

PFA-100 Test in the Detection of Platelet Dysfunction and Monitoring DDAVP in a Patient with Liver Cirrhosis Undergoing Inguinal Hernia Repair

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SUMMARY

Introduction Hemostatic abnormalities in liver cirrhosis are complex and multifactorial and may predispose to prolonged hemorrhage following invasive procedures. Due to increased perioperative bleeding risks, patients with cirrhosis should undergo elective surgery after making medical preparations. It has been shown that 1-deamino-8-D-arginine vasopressin (DDAVP), desmopressin, can be used as a safe and effective remedy in preventing and treating bleeding in cirrhotics. However, there is still scarce information of adequate test(s) for assessing effects of DDAVP in platelet dysfunction. The use of platelet function analyzer-100 (PFA-100) allows more reliable assessment of impaired primary hemostasis as well as follow-up of hemostatic changes induced by DDAVP effects.

Case Outline In a 49-year-old male with ethylic liver cirrhosis and prolonged bleeding time scheduled for elective left side inguinal hernia repair, we carried out PFA-100 testing to investigate the patient's platelet functional status. Results were affirmative for the presence of platelet functional problems. By standard coagulation tests the patient was also identified as having secondary hemostasis. Preoperatively, PFA-100 was used to test the patient's response to a standard dose of DDAVP, which was favorable. The patient was operated after medical preparations with DDAVP and vitamin K. Neither bleeding complications nor side effects of DDAVP were recorded in the perioperative period.

Conclusion The PFA-100 is a simple and reliable test for the assessment of primary hemostasis as well as in monitoring of DDAVP therapy.

Keywords: platelet dysfunction; liver cirrhosis; surgery; PFA-100; DDAVP

INTRODUCTION

The liver plays a central and complex role in hemostasis being involved in both primary and secondary hemostasis. With the exception of von Willebrand factor (vWF), which is synthesized by the endothelium, the liver is the sole or major site of all of the recognized blood coagulation factors, several important regulatory proteins of the coagulation system (antithrombin, protein C, protein S, C₁ inhibitor, α_2 macroglobulin) and fibrinolytic proteins and their inhibitors. Furthermore, the reticuloendothelial system of the liver is responsible for clearing from the circulation all activated clotting factors, the activation complex of both coagulation and fibrinolysis, and the degradation products of fibrin and fibrinogen [1, 2, 3].

In patients with liver cirrhosis, most coagulation factors and inhibitors of the coagulation and fibrinolytic systems are markedly reduced because of impaired protein synthesis, except for vWF, factor (F) VIII:C and fibrinogen levels, which may be normal or increased. FVIII is synthesized mainly by the hepatic sinusoidal endothelial cells. Because fibrinogen is an acute-phase reactant, its synthesis tends to be preserved in patients with stable cirrhosis. Fibrinogen levels may even be el-

evated because of abnormal non-functional fibrinogen (dysfibrinogenemia) related to defective polymerization. In addition to impaired synthesis of clotting factors, excessive fibrinolysis, disseminated intravascular coagulation (DIC), thrombocytopenia and platelet dysfunction account for the diverse spectrum of hemostatic defects in chronic liver disease. Reduced hematocrit, vitamin K deficiency, enhanced production of nitric oxide (NO) and prostacyclin, and elevated levels of tissue plasminogen activator (t-PA) produced in the endothelium, all contribute to bleeding tendency. At the same time, liver disease alters the pathways of anticoagulation (decreased levels of protein C, protein S, and antithrombin). Thus patients who have advanced disease can experience severe bleeding or thrombotic complications [3-6].

Patients with cirrhosis may carry a significant risk of adverse outcome after hernia repair compared to non-cirrhotics with regard to complications caused by the deterioration of both primary and secondary hemostatic potential. Therefore, patients with cirrhosis should undergo elective hernia repair after applying adequate medical preparations [7].

