Whole Blood Platelet Activity Measurements Multiplexed with Quantitative Fibrinogen, Clot Time and Fibrinolysis Measurements by T2MR®

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INTRODUCTION

Background

Hemostasis disorders affect millions of people in the United States each year. These disorders require diagnostic identification and monitoring for both acute and chronic conditions that range from traumatic injury to routine monitoring of anticoagulant therapy, evaluation of clotting diseases and evaluation of bleeding disorders. In chronic cases, these disorders require diagnostic information to manage the disease, and in acute episodes, such as trauma, the need exists to quickly diagnose the condition and subsequently monitor transfusion protocols. In most cases, testing for these conditions requires multiple instruments and highly trained laboratory personnel, along with significant volumes of blood from each patient. The delay in results associated with these tests can be a significant impediment to timely patient care. A more rapid determination of coagulation test results may allow healthcare practitioners to manage their patients more effectively during many important healthcare procedures, including surgery, trauma, transfusion protocols, and therapeutic monitoring.

A comprehensive assessment of a patient's hemostasis status often requires the determination of multiple parameters including clotting times, platelet function, fibrinogen, and fibrinolysis. In many acute and transient clinical settings, the most effective patient care decisions require rapid and accurate measurement of these parameters. In fact, more rapid delivery of directed therapy for patients with impaired hemostasis has been shown to deliver a reduction of mortality from 45% to 19% and a decrease in length of hospital stay by 20%.[1] To date, there has not been a singular solution to accurately measure these parameters within the necessary time-frame to improve patient outcomes while reducing the overall cost to the hospital. T2 Biosystems' Magnetic Resonance (T2MR) device, an investigational medical device, can measure all four parameters from a single sample and deliver results in less than one hour at the point of care. By providing a rapid and accurate global assessment of a patient's hemostasis state, the T2 Biosystems' T2MR device could significantly assist with the active management of hemostasis disorders while reducing both healthcare costs and mortality.

T2MR Measurement of Hemostasis Parameters

- T2MR provides a rapid, accurate and comprehensive global assessment of a patient's hemostasis status.
- T2MR relies on proven magnetic resonance technology and has already been successfully implemented in FDA-cleared products including the T2Dx® Instrument and the T2Candida® Panel.
- T2MR measures the spin-spin (T2) relaxation times of hydrogen nuclei in water molecules in a whole blood sample.
- Water serves as a microscopic probe within changing micro environments (such as a coagulating blood sample).
- Measurement of these parameters in a single sample represents a multiplex hemostasis panel to diagnose and monitor related chronic and acute disorders
- A series of studies were conducted in which components of the clot were separated and T2 values determined. This "reductionist" approach was used to assign functional characterizations to the T2 signatures.[3]
- After initiation of blood coagulation with an activating reagent, T2MR monitors changes in water microenvironments. The experience of each water molecule is measured via the T2 values. Water molecules experience different microscopic environments for the following clinically significant conditions:

1. Unclotted blood and hematocrit
2. Formation of fibrin mesh
3. Restriction of water diffusion due to platelet induced clot contraction
4. Release of water and red blood cells due to clot lysis

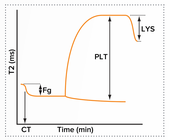

Figure 1. T2MR signatures of a coagulating blood sample as a function of time. Signatures are used to report:
1. CT: Clotting Time
2. Fg: Fibrinogen Level
3. PLT: Platelet activity
4. LYS: Fibrinolysis

METHODS

T2MR Measurements

Measurements were made using a small portable T2 Magnetic Resonance (T2MR) device. 5 µL of an activator reagent, either tissue factor or ellagic acid was added to a reaction tube (Fig. 2). The activator reagents contain all needed components to recalcify blood and initiate activation through either the extrinsic or contact factor pathways of coagulation. Reaction tubes were placed in the T2MR device and 35 µL of citrated whole blood was added and mixed. The addition of the blood triggered the start of T2MR device data collection.

Clot Time Studies

To simulate the range of coagulation disorders that present with altered clotting times, citrated whole blood was treated with 100 to 1000 ng/mL of Rivaroxaban (a direct Xa inhibitor). T2MR measurements were taken on these samples after activation with a tissue factor reagent formulation. Plasma was also isolated from each spiked sample and prothrombin time was measured on the Stago STA4 system using Stago Neoplastine reagent using manufacturer recommended methods. Data were compiled using prospectively collected blood samples from three different donors and collected across multiple T2MR devices.

