Quantitative Assessment of Liver Stiffness Using Ultrasound Shear Wave Elastography in Patients With Chronic Graft-Versus-Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation

A Pilot Study

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Abbreviations

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; SWE, shear wave elastography; SWV, shear wave velocity; US, ultrasound

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Objectives—The purpose of this study was to compare hepatic stiffness on ultrasound (US) shear wave elastography (SWE) in patients with chronic graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation versus patients with no underlying liver disease.

Methods—We performed a retrospective analysis of 4901 patients who underwent abdominal US examinations with adjunctive liver SWE between August 2014 and December 2016. Each patient was scanned supine with gentle breath holding on LOGIQ E9 (GE Healthcare, Waukesha, WI) or Epiq (Philips Healthcare, Andover, MA) US machines (3–6 MHz). Three to 10 measurements were made intercostally in the right hepatic lobe, following manufacturers' guidelines before release of the 2015 Society of Radiologists in Ultrasound consensus or the 2015 Society of Radiologists in Ultrasound consensus. The median and standard deviation of the shear wave velocity (SWV) were obtained. A 2-sample *t* test with the Welch approximation was used for statistical analysis.

Results—Six patients had documented hepatic chronic GVHD or a high clinical suspicion of liver chronic GVHD. All had normal pretransplant liver function test results and no pretransplant or posttransplant hepatic infection. The control group, obtained from the same database, contained 10 patients with normal liver function test results, no abdominal pain, and no history of liver disease or conditions that may have caused liver stiffness changes. The SWVs in patients with chronic GVHD were double those in the control group (1.96 ± 0.28 versus 0.98 ± 0.27 m/s; $P < .0001$).

Conclusions—Patients with chronic GVHD had substantially higher hepatic parenchymal SWVs than patients without liver disease, indicating increased tissue stiffness. To our knowledge, this phenomenon has not been previously reported in chronic GVHD and suggests potential utility of SWE for diagnosis and monitoring of disease progression and the treatment response in this cohort of patients.

Key Words—chronic graft-versus-host disease; hematopoietic stem cell transplantation; liver; shear wave elastography; ultrasound

Allogeneic hematopoietic stem cell transplantation (HSCT) is an effective therapy for leukemia and aplastic anemia, leading to more long-term survivors.[1] Chronic graft-versus-host disease (GVHD) has been shown to be a leading cause of nonrelapse mortality among long-term survivors after transplantation.[2] Chronic GVHD has been increasing for several reasons, including expansion of the donor population, peripheral blood cell transplantation, and donor lymphocyte infusion for the treatment of recurrent disease.[3] Chronic GVHD may progress directly from acute GVHD, follow a quiescent period, or arise de novo even without a history of acute GVHD.

Among long-term transplant survivors, approximately 40% to 73% have chronic GVHD.[4,5] The pathophysiologic mechanism of GVHD is that donor lymphocytes attack and damage the bile canaliculi of the recipients, causing cholestatic liver dysfunction, which may eventually progress to cirrhosis. Liver manifestation is heralded by abnormal laboratory findings, such as elevated serum bilirubin, alkaline phosphatase (AP), and transaminase (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) levels in the setting of cutaneous or gastrointestinal tract GVHD. However, abnormal liver function test results are nonspecific for the diagnosis of hepatic GVHD.

Early diagnosis and robust surveillance methods are essential to direct and assess treatment for this potentially reversible disease. Currently, valid biomarkers and specific imaging diagnoses of hepatic GVHD are largely absent, although some imaging features on ultrasound (US) imaging, computed tomography, and magnetic resonance imaging may suggest disease involvement in the liver[5]: for instance, enhancement of the biliary tract and thickened gallbladder wall, dilatation of the common bile duct, biliary sludge, and pericholecystic fluid.

