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Original research

Quantitative Assessment of Liver Stiffness in Patients with Chronic Graft-versus-host-disease (cGVHD) following Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) using Ultrasound Shear Wave Elastography: A Pilot Study

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Quantitative Assessment of Liver Stiffness in Patients with Chronic Graft-versus-host-disease (cGVHD) following Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) using Ultrasound Shear Wave Elastography: A Pilot Study

Abstract

Objectives: This study was to compare hepatic stiffness using ultrasound shear wave elastography (SWE) in patients with chronic graft-versus-host-disease (cGVHD) following allogeneic hematopoietic stem cell transplantation with patients with no underlying liver disease. **Methods:** We performed a retrospective analysis of 4901 patients who underwent abdominal ultrasound with adjunct liver SWE between August 2014 to December 2016. Each subject was scanned supine with gentle breath holding on GE Logiq E9 or Philips Epiq (3-6 MHz). 3-10 measurements were made intercostally in the right hepatic lobe following manufacturers' guideline before 2015 SRU consensus being released, or 2015 SRU Consensus. The median and standard deviation of shear wave velocity were obtained. Two-sample t-test with Welch's approximation was used for statistical analysis. **Results:** Six patients had documented hepatic cGVHD or high clinical suspicion of liver cGVHD. All had normal pre-transplant liver function tests and no pre- or post-transplant hepatic infection. The control group, obtained from the same database, contained 10 subjects with normal liver function tests, no abdominal pain or history of liver disease or conditions that may cause liver stiffness change. SWVs in patients with cGVHD were double those in the control group (1.96±0.28 m/s vs. 0.98±0.27 m/s, $p<0.0001$). **Conclusion:** Patients with cGVHD 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 cGVHD, and suggests potential utility of US SWE for diagnosis and monitoring of disease progression and treatment response in this cohort of patients.

Key words: Shear wave elastography, chronic graft-versus-host-disease, ultrasound, liver

Introduction

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 (cGVHD) has been shown to be a leading cause of non-relapse mortality among long-term survivors after transplantation [2]. cGVHD 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]. cGVHD may progress directly from acute GVHD, follow a quiescent period or arise de novo even without a prior history of acute GVHD.

Amongst long-term transplant survivors, approximately 40% –73% suffer from hepatic cGVHD[4, 5]. The pathophysiology 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 and transaminases (AST/ALT) in the setting of cutaneous or gastrointestinal (GI) tract GVHD. However, abnormal liver function tests 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, reliable biomarkers or specific imaging diagnosis of hepatic GVHD is largely absent, although some imaging features on ultrasound (US), Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) 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 gold standard for diagnosis and is required to document the disease. However, limitations to biopsy do exist[6]. First, post biopsy hemorrhage is a feared complication due to 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 may occur from sampling error given the patchy distribution of bile duct lesions. Therefore, development of a reliable non-invasive technique for the early diagnosis and active surveillance of hepatic cGVHD is desirable as a complementary or alternative approach to liver biopsy.

While 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 cGVHD, among which transient elastography, e.g. Fibroscan (Echosens™ North America, Waltham, MA) and acoustic radiation force impulse (ARFI) 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 cGVHD following allogeneic HSCT versus those patients with absent underlying liver disease.

Materials and Methods

Patients and study overview (definition of hepatic cGVHD)

This was an institutional review board approved, HIPAA compliant retrospective study, in which informed consent was waived given the retrospective nature of the study. A search of our database via EMERSE [8] was performed from August 2014 to December 2016, which yielded 4901 patients who underwent abdominal US with concomitant liver SWE measurements. From this database, two separate groups were identified, the cGVHD group and the control group.

Inclusion criteria for the cGVHD group included those patients with either biopsy-proven hepatic cGVHD or those who presented with cholestatic liver dysfunction manifested by elevated serum bilirubin, alkaline phosphatase and transaminases, and/or abdominal pain in the setting of biopsy-proven cutaneous or gastrointestinal tract GVHD. This yielded 6 patients.

