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Original Article

Individualized management of immunosuppressants in liver transplant recipients by using a novel immune score system

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Abstract: Background: There is no reliable means to evaluate the immune status of liver transplant recipients. We proposed a novel score model, namely Mingdao immune cell analysis and Mingdao immune score system, to quantify the immunity. Methods: Data from those who underwent a single liver transplant between January 2017 and June 2020 at Beijing Chaoyang Hospital, were collected. In addition, healthy volunteers were also enrolled. The score model was based on the immune cell populations determined by flow cytometry. Results: There were a total of 376 healthy controls with 376 tests and 148 liver transplant recipients with 284 tests in this study. Evaluated by Mingdao immune cell analysis and Mingdao immune score system, the mean scores of healthy controls were near zero suggesting a balanced immune system. In contrast, the mean scores of liver transplant recipients were negative both before and after surgery indicating a compromised immune system. When liver transplant recipients were given a reduced or routine first dose according to their preoperative score, they had similar recovery of liver function. Moreover, liver transplant recipients with increased scores ≥ 5 were associated with elevated aspartate transaminase and alanine amiotransferase. Finally, on multivariate analysis the score model was the only significant independent risk factor for clinical acute rejection (P = 0.021; Odds ratio, 0.913; 95% confidence interval, 0.845-0.987). Conclusion: The novel score model could be used as an indicator to reflect immunity and to regulate immunosuppressants in liver transplant recipients after surgery.

Keywords: Immunity assessment, liver transplant recipients, immune status, Mingdao immune cells analysis, Mingdao immune score system

Introduction

Organ transplantation is the only effective method to treat end-stage organ failure. However, it is continuously plagued by immune rejection. Therefore, immunosuppressive agents are needed to prevent rejection and improve the long-term survival of patients. While these agents are used, they are not without adverse effects. Of note, immunosuppression-related complications including over-immunosuppression [1-4] and under-immunosuppression [5], are known to affect the patients' survival. Hence, it is a challenge to allow individualized dosing of immunosuppressive agents to optimize their therapeutic effect. Presently, the dose of immunosuppressive agents is merely determined according to the drug levels, organ function, and clinical events. Actually, the regulatory function of immunosuppressive agents is achieved by inhibiting the immune cell activation and reaching a balance of the immune system at a relative low level. Thus, based on the evaluation of individualized immune status instead of empiric therapy, the tailored treatment for each recipient is more favored.

Nevertheless, there has not been a reliable means to assess immune status [6]. To date, the only method approved by the United States Food and Drug Administration (FDA) to evaluate the recipient immune status is the Immune Cell Function Assay (Cylex ImmuKnow assay), which

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measures the change in adenosine triphosphate (ATP) production by $CD4^+$ T cells after stimulation. Although the test could provide information on T cell alloreactivity status that cannot be obtained from the assessment of drug blood levels, conflicting results have been repeatedly reported which limits its widespread acceptance [7-9]. Other biomarkers and diagnostic measures have been proposed for immune monitoring but no method or assay has been able to meet the diagnostic and technical requirements [10].

Herein, we proposed a novel immune score model, namely the Mingdao immune cells analysis (MICA) and Mingdao immune score system (MISS), which is based on the whole immune system, including T lymphocytes, B lymphocyte, natural killer cells, natural killer T cells, and dendritic cells. After performing immune cell analysis by flow cytometry we could assess the immune status by calculating the MISS values. The present study sought to validate the efficacy of the MICA and MISS in healthy controls and liver transplant recipients.

Materials and methods

Study design

We applied the MICA and MISS in liver transplant recipients who underwent a single liver transplant or were followed up at Beijing Chaoyang Hospital, between January 2017 and June 2020. In addition, healthy volunteers were also enrolled to validate the effect of the score model. Data from healthy controls and patients were prospectively collected and analyzed. The study was approved by the Institutional Review Board of Beijing Chaoyang Hospital (No. 2016-2-19-38) in accordance with the Helsinki declaration of 1975, as revised in 1983. Written informed consent was obtained from all participants. The study was registered in the Chinese Clinical Trial Registry (registration No: ChiCTR2100044569).

Acute rejection was diagnosed using clinical and laboratory parameters in addition to the graft biopsy assessed according to the Banff schema [11]. Increased and decreased immune status were defined when the changes in two consecutive postoperative MISS values were ≥ 5 and ≤ -5, respectively. As tacrolimus-based treatment prevailed at our center, patients treated with cyclosporin A were excluded in this study.

Perioperative treatment

Immunosuppressive therapy consisted of induction with basiliximab (20 mg on day 0 and 4) and then maintenance, which was based on steroids, mycophenolate mofetil, and tacrolimus. Methylprednisolone (500 mg) was intravenously infused during operation. After surgery, it was given by 240 mg/day, and daily reduced by 40 mg till the 6^{th} postoperative day. Then it was changed to prednisolone (20 mg/day). Prednisolone was gradually withdrawn within one month. Sirolimus was used in selected patients with impaired renal function or for its antitumor effects one month after surgery.

