Optimal Biomechanical Parameters for Measuring Sclerotic Chronic Graft-Versus-Host Disease

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Skin biomechanical parameters (dynamic stiffness, frequency, relaxation time, creep, and decrement) measured using a myotonometer (MyotonPRO) could inform the management of sclerotic disease. To determine which biomechanical parameter(s) can accurately differentiate patients with sclerotic chronic graft-versus-host disease from post–hematopoietic cell transplant controls, 15 patients with sclerotic chronic graft-versus-host disease and 11 post–hematopoietic cell transplant controls were measured with the myotonometer on 18 anatomic sites. Logistic regression and two machine learning algorithms (least absolute shrinkage and selection operator regression and random forest) were developed to classify subjects. In univariable analysis, frequency had the highest overfit-corrected area under the curve (0.91). Backward stepwise selection and random forest machine learning identified frequency and relaxation time as the optimal parameters for differentiating patients with sclerotic chronic graft-versus-host disease from post–hematopoietic cell transplant controls. Least absolute shrinkage and selection operator regression selected the combination of frequency and relaxation time (overfit-corrected area under the curve = 0.87). Discriminatory ability was maintained when only the sites accessible while the patient is supine (12 sites) were used. We report the distribution of values for these highly discriminative biomechanical parameters, which could inform the assessment of disease severity in future quantitative biomechanical studies of sclerotic chronic graft-versus-host disease.

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INTRODUCTION

Chronic graft-versus-host disease (cGVHD) occurs in 30–40% of patients after allogeneic hematopoietic cell transplantation (HCT) and is the leading cause of nonrelapse mortality in patients surviving >2 years after transplantation (Arai et al., 2015; Socié et al., 1999). Among patients with cGVHD, 20% develop sclerosis within 3 years of initial systemic treatment for cGVHD, resulting in significant disability (Inamoto et al., 2013). However, the lack of quantitative and objective measurements of skin activity has restricted the advancement of treatment options for sclerotic disease (Socié and Ritz, 2014).

The MyotonPRO, a handheld myotonometer that measures soft tissue biomechanical parameters through a noninvasive mechanical impulse, has promise in sclerotic cGVHD management (Baker et al., 2021; Dellalana et al., 2019; Vain, 2012). The device simultaneously extracts five biomechanical parameters: dynamic stiffness, oscillation frequency, relaxation time, creep, and decrement. It has shown high interoperator reliability to measure dynamic stiffness in healthy controls and in patients with sclerotic cGVHD (Chen et al., 2019b; Dellalana et al., 2019). Although a previous study showed the dynamic stiffness parameter’s ability to differentiate patients with sclerosis from healthy volunteers, it did not examine other available parameters and lacked post-HCT controls (Chen et al., 2019a).

With the advent of new diagnostic technologies and quantitative methods, an effective coupling of data to statistical methods, including machine learning, could enhance informed decision making. Application of traditional regression models and machine learning algorithms in the setting of skin biomechanical parameters has the potential to differentiate patients with sclerotic cGVHD from post-HCT controls with greater accuracy.

In this cross-sectional study of myotonometry measurements in patients with cGVHD and in post-HCT controls, we investigated the diagnostic ability of all the five available biomechanical parameters in univariable analysis and explored the diagnostic impact of combinations of parameters selected by stepwise regression and machine learning...
algorithms. We also evaluated whether a convenient supine-only protocol maintains the diagnostic accuracy of total body measurements.

RESULTS
A total of 15 patients with sclerotic cGVHD and 11 post-HCT controls were included in this study. Patient characteristics are summarized in Tables 1 and 2. The median time from HCT was 1,686 days (1–3 quartile: 1,169–2,575 days) for patients with sclerotic cGVHD and 910 days (1–3 quartile: 335–2,507 days) for controls. Subjects were measured with a myotonometer, the MyotonPRO, on 18 anatomic sites (Figure 1). Two patients with cGVHD were measured only on the 12 sites that could be measured with the patient in the supine position owing to discomfort when laying in the prone position. The distribution of parameter values for each anatomic site is shown in Figure 2. Correlation across bilateral sites was high for all the five biomechanical parameters (Table 3). For each subject, we calculated the parameter averages over all the 18 measured sites and over the 12 supine sites. For the averages across all the 18 measured sites, patients with sclerotic cGVHD displayed significant increases in frequency ($P = 0.033$) and stiffness ($P = 0.043$) and decreases in relaxation time ($P = 0.001$) and creep ($P = 0.002$) compared with post-HCT controls (Figure 3). For the averages across the 12 supine sites, patients with sclerotic cGVHD had significant increases in frequency ($P = 0.033$) and decreases in relaxation time ($P = 0.001$) and creep ($P = 0.002$).

