Detection and treatment of thrombotic diseases by activating platelet

Leyu Wang [1, *, †], Jiawen Tan [2, †]

[1] Guangzhou Zhixin high school, Guangzhou, China

[2] University of Adelaide, Shenzhen, China

* Corresponding author: 1811000323@mail.sit.edu.cn

[†]These authors contribute equally.

Abstract. Nowadays thrombus has become one of the most prevalent diseases among the world, a large amount of population died of it. Thus, it is essential to improve the medical diagnose and treatment method to lower the morbidity as great as possible. Deep vein thrombus and artery thrombus, these two typical examples of thrombus will be given to illustrate the main methods of diagnose and treatment. In the stage of making diagnose, there are many traditional methods in medical fields like thrombelastogram, radiography, CT technology and X-rays. They are all proven medical technologies which can detect the thrombosis clearly through different principles. However, with the time goes by, Flow Cytometry, a new technology, has been developed through innumerable experiment and research. This technology is significant breakthrough in the history of blooding diagnose since it is more advanced than the classical method in many aspects. For instance, the thrombelastogram has low repeatability since it 's results are not always accurate; Radiography will cause risks since it will leave wounds for the patients; CT and X-rays are usually used in the phase of treatment but not in the early diagnose. Above all, it is better to combine these classical means with Flow Cytometry to make the diagnose more convenient and prevent getting thrombus as much as possible. For treatment of thrombus like Deep vein thrombus and artery thrombus, thrombolytic drugs, anticoagulant drugs, and antiplatelet drugs are mainly suggested. Other than those drugs, there are other drugs that may composed by other component are under ongoing research.

Keywords: Active platelet index, arterial thrombosis, deep vein thrombosis, antiplatelet treatments.

1. Introduction

In the contemporary society, the thrombosis is so prevalent that 1 in 4 people worldwide are dying from conditions caused by thrombosis every day. The formation of thrombosis is usually caused by the presence of blood clot in our blood vessels. Plus, the blocked vessels may place in arteries, deep veins or capillary. Therefore, the thrombosis is classified into two types: deep vein thrombosis and artery thrombosis. Ischemic heart disease and ischemic stroke comprise the major arterial thrombosis, and deep-vein thrombosis and pulmonary embolism comprise venous thromboembolism. Atrial fibrillation is a major risk factor for stroke and systemic arterial thromboembolism. According to the statistics, the annual incidence of DVT is 80 cases per 100,000, with a prevalence of lower limb DVT of 1 case per 1000 population [1]. Annually in the United States, more than 200,000 people develop venous thrombosis; of those, 50,000 cases are complicated by pulmonary embolism. The incidence and prevalence of the clinical manifestations of arterial thrombosis are high In 2016, CAD and peripheral vascular disease made up 3.8% of all cases of disease and injuries captured by the Global Burden of Disease study and 58.3% of all CV disease 5. So far, thrombosis has become a major contributor to the global disease burden. Due to this serious circumstance, it is important to catch the optimal time to make diagnose and get treatment. Thus, the platelet testing plays a crucial rule to guard against the thrombosis. The platelet activation index is one of the most necessary tests in preventing thrombosis.

2. Activating platelet Index and Thrombosis detection

Platelets are cells that help your blood clot, and too many platelets will put you at risk for blood clots or stroke. Platelet will go through three stages to function normally: Rest, activation, and gathering.

Resting platelet do not function and float freely in the blood vessel around our bodies in normal circumstances. As bleeding occurs, it will be activated by receiving various signals from our bodies and call on other cells to knit a fibrin reticulum to achieve cruor, which is the last stage—gathering. During the activation of platelets, the ∂ particles which contains P selectin will fuse with the plasma membrane and release the P selectin onto the surface of the platelet membrane. In this case, the P selectin will be easily detected by researchers and conclude whether the platelets are successfully activated. In order to calculate the index of the activated platelets, researchers use the activated platelets as numerator and the whole number of platelets as denominator. Since platelets are related to the blood clot forming, it is available for doctors to use this to determine if the patient have the related disease, embolism.