Based on SWE values in the cGVHD group, 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 <0.05, assuming equal variances. As such, we identified a control group of 10 patients from the overall database. Inclusion criteria for the control group included: (1) normal liver function tests, (2) no history of abdominal pain and (3) no history of prior underlying liver disease or conditions that may cause altered liver stiffness (i.e. hepatitis infection, cardiovascular dysfunction, etc.). Patients in the control group had abdominal ultrasound with concomitant liver SWE for one of the following reasons: follow-up appendicitis, weight loss, metastasis/malignancy screening for 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 on a Microsoft Excel file (Microsoft Corporation, Redmond, WA, USA), which included body mass index, spleen length, total bilirubin, alkaline phosphatase (AP), alanine transaminase (ALT), aspartate transaminase (AST), hemoglobin, platelets, and leukocytes. For the cGVHD group, pre and post-transplant lab values were obtained within 3 months prior to HSCT and within 7 months after HSCT, respectively. Mean time between HSCT and SWV assessment of the cGVHD group is 15.8 (±10.1) months.

Ultrasound and SWE assessment

Abdominal US with adjunct SWE were performed on GE Logiq E9 (GE Healthcare, Waukesha, WI) or Philips Epiq (Philips Healthcare, Andover, MA) ultrasound machines by registered diagnostic medical sonographers with at least 1 year of experience of SWE. Ultrasound was performed with patient fasting a minimum of 4 hours pre-ultrasound. The patient was placed in supine position with elevation of the right arm above the head. 3-6 MHz curved linear transducers were used to image the liver and static and cine grayscale and color Doppler images were obtained.

Hepatic SWVs were obtained while patient performed a short period of gentle shallow breath holding avoiding deep inspiration or Valsalva maneuver. Three to ten measurements of shear wave velocities (SWVs) were made in the intercostal position with the transducer placed over a homogenous area of parenchyma within the right hepatic lobe following manufacturers' guideline before 2015 SRU consensus being released, or 2015 Society of Radiologists in Ultrasound (SRU) Consensus [9]. Regions of interest (ROIs) measured at least 1 cm^2 in size and were placed 1.5-2 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).

Retrospective US imaging interpretation was performed images were reviewed on Syngo Dynamics workstations (Siemens Healthineers, Mountain View, CA) by an abdominal imaging fellow with 6 years of research experience on US elastography. SWVs of patients were recorded on a Microsoft Excel file (Microsoft Corporation, Redmond, WA, USA).

Statistical analysis

Two-sample t-test with Welch's approximation was used for statistical analysis of SWVs. For patients' demographic and lab findings, continuous variables were analyzed using t-tests and categorical variables were analyzed using Fisher extract test. All statistical tests were performed using Microsoft Excel (Microsoft Inc, Redmond WA), assuming $p < 0.05$ for statistical significance.

Results

Six patients (5 females and 1 male, mean age 51 years, range 21-69) were found to meet the inclusion criteria of the cGVHD group. Of note, all had biopsy-proven hepatic cGVHD. These six patients had normal pre-transplantation liver function tests and no pre- or post-transplantation hepatic infection, with laboratory values obtained within 3 months prior to and within 7 months after HSCT. The indications for liver US were: known cGVHD with elevated bilirubin, alkaline phosphatase, ALT/AST and/or right upper abdominal pain. Table 1 summarizes the history of transplantation and SWE of these 6 patients.

The control group contained 10 subjects (6 females and 4 males, mean age 46 years, range 25-69) with normal liver function tests, no right upper quadrant pain, no history of liver disease or conditions that may cause liver stiffness change.

Patients' demographic information and lab values are summarized in Table 2. Blood count demonstrated lower hemoglobin level ($p = 0.0007$) and platelet count ($p = 0.036$) in the cGVHD group that reached statistical significance. Leukocyte count was also low in the cGVHD group; however, statistical significance was not reached. Although liver function tests were abnormal in the cGVHD group, only alkaline phosphatase level was significantly lower than that of the control group ($p = 0.0064$). SWVs in patients with cGVHD were double those in the control group (1.96 ± 0.28 m/s vs. 0.98 ± 0.27 m/s, $p<0.0001$) (Figure 3).

