RESEARCH ARTICLE

Characteristics and risk of chronic graft-versus-host disease of liver in allogeneic hematopoietic stem cell transplant recipients

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Abstract

Chronic graft-versus-host-disease (cGvHD) is a serious complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Among various organ-specific cGvHD, the cGvHD of liver is less well-characterized. In this study, we applied the National Institutes of Health 2014 scoring criteria of cGvHD to analyze a retrospective cohort of 362 allo-HSCT recipients focusing on cGvHD of liver. The overall incidence of liver cGvHD with a score of 3 by 1.5 years post-transplant was 5.8% (21/362). Poor outcome, in terms of overall survival (OS), were observed in patients with scores of 3 liver cGvHD, comparing to those with scores less than 3 (hazard ratio [HR] 2.037, 95% confidence interval [CI] 1.123–3.696, P = 0.019). In multivariate analysis, male gender (HR 4.004, P = 0.042) and chronic hepatitis C virus (HCV) infection status (HR 19.087, P < 0.001) were statistically significant risk factors for scores of 3 liver cGvHD. Our results indicate that liver cGvHD with scores of 3 has a grave prognosis following allo-HSCT, and that HCV carrier status and male are risk factors. Early recognition of this devastating complication might help in prompt immunosuppressive therapy and reducing late poor outcome.

Introduction

Chronic graft-versus-host disease (cGvHD) is a serious complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and its incidence rate ranges from 30% to 70% [1]. The consequences of cGvHD include impaired patient quality of life, a greater symptom burden and medical costs, and an extended use of immunosuppressive therapy, and late morbidity and mortality [2–5]. To better analyze the association between the severity of cGvHD and
survival outcomes, the National Institutes of Health (NIH) Consensus Conference Working Group first proposed criteria for the diagnosis and scoring of cGvHD in 2005, which were refined in 2014 [6, 7]. In contrast to traditional classifications that divide cGvHD into limited or extensive types [8], the NIH criteria scores eight major organ systems on a scale of 0–3, which are attributed to global severity assessment scales (mild, moderate, or severe) [7]. The revision included changes in the cGvHD scoring of the skin, lungs, and liver. For liver cGvHD, the new NIH criteria increases the weight of bilirubin levels for categorization, with serum total bilirubin levels of 3 mg/dL and above corresponding to a score of 3. The revision also discarded the day-100 post-transplant cut-off for differentiation of acute and chronic GvHD [7]. Previous studies have validated the implications of NIH scores [1, 8], including for cGVHD of major organs, such as lung [9–11] and skin cGVHD [12–15]. However, for cGvHD of liver there is one prospective study that has used the 2005 NIH criteria to describe liver cGvHD [16]. The report demonstrated worse overall survival (OS) and higher non-relapse mortality (NRM) in patients with jaundice-type cGvHD [16]. In this study, we aimed to use the NIH 2014 scoring criteria to characterize cGvHD of liver from a retrospective cohort data and examine the risk factors for liver cGvHD and the impacts on survival outcome.

Materials and methods

Patient population

We identified consecutive patients who had undergone allogeneic HSCT between January 2003 and December 2013 at the Blood and Marrow Transplant Center in Taipei Veterans General Hospital in Taiwan. All patients were monitored by December 31, 2014. Patients who survived less than 100 days (N = 83) post-HSCT were excluded. A total of 362 patients were enrolled into analysis, including 42 patients below age 18. This study obeyed the principles of the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board of Taipei Veterans General Hospital in Taiwan (VGH IRB no.: 201703002BC). Informed written consent was waived by the approving IRB. In addition, patient record/information was anonymized and de-identified before analysis.

Clinical assessments and definitions

To diagnose liver cGvHD, patients were required to have liver dysfunctions with concomitant diagnostic or distinctive features of cGvHD of other organs [7]. Liver dysfunctions were defined as rising serum alanine transaminase (ALT) to more than 3 times or total bilirubin above the upper limit beyond day 70 after transplant [17]. Patients with serum total bilirubin levels above 3 mg/dL were given a score of 3, and the other patients were categorized as non-score 3 in this study. Abnormal liver function test findings caused by severe sepsis or septic shock [18], hemolysis, viral hepatitis B or C, acute liver GvHD, or biopsy proven liver hemochromatosis were identified in the non-score 3 group. If patients had recurrent events of liver cGvHD, the highest score was adopted. Hepatitis B virus (HBV) carrier status was confirmed by the positivity of serum hepatitis B surface antigen, and hepatitis C virus (HCV) carrier by positivity of enzyme immunoassay for anti-HCV [19].