Fibrinogen Studies

To represent a range of clinically relevant fibrinogen and hematocrit levels—both healthy and unhealthy—blood samples were prepared with a range of fibrinogen from 75 mg/dL to 850 mg/dL and hematocrit levels of 20% to 40%. Citrated whole blood was centrifuged to isolate red blood cells from plasma. Samples with varying fibrinogen levels were produced by mixing pooled fibrinogen-depleted plasma with plasma containing known fibrinogen levels. These samples were then mixed with known concentrations of red blood cells to produce samples that had different hematocrit and fibrinogen levels. T2MR measurements of each sample were done by mixing 5 µL of ellagic acid reagent with 35 µL of contrived whole blood. Plasma was isolated from each sample and Clauss Fibrinogen was determined on the Stago STA4 system using STA-Fibrinogen reagent using manufacturer recommended methods. Data was compiled from three different donors.

Platelet Activity Studies

To simulate healthy and unhealthy ranges of platelet function, citrated whole blood samples were spiked with 0 to 3 µM Cytochalasin D, which is an inhibitor of platelet contraction. T2MR measurements were taken after mixing 5 µL of ellagic acid reagent with 35 µL of the spiked whole blood.

Fibrinolysis

Citrated whole blood samples were spiked with 0 to 3 nM tissue plasminogen activator (tPA) to simulate fibrinolytic blood samples. Spiked samples were tested on the T2MR device by mixing 5 µL of ellagic acid reagent with 35 µL of blood. Aprotinin was spiked into blood to inhibit fibrinolysis.

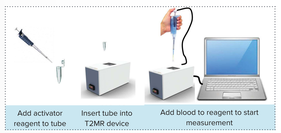

Figure 2: Schematic of procedure to obtain multiplexed hemostasis T2MR measurements.

RESULTS

Clotting Time

T2MR measures clotting times (CT) by determining the change in T2 values due to fibrin formation that occurs after the addition of an activator to a blood sample (Fig. 3). Unclotted blood has a higher T2 value than blood containing a fibrin clot. After the addition of an activator to a blood sample the fibrin clot forms and the T2 values decrease as a function of time. T2 data is fitted to determine the midpoint of the T2 values between clotted and unclotted states.

A method comparison between T2MR clot time with whole blood and plasma prothrombin time (PT) was done using normal blood samples that were spiked with Rivaroxaban and activated with tissue factor reagent. Rivaroxaban is an inhibitor of factor Xa and thus inhibits the formation of thrombin through the extrinsic and contact factor pathways. A titration of Rivaroxaban between 0 and 1000 ng/mL corresponded to a range of clot times on T2MR of 32 – 259 seconds. Blood samples were activated with tissue factor and T2MR measurements taken. Plasma from each sample was also isolated and clot time determined using Stago STA®. Precision of T2MR measurements was 3.6% compared to 2.7% on the Stago system. Correlation between the two methods gave a $R^2 = 0.98$ (Fig 4). Data was collected from analysis of three different prospective donors and 10 T2MR replicates performed across multiple devices.

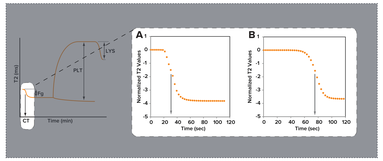

Figure 3. Examples of T2MR data. Blood samples were activated with tissue factor and evaluated by T2MR.
A) Normal blood sample.
B) Blood sample with an extended clot time.

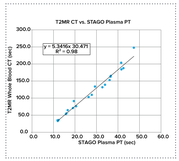

Figure 4. Correlation of T2MR clot time to plasma PT derived on the Stago STA4.

Fibrinogen

T2MR measures fibrinogen through the change in T2 values that occurs as the fibrinogen in the sample is converted to fibrin, which results in a fibrin clot (Fig. 5). T2 values decrease as the blood goes from unclotted to clotted. Higher fibrinogen in the sample will result in a larger change in T2 values. T2 data is fitted to determine the minimum and maximum T2 values before and after clotting has occurred to derive the fibrinogen measurement.

