Clinical beta-testing of a newly developed point-of-care technology shows potential to drastically reduce the time and complexity required for Fibrinolytic Activity measurements.

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Abstract: The methods used to measure fibrinolytic activity have not advanced significantly in many years despite the clinical importance of fibrinolysis and the value of its measurement to physicians' treatment decisions under many scenarios. Fibrinolysis is often assessed using viscoelastometric methods that require bulky equipment and test durations of 20 minutes or more to produce useable data. This is impractical for many settings, and the medical community has called for faster, more convenient, and more accurate methods. We introduce an innovative technological approach for fast measurement of fibrinolytic activity with a handheld point-of-care device. The technology utilizes a synthetic protein matrix to mimic a clot substrate, deposited on an electrode strip for electronic detection. The matrix is engineered to break down more rapidly than a native clot when placed in the presence of fibrinolytically active blood sample, and a quantitative reading is produced in less than 30 seconds. Clinical testing showed that the technology functioned well in a clinical setting where cardiovascular patients produced drastically different data compared to a "healthy" cohort of volunteers. The results were strong enough to justify moving forward with formal clinical trials where the device will be tested against the only approved predicate for fibrinolysis, the Euglobulin Lysis Test (ELT) test.

Clinical Importance of fibrinolysis measurement

Human haemostasis maintains blood vessel integrity through an intricate balance between coagulation (clot formation) and fibrinolysis (clot degradation). Disruption of this balance is at the root of many vascularthrombotic diseases, including leading causes of death such as acute coronary syndromes and ischemic stroke. While diagnostic tools for assessing coagulation have been becoming more robust in recent years, advancements in technology for quantitative assessment of fibrinolytic activity have been lacking, leaving a significant gap in the information required to optimize treatment for cardiovascular patients.

Heightened fibrinolytic activity is a direct signal for diagnosing acute thrombotic events such as myocardial infarctions, strokes, and pulmonary embolisms. For these events, faster diagnosis followed by appropriate treatment can have drastic impact on patient outcomes. In addition, a state of hyperfibrinolysis (HF) is a primary concern for trauma physicians as HF often triggers a series of complications – consumption of clotting factors, acidosis, hypothermia - that result in excessive hemorrhage and can rapidly erode patient conditions. The ELT test is the only approved test for measuring fibrinolysis, however, it requires several hours to conduct and is therefore impractical for use in diagnosing HF in the course of treating trauma cases or surgery.

Physicians recognize the need for this information as well as the inadequacy of ELT, so they have turned to viscoelastometric methods to gather information on this process[1,2]. These methods have only been marginally improved since their development in 1948, and they are inadequate for enabling the breadth of treatments that are now available. Viscoelastometric methods require a bulky apparatus (ROTEM/TEG) and 20-30 minutes per test to return graphical output from which parameters can then be derived to indicate

levels of fibrinolytic activity. However, patients' hemostatic conditions can change significantly in less than 3 minutes.

These methods are unable to provide rapid diagnosis of thrombotic events in the field, and they are lacking in the ability to provide true real-time feedback to physicians for optimal, case-specific administration of critical treatments to counteract hyperfibrinolysis during surgery or trauma management. Indeed, hemorrhage is recognized as the leading cause of "preventable death" post trauma. As a result, there have been calls for a faster and more advanced tool for providing feedback on this important physiological process[3].

The FibrilyzerTM technology for rapid measurement of fibrinolytic activity with a handheld point-of-care device

Fibrilyzer™ is a device based on a new technology (patent pending[4,5]) designed to address the shortcomings of the viscoelastometric devices and ELT. This technology employs disposable strips and a hand-held electronic reader for convenient use in any setting. The strips contain a synthetic fibrin matrix, which mimics a native clot, but it excludes certain structural characteristics that give native clots resilience to fibrinolytic enzymes.

With the addition of a single drop of blood, the matrix degrades at a rate proportional to the fibrinolytic activity of the sample. Upon matrix degradation, the porosity of the matrix increases, the viscosity decreases, and the diffusion of an electroactive species within the matrix is facilitated, leading to a marked increase in the measurable Faradaic current.

