Highlights

- Chronic liver disease is common in long-term survivors of allo-HCT.
- Nearly half of long-term survivors are found to have MASLD.
- MASLD may be associated with GVHD and type 2 diabetes.
Chronic Liver Disease after Allogeneic Hematopoietic Cell Transplantation

Baljit Randhawa1,2, Nikki Blosser1,2, Andrew Daly1,2, Jan Storek1,2, Abdel-Aziz Shaheen3,4 & Kareem Jamani1,2

1Alberta Blood & Marrow Transplant Program, Tom Baker Cancer Centre.
2Division of Hematology & Hematologic Malignancies, Department of Medicine, University of Calgary.
3Division of Gastroenterology and Hepatology, Department of Medicine, University of Calgary.
4Department of Community Health Sciences, University of Calgary.

Corresponding Author:
Kareem Jamani
Room 603 South Tower
Foothills Medical Centre
1403 29 St NW
Calgary, AB
T2N 2T9
Phone: 403 944 5222
Fax: 403 592 8423

Short Title for Running Header: CLD after allo-HCT

Text Word Count: 3879
Abstract Word Count: 322
Figure Count: 3
Table Count: 3
References: 51
ABSTRACT

Background

There are few descriptions of the epidemiology of chronic liver disease (CLD) after allo-HCT. Amongst those transplanted prior to 2000, viral hepatitis was the dominant cause of CLD. Recently, the prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD, previously known as non-alcoholic fatty liver disease) is rising in the general population. In addition, survivors of allo-HCT are known to be at increased risk of metabolic syndrome.

Objective

We set out to describe the epidemiology of CLD in a modern cohort of allo-HCT recipients. We hypothesized that MASLD would be the most common cause of CLD in the cohort.

Study Design

We undertook a retrospective cohort and nested case-control study of two-year survivors of allo-HCT in Alberta transplanted between 2008 and 2018.

Results

Amongst 392 two-year survivors of allo-HCT between 2008-2018, the prevalence of CLD was 41.8% and MASLD was identified in 56% of those with CLD followed by iron overload in 47% of those with CLD. The prevalence of MASLD amongst the entire cohort is 46%. While most patients developed CLD before two years post-transplant, there was a 13% cumulative incidence of new CLD after two years post-transplant. Grade 2-4 aGVHD and/or moderate-severe cGVHD and pre-transplant CLD were strongly associated with CLD. In the case-control study examining the association between cardiovascular risk factors and CLD, type 2 diabetes was associated with
CLD. Cirrhosis developed in 1.5% of survivors and MASLD was an underlying etiology in half of these cases. There was no difference in overall survival and non-relapse mortality between those who did and did not develop CLD.

**Conclusions**

MASLD is the main cause of CLD in recent long-term survivors of allo-HCT and may be associated with post-transplant corticosteroid exposure and type 2 diabetes. We note a shift in the underlying etiology of CLD post-HCT: prior studies describe viral hepatitis as the most common cause of CLD. The high prevalence of MASLD in allo-HCT recipients has important implications for survivorship care.

**Keywords:** Chronic liver disease, cirrhosis, allogeneic hematopoietic cell transplant, late effects, late toxicity, metabolic dysfunction-associated steatotic liver disease.
INTRODUCTION

As the number of long-term survivors of allo-HCT increases, an understanding of the spectrum of late effects is critical to allow for comprehensive survivorship care.[1-3] Many late toxicities of allo-HCT have been well characterized, including subsequent malignancies, premature cardiovascular events, infections, sexual dysfunction, psychosocial challenges and pulmonary disease among others.[1, 4, 5] Although the hepatic toxicity of allo-HCT was first noted decades ago, there are very few descriptions of late hepatic toxicity of allo-HCT.[6, 7]

Two reports of patients receiving allo-HCT in the 1980s and 1990s revealed that chronic hepatitis C virus (HCV) and iron overload were the major causes of chronic liver disease (CLD) in long-term survivors of allo-HCT.[8, 9] With vastly reduced rates of HCV transmission through blood products, the availability of direct acting antivirals for HCV, a downward trend in HCV prevalence and the availability of oral iron chelators, it is possible that the epidemiology and underlying causes of CLD in survivors of allo-HCT have changed in recent years.[10-13]

