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Association for Academic Surgery

Microfluidics contrasted to thrombelastography: perplexities in defining hypercoagulability

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ARTICLE INFO

Article history:
Received 2 March 2018
Received in revised form
7 April 2018
Accepted 24 April 2018
Available online xxx

Keywords:
Thrombelastography
TEG
T-TAS
Microfluidics
Hypercoagulable
Platelet

ABSTRACT

Background: Elevated clot strength (maximum amplitude [MA]) measured by thrombelastography (TEG) is associated with thrombotic complications. However, it remains unclear how MA translates to thrombotic risks, as this measurement is independent of time, blood flow, and clot degradation. We hypothesize that under flow conditions, increased clot strength correlates to time-dependent measurements of coagulation and resistance to fibrinolysis.

Materials and methods: Surgical patients at high risk of thrombotic complications were analyzed with TEG and total thrombus-formation analysis system (T-TAS). TEG hypercoagulability was defined as an r <10.2 min, angle >59, MA >66 or LY30 <0.2% (based off of healthy control data, $n = 141$). The T-TAS AR and PL chips were used to measure clotting at arterial shear rates. T-TAS measurements include occlusion start time, occlusion time (OT), occlusion speed (OSp), and total clot generation (area under the curve). These measurements were correlated to TEG indices (R time, angle, MA, and LY30). Both T-TAS and TEG assays were challenged with tissue plasminogen activator (t-PA) to assess clot resistance to fibrinolysis.

Results: Thirty subjects were analyzed, including five controls. TEG-defined hypercoagulability by MA was detected in 52% of the inflammatory bowel disease/cancer patients; 0% was detected in the controls. There were no TEG measurements that significantly correlated with T-TAS AR and PL chip. However, in the presence of t-PA, T-TAS AR determined OSp to have an inverse relationship with TEG angle (−0.477, $P = 0.012$) and LY30 (−0.449, $P = 0.019$), and a positive correlation with R time (0.441 $P = 0.021$). In hypercoagulability determined by TEG MA, T-TAS PL had a significantly reduced OT (4:07 *versus* 6:27 min, $P = 0.043$). In hypercoagulability defined by TEG LY30, T-TAS PL had discordant findings, with a significantly prolonged OT (6:36 *versus* 4:30 min, $P = 0.044$) and a slower OSp (10.5 *versus* 19.0 kPa/min, $P = 0.030$).

Presented at the Academic Surgical Congress Jacksonville FL, February 2018.

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0022-4804/$ – see front matter
https://doi.org/10.1016/j.jss.2018.04.059

Conclusions: Microfluidic coagulation assessment with T-TAS has an overall poor correlation with most TEG measurements in a predominantly hypercoagulable patient population, except in the presence of t-PA. The one anticipated finding was an elevated MA having a shorter time to platelet-mediated microfluidic occlusion, supporting the role of platelets and hypercoagulability. However, hypercoagulability defined by LY30 had opposing results in which a low LY30 was associated with a longer PL time to occlusion and slower OSp. These discordant findings warrant ongoing investigation into the relationship between clot strength and fibrinolysis under different flow conditions.

Introduction

In-hospital thrombotic complications are common in both medical and surgical patients. Although prophylactic subcutaneous heparin can reduce the risk of deep vein thrombosis,[1] higher risk patients may receive an additional benefit by adding an antiplatelet medication.[2] Despite these recommendations, screening patients for hypercoagulability has not become a standard of care to direct targeted therapies. This is partially attributable to conventional labs that assess coagulation lacking an ability to detect hypercoagulability. However, whole blood viscoelastic hemostatic assays (VHA) has been shown to predict thrombotic complications in trauma patients,[3,4] liver disease patients,[5] cardiac surgery patients,[6] inflammatory bowel disease patients,[7] and malignancy.[5,8] This is further supported by patients presenting with new onset deep vein thrombosis having hypercoagulable VHA values compared with healthy controls.[9]

Elevated clot strength, defined with maximum amplitude (MA; thrombelastography [TEG]) or maximum clot firmness (rotational thrombelastography), is a particularly important variable, as it has been repeatedly identified as a risk factor for thrombotic complications.[4-6,8] Clot strength is a function of both fibrinogen and platelets.[10] However, the majority of clot strength is attributable to platelets,[11] which continue to evolve as an important process driving thrombotic complications in patients in the intensive care unit.[12] A key limitation to using VHA to determine thrombotic complications is that measuring a stagnant pool of blood does not represent flow conditions as coagulation occurs *in vivo*. Understanding coagulation under flow conditions is becoming increasingly important for the assessment of bleeding and thrombotic complications in patients.[13] Flow information also provides information on occlusion time (OT), which has direct clinical translation and cannot be determined by measurements of clot strength.

Total thrombus-formation analysis system (T-TAS) is a novel technique that uses a microchip flow chamber system to analyze the thrombus formation process under flow conditions. In addition to measuring the clotting parameters of whole blood, T-TAS can measure platelet specific coagulation properties. Our primary aim was to correlate T-TAS measurements of microvascular occlusion properties—in both whole blood and platelet function—to TEG measurements, within a heterogeneous population of healthy controls and patients presumed to be at risk of thrombotic complications. Our secondary aim was to assess the impact of tissue plasminogen activator (t-PA) under flow *versus* nonflow conditions as recent evidence supports impaired fibrinolysis is also associated with thrombotic complications.[14] We hypothesize that increased clot strength (MA) measured by TEG correlates to flow-dependent measurements of platelet-mediated microvascular occlusion by T-TAS and impaired clot degradation in the presence of t-PA.

Materials and methods

Subjects

Surgical patients at risk of thrombotic complications were enrolled in a Colorado Multi-Institutional Review Board study to prospectively collect blood samples preoperatively before their surgery. All patients were treated at the University of Colorado Hospital. Enrollment criteria were adult (>18 y) and patients undergoing elective surgery or endoscopy for inflammatory bowel disease or pancreatic cancer; in addition to five adult healthy volunteers. These patients represented a convenient sample as they required consent and the availability of a member of the research team capable of running both TEG and T-TAS assays. The specific number of patients per cohort was based on ongoing studies of coagulation in inflammatory bowel disease and pancreatic cancer—both having a consistent and predictable enrollment potential of patients during the 30 d period, in which the microfluidic device was available to the research team (i.e., in the given 30 d, we anticipated we could enroll 15 patients with pancreatic cancer and 10 patients with inflammatory bowel disease). Inflammatory bowel disease and pancreatic cancer patients were analyzed together as a single hypercoagulable group so that direct comparisons could be made to controls, and not between the two groups themselves—which explanations for any shared or differing properties related to their coagulopathies are unknown.

Thrombelastography

TEG assays were not performed on these patients as part of their routine preoperative coagulation assessment. Both TEG and T-TAS assays were performed in separate lab environments. Preoperative blood samples were drawn in the operating room after intubation and before incision. Blood was collected in citrated tubes and assayed at room temperature between 20 min and 2 h after blood draw, as per manufacturer's guidelines. The citrated samples were recalcified and assayed using the TEG 5000 Thrombelastograph Hemostasis