Discussion

Chronic GVHD is one of the most common complications of allo-HSCT and accounts for a significant amount of non-relapsing morbidity and mortality. Despite limited understanding of the pathophysiology of post-transplant cGVHD, 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, develop following a quiescent period after prior acute GVHD flare-ups, with an intermediate prognosis, or arise de novo without prior acute GVHD which has a relatively good prognosis [1, 4].

Diagnosing and grading of chronic GVHD has been challenging due to inadequate understanding of the pathophysiology, lack of biomarkers and coexistent diseases. This can also pose challenges to

treatment in this patient population given the difficulty in diagnosing not only the disease, but the severity of the disease.

Hepatic cGVHD typically manifests as cholestasis with laboratory abnormalities showing elevated alkaline phosphatase, serum bilirubin and ALT/AST with or without right upper abdominal pain. The associated histological 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, reliable and timely diagnosis is essential for the successful treatment.

In this study, we have shown that patients with cGVHD had statistically significant 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 cGVHD and suggests potential use of US SWE for diagnosis and monitoring of disease progression and treatment response.

This preliminary retrospective study has some limitations. First, the sample size is small. However, we detected a statistical difference between the two groups, suggesting our study was sufficiently powered. Second, our study only includes SWV measured at one-time point, and thus reproducibility of the findings is not assessed. Further studies in which SWV measuring in patients with GVHD or suspected GVHD should include SWVs measured at different time points and correlate them to the laboratory findings at those specified time points to assess disease process or treatment response. Third, we did not investigate possible coexistent conditions such as hepatic venoocclusive disease

(HVOD), hepatic toxicity or systemic complications including cardiopulmonary distress which may alter hepatic function.

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

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Table 1: History of HSCT and shear wave imaging of the six patients with hepatic cGVHD.

Patients with cGVHD	Age	Gender	Initial Diagnosis	Transplantation Date	Date of Biopsy-proven cGVHD	Date of SWE	SWVs (m/s)
1	69	F	AML	1/1/2015	9/18/2015	10/19/2015	2.1
2	60	F	AML	4/1/2015	12/3/2015	3/13/2016	1.9
3	54	F	AML	1/1/2014	OSH 2014	12/2/2016	2.3
4	43	F	AML	2/1/2015	3/11/2015	8/5/2016	2.1
5	60	M	AML	4/1/2014	11/14/2014	6/12/2015	1.8
6	21	F	ALL	3/1/2016	10/19/2016	11/22/2016	1.5

Table 2: Patients' demographic information and lab values.

AP-alkaline phosphatase; ALT-alanine transaminase; AST-aspartate transaminase; Hb-hemoglobin.