When surgery is indicated, a prompt evaluation and correction of hemostatic alterations

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in liver disease are indicated [2]. In the last couple of decades, the search for pharmacological agents that can reduce perioperative blood loss has been intensive. Among pharmacological options, DDAVP, given in a single dose, is the first agent without major side effects that has been shown to shorten skin bleeding time (BT) significantly in patients with cirrhosis [8]. However, there is still no validated concept of when to use this drug and how to assess its effect in general clinical situations as well as before or during surgery [9, 10]. These uncertainties reflect insufficient knowledge of the mechanisms through which DDAVP improves platelet function and, consequently, of the adequate test(s) or mediator(s) to be evaluated for assessing biological and clinical effects of DDAVP in platelet function abnormalities [10].

Even the oldest in vivo test of platelet function, the BT has failed to predict clinical gastrointestinal bleeding or bleeding after major hepatectomy and has been largely abandoned as a routinely used test [11]. Despite attempts at standardization, the test remains poorly reproducible and subject to a large number of variables. Skin BT has considerable limitations due to technical factors (location and direction of incision), insensitivity to some significant bleeding disorders and sensitivity to some common abnormalities without any clear relationship to bleeding risk [12].

The aim of this report was to present a possible approach using PFA-100 test for reliable preoperative screening of patients with impaired primary hemostasis. We also aimed to demonstrate the utility of this test in monitoring DDAVP therapy in the responder who underwent elective surgery.

CASE REPORT

A 49-year-old male was admitted to hospital for elective surgery of the left side inguinal hernia. Ten days before admission he experienced an episode of incarcerated hernia solved in the Emergency Care by hernia reduction. Five years ago ethylic liver cirrhosis had been diagnosed. He had no personal and family history of bleeding. The patient denied of using drugs that could have affected platelet function. On admission, no signs of decompensated liver disease were detected. There were no signs of bleeding. The patient experienced a tendency to prolonged bleeding from venipuncture sites. His BT (Duke) was 6 minutes (normal range: 1-3 minutes).

We suspected that platelets might be affected and performed the platelet functional testing by the PFA-100; collagen-epinephrine (Col/Epi); collagen-ADP (Col/ADP) (Dade-Behring, Germany), and the results confirmed the presence of platelet dysfunction (Table 1). The patient was identified preoperatively as having combined hemostatic defects; acquired impaired primary hemostasis, deteriorated coagulation with prolonged activated partial thromboplastin time (APTT) and also, according to the International Normalized Ratio (INR), and also a decreased level of coagulation factors II, V and VII (Table 1).

In order to minimize blood loss and avoid allogeneic blood transfusion, we created bloodless treatment strategy

Table 1. Hemostatic parameters before and one hour after DDAVP test infusion

| Hemostatic test | Results before DDAVP | Results 1 hour after DDAVP | Normal value |
|-----------------------|------------------------|----------------------------|----------------------------|
| Bleeding time | 6 min | 150 s | 1-3 min (Duke) |
| Col/Epi | 199 s | 118 s | 94-193 s |
| Col/ADP | 139 s | 96 s | 71-118 s |
| Platelet count | 103×10 ⁹ /L | 101×10 ⁹ /L | 158-425×10 ⁹ /L |
| Hemoglobin | 117 g/L | 117 g/L | 119-175 g/L |
| FVIII:C | 84% | 153% | 50-150% |
| vWF:RcoF | 100% | 115% | 50-100% |
| Euglobulin lysis time | >2 h | >2 h | >2 h |
| FII | 49% | 50% | 70-150% |
| FV | 34% | 38% | 70-150% |
| FVII | 30% | 30% | 70-50% |
| APTT | 48.2 s | 42.3 s | 24.3-36.8 s |
| INR | 1.61 | 1.59 | 0.8-1.2 |
| Fibrinogen | 2.6 g/L | 2.5 g/L | 1.69-3.92 g/L |
| D-dimer | <125 µg/L | <125 µg/L | <125 µg/L |

Col/Epi – collagen-epinephrine; Col/ADP – collagen adenosine diphosphate; FVIII:C – coagulation factor VIII activity; vWF:RcoF – von Willebrand factor ristocetin cofactor; APTT – activated partial thromboplastin time; INR – International Normalized Ratio

with preoperative administration of DDAVP (EmosintR, Kedrion S.p.A, Italy) and vitamin K (Konaktion MM*, Roche, France).

