Immunosuppression Modifications Based on an Immune Response Assay: Results of a Randomized, Controlled Trial

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Background. An immune function assay shows promise for identifying solid organ recipients at risk for infection or rejection. The following randomized prospective study was designed to assess the clinical benefits of adjusting immunosuppressive therapy in liver recipients based on immune function assay results. **Methods.** Adult liver recipients were randomized to standard practice (control group; n = 102) or serial immune function testing (interventional group; n = 100) performed with a commercially available in vitro diagnostic assay (ImmuKnow; Viracor-IBT Laboratories, Lee's Summit, MO) before transplantation, immediately after surgery and at day 1, weeks 1 to 4, 6, and 8, and months 3 to 6, 9, and 12. The assay was repeated within 7 days of suspected/confirmed rejection/infection and within 1 week after event resolution. **Results.** Based on immune function values, tacrolimus doses were reduced 25% when values were less than 130 ng/mL adenosine triphosphate (low immune cell response) and increased 25% when values were greater than 450 ng/mL adenosine triphosphate (strong immune cell response). The 1-year patient survival was significantly higher in the interventional arm (95% vs 82%; $P < 0.01$) and the incidence of infections longer than 14 days after transplantation was significantly lower among patients in the interventional arm (42.0% vs. 54.9%, $P < 0.05$). The difference in infection rates was because of lower bacterial (32% vs 46%; $P < 0.05$) and fungal infection (2% vs 11%; $P < 0.05$). Among recipients without adverse events, the study group had lower tacrolimus dosages and blood levels. **Conclusions.** Immune function testing provided additional data which helped optimize immunosuppression and improve patient outcomes.

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Most solid organ transplant recipients require lifelong treatment with potent immunosuppressant medications which reduce the risk of allograft rejection but increase patient morbidity and mortality after long-term use. Commonly reported adverse events associated with immunosuppressive therapy include hypertension (77%), hyperlipidemia (66%), renal toxicity (50%), obesity (40%), cancer (26%), and diabetes mellitus (22%).[1] During the immediate postoperative period, overimmunosuppression is associated with a greater incidence of infection and sepsis. Optimal immunosuppressive therapy balances the risk of rejection caused by an inadequately suppressed immune system and the risk of infection, cancer, and drug toxicity caused by overimmunosuppression. Drug therapy must be carefully tailored to each transplant recipient because of the differences in race, sex, metabolism, multiple drug regimens, and type of allograft transplant.

An immune function assay has been cleared by the U.S. Food and Drug Administration for measuring changes in cell-mediated immunity in solid organ transplant recipients undergoing immunosuppressive therapy (ImmuKnow; Viracor-IBT Laboratories, Lee's Summit, MO).[2] Numerous retrospective and prospective studies have demonstrated the ability of this immune function assay—when used with

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other biomarkers—to identify patients at risk of organ rejection[3–7] and infection[5,8–15] across a range of organs. The assay can also distinguish between rejection and recurrent hepatitis C infection,[16] facilitate conversion from one immunosuppressant to another,[17,18] and may be useful for identifying patients with increased risk of short-term mortality,[19] predicting posttransplant recurrence of hepatocellular carcinoma (HCC)[12] and evaluating immune status of patients with de novo malignancy.[20]

Previously, the role of this immune function test was retrospectively assessed for monitoring and adjustment of immunosuppression in patients after orthotopic liver transplant.[21] Based on those promising results, a prospective randomized study was designed to assess the clinical benefit of adjusting immunosuppressive therapy based on immune function assay results compared to a group managed with the standard clinical practice. The outcome data of this analysis are reported here.

MATERIALS AND METHODS

Study Design

This was a prospective, randomized, parallel, blinded, interventional trial comparing the outcomes of adult liver transplant recipients whose immunosuppressive therapy is managed by previous standard practice (control group) or by adjusting therapy based on cell-mediated immune responses determined by the immune function assay (interventional group). Outcomes were assessed 12 months after transplantation among liver recipients enrolled between July 2008 and March 2013. This study was approved by the local hospital ethics committee.

Patients

Consecutive adult liver transplant recipients not participating in other studies and who provided informed consent were enrolled. There were no exclusions pertaining to the cause of end-stage liver disease (ESLD). To ensure treatment groups were balanced, enrolled patients were randomized 1:1 based on the cause of ESLD, model for end-stage liver disease (MELD) score, the presence of HCC, and sex. A significant number of liver recipients with ESLD secondary to hepatitis C virus (HCV) infection were not included because of a parallel study.

