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Evidence of Clinical Utility: An Investigator Assessment of a Novel Blood-Based Biomarker of Liver Transplant Rejection to Guide Immunosuppression Decisions

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Abstract

Background: We have previously discovered and validated a microarray-based test that analyzes blood gene expression profiles (GEP) as an indicator of immune status in liver transplant recipients with stable liver function.

Methods: In this study, investigators (7 transplant hepatologists) assessed clinical utility of the TruGraf Liver test in patient management. In a retrospective study, simultaneous blood tests and liver function tests (882 serial time points within the first year of liver transplant) were performed in 29 patients at 7 transplant centers.

Results: When queried regarding whether a single initial TruGraf Liver test result impacted their decision regarding patient management, in 455/882 (51.5%) of serial time points, the investigator responded in the affirmative. Of the 455 affirmative responses, nearly 70% were related to the test result supporting a decrease or increase in immunosuppressive therapy. Of the responses that TruGraf liver did not alter care, nearly 40% were related to the need to see the next serial test before modifying patient management. All seven providers (100%) stated the affirmative when asked if this blood test would be useful in general for future liver transplant patient management.

Conclusion: The previously published biomarker is the first non-invasive test to demonstrate clinical utility in assessing immune status of LT recipients with stable liver function and shows promise as a reasonable and necessary tool supporting clinical decisions to personalize immunosuppressive therapy.

Key Words: Clinical utility, Gene expression biomarker, Liver transplantation

Introduction

The survival benefits of solid organ transplants in the United States are well documented (1). Improvements in immunosuppression (IS), better anti-microbial agents, and other aspects of ancillary care have resulted in significant improvements in short-term outcomes; however, there has been little improvement in long-term graft survival, particularly in liver transplant (LT).

Importantly, longer-term transplant outcomes are limited in large part by the very key factor that made LT successful in the first place: the lifetime need for IS. Despite incremental improvements in both efficacy and effectiveness of IS drugs over the past 3 decades, their long- term sequelae include, but are not limited to a higher incidence of life-threatening opportunistic infections, cancer, chronic kidney disease, metabolic disorders, cardiovascular disease, and osteoporosis. In order to mitigate the risk of their use, most clinicians actively minimize IS medications as the interval from the time of LT increases. However, other than clinician judgement, there are currently no reliable objective tools to help inform IS reduction. IS drug levels are poorly associated with efficacy, and therapeutic windows were largely targeted to limit severe toxicity. Even with that guidance, clinicians are generally required to use their own clinical experience to individualize each patient's therapeutic level to avoid adverseeffects.

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Current best practices in the management of IS following LT include 1) routine pre- emptive reduction of IS over time to prevent IS complications, and 2) therapeutic reduction of IS in response to specific IS complications. In either situation, current practice is to minimize IS over time while monitoring liver function tests (LFTs) closely. In the event of a rise in LFTs as a result of IS minimization, the immediate response is to perform an invasive liver biopsy for presumed Acute Cellular Rejection (ACR) and, if diagnosed, treat the ACR episode with high dose corticosteroids and escalation of IS until LFTs normalize and for an undetermined period of time afterwards. Occasionally escalation of IS is performed without a liver biopsy, but this is becoming less common. Either way, escalation of IS is associated with significant complications, particularly related to infections and the risk of other short and long term IS complications. As a result of this, there is significant evidence that treatment of ACR is associated with worse graft and patient survival, putting clinicians into a difficult circuitous predicament.

Therefore, developing, validating and ultimately integrating biomarkers into clinical practice for the purpose of informing decisions, specifically regarding IS reduction in the management of LT recipients, would be extremely welcome by both clinicians and their patients. More specifically, enhanced information regarding the immune status of the patient, i.e., adequately immunosuppressed (immune quiescent - IQ) versus not adequately immunosuppressed (immune activation - IA), would potentially inform and guide a decision to reduce IS, whether pre-emptively or therapeutically.

The Transplant Genomics TruGraf Liver blood test uses DNA microarray technology that measures differentially expressed genes in the blood of stable liver transplant recipients to determine whether a patient's blood gene expression profile is similar to that obtained from one reference population versus another. A negative test classified as Transplant eXcellence (TX) correlates with IQ, whereas a positive test, reported as Acute Rejection (AR) correlates with IA. Thus, this minimally invasive biomarker can be used to reassure clinicians that IS reduction is less likely to trigger ACR if the test is

negative, or more likely to trigger ACR if the test is positive, thereby, guiding, informing, and supporting management decisions for both pre- emptive and therapeutic IS reductions. TruGraf Liver provides clinicians with actionable information that will be combined with standard assessments to allow IS adjustments proactively instead of reactionary.