Anticoagulation was given on the second postoperative day if no sign of bleeding occurred. Cefoperazone/Sulbactam was administered to prevent bacterial infection. Cytomegalovirus and Epstein-Barr virus reactivations were monitored by measuring quantitative nucleic acid testing in plasma. All patients were routinely screened for fungal infection. The decision to reduce the dose of immunosuppressive agents after infections was at the discretion of the transplant surgeon who was in charge of patient care. The vascular color doppler ultrasound and the contrast-enhanced multidetector row helical computed tomography scan combined with angiography were used to check the patency of anastomosed vessels at regular intervals.

The MICA and MISS

According to the role of lymphocytes in immune system, we selected 25 populations of lymphocytes including T cells, B cells, natural killer (NK) cells, and dendritic cells, and measured the percentage and absolute count of each cell population with a fluorescence activated cell sorter. Then, we got 28 values of the immune cell populations and ratios, and formed a report of immune status, namely MICA (**Table 1**).

The MISS value was calculated according to a mathematical equation. *X* and *W* represent the percentage and the weighting of each immune cell population, respectively, while *n* means each immune cell population. Then, we used

Table 1. Lymphocyte subpopulations to be measured

Mingdao immune cell analysis
T lymphocytes
Helper/inducer T lymphocyte
Suppressor/cytotoxic T lymphocyte
Ratio of helper/inducer T lymphocyte to suppressor/cytotoxic T lymphocyte
Regulatory helper/inducer T lymphocyte I
Regulatory helper/inducer T lymphocyte II
Regulatory helper/inducer T lymphocyte III
Regulatory helper/inducer T lymphocyte IV
Effector helper/inducer T lymphocyte I
Effector helper/inducer T lymphocyte II
Effector helper/inducer T lymphocyte III
Effector helper/inducer T lymphocyte IV
Regulatory suppressor/cytotoxic T lymphocyte I
Regulatory suppressor/cytotoxic T lymphocyte II
Regulatory suppressor/cytotoxic T lymphocyte III
Regulatory suppressor/cytotoxic T lymphocyte IV
Effector suppressor/cytotoxic T lymphocyte I
Effector suppressor/cytotoxic T lymphocyte II
Effector suppressor/cytotoxic T lymphocyte III
Effector suppressor/cytotoxic T lymphocyte IV
$CD19^+$ B lymphocytes
$CD20^+$ B lymphocytes
Ratio of $CD19^+$ B lymphocyte to $CD20^+$ B lymphocyte
Natural killer cells
Natural killer T cells
Plasmacytoid dendritic cells
Myeloid dendritic cells
Ratio of Plasmacytoid dendritic cells to Myeloid dendritic cells

Figure 1. Mingdao immune score system. Zero means a balanced immune status while plus or minus values mean activated or suppressed immune status, respectively.

the equation to quantify the whole immunity by MISS values (**Figure 1**). On a scale of -25 to +25, the ideal MISS value of a healthy individual was supposed to be around 0 according to the principle of ancient Chinese yin-yang theory, which meant a balance between over- and under-immunosuppression. Negative MISS values meant an immunosuppressive status while positive MISS values indicated immune activation.

$$f(y) = \sum (X_n \times W_n)$$

Antibodies

The following reagents were all obtained from BD Biosciences: FITC-anti-CD3, CY5.5-anti-

CD4, CY5.5-anti-CD8, PE-anti-CD19, PerCP-CY5.5-anti-CD20, APC-anti-CD16, PE-anti-CD-56, PE-anti-CD4, FITC-anti-Lin1, PerCPCY5.5-anti-CD123, APC-anti-CD11C, antibody I, antibody II, antibody III and antibody IV.

Flow cytometry

Five mL of heparinized whole blood for flow cytometric analysis was taken from healthy controls and from patients immediately before transplant surgery and 10 days, 1 month, 3 months, 6 months, and 1 year after surgery or in case of clinical acute rejection.

Peripheral blood mononuclear cells (PBMC) were isolated by ficoll density gradient centrifugation and resuspended in phosphate-buffered saline (PBS). Then, PBMC were stained with antibodies mentioned above at 4°C in the dark for 20 min. After that, PBMC were washed once with 2 mL PBS and resuspended in 400 μl PBS for flow cytometry analysis.

Flow cytometry was performed in NovoCyte D2060R (ACEA Biosciences Inc.). NovoEXpress software (San Diego, CA, USA) was used for analysis. Flow cytometry characterization of lymphocyte subsets is presented in **Figure 2**.

Statistical analysis

Data analyses were carried out by using SPSS 19.0 computer software (IBM Corp., Armonk, NY, USA). All values compared were expressed as mean ± standard deviation and the independent samples t test was employed for quantitative variables. Significance for the difference between unpaired groups was determined using the Mann-Whitney U test due to non-normal distribution. Spearman's rank correlation was applied for detecting correlations between different study values. Variables on univariate analysis with *P* values less than 0.05 were subjected to further analysis to identify independent predictive factors for acute rejection. Relative risks were expressed as odds ratios with a 95% confidence interval. A *P*-value < 0.05 was considered significant.

