T2MR® Platelet Analysis Correlates With LTA and Reveals Unique Details of ADP-Mediated Platelet Activation in Whole Blood

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INTRODUCTION

- There is a need for a simple, rapid platelet activity diagnostics with similar or improved performance relative to the established reference method, light transmission aggregometry (LTA).
- Recent reviews highlight the need for platelet function tests to demonstrate sensitivity to anti-platelet medications and to predict clinical bleeding and thrombotic events.[1,2]
- For these purposes, we present new data characterizing the T2 magnetic resonance (T2MR) hemostasis methodology for the assessment of platelet-mediated clot contraction in whole blood.[3]
- Our aim was to assess the ability of T2MR to discriminate platelet activity between donors with normal and impaired platelet function, defined by LTA.

METHODS

T2MR Measurement of Hemostasis Parameters

- Water serves as a microscopic probe within changing micro environments (such as a coagulating blood sample).
- T2MR measures the spin-spin (T2) relaxation times of hydrogen nuclei in water molecules in a sample.
- 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.
- A series of studies were done where components of the clot were separated and T2 values determined. This "reductionist" approach was used to assign functional characterizations to the T2 signatures.[1]
- A series of foundational experiments were conducted in T2 Biosystems and University of Pennsylvania laboratories to assign T2 relaxation times for water in each of these environments.[3]

T2MR Measurements

T2MR measurements were made using a small portable T2MR device (Fig. 1). A 5 µL volume of "activator solution" was added to a reaction tube to generate a fibrin mesh, inhibit thrombin, calcify the citrated blood, and activate platelets with a specific platelet agonist, e.g., arachidonic acid. Reaction tubes were placed in the T2MR device and 35 µL of citrated whole blood was added and mixed. Addition of the blood triggered the start of T2MR data collection.

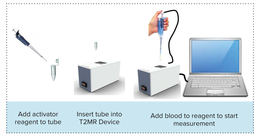

Figure 1: Schematic of procedure to measure platelet-induced clot contraction.

Blood Draw Methods

- Whole blood was collected in citrated vacutainers from 21 healthy donors not taking anti-platelet medications.
- A 19G needle was used during blood collection with an applied tourniquet. The first 5 mL Vacutainer® drawn was discarded and not included in the analysis.

T2MR Platelet Activity Measurements

- Platelets were then activated by adding 10 µM ADP, 0.5 mM arachidonic acid (AA), 10 µM thrombin receptor activator peptide (TRAP), or 10 µM epinephrine (EN) (final concentrations).[3] Clotting was initiated with a formulation containing reptilase to generate fibrin mesh.
- To assess specificity, agonists were combined with specific cognate receptor antagonists, which were:
  - AA: acetylsalicylic acid (600 µM; aspirin)
  - ADP: MeSAMP (100 µM) and/or MRS2279 (10 µM)
  - EN: yohimbine (10 µM)
  - TRAP: vorapaxar (5 µM)
- All antagonists were incubated for at least 15 minutes in whole blood prior to addition of agonists.
- The "activator solution" with platelet agonist was added in a 0.2 mL tube, which was placed into the T2MR device. Thirty-five µL of whole blood was added at 37° C and the T2MR measurements were initiated.
- A platelet activity metric (PAM) was devised to quantify platelet induced clot contraction by evaluating the formation of serum (T2 difference) and how much serum has formed (intensity) from time 0 to 20 min. (Fig. 2A).

LTA Aggregation Measurements

- LTA measurements were performed using the Chrono-log instrument in parallel with T2MR, from the same specimen measured by T2MR, using the same whole blood sample.
- LTA was performed using platelet rich plasma (PRP) prepared by centrifuging blood at 200 x g.
- Platelet poor plasma (PPP) was used as the blank control for LTA by centrifuging blood at 2000 x g. Platelet count was used unadjusted for LTA measurements.

RESULTS

- T2MR provides dynamic measurements of clot formation and platelet-mediated clot contraction.[4] At the initiation of clot retraction, T2 values from the serum and blood clot separate rapidly, and the intensity values or relative moles of water increases in the serum phase relative to the clot phase (Fig. 2A). The T2MR platelet activity metric (PAM) utilizes both of these measures to quantify functional platelet-induced clot contraction.
- T2MR detects contraction after addition of TRAP, an activator of the PAR-1 platelet receptor. However, when TRAP and a PAR-1 inhibitor (vorapaxar) are added, no retraction is detected over 20 minutes (Fig. 2B).
- A patient with Glanzmann's thrombasthenia shows no contraction response on T2MR to activation by TRAP, as expected, due to a defect in glycoprotein IIb/IIIa. The response is similar to that of a healthy donor following addition of vorapaxar, a PAR-1 inhibitor (Fig. 2C).