2.1. Embolism diseases

There are two main types of embolism, one is arterial thrombosis, the other is deep vein thrombosis.

2.1.1. Arterial thrombosis

Arterial thrombosis usually happens in small arteries as the blood pressure in arteries are higher than veins. Diseases like heart attack and stroke are related to arterial thrombosis. The most known thrombosis related disease is stroke, as the blood vessel is small, even a small blood clot can block that blood vessel, causing ischemic stroke. According to CDC, although stroke does not cause many deaths like coronial artery disease, but it can happen in any age and causing patients to suffer a long-term disability in life [2]. The major symptoms are paralysis in legs or arms, headache, sight problems in eyes.

2.1.2. Deep vein thrombosis

Another major thrombosis type is deep vein thrombosis. This thrombosis can be caused by many different reasons, but research shows it is heavily related to long time sitting and other cardiovascular diseases. Unlike arterial thrombosis, most deep vein thrombosis will not fatal and only have swelling, throbbing and pain [3]. However, there is one disease that might be fatal as the thrombosis move up to the pulmonary artery, causing pulmonary embolism, which can be deadly if patient does not get support in time [4]. If patient arrive in hospital with the above or even worse symptoms, doctors will have to do specific test on the person to determine the disease. In this case, we will use active platelet index to diagnose if there is blood clot causing thrombosis in blood vessels.

2.2. Prevalence of the two Thrombosis

Thrombosis often takes place in veins and arteries. Deep vein thrombus and artery thrombus are two of the most prevalence diseases in thrombosis. Deep vein thrombosis (DVT) occurs when a blood clot (thrombus) forms in one or more of the deep veins in the body. The precise number of people affected by deep vein thrombosis (DVT) or pulmonary embolism (PE) is unknown, although as many as 900,000 people could be affected each year in the United States, and estimates suggest that 60,000-100,000 Americans die of DVT. Similarly, artery thrombus is a blood clots happened in arteries. The incidence of cerebrovascular events is 2,900 cases per million inhabitants per year, of which 500 are transient ischemic attacks and 2,400 are strokes, representing an extremely serious trend. Therefore, experts are calling for early diagnosis to prevent further deterioration of the disease. Many traditional diagnostic methods have their unique advantages in helping to detect blood clots.

2.3. Active Platelet Index

During the recent years, a company in China has been focused on the project of platelet testing. In their experiments, researchers draw 1ml blood samples from normal people and use Flow Cytometry to partition platelets from the red blood cells, white blood cells and some cell fragments. The FC strategy includes Forward scatter (FC) and side scatter (SC), which is to use a laser beam to project the blood cells on the Photomultiplier tubes (PMT). FSC and SSC gating are commonly used to identify cells of interest based on size and granularity (complexity). It is often suggested that forward scatter indicates cell size whereas side scatter relates to the complexity or granularity of the cell. In this way, red blood cells, white blood cells, debris and platelet can be distinguished by their particular size and complexity (Fig1). However, since the platelet sometimes cannot be divided from other cells comprehensively by observing the cells projection, antibodies CD-41 and CD-61(in the reagent) with fluorescein will combine with antigens CD-41 and CD-61 on the platelets to detect the accurate ranges of real platelets. To count the number of activated platelets, researchers in the company use a special P-selectin, CD62P, marking with fluorescein, and the platelets are supposed to be activated successfully if CD62P antigens are expressed well. With the number of activated platelets and total platelets, platelets activation index can be calculated. The conclusion shows that if a person's platelets activation index exceeds 3%, will have the risk for thrombosis.