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as nonalcoholic fatty liver disease or NAFLD, has become the dominant cause of CLD in the general population, affecting approximately 30% of individuals worldwide and accounting for 75% of CLD cases.[14-16] MASLD represents a spectrum of disease, ranging from simple steatosis to steatohepatitis with or without fibrosis and finally to cirrhosis.[17] Importantly, MASLD is strongly associated with cardiovascular risk factors including hypertension, diabetes, hyperlipidemia and obesity.[18-20] Long-term survivors of allo-HCT are known to accumulate cardiovascular risk factors at a younger age and in excess to the general population, thus raising the possibility that MASLD could be an important cause of CLD in allo-HCT survivors.[21, 22]
We set out to the hypothesis that MASLD is now the primary cause of CLD in allo-HCT recipients.

METHODS

Study Cohort

We included all adult two-year survivors of a first allo-HCT in Alberta who were transplanted between January 1, 2008 and December 31, 2018 and who remained in Alberta for follow-up. Those who remained on systemic or topical immunosuppression for chronic GVHD (cGVHD) beyond two years post-transplant were excluded from the main analysis because of the challenge of excluding cGVHD itself as the single underlying cause of CLD in these patients. The cohort was analyzed for the prevalence and etiologies of CLD that either persisted beyond two years post-HCT or that began at ≥ 2 years post-HCT. The subset of the cohort who did not have CLD at two years post-HCT were further analyzed separately and are referred to as the “incidence cohort”. During the entire study period, standard practice in Alberta was to use ursodiol prophylaxis for all patients at 15-20 mg/kg daily until day 100 post-HCT.

Definitions of Chronic Liver Disease and Other Covariates

CLD was defined as at least one of the following: one or more liver enzyme above the upper limit of normal on >1 measurement at least 6 months apart without intervening normalization, liver imaging consistent with cirrhosis, or liver pathology consistent with CLD.[9, 23] In Alberta, liver enzymes are measured as part of routine labs every 1-3 months in the first two years post-HCT and at least every 3-6 months after the two year mark. Pre-HCT CLD was
defined as CLD occurring within the 12 months prior to HCT. All investigations were retrieved from a province-wide electronic medical record.

MASLD was defined by the demonstration of hepatic steatosis by imaging or biopsy and exclusion of other causes of hepatic steatosis (particularly alcohol use, hepatitis C and medications).[24] Workup for CLD in Alberta typically includes evaluation of alcohol use, viral hepatitis serologies, biochemical testing and/or imaging for iron overload, ceruloplasmin and autoimmune hepatitis serologies. Liver biopsies are done at clinician discretion. Lipid panel, hemoglobin A1C, ferritin and transferrin saturation are obtained at least yearly at ≥2 years post-HCT as part of routine post allo-HCT survivorship care.

Recurrent or de novo GVHD of the liver occurring beyond 2 years post-HCT was identified based on liver biopsy, clinician impression in progress notes and/or re-initiation of immunosuppression for abnormal liver enzymes.

The diagnosis of iron overload was established by one of the following: increased serum ferritin in a patient without significant inflammation or infection plus increased transferrin saturation (≥45%), evidence of iron overload by hepatic MRI, or evidence of iron overload on tissue biopsy.[25] Hypertension was defined as current therapy with antihypertensive(s). Type 2 diabetes was defined by current therapy with insulin or other antidiabetic drug, a fasting plasma glucose of ≥ 7.0 mmol/L and/or HgbA1c ≥ 6.5%.[26] Hyperlipidemia was defined as LDL-C ≥ 3.5 mmol/L or non-HDL-C ≥ 4.2 mmol/L.[27] Hypertriglyceridemia was defined as triglycerides > 1.7 mmol/L, typically on a fasting measurement.[28]