Immune Function Testing

Immune function testing was performed with a commercially available in vitro diagnostic assay cleared by the U.S. Food and Drug Administration that uses whole blood samples collected in sodium heparin vacuum tubes (ImmuKnow; Cylex, Inc., now part of ViraCor-IBT Laboratories, Inc., Lee's Summit, MO). The assay is designed to detect cell-mediated immunity by measuring the concentration of ATP from CD4+ cells after stimulation and was performed as directed by the manufacturer.[2] Values of 225 ng/mL or lower ATP suggest a low immune cell response and values of 525 ng/mL or higher ATP suggest a strong immune cell response.[22]

To prospectively evaluate the management of immunosuppression based on their immune function assay values, all patients were tested with the assay before liver transplantation, immediately after surgery, and at each clinic visit occurring at approximately day 1, weeks 1 to 4, 6 and 8, and months 3 to 6, 9, and 12.[2] The assay was repeated within 7 days of a suspected or confirmed rejection or infection and again within 1 week after resolution of the event.

Intervention

Immunosuppressive therapy consisted of tacrolimus and a steroid taper according to standard practice at our center. The steroid tapering was planned as follows: 1 g methylprednisolone before liver reperfusion, 200 mg at day 1, 160 mg at day 2, 120 mg at day 3, 80 mg at day 4, and 40 mg at day 5. Subsequently, oral prednisone 25 mg was administered daily and gradually stopped during the next 6 months.

The target tacrolimus plasma levels were 8 to 12 ng/mL at 4 months and 6 to 10 ng/mL after 4 months.[23] Steroids were not tapered in patients transplanted for primary biliary cirrhosis, autoimmune cirrhosis, or primary sclerosing cholangitis. In cases of renal impairment (defined as plasma creatinine > 1.5 mg/dL), tacrolimus levels were reduced to 4 to 6 ng/mL and mycophenolic acid or a mechanistic target of rapamycin (mTOR) inhibitor was added.

Based on immune function assay values and our clinical experience, the following was used to adjust therapy in the Interventional group: the tacrolimus dose was reduced by 25% when immune function values were less than 130 ng/mL ATP (low immune response) and increased by 25% when assay values were greater than 450 ng/mL ATP (strong immune response). Tacrolimus dosage changes were made until the assay values stabilized between 130 and 450 ng/mL ATP. These values were previously documented as thresholds for risks of infection and rejection, respectively, with a value of 280 ng/mL corresponding with the greatest negative predictive value for either event.[7] Treating physicians in the control group were blinded to the immune assay results, and immunosuppression was adjusted according to standard practice.

Adverse Events

Rejection was diagnosed by biopsy and graded as indeterminate, mild, moderate, or severe according to the Banff schema.[24] Infection was defined as a microbial phenomenon characterized by an inflammatory response (pain, heat, redness, swelling)[25] to the presence of microorganisms and were classified according to standardized criteria.[26] In this sense, bacterial, viral, parasitic, and mycotic infections were included.

Systemic inflammatory response syndrome was defined as a systemic inflammatory response to a variety of severe clinical insults, and it was considered to be manifested by the presence of 2 or more of the following conditions: (1) body temperature greater than 38 °C or less than 36 °C; (2) heart rate greater than 90 beats/min; (3) respiratory rate greater than 20/min or $PaCO_2$ less than 32 mm Hg; (4) white blood count greater than 12,000/mm^3 or less than 4000/mm^3. Sepsis was defined as a systemic response to infection manifested by systemic inflammatory response syndrome,[27] and it was deemed severe in presence of organ dysfunction or when requiring hospitalization. Cytomegalovirus (CMV) viral syndrome was defined when CMV-DNA or CMV-antigen positivity was associated with fever greater than 38°C for 2 days of unexplained origin, leucopenia, myalgia, or arthralgia.[28]