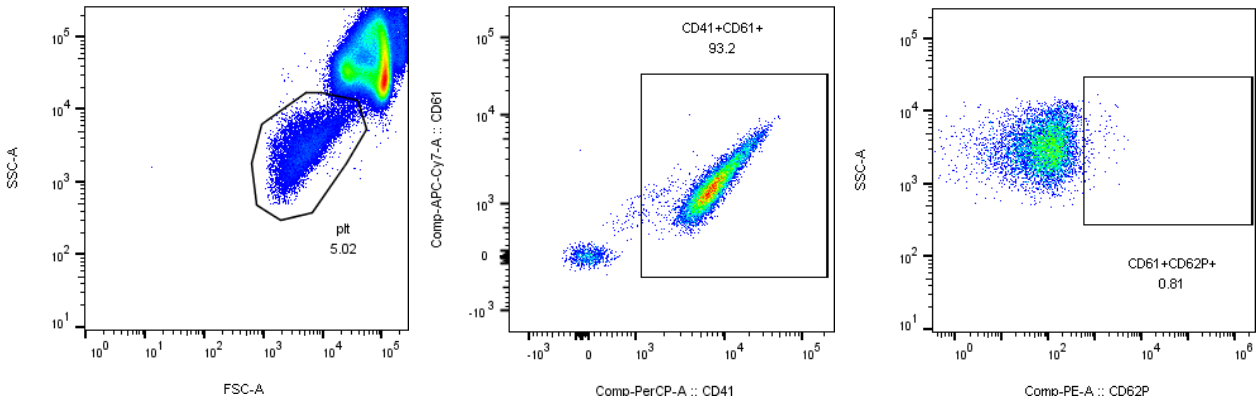

Figure 1. Detect the accurate ranges of real platelets

2.4. Conventional diagnose methods

To begin with, the angiogram, which is an interventional test in which a developer is injected into a blood vessel. Moreover, in the further stage of surgery, doctors may open clogged heart arteries (angioplasty) during coronary angiogram. Admittedly, Angiography has an immediate effect on thrombus, and it has great significance on clinical medicine. Nevertheless, it is a conventional method which exists some potential risks, such as allergic reaction to contrast dye, pain at the site of surgery, damage to arteries, stroke, heart attack, restenosis, and blood clots. Also, CT technology, is a conventional diagnose methods used to make a further confirmation of the disease by forming images inside bodies. CT scan combines a series of X-ray images taken from different angles of the body, and uses computer processing to create cross-sectional images (slices) of your bones, blood vessels and soft tissues so that clots in your blood vessels can be detected and located. CT scan images provide more detailed information than regular X-rays does. But in general, these methods (CT and X-rays) are mainly serve for treatment after diagnose, they can not used to predict the risks in human bodies.

2.5. Flow cytometry

Comparing to conventional diagnose method, many new technologies has greatly deal with those problems, and greatly improve the diagnosis efficiency. For instance, the Flow Cytometry, which is one of the large achievement in the field of clinical medicine. Weimi—a biotechnology company in China, has done many researches of testing activation of platelet by using Flow Cytometry. As

mentioned in the introduction, the principle of the whole experiment is to add CD61-APC-Cy7, CD41-PerCP and cd62-pe monoclonal antibody reagents into human blood samples, which are specifically bound to platelet surface antigen, and analyzed and detected by flow cytometry.

After numerous times of experiments, researchers has drawn a conclusion of each indexs' normal ranges.

Table 1. Diagnostic indicators

Order	Name	Abbreviation	unit	Reference range
1	CD41 plasma membrane glycoprotein	CD41+/CD61+	%	83.60~100
2	CD62P activated platelet	CD62P+/CD61+	%	0.01~3.05
3	CD61plasma membrane glycoprotein	CD61+/PLT	%	89.83~100

To analyse the result of the research, the detection of platelet membrane glycoprotein is used for the diagnosis of platelet asthenia. CD41 and CD61 are diagnostic indicators of platelet asthenia (Table 1). Thombothenia is an agglutinative state or reduced response to platelet membrane glycoprotein Ib/Ia due to a reduction or abnormal quality of the gene GPIIb (CD41) or GPIIIa (CD61). Platelet GP detection is of great value in the diagnosis of platelet dysfunction diseases such as thrombothenia.