Univariable analysis
For each parameter, averages across all sites and averages across supine sites were used to develop univariable logistic regressions that modeled the diagnosis of sclerotic cGVHD as a dichotomous variable. For parameter averages over all the 18 measured sites, the univariable overfit-corrected areas under curve (AUCs) for frequency, relaxation time, stiffness, and creep were 0.91, 0.89, 0.88, and 0.86, respectively (Table 4). For parameter averages across the 12 supine sites, the overfit-corrected AUCs for frequency, relaxation time, stiffness, and creep were 0.87, 0.87, 0.84, and 0.76, respectively.

Backward stepwise selection
Backward stepwise selection using the Akaike information criterion (AIC) was applied to a starting multivariable logistic regression model that consisted of all the five biomechanical parameters. The final model for averages across all the sites consisted of the single parameter frequency, whereas the final model for averages across the supine sites consisted of the single parameter relaxation time.

Machine learning models and bivariable logistic regression
Regression with least absolute shrinkage and selection operator (LASSO) is a machine learning algorithm that performs autonomous variable selection to create reduced models, which can include either a single parameter or a combination of parameters. For both averages across all the sites and averages across the supine sites, LASSO regression selected the combination of frequency and relaxation time as the most important variables in differentiating patients with sclerotic cGVHD from post-HCT controls (overfit-corrected AUC = 0.87 for averages across all the sites and 0.78 for averages across the supine sites) (Table 5).

A random forest classification algorithm ranked the five biomechanical parameters by their importance in differentiating patients with sclerotic cGVHD from post-HCT controls. Ten of 10 iterations (Table 6) identified frequency as the most important variable, followed by relaxation time, consistent with both logistic regression and LASSO regression models.

The overfit-corrected AUCs from bivariable logistic regressions of frequency and relaxation time for averages across all the sites and averages across the supine sites were 0.89 and 0.85, respectively (Table 4).

Analysis of anatomic sites
LASSO regression was performed on each anatomic site. The combinations of parameters determined by LASSO as the most important variables for each site are shown in Table 7. Additional univariable analyses examined the discriminatory ability of each of the five biomechanical parameters for individual anatomic sites (Table 8). Both frequency and relaxation time trended toward a higher discriminatory ability in the shin, upper back, chest, upper arm, and abdomen (Figure 4). Stiffness demonstrated a higher discriminatory ability in the chest, shin, and upper arm, whereas creep had a higher discriminatory ability in the chest and upper back. Decrement had a high discriminatory ability in the dorsal forearm (Table 8).

Correlation between biomechanical parameters
Spearman’s correlation ($r$) between frequency and relaxation time was $-0.94$ for both averages across all the sites and averages across the supine sites (Table 9). High correlation (absolute value of $r$ close to 1) between frequency and relaxation time suggests the presence of redundant information provided by the two parameters. Similarly, stiffness was highly correlated with frequency ($r = 0.95$ for both averages across all the sites and averages across the supine sites) and negatively correlated with relaxation time ($r = -0.93$ and $-0.94$ for averages across all the sites and averages across the supine sites, respectively).

DISCUSSION
Objective measurement of skin sclerosis is an unmet need for many sclerosing diseases, including cGVHD and systemic sclerosis (Carpenter et al., 2015; Odell et al., 2020). The myotonometer is a promising potential response measure instrument, but many questions must be answered before clinical implementation (Table 10). The device has demonstrated high interoperator reliability in measuring stiffness in healthy controls and in patients with sclerotic cGVHD (Chen et al., 2019b; Dellalana et al., 2019). A recent longitudinal study in patients with sclerotic cGVHD showed that stiffness measurements over time correlated with clinically important changes in disease (Baker et al., 2021). Our previously published cross-sectional analysis of myotonometry measurements lacked post-HCT controls and only evaluated the discriminatory ability of the stiffness parameter (Chen et al., 2019a). By investigating all the five available parameters using logistic regression and machine learning models, this study found that measuring frequency...
## Table 1. Demographic and Clinical Characteristics of the 15 Patients with Sclerotic cGVHD