Results

Validation of the MICA and MISS in healthy controls

A total of 376 healthy controls (187 males, 189 females) with a mean age of 45.7 years (20-85 years), who were ensured healthy via medical check-ups, were enrolled in this study. There were 7 males and 22 females in the age group of 20-29 years, 19 males and 47 females in the age group of 30-39 years, 99 males and 69 females in the age group of 40-49 years, 42 males and 34 females in the age group of 50-59 years, and 20 males and 17 females in the age group of > 59 years, respectively. The test results from healthy controls were collected and analyzed. Evaluated by the MICA and MISS, the score distribution of healthy population was normal with an average score of near zero. Subsequently, we compared the MISS values among males, females and all healthy controls while no difference was observed (**Figure 3A**, P > 0.05). Then, we wanted to check the difference among various age groups. The difference did not reach statistical significance (**Figure 3B**, P > 0.05). After that, the gender difference in MISS values was further compared within each age group (**Figure 3C-G**, P > 0.05). The results were similar between males and females among different age groups although females over sixty had a positive mean. Collectively, a balanced immune system in healthy controls suggested the efficacy of our proposed MICA and MISS.

General characteristics of the MICA and MISS in liver transplant recipients

There were 148 liver transplant recipients included in our study, among which 65 preoperative tests in 65 patients and 219 postoperative tests in 95 patients were performed, respectively. Characteristics of liver transplant recipients are listed in **Table 2**. No death and graft loss occurred during the follow-up period. 26 patients developed clinical acute rejection.

When the preoperative MISS values were analyzed we found there was no difference among three age groups (**Figure 4A**, P > 0.05). Besides, the preoperative MISS values were higher in patients with malignant diseases than in patients with benign diseases (**Figure 4B**, P = 0.000). Moreover, the preoperative MISS values were higher in female patients than in male patients (**Figure 4C**, P = 0.034). Then, we calculated the postoperative MISS values and found most patients had negative MISS values demonstrating an inhibited immune status. The postoperative MISS values were the lowest tested one month after surgery compared with

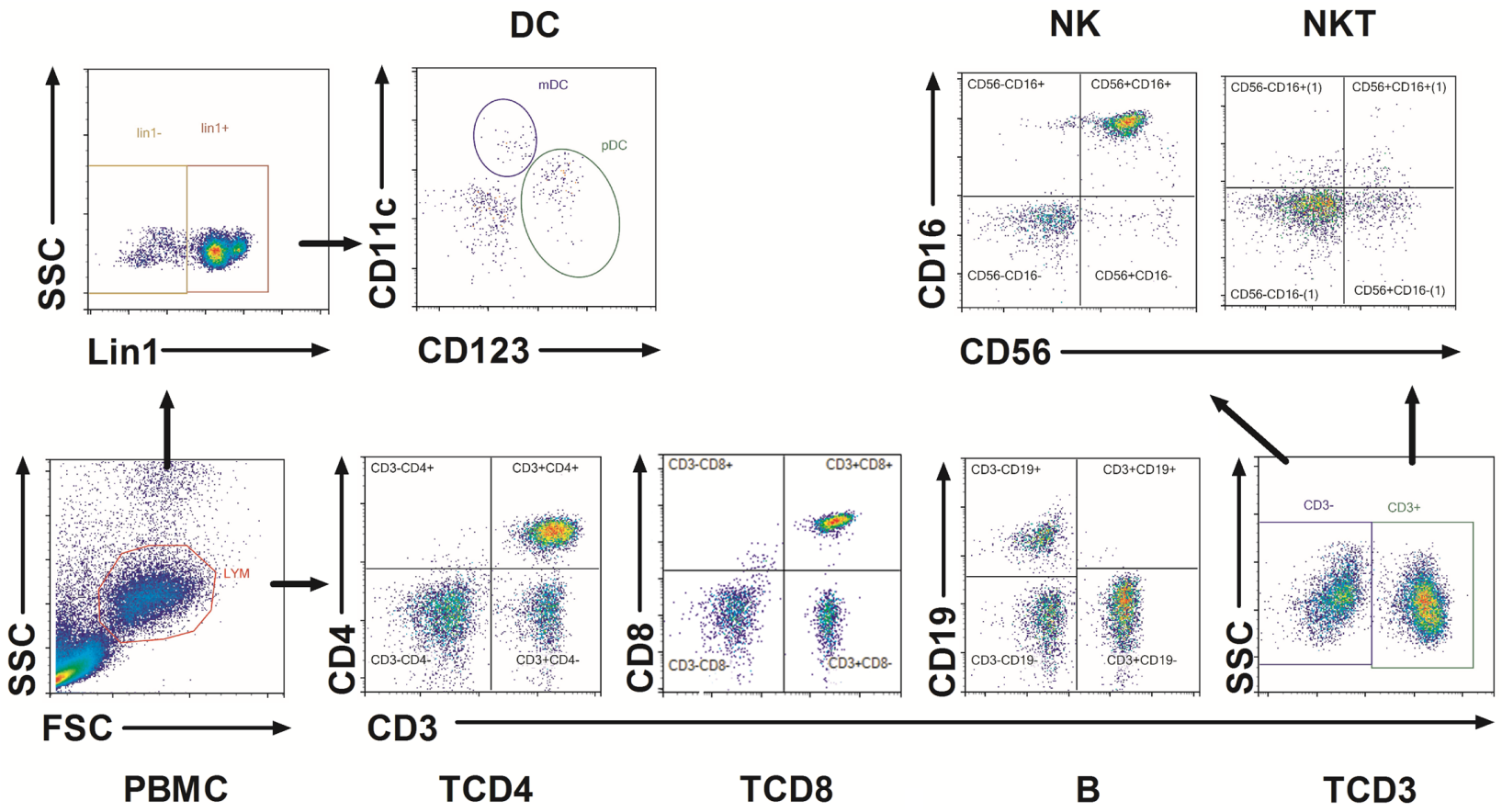

Figure 2. The lymphocyte subsets evaluated. $CD3^+$ T cells (TCD3), $CD3^+CD4^+$ T cells (TCD4), $CD3^+CD8^+$ T cells (TCD8), $CD19^+$ B cells (B), $CD3^-CD56^+CD16^+$ Natural Killer cells (NK), $CD3^+CD56^+CD16^+$ Natural Killer T cells (NKT), and $lin1^-CD11c^+$ dendritic cells and $lin1^-CD123^+$ dendritic cells (DC).