Infectious Screening Protocol

The CMV-DNA was routinely measured once weekly during the hospital stay, as well as hepatitis B surface antigen and hepatitis B surface antibody in patients who underwent liver

transplantation for hepatitis B virus (HBV) infection or who received a graft from a HBcAb donor. After discharge, patients were followed in a dedicated outpatient clinic. Complete blood count, renal and hepatic function, urinalysis, and CMV-DNA were measured at every visit. Hepatitis B surface antigen, hepatitis B surface antibody, HBV-DNA, and HCV-RNA were also measured if clinically indicated (ie, HBV-positive patients, HCV-positive patients, and recipients of HBcAb-positive graft). Blood, urine, ascites, and bronchoalveolar cultures were performed any time there was clinical suspicion of infection but surveillance cultures were not routinely performed. More specific microbiological tests, such as galactomannan Ag determination and interferon- γ release assay for latent tuberculosis infection, were performed as second-line tests. Chest roentgenograms were performed any time there was the clinical suspicion of pulmonary infection. Computed tomography (CT) was the second-line test if the roentgenogram was dubious. Abdominal ultrasound or CT scan was performed any time there was a clinical suspicion of intra-abdominal infections and to rule out arterial/biliary graft complications.

Infection Prophylaxis Protocol

The following medications were administrated as infection prophylaxis:

- Trimethoprim/sulfamethoxazole tablets 60 mg/800 mg, 3 times weekly for 12 months after liver transplantation as *Pneumocystis carinii* prophylaxis,
- Nystatin oral suspension 100,000 IU/mL, 5 mL 4 times daily until steroid treatment interruption as candidiasis prophylaxis,
- Intravenous ganciclovir at a dose based on creatinine clearance until postoperative day 7 in cases of donor/recipient CMV serology mismatch (donor IgG+/recipient IgG–); subsequently, oral valganciclovir at a dose based on creatinine clearance for 12 months after liver transplantation. No CMV infection prophylaxis is routinely administered to IgG+ recipients.

Infection Treatments

An empiric antimicrobial therapy was started in case of clinical suspicion of infection. Blood and urine cultures were performed when clinically indicated and ascites and bronchoalveolar lavage cultures were performed when available. More specific microbiological tests were performed as second line tests (galactomannan Ag culture; QuantiFERON, Cellestis LTD, Chadstone, Australia). Radiological tests (chest X-ray, abdominal CT scan) were used to identify infection sites. If the cultures were positive, the antimicrobial treatment was modified according to the antibiograms.

Outcomes

The primary study outcome was a comparison of patient survival, infections, allograft rejection, and graft loss between the control and interventional groups. Secondary outcomes were differences in the dose of primary immunosuppressants among patients without adverse events, posttransplant renal failure (considering dialysis, plasma creatinine level and glomerular filtration rate according to the formula: GFR (mL/min/1.73 m^2) = 175 × $(S_{cr})^{-1.154}$ × $(Age)^{-0.203}$ × (0.742 if female) × (1.212 if African American), diabetes (treatment with insulin or oral antidiabetic agents), HCC recurrence, and the addition of adjunctive immunosuppressants, such as an antiproliferative, mTOR, or steroid.

Statistical Analysis

Published data of a 20% incidence of rejection[29,30] and 30% to 65% incidence of bacterial and CMV infections[31,32] were used to calculate the required number of enrolled patients. We hypothesized that immune function testing may lower the risk of these events by 30%. Using Fisher exact test with an α of 0.05 and β of 0.80, a sample size of 103 patients was required for each group. Univariate analysis was performed using Student *t* test for continuous variables and Fisher exact test for categorical variables. For 2-tailed calculations, differences were considered significant for *P* less than 0.05.

The patient survival after liver transplantation was calculated by the Kaplan-Meier method starting from the day of LT to the day of death or to the most recent follow-up visit. Differences were compared by the log-rank test, and variables were evaluated in the multivariate analysis using Cox proportional hazard model (Figure 1).

Ethics

The study was conducted in accordance with the declaration of Helsinki and followed the Good Clinical Practice guidelines of the International Conference on Harmonization. The protocol used in this study was approved by the Independent Ethics Committee at Sant'Orsola-Malpighi Hospital of the Università di Bologna. ClinicalTrials.gov Identifier: NCT01764581.

RESULTS

Patient Characteristics and Allocation

A total of 202 de novo liver transplant patients 18 years or older received a deceased or extended criteria donor liver between July 2008 and March 2013. Patients were randomized 1:1 to the control (standard practice) group (n = 102) and the interventional (immune function testing) group (n = 100). There were no statistical differences between the groups with respect to group allocation, posttransplantation follow-up, and analysis.