Moreover, the detection of platelet activation is used for the diagnosis of pre-thrombotic state and thrombotic diseases. Platelet activation plays an important role in the formation of arterial thrombosis. Activated platelet increase, suggesting that the body is in a pre-thrombotic state or local thrombosis, such as ischemic stroke, myocardial infarction, peripheral vascular disease. The examination of platelet activation also used for monitoring of cardiovascular and cerebrovascular diseases complicated by atherosclerosis, diabetes, hypertension, hyperlipidemia, arterial hyperviscosity, etc. Furthermore, it applies to the dynamic monitoring of clinical antiplatelet therapy, and guide the treatment of thrombocytopenic diseases.

Comparing with the conventional diagnose method, Flow Cytometry helps Fusing different data sources continuously, like the thrombelastogram can combine with Flow Cytometry to achieve mutual complementarity. Also, it is Highly personalized care that it helps detect signs earlier to improve patient outcomes and reduce the length of hospital stays. Flow cytometry has a bright future because it is the only viable technology for comprehensive and multiplexed analysis of single cells. Furthermore, with the latest spectral cell sorting technology, a single cell from a multicellular mixture, identifiable only by a complex algorithm, can be separated for cloning or sequencing.

After testing the index, patients are allowed to get treatment as soon as possible. The drugs used to treat thrombus can be classified into the following categories: thrombolytic, anticoagulant, and antiplatelet. Drugs that treat blood clots use different mechanisms to treat different blood clots, and we should use different treatment according to the locations where thrombosis taken place as well. In short, we are aimed to test the patients' activation platelet index more accurately, so that they can make diagnose and get treatment as early as possible.

3. Treatments of thrombosis

3.1. Thrombolytic drugs

This is one of the most common treatments used in hospital for thrombosis. This type can completely dissolve the blood clot that is blocking the blood vessel by breaking down proteins (fibrins) to quickly stop the clotting process [5]. There are several commonly used drugs, such as alteplase and reteplase.

3.1.1. Alteplase

Alteplase, manufactured by recombinant DNA technology, and being approved by FDA for treating stroke, pulmonary embolism, and other thrombosis related diseases [6]. It is a fibrinolytic agent, converts plasminogen to the proteolytic enzyme plasmin, and will be cleared by liver between 5 minutes to 1 hour [7].

3.1.2. Reteplase

Reteplase, an alter type of alteplase, manufactured by non-glycosylated recombinant form of tissue plasminogen activator (tPA) [8]. Like alteplase, it also has the function as converting the plasminogen into the active plasmin, which will dissolve the fibrin into small, degraded products [9]. However, reteplase has a lower binding affinity compared to alteplase due to a deletion in the structure [10-12].

3.2. Anticoagulant drugs

Another commonly used type for treating thrombosis. Instead of rapidly dissolve the blood clot, these drugs decrease the blood's ability to form a clot to prevent the blood clot into critical blood vessels and become a fatal disease [2]. While preventing forming of new clots, these drugs also break down previously formed clots. There are two types of anticoagulant drugs, heparin, and warfarin [13].

3.2.1. Heparin

Heparin, also known as unfractionated heparin, being considered as anticoagulant since it can decrease the blood clotting ability to prevent harmful blood clots to form [14]. The only way to take heparin is injection under skin. There is also a lower molecule weight derivative called enoxaparin, which have the similar function as heparin. Although heparin and the low-molecule weight derivatives are effective preventing blood clot forming, there is no evidence showing it can prevent mortality [15]. There is an antidote called protamine sulfate, which can reverse the effect of heparin.