| cGVHD Subjects (n = 15) | Subject ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Summary |
|-------------------------|------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---------|
| Demographics | Age, y | 55 | 52 | 40 | 28 | 72 | 59 | 47 | 47 | 71 | 60 | 14 | 65 | 60 | 35 | 70 | 55 (40–65) |
| | Race | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C | 100% C |
| | Sex | M | M | M | F | M | M | M | F | F | M | M | M | M | M | M | 80% M |
| | BMI | 28 | 24 | 27 | 14 | 31 | 17 | 23 | 34 | 21 | 20 | 22 | 33 | 18 | 23 | 32 | 23 (20–31) |
| Disease characteristics | Disease histology | AML | MDS | MM | HL | ALL | AML | NHL | ALL | AML | MDS | AML | NHL | ALL | CLL | 33% AML |
| | Transplant characteristics | Transplantation source | BM | PB | PB | PB | PB | PB | BM | PB | PB | PB | PB | PB | PB | PB | 12/14 PB |
| | cGVHD characteristics at study entry | NIH score BSA involvement | 3 | 3 | 2 | 3 | 2 | 3 | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 1 | — | 4/14 NIH 1 |
| | | NIH score skin feature | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 80% NIH 3 |
| | MyotonPRO session | Days from HCT | 1,706 | 4,604 | 1,084 | 1,169 | 2,030 | 2,575 | 4,429 | 1,256 | 1,119 | 1,339 | 3,660 | 1,061 | 1,686 | 2,132 | 1,706 (1,169–2,575) |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; BMI, body mass index; BSA, body surface area; C, Caucasian; cGVHD, chronic graft-versus-host disease; CLL, chronic lymphoblastic leukemia; F, female; HCT, hematopoietic cell transplantation; HL, Hodgkin’s lymphoma; ID, identification; M, male; MDS, myelodysplastic disorder; MM, multiple myeloma; NHL, non–Hodgkin’s lymphoma; NIH, National Institutes of Health; PB, peripheral blood.

1Values are shown as median (interquartile range) for continuous variables and n (%) for categorical variables.

2Demographics: C, M, and F.

3Disease characteristics: AML, ALL, CLL, MDS, MM, NHL, and HL.

4Transplant characteristics: PB and BM.

5NIH Score BSA involvement: 1: 1–18%, 2: 19–50%, and 3: >50%; NIH score skin feature: 2 for superficial sclerosis and 3 for deep sclerosis.

6Days from HCT: days between HCT and MyotonPRO measurement session.
Table 2. Demographic and Clinical Characteristics of the 11 Post-HCT Controls

| Post-HCT Controls (n = 11) | Subject ID | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | Summary |
|---------------------------|------------|----|----|----|----|----|----|----|----|----|----|----|---------|
| Demographics<sup>2</sup>  | Age, y     | 66 | 25 | 71 | 52 | 65 | 59 | 59 | 65 | 62 | 63 | 57 | 62 (57–65) |
| Race                      | C          | C  | C  | C  | C  | C  | C  | C  | C  | AAn | C  | AAn | 82% C 82% AAn |
| Sex                       | M          | F  | M  | F  | M  | M  | M  | M  | M  | F  | M  | 73% M       |
| BMI                       | 26         | 40 | 26 | 43 | 31 | 24 | 27 | 26 | 46 | 35 | 31 | 26 (26–40)  |
| Disease characteristics<sup>3</sup> | Disease histology | MF | AA | AML | MDS | NHL | AML | CLL | MDS | AML | AML | ALL | 36% AML 18% MDS 9% ALL 9% MDS 9% AA 9% NHL 9% CLL |
| Transplant characteristics<sup>4</sup> | Transplant source | PB | BM | PB | CB | PB | PB | PB | PB | -  | PB | PB | 8/10 PB 1/10 BM 1/10 CB |
| MyotonPRO session         | Days from HCT<sup>5</sup> | 329 | 1,785 | 1,301 | 3,276 | 910 | 411 | 2,507 | 4,046 | 857 | 335 | 148 | 910 (335–2,507) |

Abbreviation: AA, aplastic anemia; AAn, African American; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; BMI, body mass index; C, Caucasian; CB, cord blood; CLL, chronic lymphoblastic leukemia; F, female; HCT, hematopoietic cell transplantation; ID, identification; M, male; MDS, myelodysplastic disorder; MF, primary myelofibrosis; NHL, non–Hodgkin’s lymphoma; PB, peripheral blood.

1Values are shown as median (interquartile range) for continuous variables and n (%) for categorical variables.
2Demographics: C, AAn, M, and F.
3Disease characteristics: AML, ALL, CLL, MDS, MF, AA, and NHL.
4Transplant characteristics: PB, BM, and CB.
5Days from HCT: days between HCT and MyotonPRO measurement session.

Figure 1. The 18 measured sites.
Green circles represent the 12 sites that are measured in the supine position.
and relaxation time offers the highest diagnostic yield in differentiating patients with sclerotic cGVHD from post-HCT controls.

Although all models placed importance on frequency and relaxation time, whether these parameters should be used alone or in combination requires further consideration.