The development and validation of the TruGraf Liver test was based on blood gene profiling paired with biopsy samples consisting of ACR (elevated LFTs) following LT compared with blood samples of patients with stable LFTs and no clinical evidence of ACR (2). The biomarker was developed and validated using these 2 phenotypes. Moreover, serial samples collected prior to the development of the ACR phenotype also exhibited a positive TruGraf test result consistent with ACR. Conversely, samples collected following successful treatment of ACR with normalization of LFTs demonstrated conversion to a negative TruGraf test result (TX).

Crucially, the TruGraf Liver test was developed and validated as a 'rule out' test to provide clinicians with a high level of confidence that the patient is IQ versus IA. Thus, if the test result is negative (TX) which correlates with IQ, the clinician may feel more comfortable proceeding with IS reduction with a high level of confidence that the patient is in an IQ state. In contrast, if the test result is positive, the clinician may choose to not reduce IS, or to reduce it more cautiously. In addition, the clinician can repeat the test after IS reduction to monitor the patient's phenotype and potentially avoid the onset of ACR. Stated a different way, a finding of the LT recipient being adequately immunosuppressed (immune quiescent - IQ) versus not adequately immunosuppressed (immune activation - IA), would help inform and guide a decision to reduce IS, whether pre-emptively or therapeutically.

We have developed TruGraf Liver for LT recipients such that modifications to lower (TX result) immunosuppressive therapy could be guided by the TruGraf Liver test instead of clinical 'guessing' – the current approach. This innovative approach has the potential to personalize the treatment of LT recipients to improve outcomes. However, before TruGraf Liver is readily available as a commercial test, we believe that it would be important determine the clinical utility of the test and further understand if liver transplant providers would use the test in managing patients and in what circumstances. Similar studies for other diagnostic tests have investigated the performance of a test in clinical practice and performed a retrospective evaluation of its impact on treatment decisions, there by demonstrating clinical utility after establishing the clinical validity.

To this end, we administered a retrospective survey to transplant hepatologists that participated in the CTOT-14 clinical study that generated the analytical and clinical validity of the TruGraf Liver test. The goal of this survey was to determine whether the results of serial TruGraf Liver tests would have affected or informed their clinical decision and management of LT recipients, particularly in guiding modifications of immunosuppressive therapy to reduce complications of over- or under-immunosuppression. Furthermore, while the context of use for IS minimization was not included in the CTOT-14 directive, we demonstrate that the information provided by TruGraf Liver would have played a significant role in patient management and remove the 'trial and error' approach that physicians often have to use when determining standard care for IS reduction. Overall, when paired with the clinical validity of the test as determined by the CTOT-14 study, we demonstrate that the results presented here firmly provide strong evidence for the clinical utility of TruGrafLiver.

Materials & Methods

The Northwestern University Institutional Review Board approval was obtained prior to commencement of the research. Transplant hepatology physicians at seven large academic LT centers were asked to complete a one-time survey via email. To better support the physicians in their assessments, they were also provided with the recent AJT publication describing the performance of the TruGraf Liver in distinguishing AR vs. TX in the serial management of recipients (2). Informed consent was required at the start of the survey to indicate their willingness to proceed with the study survey. In the survey, the participants were presented with a series of actual patient case studies which were created using de-identified data from a completed study (CTOT-14, NCT01672164), including results for liver function and TruGraf Liver tests over the course of the first-year post-transplant. The liver function results were required to be within pre-defined normal range: Total Bilirubin (TB) ≤1.5 mg/dL and Direct Bilirubin (DB) <0.5 mg/dL, Alkaline Phosphatase (AP) ≤200 U/L, and Alanine Transaminase (ALT) ≤60 U/L (males) ≤36 U/L (females). Participants were asked a series of questions regarding how they would treat the patient at each post-transplant timepoint based on the simultaneous liver function and TruGraf Liver test results.

Table 1 shows the basic structure of the questionnaire. Full detail of the survey is included in Supplemental Information. Once the participant completed the survey, their participation was complete. The survey was administered via REDCap and final data were assembled after the surveys were completed.