The demographics, cause of ESLD, and surgical procedures of the enrolled patients are summarized in Table 1. There were no significant differences with respect to age, sex, race, MELD score, donor MELD (D-MELD) score,[33] or reason for liver transplantation. All donors were deceased, and no differences were seen between standard or extended criteria donors.

Notably, there was an inverse correlation between immune function assay values and MELD scores prior to transplantation. Patients with MELD scores greater than 20 had statistically lower immune function values than patients with lower MELD scores (median 58 ng/mL adenosine triphosphate [ATP] vs. 114 ng/mL ATP; $P < 0.05$).

Patient Survival

The actuarial survival rate of patients was 89% in the interventional group compared to 78.4% in the control group ($P < 0.05$) and 1-year patient survival was significantly higher in the interventional group (95% vs 82%, $P < 0.01$), as reported in Figure 2.

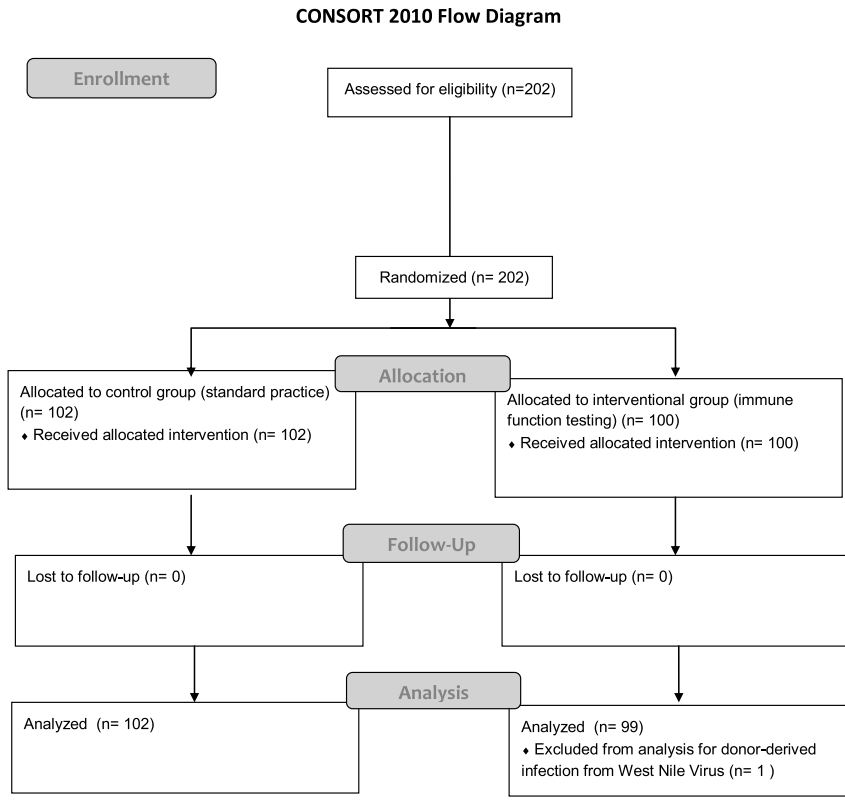

FIGURE 1.

Among the 33 patients who died, 11 were in the interventional group (33%) and 22 were in the control group (67%). Eleven patients died from infections (33.3%), 9 patients from multiorgan failure (27.3%), 6 from recurrent HCC or de novo tumor (18.2%), 4 from surgical complication (12.1%), and 3 from recurrent HCV hepatitis (9.1%).

One patient in the control group and 2 patients in the interventional group were retransplanted because primary graft nonfunction.

The univariate analysis showed a statistical correlation with a lower patient survival at 1 year for the cases with D-MELD greater than 1600 (81.5% vs. 91%, $P < 0.05$) and with infection events (82.6% vs 96%, $P < 0.01$), whereas the presence of HCC or HCV-positive, acute rejection, or other recipient and donor features did not demonstrate any correlation with the patient survival.

The multivariate analysis, including the D-MELD greater than 1600 and the study groups, showed that the interventional arm was independently associated with a higher 1-year patient survival ($P < 0.01$; 95% confidence interval, 1.3–9.5).

Adverse Events

The numbers of adverse events in the 2 study groups are shown in Table 2. There was no statistical difference between cohorts with respect to acute rejection events, which were mild in most cases according to Banff scores with no occurrence of steroid-resistant or chronic rejection.