Figure 2. Box and whisker plots of the parameter values by sites in patients with sclerotic cGVHD (red) and in post-HCT controls (blue). Sites are displayed in descending order of the frequency AUCs shown in Figure 4. Bilateral sites are displayed next to each other. The bottom and top boundaries of each box represent the 25th and 75th quartiles, respectively; the horizontal line within the box represents the median; and the whiskers represent the minimum and maximum values. AUC, area under the curve; cGVHD, chronic graft-versus-host disease; HCT, hematopoietic cell transplantation; L, left; R, right.
Possible redundant information between frequency and relaxation time, suggested by the high Spearman's correlation, may limit the added explanatory value provided by the inclusion of the second parameter. Accordingly, with overfit correction, bivariable frequency and relaxation time did not outperform either univariable frequency or univariable relaxation time analyses. Similarly, backward elimination selected single parameter models (frequency for averages across all the sites and relaxation time for averages across the supine sites) over models with combinations of parameters. In contrast, the LASSO algorithm, which also had the ability to select either a single variable or a combination of variables, favored models using the combination of frequency and relaxation time over single-variable models. An independent data set is needed to validate and compare the classification performance of frequency, relaxation time, and the combination of the two parameters.

This study also informs the development of future myotonometer measurement protocols. Because the AUCs of the parameter averages across the 12 supine sites did not differ significantly from those across all the 18 measured sites, an expedient supine-only measurement protocol may be implemented to reduce measurement time and improve patient experience while maintaining diagnostic accuracy. In addition, the dorsal forearm, which had previously been found to have low interobserver repeatability (Dellalana

### Table 3. Spearman’s Correlation Coefficients between Left and Right Side Parameter Measurements for all the Five Biomechanical Parameters in Patients with Sclerotic cGVHD and in Post-HCT controls

| Subject Group | Frequency | Relaxation Time | Stiffness | Creep | Decrement |
|---------------|-----------|----------------|-----------|-------|-----------|
| Sclerotic cGVHD | 0.812 | 0.879 | 0.859 | 0.847 | 0.838 |
| Post-HCT controls | 0.868 | 0.915 | 0.955 | 0.842 | 0.843 |

Abbreviations: cGVHD, chronic graft-versus-host disease; HCT, hematopoietic cell transplantation.
et al., 2019), exhibited the lowest AUCs for both frequency and relaxation time in this study. Thus, the dorsal forearm may be omitted or substituted with another site, such as the volar forearm, in future measurement protocols. Finally, we report the distribution of values of biomechanical parameters for patients with sclerotic cGVHD and for post-HCT controls, which could inform the design of future myotonometer studies.

This study has several limitations. Of the 11 post-HCT controls, 3 subjects were measured within 12 months of HCT (Table 2) and could have had subclinical cGVHD at the time of measurement. In addition, the myotonometer does not distinguish between cGVHD changes in the skin, changes in subcutaneous tissue, and changes in fascia and skeletal muscle (Hu et al., 2018; Oda et al., 2009). The measurements represent contributions from all underlying tissues.

Table 4. Univariable and Bivariable Logistic Regression AUCs (with and without Overfit Correction) and 95% CIs for Averages across all the Measured Sites (n for Sclerotic cGVHD = 13, n for Post-HCT controls = 11) and Averages across the 12 Supine Sites (n for Sclerotic cGVHD = 15, n for Post-HCT Controls = 11)

| Parameter(s)          | Across all the 18 Measured Sites | Across the 12 Supine Sites | P-Value | Across all the 18 Measured Sites | Across the 12 Supine Sites | P-Value |
|-----------------------|----------------------------------|----------------------------|---------|----------------------------------|----------------------------|---------|
| Frequency             | 0.909 (0.786–1.000)              | 0.890                      | 0.014   | 0.873 (0.725–1.000)              | 0.873                      | 0.011   |
| Relaxation Time       | 0.888 (0.756–1.000)              | 0.875                      | 0.011   | 0.873 (0.737–1.000)              | 0.874                      | 0.011   |
| Stiffness             | 0.874 (0.734–1.000)              | 0.875                      | 0.012   | 0.842 (0.692–0.993)              | 0.840                      | 0.012   |
| Creep                 | 0.860 (0.711–1.000)              | 0.862                      | 0.014   | 0.767 (0.581–0.953)              | 0.763                      | 0.026   |
| Decrement             | 0.615 (0.361–0.869)              | 0.594                      | 0.184   | 0.576 (0.347–0.804)              | 0.503                      | 0.497   |
| Frequency and relaxation time | 0.916 (0.801–1.000) | 0.890 |       | 0.879 (0.732–1.000)              | 0.851                      |         |

Abbreviations: AUC, area under the curve; cGVHD, chronic graft-versus-host disease; CI, confidence interval; HCT, hematopoietic cell transplantation.