Transplantation and post-HSCT care

We used low- to intermediate-resolution HLA (human leukocyte antigen) tests to detect six to eight alleles (HLA-A, -B, -DR, and/or -C). Patients were classified as either fully matched or mismatched. Donors were divided into matched sibling donor (MSD), matched/mismatched unrelated donor (MUD), umbilical cord blood (UCB), and haplo-identical donor. MUD,
UCB, and haplo-identical donors are categorized as non-MSD in analysis. Conditioning regimens were categorized as total body irradiation- (TBI-, 12 Gy divided into six fractions), Busulfan-, Cyclophosphamide (total 120 mg/kg)-based regimens, and as myelo-ablative or reduced-intensity regimens.

To prevent acute GvHD, we administered 3 mg/kg/day cyclosporine in two split doses, with adjusted trough plasma levels of 100–250 μg/L. In general, cyclosporine was tapered starting 2 months post-HSCT over a 3-month period and may be individualized at the discretion of attending physicians. Anti-thymocyte globulin (ATG) were given at a dose of 8mg/kg in 4 days for selected cases transplanted with unrelated or haplo-identical donors. Short-term methotrexate was administered on the first (15 mg/m²), third, sixth, and eleventh (10 mg/m², respectively) days after HSCT. One patient received alemtuzumab for in-vivo T cell depletion. No patients had ever undergone post-transplanted cyclophosphamide or pre-transplant graft T cell depletion. Real-time quantitative polymerase chain reaction was performed weekly to test for cytomegalovirus (CMV). Ganciclovir was administered pre-emptively to patients positive for CMV viremia.

The principle protocols, such as GvHD prevention and post-transplant care for allogeneic hematopoietic stem cell transplant recipients in Taipei Veterans General Hospital have been well-defined [20]. Treatment of cGvHD was in general consistent with the guidelines [21, 22]. Systemic steroids, usually methylprednisolone at doses of 1–2 mg/kg/day, were the mainstay treatment. Cyclosporine or other immunosuppressive agents such as mycophenolate mofetil were used on an individual basis when persisted/worsened cGvHD despite steroid treatment.

**Evaluation of transplant risk**

Patients with European Group for Blood and Marrow Transplantation (EBMT) risk scores [23] of 3 were considered to be at intermediate risk, those with scores between 0 and 2 were considered to be at low risk, and those with scores between 4 and 7 were at high risk. OS was defined as the duration between transplantation to death or the last follow-up.

**Statistical methods and study endpoints**

We retrospectively collected clinical data, including patient age at transplant, diagnosis, recipient-donor gender combination, disease status at transplant, hepatitis virus carrier status, conditioning regimen, incidence of GvHD, transplant type, degree of HLA matching, date of death, relapse, and last follow-up. Only data from the last allogeneic HSCT were obtained.

We first analyzed risk factors associated with liver cGvHD scores of 3. The potential variables included age at HSCT, gender, underlying disease, donor types, transplant types, EBMT risk scores, stem cell sources, donor-recipient gender combination, conditioning regimen, TBI dosage, GvHD prophylaxis regimen, and hepatitis B and C carrier status. In Cox regression univariable analysis, significant factors (P < 0.1) were included in the multivariable model. A P value less than 0.05 was considered statistically significant. In the analysis, we used 30 years of age as the cut-off because it was the median age of the subjects in our study.

We next analyzed survival outcome in patients with score 3 liver cGvHD compared to patients with non-score 3 disease. Eight factors were considered, including age, gender, malignant disease, transplant type, conditioning regimen intensity, transplant number, EBMT score, and liver cGvHD score of 3. Univariate and multivariate Cox regression analysis were used to determine the survival outcome for score 3 liver cGvHD. Because most patients undergoing allo-HSCT had scores of 0 to 1 based on the Eastern Cooperative Oncology Group (ECOG) scale, performance status was not included in our survival evaluation. All analyses
were performed using SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

**Results**

**Patient characteristics and incidence of score 3 liver cGvHD**

A total of 362 patients (median age, 30 years; range, 0.67–67) were categorized into groups with hematological malignancies (N = 308) and non-malignant disease (N = 54). Fifty-nine percent of patients were male. There were 176 MSD (48.6%), 178 MUD (49.2%), and (2.2%) haplo-identical or cord blood donors. The median follow-up time after HSCT was 1,039 days (range: 102–4440 days). A total of 37 patients underwent liver biopsy for differential diagnosis of liver cGvHD, including 33 in the sub-cohort having cGvHD (N = 190), and 4 in the sub-cohort not having cGvHD (N = 172). Among the patients with HCV infection, two patients underwent liver biopsy (Table in S1 Table). The median day of score 3 liver cGvHD occurrence was 147 days (range: 90–546 days) after transplant. The clinical characteristics are shown in Table 1. Among 58 patients with liver cGvHD, 21 cases had a score of 3 (Table 2), and 11 patients progressed from previous scores of 1 or 2.