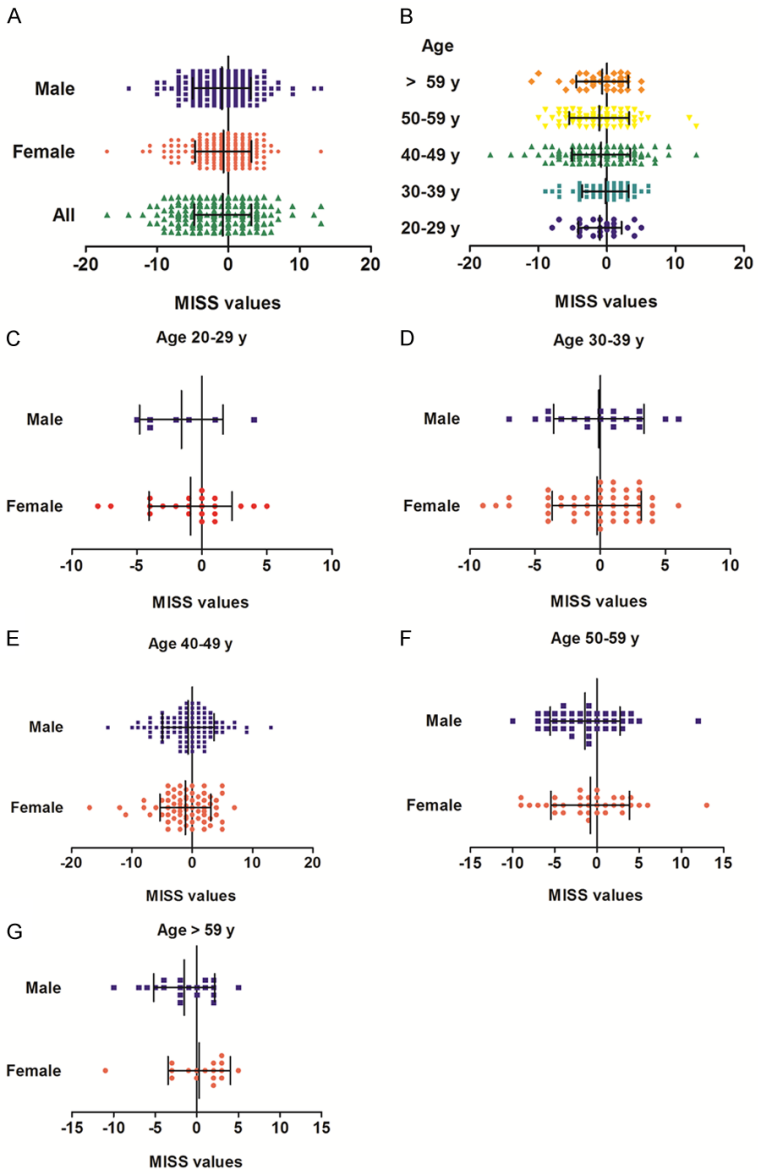

Figure 3. Distribution of the MISS values in healthy controls. Comparison of the MISS values among different groups did not reach statistical significance (P > 0.05). MISS, Mingdao immune score system.

those tested three months (P = 0.008), six months (P = 0.001) and one year (P = 0.002) after surgery while similar to those tested on the 10th postoperative day (**Figure 4E**, P > 0.05). Finally, we found there was no difference among preoperative, postoperative, and overall MISS values (**Figure 4D**, P > 0.05). Taken together, our proposed MICA and MISS indicated an impaired immune status in liver transplant recipients before and after surgery.

Effect of preoperative MISS values on the first dose of immunosuppressive agents

Subsequently, we wanted to determine whether the preoperative MISS values could be used to as an indicator to regulate the first dose of immunosuppressive agents since the score model was found to reflect the immune status. The patients were treated with a reduced first dose of immunosuppressive agents, mainly tacrolimus, to prevent the opportunity infections and severe immune inhibition when the preoperative MISS values were ≤ -5; otherwise, a routine first dose was given. After analysis, we found the preoperative MISS values correlated positively with the levels of tacrolimus (**Figure 5A**, P = 0.000). Accordingly, patients with low MISS values had lower levels of tacrolimus compared to those with high MISS values (**Figure 5B**, P = 0.000). Notably, there was no statistical difference between patients with low and high MISS values with respect to aspartate transaminase (AST; **Figure 5C**, P > 0.05), alanine amiotransferase (ALT; **Figure 5D**, P > 0.05) or total bilirubin (TBIL; **Figure 5E**, P > 0.05) on the 1st, 3rd, 5th, 7th, and 9th postoperative day when treated with different first doses of immunosuppressive agents. Taken together, patients with low MISS values had a more suppressed immune system.