Statistical Analyses
The prevalence of CLD in the 12 months before transplant and the prevalence of MASLD at any time pre-transplant are described. Patients were followed for CLD until the earliest of relapse, death without CLD, or last follow-up. The prevalence of CLD and underlying etiologies of CLD in the cohort are described. A multivariable logistic regression with backwards stepwise selection (removing terms with p≥0.2 and adding terms with p≤0.1) was used to evaluate the associations between CLD and transplant-related characteristics and demographics. Covariables included age at HCT, sex, indication for HCT (lymphoid disease vs all others), presence of pre-HCT CLD, stem cell source (PBSC vs. others), history of grade 2-4 acute GVHD (aGVHD) or history of moderate-severe cGVHD and receipt of total body irradiation and ATG with conditioning. We combined acute aGVHD and cGVHD into a single variable to avoid collinearity in the model (as some patients experienced both) and to enhance statistical power given small numbers of each. Ultimately, both aGVHD and cGVHD are associated with post-HCT corticosteroid exposure which may impact the subsequent risk of MASLD.

In the incidence cohort, the cumulative incidence of CLD beginning at ≥2 years post-transplant was modeled, accounting for relapse or death without CLD as competing risks. Non-relapse mortality and overall survival were compared in those with CLD and without CLD by Cox regression, adjusting for age.

In the nested case-control study, for each case of CLD in the incidence cohort regardless of etiology, two controls without CLD were identified for detailed chart review to evaluate the association between CLD and cardiovascular risk factors (hypertension, type 2 diabetes, hyperlipidemia, low HDL cholesterol and elevated triglycerides) by conditional logistic regression. Controls were matched with cases on age, year of transplant and any significant
associations found in the multivariable analysis. All analyses were performed with STATA 17.0 (StataCorp, College Station, Texas).

RESULTS

Patients

Seven hundred eighty-two adults received a first allo-HCT in Alberta between January 1, 2008 and December 31, 2018. Amongst these, 459 were two-year survivors. We excluded 8 patients who moved away from Alberta or were lost to follow-up. Of the remaining patients, 59 remained on systemic or topical immunosuppression beyond two years post-HCT for cGVHD. Thus, the cohort consists of 392 patients (figure 1). Characteristics of the cohort, including those who did and did not develop CLD are presented in table 1.

Pre-Transplant CLD & MASLD

Of the 390 patients with available liver enzyme measurements before HCT, 103 (26%, 95% CI 22-31%) met criteria for CLD. Of these 103 with pre-HCT CLD, 45 (44%, 95% CI 34-54%) experienced resolution of CLD by 2-years post-HCT. Of the 363 patients with hepatic imaging or biopsy before HCT, 126 (35%, 95% CI 30-40%) were noted to have MASLD.

Prevalence & Etiology of CLD

Amongst the entire cohort, 164 patients developed CLD for a prevalence of 41.8% (95% CI 37.0-46.8%). The median follow-up of those who did and did not develop CLD was 6.6 years (IQR 4.0-8.5) and 6.0 years (IQR 3.5-8.1) post-transplant, respectively. Of the 164 patients with CLD, 138 developed CLD before two years post-transplant with persistence of CLD beyond two
years post-transplant, while 26 developed CLD after two years post-transplant. All etiologies of CLD are described in table 2. Approximately half of patients had more than one underlying cause of CLD identified (supplementary table 1). MASLD is the most common underlying etiology of CLD, affecting 56% of patients and was the only identified underlying etiology in 29% of patients. Iron overload was identified in 47% of patients and was the single underlying etiology in 14% of patients. Hepatic GVHD was noted in a minority (9%) of patients: These cases represented either new onset or recurrent GVHD after 2-years post-transplant, as was drug-induced liver injury (7%) and alcohol abuse (4%). Hepatitis B or C were noted in only 3 patients in the cohort. Three patients in the cohort experienced hepatic sinusoidal obstructive syndrome (SOS), all were biopsy proven and retrospectively graded as 1 mild, 1 severe and 1 very severe.[29] Only 1 of these 3 patients, who developed multifactorial cirrhosis, was noted to have prior SOS as a cause of CLD. The remaining 2 experienced complete resolution of SOS and later developed alternative causes of CLD. No underlying cause of CLD was found in 11% of patients.

**Associations with CLD**

Results of multivariable logistic regression are shown in table 3. A history of grade 2-4 aGVHD or moderate-severe cGVHD and the presence of pre-HCT CLD were strongly associated with CLD. Conversely, receipt of 4 Gy TBI with conditioning and older age at transplant were associated with lower odds of CLD. Sex, indication for transplant, and stem cell source were not associated with CLD.

