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Use of immune function test in monitoring immunosuppression in liver transplant recipients

Te HS, Dasgupta KA, Cao D, Satoskar R, Mohanty SR, Reau N, Millis JM, Jensen DM. Use of immune function test in monitoring immunosuppression in liver transplant recipients.

Abstract: Immune function test (Immuknow^{TM}) is a measure of cell-mediated immunity based on peripheral CD4^{+} T cell adenosine triphosphate activity (desired range, 225–525 ng/mL). We evaluated the role of immune function test (IFT) in monitoring and adjustment of immunosuppression in orthotopic liver transplant (OLT) recipients. A total of 289 IFTs were obtained from 171 patients from March 2007 to June 2008. Graft/patient status was classified as stable, serious infection, or malignancy. IFT levels were analyzed with duration of follow-up after OLT, graft/patient status, and the presence of hepatitis C (HCV) infection. The mean age was 54 ± 14 yr, with 62% men. The median follow-up was 65 (2–249) months. Mean IFT levels were significantly lower in patients who were <24 months than in those ≥ 24 months post-OLT (220 ± 19.5 vs. 257 ± 11.3 ng/mL, p = 0.03). Clinically stable patients had higher IFT levels than those with serious infection or malignancy (254 ± 11.1 vs. 162.5 ± 23.9, p < 0.001). HCV-infected patients had lower IFT levels than uninfected patients (206.7 ± 15.7 vs. 273 ± 12.0 ng/mL, p < 0.001). Immunosuppression was reduced in 58 patients with IFT levels <225 ng/mL, and 90% maintained stable graft function after a median follow-up of 22 (1–39) months. IFT may be a useful tool in monitoring and lowering of immunosuppression in long-term OLT recipients.

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Key words: immune function test – immunosuppression – liver transplantation

Abbreviations: IFT immune function test; OLT orthotopic liver transplantation; HCV hepatitis C; CSA cyclosporine; TC tacrolimus; LCTs liver chemistry tests; PHA phytohemagglutinin-L; CMV cytomegalovirus

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The growing number of long-term survivors of orthotopic liver transplantation (OLT) has prompted liver transplant professionals to take measures to improve the well-being of such patients by tailoring immunosuppressive regimens to minimize complications. Currently, most immunosuppressive regimens include either cyclosporine (CSA) or tacrolimus (TC). Initially given in higher doses for induction, these medications are slowly tapered to minimize adverse effects while preventing graft rejection. Nonetheless, side effects still occur despite lowered doses and may require aggressive therapy in some cases.

Monitoring of immunosuppression in OLT patients is routinely performed with periodic measurements of serum liver chemistry tests (LCTs) and serum immunosuppressive drug levels when applicable. However, responses to immunosuppressive drugs vary among individual patients. In fact, patient-specific factors such as age, weight, comorbidities, and race are known to affect the choice of and response to immunosuppressive agents. Determination of individual responses to immunosuppression by a clinical test has not been feasible until recently, when the immune function test (IFT, Immuknow^{TM},

Cyclex Incorporated, Columbia, MD, USA) was introduced (1).

Immune function test was approved by the US Food and Drug Administration for detection of cell-mediated immunity in an immunosuppressed population. It measures the response of T-helper lymphocytes to mitogenic stimulation by phytohemagglutinin-L (PHA) in vitro by quantitating the amount of adenosine triphosphate produced by $CD4^+$ T cells following stimulation (1). Immune responses are classified as strong (ATP $\geq$ 525 ng/mL), moderate (225–524 ng/mL), or low ($\leq$225 ng/mL) (2). A strong immune response is associated with a higher risk for graft rejection, while a low immune response is associated with a higher risk for infection (1). The intersection point of the odds ratio curves for infection and rejection is in the moderate immune response zone at 280 ng/mL. These ranges were determined from a combined pool of kidney, liver, heart, and small bowel recipients who were all within three yr of transplantation, with majority of the population (75%) being within the first year of transplantation (1). Whether these ranges hold true for longer-term OLT recipients is not known.

