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PRACA POGLĄDOWA
REVIEW

Multiple electrode aggregometry – only for cardiologists?

Metoda agregacji impedancyjnej w ocenie funkcji płytek krwi – czy tylko dla kardiologów?

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ABSTRACT

Multiple electrode aggregometry is one of the newest technologies in platelet function monitoring. The idea of this assay is based on whole blood impedance aggregometry measurements. The main advantages of this device are rapid and easy use, no necessity of sample pre-processing or requiring a specialized laboratory. These features allow one to include this methodology in point-of-care testing methods that can be performed at the patient bedside. Five different pathways of platelet activation can be investigated by adding specific reaction agonists. Antiplatelet drugs, such as acetylsalicylic acid or clopidogrel, inhibit arachidonic acid-dependent and adenosine diphosphate-dependent pathways of platelet activation. Individual patient response to these drugs can be estimated using multiple electrode aggregometry. The identification of low-responders may result in reducing thrombosis events in this group and make antiplatelet treatment more effective. Furthermore, it is supposed to be a reliable method of estimating the risk of perioperative bleeding in adults undergoing cardiac surgery. Other potential clinical applications for this technology are being found. Many studies report its use in determining prognosis in severe sepsis, detecting heparin-induced thrombocytopenia and diagnosing von Willebrand disease. Although multiple electrode aggregometry seems to have great diagnostic potential, more tests need to be performed before it becomes standard hospital equipment.

KEY WORDS
multiple electrode aggregometry, platelet function monitoring, antiplatelet therapy, platelet tests

STRESZCZENIE

Metoda agregacji impedancyjnej jest jedną z najnowszych technik stosowanych w ocenie funkcji płytek krwi, wykorzystującą krew pełną jako środowisko reakcji. Zasada jej działania opiera się na pomiarach zmian impedancji, jakie następują na skutek agregacji płytek krwi po dodaniu egzogennego aktywatora. Najważniejszymi zaletami tej metody są: łatwość jej wykonania bez specjalistycznego laboratorium, brak konieczności wcześniejszego przetwarzania pobranej do badania próbki oraz szybkość w uzyskaniu wyników. Wszystkie te cechy pozwalają na wykonanie tego badania przy łóżku pacjenta. Metoda agregacji impedancyjnej pozwala na ocenę pięciu różnych szlaków aktywacji

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płytek krwi w zależności od zastosowanego agonisty. Leki przeciwpłytkowe, takie jak kwas acetylosalicylowy czy klopidogrel, powodują blokadę aktywacji trombocytów zależną kolejno od kwasu arachidonowego i adenozynodifosforanu. Zastosowanie tych związków, jako aktywatorów agregacji płytek krwi, pozwala na ocenę indywidualnej odpowiedzi pacjentów na terapię tymi lekami. Identyfikacja osób „odpornych" na leczenie przeciwpłytkowe może spowodować zmniejszenie liczby powikłań zakrzepowych u tej grupy chorych oraz pozwoli na zwiększenie efektywności leczenia. Obecnie poszukiwane są także inne kliniczne zastosowania agregacji impedancyjnej. Technika ta może być również stosowana do oceny ryzyka krwawienia okołooperacyjnego w kardiochirurgii. Trwają badania dotyczące jej potencjalnego użycia przy określaniu rokowania u pacjentów z ostrą sepsą, wykrywaniu trombocytopenii indukowanej heparyną czy diagnostyce choroby von Willebranda. Pomimo że technika ta ma potencjał, aby stać się metodą przyszłości w ocenie funkcji płytek krwi, wiele badań musi potwierdzić jej przydatność zanim stanie się standardową procedurą szpitalną.