Pre-HCT CLD and pre-HCT MASLD were not associated with the occurrence of aGVHD or cGVHD when controlling for patient age, graft type, HLA mismatch, and ATG use.
Incidence & Etiology of New CLD after two years Post-Transplant

Of the 392 patients in the cohort, 254 were not experiencing CLD at two years post-transplant (the incidence cohort). Of these patients, 26 went on to develop CLD at a median of 3.1 years post-transplant (range 2-7.8 years). The cumulative incidence of new CLD beginning at two years post-HCT is 13.1% (95% CI 8.6-18.5%) at 10 years post-transplant (figure 2). All etiologies of CLD in the incidence cohort are detailed in supplementary tables 2 and 3. MASLD was the most common underlying etiology, affecting 73% of patients, followed by iron overload affecting 42%. Additionally, MASLD was the most common single underlying etiology, affecting 50% of patients. Other etiologies, including drug-induced liver injury, GVHD and alcohol were rare, affecting less than 10% of patients each.

Association of CLD with Cardiovascular Risk Factors

For each of the 26 patients with CLD in the incidence cohort, we selected two matched controls from those in the cohort who did not develop CLD. Cases and controls were matched for age, year of transplant, pre-transplant CLD, history of grade 2-4 aGVHD and receipt of TBI with conditioning. The clinical characteristics of cases and controls are detailed in supplementary table 4. Type 2 diabetes was strongly associated with CLD (OR 5.9, 95% CI 1.6-21.8, p=0.007), while hypertension, hyperlipidemia, low HDL cholesterol and elevated triglycerides were not. Results of the case control analysis are presented in supplementary table 5. Of the 63 total cardiovascular risk factors identified amongst the 26 patients with CLD in the incidence cohort, 46 (73%) were diagnosed post-HCT. Of the 110 total cardiovascular risk factors identified amongst the 52 patients in the control group, 65 (59%) were diagnosed post-HCT. A detailed description of these risk factors is found in supplementary table 6.
**Prevalence of MASLD Post-Transplant in the Entire Cohort**

Of the 351 patients with hepatic imaging or biopsy after HCT, 160 (46%, 95% CI 40-51%) were noted to have MASLD. Of these 160 with MASLD post-transplant, all but 8 patients had pre-transplant hepatic imaging or biopsy: 68 were noted to have MASLD before transplant.

**CLD in Those with Active Chronic GVHD**

Of the 59 patients with active cGVHD beyond two years post-transplant, 40 (68%, 95% CI 54-79%) developed CLD. GVHD was noted to be a cause of CLD in 15 patients, while MASLD was noted in 20, iron overload in 10, drug-induced liver injury in 7, prior hepatic SOS in 1, alcohol in 1 and unknown in 4.

**Cirrhosis**

Six patients, or 1.5% (95% CI 0.6-3.3%), developed cirrhosis at a median of 3 years post-transplant. MASLD was noted as an underlying cause of cirrhosis in 3 of 6 cases: MASLD was the single underlying cause of cirrhosis in two cases and a contributor in addition to prior hepatic SOS in a third case. One of these 3 were noted to have MASLD pre-HCT. Of the 3 remaining cases, cirrhosis was due to hepatitis C and iron overload in one case, hepatitis B in one case, and iron overload plus alcohol-related liver disease in one case.

**Survival and Non-Relapse Mortality**

There was no difference in overall survival between those with and without CLD (figure 3) in univariable and multivariable analysis adjusting for age (HR 1.2, 95% CI 0.6-2.4, p=0.7). There was no difference in non-relapse mortality in between those with and without CLD in univariable and multivariable analysis adjusting for age (HR 2.0, 95% CI 0.5-6.0, p=0.2). In the CLD group,
1 patient died of CLD (cirrhosis related to chronic hepatitis C). In the group without CLD, there were no deaths related to liver disease.

