Case Report

Recurrent arterial thrombosis of the lower extremity with secondary thrombocythemia due to reperfusion injury: a case report

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Abstract: Thrombocythemia is an important cause for thrombogenesis and can be classified as essential or secondary according to the etiology. Secondary thrombocythemia (ST), also called reactive thrombocytosis, is caused by a disorder that triggers increased production by normal platelet-forming cells and is characterized in terms of abnormal increased number of platelet in blood and megakaryocytes in bone marrow. Previous reports have found that complications from malignant tumors, chronic inflammation, acute inflammation, acute hemorrhage, spleen resection etc. to be the common causes of ST. A 53-year-old Chinese male with right lower limb arterial ischemic embolism developed recurring arterial thrombosis at the previous site after operation. During his hospitalization, the patient had a platelet count that was positively correlated with alanine transaminase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (α-HBDH), creatine kinase (CK), and creatine kinase isoenzyme MB (CK-MB) while his thromboelastogram (TEG) and platelet aggregation test obtained by sequential platelet count showed inconsistent platelet function. We describe a case in which ischemia–reperfusion injury caused ST and recurrent thrombosis and analyse the probable cause of contradictory results of different platelet function tests. In thrombolytic therapy, we recommend adding platelet count and two more platelet aggregation tests to the routine laboratory items to aid in the prevention of recurrent thrombosis.

Keywords: Secondary thrombocythemia (ST); recurrent thrombosis; reperfusion injury; platelet aggregation function; case report

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Introduction

Thrombocythymia is an important cause for thrombogenesis and is characterized by peripheral platelet count more than the upper limit of normal or more than 450×10^9/L (1), affecting one fifth of trauma patients and up to one third of patients in intensive care unit (2,3). Thrombocytosis predicts an underlying solid tumor diagnosis in 11.6% of men and 6.5% of women (4) while pretreatment thrombocytosis was significantly related to a decreased overall survival and disease-free survival (5). The condition can be classified as primary or essential thrombocytosis resulting from a clonal bone marrow abnormality such as myeloproliferative neoplasm (MPN), secondary thrombocytosis (ST) and inherited thrombocytosis according to the etiology (1). The most common form of thrombocytosis is ST. ST, also known as reactive thrombocytosis, is caused by a disorder that triggers increased production by normal platelet-forming cells. It is known to commonly result from malignant tumors, chronic inflammation, acute inflammation, acute hemorrhage, spleen resection, and other conditions. Most patients with ST show no symptoms, but a few,

usually older patients, have atherosclerosis (6,7). In the case discussed here, our patient developed recurring arterial thrombosis at the previous site of his right lower extremity artery after balloon-catheter thrombectomy in the period of hospitalization. During his admission, his thromboelastogram (TEG) and platelet aggregation test showed inconsistent platelet function, while laboratory examinations showed that the patient's platelet (PLT) count and concentrations of alanine transaminase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (α-HBDH), creatine kinase (CK), and creatine kinase isoenzyme MB (CK-MB) were fluctuating. However, following the laboratory findings came to normality, the patient's thrombosis disappeared, and he was discharged.

We present the following article in accordance with the CARE reporting checklist (available at http://dx.doi.org/10.21037/apm-20-1649).

Case presentation

A 53-year-old Chinese male presented to our hospital with a 2-year history of intermittent claudication that limited his ability to walk 300 meters and a 4-hour history of sudden pain and numbness. Physical examination found no abnormalities of the heart, lung, or abdomen. Both lower limbs had normal length and shape. His bilateral femoral artery pulse was palpable, with the right one being weaker. The 10-cm distal skin of his right knee was cool and pale, while his right ankle and foot had hypoesthesia. Laboratory findings were normal for complete blood cell count, coagulation, liver function, renal function, cholesterol, and blood glucose. Doppler ultrasound (DUS) showed no vascular blood flow in the right acrotarsium and posterior tibial artery, and echogenic content was consistent with embolism. Computed tomography (CT) revealed bilateral femoral artery thrombosis. According to the presenting symptoms, signs, and imaging findings, he was diagnosed with right lower limb arterial ischemic embolism and thrombosis, with-rest pain of the right limb. Under general anesthesia, the patient underwent right lower limb artery balloon-catheter thrombectomy and right iliac artery stent implantation which resulted in good distal perfusion. This was followed with conventional administration with continuous heparin for anticoagulation and urokinase for thrombolysis in order to avoid recurrent thrombosis. Routine laboratory tests and physical examination monitored the antithrombotic effect.