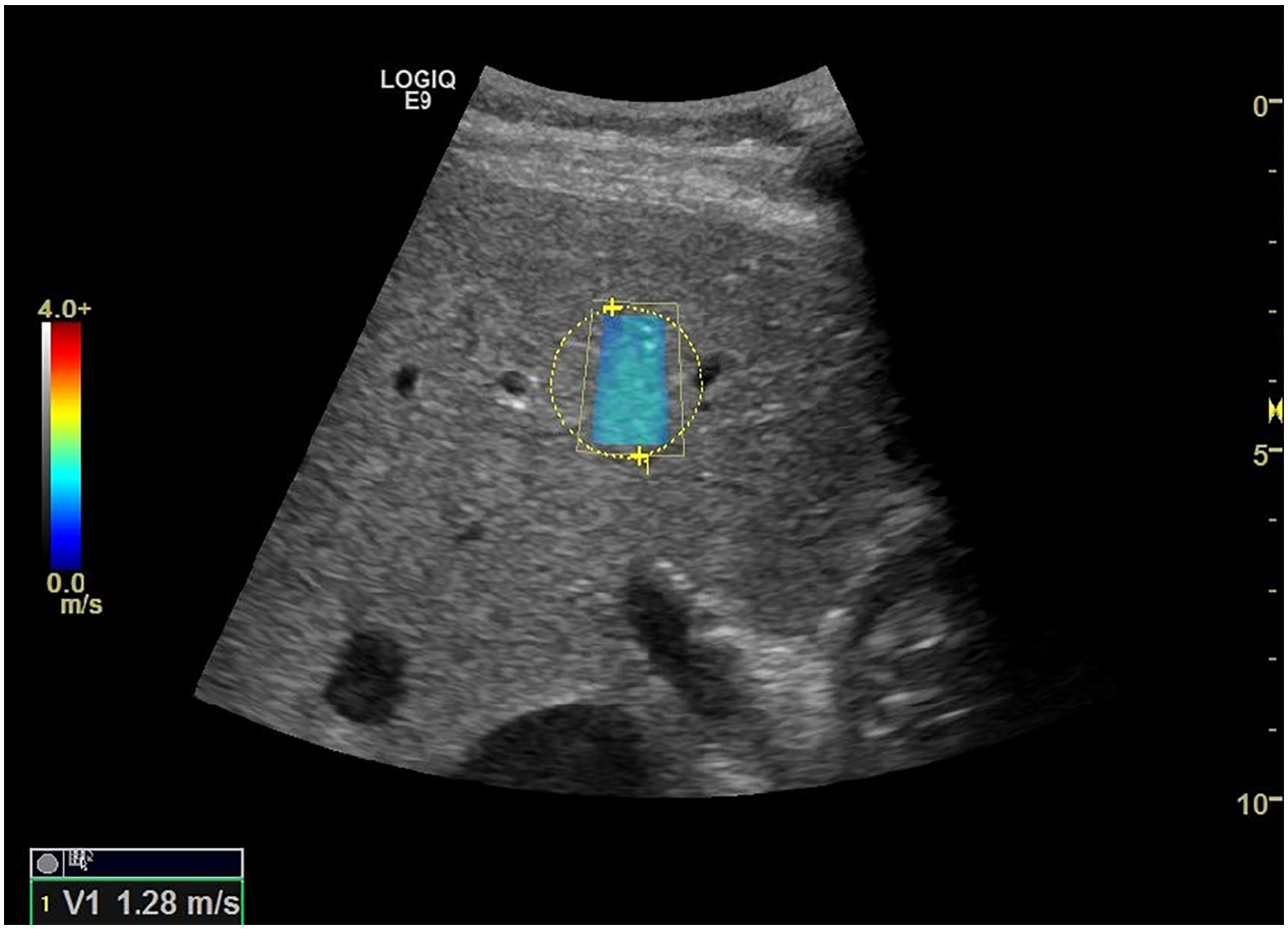
Parameter	Disease Group	Control Group	p-value
No. of patients (n)	6	10	
Age (years)	57±17	46±17	0.23
Gender (male/female)	1/5	4/6	0.33
Body mass index (kg/m^2)	29.0±12.8	26.5±3.0	0.56
Spleen length (cm)	11.8±3.12	10.8±1.7	0.42
AP (U/L)	427.8±348.1	81.0±35.2	**0.0064**
Total bilirubin (mg/dL)	1.3±1.3	0.7±0.1	0.16
ALT (U/L)	140.3±181.6	21.4±8.0	0.053
AST (U/L)	137.3±199.7	22.8±3.3	0.084
Hb (g/dL)	9.7±2.4	13.8±1.4	**0.0007**
Platelets (x10^3/µL)	127.5±116.6	247.5±89.9	**0.036**
Leukocytes (x10^3/µL)	5.0±1.7	8.6±4.4	0.078

Figure Legends:

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

Figure 2: Shear wave elastography of the liver of a patient with hepatic cGVHD.