Table 5. LASSO Regression Coefficients and AUCs of 10 Repeats of 3-Fold Cross-Validation for Averages across All the 18 Measured Sites (n for Sclerotic cGVHD = 13, n for Post-HCT Controls = 11) and Averages across the 12 Supine Sites (n for Sclerotic cGVHD 15, n for Post-HCT Controls 11)

| Rep | AUC without Overfit Correction (95% CI) | AUC with Overfit Correction (95% CI) | Frequency | Relaxation Time | Stiffness | Creep | Decrement |
|-----|----------------------------------------|-------------------------------------|-----------|----------------|-----------|-------|-----------|
| 1   | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.722     | –0.050         | –         | –     | –         |
| 2   | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.592     | –0.030         | –         | –     | –         |
| 3   | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.300     | –              | –         | –     | –         |
| 4   | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.456     | –0.008         | –         | –     | –         |
| 5   | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.592     | –0.030         | –         | –     | –         |
| 6   | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.524     | –0.019         | –         | –     | –         |
| 7   | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.904     | –0.076         | –         | –     | –         |
| 8   | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.040     | –              | –         | –     | –         |
| 9   | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.383     | –              | –         | –     | –         |
| 10  | 0.909 (0.786–1)                        | 0.871 (0.641–0.998)                | 1.592     | –0.030         | –         | –     | –         |

| Rep | AUC without Overfit Correction (95% CI) | AUC with Overfit Correction (95% CI) | Frequency | Relaxation Time | Stiffness | Creep | Decrement |
|-----|----------------------------------------|-------------------------------------|-----------|----------------|-----------|-------|-----------|
| 1   | 0.873 (0.725–1)                        | 0.778 (0.429–0.978)                | 1.161     | –0.139         | –         | –     | –         |
| 2   | 0.873 (0.725–1)                        | 0.778 (0.429–0.978)                | 0.970     | –0.014         | –         | –     | –         |
| 3   | 0.873 (0.725–1)                        | 0.778 (0.429–0.978)                | 1.040     | –0.061         | –         | –     | –         |
| 4   | 0.867 (0.721–1)                        | 0.777 (0.429–0.978)                | 1.627     | –2.367         | –0.542    | 1.500 | –0.095    |
| 5   | 0.873 (0.725–1)                        | 0.778 (0.429–0.978)                | 1.005     | –0.038         | –         | –     | –         |
| 6   | 0.873 (0.725–1)                        | 0.778 (0.429–0.978)                | 1.161     | –0.139         | –         | –     | –         |
| 7   | 0.873 (0.725–1)                        | 0.778 (0.429–0.978)                | 1.040     | –0.061         | –         | –     | –         |
| 8   | 0.873 (0.725–1)                        | 0.778 (0.429–0.978)                | 1.161     | –0.139         | –         | –     | –         |
| 9   | 0.873 (0.725–1)                        | 0.778 (0.429–0.978)                | 0.923     | –              | –         | –     | –         |
| 10  | 0.873 (0.725–1)                        | 0.778 (0.429–0.978)                | 1.040     | –0.061         | –         | –     | –         |

Abbreviations: AUC, area under the curve; cGVHD, chronic graft-versus-host disease; HCT, hematopoietic cell transplantation; LASSO, least absolute shrinkage and selection operator; Rep, repetition.
soft tissue layers, and the proportional contribution of each layer is unknown. Our study design lacked an independent validation data set. Although bootstrapping was used to generate confidence intervals, an independent validation set is required to assess model accuracy. Finally, the device’s ability to differentiate clinically sclerotic sites from clinically normal sites is an important question precluded by our study design. For clinical implementation, a future study that follows patients before clinical signs of sclerosis through the subsequent appearance of sclerosis is required.

In conclusion, this study shows that a brief clinical protocol measuring the frequency and relaxation time of patients’ skin can effectively differentiate patients with sclerotic cGVHD from post-HCT controls. A larger follow-up study is needed for model validation.