SŁOWA KLUCZOWE
metoda agregacji impedancyjnej, terapia przeciwpłytkowa, trombocyty, ocena funkcji płytek krwi

INTRODUCTION

Platelets, next to their coagulation and fibrinolytic factors, protein inhibitors and endothelial cells, are an essential part of hemostasis in the human organism. They are involved in physiological primary hemostasis as well as in some pathological processes such as bleeding disorders, thrombosis or atherosclerosis. Their role in the hemostatic process starts after vessel endothelium injury. Activation factors exposed on the damaged wall of the vessel, such as ADP, collagen, thromboxane A_2, epinephrine, serotonin, von Willebrand factor and thrombin, recruit platelets from circulation. They bind to the activators by means of their surface receptors, which leads to a change in their shape from discoid to a spherical form. Then platelets in sequence undergo several changes which are activation, adhesion, aggregation and release of their granule contents [1]. Increased risk of bleeding can be observed when the platelet count is reduced or their function is incorrect. Conversely, due to an excess platelet amount or their hyperactivity, thrombosis may occur. These different functions of platelets can be detected using a wide spectrum of tests. As the importance of the role of platelets in the mentioned processes has been emphasized, platelet functions have also become a therapeutical target in cardiology. Three groups of antiplatelet drugs (acetylsalicylic acid, $P2Y_{12}$ – receptor antagonists and glycoprotein IIb/IIIa receptor antagonists) have the ability to inhibit platelet aggregation by blocking specific receptors on the platelet surface. Monitoring individual patient response to this therapy is nowadays an important issue of treatment after cardiovascular events.

Platelet function testing history started in 1910. The Duke bleeding time test was the first test ever used *in vivo* in diagnosing disorders of hemostasis caused by the inability of platelets to form a plug. Interpretation of the results depends on the time it takes from the beginning till the end of bleeding after pricking the fingertip. The normal values range is between 2–5 minutes. Although it has been successfully replaced with new methods, bleeding time can still be used as a screening method in diagnosing platelet dysfunction [2]. The revolution in the development of platelet function monitoring came 50 years later. It was in the 1960s when Born [3] designed light transmission aggregometry (LTA), which is still currently the golden standard for: assessing various platelet functions:

– diagnosing inherited and acquired platelet disorders,
– monitoring individual response to dual antiplatelet therapy by acetylsalicylic acid and clopidogrel [1,3].

The idea of this test is based on the increase in light transmission after the addition of an exogenous platelet agonist to platelet-rich-plasma (PRP). The exogenous agonist (e.g. ADP, arachidonic acid, collagen) has the ability to activate platelets by reacting with their surface receptors. In that mechanism platelets aggregate and form a plug. As a result of this reaction, the previously dense PRP sample becomes clearer and an increase in light transmission is observed by a photometer [4,5]. LTA presents this signal as a graphic curve and measures the extent of aggregation percentage. By adding different agonists, various pathways of platelet activation can be investigated. Although LTA remains the most useful technique for assessing platelet functions, it is not perfect. The main limitations of this device are connected with the use of PRP instead whole blood which makes this test more time-consuming, more difficult to perform and requiring a specialized laboratory. Furthermore, LTA is still a poorly-standardized technique [4].

As the role of platelet function monitoring has become a more important issue in cardiology, better technologies have been developed. Some of the currently performed tests are presented in Table I.

Table I. Characteristics of currently used tests for platelet monitoring
Tabela I. Charakterystyka obecnie stosowanych testów do oceny funkcji płytek krwi

Test	Methodology and limitations	Clinical use
Plateletworks system	Based on measurement of platelet count before and after platelet aggregation; POCT assay Sample: citrated WB Limitations: strict time from sample collection to performing test	– analysis of platelet function during cardiac surgery – antiplatelet therapy monitoring
The Platelet Function Analyzer (PFA-100)	Based on platelet adhesion under shear stress and aggregation after agonist addition; POCT assay Sample: citrated WB Limitations: sensitive to hematocrit and thrombocytopenia	– antiplatelet therapy monitoring – bleeding risk prediction – blood transfusion management – VWD diagnosis
Flow cytometry	Laser-based platelet physical and chemical estimation (e.g. size, biomarker detection) Sample: citrated WB/PRP/W-Plt Limitations: expensive, requires specialized laboratory and devices	– diagnosing inherited and acquired thrombocytopathies – platelet counting – antiplatelet therapy monitoring

Abbreviations: POCT (point-of-care testing); WB (whole blood); VWD (von Willebrand disease); PRP (platelet-rich plasma); W-Plt (washed platelets).