Figure 3: Shear wave velocities in the control and disease groups.



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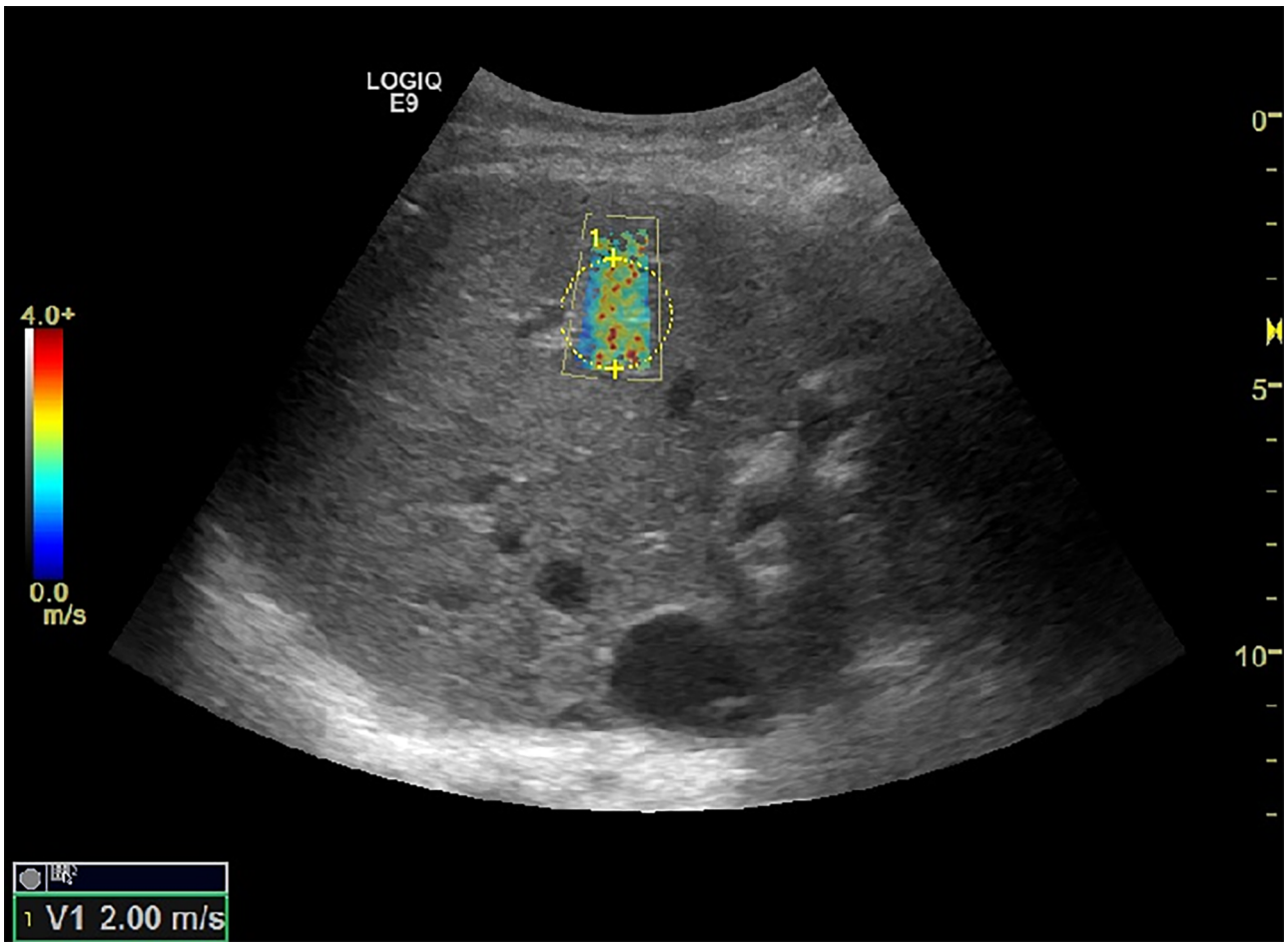