Table 1. Survey Questions.

Case Study-N Question 1 "Would the TruGraf test result modify your decision on how to manage the patient at this time point?"	
Time Point1	Yes/No
Time Point2	Yes/No
Time Point3	Yes/No
Time Point4	Yes/No
Time Point5	Yes/No
Case Study N – Question 2 (If "Yes" to Question 1 for each Time Point) Please select the most appropriate answer for each time point (1-5). "The TruGraf test result and laboratory tests indicate the patient…."	
1. Is stable and no intervention isneeded	
2. Is stable and support a decrease in immunosuppressiontherapy	
3. Has signs of acute rejection and support an increase in immunosuppressiontherapy	
4. Has signs of acute rejection and needs a liverbiopsy	
Case Study N – Question 3 (If "No" to Question 1 for each Time Point) Please select the most appropriate answer for each time point (1-5). "Why would you not use the TruGraf result in managing the patient?"	
1. The result conflicted with my clinical opinion on the patient'sstatus	
2. The result did not change my opinion on how to manage thepatient	
3. I wanted to see the next test result before I make my managementdecision	
Final Summary Question	
"From all of these cases, taken together, do the TruGraf results encourage you to use the test in future liver transplant patient management?"Yes/No	

Footnote:
TruGraf Liver described in the paper = TruGraf test in the survey

Descriptive statistics are reported in the results of this manuscript.

Results

Seven liver transplant providers completed the survey of 29 cases, each with up to 5 data points. Overall, 882 time points were evaluated by the providers by answering Question 1 and 2 (if "yes" to Question 1) or Question 1 and 3 (if "no" to Question 1). In 455 situations (51.59%), the respondents indicated that the TruGraf Liver test result modified their decision in regard to patient management (Question 1 = "yes"). This ranged from 34.9% to 78.6% among individual respondents.

To better understand how TruGraf Liver impacted the clinical management of IS therapy, we looked further into the situations where the provider indicated the TruGraf Liver test supported an adjustment to IS therapy. Almost 70% of respondents showed that TruGraf Liver results supported the physicians' change in IS therapy in either direction (decrease (39%) of IS or increase (29%) of IS). The remainder of respondents, while they did not adjust the IS therapy, stated the TruGraf Liver result was supportive because it confirmed stability and therefore no intervention (15%) was necessary or demonstrated a state of IA and signs of acute rejection therefore, prompting a liver biopsy (15%).

Interestingly, in situations where the TruGraf Liver result did not alter the physicians' course of action, 37.7% of the time respondents still indicated they wanted to see the next test result before making another management decision. This was most common in early post-transplant timepoints, suggesting inherent value in repeat testing and monitoring TruGraf Liver results as a function of time. In addition, the TruGraf Liver result conflicted with clinical opinion in less than 20% (19.44%) of overall responses in which the result was not considered helpful. The majority of respondents found TruGraf Liver results helpful in guiding the management of LT patients, the remainder usually wanted to see another test, or the result did not change their managementopinion.

At the end of the survey, after reviewing all time points of the 29 cases, all seven respondents reported "yes" that the TruGraf Liver results encouraged them to use the test in future liver transplant patient management and support of IS therapy adjustments.

Discussion

The generally accepted definition of clinical utility for an assay is that the results of the assay lead to a clinical decision that has been shown with a high level of evidence to improve patient outcomes (3). Often the demonstration of clinical utility is commonly a two-step process designed to show the clinical validity first (that the test provides accurate diagnostic information) and second, that the diagnostic information, when used in managing patients, helps physicians to improve patient outcomes when compared to current standard of care. In general, an assay may be considered useful if its results are actionable, provoking a treatment decision that leads to a better outcome. In organ transplantation, numerous studies have demonstrated that the development of body fluid (blood, urine) and tissue-based biomarkers correlates with patient outcomes (i.e. rejection events). Some have focused on diagnosing rejection to avoid invasive biopsies or improve diagnostic accuracy , while others have developed serial biomarkers to predict rejection (4-7). Additionally, the 'quiescent state' of healthy transplant function ("TX"), as we have examined in both liver and kidney recipients , is also an important signature when considering the potential for using such markers in IS optimization.