The patient was initially stable, but on postoperative day 3, began to have cool skin of the right lower limb extremity, which was confirmed with DUS as right femoral artery thrombosis at the previous site. An emergency reoperation was performed for resolution of the thrombosis. To determine the cause of patient's excessive thrombotic tendency before balloon-catheter thrombectomy, an additional laboratory review was conducted and showed that he had higher PLT, ALT, AST, LDH, α-HBDH, CK, and CK-MB than baseline levels (*Figure 1*). Clopidogrel and aspirin were added to the therapeutic schedule for anti-platelet aggregation. On postoperative day 5, an anti-platelet drug effect was evaluated with a platelet aggregation test obtained by sequential platelet count (Aggrestar PL-16, Sinnowa, China) and TEG (Thrombelastography, Haemoscope, US). His platelet aggregation test (*Figure 2*) and TEG (*Figure 3*) showed unusually inconsistent platelet function. The platelet aggregation test by counting platelet number revealed that the platelet aggregation rate was 54.4% for arachidonic acid (AA)-induced antiplatelet effect of aspirin and 15.2% for adenosine diphosphate (ADP)-induced antiplatelet effect of P2Y12 inhibitor of clopidogrel, which suggested that the inhibition rate of AA and ADP were 45.6% and 84.8%, respectively. Meanwhile TEG showed no inhibition effects for AA- and ADP-induced antiplatelet agents, the inhibition rates being 0. Later that same day, after heparin was switched to argatroban and clopidogrel and aspirin were switched to ticagrelor, his thrombosis symptoms gradually disappeared. It was very lucky that there were no complications such as bleeding and hyperkalemia during antithrombotic therapy. From postoperative day 2 onward, the patient showed persistent thrombosis at the same site of his right lower extremity artery while his PLT, ALT, AST, LDH, and CK fluctuated up and down (*Figure 1*). During the hospitalization, his PLT count was positively correlated to ALT, AST, LDH, HBDH, CK, and CK-MB (r=0.4442, 0.6303, 0.4892, 0.4622, 0.6154, 0.4618, respectively). On postoperative day 23, his symptoms and laboratory results showed no obvious abnormalities, and the patient was discharged.

Written informed consent was obtained from the patient for publication of this study and any accompanying images. And all procedures performed in this study was in accordance with the ethical standards of the institutional