Effect of postoperative MISS values on dose of immunosuppressive agents

Next, we wanted to know whether a change in the MISS values could reflect a change in the immune status. According to the definitions of increased and decreased immune status we

Table 2. Characteristics of liver transplant recipients

Data	Liver transplant recipients
Preoperative	N = 65
Age (y, median, range)	55, 28-74
Male	48
Maligant	33
Postoperative	N = 95
Age (y, median, range)	53.5, 24-78
Male	78
Maligant	36
Operative time (min, median, range)	485, 330-1200
Blood loss (mL, median, range)	800, 200-4000
Warm ischemia time (min, median, range)	3, 0-8
Cold storage time (min, median, range)	480, 60-660
Bile leak	1
Delayed graft function	2
Respiratory infection	1
Abdominal infection	3
Bleeding	5

regrouped the patients (**Figure 6A** and **6E**). The patients took the same dose of immunosuppressive agents as before. Comparison of liver function did not reach statistical significance on different days following the second test when the changes in two consecutive postoperative MISS values were ≤ -5 (**Figure 6B-D**, P > 0.05). However, the levels of AST and ALT on the 10th day were higher than those on the 1st day (P = 0.001 and P = 0.001) and the 4th day (P = 0.012 and P = 0.014) following the second test in patients with increased MISS values ≥ 5, respectively, while similar to those on the 7th day (**Figure 6F**, **6G**, P > 0.05). However, the levels of TBIL remained similar at different time points (**Figure 6H**, P > 0.05). We consider it an early stage of acute rejection. After adding to the patients' increased dose of tacrolimus, the levels of AST and ALT decreased. The results indicated an increased dose of immunosuppressive agents might fit an activated immune system according to the MISS values.

Predictors for clinical acute rejection

Finally, we wanted to know whether the score model could predict the occurrence of acute rejection. Data from liver transplant recipients were collected and compared, broken into preoperative, operative and postoperative values (**Table 3**). There was no significant difference in preoperative parameters such as sex, age, primary diseases, model for end-stage liver disease score, diabetes, smoking, drinking, heart disease, respiratory disease, albumin, creatinine, bilirubin and international normalized ratio (P > 0.05). Then, comparison of operative data such as operating time, warm ischemia time, cold storage time, bleeding, transfusion, and anhepatic phase did not reach statistical significance (P > 0.05). Finally, postoperative data such as respiratory infection, bile leak, delayed graft function, bleeding, abdominal infection, white blood cell counts, neutrophil lymphocyte ratio, lymphocyte counts, lymphocyte percentage, $CD4^+$ T-cell counts, $CD8^+$ T-cell counts, $CD8^+$ T-cell percentage, NK cell counts, and NK cell percentage were similar between patients with and without clinical acute rejection (P > 0.05).

However, the levels of tacrolimus were significantly lower in patients with acute rejection, who also had higher MISS values and $CD4^+$ T-cell percentages when compared to those without rejection (P < 0.05).

Using multivariable logistic regression, we found that the MISS values (P = 0.021) were the only significant independent risk factor for acute rejection.

Discussion

An estimated 40%-70% of the causes of short and late mortality are related to immunosuppressive therapy following liver transplantation [12, 13]. The available standard in most centers to monitor immune status relies heavily on drug levels, liver biochemistry, and clinical events, which do not adequately assess the immune system. Moreover, the challenge in balancing under- and over-immunosuppression is further complicated by the lack of a reliable means of predicting patients' immunosuppressive needs.

Our proposed MICA is based on the role of each cell population in the immune system, thus it