3.2.2. Warfarin

Warfarin, the other common type of anticoagulant drug. Unlike heparin which must doze into body, warfarin, also as vitamin K antagonist, can only be taken from oral [16]. Usually, vitamin K will be transformed into vitamin K epoxide in the liver and being reduced by reductase, warfarin will inhibit the reductase to lower the clotting ability. There are some conditions like disease in the mitral valve in the heart, having a mechanical heart valve, or kidney disease, cannot use heparin as treatment. Therefore, warfarin will be their only choice. Also, there are research showing taking vitamin E will increase the effect while taking vitamin K will reduce it.

3.3. Antiplatelet drugs

Besides from thrombolytic drugs and anticoagulant drugs, there is another type of drugs called antiplatelet drug. Unlike anticoagulant drugs which interfere with the proteins in the blood, antiplatelet drugs preventing the platelet stick together to form blood clot [17]. Sample drugs like ADP-receptor inhibitors, adenose reuptake inhibitors, etc. The most used and first antiplatelet drug is aspirin. Most of the antiplatelet drugs can be taken from oral, but some may require dose.

3.3.1. Aspirin

Although aspirin does not mainly used for treating blood clots, but it does have effect on lowering the risk of blood clots. It is usually used for patients who have artificial heart valves or other heart conditions [18].

3.3.2. Clopidogrel

This usually used alone or with aspirin to prevent blood clots forming in critical blood vessels. This drug has similar effect like aspirin, but it can also be used on treating peripheral arterial disease [19].

3.4. Advantage and disadvantage

Thrombolytic drugs can function very fast as it directly dissolves the blood clot causing the thrombosis. Because of this, it is being used commonly in heart attacks or strokes caused by thrombosis. As this type can react to the thrombosis fast, it is suggested to take the medicine within 30 minutes after arriving for the treatment if the patient fits the condition.

Unlike thrombolytic drugs, anticoagulant drugs prevent the formation of blood clots. It can be found in pharmacy and used daily. Also, there are antidotes to reduce the side effects for overdose or overtake, means anticoagulant drugs will not cause serious, unreversible condition for patients if they can have the antidote in time.

Although thrombolytic drugs and anticoagulant drugs are highly effective, they also have similar significant adverse effects. These could be bleeding, angioedema, anaphylaxis, and fever. Data shows the chance of bleed from the gums and noses after taken the drug is around 25%, and severe bleeding, such as bleeding in brain, is approximately 1%. There will be other adverse effects depending on the type of thrombolytic drugs [20]. It is not suggested to overdose thrombolytic drugs as it does not have antidote, otherwise, it will cause serious bleeding which need supportive care.

Anticoagulant drugs have antidotes to reverse the overdose. However, overtaking these drugs, especially heparin, will still lower down the clot ability too much. This means, a small injury may cause continuous bleeding. There is also a chance which heparin will cause a specific side effect called heparin-induced thrombocytopenia. There are two types of this side effect which chance is 10% and 1%, cause either a slightly decrease of platelet or a fatal, widespread clotting in the body [21]. Also, long tern using of heparin will cause osteoporosis, caused by decreasing of the formation of new bone cells.

There are multiple side effects for taking antiplatelet drugs, but most of those side effects are mild and will not cause serious problems like thrombolytic drugs. The main side effects are extremely tiredness, nose bleeding, vomiting, etc. There are some serious side effects, if patients find themselves have these side effects, they will need to see the doctor immediately as there are no antidotes for current antiplatelet drugs. Overdose antiplatelet drugs can cause several serious side effects and even be life-threatening.

3.5. Ongoing research

There are researching about antiplatelet drugs which may not have these heavy side effects. Sample medicines, or herb are like panax ginseng, an herb known as “renshen” in China, contains several similar pharmacological compounds and therapeutic applications [22]. There are multiple compounds have been studied as it does have effects on inhibiting platelet aggression and can be mixed with epinephrine to evaluate antithrombotic drugs. Another study of panax japonicus, another type of ginseng, shows the concentration around 5mg/L can significantly increase the blood clot forming time, which means it can decrease the clotting ability of blood to prevent forming of blood clot [23].