Currently, liver biopsy is the reference standard for diagnosis and is required to document the disease. However, limitations to biopsy do exist.[6] First, postbiopsy hemorrhage is a feared complication because of hemodynamic instability and coagulopathy, which may be life threatening. Second, these immunocompromised patients have an increased propensity to biopsy-related infections. Finally, false-negative biopsy results may occur from sampling errors, given the patchy distribution of bile duct lesions. Therefore, development of an effective noninvasive technique for the early diagnosis and active surveillance of hepatic chronic GVHD is desirable as a complementary or alternative approach to liver biopsy.

Although there is a plethora of research documenting the utility of US elastography in evaluation of liver stiffness, there is limited research documenting its utility for hepatic disease processes such as chronic GVHD, among which transient elastography (e.g., FibroScan; Echosens North America, Waltham, MA) and acoustic radiation force impulse imaging[7] are the main techniques being investigated. Thus, the goal of this study was to compare hepatic tissue stiffness using US shear wave elastography (SWE) in patients with chronic GVHD after allogeneic HSCT versus patients with no underlying liver disease.

Materials and Methods

Patients and Study Overview (Definition of Hepatic Chronic GVHD)

This work was an Institutional Review Board–approved, Health Insurance Portability and Accountability Act–compliant retrospective study, in which informed consent was waived given the retrospective nature of the study. A search of our database via the Electronic Medical Record Search Engine[8] was performed from August 2014 to December 2016, which yielded 4901 patients who underwent abdominal US examinations with concomitant liver SWE measurements. From this database, 2 separate groups were identified: a chronic GVHD group and a control group.

Inclusion criteria for the chronic GVHD group included those patients with either biopsy-proven hepatic chronic GVHD or those who presented with cholestatic liver dysfunction manifested by elevated serum bilirubin, AP, or transaminase levels, and abdominal pain in the setting of biopsy-proven cutaneous or gastrointestinal tract GVHD, or both. These criteria yielded 6 patients.

Based on SWE values in the chronic GVHD group, a power calculation was performed and indicated that a control group of 10 patients would provide 90% power for detecting a difference of 0.5 m/s at a significance level of less than .05, assuming equal variances. As such, we identified a control group of 10 patients from the overall database. Inclusion criteria for the control group included: normal liver function test results, no history of abdominal pain, and no history of underlying liver disease or conditions that may

have caused altered liver stiffness (ie, hepatitis infection and cardiovascular dysfunction). Patients in the control group had abdominal US examinations with concomitant liver SWE for one of the following reasons: follow-up of appendicitis, weight loss, metastasis/malignancy screening for ocular melanoma or breast cancer/Li-Fraumeni syndrome, or evaluation of cholelithiasis.

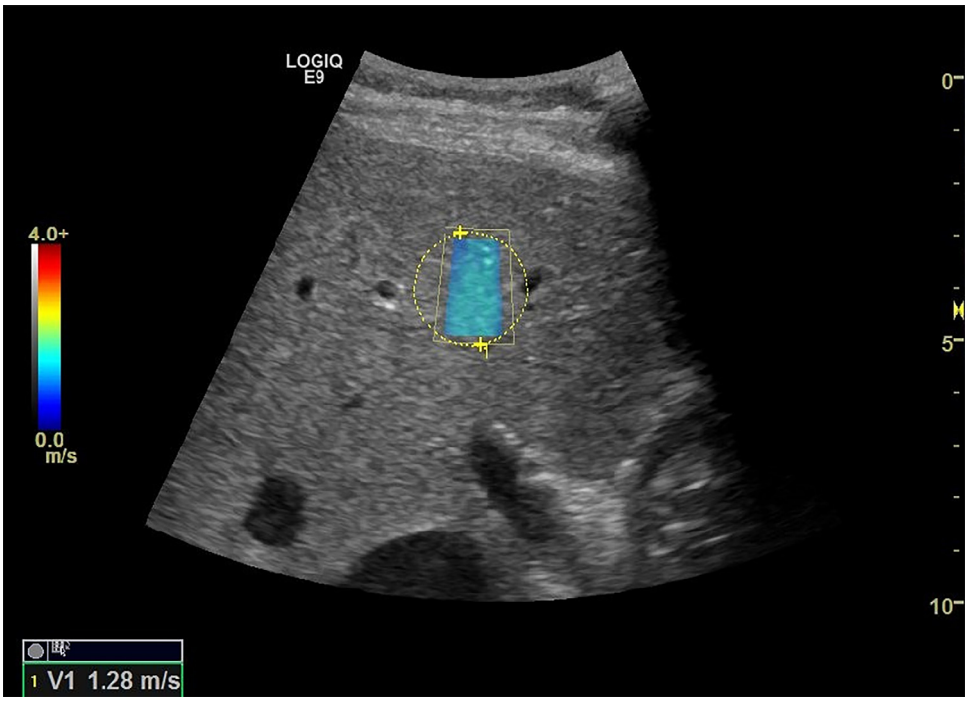
Pertinent clinical data and laboratory values within 3 months of the date of SWE were recorded per patient in a Microsoft Excel file (Microsoft Corporation, Redmond, WA), which included body mass index, spleen length, total bilirubin, AP, ALT, AST, hemoglobin levels, and platelet and leukocyte counts. For the chronic GVHD group, pretransplant and posttransplant laboratory values were obtained within 3 months before HSCT and within 7 months after HSCT, respectively. The mean time ± SD between HSCT and SWE in the chronic GVHD group was 15.8 ± 10.1 months.