Point-of-care-testing (POCT) includes the newest methodologies that step towards expectations such as easy use or fast and simple specimen handling (whole blood, urine) without the necessity of its preprocessing [6]. By definition, these tests can be performed at the patient bedside and some of them even by the patient himself. Nowadays, POCT methods are commonly used in urinalysis, blood glucose testing and coagulation monitoring (international normalized ratio – INR). POCT is supposed to be as effective as the previously used methods and available not only in specialized teaching hospitals but in general hospitals as well.
Multiple Electrode Aggregometry (MEA) is a method of POCT based on whole blood impedance aggregometry measurements, which is useful in various clinical purposes. Here we present our review on its topic.

Multiple Electrode Aggregometry – basic information

Multiple electrode aggregometry, firstly introduced to the world in 2006, is a new methodology in platelet function monitoring. It allows one to assess platelet functions with one device using whole blood as the milieu [4]. This is a crucial aspect for the measurements due to imitation of the physiological conditions of platelet activation and takes under consideration the effect of other blood elements e.g. red blood cells, on the process [4]. Furthermore, there is no need for sample processing either, which makes this test rapid and easy to perform.
Before starting the analysis, the whole blood sample has to be anticoagulated. Recommended anticoagulants for this process are sodium heparin or r-hirudin which guarantee stability and allow the longest storage time for impedance aggregometry [7]. The device consists of two main parts: the sensors and the instrument. The first of them is made by two independent electrodes placed in disposable cuvettes. After the addition of an exogenous activator to the anticoagulated whole blood sample, activated platelets adhere on the surfaces of the sensors. Platelet aggregates form an insulation layer on the sensors, which results in a rise in impedance between the electrodes. Platelet aggregation on each of them is measured doubly and calculated automatically. The measurement outcomes are presented on the monitor as a graphic curve of platelet aggregation in time. A computer analyzes three parameters, which are in the area under the curve, aggregation and velocity.
The instrument is a five-channel device able to perform parallel tests (up to 30 tests per hour). Each of them examines the different pathways of platelet activation, which makes this device a valid tool for complete platelet function evaluation [4]. In order to carry out a defined test, a specific activator needs to be used. Agonists and diluents are prepared exactly for the reaction and they are pipetted automatically. Available activators are arachidonic acid, ADP, collagen, thrombin receptor activating peptide (TRAP) and ristocetin. The most commonly used tests with the activators and their antiplatelet inhibitors are presented in Figure 1.
In comparison to LTA, multiple electrode aggregometry is not a complicated nor time-consuming method. It can be performed at or near the patient bedside, which allows one to include this technology in POCT methods. MEA presents many potentially useful features with relatively few limitations (Tab. II). Its clinical use is especially highlighted in cardiology although it might also prove to be a useful device in different areas.

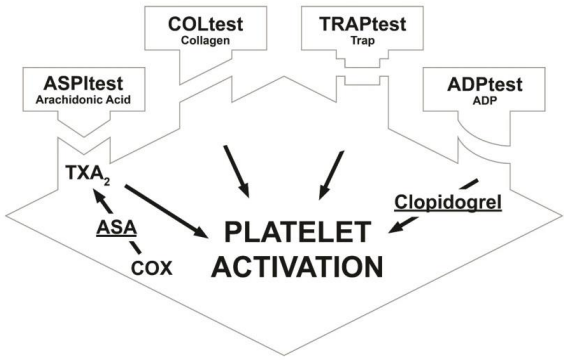

Fig. 1. Multiple tests.
Ryc. 1. Testy stosowane w impedancji agregacyjnej.