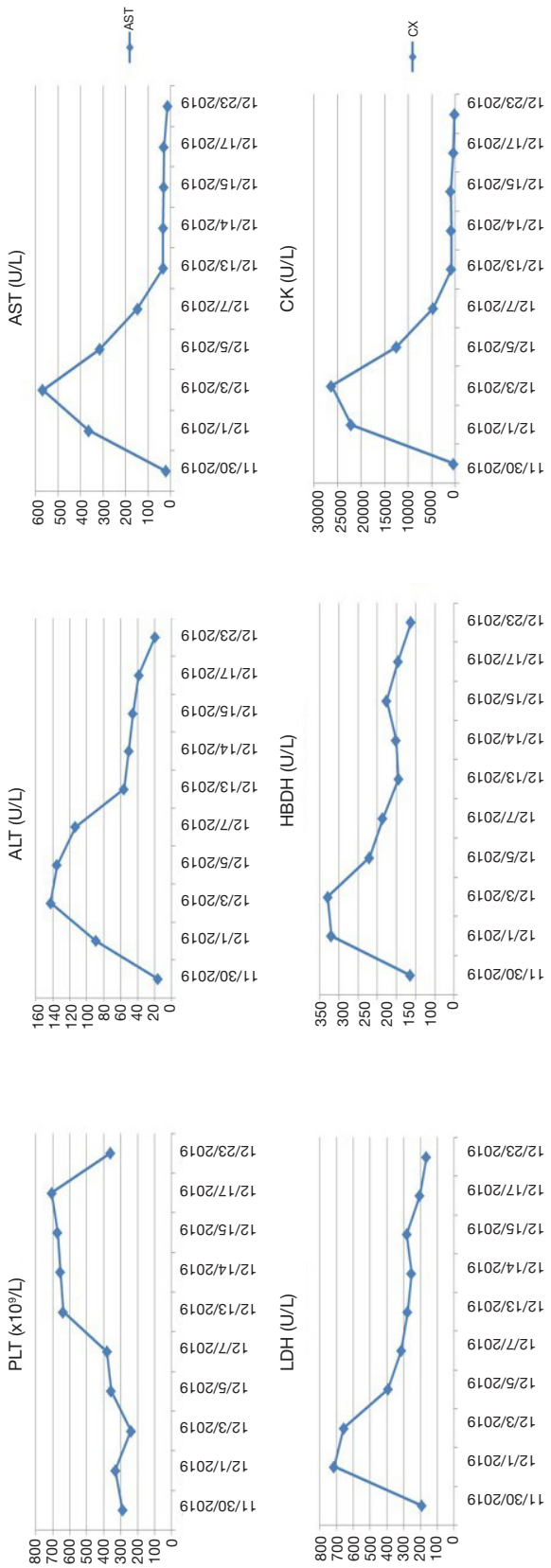

Figure 1 Several blood marker changes during the total hospitalization following the changes of patient's condition and therapy administration. PLT, patient's platelet; ALT, alanine transaminase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase isoenzyme MB.

Platelet Aggregation Test (ADP)

Serial No.	Items	Results	Unit	Reference values		Hint
1	PLT-O	626	10^9/L	100	300	↑↑
2	MPV-O	7.16	fL	7.00	11.00	
3	PDW-O	8.78	fL	7.00	17.00	
4	MAR	17.1	%	30.0	70.0	↓
5	MAT	545	s	210	600	
6	AAR	15.2	%	20	60	↓
7	RBC-O	2.66	10^12/L	3.50	5.50	↓
8	MCV-O	89	fL	80	100	
9	RDW-O	74.50	fL	35.00	56.00	↑

Platelet Aggregation Test (AA)

Serial No.	Items	Results	Unit	Reference values		Hint
1	PLT-O	626	10^9/L	100	300	↑↑
2	MPV-O	7.16	fL	7.00	11.00	
3	PDW-O	8.78	fL	7.00	17.00	
4	MAR	56.2	%	40.0	80.0	
5	MAT	487	s	210	600	
6	AAR	54.4	%	30	70	
7	RBC-O	2.66	10^12/L	3.50	5.50	↓
8	MCV-O	89	fL	80	100	
9	RDW-O	74.50	fL	35.00	56.00	↑

Figure 2 Platelet aggregation test revealed that part platelet function was inhibited. PLT-0, initial Platelet count; MPV-0, initial mean platelet volume; PDW-0, initial platelet distribution width; MAR, maximum platelet aggregation ratio; MAT, maximum platelet aggregation time; AAR, average platelet aggregation rate; RBC-0, initial red blood cell count; MCV-0, initial mean red blood cell volume; RDW-0, initial red blood cell distribution width.

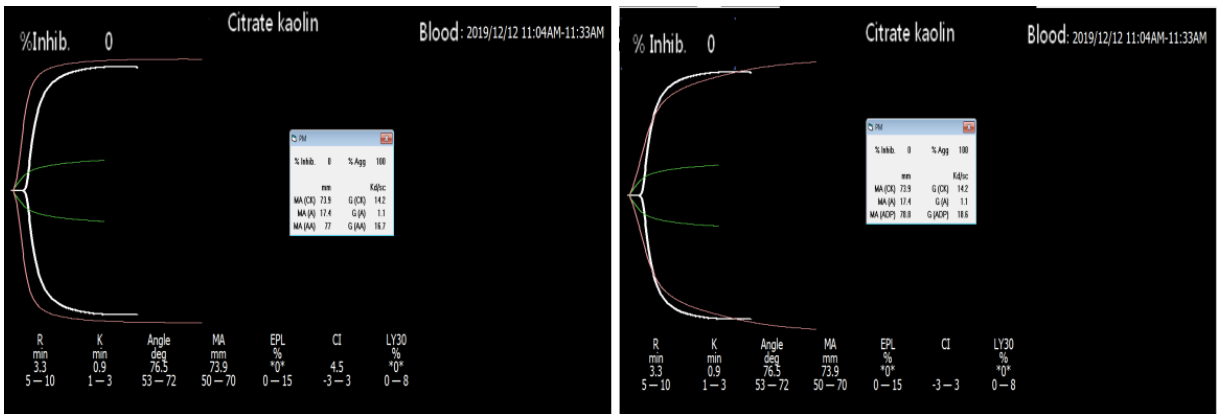

Figure 3 TEG showed no platelet inhibition of AA-induced antiplatelet effect for aspirin and ADP-induced antiplatelet effect for clopidogrel in the patient. TEG, thromboelastogram; AA, arachidonic acid; ADP, adenosine diphosphate.

and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013).