Ultrasound and SWE Assessments

Abdominal US examinations with adjunctive SWE were performed on LOGIQ E9 (GE Healthcare, Waukesha, WI) or Epiq (Philips Healthcare, Andover, MA) US machines by registered diagnostic medical sonographers with at least 1 year of experience with SWE. The US examination was performed with the patient fasting for a minimum of 4 hours before the examination. The patient was placed in the supine position with elevation of the right arm above the head. Three- to 6-MHz curved linear transducers were used to image the liver, and static and cine grayscale and color Doppler images were obtained.

Hepatic shear wave velocities (SWVs) were obtained while the patient performed a short period of gentle shallow breath holding, avoiding deep inspiration or the Valsalva maneuver. Three to 10 SWV measurements were made in the intercostal position with the transducer placed over a homogeneous area of parenchyma within the right hepatic lobe, following manufacturers' guidelines before release of the 2015 Society of Radiologists in Ultrasound consensus or the 2015 Society of Radiologists in Ultrasound consensus.[9] Regions of interest measured at least 1 cm^2 and were placed 1.5 to 2 cm below and perpendicular to the hepatic capsule and at least 1 cm apart from the gallbladder and any adjacent large vessels (Figures 1 and 2).

Figure 1. Shear wave elastography of the liver of a patient in the control group.



Retrospective US imaging interpretation was performed, in which images were reviewed on Syngo Dynamics workstations (Siemens Healthineers, Mountain View, CA) by an abdominal imaging fellow with 6 years of research experience in US elastography. Shear wave velocities of the patients were recorded in a Microsoft Excel file.

Statistical Analysis

A 2-sample *t* test with the Welch approximation was used for statistical analysis of SWVs. For patients' demographic and laboratory findings, continuous variables were analyzed by *t* tests, and categorical variables were analyzed by the Fisher exact test. All statistical tests were performed with Microsoft Excel, assuming $P < .05$ for statistical significance.

Results

Six patients (5 female and 1 male; mean age, 51 years; range, 21–69 years) were found to meet the inclusion

Figure 2. Shear wave elastography of the liver of a patient with hepatic chronic GVHD.