**MATERIALS AND METHODS**

**Data collection**

The study protocol was approved by the Institutional Review Board at Vanderbilt (Institutional Review Board number: 170456) and the U.S. Department of Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN (Institutional Review Board number: 1170055). A total of 15 patients with sclerotic cGVHD (National Institutes of Health skin features scores 2–3) and 11 post-HCT controls were recruited. All participants provided written informed consent. Subjects underwent MyotonPRO measurement by a single observer (F.C.) according to our previously published protocol (Chen et al., 2019a) with a 12 mm disk attachment. Each subject was measured on 9 anatomic sites bilaterally over completely relaxed muscles (shin over tibialis anterior, dorsal forearm over extensor digitorum, upper arm over biceps brachii caput longum, shoulder over medial deltoideus, chest over pectoralis major, abdomen over rectus abdominus, calf over gastrocnemius caput laterale, upper back over medial trapezius, and lower back over erector spinae), resulting in 18 total measurement sites. Each measurement session

Table 6. MyotonPRO Biomechanical Parameters in Descending Order of Feature Importance (1 = most important, 5 = least important) Selected by Random Forest Models (10 iterations) for Averages across All the Sites (n for cGVHD = 13, n for control = 11) and Averages Across the Supine Sites (n for cGVHD = 15, n for control = 11)

| Parameter    | Feature Importance |
|--------------|--------------------|
| Frequency    | 1                  |
| Relaxation time | 2                |
| Stiffness    | 4                  |
| Creep        | 3                  |
| Decrement    | 5                  |

Averages Across the Supine Sites

| Parameter    | Feature Importance |
|--------------|--------------------|
| Frequency    | 1                  |
| Relaxation time | 2                |
| Stiffness    | 3                  |
| Creep        | 4                  |
| Decrement    | 5                  |

Abbreviation: cGVHD, chronic graft-versus-host disease. Note the conserved order apart for the position of stiffness and creep.

Table 7. Representative Combinations for Parameters Selected by LASSO Regression as the Most Important Variables in Differentiating Patients with Sclerotic cGVHD from Post-HCT Controls for Each Site

| Sites            | Frequency | Relaxation Time | Stiffness | Creep | Decrement | AUC with Overfit Correction (95% CI) |
|------------------|-----------|-----------------|-----------|-------|-----------|-------------------------------------|
| Abdomen L        | +         | +               | +         | +     | +         | 0.884 (0.674–0.996)                  |
| Abdomen R        | +         | +               | +         |       |           | 0.902 (0.737–0.999)                  |
| Upper Arm L      | +         |                 | +         |       |           | 0.773 (0.431–0.981)                  |
| Upper Arm R      | +         |                 | +         |       |           | 0.773 (0.422–0.983)                  |
| Chest L          | +         | +               | +         | +     |           | 0.801 (0.437–0.992)                  |
| Chest R          |           | +               |           |       |           | 0.829 (0.546–0.992)                  |
| Upper back L     | +         |                 |           |       |           | 0.840 (0.533–0.994)                  |
| Upper back R     | +         |                 |           |       |           | 0.756 (0.358–0.987)                  |
| Shin L           |           | +               |           |       |           | 0.855 (0.606–0.992)                  |
| Shin R           | +         | +               |           |       |           | 0.761 (0.407–0.978)                  |
| Calf L           | +         |                 |           |       |           | 0.741 (0.404–0.964)                  |
| Calf R           | +         | +               | +         | +     |           | 0.780 (0.411–0.986)                  |
| Lower back L     | +         | +               |           |       |           | 0.812 (0.445–0.988)                  |
| Lower back R     | +         |                 | +         |       |           | 0.740 (0.327–0.979)                  |
| Shoulder L       | +         | +               | +         | +     |           | 0.725 (0.381–0.955)                  |
| Shoulder R       | +         | +               | +         | +     |           | 0.757 (0.392–0.981)                  |
| Dorsal forearm L | +         |                 |           |       |           | 0.782 (0.427–0.985)                  |
| Dorsal Forearm R |           |                 |           |       |           | 0.846 (0.559–0.995)                  |
| Average across all sites | +         | +               |           |       |           | 0.871 (0.641–0.998)                  |
| Average across supine sites | +         | +               |           |       |           | 0.778 (0.429–0.978)                  |