**DISCUSSION**

Here we set out to describe the causes of CLD in a modern cohort of allo-HCT recipients. We found that approximately 40% of two-year survivors experienced CLD. While most patients developed their CLD prior to two years post-HCT, there was a 13% 10-year cumulative incidence of new CLD after two-years post-HCT. The dominant underlying cause of CLD was MASLD, affecting 56% of those with CLD. Amongst the entire cohort, the prevalence of MASLD before transplant was 35%, similar to recent general population estimates.[14] After transplant, the prevalence of MASLD in the cohort was 46%, exceeding general population estimates. As expected in this cohort of 2-year survivors without active cGVHD at the 2-year mark, GVHD was not a major cause of CLD.

A single prior study has evaluated the prevalence and underlying causes of CLD in survivors of allo-HCT: Tomas and colleagues reported a prevalence of CLD of 57.5% amongst 106 two-year survivors who were transplanted in the 1980s-1990s.[9] We report a slightly lower prevalence of CLD, likely owing to a lower prevalence of hepatic cGVHD in our cohort (<20% vs. 38%) in the setting of prophylaxis with ATG.[30] Importantly, we note a significant shift in the leading underlying cause of CLD: Tomas and colleagues reported that MASLD contributed to CLD in only 5% of patients while hepatitis B (6.5%) and C (47.5%) were contributors in over 50% of CLD cases. In contrast, we report that MASLD was associated with CLD in over 50% of cases, while hepatitis B and C were noted in 2% of CLD cases. With respect to cirrhosis, Strasser and
colleagues described a 0.6% 10-year cumulative incidence in patients transplanted before 2000, with nearly all cases associated with hepatitis C.[8] We found a similar rate of cirrhosis in our cohort; however, half of the cirrhosis cases were associated with MASLD. The median onset of cirrhosis in the Strasser cohort was 10 years post-HCT while the median follow-up of our cohort was ~7 years, suggesting that we might expect further cases of cirrhosis in our cohort in the coming years.

The rise of MASLD in long-term survivors of allo-HCT is perhaps unsurprising. First, the prevalence of MASLD in the general population is rising and MASLD is now a major underlying cause of CLD. Depending on the methodology used to identify MASLD, the prevalence either doubled from 5.5% in 1988-1994 to 11% in 2005-2008 or increased from 25.5% before 2005 to 37.8% in 2016 or later.[14, 15] In the former study, MASLD accounted for 47% of CLD in 1988-1994 and 75% of CLD in 2005-2008. Second, the rise in MASLD in the general population has been accompanied by a rise in associated cardiovascular risk factors or the metabolic syndrome.[19, 20] Recipients of allo-HCT are known to develop cardiovascular risk factors such as hypertension, dyslipidemia, and diabetes, earlier and in excess to the age and sex matched general population,[31] possibly placing them at higher risk of MASLD. Indeed, we found MASLD to be associated with type 2 diabetes and our estimated prevalence of MASLD of 46% appears to exceed the global prevalence estimate of 37.8% noted in a recent large systematic review.[14] Further, it is conceivable that the prevalence of MASLD is even higher in subsets of allo-HCT recipients that we have not studied here, particularly those who received allo-HCT in childhood with high dose total body irradiation, who are risk of sarcopenic obesity and metabolic dysregulation.[32, 33]
The identification of MASLD as highly prevalent and as the dominant etiology of CLD in long-term survivors of allo-HCT is important for several reasons. First, MASLD, even in the absence of steatohepatitis and fibrosis, is associated with excess mortality.[34] Our cohort likely had inadequate follow-up to demonstrate excess mortality. Second, those with MASLD are at risk of progression to advanced liver disease, specifically steatohepatitis, fibrosis, and cirrhosis. Accordingly, cirrhosis related to MASLD is now a leading indication for liver transplantation.[17] Importantly, lifestyle modification such as weight loss, dietary modification, limiting alcohol intake, exercise, and certain medications can lead to improvements the histologic changes of MASLD.[17, 35] Third, MASLD appears to be an independent risk factor for atherosclerotic cardiovascular disease.[36] Indeed, cardiovascular disease is the leading cause of death in those with MASLD.[17, 37] Additionally, MASLD is associated with an increased risk of incident type 2 diabetes and hypertension.[20] Thus, MASLD may present an additional risk factor for cardiovascular disease in allo-HCT recipients who are already at higher risk than the general population.[31] Fourth, MASLD, including simple steatosis, has been associated with an increased risk of non-hepatic cancers.[34] Additionally, patients with more advanced MASLD are at risk of hepatocellular carcinoma, which may occur in the absence of cirrhosis.[19, 38] Therefore, MASLD may present an additional risk factor for subsequent malignancy in allo-HCT recipients who are already at higher risk than the general population.[39, 40]

