-200 were analyzed considering the results from VerifyNow as true positive and negative values. Moreover, the comparison between VerifyNow and Anysis-200 was made only with respect to aspirin. In the future, other antiplatelet drugs, such as clopidogrel and abxicimab, should be used for comparison. Lastly, in the elderly (>75 years), agonist induced platelet aggregation is reduced due to chronic increase in the activation of circulating platelets [30]. Therefore care should be taken in interpreting the analysis of platelet function in these populations.

Acknowledgments

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Conflicts of interest

The authors report no potential conflicts of interest relevant to this article.

Ethical approval

This study was approved by Institutional Review Board, Yonsei University Gangnam Severance Hospital (approval no. 3-2018-0199) and performed in accordance with the declaration of Helsinki. The participants provided informed consent for participation in the study.

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