Whole blood samples were produced that contained differing levels of fibrinogen and red blood cells. T2MR fibrinogen measurements were determined after ellagic acid activation by calculating the normalized change in T2 values. Clauss fibrinogen measurements were used to assign fibrinogen (mg/dL) levels for each sample. Data was collected over a 20% to 40% range of hematocrit levels and 75 mg/dL to 850 mg/dL fibrinogen. T2MR data had a correlation to Clauss Fibrinogen of $R^2 = 0.98$ and the calibration curve had a ±10% variation (Fig. 6).

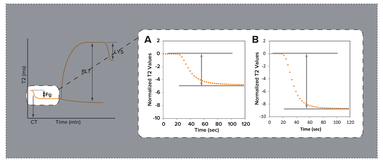

Figure 5. Examples of T2MR data. Blood was activated with ellagic acid and evaluated by T2MR. (Orange points are actual data.)
A) Sample with low fibrinogen.
B) Sample with high fibrinogen.

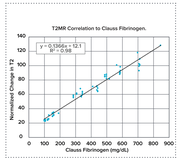

Figure 6. Calibration curve of T2MR fibrinogen measurements over a range of hematocrit and fibrinogen levels.

Platelet Activity

After coagulation is activated, thrombin is produced, which activates platelets. Activated platelets bind to the fibrin clot and induce clot contraction. As the clot contracts, serum is extruded from the clot, creating two phases within the sample (Fig. 7). Serum has higher T2 values compared to the fibrin clot. T2MR is able to differentiate the T2 values associated with the fibrin clot and the extruded serum and two distinct peaks are detected (Fig. 7A). As more serum is extruded from the clot, the intensity or relative quantification of water associated with serum increases and that of the fibrin clot decreases (Fig. 7B). The presence of the higher T2 values and intensity associated with serum is a measure of platelet function, which can be normalized to give a relative platelet activity metric (PAM) and be used to distinguish normal from abnormal platelet activity.

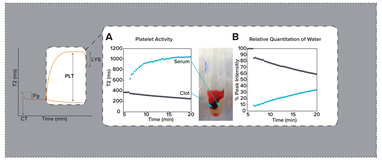

Figure 7. Example of T2MR data. A whole blood sample was activated with an ellagic acid reagent.
A) Activation of platelet results in distinct T2 values associated with the fibrin clot (gray) and extruded serum (blue).
B) T2MR detected intensities (relative quantification of water) associated to the fibrin clot (gray) and extruded serum (blue).

To demonstrate the ability to detect differing levels of platelet function by T2MR, varying concentrations of Cytochalasin D were mixed into citrated whole blood samples (Fig. 8A). Cytochalasin D is a potent inhibitor of actin polymerization and thus inhibits the platelet contractile function. Blood samples with or without Cytochalasin D were activated with ellagic acid and tested by T2MR. With increasing levels of Cytochalasin D the time at which T2 values associated with serum are detected and maximum amplitude of these T2 values were decreased. PAM was determined at 20 minutes after activation and showed a titration of platelet function with increasing Cytochalasin D concentrations (Fig. 8B). Inhibition of platelets with Cytochalasin D did not alter the detected clot time and fibrinogen by the T2MR device.

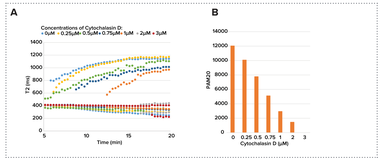

Figure 8. Inhibition of platelet function was detected by T2MR.
A) T2MR data from whole blood samples spiked with increasing concentrations of Cytochalasin D.
B) PAM calculated at 20 minutes after activation.

Fibrinolysis

In cases of severe trauma, high levels of tissue plasminogen activator (tPA) can be released into the blood. tPA converts plasminogen to active plasmin, which cleaves fibrin strands. This cleavage can lead to the dissolution of the fibrin clot and result in massive bleeding if untreated. T2MR detects fibrinolysis by monitoring the changes that occur in the T2 values associated with the serum (Fig. 9). As the clot is broken down and RBCs are released back into the serum environment, the T2 values associated with the serum will decrease. The percentage difference in the maximum T2 value and the final T2 value of the serum peak were used to determine percentage lysis.