In multivariable analysis, we found that aGVHD and/or cGVHD was associated with CLD. We hypothesize that the metabolic toxicity of corticosteroid exposure in GVHD is associated with development of MASLD. Consistent with this, a history of aGVHD has previously been associated with the development of cardiovascular risk factors.[41] The possible role of
corticosteroids in the pathophysiology of MASLD has been demonstrated in other clinical contexts and in basic science studies.[42, 43] Those with cGVHD typically receive very long-term exposure to corticosteroids: Consistent with this, in our separate description of CLD in those with active cGVHD beyond 2 years post-HCT, we found MASLD in 50%. Interestingly, we found low dose TBI (4 Gy) and older recipient age to be associated with a lower risk of CLD. We hypothesize that both associations are related to a lower risk of cGVHD (thus less corticosteroid exposure) and, in the case of older patient age, patient selection. With respect to low dose TBI, we have previously shown in a multicentre study that 4 Gy TBI as part of conditioning is associated with a lower risk of cGVHD.[44] We hypothesize that low dose TBI may eliminate recipient myeloid-lineage antigen presenting cells (APCs) that contribute to the pathogenesis of cGVHD. Indeed, we have recently found mixed myeloid chimerism (i.e. residual recipient APCs) to be associated with a higher risk of cGVHD versus full donor chimerism (Puckrin, R, manuscript in preparation). With respect to age, we have paradoxically found older age to be associated with a lower risk of cGVHD in a multicentre cohort of ATG-conditioned recipients: We hypothesize that age-related variation in ATG pharmacodynamics may underlie this association.[44] Alternatively, patient selection may have played a role: older patients with pre-existing MASLD or cardiovascular risk factors (who would be at higher risk of post-transplant MASLD) are likely to have associated cardiovascular comorbidities and may have been less likely to receive allo-HCT due to these comorbidities.

There are important clinical implications of our findings. Providers caring for long-term survivors of allo-HCT should have a low threshold to screen for MASLD via ultrasound when liver enzymes are abnormal or when risk factors are present, such as a history of grade 2-4 acute or moderate-severe chronic GVHD or type 2 diabetes. Those with identified MASLD may be
referred to hepatology and/or risk stratified for MASLD-associated hepatic fibrosis and cirrhosis with transient elastography per local practice.[45, 46] In addition, those with MASLD should have regular thorough assessment and management of cardiovascular risk factors.[17, 45, 47] Lifestyle modification through dietary modification, regular exercise and reduction in alcohol intake are cornerstones of MAFLD treatment to reduce the risk of progression to advanced liver disease.[17, 45, 47]

Our study has limitations. Although we aimed to be thorough in evaluating charts for causes of CLD, as in any retrospective study, we cannot be certain that all underlying causes of CLD were ascertained due to investigations possibly missed at the time of CLD. Yet, we were able to review a single, comprehensive province-wide electronic medical record for all patients, limiting errors/misses in retrospective data collection. Without a liver biopsy in all patients, we cannot be certain that additional causes of CLD were missed, or of the relative contribution of each cause of CLD identified. These limitations can only be overcome with a multi-year prospective study that standardizes workup of CLD after allo-HCT, including liver biopsy for most patients. We may have underestimated the prevalence of MASLD in our cohort, given that most patients had MASLD identified by ultrasound which may be of lower sensitivity in the setting of obesity.[17] Additionally, we cannot be certain of the degree of MASLD (steatosis, steatohepatitis or fibrosis) in our cohort given that most patients did not have a liver biopsy. We used a relatively liberal definition of CLD (any degree of liver enzyme elevation was sufficient) to enable a direct comparison to previous studies of CLD in recipients of allo-HCT. However, the requirement that liver enzymes were elevated for at least 6 months likely eliminates many transient or unimportant etiologies. Because we only studied adults who mostly received a single conditioning regimen, our results cannot be generalized to those who underwent allo-HCT in
childhood and we could not study the effect of various conditioning regimens or doses of TBI. Finally, our results are most generalizable to regions with low hepatitis B/C prevalence: Viral hepatitis remains a global public health concern, although MASLD is a growing global public health concern.[14, 48, 49]