Physiological native clots have evolved as difficult to degrade structures, in order to allow sufficient time for blood vessel repair. This is naturally achieved through the following factors: (i) covalent crosslinking of the fibrin filaments by Factor XIII to provide structural stability and rigidity, and (ii) crosslinking of plasmin inhibitors (antiplasmin) into the clot structure to inactivate the lytic activity of the main fibrinolytic enzyme. In contrast, the synthetic Fibrilyzer™ clots have features that allow for fast degradation to consequently allow for enhanced sensitivity in detecting of elevated fibrinolytic activity in a blood sample. These include: (i) non-covalent crosslinking of fibrin, (ii) absence of any inhibitors in the clot, and (iiI) optimized fibrin content for efficient binding of tPA and plasmin to the clot surface.

These features of Fibrilyzer™ allow it to return a quantitative measurement of fibrinolytic activity within 30 seconds of applying the blood sample. This information can be used to aid in diagnosis, determine case-specific dosing of thrombolytics in the case of an acute event (ischemic stroke, pulmonary embolism), or guide dosing of TXA in the case of post-trauma hyperfibrinolysis.

Clinical pilot test design

A clinical beta test was performed as an initial assessment of device performance in a clinical setting. The testing was performed on a random sampling of subjects, most of them deemed "heart failure" patients in the cardiology ward (not in a ER or ICU) at University Hospital "Queen Yoanna" in Sofia, Bulgaria. Detailed case history was often not available, and none of the subjects were representative of an acute thrombotic ER case due to their length of stay in the ward, medication, or conditions unrelated to thrombotic events. Most of the patients, but none of the healthy individuals, were being treated with anticoagulants. The "gold standard" ELT test was not available in Bulgaria to compare against the Fibrilyzer™ readings.

Nevertheless, the rationale for the test was that some of the subjects in any cardiology ward would likely have elevated fibrinolysis so that important fundamental assumptions could be verified:

1) There would be no technical difficulties with the test when run by healthcare professionals
2) A "normal" fibrinolysis range could be established per the device readings
3) The device could clearly detect elevated fibrinolysis in a clinical setting

The trial included 30 healthy volunteers and 62 patients from the cardiology ward and was managed by Prof. Assan Goudev, Dept. Chair. After ethanol swipe, subjects were given a fingerstick with a regular syringe needle or a lancet. Care was taken to make sure that the ethanol was dry before the stick to prevent ethanol contamination. Upon touching the blood drop formed on the finger, Fibrilyzer™ devices start an automatic incubation time counter, and two measurements were taken after preset time intervals. The electrochemical Faradaic currents were measured for three milliseconds in the middle of a 0.5 second voltage step. The incubation and measurement steps were performed in a small Merck-Serono incubator set at 37°C. All patients were tested in triplicate with three separate devices working simultaneously.

After examining the data for the cardiovascular patients, those cases demonstrating highest values of fibrinolytic activity relative to the healthy sample were investigated in much greater detail to determine if their readings were truly indicative of thrombotic conditions.

Results

The initial rationale behind having two measurement time points was to establish a baseline at 10 seconds and then an actual reading of a digested sample at 90 seconds. However, it was discovered early in the study that lysis occurs very quickly, so even the 10-second sample was digested. Accordingly, the protocol was changed to reflect two actual measurements at 20 seconds and 90 seconds.

The results of the 20 sec time point are shown in Figure 1 below. Healthy volunteers registered values between 0.50 and 2.00 uA while the subjects in the Cardiology ward produced readings ranging from 1.00 to 4.50 uA.

Those patients demonstrating the highest levels of fibrinolysis as measured by the device had the following clinical diagnoses:

#1 - Systolic Heart Failure with Pulmonary Edema
#2 – Myocardial Infarction followed by Systolic Heart Failure
#3 – Pulmonary Embolism, right ventricular HF, high D-dimer
#4 – Systolic Hear Failure Thrombocytopenia

Conclusions

All three goals of the trial were accomplished. Medical personnel easily managed the administration of the tests, and samples from the healthy volunteers produced fibrinolysis readings that demonstrated a grouping from which a "normal range" could be derived. After only 20 seconds, the samples taken from the cardio patients yielded a scattered distribution that was very different than the comparatively tight distribution for the healthy sample, demonstrating the cardiovascular patients' varying degrees of elevated fibrinolysis. A detailed review of higher readings indeed indicated thrombotic symptoms.