Abbreviations: AUC, area under the curve; cGVHD, chronic graft-versus-host disease; CI, confidence interval; HCT, hematopoietic cell transplantation; L, left; LASSO, least absolute shrinkage and selection operator; R, right. Representative: of 10 repetitions, the most commonly selected combination of parameters for each site is shown.
| Site                | Frequency AUC (95% CI) | Overfit-Corrected AUC | Relaxation Time AUC (95% CI) | Overfit-Corrected AUC | Stiffness AUC (95% CI) | Overfit-Corrected AUC | Creep AUC (95% CI) | Overfit-Corrected AUC | Decrement AUC (95% CI) |
|---------------------|------------------------|-----------------------|-------------------------------|-----------------------|------------------------|-----------------------|---------------------|----------------------|------------------------|
| Abdomen L           | 0.824 (0.665–0.983)    | 0.825                 | 0.873 (0.737–1)               | 0.871                 | 0.661 (0.437–0.884)   | 0.652                 | 0.739 (0.543–0.936) | 0.728                | 0.661 (0.436–0.885)   |
| Abdomen R           | 0.939 (0.845–1)        | 0.949                 | 0.873 (0.725–1)               | 0.878                 | 0.745 (0.550–0.941)   | 0.743                 | 0.758 (0.567–0.949) | 0.753                | 0.606 (0.376–0.836)   |
| Upper arm L         | 0.861 (0.715–1)        | 0.852                 | 0.788 (0.610–0.966)           | 0.778                 | 0.855 (0.702–1)      | 0.848                 | 0.667 (0.452–0.882) | 0.627                | 0.600 (0.373–0.827)   |
| Upper arm R         | 0.824 (0.656–0.992)    | 0.818                 | 0.776 (0.593–0.959)           | 0.766                 | 0.842 (0.693–0.992)   | 0.833                 | 0.648 (0.431–0.866) | 0.607                | 0.582 (0.350–0.814)   |
| Chest L             | 0.861 (0.718–1)        | 0.853                 | 0.836 (0.675–0.998)           | 0.828                 | 0.812 (0.642–0.983)   | 0.801                 | 0.779 (0.599–0.958) | 0.769                | 0.491 (0.260–0.722)   |
| Chest R             | 0.812 (0.645–0.979)    | 0.810                 | 0.885 (0.755–1)               | 0.884                 | 0.812 (0.646–0.978)   | 0.804                 | 0.873 (0.730–1)     | 0.874                | 0.588 (0.359–0.817)   |
| Upper back L        | 0.851 (0.697–1)        | 0.852                 | 0.877 (0.739–1)               | 0.877                 | 0.779 (0.594–0.965)   | 0.777                 | 0.877 (0.731–1)     | 0.877                | 0.799 (0.622–0.976)   |
| Upper back R        | 0.821 (0.648–0.995)    | 0.819                 | 0.818 (0.624–1)               | 0.815                 | 0.727 (0.515–0.939)   | 0.706                 | 0.825 (0.632–1)     | 0.824                | 0.773 (0.583–0.963)   |
| Shin L              | 0.8 (0.609–0.991)      | 0.788                 | 0.836 (0.662–1)               | 0.829                 | 0.824 (0.643–1)      | 0.815                 | 0.818 (0.639–0.997) | 0.811                | 0.733 (0.533–0.933)   |
| Shin R              | 0.788 (0.595–0.98)     | 0.779                 | 0.824 (0.661–0.987)           | 0.816                 | 0.861 (0.719–1)      | 0.853                 | 0.770 (0.584–0.956) | 0.762                | 0.664 (0.443–0.884)   |
| Calf L              | 0.782 (0.593–0.97)     | 0.771                 | 0.764 (0.563–0.964)           | 0.747                 | 0.782 (0.583–0.980)   | 0.762                 | 0.721 (0.510–0.932) | 0.7       | 0.582 (0.349–0.814)   |
| Calf R              | 0.818 (0.631–1)        | 0.808                 | 0.800 (0.611–0.989)           | 0.788                 | 0.861 (0.690–1)      | 0.857                 | 0.752 (0.553–0.95)  | 0.738                | 0.491 (0.251–0.731)   |
| Lower back L        | 0.804 (0.623–0.986)    | 0.801                 | 0.776 (0.586–0.966)           | 0.773                 | 0.790 (0.605–0.975)   | 0.786                 | 0.657 (0.427–0.888) | 0.628                | 0.713 (0.492–0.935)   |
| Lower back R        | 0.685 (0.465–0.906)    | 0.665                 | 0.755 (0.556–0.955)           | 0.75                  | 0.783 (0.593–0.973)   | 0.781                 | 0.699 (0.484–0.914) | 0.671                | 0.678 (0.453–0.904)   |
| Shoulder L          | 0.594 (0.360–0.828)    | 0.517                 | 0.655 (0.428–0.881)           | 0.607                 | 0.673 (0.449–0.896)   | 0.627                 | 0.630 (0.398–0.862) | 0.554                | 0.567 (0.334–0.799)   |
| Shoulder R          | 0.691 (0.478–0.904)    | 0.681                 | 0.752 (0.561–0.942)           | 0.741                 | 0.776 (0.595–0.956)   | 0.779                 | 0.733 (0.528–0.938) | 0.706                | 0.515 (0.279–0.751)   |
| Dorsal forearm L    | 0.594 (0.350–0.838)    | 0.545                 | 0.570 (0.323–0.817)           | 0.515                 | 0.503 (0.260–0.746)   | 0.433                 | 0.618 (0.370–0.866) | 0.568                | 0.824 (0.663–0.985)   |
| Dorsal forearm R    | 0.685 (0.465–0.905)    | 0.671                 | 0.648 (0.427–0.870)           | 0.618                 | 0.594 (0.367–0.821)   | 0.555                 | 0.673 (0.458–0.887) | 0.650                | 0.915 (0.788–1)      |
| Average across 18 sites | 0.909 (0.786–1)       | 0.910                 | 0.888 (0.756–1)               | 0.890                 | 0.874 (0.734–1)      | 0.875                 | 0.860 (0.711–1)     | 0.862                | 0.615 (0.361–0.869)   |