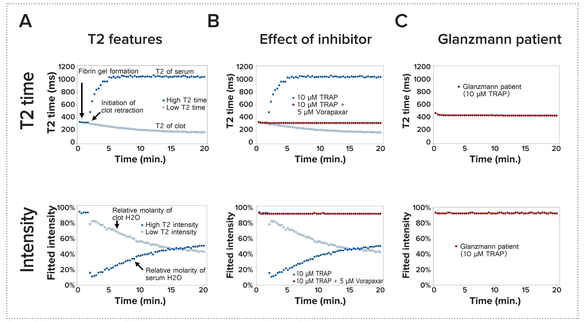

Figure 2: Raw data curves from T2MR platelet activity measurements: Effect of PAR-1 inhibitor and defect in glycoprotein IIb/IIIa.
(A) Sample from normal donor showing initiation of clot contraction and changes in T2 time and intensity during clot contraction.
(B) Effect of TRAP and inhibitor of PAR-1 activation on clot formation and contraction compared to normal donor response.
(C) Absence of contraction in sample from patient with Glanzmann's thrombasthenia.

- Using platelet receptor specific agonists and antagonists, we found a 100% positive percent agreement (PPA) and 100% negative percent agreement (NPA) between T2MR and LTA using each of the four platelet agonists. LTA response is shown in Fig 3A by green for activated and red for inhibited.
- After activation with ADP, only partial inhibition with either 100 µM MeSAMP (P2Y12 receptor inhibitor) or 10 µM MRS2279 (P2Y1 receptor inhibitor) alone was seen using T2MR. However, complete inhibition was seen when both compounds were present, indicating activation through both receptor pathways contribute to the T2MR signal (Fig. 3B).
- This finding is in contrast to platelet aggregometry, where MeSAMP gave complete inhibition, suggesting ADP-mediated platelet clot retraction in whole blood is more reliant on P2Y1 signaling than platelet aggregation in plasma.

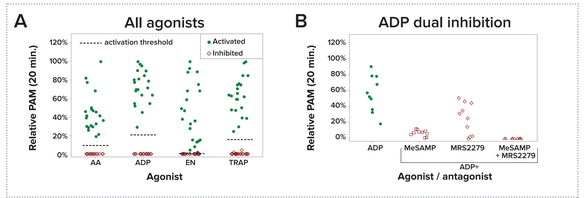

Figure 3: Specificity of T2MR platelet agonist assays.
(A) T2MR relative PAM for four agonists and corresponding thresholds. Data points are color coded to indicate LTA responsiveness.
(B) Dual-inhibition of response to ADP using either 100 µM MeSAMP or 10 µM MRS2279 alone, or both compounds together (N = 9).

- The feasibility of T2MR to measure agonist specific platelet activity and inhibition in vivo was investigated by measuring platelet activity in response to arachidonic acid using T2MR and LTA after ingestion of 325 mg of aspirin over 5 days.
- T2MR demonstrated partial recovery of platelet activity 29 hours after ingestion of ASA. Using LTA, partial recovery was first evident at 78 hours (Fig. 4A). This demonstrates that T2MR, like LTA, can assess restoration of platelet activity after therapeutic platelet inhibition.
- Although the raw data curves (Fig. 4B) may indicate more rapid results for platelet function with T2MR compared to LTA, additional studies are needed to verify this difference.

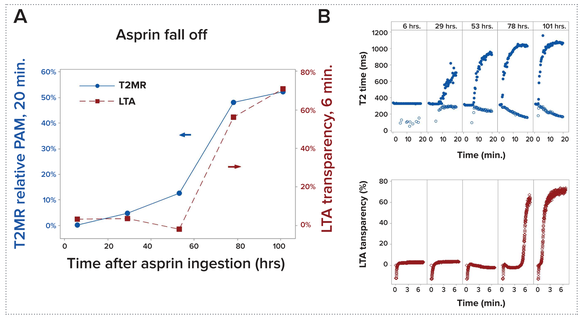

Figure 4: Assessment of platelet function in response to arachidonic acid after ingestion of aspirin measured using T2MR and LTA.
(A) Comparison of platelet activity measured by each platform, where T2MR indicates recovery of platelet activity earlier than LTA.
(B) Raw data curves demonstrating T2MR detects partial platelet activity at 29 hours, compared to 78 hours using LTA. LTA curves begin with negative transparency due to initial addition of oil-based AA to PRP.

- "Reconstructed" blood samples were generated using autologous RBCs, platelets and plasma. ADP was then added to activate platelets. Clotting was initiated with a formulation containing reptilase to generate fibrin mesh.
- Zero or near-zero response was seen at platelet counts below 100 k/µL using LTA, whereas platelet activity was detectable at platelet counts as low as 40 k/µL using T2MR (Fig. 5).
- PPP was used to adjust platelet count in LTA and T2MR samples
- This result is consistent with high sensitivity to less intense platelet function and lower platelet counts using T2MR. Additional studies will investigate platelet function on T2MR in thrombocytopenia.