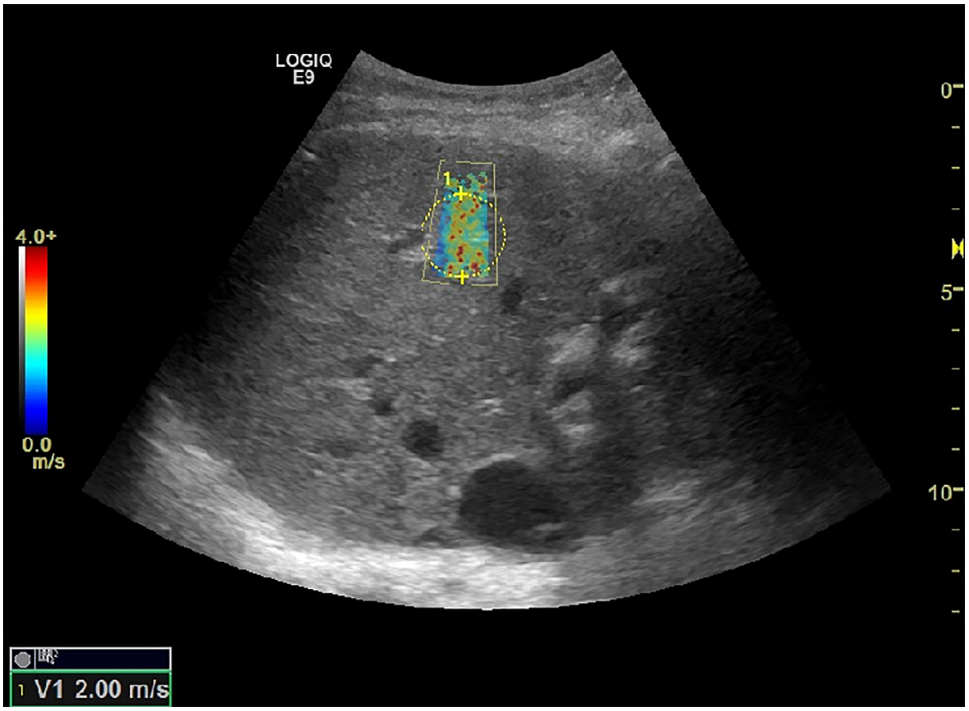
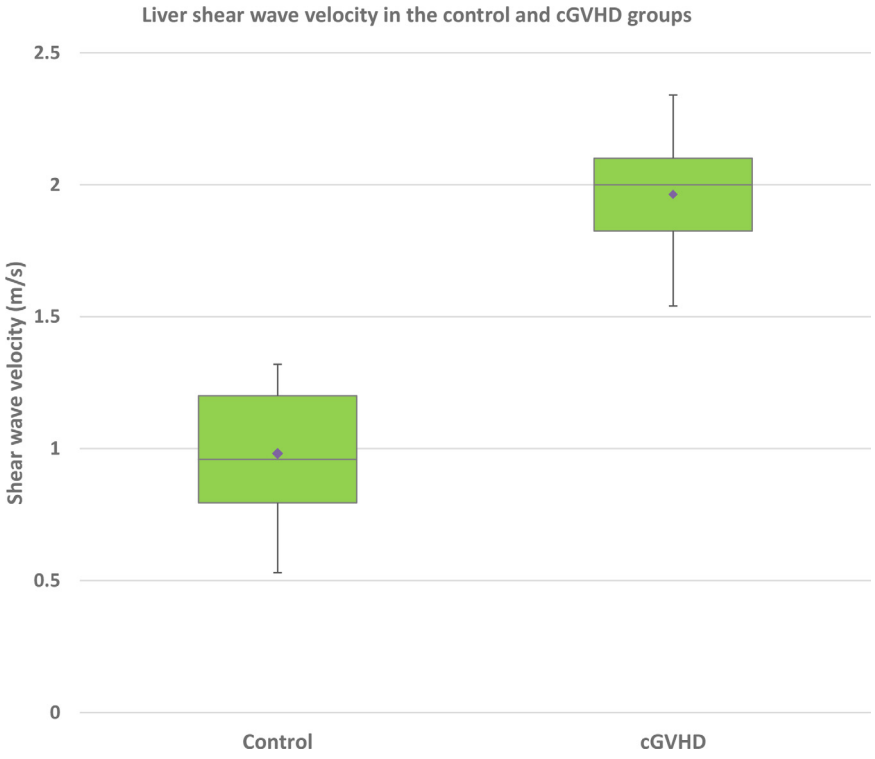