The overall cumulative incidence of score 3 liver cGvHD plateaued at 5.8% (21/362) by 1.5 years among all patients (Fig 1), and 11% (21/190) among patients with cGvHD.

**Risk factors for development of score 3 liver cGvHD**

Univariate analysis revealed that younger age (less than 30 years), male gender, female-to-male (F-M) donor-recipient gender combination, and HCV carrier status were significantly associated with liver cGvHD scores of 3. In multivariate Cox regression analysis, male gender and chronic HCV infection remained statistically significant predictive factors, while younger age at transplantation had a trend toward more frequent liver cGvHD scores of 3 (Table 3).

**Survival outcome in patients with score 3 liver cGvHD**

The impact of liver cGvHD scores of 3 on survival was adjusted in multivariable Cox regression analysis (Table in S2 Table). In the score 3 group, there were 6 relapses and 13 mortalities, including 4 disease-related and 9 non-relapse deaths. All relapses (6/6) occurred before 31st month, compared to 23rd month for most relapses (84/87) in the non-3 group. The relapse rate was comparable (23% vs 25%), giving it a non-significant difference of relapse-free survival (median RFS, 14.9 versus 13.8 months, HR 0.955, 95% CI 0.411–2.217, P = 0.914) (Fig 2). However, there was a statistically significant difference in survival (median OS, 37 vs 19.4 months, HR 2.037, 95% CI 1.123–3.696, P = 0.019) between patients with score 3 liver cGvHD and those without (Fig 3). The OS curve had a steady decline until 43rd months. Most mortalities (102/109) occurred by this point.

**Discussion**

In our analysis, the incidence of liver cGvHD scores of 3 was 5.8%, slightly lower than that reported by Pidala et al[16] (8%) and Bresters et al[24] (8%) in pediatric patients. Using 2005 NIH criteria, Pidala et al[16] reported liver cGvHD to be a poor prognostic factor for OS (HR 2.46, 95% CI 1.48–4.09, P = 0.001) and higher NRM (HR 2.15, 95% CI 1.13–4.11, P = 0.02). Comparing patients with score 3 cGvHD to those with score of less than 3 cGvHD, there was no difference in RFS but significant OS difference (Figs 2 & 3), after considering competing risk factors, including whether patients received second transplant (Table in S2 Table). In the group with score of less than 3, patients might tolerate better to salvage chemotherapy or
Table 1. Clinical characteristics of study population.

| Patient characteristics | Cohort, N = 362 |
|-------------------------|----------------|
| Median patient age at transplantation, y (range) | 30 (0.67–67) |
| Patient Gender, no.(%) | |
| Male | 213(59) |
| Female | 149(41) |
| Diagnosis, no.(%) | |
| AML/MDS | 145(40) |
| ALL | 75(21) |
| CML | 16(5) |
| MPN | 3(0.8) |
| CLL | 4(1) |
| Lymphoma | 51(14) |
| MM | 13(4) |
| SAA | 49(13) |
| Other | 7(2) |
| Transplant type, no. (%) | |
| MSD | 176(49) |
| Non-MSD | 186(51) |
| MUD | 178(49) |
| UCB | 3(0.8) |
| Haplo-identical | 5(1.4) |
| Disease risk, no. (%) | |
| Low (EBMT score \(\leq 2\)) | 170(47) |
| Intermediate (EBMT score 3) | 92(25) |
| High (EBMT score \(\geq 4\)) | 100(28) |
| Stem cell source, no.(%) | |
| Mobilized blood cells | 359(99) |
| Donor-Recipient gender combination, no. (%) | |
| Female to male | 78(22) |
| Others | 284(78) |
| Conditioning Regimen, no. (%) | |
| Busulfan-based | 164(45) |
| TBI-based (12Gy) | 118(33) |
| Cyclophosphamide-based (total 120mg/kg) | 49(13.5) |
| Others | 31(8.5) |
| Intensity of conditioning regimen, no. (%) | |
| Myeloablative | 244(67.4) |
| Reduced-intensity | 118(32.6) |
| TBI dose in conditioning regimen, no. (%) | |
| \(\leq 450\) cGy | 44(12) |
| \(\geq 1200\) cGy | 121(33) |
| GvHD prophylaxis regimen, no. (%) | |
| CsA plus MTX | 360 (99) |
| ATG | 65 (18) |
| Patient with chronic GvHD, no. (%) | |
| 190(52) |
| Sites° involved with chronic GvHD, no. (%) | |
| Skin | 43(12) |
| Lung | 25(7) |
| Liver | 58(16) |
| score 1 | 5(1) |
| score 2 | 32(9) |
| score 3 | 21(6) |
| Eye | 72(20) |