In summary, MASLD is now the dominant cause of CLD after allo-HCT in non-hepatitis B endemic parts of the world, affecting almost 50% of long-term survivors, and is associated with a history of aGVHD and/or cGVHD and type 2 diabetes. MASLD may place survivors of allo-HCT at increased risk of advanced liver disease, cardiovascular events and subsequent malignancies. Further work is required to replicate this finding in other allo-HCT settings, to evaluate whether there is regional variability to the prevalence of MAFLD after allo-HCT (for example, in the general population, MASLD is highly prevalent in North America, Europe, the Middle East and throughout Asia while somewhat less prevalent in Africa and Latin America),[50, 51] to describe the distribution of MASLD-related liver disease (steatosis vs. steatohepatitis vs. fibrosis vs. cirrhosis), to delineate the association between MASLD and clinical outcomes in studies with longer follow-up, and to describe the efficacy of interventions directed at MASLD in allo-HCT recipients.

**Author Contributions**

BR and KJ conceived and designed the study, collected data, interpreted the results and wrote the manuscript. KJ analyzed the data. AS assisted in designing the study, interpreted the results and critically reviewed/edited the manuscript. NB, AD and JS critically reviewed/edited the manuscript.
Conflict of Interest

The authors have no relevant conflict of interests to disclose.
REFERENCES


Figure Titles

Figure 1. Description of the cohort.

782 patients underwent allo-HCT 2008-2018

323 died before 2 years

459 2-year survivors

8 patients lost to follow up or moved outside Alberta

59 patients remained on systemic immunosuppression for cGVHD

392 patients - 103 with pre-HCT CLD

138 patients developed CLD before 2 years that persisted

254 Patients did not have CLD at 2 years. "Incidence Cohort"

26 patients developed CLD after 2 years
Figure 2. Cumulative incidence of chronic liver disease beginning at 2 years post-transplant.
Figure 3. Overall survival in those who did and did not develop chronic liver disease.
Table 1
Clinical Characteristics of Patients with and without Chronic Liver Disease (CLD).

<table>
<thead>
<tr>
<th></th>
<th>CLD (n=164)</th>
<th>No CLD (n=228)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>70 (43)</td>
<td>107 (47)</td>
</tr>
<tr>
<td>Median Age at Transplant (range)</td>
<td>50 (18-66)</td>
<td>51 (18-72)</td>
</tr>
<tr>
<td>Pre-Transplant CLD (%)</td>
<td>58 (36)</td>
<td>45 (20)</td>
</tr>
<tr>
<td>HCT-Comorbidity Index (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>65 (40)</td>
<td>98 (43)</td>
</tr>
<tr>
<td>1-2</td>
<td>51 (31)</td>
<td>68 (30)</td>
</tr>
<tr>
<td>≥3</td>
<td>27 (16)</td>
<td>25 (11)</td>
</tr>
<tr>
<td>Missing</td>
<td>21 (13)</td>
<td>37 (16)</td>
</tr>
<tr>
<td>Primary Disease, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>77 (47)</td>
<td>121 (53)</td>
</tr>
<tr>
<td>ALL/Biphenotypic</td>
<td>29 (18)</td>
<td>34 (15)</td>
</tr>
<tr>
<td>MDS/MPN</td>
<td>29 (18)</td>
<td>38 (17)</td>
</tr>
<tr>
<td>CLL</td>
<td>15 (9)</td>
<td>12 (5)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>11 (7)</td>
<td>13 (6)</td>
</tr>
<tr>
<td>Severe Aplastic Anemia</td>
<td>3 (2)</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Donor Type, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matched Unrelated</td>
<td>74 (45)</td>
<td>108 (47)</td>
</tr>
<tr>
<td>Matched Sibling</td>
<td>57 (35)</td>
<td>82 (36)</td>
</tr>
<tr>
<td>Mismatched Unrelated</td>
<td>29 (18)</td>
<td>35 (15)</td>
</tr>
<tr>
<td>Haploidentical</td>
<td>4 (2)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Stem cell source, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBSC</td>
<td>158 (96)</td>
<td>216 (95)</td>
</tr>
<tr>
<td>BM</td>
<td>3 (2)</td>
<td>7 (3)</td>
</tr>
<tr>
<td>Cord</td>
<td>3 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Conditioning, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FluBu + 4 Gy TBI +/- ATG</td>
<td>136 (83)</td>
<td>209 (92)</td>
</tr>
<tr>
<td>FluBu + ATG</td>
<td>21 (13)</td>
<td>11 (5)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (4)</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Myeloablative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIC/non-myeloablative</td>
<td>159 (97)</td>
<td>220 (96)</td>
</tr>
<tr>
<td></td>
<td>5 (3)</td>
<td>8 (4)</td>
</tr>
<tr>
<td>GVHD Prophylaxis (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine/methotrexate</td>
<td>160 (98)</td>
<td>224 (98)</td>
</tr>
<tr>
<td>PTCy/Tacrolimus/MMF</td>
<td>4 (2)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Grades II-IV Acute GVHD, n (%)</td>
<td>38 (23)</td>
<td>25 (11)</td>
</tr>
<tr>
<td>Mod-Severe Chronic GVHD, n (%)</td>
<td>33 (20)</td>
<td>33 (14)</td>
</tr>
</tbody>
</table>