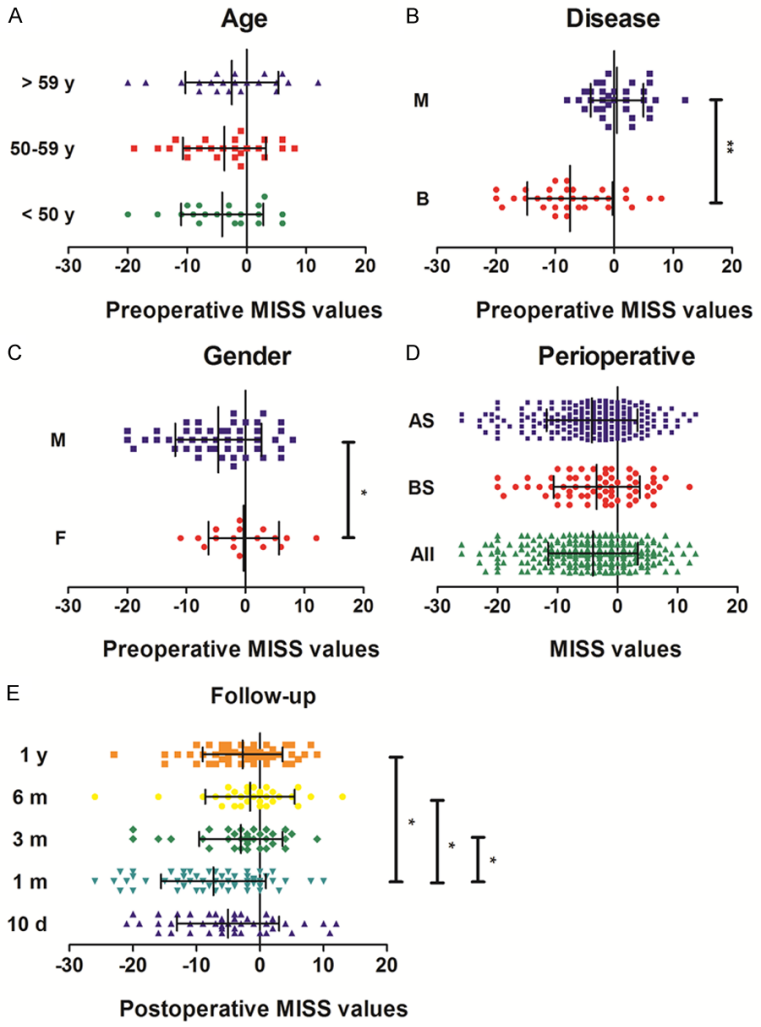

Figure 4. Distribution of the MISS values in liver transplant recipients. A. Comparison of preoperative MISS values among age groups; B. Comparison of preoperative MISS values between patients with malignant and benign diseases; C. Comparison of preoperative MISS values between female and male patients; D. Comparison of preoperative MISS values, postoperative MISS values and all MISS values; E. Comparison of postoperative MISS values at different time points. MISS, Mingdao immune score system; M, malignant; B, benign; M, male; F, female; AS, after surgery; BS, before, surgery; *, < 0.05; **, < 0.01.

can give a thorough evaluation of the immune status. The MISS values are closely associated with the immune responses and vice versa. Hence, the MISS values in essence reflect the immune status of liver transplant recipients as the changes in the percentages of immune cell populations from the peripheral blood can result in the changed MISS values.

In our study, we first evaluated the efficacy of the score model in healthy individuals, who were found to be in an almost balanced immune state according to the mean MISS values. Nowadays, healthy individuals have been under a number of pathophysiologic stresses, such as hard work, fatigue, spiritual stress, intensive exercises, and sleep disturbances, which pertains to the piling up of biowaste produced by biochemical side-reactions [14-18]. The consequential accumulation of metabonomic byproducts in human body will finally harm the immune system, leading to a subhealthy condition [19, 20]. Therefore, we assume that it is a state characterized by some disturbances in psychological behavior or physical characteristics, or in some indices of medical examination, with no typical pathologic features [21].

Next, we found the immunity was greatly impaired in liver transplant recipients before surgery, especially in patients with benign diseases. Innate and adaptive immune dysfunctions are significantly compromised in patients with chronic liver disease on the waiting list as cirrhosis-associated immune dysfunction contributes to immune paresis and impaired anti-microbial response [22, 23]. Therefore, patients with cirrhosis are at increased risk of infections due to impaired immunity and increased gut permeability, leading to bacterial translocation in the setting of portal hypertension [24]. On the other hand, bacterial translocation can lead to spontaneous bacterial peritonitis, and subsequent systemic inflammatory response syndrome, sepsis, and multiorgan failure, accelerating immune suppression [25]. Patients with malignant diseases on the waiting list usually have normal organ functions at our department. The possible explanation might be that only the anti-tumor function of lymphocytes is inhibited sig-

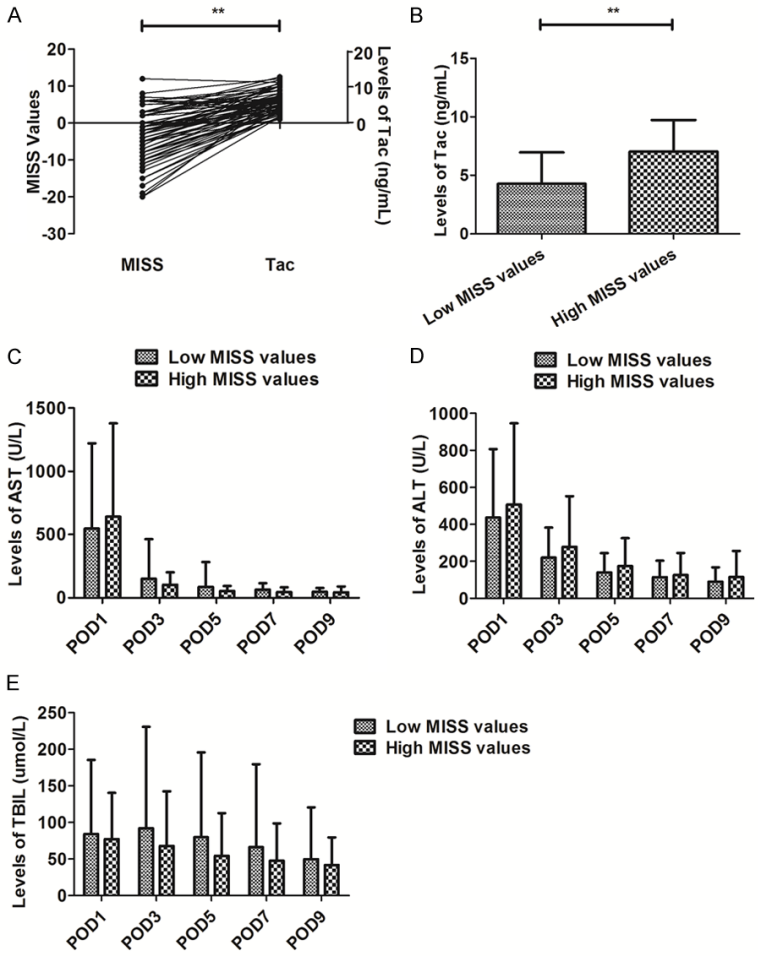

Figure 5. Effect of preoperative MISS values on the first dose of immunosuppressive agents. (A) Correlation between preoperative MISS values and tacrolimus levels; Comparison of the levels of tacrolimus (B), AST (C), ALT (D), and TBIL (E) between patients with low and high MISS values. MISS, Mingdao immune score system; AST, aspartate transaminase; ALT, alanine amiotransferase; TBIL, total bilirubin; POD, postoperative day; **, < 0.01.