(Continued)
Table 1. (Continued)

| Patient characteristics          | Cohort, N = 362 |
|----------------------------------|----------------|
| Mouth                            | 81(22)         |
| GI tract                         | 22(6)          |
| Sclerodermatous feature          | 15(4)          |
| LONIPCs                          | 21(6)          |

MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; MM, multiple myeloma; SAA, severe aplastic anemia; MSD, matched sibling donor; MUD, matched/mismatched unrelated donor; UCB, umbilical cord blood; EBMT, European Group for Blood and Marrow Transplant; CsA, cyclosporine A; MTX, methotrexate; ATG, anti-thymocyte globulin; GI, gastro-intestine; LONIPCs, late onset non-infectious pulmonary complications

*61 patients had multiple sites (≥3) involvement, another 93 patients with 2 organs involved.

https://doi.org/10.1371/journal.pone.0185210.t001

Table 2. Characteristics of patients with score 3 liver cGvHD.

| Patient | Age (years) | Diagnosis | Donor type | EBMT score | Donor-recipient sex combination | HCV carrier | Time to score 3 liver cGvHD (days) | Relapse (days after HSCT) | Survival outcome (days after HSCT) |
|---------|-------------|-----------|------------|------------|---------------------------------|-------------|-----------------------------------|--------------------------|-----------------------------------|
| 1       | 33          | CML       | MSD        | 1          | M–M                             | no          | 126                               | no                       | 4352, alive                       |
| 2       | 24          | ALL       | MUD        | 5          | F–M                             | no          | 545                               | yes, 535                 | 580, died of relapse               |
| 3       | 18          | SAA       | MUD        | 1          | M–M                             | no          | 133                               | no                       | 725, alive                        |
| 4       | 46          | MM        | MUD        | 3          | M–M                             | no          | 147                               | yes, 528                 | 663, died of relapse               |
| 5       | 15          | MDS       | MUD        | 2          | M–M                             | no          | 121                               | no                       | 156, died of CMV pneumonitis       |
| 6       | 48          | CML       | MUD        | 4          | F–M                             | no          | 102                               | no                       | 785, died of HBV reactivation      |
| 7       | 41          | MM        | MSD        | 6          | F–M                             | no          | 156                               | yes, 365                 | 944, died of relapse               |
| 8       | 43          | MM        | MSD        | 5          | F–F                             | no          | 165                               | no                       | 180, died of PJP infection & intracranial hemorrhage |
| 9       | 28          | CML       | MUD        | 3          | F–M                             | no          | 147                               | yes, 902                 | 1694, died of GvHD-related cardiac tamponade |
| 10      | 16          | ALL       | MUD        | 1          | M–M                             | no          | 121                               | no                       | 4440, alive                       |
| 11      | 27          | AML       | MSD        | 1          | F–M                             | no          | 174                               | no                       | 1341, alive                       |
| 12      | 11          | lymphoma  | MSD        | 3          | F–M                             | no          | 126                               | yes, 260                 | 369, died of relapse               |
| 13      | 50          | ALL       | MSD        | 3          | M–F                             | yes         | 283                               | no                       | 352, died of liver cGvHD and sepsis |
| 14      | 58          | AML       | MSD        | 4          | F–M                             | no          | 289                               | no                       | 379, alive                        |
| 15      | 29          | AML       | MSD        | 2          | M–M                             | no          | 124                               | no                       | 182, died of lung infection        |
| 16      | 67          | AML       | MSD        | 2          | M–M                             | no          | 90                                | no                       | 266, died of lung infection        |
| 17      | 56          | ALL       | MSD        | 2          | M–M                             | no          | 339                               | no                       | 415, died of pneumonia and CMV pneumonitis |
| 18      | 29          | ALL       | MSD        | 3          | M–F                             | no          | 123                               | no                       | 3326, alive                       |
| 19      | 24          | HD         | MSD        | 2          | M–M                             | no          | 236                               | yes, 218                 | 582, alive                        |
| 20      | 28          | CML       | MSD        | 2          | F–M                             | yes         | 100                               | no                       | 3811, alive                       |
| 21      | 27          | AML       | MSD        | 5          | F–M                             | no          | 236                               | no                       | 248, died of PJP infection         |