Abbreviation: AUC, area under the curve; CI, confidence interval; L, left; R, right.
lasted ~20 minutes. Two of the patients with cGVHD were measured only on the 12 sites that could be measured with the patient in the supine position (bilateral shin, dorsal forearm, upper arm, shoulder, chest, and abdomen) owing to discomfort when laying in the prone position. These supine-only measurement sessions lasted ~10–15 minutes.

**Statistical analysis**

For each subject, we calculated the parameter averages over all the 18 measured sites and over the 12 supine sites. The Wilcoxon rank-sum test was performed to compare the distributions of parameter averages between patients with sclerotic cGVHD and post-HCT controls. Spearman’s correlation coefficient was calculated for each pair of the parameters. We evaluated the discriminatory ability of the parameters using (i) univariable logistic regression for each parameter, (ii) backward stepwise selection on the five-parameter logistic regression model, (iii) bivariable logistic regression consisting of frequency and relaxation time, (iv) LASSO regression, and (v) random forest. The AUC was used to compare model performances. Overfit-corrected AUCs were calculated from bootstrap 0.632+ method with 100 bootstrap replications (Efron and Tibshirani, 1997). All analyses were completed using R, version 3.6.1.

A backward stepwise selection was performed by eliminating one parameter at a time from the full five-parameter model to minimize the AIC. The AIC assesses the models’ goodness of fit while penalizing model complexity. A smaller AIC value suggests a simpler model with a better fit. The final model is reached when the elimination of any remaining parameter no longer reduces the AIC.

LASSO is a machine learning algorithm that extends standard logistic regression models by enabling variable selection in the fitting process (Friedman et al., 2010). All variables were standardized before LASSO regression. The R package glmnet was applied to build a LASSO regression (Friedman et al., 2021). Each analysis

| Parameter | Frequency | Relaxation Time | Stiffness | Creep | Decrement |
|-----------|-----------|----------------|-----------|-------|-----------|
| Averages Across All Sites | Frequency | 1 | -0.94 | 0.95 | -0.86 | -0.28 |
| | Relaxation time | -0.94 | 1 | -0.93 | 0.94 | 0.41 |
| | Stiffness | 0.95 | -0.93 | 1 | -0.82 | -0.22 |
| | Creep | -0.86 | 0.94 | -0.82 | 1 | 0.54 |
| | Decrement | -0.28 | 0.41 | -0.22 | 0.54 | 1 |

| Parameter | Frequency | Relaxation Time | Stiffness | Creep | Decrement |
|-----------|-----------|----------------|-----------|-------|-----------|
| Averages Across Supine Sites | Frequency | 1 | -0.94 | 0.95 | -0.87 | -0.10 |
| | Relaxation time | -0.94 | 1 | -0.94 | 0.94 | 0.15 |
| | Stiffness | 0.95 | -0.94 | 1 | -0.87 | -0.04 |
| | Creep | -0.87 | 0.94 | -0.87 | 1 | 0.32 |
| | Decrement | -0.10 | 0.15 | -0.04 | 0.32 | 1 |

Abbreviation: cGVHD, chronic graft-versus-host disease.

Higher absolute values of Spearman’s correlation coefficient represent a greater correlation between the two parameters.
Random forest is an ensemble learning algorithm that uses sets of classification or regression trees to evaluate the potential variables and rank them according to their relative strength in predicting the outcome (Strobl et al., 2009). The R package randomForest was applied to build the random forest classification algorithm (Breiman et al., 2018). Random forest analysis ranked the five biomechanical parameters by their importance in differentiating patients with sclerotic cGVHD from post-HCT controls.

Data availability statement

Datasets related to this article can be found at https://doi.org/10.17632/6mdy96dkxz.1, an open-source online data repository hosted at Mendeley Data (Baker et al., 2021).

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CONFLICT OF INTEREST

AV is the inventor of the Myoton but has no financial interest in the device. The remaining authors state no conflict of interest.

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