3.6. Other treatment ways other than drugs

However, if the patient must take surgery to remove the blood clot form a very critical blood vessel, they will not be suggested to take any of these antithrombotic drugs few days before the surgery due to the common adverse effect. There are different surgeries depending on the blood clot location, but all surgeries have risk and may cause other side effects after the treatment [24]. The best way to prevent having thrombosis is keep a healthy lifestyle, do not smoke, do exercise, moving around will significantly reduce the chance of blood clot forming [25].

4. Conclusion

The research on using active platelet index to diagnose the embolism is being proved and still under development for a finalization in hospital. Patients who have confirmed for embolism have to use either thrombolytic drugs to quickly remove the thrombosis or use anticoagulant drugs or antiplatelet drugs to maintain the healthy body from have the risk of embolism. However, all those drugs have relatively serious adverse effects and patients will have to consider their own conditions if they are available to those choices. Therefore, there are antiplatelet drugs make by traditional herbs which may not have such serious side effects to patients are under researching. We believe the active

platelet index testing will be used more on detecting thrombosis and those drugs will be proved one day and be used on patients who suffers embolism.

References

[1] Mirembe R, Connor C. K, Diala N, Alteplase [Internet]. Treasure Island (FL): StatPearls Publishing; 2023.

[2] Cleveland clinic health library, Anticoagulants, latest review on 10th Jan 2022, accessed on 20th Mar 2023.

[3] Elmira M, Hooria S, Karim M, Ali J, Hamid Mir Mohammad S, Reteplase: structure, function, and production, Adv biomed Res, 2019, 8: 19.

[4] Cleveland clinic health library, Thrombolytics, accessed in 2023.

[5] Mayo Clinic, Reteplase, Recombinant (Intravenous Route), accessed on 2023.

[6] National library of medicine, Thrombolytic therapy, accessed in 2023.

[7] Shu Pan-pan, Zhu Peng-fei, Yang Xin-long, Li Lu-xi, Yu Wei-bo, Yang Ye, Qu Yuan, Correlation between anticoagulant activity and ginsenoside content in six Panax species, Chinese traditional and herbal drugs, 2019, 50: 4.

[8] Muhammad I, Minki K, Man Hee R, Anti-platelet role of Korean ginseng and ginsenosides in cardiovascular diseases, journals of ginseng research,2020, 44 (1): 24 - 32.

[9] University of Rochester medical Center, Surgical thrombectomy, accessed on 2023.

[10] University of California San-Francisco, Deep vein thrombosis, conditions and procedures, accessed in 2023.

[11] Cian P McCarthy, Gabriel Steg, Deepak L Bhatt, the management of antiplatelet therapy in acute coronary syndrome patients with thrombocytopenia: a clinical conundrum, 2017, 38 (47): 3488 – 3492.

[12] Penn Medicine, Arterial embolism, latest reviewed on 5th Aug 2022, accessed in 2023.

[13] health direct, Thrombosis, accessed on2023.

[14] National Health Service, Deep vein thrombosis, latest reviewed on 2023.

[15] Center for Disease Control and Prevention, Stroke facts, accessed on 2023.

[16] Mayo clinic, Stroke, accessed on 26th Mar 2023.

[17] Cleveland clinic health library, Pulmonary embolism, accessed in 2023.

[18] D Lays, Atherothrombosis: a major health burden, cerebrovascular diseases, 2001, 1 (S2): 1 - 4.

[19] Data and Statistics on Venous Thromboembolism accessed in 2023.

[20] Mayo clinic, Coronary angiogram, accessed in 2023.

[21] Mayo clinic, CT scan, accessed on 17th Mar 2023.

[22] Thrombosis advisor, Arterial thrombosis, accessed on 18th Mar 2023.

[23] J Paul Robinson, Flow cytometry: past and future, Biotechniques, 2022, 72: 4.

[24] Mayo clinic, Heparin, accessed on 23rd Mar 2023.

[25] MedlinePlus, Clopidogrel, accessed on 26th Mar 2023.