The primary aim of this study was to evaluate the role of IFT as an adjunct tool to immunosuppressive drug levels in monitoring and adjustment of immunosuppressive drug dosages in OLT recipients. The secondary aims included the following: (i) to compare the IFT and immunosuppressive drug levels of OLT recipients who are <24 months from OLT and those who are $\geq$24 months out from OLT; (ii) to compare the IFT levels of patients with stable graft function and patient status vs. those with serious infection or malignancy, (iii) to compare the IFT levels of patients with recurrent hepatitis C (HCV) vs. those without HCV, and (iv) to evaluate the utility of IFT as a guide in reduction in immunosuppression. If found useful, IFT may allow for tailoring of immunosuppression drug regimens at the cost of a low risk for graft rejection to decrease the risk for immunosuppression-related complications in OLT recipients.

Patients and methods

Liver transplant recipients who have had random IFT levels drawn and processed at the University of Chicago Medical Center (UCMC) from March 1 2007 to June 30 2008 were retrospectively identified from the UCMC Liver Transplant Program Database. Demographic characteristics such as age, gender, and race, and clinical data including indication for transplantation, duration of post-OLT follow-up, most current patient status and graft function, most current immunosuppressive agents, and complications in terms of serious infection (defined as the presence of an opportunistic infection or severe recurrent HCV [e.g., with clinical sign of jaundice or fibrosing cholestatic hepatitis]) or malignancy within six months were noted. Laboratory data such as IFT levels, serum LCTs, and serum immunosuppressive drug levels were also obtained. Blood samples for IFT and serum trough TC or CSA levels were drawn at the same time.

Patients included in this study received their OLT from 1987 to 2008, a span of 21 yr in which various immunosuppression protocols have been adopted at different time points within our program, except for the use of corticosteroids that has been a mainstay in the initial immunosuppression protocol throughout these two decades. In general, CSA and azathioprine (AZA) were the immunosuppressive regimen utilized in the late 1980s to early 1990s and were replaced with TC in the later 1990s to present, with the addition of mycophenolic acid or sirolimus when adverse effects from TC require lowering of its dose. Induction therapy was administered in some patients in whom renal dysfunction required lower TC dosing in the immediate post-transplant period. Corticosteroids were weaned within the first year following OLT in most cases, and CSA and TC doses were lowered at 6–12 months following OLT. However, immunosuppression may have been altered at any point in the follow-up period as mandated by graft function such as rejection or adverse effects. Hence, patients' long-term immunosuppression regimen may differ significantly from the initial regimen that was started immediately post-OLT.

Immune function test levels were analyzed according to duration of follow-up from OLT (<24 months from OLT vs. $\geq$24 months from OLT), patient and graft status, age, gender, race, and indication for OLT (HCV vs. other indications). Twenty-four months was arbitrarily chosen as a cutoff to distinguish shorter-term (<24 months) and longer-term ($\geq$24 months) OLT recipients, because immunosuppression dosages are typically lowered after the first year of OLT; thus, dosages should be at stable, long-term maintenance levels by 24 months following OLT. A correlation between IFT levels and serum TC levels for patients on TC monotherapy was also evaluated. Patients who had reductions in immunosuppressive dosages following the measurement of IFT levels were identified, and graft functions on subsequent follow-ups were noted.

Statistical analysis

Continuous variables were expressed as means and standard errors or as medians and ranges, while discrete variables were expressed in percentages. For patients with multiple IFT and LCT measurements, only the first ones were used for descriptive statistics. Data were analyzed using generalized estimating equation (GEE) (3) models that can account for correlation between multiple measurements within patients and using Stata 10 (Stata Corp LP, College Station, TX, USA). The study was approved by the institutional review board of the UCMC.

Results

A total of 289 IFTs were obtained from 171 patients (mean, 1.69 per patient; range, 1–6 IFTs per patient) within a median of 65 months (range, 2–249 months) from the time of OLT. Most patients (n = 128, 75%) were at least 24 months out from OLT. All but seven patients received cadaveric grafts.