HCV, hepatitis C virus; HBV, hepatitis B virus; HD, Hodgkin disease; MUD, matched/mismatched unrelated donor; PJP, pneumocystis jiroveci pneumonia

https://doi.org/10.1371/journal.pone.0185210.t002
lymphocyte infusion, which might contribute to more durable post-relapse survival and to significant OS difference.

The potential risk factors of post-transplant liver dysfunction in pediatric patients months or years after undergoing allo-HSCT include pre-transplant liver injury[25] and underlying benign hematological disease[24]. The former study did not analyze HCV carrier as a risk factor, while another showed non-significant findings due to a limited number of patients with HCV (N = 3). However, in an analysis of a Japanese transplant registry database, HCV positivity was associated with higher NRM, inferior OS in patients undergoing allo-HSCT[26, 27], and was a risk factor for acute liver GvHD in adults[28]. Thus, HCV carrier status has been persistently implicated in both liver aGvHD and cGvHD. Indeed, HCV is associated with immune dysfunction[29] and has been shown to prime the liver with a pro-inflammatory environment[27]. Though there is no consensus if HCV carriers should be treated with novel antiviral agents (Ledipasvir/Sofosbuvir) prior to transplant, we suggest that viral eradication might mitigate the risks of score 3 disease. Furthermore, for patients with pre-existing HCV infection and post-transplant hepatic dysfunction, liver biopsy help in differentiate abrupt onset of liver cGvHD from acute viral hepatitis[17].

The F-M donor-recipient gender combination is a risk factor for grade II to grade IV acute GvHD[30, 31] (aGvHD) and cGvHD[30, 32]. In our analysis, the predictive effect of F-M donor-recipient combination for score 3 liver cGvHD was offset after adjusting for male gender in multivariable analysis. The small sample size might prevent several established risk factors (F-M combination, MAC preparations, etc) from reaching statistical significance. Host innate immunity in different gender might play a role. A xenograft model showed clear sex differences in intestinal and peripheral innate immune cell populations[33].

Inconsistent with previous studies[34, 35], our results indicate that recipients less than 30 years of age tended to have a higher incidence of liver cGvHD. Lim et al.[36] hypothesized that
the age-related variation in thymoglobulin pharmacokinetics may play a role in these findings, though the result was inconclusive. In our practice, physicians tend to maintain some degree of cGvHD without increasing cyclosporine dosage, especially in younger patients with higher-risk disease, which could partly explain our findings.

Apart from Pidala et al. [16], who reported poorer OS (HR 3.73, P < 0.01) in patients with jaundice-type liver cGvHD (scores of 2 and 3 by 2014 NIH criteria), our results suggest lower