As previously mentioned, the management of LT recipients includes a lifetime provision of IS as well as lifelong monitoring of the graft and the potential life-threatening complications of IS. If the natural allo-immune response of the recipient to the graft is not managed and controlled by IS, ACR will inevitably cause graft injury (allo-immune inflammation), graft dysfunction, graft failure, and ultimately result in graft loss. Given the lack of a liver support machine like dialysis for kidneys, graft loss will result in patient death unless a successful re- transplant is performed. And yet, outcomes of liver re-transplants are associated with significantly worse outcomes than primary transplants (6). Moreover, ACR, even if treated successfully, is also associated with worse graft and patient outcomes (1). In addition, IS agents are associated with potential life-threatening complications in LT recipients, not only as a result of their immunosuppressive action, but also as a result of other side-effects inherent to each IS drug. Therefore, in order to achieve successful short- and long-term LT outcomes, it is essential to maintain, to the extent possible, a perfect immune balance between the recipient's immune system and the presence of an allograft. In other words, it is imperative to maintain a LT recipient not only on the right dose of IS drugs, but also on the right combination of drugs to allow for adequate IS (sufficient IS to maintain a state of IQ and avoid a state of IA that would lead to ACR), while at the same time being constantly concerned about a state of over- immunosuppression that could potentially lead to the life-threatening complications.

There are currently no monitoring strategies designed to overcome the limitations inherent to monitoring LT recipients outlined above. The CTOT-14 multi-center NIH sponsored clinical trial (NCT01672164) was specifically designed to validate a peripheral blood signature in LT recipients that correlates with clinical phenotypes of ACR versus no ACR. Previous work had led to the development of such a molecular profile but there was a need to validate the profile in an external cohort of patients and test the predictive value of these molecular signals in stable LT recipients with normal LFTs preceding documented episodes of biopsy-proven ACR. Thus, the development and validation of the TruGraf Liver test are the result of scientific evidence based on blood gene profiling paired with biopsy samples reflecting ACR (elevated LFTs) following LT compared with blood samples of patients with stable LFTs and no clinical evidence of ACR.

This retrospective study strongly demonstrates the clinical utility for the TruGraf Liver assay in assessing immune status in patients with stable liver function and in supporting clinical decisions to reduce or augment IS therapy. In over 50% of the time points, the survey respondents reported the TruGraf Liver result (either AR or TX) would support their management decision compared to just using the liver function test results alone, which is the current practice. The majority also reported that the main reason the test helped was that it supported decreasing or increasing immunosuppression therapy. These were all situations in which the liver function tests were in a stable range therefore, these clinicians would likely not have modified immunosuppression if not for the TruGraf Liver result. The potential here is to provide additional clinical information for decision-making to reduce immunosuppression complications more proactively (by using the test to guide IS reductions) as well as minimize the acute rejection ahead of time (by using the test to guide appropriate IS increases).

Interestingly, when the providers reported the TruGraf Liver did not alter their preferred standard of care and chose to not adjust the IS therapy at a particular time point, nearly 40% reported wanting to see the next TruGraf Liver result before making a management decision. This phenomenon decreased over time in conjunction with an increase in providers reporting the test supported IS modifications and alterations, suggesting that there was a learning curve with more experience and more serial test results over time. Indeed, transplant clinicians are accustomed to monitoring the trend of laboratory tests such as liver function, kidney function, and serum IS trough levels, rather than react to single time point results. However, the vast majority of affirmative responses resulted in an immediate action (decrease or increase immunosuppression or perform a liver biopsy), rather than waiting for the next test. Most importantly, all respondents felt that their experience using the TruGraf Liver test in this survey encouraged them to utilize it for future LT recipients. Thus, our recent publication and this clinical utility survey represent an advance toward commercializing the TruGraf Liver test for use in liver transplant recipients, similar to the TruGraf blood test for kidney transplant recipients.

The study is limited by the fact that these were historical LT cases and not prospectively managed patients. Also, TruGraf Liver is a new test, and the clinicians did not have prior experience with the test before the survey. This

might not accurately estimate the changes in patient management once the test is fully integrated into clinical practice. We plan to perform interventional studies in which actual clinician decisions are made based on the TruGraf Liver test result provided in real time, and opinions on the test and outcomes are assessed.