In preoperative assessment of individual response to desmopressin several days prior to the operation, the patient was administered a test dose of 0.3 µg/kg DDAVP diluted in 100 ml of isotonic saline and infused for 30 minutes. BT and PFA-100 test were carried out before and one hour after test drug administration. The patient responded to DDAVP with normalization of both BT and PFA-100 platelet function tests. DDAVP resulted in the correction of platelet dysfunction as determined by normalization of PFA-100:Col/Epi and PFA-100:Col/ADP. Similarly, after DDAVP infusion BT was lowered to below 3 min. This value we consider the upper clinical threshold for normal primary hemostasis. Increase of FVIII activity (FVIII:C) from 84% to 153% and of VWF ristocetin cofactor (vWF:RcoF) from 110% to 115% were measured. A decrease of APTT from 48.2 sec to 42.3 sec was achieved, and no change in INR level (Table 1).

Preoperatively, we also tested response to vitamin K. Over a three-day period, the patient received vitamin K in a total dose of 60 mg. There was no change in APTT value. However, a shortened INR to 1.40 was recorded (results are not shown in Table 1).

Before surgery, the patient was prophylactically treated with three daily intravenous infusions of 20 mg/day of vitamin K. DDAVP was used at a standard dose of 0.3 µg/kg in infusion one hour before operation and then once daily until the end of the second post-operative day. Coagulation parameters were checked postoperatively for two days, and results remained within similar range.

No bleeding complications and no side effects as a result of DDAVP administration were recorded during or after surgery. At the time of operation, the baseline hemoglobin level was 117 g/L, and on discharge it was 114 g/L. There was no need for allogeneic blood products

administration during perioperative period. The patient had an uneventful postoperative course and left hospital on 4th postoperative day.

DISCUSSION

Not only does cirrhosis increase the risk of significant perioperative complications such as infection, recurrence of the inguinal hernia and ascites leak, but it also occurs associated with thrombocytopenia and thrombocytopenia that are a predisposition for prolonged hemorrhage, especially when invasive procedures are performed [7, 8, 13].

DDAVP, a synthetic analogue of the antidiuretic hormone vasopressin, induces increase in plasma levels of vWF and FVIII three to fivefold above the baseline level by exocytosis of these factors from the Weibel-Palade bodies (WPB) in the endothelial cells. In this way, DDAVP promotes the platelet adhesion to the subendothelium and platelet-platelet interactions as well as the coagulation process. In addition, DDAVP causes a co-secretion of t-PA from WPB. Moreover, DDAVP exerts a vasodilator effect via activation of endothelial NO synthase, and consequently NO production. These effects of DDAVP could be explained by a direct action on the endothelium via activation of endothelial vasopressin V2 receptor and by activating cyclic adenosine monophosphate (cAMP)-mediated signaling [14].

DDAVP is the first agent that has been shown to shorten BT significantly in patients with liver cirrhosis despite the fact that vWF and FVIII are within normal range [8]. In our patient, the normalization of BT after DDAVP infusion was not accompanied by a significant increase of either vWF or FVIII level. These results are in line with findings in the literature reporting that DDAVP-induced increase of vWF/FVIII in patients with liver cirrhosis is much lower than in healthy individuals [8, 15, 16]. Our results might be consistent with reports that postulated several putative mechanisms or mediators for the action of DDAVP other than vWF [10, 17, 18, 19]. In this respect, after DDAVP administration the significant increase of the expression of the endothelial-derived P-selectin (another major constituent of WPB) or a tissue factor (TF) suggests a role of other endothelial mediators, with a possible involvement of monocyte-dependent mechanisms (platelets activation by increasing the expression of P-selectin ligand on their membrane) [10]. Additionally, the formation of microparticles and generation of procoagulant activity following DDAVP stimulation has been described [17]. It has been suggested that the final effect of DDAVP on platelets involves the facilitation of glycoprotein (Gp) IIb-IIIa ligand interactions through modification of the cy-

toskeleton focal adhesion protein VASP. This molecular mechanism modulates platelets binding to fibrinogen and vWF [18]. DDAVP is supposed to induce a release of other WPB constituents such as interleukin (IL)-8, CD63 and endothelin-1 [19].