Demographic data

The mean age of the OLT recipients was 54 ± 14 yr, with 106 (62%) men. There were 108 (63%) Caucasians, 28 (16%) African Americans, 24 (14%) Hispanics, and 11 (7%) Asians or other races. These demographic characteristics in terms of age (53 ± 13 vs. 55 ± 14 yr, p = 0.21),% African Americans (12% vs. 18%, p = 0.11), and% HCV infection (44% vs. 38%, p = 0.21) were similar among those who were within 24 months from OLT and those who were $\geq$24 months out. The most common indication for OLT was HCV-related cirrhosis (39%), followed by alcohol-related cirrhosis and cryptogenic cirrhosis (10.5% each), primary biliary cirrhosis and primary sclerosing cholangitis (6.4% each), and fulminant hepatic failure (5.8%). Hepatocellular carcinoma cases were classified according to the underlying liver disease unless no underlying liver disease was identified.

The median serum liver chemistry test values were as follows: albumin, 4.1 mg/dL (range, 2.6–5.1); total bilirubin, 0.6 mg/dL (range, 0.1–16.3); alkaline phosphatase, 107 IU/mL (range, 38–1320); alanine aminotransferase, 29 IU/mL (range, 12–429); and aspartate aminotransferase, 32 IU/mL (range 8–484).

Immune function tests

The mean IFT level for patients with OLT duration <24 months (n = 43) was lower than for those with OLT duration $\geq$24 months (n = 128) (220 ± 19.5 vs. 257 ± 11.3 ng/mL, p = 0.03), representing a weaker immune response (Fig. 1). In the group with OLT duration $\geq$24 months alone, 41% of patients had IFT levels <225 ng/mL or within the low immune response zone (Fig. 2). Patients who had stable graft function status (n = 137) had a higher IFT level than those with serious infection or malignancy (n = 20) (254 ± 11.1 vs. 162.5 ± 23.9, p < 0.001), representing a stronger immune response (Fig. 3A). This difference remains statistically significant even when the presence of HCV and OLT duration were controlled for (p = 0.003). Serious infections (fungal and mycobacterial infections and severe recurrent HCV) occurred within $\leq$14 months of OLT in 6/8 patients, while malignancies (skin cancers, lymphoma, breast cancer, hepatocellular carcinoma, tongue cancer, and myelodysplastic syndrome) were diagnosed $\geq$58 months from OLT in 13/14 patients.

Patients with HCV infection (n = 67) had lower IFT levels than those without (n = 104) (206.7 ± 15.7 vs. 273 ± 12.0 ng/mL, p < 0.001), representing a weaker immune response in HCV-infected patients (Fig. 3B). There was no relationship between age, gender or race, and IFT levels.

Immunosuppressive agents

Majority of the patients (n = 127, 74%) were on TC, 21% (n = 36) were on CSA, and a minority was on a calcineurin-sparing regimen. Forty-one percent (n = 70) were on TC monotherapy, while only 12% (n = 20) were on CSA monotherapy. There were more patients taking TC within the first

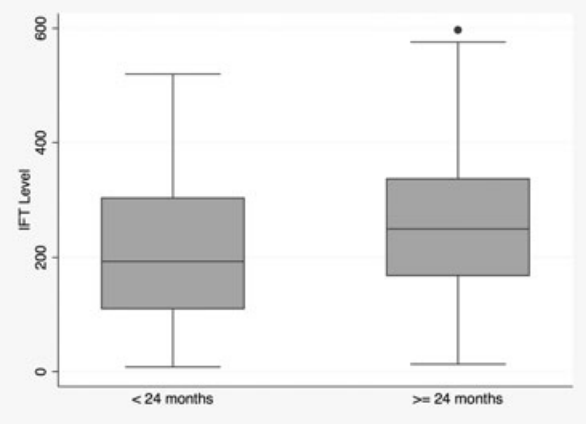

Fig. 1. Immune function test levels (mean ± SE) and duration from orthotopic liver transplantation. Mean immune function test level was at 220 ± 19.5 ng/mL in patients who were within 24 months vs. 257 ± 11.3 ng/mL in those who were at least 24 months out (p = 0.03).