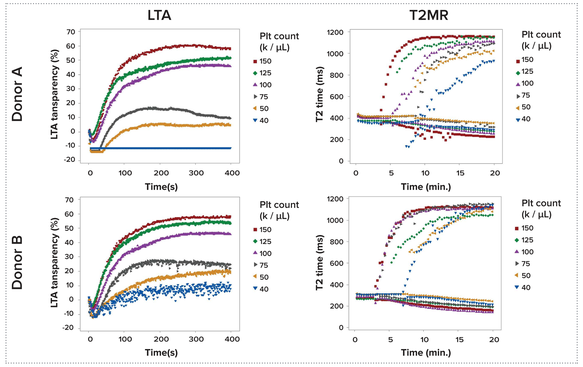

Figure 5: Measurement of LTA and T2MR at low platelet counts in two donors using 10 µM ADP to activate platelets. LTA was measured in platelet rich plasma diluted with autologous plasma and T2MR in reconstructed whole blood.

CONCLUSIONS

These studies show that the T2MR device detects a range of clinically valuable parameters to provide a rapid and accurate assessment of platelet activity. Further, the T2MR device provided highly accurate results when compared to gold standards methods (100% PPA and 100% NPA). In previous studies, the T2MR device had predicted thrombotic events otherwise missed by established diagnostic methods.[3] Clinical-lab quality measurements could impact patient management by providing rapid, comprehensive coagulation data at point of care. Future studies will include assessment of these T2MR tests in the intent to treat clinical patient populations. Advantages to T2MR device include:

- High accuracy: T2MR requires 40 µL of whole blood and provides a quantitative measure of platelet activity that correlates closely with the results of LTA using "activated" and "inhibited samples" (100% PPA and NPA).
- Rapid results: Turn-around times with T2MR are shorter than with LTA (20 minutes vs. 3 hours).
- Ease of use: T2MR measurements require less laboratory expertise than LTA, since they are a true mix-and-read measurement using whole blood.
- High sensitivity: Using ADP and its antagonists to study platelet activation, T2MR showed dependence on P2Y1 signaling, suggesting the potential importance of evaluating platelet contraction in whole blood.
  - T2MR detected "recovery" from aspirin earlier than LTA, suggesting more rapid detection to platelet function on T2MR.
  - Platelet activity could be measured by T2MR at platelet counts 100 k/µL and below, supporting the sensitivity of T2MR to residual platelet function and potential applications in which assessment of even weak platelet activity levels is important, such as in thrombocytopenia.
  - These observations suggest that the ADP-effects seen in T2MR are more evenly dependent on both P2Y1 and PY12 signaling than LTA. These results using T2MR are consistent with experiments using genetically engineered mice that have demonstrated in vivo thrombosis formation is substantially (and equally) dependent on both P2Y1 and P2Y12. Whether this has clinical significance in humans will require further study.

In this study, T2MR has demonstrated the rapid and accurate assessment of platelet function on a simple-to-use device. Taken together, the quantitative readout of T2MR in whole blood, close correlation with LTA, and apparent higher sensitivity to platelet activity may open the door to new opportunities in patient management and drug development with T2MR technology. The application of this technology to the diagnosis and monitoring of 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.

Proven Detection Platform
T2MR device relies on proven magnetic resonance technology and has already been successfully implemented in FDA-cleared products including the T2Dx® Instrument and the T2Candida® Panel.

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

References

1. Jae-Lim Choi, Shuhua Li, and Jin-Yeong Han, "Platelet Function Tests: A Review of Progresses in Clinical Application," *BioMed Research International*, vol. 2014, Article ID 456569, 7 pages, 2014. doi:10.1155/2014/456569
2. Angiolillo DJ. Applying Platelet Function Testing in Clinical Practice: What Are the Unmet Needs? *JAMA*. 2011;306(11):1260-1261. doi:10.1001/jama.2011.1349.
3. Skewis, L. R., Lebedeva, T., Papkov, V., Thayer, E. C., Massefski, W., Cuker, A., … & Cines, D. B. (2014). T2 magnetic resonance: a diagnostic platform for studying integrated hemostasis in whole blood—proof of concept. *Clinical chemistry*, 60(9), 1174-1182.
4. Cines, D. B., Lebedeva, T., Nagaswami, C., Hayes, V., Massefski, W., Litvinov, R. I., … & Weisel, J. W. (2014). Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. *Blood*,123(10), 1596-1603.
5. Vinagre, Y. M., et al. (2013). A Clinical Pilot Study to Compare T2MR and VerifyNow P2Y12 Platelet Activity Measurements in Cardiovascular Patients. International Society for Thrombosis and Hemostasis 2013 Amsterdam, NL.

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

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