The infection events after the first 2 weeks posttransplant were significantly lower in the interventional group (42% vs. 54.9%, $P < 0.05$) corresponding with improved patient

TABLE 1.

Demographics and clinical features of the study populations

	All patients (N = 202)	Intervention group (n = 100)	Control group (n = 102)	*P*
Recipients				
Mean age (SD), y	54 (11)	55 (11)	53 (12)	n.s.
Male sex, n (%)	127 (62.9)	66 (66)	61 (59.8)	n.s.
Viral hepatitis, n (%)				
HCV-positive	53 (26.2)	25 (25)	28 (27.5)	n.s.
HBV-positive	81 (40)	39 (39)	42 (41.2)	n.s.
Virus negative	68 (33.7)	36 (36)	32 (31.4)	n.s.
HCC, n (%)	70 (34.7)	32 (33)[a]	38 (37.3)[b]	n.s.
Mean MELD score (SD)	20 (10)	19 (9)	21 (10)	n.s.
MELD > 20, n (%)[c]	99 (49)	44 (44)	55 (53.9)	n.s.
Mean BMI (SD), kg/m^2	25.5 (4.6)	25.4 (4.5)	25.5 (4.7)	n.s.
Donors				
Mean age (SD), y	54.54 (20.3)	53.22 (19.3)	55.7 (21.3)	n.s.
Male sex, n (%)	106 (52.5)	57 (57)	49 (48)	n.s.
Cause of death, n (%)				
Cerebrovascular accident	132 (65.3)	63 (63)	69 (67.6)	n.s.
Trauma	39 (19.3)	21 (21)	18 (17.6)	n.s.
Other	31 (15.3)	16 (16)	15 (14.7)	n.s.
Mean BMI (SD), kg/m^2	26.1 (5.2)	25.9 (5.1)	26.5 (5.3)	n.s.
Mean ischemia time (SD), min	396 (102)	400 (127)	383 (69)	n.s.
D-MELD score (SD)	1162 (708)	1090 (705)	1234 (706)	n.s.
D-MELD >1600, n (%)	54 (27.1)	22 (22.2)	32 (32)	n.s.

[a] (T1 = 13, T2 = 11; T3 = 8).
[b] (T1 = 17, T2 = 12, T3,=9).
[c] MELD score was measured 1 day before liver transplantation.
BMI, body mass index.

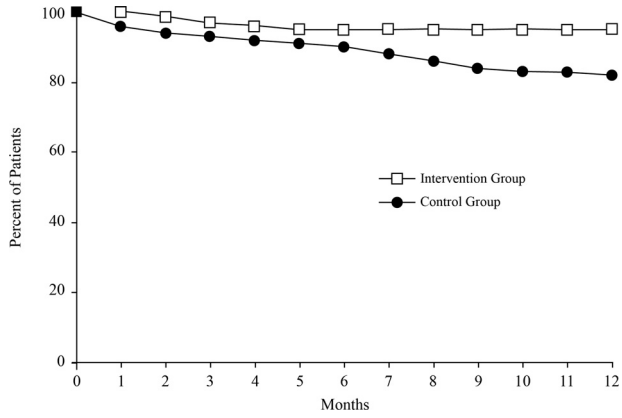

FIGURE 2. Patients survival 1 year after transplantation for intervention and control groups. The patient survival after liver transplantation was calculated by the Kaplan-Meier method starting from the day of LT to the day of death or to the most recent follow-up visit. The 1-year patient survival was significantly higher in the Interventional group (95% vs. 82%, $P < 0.01$).

survival in that group. This difference was further increased when selecting the recipients with MELD scores greater than 20 (Table 2).

Among the laboratory-confirmed infectious episodes that occurred after postoperative day 14, bacterial infections were responsible for most cases (57.1%), specifically, bacteremia (n = 45), pneumonia (n = 15), biliary tree infections (n = 6), urinary tract infections (n = 5), and ascites infection (n = 6). Viral infections were involved in 33.3% of cases including herpes simplex (n = 3), varicella zoster (n = 2), human herpes virus 6 (n = 1), human herpes virus 1 (n = 2), human herpes virus 8 (n = 2), West Nile virus (n = 1), and CMV (N = 33). Fungal infections were responsible for the remaining cases (9.6%): *Aspergillus* pneumonia (n = 3), cerebral *Aspergillosis* (n = 1), and candidemia (n = 9).