Discussion

Peripheral arterial disease (PAD) is a progressive disease most often resulted from atherosclerosis and characterized by obstruction of arterial blood flow in the lower limbs. Lower limb peripheral artery disease (PAD) is highly prevalent throughout the world. Anyway, it is uncommon before the age of 50 years. A higher prevalence of PAD among Americans 60 years and older has been reported to range from 12% to 20%. Strikingly, the rate for African Americans was about twice that of non-Hispanic whites at any given age (8). Furthermore, the National Health and Nutrition Examination Survey (NHANES) has estimated that the prevalence of PAD to be 4.3% among adults aged 40 years and over and 14.5% among those aged 70 years of age and over (9). Global data on trends in PAD prevalence between the years 2000 and 2010 demonstrated that the number of individuals with PAD increased by 28.7% in low-income and middle-income countries and by 13.1% in high-income countries (10). During the past decades, the consensus-based venous thrombosis mechanism was the combination of stasis and hypercoagulability, much more than endothelial damage, and less by platelets. Instead, platelets are essential for primary haemostasis, repair of damaged endothelium and play an important role in the arterial thrombosis. Inflammation, lipids and the immune system, through a complex interplay, are also

vital contributors to arterial thrombosis (11). Cigarette smoking, diabetes mellitus, hypertension, dyslipidemia, obesity, alcohol consumption, race, homocysteine, C-reactive protein, fibrinogen, chronic kidney disease, genetic factors, and so on are probable risk factors of PAD, although the onset of PAD is often asymptomatic and, in any case (12). Critical lower limb ischemia (CLI) is a manifestation of PAD that is seen in patients with typical chronic ischemic rest pain or with ischemic skin lesions, ulcers, or gangrene persisting longer than 2 weeks. Medical interventions are important to improve arterial perfusion. In the lower extremities, PAD affects three major arterial segments including the femoropopliteal arteries. Among the therapeutic options available, surgical revascularization is considered superior for resolving acute or limb-threatening ischemia, especially for those patients with inadequate response to the aforementioned therapies (8).

In terms of drug-induced thrombocytosis, there are three types of drugs with relatively high composition: antitumor drugs, blood system drugs and antibacterial drugs. Clinical manifestations: progressive increase of platelets, severe thrombosis, hematoma thrombosis and so on. The mechanism is as follows: promote the secretion of cytokines and induce platelet formation; destroy the regulatory system of PLT production, promote the maturation of megakaryocytes and increase the production of PLT; change gene expression to promote the maturation of megakaryocytes (13,14).

During perioperative periods, thrombosis is a major cause of morbidity and, even with anticoagulant therapy, is reported to be as high as 36% (15). Although advanced age, pregnancy, surgery, immobilization, malignancy, anticoagulation resistance, and oral contraceptive use are considered to be important clinical risk factors, the cause of many venous thrombotic events remains unknown (16). However, due to the rarity of arterial thromboembolism (aTE) and the inherent limitations imposed by the Taiwan registry data (17), few studies have explored the recurrence of arterial thrombosis. It has been reported that BMI >23 kg/m^2, hypertension, cerebrovascular disease, atrial fibrillation, stage IV disease, and AST level are risk factors of aTE. Among these, however, only hypertension, atrial fibrillation, and high AST level have proven to be independent risk factors (18). The patient presented here also had arterial thrombosis of the right lower limb. Unfortunately, his arterial thrombosis recurred. A case report demonstrated that the drug of hydroxyurea promoted secondary severe thrombocytosis in a patient with hereditary spherocytosis after splenectomy, but no literatures showed that antithrombotic drugs can cause ST (19). We were convinced that no injury occurred during surgical procedure, and we concluded that arterial thrombosis recurred due to reperfusion mechanisms. This phenomenon is mentioned in some literature and is due to oxidative stress, although the exact cause is not known (20,21).