Table II. Advantages and limitations of multiple electrode aggregometry
Tabela II. Zalety i ograniczenia agregacji impedacyjnej

Pro	Contra
– Small sample volume (only 0.3 ml pertest) – Standardized procedures – Minimal technical knowledge necessary – Sensitive to antiplatelet therapy – Results not influenced by hematocrit orred blood cell count [8] – Potential new clinical applications	– results influenced by thrombocytopenia [9] – necessity of further prospective studies to define new applications for MEA – not experienced in clinical use

Clinical use of MEA in cardiology

Multiple electrode impedance aggregometry has acquired a clinical value in cardiology nowadays. The ability of investigating various platelet activation pathways has made this technology a reliable method for:

1. Monitoring individual patient response to dual antiplatelet therapy.
2. Identifying patients who are non-responders to antiplatelet drugs.
3. Estimating risk bleeding during and after cardiac surgery in adults.

Dual antiplatelet therapy (DAPT) is an antithrombotic treatment using acetylsalicylic acid (cyclooxygenases inhibitor) and one of the $P2Y_{12}$-receptor antagonists (clopidogrel, ticagrelor or prasugrel). According to the ESC/EACTS Guidelines on Myocardial Revascularization 2014, patients after percutaneous coronary intervention (PCI) in stable coronary artery disease and acute coronary syndrome (both non-ST-segment elevation and ST-segment elevation) should be treated with these groups of drugs in order to reduce the risk of stent thrombosis. The duration of treatment, combination and doses of antiplatelet drugs depend on the clinical setting and mode of intervention (PCI or CABG) [10]. In order to maximize the effectiveness of therapy and simultaneously minimalize the hazard of bleeding, DAPT should be tailored individually to each patient. MEA can be successfully used to assess the influence of aspirin (ASA) and $P2Y_{12}$--receptor antagonists to platelet functions [11]. These two groups of drugs have an impact on platelet activity using different pathways. Arachidonic acid-induced aggregation (ASPI-test) is the blocking target for ASA while clopidogrel and other thienopyridines inhibit ADP-induced aggregation (see Fig. 1). Several studies, using MEA, proved that:

1. ADP-induced aggregation is not disturbed by acetylsalicylic acid either in vitro or in vivo.
2. Isolated $P2Y_{12}$ inhibition is capable of blocking ADP-induced aggregation, which makes this method a sensitive device to monitor thienopyridine intake.
3. In some groups of patients when ADP-induced aggregation was fully blocked, after oral clopidogrel intake, AA-induced aggregation was reduced [11].
4. Acetylsalicylic acid effectively inhibited more than 95% AA-induced aggregation in vitro and in vivo [11].

According to these results, multiple electrode aggregometry is reliable technology for monitoring patient response to dual antiplatelet therapy. The interactions between these two groups of medicines can be different for everyone. Furthermore, many other factors influence individual response to treatment. High platelet count, diabetes mellitus type 2, high body mass index and prior myocardial infarction are confirmed as independent determinants of increased platelet aggregation [12]. The mentioned characteristics are supposed to be key factors in lowering response to antiplatelet treatment. Genetic aspects, such as variability in metabolism and absorption of clopidogrel caused by dysfunctional hepatic cytochrome P450, ADP-receptor defect or cyclooxygenases polymorphisms are also connected with the ineffectiveness of DAPT [10,13].

Although many studies have been carried out, the "acetylsalicylic acid resistance" definition is still not clear. In clinical medicine it can be defined as the occurrence of thromboembolic events during ASA intake while in laboratory medicine it is known as failure in platelet function inhibition despite therapy [14]. These statements are still not universal. Regardless of the definition, multiple electrode aggregometry may help in recognizing patients who are low-responders to antiplatelet treatment. Research performed with multiple electrode aggregometry on patients with acute coronary syndrome showed that almost 5% of this group was acetylsalicylic acid-resistant while almost 22% did not react to clopidogrel intake [15]. The same studies revealed that a high level of triglycerides is associated with resistance to clopidogrel.