These results are even more promising, considering that: (i) in a Cardiology ward – as opposed to a ICU or ER unit - there were no nascent acute cases where one would expect the fibrinolytic burst to be most pronounced; and (ii) most patients were treated with anticoagulants, which suppress global hemostasis. With regard to the last point, Fibrilyzer™ measures fibrinolysis in a peripheral sample, and thus any fibrinolytic burst due to a thrombotic event is attenuated by the global inhibition of hemostasis from

anticoagulants. Fibrilyzer™ readings upon admission or in the field, prior to administration of anticoagulants, would thus be expected to be even higher.

The measurements from the triplicate tests, where successful, showed very high concordance indicating excellent reproducibility of the technology. However, some of the tests failed to produce any reading due to slight mismatch between the strip width and the reader slot, which prevented making electrical contact. This defect has been since remedied.

This trial showed that the technology to perform as expected in a clinical setting and confirmed that it should move to formal clinical trials for detailed comparison to the predicate test (ELT). It produces data with speed and accuracy that enables a range of functions appropriate for field diagnostics, surgical monitoring, and dosing adjustments. Upon successful completion of formal clinical trials, these numerous applications will enable the technology to materially advance the standards of care.

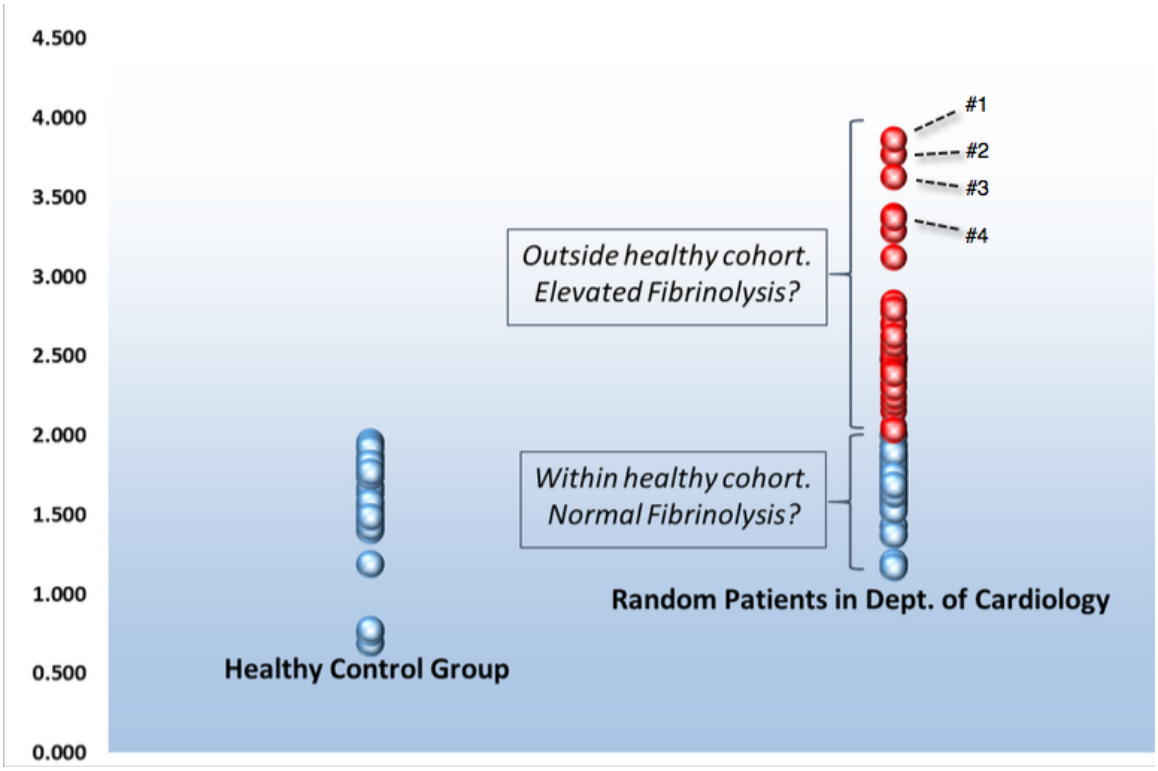

Figure 1

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