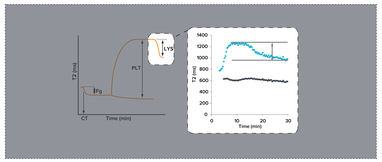

Figure 9. Example data from a whole blood sample that was spiked with tPA. After the initial formation of the contracted clot the T2 values associated with the serum start to decrease as the contracted clot is broken down and RBCs are released into the serum.

Detection of fibrinolysis was demonstrated by spiking citrated whole blood samples with 0 to 3nM concentrations of tPA (Fig. 10A). Maximum T2 values associated with serum occurred approximately 20 – 25 minutes after activation of blood samples with ellagic acid. After 30 minutes the T2 values of samples that contained tPA started to decrease. There was no change in T2 values in samples that did not contain tPA or were spiked with the fibrinolysis inhibitor Aprotinin. The higher the concentration of tPA, the larger the change in T2 values corresponding to more lysis (Fig. 10B).

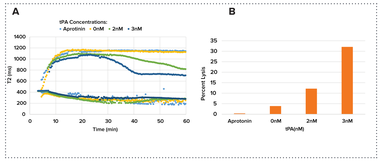

Figure 10. Fibrinolysis was induced in citrated whole blood samples by adding tPA to samples.
A) Example T2MR data of blood samples containing 0 – 3nM tPA or the fibrinolysis inhibitor Aprotinin.
B) Percent lysis was determined by calculating the percent change in T2 values of the serum peak at 45 minutes after activation of coagulation.

CONCLUSIONS

These research studies show that T2MR detects a range of clinically valuable parameters to provide a global assessment of a patient's coagulation, platelet and fibrinolysis status. Further, T2MR provided highly accurate results when compared to the gold standard methods ($R^2 = 0.98$). In previous studies, T2MR had predicted thrombotic events otherwise missed by established diagnostic methods.[4] Providing these clinical-lab quality measurements as an integrated assessment of global hemostasis status could impact patient management by providing rapid, comprehensive coagulation data closer to the patient. Future studies will include assessment of T2MR tests in clinical patient populations. The potential advantages to the T2MR device include:

1. **Faster:** Clot time and fibrinogen data within 3 minutes and platelet function within 20 minutes.
2. **Low blood volumes:** Assay uses 35 µL whole blood.
3. **Easier:** Simple mix-and-read approach requiring no sample processing or calculation of data.
4. **Sensitive:** Earlier detection of hemostasis abnormalities with less analyte present.
5. **Accurate:** High correlation with standard methods.
6. **Novel biomarkers and data:** T2MR data provides information rich signatures that have led to the discovery of novel biomarkers that may have significant clinical impact.[5]

In this study, the T2MR device has demonstrated the rapid and accurate assessment of potential bleeding disorders through a single multiplex analysis of global hemostasis. The application of this technology to the diagnosis and monitoring to bleeding disorders may significantly reduce patient mortality and associated healthcare costs.

T2MR's performance attributes are consistent with enabling significant research applications, drug development and biomarker studies.

Biomarker Discovery

T2MR can identify novel biomarkers, as evidenced by the recent discovery of novel clot biology of polyhedral red cells that may have significant clinical impact.[5]

The robust and sensitive data generated by T2MR may help in the discovery and development of novel drug targets, as well as the monitoring of these compounds in pre-clinical animal testing and patients to enable more efficient discovery and clinical trials for new and existing therapies.

Small Blood Volume

T2MR requires very little blood for its hemostasis measurements, as little as a fingerstick in many cases. The reduced blood requirements facilitates more effective animal studies, clinical research, and patient monitoring for coagulation disorders.

Rapid Results

T2MR generates rapid results to provide clinicians actionable results, while enabling more frequent testing of samples in the clinical and research setting.

Simplicity

T2MR is simple to use, allowing analysis to be done in virtually any setting, including research laboratories and core and satellite laboratories, as well as the point of care.

Multiplexing

T2MR generates multiple hemostasis measurements from a single blood sample in a single analysis, providing comprehensive and actionable results for both clinicians and researchers.

High Sensitivity

T2MR is a highly sensitive detection method that provides results at much lower thresholds that other devices to provide more robust and faster data for hemostasis testing.

Versatility

T2MR is a versatile system that can be used for hemostasis, infectious diseases, oncology, biosafety, drug development and many other clinical and research markets.

References

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The T2MR device in this study is investigational and for research use only.

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