nificantly in the tumor microenvironment to result in immune escape of malignant tumors [26, 27]. Notably, female patients had higher preoperative MISS values than males. That might be a result of a higher ratio of malignant diseases in females (58.8%) than in males (47.9%). Following liver transplantation the immunity is suppressed due to the combined effects of surgery and immunosuppressive therapy [28, 29]. Thus, patients had the lowest MISS values at the first postoperative month. Then, there might be an immune homeostasis reached between the graft and the recipient, resulting in a gradual recovery of immunity.

Since the MISS values could be used to reflect the immunity of healthy controls and patients, we applied the preoperative MISS values as an index to regulate the first dose of immunosuppressive agents. Tailored immunosuppressive therapy should be provided as the immune status varies among different patients. Therefore, we should not pursue the target trough levels recommended by the guidelines, which in fact is not proper for each liver transplant recipient. We found patients with low MISS values treated with reduced first doses of immunosuppressive agents had the similar recovery of liver function to those with high MISS values treated with a routine first dose, suggesting that regulating the first dose of immunosuppressive agents based on the MISS values could be an alternative.

Currently, ImmuKnow has been the only assay approved by FDA to quantify cell-mediated immunity by measuring the concentration of ATP from $CD4^+$ T cells [30]. However, contradictory results have been reported in predicting acute rejection [7-9], which affect its widespread usage. There have been repeated reports that both cellular immunity and humoral immunity play an important role in rejection following liver transplantation [31-33]. The most commonly used immunosuppressive agents, such as cyclosporine and tacrolimus, only inhibit T lymphocytes such as $CD4^+$ T cells [34]. Thus, a possible explanation for above phenomenon is that ImmuKnow focuses on the cellular immunity. By only stimulating the cell-mediated immunity, the poor sensitivity may reflect ImmuKnow failing to recognize and therefore measure the contribution made by humoral immunity to rejection processes.

Acute graft rejection is a response of the adaptive (cellular immunity) and humoral immune system (secreted antibodies by activated B

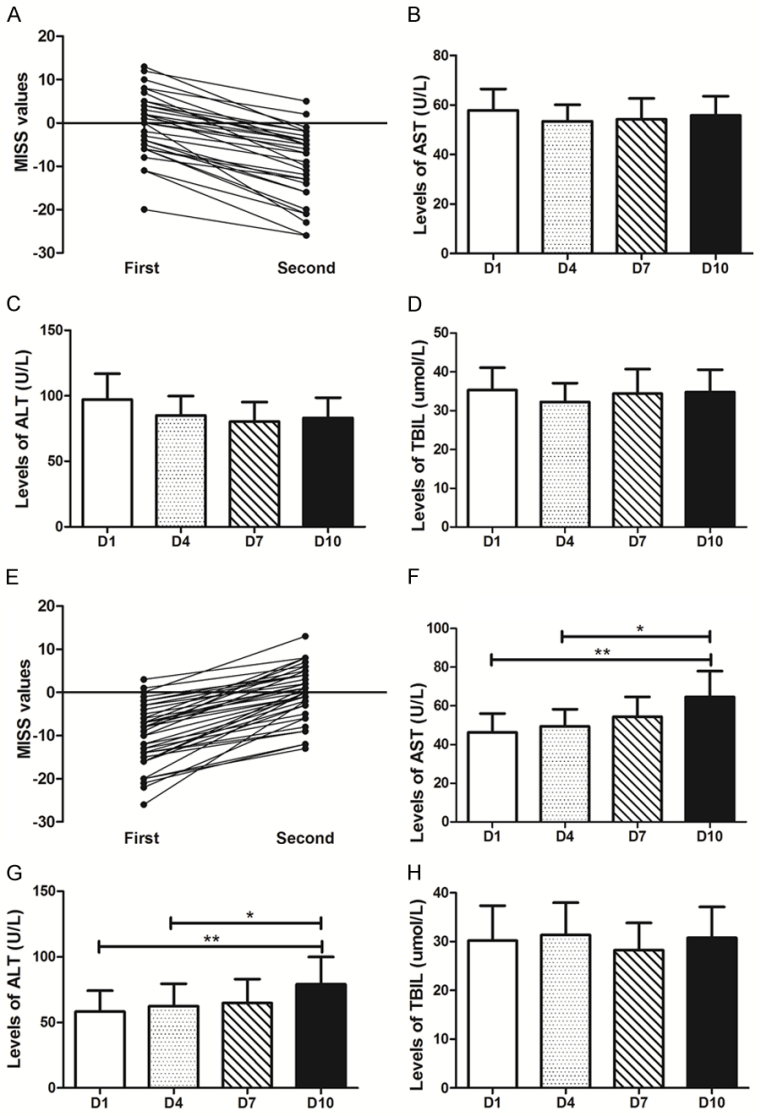

Figure 6. Increased MISS values suggest an activated immune status. (A) The changes ≤ -5 in two consecutive postoperative MISS values; Comparison of levels of AST (B), ALT (C), and TBIL (D) at different time points following the second detection; (E) The changes ≥ 5 in two consecutive postoperative MISS values; Comparison of levels of AST (F), ALT (G), and TBIL (H) at different time points following the second detection. MISS, Mingdao immune score system; AST, aspartate transaminase; ALT, alanine amiotransferase; TBIL, total bilirubin; *, < 0.05; **, < 0.01.