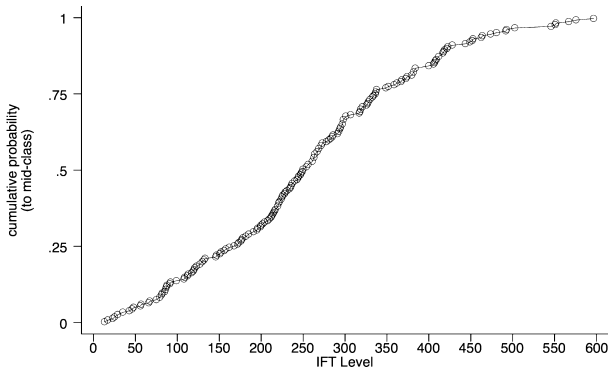

Fig. 2. Immune function test level distribution for orthotopic liver transplantation recipients who are at least two yr out from transplant. Of this population, 41% of patients had immune function test levels <225 ng/mL and may be over-immunosuppressed.

two yr after OLT than those who were at least two yr out (95% vs. 68%, $p < 0.0001$). Conversely, there were fewer patients taking CSA within two yr of OLT than those farther out from OLT (26% vs. 5%, $p < 0.0001$). Other immunosuppressive agents were used at similar rates in both groups (48% vs. 53%, $p = 0.31$). Approximately half of the patients who were farther out from OLT were on more than one immunosuppressive agent (Table 1). There was no correlation between the number of immunosuppressive agents and the IFT levels in this subgroup.

There was no correlation between the serum TC levels and the IFT levels of patients on TC monotherapy ($n = 70$) (Fig. 4). Despite similar TC dosages at 5 ± 3 mg/d ($n = 20$) and 4 ± 3 mg/d ($n = 50$) in both groups ($p = 0.30$), trough serum TC levels were higher in patients within 24 months of OLT than those who were farther out from OLT (7 ± 3 vs. 5 ± 2 ng/mL, $p = 0.03$). However, the IFT levels were not statistically significant between the two groups (211 ± 117 vs. 250 ± 124 ng/mL, $p = 0.23$).

Adjustments in immunosuppression

There were 136 samples drawn from 83 patients with IFT levels <225 ng/mL; a third of these patients were within 24 months post-OLT. From this pool, immunosuppression was reduced in a stepwise fashion in 58 patients in response to each of 65 IFT levels. In these 58 individuals, immunosuppression reduction was performed within two wk in response to the first IFT level when an indication such as severe infection or malignancy ($n = 8$) or drug-related adverse event was present, or in response to two consecutive IFT levels <225 ng/mL in patients who had stable graft function and status. Tacrolimus levels were reduced by 1 mg/d increments (0.5 mg increments when the baseline dose was at 2 mg/d or lower), whereas CSA levels were reduced by 25 mg/d increments, with the goal of increasing IFT to >225 ng/mL. Attempts to lower or discontinue prednisone, mycophenolic acid, or sirolimus dosages were made first in patients who were taking these immunosuppression, if adverse effects of calcineurin inhibitors were not the main indication for the reduction.

Repeat IFT levels following reduction in immunosuppression were significantly higher than the baseline levels (227 ± 109 vs. 134 ± 58 ng/mL, $p < 0.0001$). Reduction in immunosuppression led to elevations in LCTs above normal or >20% above baseline levels in six patients (10%), all of whom were within 24 months post-OLT except for one, while the rest continued to maintain stable graft function on the lower immunosuppressive dosages after a median follow-up of