Other investigations also confirmed MEA as an applicable technology for identifying patients with an ineffective response to antiplatelet treatment. The percentage of low-responders is different in various groups and depends on individual predispositions as well as different cut-off values, but it may even reach 30% for clopidogrel and almost 20% for acetylsalicylic acid [13]. Identifying these patients allows one to change the treatment strategy for their own needs in order to reduce the risk of thrombotic or ischemic events. Data suggest the addition of an extra dose of clopidogrel or switching this drug for ticlopidine or prasugrel in clopidogrel-resistance and increasing the dose of acetylsalicylic acid in ASA-resistance [13]. Although antiplatelet therapy monitoring seems to be effective technology, the actual guidelines recommend it only in specific high-risk situations like a history of stent thrombosis, a compliance issue or high bleeding risk [10].

Platelet function monitoring plays an important role in balancing between the risk of thrombotic events (characteristic for antiplatelet therapy resistance) and perioperative bleeding. Surgeries requiring a cardiopulmonary bypass are often complicated by perioperative massive bleeding which results in increased mortality, morbidity, transfusion requirements and reinterventions [16]. Here the hopes for using MEA start, as this POCT method seems to be a useful device for assessing the risk of postoperative hemorrhage by performing ASPI- and ADP-tests mentioned earlier [17]. Present studies on patients undergoing coronary artery surgery used MEA in defining the therapeutic window that fits exactly between the risks of thrombosis and hemorrhage. It was the first research ever performed that used MEA for the perioperative management of antiplatelet therapy based on this concept [18].

Using MEA in predicting bleeding becomes questionable when it comes to pediatric patients. Studies carried out on fifty children with congenital heart disease undergoing surgery did not confirm the relationship between the platelet aggregation parameters assessed by the ASPI-, ADP- or TRAP-test and high blood loss [19]. The same results were obtained in different research on pediatric patients after cardiopulmonary bypass surgery, using the TRAP-test [20]. The different behaviour of MEA in children may be justified by the fact that antiplatelet drugs are not commonly used in congenital heart paediatric patients. However, it still remains in contrast to adults who showed the association between MEA measurements and bleeding with an absence of antiplatelet therapy [21]. Furthermore, the results of the mentioned research showed that low aggregation values are still not associated with blood loss as long as the platelet count is within the normal limits [20]. The authors of the analysed studies suggest that platelet aggregability is not a relevant determinant of perioperative bleeding or transfusion requirements in children and should never be used as the only device in this case [19]. Better factors for this purpose are low platelet count and increased INR [20].

Other potential applications for MEA

Hip fracture

Hip fracture is currently one of the most important challenges for orthopedics. This injury is common in people over 65 years of age, especially females, and is associated with high mortality in this group of patients. Some studies report that almost 50% of hip fractured elderly will die within six months and even those who survive may not fully recover [22]. Complications such as pulmonary embolism, a major cardiac event and sepsis [23] are the main causes of death.

According to the American Academy of Orthopaedic Surgeons guidelines, surgery should be performed within 48 hours after admission to hospital, which is associated with better outcomes and fewer complications. A delay may increase all-cause mortality by 41% in 30 days after surgery [24]. Elderly patients who are often treated with antiplatelet therapy after cardiovascular or cerebrovascular events have an increased risk of serious bleeding during and after surgery. Patients taking clopidogrel can be operated at least 5 days after discontinuing antiplatelet treatment [25] in order to avoid bleeding complications. Such a long period is crucial for hip fracture recovery and significantly worsens the prognosis. A current retrospective case-controlled study performed on 112 patients used multiple electrode aggregometry to identify hip fractured patients on antiplatelet therapy who were non-responders to clopidogrel. Almost one third of the patients showed no response to this drug and could be operated on without delay [26], which is a crucial aspect for recovery. Patients with good response to the drug could be optimized with platelet transfusion and operated immediately.