In our case, platelet aggregation was modified by DDAVP with PFA-100 Col/Epi to a greater extent compared to PFA-100 Col/ADP. These findings are in accordance with some other [20, 21] which showed a higher sensitivity of PFA-100 Col/Epi test in assessing the effect of DDAVP.

In a prospective study by Koscielny et al. [9], 254 of 5649 unselected patients scheduled for surgery were identified preoperatively as having either acquired (n=182) or inherited (n=72) impaired primary hemostasis. The hemostatic defect was most frequently identified based on PFA-100 Col/Epi (97.7%) and followed by PFA-100 Col/ADP (77.7%). DDAVP administration resulted in the correction of platelet dysfunction in 229 of 254 patients (90.2%). In a subgroup of 13 patients with liver cirrhosis, platelet dysfunction was corrected in 11 (84.6%) cases, as demonstrated by PFA-100 Col/Epi in 11 patients and by PFA-100 Col/ADP in 11 patients. After DDAVP administration, the correction of platelet dysfunction by Col/ADP, but not by Col/Epi was detected in two patients. The correction of BT after DDAVP administration was observed in 9 of 13 patients with liver cirrhosis.

Considerable interindividual variations in response to DDAVP may account for some of the controversies on hemostatic benefits of DDAVP. Thus, the ability of DDAVP to improve the platelet function is unpredictable, and a test dose of a remedy is recommended to establish the individual patterns of biological response and to predict clinical efficacy during bleeding and surgery [14].

We want to emphasize the usefulness of the PFA-100 test both in the detection of platelet functional abnormalities and in monitoring DDAVP therapy that is in accordance with data in the literature [9, 20]. It is not usually necessary to monitor skin BT because this test is a poor predictor of hemostasis in soft-tissue and postoperative bleeding [12].

Prophylactic treatment with DDAVP should be administered approximately 1 hour before the planned surgical intervention. Postoperative treatment with repeated doses 12-24 hours after the procedure should be considered until progression of wound healing [22].

The PFA-100 has shown to be useful preoperatively in identifying functional platelet abnormalities in liver cirrhotic patients as well as in monitoring DDAVP therapy. This represents a useful approach to indiscriminate use of allogeneic replacement therapy, allowing for substantial avoidance of the risks of blood supply and precocious platelet alloimmunization inherent to replacement therapy.

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Значај теста *PFA-100* у утврђивању дисфункције тромбоцита и ефикасности *DDAVP* код болесника с цирозом јетре подвргнутог репарацији препонске киле

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КРАТАК САДРЖАЈ

Увод Болесници с цирозом јетре се подвргавају хируршкој интервенцији након припреме због повећаног периоперационог ризика за настанак крварења условљеног поремећајима хемостаза. Показано је да је 1-деамино-8-D-аргинин вазопресин (*DDAVP*), дезмопресин, безбедан и делотворан лек у превенцији, односно збрињавању крварења код ових болесника. Мало се зна о одговарајућем тесту (или тестовима) за процену ефеката *DDAVP* на дисфункцију тромбоцита. Тест *PFA-100* (енгл. *Platelet Function Analyzer-100*) омогућава поузданију процену поремећаја примарне хемостаза, као и праћење хемостатских промена изазваних деловањем *DDAVP*.

Приказ болесника У раду приказујемо болесника старог 49 година с алкохолном цирозом јетре који је смештен у

болницу због планиране операције препонске киле. Због продуженог времена крварења, тестом *PFA-100* обављено је испитивање функције тромбоцита, које је указало на њихову дисфункцију. Стандардним коагулационим тестовима утврђен је поремећај секундарне хемостаза. Пре операције је истим тестом процењен болесников одговор на уобичајену дозу *DDAVP*, и он је био повољан. Болесник је оперисан након примене *DDAVP* и витамина К пре операције. Током периоперационог периода није било крварења, ни нежељеног дејства лека.

Закључак *PFA-100* је једноставан и поуздан тест за процену примарне хемостаза и праћење ефеката *DDAVP*.

Кључне речи: дисфункција тромбоцита; цироза јетре; хирургија; *PFA-100*; *DDAVP*