**Table 3. Risk factors for score 3 liver cGvHD.**

| Risk factor          | Patient (N) | Score 3 liver cGvHD | Univariate   | Multivariate |
|----------------------|-------------|---------------------|--------------|--------------|
|                      | N | % | HR (95% CI) | P | HR (95% CI) | P |
| **Gender**           |   |   |            |   |            |   |
| female               | 149 | 3 | 2 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| Male                 | 213 | 18 | 8.5 | 4.464 (1.315–15.158) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| **Age**              |   |   |            |   |            |   |
| ≥ 30                 | 237 | 9 | 3.8 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| < 30                 | 125 | 12 | 9.6 | 2.476 (1.043–5.586) | 0.040 | 2.445 (0.979–6.107) | 0.056 |
| **Diagnosis**        |   |   |            |   |            |   |
| Non-malignant        | 54 | 2 | 3.7 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| Malignant            | 308 | 19 | 6.2 | 1.788 (0.416–7.680) | 0.434 |
| **Transplant type**  |   |   |            |   |            |   |
| Non-MSD              | 185 | 8 | 4.3 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| MSD                  | 176 | 13 | 7.4 | 1.811 (0.751–4.370) | 0.186 |
| **EBMT score**       |   |   |            |   |            |   |
| ≤2                   | 170 | 10 | 5.9 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| >2                   | 192 | 11 | 5.7 | 1.054 (0.448–2.484) | 0.903 |
| **Conditioning regimen** |   |   |            |   |            |   |
| Others               | 198 | 9 | 4.5 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| Busulfan-based       | 164 | 12 | 7.3 | 1.698 (0.715–4.030) | 0.230 |
| **Conditioning regimen** |   |   |            |   |            |   |
| Others               | 238 | 14 | 5.9 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| TBI-based (≥12Gy)    | 121 | 6 | 5.0 | 0.848 (0.326–2.207) | 0.735 |
| **Conditioning regimen** |   |   |            |   |            |   |
| Reduced intensity    | 118 | 7 | 5.9 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| Myeloablative        | 244 | 14 | 5.7 | 0.928 (0.374–2.299) | 0.871 |
| **GvHD prophylaxis** |   |   |            |   |            |   |
| Without ATG          | 288 | 17 | 5.9 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| With ATG             | 65 | 2 | 3 | 0.481 (0.111–2.080) | 0.327 |
| **Gender combination** |   |   |            |   |            |   |
| Others               | 284 | 12 | 4.2 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| Female to male       | 78 | 9 | 11.5 | 2.873 (1.210–6.821) | 0.017 | 1.739 (0.680–4.445) | 0.248 |
| **HBV status**       |   |   |            |   |            |   |
| Non-carrier          | 313 | 20 | 6.4 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| carrier              | 49 | 1 | 2.0 | 0.329 (0.044–2.449) | 0.277 |
| **HCV status**       |   |   |            |   |            |   |
| Non-carrier          | 356 | 19 | 5.3 | 1.00 (reference) | 0.016 | 4.004 (1.049–15.274) | 0.042 |
| carrier              | 6 | 2 | 33.3 | 6.684 (1.556–28.705) | 0.011 | 19.087 (3.931–92.672) | <0.001 |

95% CI, 95% confidence interval; HR, hazard ratio; HBV, hepatitis B virus

https://doi.org/10.1371/journal.pone.0185210.t003
Fig 2. Relapse-free survival (RFS) after HSCT for patients with score 3 liver cGvHD or not. The relapse rate was comparable (23% vs 25%), giving it a non-significant difference of RFS (median RFS, 14.9 versus 13.8 months, HR 0.955, P = 0.914).

https://doi.org/10.1371/journal.pone.0185210.g002

Fig 3. Overall survival (OS) after HSCT for patients developing score 3 liver cGvHD or not. There was a statistically significant difference in OS (median OS, 37 vs 19.4 months, HR 2.037, P = 0.019).

https://doi.org/10.1371/journal.pone.0185210.g003
OS in the group with score of 3, which might be driven by more durable post-relapse survival in the non-3 group. In this category, patients probably had higher potential of immune suppression and organ injury, making them prone to treatment related death after relapse. However, we have several limitations in our study, including the limited number of patients receiving bone marrow transplants, few HCV carriers, scarce relapse events, and the possible underestimation of GvHD prevalence in the setting of out-patient visits. The associations between liver dysfunctions and drugs or parenchymal liver disease were difficult to prove in the absence of adequate biopsy, and might introduce bias in identifying liver GvHD. The histologic changes of HCV infection share many features with those of liver GvHD, especially when fibrosing cholestatic hepatitis is a possibility [37, 38]. During data collection, there may have been an overlap between acute and chronic liver GvHD, despite differentiation based on the 2014 NIH criteria. In addition, we lacked complete data on cyclosporine concentrations throughout the GvHD course, HLA alleles C/DQ, and causes of death.

In our study, patients in the earlier era were not analyzed because of incoherent follow-up on liver function panel, ambiguous depiction of symptoms and signs relating to cGvHD in each patient’s visit, and missing record on transplant-related information. Thus, our result should be interpreted cautiously.

Conclusion
Based on 2014 NIH consensus criteria, patients with liver cGvHD with a score of 3 had inferior outcome in overall survival. HCV carrier status and male gender were risk factors of developing cGvHD. Early recognition of this devastating complication might help in prompt immunosuppressive therapy and reducing late poor outcome.

Supporting information
S1 Table. Characteristics of 6 HCV carriers.
(DOCX)

S2 Table. Impact of score 3 liver cGvHD on overall survival.
(DOCX)

Acknowledgments
We thank Dr. Yao-Chung Liu for providing a complete database.

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