Determining Prognosis in Sepsis Patients

Sepsis, defined as systemic inflammatory response syndrome (SIRS) with proven or probable infection, is the most common cause of death among critically ill patients in non-coronary intensive care units [27]. Severe sepsis, with an additional organ dysfunction, occurs in almost 40% of the patients hospitalized on these wards [28]. The infection associated with the highest mortality is pneumonia [27]. The challenges for sepsis therapy are both better diagnosis and more effective treatment.

Currently, routinely used sepsis biomarkers are acute phase proteins (CRP and procalcitonin), IL-6 and

organ dysfunction biomarkers for example troponin, natriuretic peptides (ANP, BNP) or cytokeratin-18 [28]. Studies on the pathophysiology of sepsis proved that coagulation abnormalities and progress of the disease are strongly connected.

Platelets, which are one of the mediators of immunological response to infection, aggregate while presenting lipopolysaccharide (LPS) to the reticuloendothelial system, which leads to disseminated intravascular coagulation (DIC) [29]. On the other hand, the incubation of LPS with whole blood revealed a markedly inhibited platelet function in both in vivo and in vitro studies [30,31]. That is why new research reported that multiple electrode impedance aggregometry may become a better device for diagnosis and to predict survival even than conventional biomarkers and platelet count [30,31]. The analysis of previously mentioned studies proved remarkably higher mortality in the group of sepsis patients with a low platelet function. The results revealed that impedance aggregometry using collagen as an activator was the most compatible predictor of sepsis while arachidonic acid failed in this use [30]. The cause of this phenomenon is still not clear but may become the object of interest in the near future.

However, other research did not find any association between MEA measurements and mortality [29,32]. The authors suggest that platelet aggregation can be influenced by actual platelet count, which makes these tests unreliable [32]. They also proved that platelet count and hemoglobin are better diagnostic and prognostic factors [29]. Nevertheless, this topic is becoming one of the most popular aspects of determining prognosis in sepsis patients nowadays.

Detection of heparin-induced thrombocytopenia

Multiple electrode impedance aggregometry turned out to be a useful tool for hematology laboratories in detecting heparin-induced thrombocytopenia (HIT) [33,34,35]. HIT, a severe complication during treatment with heparin, is connected with the synthesis of IgG antibodies that activate platelets. Thrombotic events happen in even 50% of these patients [36]. Platelets activated by the mentioned antibodies can be measured more successfully using MEA than by the ELISA test. MEA detected almost 3% more patients with HIT than the ELISA test. Furthermore, it turned out to be not only more specific but also easier to perform [35]. MEA proved to be a more sensitive method than the current gold standard – the ^{14}C-serotonin release assay for detecting patients with HIT [34].

von Willebrand Disease Diagnosis

Multiple electrode impedance aggregometry has also proven to be a new tool useful in diagnosing von Willebrand disease (both inherited and acquired). In comparison to standard methods, which are light transmission aggregometry (LTA) and ristocetin-induced platelet aggregation (RIPA), MEA turned out to be as sensitive as the mentioned tests [37]. It also correctly identified patients with the challenging type 2B of this disease with additional thrombocytopenia. The authors of this research also emphasize the simplicity in taking measurements while using MEA, but mention the necessity of enlarging this research.

CONCLUSIONS

Multiple electrode aggregometry is a new step towards complete platelet function assessment. It presents many potential opportunities connected with cardiology as well as other fields of medicine. The main advantages of this method could be summarized as follows:

– using whole blood as a milieu makes this method rapid and simple to perform,
– assessing different pathways of platelet activation gives opportunities for complete platelet function monitoring.

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