1,2 Missing for 1 patient each.
3 One each of hemophagocytic lymphohistiocytosis and sickle cell anemia.
4 ATG omitted for haploidentical transplants.
5 In the CLD group, FluCy + ATG (n=3), FluMel + ATG (n=1), VP16 + ATG + 5 Gy TBI (n=2), FluThiotepa + ATG (n=1). In the no CLD group, FluCy + ATG (n=6), alemtuzumab + 3 Gy TBI (n=1), Cy + ATG (n=1).

Table 2.
All Etiologies of Chronic Liver Disease.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MASLD</td>
<td>92 (56)</td>
</tr>
<tr>
<td>Iron Overload</td>
<td>77 (47)</td>
</tr>
<tr>
<td>Unknown</td>
<td>18 (11)</td>
</tr>
<tr>
<td>GVHD</td>
<td>15 (9)</td>
</tr>
<tr>
<td>Drug-Induced Liver Injury</td>
<td>11 (7)</td>
</tr>
<tr>
<td>Alcohol Abuse Disorder</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Obstruction</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Sinusoidal Obstructive Syndrome</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Primary Biliary Cirrhosis</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

Table 3. Results of Logistic Regression for Associations with Chronic Liver Disease (CLD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBSC Graft</td>
<td>2.77</td>
<td>0.09</td>
<td>0.84-9.14</td>
</tr>
<tr>
<td>4 Gy TBI with Conditioning</td>
<td>0.36</td>
<td>&lt;0.01</td>
<td>0.17-0.75</td>
</tr>
<tr>
<td>ATG with Conditioning</td>
<td>0.31</td>
<td>0.11</td>
<td>0.07-1.29</td>
</tr>
<tr>
<td>Age at Transplant</td>
<td>0.98</td>
<td>0.03</td>
<td>0.97-1.00</td>
</tr>
<tr>
<td>Hx of Grade 2-4 aGVHD or Moderate-Severe cGVHD</td>
<td>1.97</td>
<td>&lt;0.01</td>
<td>1.23-3.14</td>
</tr>
<tr>
<td>Pre-Transplant CLD</td>
<td>2.14</td>
<td>&lt;0.01</td>
<td>1.33-3.46</td>
</tr>
</tbody>
</table>
Graphical Abstract

Chronic Liver Disease in 2-year survivors of allo-HCT

- Prevalence of CLD: 82%
- MSLD in 56%
- Prevalence of MSLD: 16%

CLD: chronic liver disease
MSLD: metabolic dysfunction-associated steatotic liver disease