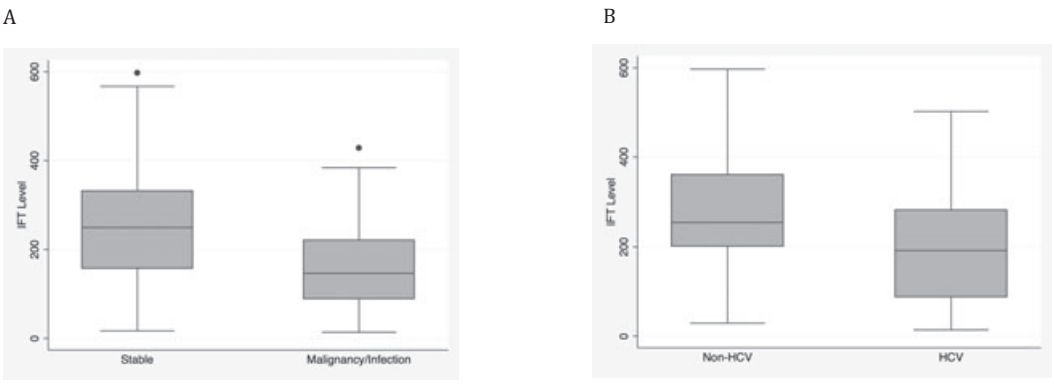

Fig. 3. (A) Immune function test levels (mean ± SE) and patient/graft status. Mean immune function test (IFT) level in stable orthotopic liver transplantation (OLT) recipients was at 254 ± 11.1 vs. 162.5 ± 23.9 ng/mL in OLT recipients with malignancy or serious infection ($p < 0.001$). (B) Immune function test levels and disease etiology. Mean IFT level in hepatitis C (HCV)-infected recipients was at 206.7 ± 15.7 vs. 273 ± 12.0 ng/mL in non-HCV-infected recipients ($p < 0.001$).

Table 1. Number of immunosuppressive agents taken by liver transplant recipients >24 months from orthotopic liver transplantation (n = 128)

No of immunosuppression	n (%)
Single immunosuppression	66 (51.6)
Double immunosuppression	60 (46.9)
Triple immunosuppression	2 (1.5)

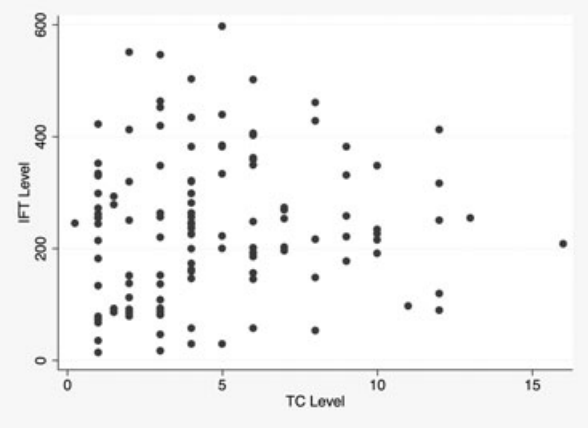

Fig. 4. Immune function test levels and serum tacrolimus (TC) levels. There was no correlation amongst the immune function test levels and serum TC levels for patients who were on TC monotherapy (p = 0.18).

23 months (range, 1–35 months). When reduction in immunosuppression was performed within 24 months post-OLT, 5/22 patients (23%) developed elevated LCTs, whereas only 1/36 patients (3%) who were $\geq$24 months had the adverse result (p = 0.0001) (Table 2). Of all six patients who developed elevated LCTs, liver biopsies showed hepatic steatosis in one and recurrent HCV in another, and a third was diagnosed with biliary strictures. No liver biopsies were performed in the other three, but the LCT elevations resolved in response to increased immunosuppression in all of them, two of whom received OLT for primary sclerosing cholangitis and autoimmune hepatitis, respectively.