In summary, the TruGraf Liver test was developed and validated as a 'rule out' test to provide clinicians with a high level of confidence that the patient is immune quiescent (IQ) versus immune activated (IA). Thus, if the test result is negative (TX) which correlates with IQ, the clinician may feel more comfortable proceeding with IS reduction with a high level of confidence that the patient is in an IQ state. In contrast, if the test result is positive, the clinician may choose to not reduce IS, or to reduce it more cautiously. In either case, the patient can be subsequently monitored to inform on persistent IA or a transition back to IQ. Stated a different way, a finding of the LT recipient being adequately immunosuppressed (immune quiescent - IQ) versus not adequately immunosuppressed (immune activation - IA), would inform and guide a decision to reduce IS, whether pre-emptively or therapeutically. Based on this survey, the clinical utility of the TruGraf Liver test is very clear and firmly established: when comparing blind 'trial and error' and reactive standard of care practices, TruGraf provides insight and valuable reassurance to clinicians to allow a more proactive and personal touch in optimizing patient and graft health.

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References

1. Rana A, Gruessner A, Agopian VG, Khalpey Z, and RiazIB, et al. "Survivalbenefit of solid-organ transplant in the united states". *JAMA Surg*. (2015): 150(3), 252-9.
2. Muller BM, Keil E, Lehmann A, Winzer KJ, and Richter-Ehrenstein C, et al. "The Endopredict gene-expression assay in clinical practice - performance and impact on clinical decisions". *PLoS One*. (2013): 8(6), e68252.
3. Levitsky J, Asrani S, Schiano T, Moss A, and Chavin K, et al. "Discovery and validation of a novel blood-based molecular biomarker of rejection following liver transplantation". *Am J Transplant*. (2020).
4. Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, and Deng MC, et al. "Gene- expression profiling for rejection surveillance after cardiac transplantation". *N Engl J Med*. (2010): 362(20), 1890-900.
5. Modena BD, Kurian SM, Gaber LW, Waalen J, and Su AI, et al. "Geneexpression in biopsies of acute rejection and interstitial fibrosis/ tubular atrophy reveals highly shared mechanisms that correlate with worse long-term outcomes". *Am J Transplant*. (2016): 16(7), 1982-98.
6. Kurian SM, Williams AN, Gelbart T, Campbell D, and Mondala TS, et al. "Molecular classifiers for acute kidney transplant rejection in peripheral blood by whole genome gene expression profiling". *Am J Transplant*. (2014): 14(5), 1164-72.
7. Mengel M, Sis B, Kim D, Chang J, and Famulski KS, et al. "The molecular phenotype of heart transplant biopsies: relationship to histopathological and clinical variables". *Am J Transplant*.(2010):10(9), 2105-15.
8. Sellares J, Reeve J, Loupy A, Mengel M, and Sis B, et al. "Molecular diagnosis of antibody-mediated rejection in human kidney transplants". *Am J Transplant*. (2013): 13(4), 971-83.
9. Li L, Khatri P, Sigdel TK, Tran T, and Ying L, et al. "A peripheral blooddiagnostic test for acute rejection in renal transplantation". *Am J Transplant*. (2012): 12(10), 2710-8.
10. Suthanthiran M, Schwartz JE, Ding R, Abecassis M, and Dadhania D, et al. "Urinary-cell mRNA profile and acute cellular rejection in kidney allografts". *N Engl J Med*. (2013): 369(1), 20-31.
11. Muthukumar T, Dadhania D, Ding R, Snopkowski C, and Naqvi R, et al. "Messenger RNA for FOXP3 in the urine of renal-allograft recipients". *N Engl J Med*. (2005): 353(22), 2342-51.
12. Li B, Hartono C, Ding R, Sharma VK, and Ramaswamy R, et al. "Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine". *N Engl J Med*. (2001): 344(13), 947-54.
13. Shaked A, Chang BL, Barnes MR, Sayre P, and Li YR, et al. "An ectopically expressed serum miRNA signature is prognostic, diagnostic, and biologically related toliver allograft rejection". *Hepatology*. (2017): 65(1), 269-80.
14. Friedewald JJ, Kurian SM, Heilman RL, Whisenant TC, and Poggio ED, et al. "Development and clinical validity of a novel blood-based molecular biomarker for subclinical acute rejection following kidney transplant". *Am J Transplant*. (2019): 19(1), 98-109.
15. First MR, Peddi VR, Mannon R, Knight R, and Marsh CL, et al. "Investigator sssessment of the utility of the trugraf molecular diagnostic test in clinical practice". *Transplant Proc*. (2019): 51(3), 729-33.

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