cells) in combination with the innate immune system (phagocytosis) [35-37]. These immune cell populations are included in our MICA. The lymphocytes clonally expand and differentiate at different timing and rates in case of acute rejection, which can accordingly lead to different MISS values. From this perspective, the score model can predict rejection, which was proven in our study. At the same time a significant increase in MISS values might also indicate acute rejection. After clonal expansion, activated lymphocytes will cause organ damage, leading to increased levels of aspartate transaminase and alanine amiotransferase. Therefore, the MISS values change theoretically a few days ahead of the liver function, allowing us to add the dose of immunosuppressive agents before the situation gets worse.

There are some limitations in our study. First, the sample size of patients with rejection in this study is small, and the results obtained are only for functional clinical reference. Although we extended our study to three years, the occurrence rate was pretty low. Besides, some patients dropped out in the study due to various reasons. Moreover, this represents the experience of a single center. Future studies, preferably randomized controlled trials in multiple centers, are needed to further validate our initial report. In addition, patients with decreased MISS values were in an over-immunosuppressive condition theoretically according to our score model. As there were no signs of infections or toxic and side effects of immunosuppressive agents their dose remained the same. Thus, accumulated experience will likely be needed in order to reduce clinical outcomes, but also to identify the appropriate dose changes to minimize the risk of a rebound effect.

In conclusion, the MICA and MISS could be used as an alternative to reflect the immunity of healthy controls and liver transplant recipients as well as an index to regulate the dose of immunosuppressive agents.

Table 3. Risk factors for clinical acute rejection

Value	Univariate analysis			Multivariate analysis		
	With CAR (N = 26)	Without CAR (N = 69)	P	OR	CI	P
Preoperative						
Sex (male/female)	22/4	56/13	0.927			
Age	50.62±10.38	51.46±11.31	0.652			
Disease (benign)	13	46	0.135			
MELD score	16.69±9.97	16.82±9.97	0.802			
Diabetes	4	13	0.835			
Smoking	11	25	0.586			
Drinking	10	26	0.944			
Heart disease	1	6	0.714			
Respiratory disease	12	23	0.248			
Albumin (g/L)	33.80±5.81	32.06±7.37	0.110			
Creatinine (μmol/L)	93.87±80.10	82.61±51.46	0.764			
Total bilirubin (μmol/L)	145.34±160.55	180.45±213.96	0.767			
INR	1.73±0.71	1.89±1.55	0.874			
Operative						
Operating time (min)	479.62±89.37	530.97±151.70	0.137			
Warm ischemia time (min)	1.85±1.01	2.46±1.41	0.059			
Cold storage time (min)	420.00±105.98	446.96±101.01	0.234			
Bleeding (ml)	738.46±456.14	1066.67±761.13	0.060			
Transfusion (ml)	1032.69±799.37	1498.55±1277.69	0.080			
Anhepatic phase (min)	70.31±21.26	68.48±15.94	0.771			
Postoperative						
Respiratory infection	0	1	1.000			
Bile leak	1	0	0.610			
Delayed graft function	0	2	0.939			
Bleeding	2	3	0.892			
Abdominal infection	1	2	1.000			
Levels of tacrolimus (ng/ml)	6.25±3.34	7.97±3.77	0.046	1.153	0.996-1.336	0.057
MISS values	-0.50±5.64	-5.41±8.42	0.008	0.913	0.845-0.987	0.021
White blood cell counts (10^9/L)	5.62±2.39	6.76±4.79	0.211			
Neutrophil lymphocyte ratio	4.18±.03	6.92±10.50	0.773			
Lymphocyte counts (10^9/L)	1.26±0.59	1.38±1.17	0.596			
Lymphocyte percentage (%)	23.01±10.33	24.06±16.82	0.713			
$CD4^+$ T-cell counts (/μL)	382.36±350.91	353.91±337.25	0.695			
$CD4^+$ T-cell percentage (%)	31.48±12.06	25.38±12.88	0.041	0.976	0.936-1.017	0.243
$CD8^+$ T-cell counts (/μL)	331.28±315.50	373.71±341.86	0.590			
$CD8^+$ T-cell percentage (%)	28.83±10.89	27.65±10.95	0.977			
NK cell counts (/μL)	112.64±94.33	146.02±182.19	0.548			
NK cell percentage (%)	9.79±5.07	10.74±7.37	0.858			

CAR, clinical acute rejection; OR, odds ratio; CI, confidence interval; MELD, model for end-stage liver disease; INR, international normalized ratio; MISS, Mingdao immune score system.

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Informed consent was obtained from all individual participants included in the study.

Disclosure of conflict of interest

None.

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