Table 2. Reduction in immunosuppression in liver transplant recipients with immune function test (IFT) level <225 ng/mL according to duration from orthotopic liver transplantation

	<24 months (n = 22)	$\geq$24 months (n = 36)	p Value
Age (yr)	57 ± 12	54 ± 14	0.40
Mean IFT (ng/mL)	127 ± 50	138 ± 63	0.40
Mean follow-up after reduction (months)	6 ± 4	6 ± 4	0.40
No. of patients with increased liver chemistry tests following reduction	5 (23%)	1 (3%)	0.0001

Discussion

The goal of most OLT professionals is to promote good graft function while minimizing adverse effects from immunosuppression. Whereas serum drug levels have served as routine guides in adjustments of immunosuppression, individual responses to the amount of immunosuppressives being administered are highly variable. Genetic polymorphisms that encode for CYP3A and CYP3A5 impact the pharmacokinetics of calcineurin inhibitors (4). Elderly recipients may demonstrate more immune tolerance (5, 6), and body weight and renal function may also affect individual responses to immunosuppression. Ideally, a more reliable means of measuring the balance between alloimmune activation and the immunosuppressive effects of the drugs would be useful to complement the serum immunosuppressive drug levels.

The IFT (Immuknow^{TM}) measures the net immune response via quantitation of ATP release following T-helper lymphocyte stimulation with PHA. Healthy adults were reported to have a mean IFT of 432 ng/mL (median, 408 ng/mL). Recipients of liver, kidney, and pancreas transplants had mean IFT level of 282 ng/mL (median, 259 ng/mL) (p < 0.0001). In adult transplant recipients, immune responses are classified as strong (IFT level $\geq$525 ng/mL), moderate (IFT level = 225–524 ng/mL), or low (IFT level $\leq$ 225 ng/mL) (2).

Low IFT levels have been reported to be frequently associated with infections such as lymphocytic choriomeningitis virus (7, 8), Epstein–Barr virus (7, 9), BK virus (7, 10), cytomegalovirus (CMV), as well as HCV infections in kidney and OLT recipients. In addition, increases in the IFT levels following lowering of immunosuppression and treatment for the viral infection have been observed (7). Pancreas and lung transplant recipients who had either significant infections or post-transplant lymphoproliferative diseases were similarly found to have lower mean IFT levels (11). The correlation between IFT and risk for infection was best demonstrated in a meta-analysis that included 504 kidney, liver, heart, and small bowel recipients, which also led to the establishment of the odds ratio curves for rejection and infection, where the risk of infection was seen to rise more steeply with IFT levels <225 ng/mL (1). Our study confirmed these findings, with lower IFT levels observed in patients with serious infection or malignancy as compared to those with stable graft function. Furthermore, very low IFT levels

(<50 ng/mL) have also correlated with a higher risk of sepsis and death within the first year after OLT (12).

In this study, patients who are <24 months from OLT tend to have lower IFT levels compared with those $\geq$24 months from OLT, suggesting more intense immunosuppression in former group. This is expected, as most OLT recipients undergo gradual tapering of their immunosuppression as they go farther out from OLT. The higher mean IFT level in long-term OLT recipients suggests that long-term OLT recipients can tolerate less immunosuppression while preserving graft function, and the target immune response zones may be different for this patient population.

In the TC monotherapy group, there was no correlation between serum trough TC levels and the IFT levels. Such discordance between trough serum TC levels and IFT levels has been reported by other authors in pediatric OLT recipients (13, 14) and in adult lung transplant recipients (15). Tacrolimus dosages were similar for patients within 24 months from OLT and those beyond 24 months, but trough serum TC levels were higher in those within 24 months. Interestingly, although the mean IFT level was lower in those <24 months from OLT than in those $\geq$24 months, it did not reach statistical significance. This may have been because of a type II error, because there were only a small number of patients who were on TC monotherapy within 24 months from OLT. Nevertheless, the lack of correlation between trough serum TC levels and IFT levels on the plot graph suggests that serum drug levels may not be sufficient in measuring the degree of immunosuppression in individual patients.