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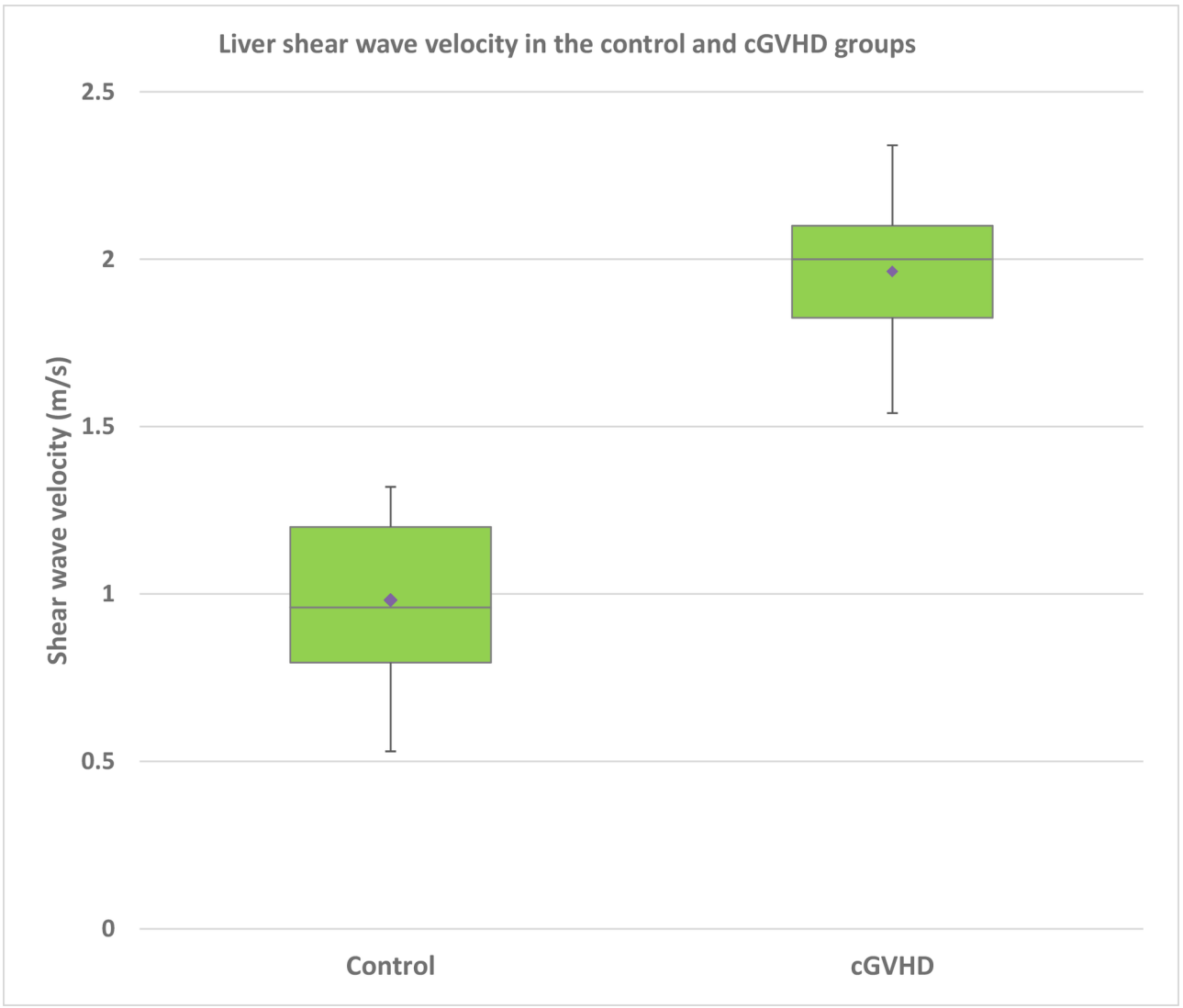

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