Table 1. History of HSCT and SWE of the 6 Patients With Hepatic Chronic GVHD

Patient	Age, y	Sex	Initial Diagnosis	Transplantation Date	Date of Biopsy-Proven Chronic GVHD	Date of SWE	SWV, m/s
1	69	Female	AML	1/1/2015	9/18/2015	10/19/2015	2.1
2	60	Female	AML	4/1/2015	12/3/2015	3/13/2016	1.9
3	54	Female	AML	1/1/2014	OSH 2014	12/2/2016	2.3
4	43	Female	AML	2/1/2015	3/11/2015	8/5/2016	2.1
5	60	Male	AML	4/1/2014	11/14/2014	6/12/2015	1.8
6	21	Female	ALL	3/1/2016	10/19/2016	11/22/2016	1.5

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; and OSH, outside hospital.

Figure 3. Shear wave velocities in the control and disease groups. cGVHD indicates chronic GVHD.



criteria of the chronic GVHD group. Of note, all had biopsy-proven hepatic chronic GVHD. These 6 patients had normal pretransplantation liver function test results and no pretransplantation or posttransplantation hepatic infection, with laboratory values obtained within 3 months before and within 7 months after HSCT. The indications for liver US were known chronic GVHD with elevated bilirubin, AP, ALT, and AST levels, right upper abdominal pain, or both. Table 1 summarizes the histories of transplantation and SWE of these 6 patients. The control group contained 10 patients (6 female and 4 male; mean age, 46 years; range, 25–69 years) with normal liver function test results, no right upper quadrant pain, and no history of liver disease or conditions that may have caused liver stiffness changes.

Patients' demographic information and laboratory values are summarized in Table 2. The blood count showed a lower hemoglobin level (P = .0007) and platelet count (P = .036) in the chronic GVHD group, which reached statistical significance. The leukocyte count was also low in the chronic GVHD group; however, statistical significance was not reached. Although liver function test results were abnormal in the chronic GVHD group, only the AP level was significantly higher than that of the control group (P = .0064). The SWVs in patients with chronic

Table 2. Patients' Demographic Information and Laboratory Values

Parameter	Disease Group	Control Group	*P*
Patients	6	10	
Age, y	51 ± 17	46 ± 17	.23
Male/female	1/5	4/6	.33
Body mass index, kg/m^2	29.0 ± 12.8	26.5 ± 3.0	.56
Spleen length, cm	11.8 ± 3.12	10.8 ± 1.7	.42
AP, U/L	427.8 ± 348.1	81.0 ± 35.2	.0064
Total bilirubin, mg/dL	1.3 ± 1.3	0.7 ± 0.1	.16
ALT, U/L	140.3 ± 181.6	21.4 ± 8.0	.053
AST, U/L	137.3 ± 199.7	22.8 ± 3.3	.084
Hemoglobin, g/dL	9.7 ± 2.4	13.8 ± 1.4	.0007
Platelets, × 10^3/μL	127.5 ± 116.6	247.5 ± 89.9	.036
Leukocytes, × 10^3/μL	5.0 ± 1.7	8.6 ± 4.4	.078

Data are presented as mean ± SD where applicable.

GVHD were double those in the control group (median ± SD, 1.96 ± 0.28 versus 0.98 ± 0.27 m/s; $P < .0001$; Figure 3).

Discussion

Chronic GVHD is one of the most common complications of allo-HSCT and accounts for a substantial amount of nonrelapsing morbidity and mortality. Despite a limited understanding of the pathophysiologic mechanism of post-transplant chronic GVHD, it appears to be related to inflammation secondary to cell-mediated and humoral immunity, eventually leading to fibrosis. Chronic GVHD may progress directly from acute GVHD, which carries a grim prognosis, may develop after a quiescent period after prior acute GVHD flare-ups, with an intermediate prognosis, or may arise de novo without prior acute GVHD, which has a relatively good prognosis.[1,4]

Diagnosing and grading of chronic GVHD have been challenging because of an inadequate understanding of the pathophysiologic mechanism, lack of biomarkers, and coexistent diseases. These factors can also pose challenges to treatment in this patient population, given the difficulty in diagnosing not only the disease but also the severity of the disease.