Hepatitis C-infected patients have lower IFT levels than those without HCV in our study population, a finding also observed in other studies. Sebastian et al. (16) reported lower median IFT level in patients with HCV reactivation as compared to stable patients (38 vs. 286 ng/mL, $p < 0.01$). Similarly, Mendler et al. (17) noted the mean IFT level to be lower in HCV-positive than in the HCV-negative OLT recipients (151 ± 109 vs. 211 ± 139 ng/mL, $p < 0.0001$). Furthermore, progression of fibrosis in HCV-infected recipients has also been correlated with lower IFT levels (152 vs. 264 ng/mL; $p = 0.008$) (18). The IFT levels in our population are not as low as those found in these studies, and this is likely due to our aggressive protocol of immunosuppression reduction in HCV-infected OLT recipients within the first year from OLT. Immune function test levels have also been reported to distinguish HCV recurrence from acute cellular rejection, with lower IFT levels associated with HCV recurrence (19, 20). It is likely that HCV itself exerts immunosuppressive effects, as immunosuppression is particularly reduced at an earlier time and to lower doses in HCV-infected OLT recipients than in those without HCV. Indeed, a small pilot study reported lower IFT levels in HCV-infected cirrhotic patients as compared to non-HCV-infected patients and healthy controls (mean IFT levels of 127.9 vs. 264.6 vs. 468.9 ng/mL, respectively) (21).

Reduction in immunosuppression can be successful in 90% of OLT recipients with weak immune responses as measured by IFT, and those $\geq$24 months out appear to be at a lower risk for developing increased LCTs. Of the six patients who developed LCT elevations, three cases were proven to be due to non-rejection causes. The other three did not have liver biopsies but responded well to an increase in immunosuppression. Two of the three patients had primary sclerosing cholangitis and autoimmune hepatitis as indications for OLT, suggesting that patients with immune-mediated diseases may not tolerate lowering of immunosuppression as well despite relatively low IFT levels. Of note, 40% of the patients who are at least two yr from OLT receiving conventional immunosuppression management had low immune responses, and majority of these patients may potentially be able to tolerate lowering of their immunosuppression. This will not only lower the risk of adverse effects from the drugs, but it will also lower the financial burden. Finally, it may also bring the possibility of complete discontinuation of immunosuppression closer to reality. Whether or not IFT would be able to reflect allograft-specific T-cell response adequately to allow the identification of OLT recipients who have developed immunotolerance is not known.

We acknowledge the limitations that are inherent in our study because of its retrospective nature and the use of single time point measurements of IFT levels in some patients. Some long-term recipients only had a single IFT level drawn because of the long interval between their routine clinic visits, as the IFT test samples had to be drawn and processed at our center to avoid the variability in test results that can stem from sample storage and shipping. It should also be noted that these long-term recipients have remained on stable dosages of immunosuppression for long periods of time and were expected to demonstrate a stable degree of immunosuppression. However, a single IFT value in time may not necessarily be an accurate determination of the actual status of the patient. We also recognize that target IFT ranges that represent stable graft and patient condition in OLT recipients

may potentially differ among individuals according to variability in individual immunologic constitution and to the time interval since OLT. Despite these limitations, the data presented in this study are intriguing and provide a foundation from which larger prospective longitudinal studies can be designed.

In conclusion, IFT may be a useful tool to monitor and guide the lowering of immunosuppression in long-term OLT recipients. Its utility may even be more magnified in patients with HCV infection, as reduction in immunosuppression while preventing rejection in such cases may potentially ameliorate the progression of HCV disease. The effect of HCV itself on the immune response of OLT recipients deserves further investigation, and prospective studies need to be carried out to evaluate the utility of IFT in improving the outcomes of OLT recipients.

Author contributions

Helen S. Te, MD, is the principal investigator and was involved in research design, data collection, data analysis, and paper writing; Kathleen A. Dasgupta, RN, MSN, collected the data; Dingcai Cao, PhD, analyzed the data; and Dingcai Cao, PhD, Rohit Satoskar MD, Smruti R. Mohanty, MD, Nancy Reau, MD, J. Michael Millis, MD, and Donald M. Jensen, MD, were involved in paper writing.

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