Overall, patients in the control group experienced a higher incidence of infections. Among patients with bacterial infections (N = 77; 57.1%), 47 (46.1%) occurred in the control group versus 32 (32%) in the interventional group ($P < 0.05$). Similarly, among patients with fungal infections (n = 13; 9.6%), 11 (10.8%) occurred on the control group versus 2 (2%) in the interventional group ($P < 0.05$). There was no significant difference in the number of patients with viral infections in the control and intervention groups (23 vs 22, respectively). Patients in the control group were twice as likely to be hospitalized for infections with longer stays compared to patients in the interventional group (relative risk = 2.1).

Immunosuppression Adjustment: Patient Example

An example case of immunosuppression adjustment based on immune function assay values and clinical condition is described in an immune monitoring profile. This 54-year-old man was transplanted in June 2009 for cryptogenic cirrhosis. His immunosuppressive therapy was managed according to immune function testing as described above. Immune function values increased from 23 to 137 ng/mL ATP during the first posttransplant week and by week 2 had reached 285 ng/mL ATP. During this time, his immunosuppression therapy followed routine practice as these values did not persist below 130 ng/mL or exceed 450 ng/mL ATP. His immune function assay values decreased to 32 ng/mL ATP between days 14 and 50 and his tacrolimus (Prograf; Astellas Pharma S.p.A., Carugate, IT) was reduced from 12 mg per day (6 mg twice per day) to 9 mg per day (5 mg in the morning, 4 mg in the evening). As a result, his immune function value increased to 166 ng/mL ATP at 3 months. His immune function assay value decreased to 106 ng/mL at his 6-month clinic visit, and his tacrolimus was further adjusted to 4 mg per day (2 mg twice per day). At 9 months after transplantation, he was diagnosed with chronic renal failure and at 12 months his immune function assay value was 65 ng/mL ATP. Tacrolimus was further reduced to 2 mg per day and then to 1 mg per day and mycophenolate mofetil (CellCept; Roche Registration Limited, Welwyn Garden City, UK) was added at a dose of 1000 mg per day (500 mg twice per day). No infections or rejections occurred during the course of this patient's first posttransplant year.

TABLE 2.

Comparison of outcomes at 12 months of follow-up

	All patients (N = 202)	Intervention group (n = 100)	Control group (n = 102)	*P*
Patient survival, %	170 (84.2)	89 (89.0)[a]	80 (78.4)[b]	<0.05
Event-free recipients, %	83 (58.9)	41 (41.0)	42 (41.2)	n.s.
Infectious episodes >14 d after transplantation, %	98 (48.5)	42 (42.0)	56 (54.9)	<0.05
Acute rejections, %	33 (16.3)	19 (19.0)	14 (13.7)	n.s.
Recipients with >3 infection, %	21 (10.4)	11 (11.0)	10 (9.8)	n.s.
Bacterial infections, %	77 (57.1)	32 (32.0)	47 (46.1)	<0.05
Fungal infections, %	13 (9.6)	2 (2.0)	11 (10.8)	<0.05
Viral infections, %	45 (33.3)	22 (22.0)	23 (22.5)	n.s.
	All patients, MELD >20 (N = 99)	Intervention group, MELD >20 (n = 44)	Control group, MELD >20 (n = 55)	
Patient survival, %	81 (81.8)	41 (93.2)	40 (72.7)	<0.01
Infectious episodes >14 d after transplantation, %	60 (60.6)	22 (50.0)	38 (69.1)	<0.05

[a] Cause of 11 deaths: multiple organ failure (n = 2, 18%), infections (n = 3, 27%), recurrent HCV hepatitis (n = 1, 9.1%), surgical complication (n = 2, 18%), and tumor-related causes (n = 3, 27%).
[b] Cause of 22 deaths: multiple organ failure (n = 7, 32%), infections (n = 8, 36%), recurrent HCV hepatitis (n = 2, 9.1%), technical reasons (n = 2, 9.1%), and tumor-related causes (n = 3; 14%). MELD scores were measured the day before liver transplantation.

Immune Function Assay, Tacrolimus Level, and Infections

Among recipients without adverse events during the first 3 months after transplantation, patients in the interventional group had significantly lower median tacrolimus doses (6 mg vs 8 mg, $P < 0.01$) and tacrolimus trough levels (8 mg/dL vs 9 mg/dL, $P < 0.01$). At 6 and 12 months after transplantation, the median tacrolimus level were 7 and 6 mg/dL, respectively, in the interventional group compared to 8 and 7 mg/dL in the control group (for each, $P < 0.05$).