Hepatic chronic GVHD typically manifests as cholestasis with laboratory abnormalities, showing elevated AP, serum bilirubin, ALT, and AST levels with or without right upper abdominal pain. The associated histologic findings include lymphocytic infiltration of bile ducts, damage to and loss of small bile ducts, portal fibrosis, and necrosis. Ductopenic GVHD is potentially reversible if immunologic destruction of ductal epithelial cells is halted.[10] Therefore, effective and timely diagnosis is essential for successful treatment.

In this study, we have shown that patients with chronic GVHD had statistically significantly higher hepatic parenchymal SWVs than patients with no underlying liver disease, indicating increased tissue stiffness, which may relate to the effects of chronic inflammation and portal fibrosis. To our knowledge, this phenomenon has not been previously reported in hepatic chronic GVHD and suggests the potential use of US SWE for diagnosis and monitoring of disease progression and the treatment response.

This preliminary retrospective study had some limitations. First, the sample size was small. However, we detected a statistical difference between the groups, suggesting that our study was sufficiently powered. Second, our study only included SWV measurement at a single time point; thus, the reproducibility of the findings was not assessed. Further studies with SWV measurements in patients with GVHD or suspected GVHD should include SWVs measured at different time points and should correlate them with the laboratory findings at those specified time points to assess the disease process or treatment response. Third, we did not investigate possible coexistent conditions such as hepatic veno-occlusive disease, hepatic toxicity, and systemic complications, including cardiopulmonary distress, which may alter hepatic function.

In summary, our results indicate that patients with liver chronic GVHD have greater liver stiffness and resulting elevations in SWVs compared to healthy controls. Given the importance of chronic GVHD as a cause of treatment-related morbidity and mortality in patients with leukemia or aplastic anemia after stem cell transplantation, the need for noninvasive disease monitoring is substantial. Although preliminary, our results support the potential utility of US SWE for use in guidance of diagnosis and treatment assessment in this population of patients.

References

1. Carpenter PA, Kitko CL, Elad S, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-Versus-Host Disease, V: the 2014 Ancillary Therapy and Supportive Care Working Group report. *Biol Blood Marrow Transplant* 2015; 21:1167–1187.
2. Socie G, Stone JV, Wingard JR, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation: Late Effects Working Committee of the International Bone Marrow Transplant Registry. *N Engl J Med* 1999; 341:14–21.
3. Linhares YP, Pavletic S, Gale RP. Chronic GVHD: where are we? Where do we want to be? Will immunomodulatory drugs help? *Bone Marrow Transplant* 2013; 48:203–209.
4. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 2003; 9:215–233.
5. Mahgerefteh SY, Sosna J, Bogot N, Shapira MY, Pappo O, Bloom AI. Radiologic imaging and intervention for gastrointestinal

and hepatic complications of hematopoietic stem cell transplantation. *Radiology* 2011; 258:660–671.

6. Sharma P, McDonald GB, Banaji M. The risk of bleeding after percutaneous liver biopsy: relation to platelet count. *J Clin Gastroenterol* 1982; 4:451–453.
7. Nightingale K. Acoustic radiation force impulse (ARFI) imaging: a review. *Curr Med Imaging Rev* 2011; 7:328–339.
8. Hanauer DA, Mei Q, Law J, Khanna R, Zheng K. Supporting information retrieval from electronic health records: a report of University of Michigan's nine-year experience in developing and using the Electronic Medical Record Search Engine (EMERSE). *J Biomed Inform* 2015; 55:290–300.
9. Barr RG, Ferraioli G, Palmeri ML, et al. Elastography assessment of liver fibrosis: Society of Radiologists in Ultrasound consensus conference statement. *Radiology* 2015; 276:845–861.
10. McDonald GB. Hepatobiliary complications of hematopoietic cell transplantation, 40 years on. *Hepatology* 2010; 51: 1450–1460.