After the first operation, the patient's PLT count was markedly increased, indicating thrombocytosis. However, thrombopoiesis occurs in the bone marrow, and it is regulated by the hormone, thrombopoietin (TPO). Thrombocytosis may be a secondary phenomenon, reflecting an inflammatory state, infection, iron deficiency, recent surgery, haemolysis, or resulting from underlying neoplasms while increased concentration of peripheral thrombopoietin and pro-inflammatory cytokines (for example IL-6) are included in the pathogenesis of ST (22). This case was clearly secondary thrombocythemia (ST) because the patient improved after his PLT count returned to normal.

Ischemia-reperfusion injury is a well-known pathological condition which may lead to disability and mortality (23). Tissue injury and/or death occur as a result of the initial ischemic events, and are primarily caused by the magnitude and duration of the interruption in the blood supply, and the subsequent damage induced by reperfusion (24). The gut, lung, liver, kidney, skeletal muscle, heart, and liver are the commonly injured organs (25). We found there to be a moderate to high correlation between PLT count and the levels of ALT, AST, LDH, α-HBDH, CK, and CK-MB. It is generally agreed that ALT and AST reflect liver function while LDH, α-HBDH, CK, and CK-MB reflect myocardial function. During reperfusion, liver cells produce not only more ALT and AST but also more TPO which is the primary and vital regulator of megakaryocyte progenitor expansion and differentiation (26). No reports from the literature suggest that myocardial injury contributes to PLT production, although our results showed a slight relationship between PLT count and myocardial markers. Moreover, none of the current antiplatelet therapeutics protect against reperfusion injury, which is defined as myocardial injury caused by reoxygenation of a previously ischemic myocardium (27). Reperfusion injury accounts for upwards of 50% of the final size of a myocardial infarct and is characterized by impaired microvascular

perfusion (27). Some studies found the levels of both serum malondialdehyde and glutathione to be significantly higher in a lower limb ischemia-reperfusion group and liver ischemia-reperfusion group, while the damage score from histological evaluation was found to be higher in a liver ischemia–reperfusion group and lower limb ischemia–reperfusion group (28). Our case showing an abnormally high platelet count and having other blood makers gradually return to normal, suggests that reperfusion injury is a key cause of ST and thrombosis. Moreover, one study found that ST was associated with *in vitro* prothrombotic tendency correlated to thrombin generation, thrombin activity level depending on coagulation factors. Patients with moderate-to-severe thrombocytosis (platelet count >700×10^9/L) have significantly higher fibrinogen, factor VII, and VWF antigen and activity level than those with mild thrombocytosis (platelet count 500–700×10^9/L) (29). It is suggested that using both antiplatelet drug and anticoagulants may be a better way for prevention and treatment of ST, especially after postoperative reperfusion.

In order to evaluate antiplatelet effect of aspirin and clopidogrel, AA and ADP platelet function tests were carried out with TEG and platelet aggregation test by counting platelet number. The results of TEG and platelet aggregation test showed contradictory, the former being 0 of the degree of AA and ADP inhibition, the latter being 45.6% and 84.8%, respectively. There are 13 methodologies for assessment of platelet function with various methodological principles and each has its own pros and cons (30). This case revealed poor correlations between TEG and platelet aggregation test after a rapid rising postoperative platelet count over 350×10^9/L, so we speculate that TEG is less sensitive to platelet aggregation function when a patient has a higher platelet number. Gremmel *et al.* reported that the influencing factors for platelet reactivity during clopidogrel therapy were assay-dependent (31). Therefore, in antiplatelet treatment, two more assays each sensitive to targeted drugs are available for evaluating platelet function in order to complement each other pros and cons.

Conclusions

Although the recurrent thrombogenesis in this case was successfully resolved, careful attention must be paid to the rational administration of ST and it is suggested to add platelet count and two more platelet aggregation tests to the routine laboratory items during thrombolysis.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at http://dx.doi.org/10.21037/apm-20-1649

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-20-1649). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Written informed consent was obtained from the patient for publication of this study and any accompanying images. And all procedures performed in this study was in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013).

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