At postoperative day 30, the number of infection events was statistically correlated with lower level of immune function assay values (median, 83 vs 178 ng/dL ATP; $P < 0.001$), as well as the tacrolimus daily dosage (median, 5 vs 4 mg/day, $P < 0.01$) and tacrolimus level (median, 7 vs 8 ng/mL; $P < 0.05$), which were lower in the infection group and therefore not effective to prevent them.

Dividing the patients according to the presence or the absence of infection, we found a correlation with a lower immune function assay at each timepoint during the 6-month posttransplant period, whereas the tacrolimus level showed a correlation only in the first month after transplantation (Figure 3).

The patients with rejection had a higher level of immune function assay values without reaching statistical difference (median, 211 vs 163 ng/dL; $P = 0.09$), while they had a significantly higher tacrolimus daily dosage and plasma level (median 8 vs 5 mg/day, $P < 0.001$ and median 9 vs 8 ng/mL; $P < 0.001$). Conversely, the tacrolimus dosage and levels were higher in the patients with rejection.

There were no between-group differences in the use of antiproliferatives (mycophenolic acid, mycophenolate mofetil, or azathioprine (GlaxoSmithKline S.p.A., Verona, IT), steroids or mTOR inhibitors.

DISCUSSION

The present randomized trial demonstrated the benefit of managing immunosuppression in adult liver transplant recipients using the ImmuKnow immune function assay. The ImmuKnow assay measures the ability of CD4+ cells to respond to mitogenic stimulation by phytohemagglutinin-L in vitro by quantifying the amount of ATP produced in CD4+ cells after stimulation.[22] The most commonly used immunosuppressive medications, such as cyclosporine and tacrolimus, inhibit lymphocytes and especially T lymphocytes such as CD4+ cells.[2] As the proliferation of CD8+ cells and other components of the cell-mediated immune response are largely under the control of CD4+ cells, the measurement of CD4+ activation is a more accurate reflection of global cell-mediated immune function. By combining sequential immune assay values with other routine tests, the treating transplant physician has a more complete immunological picture helpful in making more pondered decisions regarding therapy adjustments.

Similar to other reports, we found bacterial infections to be the most common type of infections[26]; however, using the immune function assay, the patients in the interventional arm of the study had a reduced number of bacterial and fungal infection events and improved the patient survival during the first year after transplantation.

These results are in agreement with other studies that demonstrated a correlation between low assay values and the incidence of recurrent hepatitis C viral infections,[7,10,21,29] human immunodeficiency virus/HCV-coinfected patients,[30] bacterial infections,[31] and invasive fungal infections[32] among liver transplant recipients. In 1 observational study, liver recipients with an immune function value less than 130 ng/mL ATP were 12 times more likely to develop an infection ($P < 0.001$),[9] and the results of a large meta-analysis concluded that the immune function test is a valid tool for determining the risk of further infection in adult liver transplant recipients.[8]

Similar to several previous studies, the immune function test values did not correlate with episodes of rejection in liver recipients[34] although some investigators have reported it to be useful for monitoring rejection.[6,31] The results of a recent study suggest the predictive power of the immune function assay may be improved by combining it with the CD4+ T lymphocyte count and CD4+/CD8+ ratio.[35]

The multivariate analysis including the variable D-MELD score, which summarizes the donor and recipient features, confirmed that the interventional arm had an independently higher survival (19% improvement) at the first year after transplantation compared to the control group with conventional postoperative management.

Early during the study, it was observed that patients with pretransplant MELD scores greater than 20 had significantly lower ImmuKnow values. This demonstrates that many patients are highly immunosuppressed before receiving any immunosuppressive therapy. As a result of this finding,

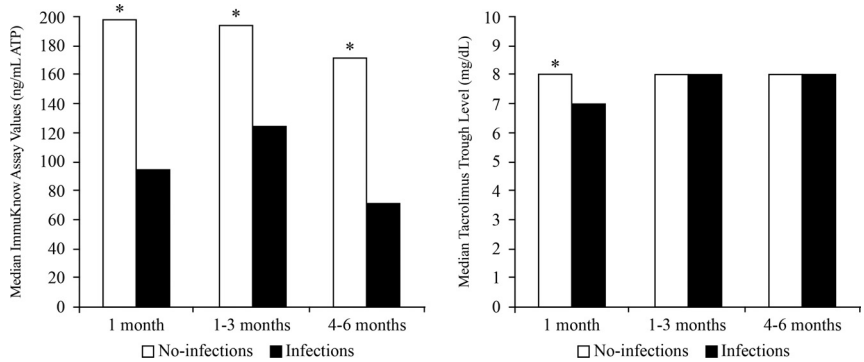

FIGURE 3. Plasma ImmuKnow and Tacrolimus values among with and without infections. Left, Median ImmuKnow assay values were significantly higher (Stronger immune response) among patients without infections at each time period after transplantation. *$P < 0.01$. Right, Median tacrolimus trough levels were significantly higher among patients without infections during the first month following transplantation. *$P < 0.01$.

we adjusted the amount of immunosuppression initially given to patients during transplant surgery by combining ImmuKnow values with MELD scores. These very sick patients, transplanted earlier with an allocation system based on MELD score,[36–38] are at higher risk of mortality due to infection,[39] demanding a strategy to reduce their immunosuppression.

Utilization of the ImmuKnow test results to optimize immunosuppression yielded improved patient survival and reduced infectious episodes in the interventional group as shown in Table 2. These findings were even more striking for the recipients with MELD scores greater than 20 in the interventional group who had a patient survival nearly 20% higher than that in the control group with the same MELD score.

Drug levels are often used to guide immunotherapy; however, this approach often results in drug toxicity, infection, or graft rejection.[40] We showed that the measurement of plasma tacrolimus levels is not sufficient to prevent infection as demonstrated in Figure 3. After the first month posttransplant, patients with or without infection had the same tacrolimus level, whereas the ImmuKnow values were significantly lower in the cases with infections. On the other hand, the immune assay should not be considered an alternative to the tacrolimus level, but it should be a piece of a complex puzzle of the immunosuppression of a transplant recipient. By using this assay, we were able to reduce the daily tacrolimus dosage and the trough level in the interventional group. This was demonstrated among the patients without adverse events because patients with infection were managed by reducing immunosuppression while increasing immunosuppression in patients with rejection. Therefore, if 1 group had higher rates of infection, an analysis of tacrolimus dose and plasma level would have suggested reducing immunosuppression in patients with infection.

The analysis of tacrolimus dosage and plasma levels was focused on recipients without adverse events to limit biases that may be caused from treating patients with various adverse events (increased immunosuppression for apparent rejections and decreased immunosuppression for diagnosed infections). The lower exposure to tacrolimus will likely improve the long-term renal function of patients when managed by ImmuKnow,[41] even if our data did not show any statistical significant difference of creatinine level and glomerular filtration rate at 6 and 12 month after transplantation (data not reported).

Because the use of steroids and other immunosuppressants were not subject to change based on ImmuKnow values, there were no differences in the amount of these agents used between the 2 groups.

There was higher patient survival among HCV-positive patients and those with HCC in the Interventional group (data not reported). These patients are supposed to benefit from lower immunosuppression to prevent viral and tumor recurrence of disease, but there were no statistical differences, and the number of cases was insufficient for any conclusive subanalysis.

It may be suggested that the same lower levels of immunosuppression among patients in the interventional group without adverse events can be achieved without using the ImmuKnow assay[42] and that the assay was less effective in preventing rejection events compared to infections. Performing another randomized study in which immunosuppression is minimized by using conventional criteria of clinical practice may provide additional data. Although the literature does not provide any suggestions for implementing this strategy,[47,48] the results of our study offers an effective tool to minimize immunosuppression, especially in patients with high MELD scores.

Our results showed a significant difference in patient survival and significantly lower bacterial and fungal infection rates between the study groups, which were otherwise comparable according to the donor and recipient features and the multivariate analysis confirmed this important data. Furthermore, the sample size and the statistical power of the study prevented any statistical bias.

In conclusion, the present prospective, interventional, controlled, and randomized study demonstrates that ImmuKnow provides a useful biomarker which enables optimizing immunosuppression to improve patient outcomes by preventing bacterial and fungal infections, reducing immunosuppressant drug use and improving 1-year patient survival after liver transplantation. These results are even more compelling in cases with MELD scores greater than 20.

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