RESEARCH ARTICLE

Evaluation of treatment response in oral chronic graft-versus-host patients by power doppler ultrasound: a prospective study

¹Yiqun Liu, ¹Jiaan Zhu, ²Huijie Guo, ¹Wenxue Li, ¹Xiuming Wang, ¹Zheng Chen, ¹Fang Liu and ¹Ariun Bilig

¹Department of Ultrasound, Peking University People's Hospital, 11 Xizhimen South Street, Beijing 100044, People's Republic of China; ²Department of Stomatology, Peking University People's Hospital, 11 Xizhimen South Street, Beijing 100044, People's Republic of China

Objective: To date, imaging and non-invasive examination methods for evaluating the oral tissue of patients who with oral chronic graft-versus-host disease (GVHD) are still lacking. Herein, we assessed the ultrasonography imaging characteristics of chronic GVHD patients' buccal soft tissue and evaluated the response to treatment using gray-scale and power Doppler (PD) ultrasound. The overall objective of the study was to determine the potential of ultrasonography to provide an early and objective indication of a therapeutic response to treatment intervention in oral chronic GVHD.

Methods: Buccal mucosa in 21 patients with clinically diagnosis of oral chronic GVHD were assessed before and after 14day treatment between 2016 and 2018. Ultrasonography assessment included thickness and echogenicity evaluation, and PD. Echogenicity and PD were measured and scored according to a semi-quantitative method. Ultrasonography parameters were compared with clinical activity assessments of disease activity and patient-reported measures.

Results: Following 14 day therapy, a significant decrease in PD scores and patient-reported measures was observed ($p < 0.01$), and clinical assessment scores also decreased ($p < 0.05$). Changes from baseline PD scores correlated significantly with patient-reported measures ($r = 0.85$, $p < 0.01$). Changes in PD scores correlated moderately with the changes in the clinical assessments from baseline ($r = 0.46$, $p < 0.05$). A significant decrease in PD scores was observed in the buccal soft tissue of patients that responded clinically to the treatment. A significant decrease in PD scores was also observed in patients that were failed to show clinical improvement ($p < 0.05$).

Conclusion: Ultrasonography is a novel and feasible imaging technique for measurement of the response of oral chronic GVHD to therapy. Ultrasonography yields additional information about buccal mucosa and is a complementaty to clinical examination.

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Keywords: Graft-versus-host disease; Mouth mucosa; Ultrasonography

Introduction

Chronic graft-versus-host disease (GVHD) is a serious complication that commonly occurs after allogeneic haematopoietic stem cell transplantation (HSCT),

affecting 30–70% of survivors.¹ Although chronic GVHD can affect many organs, including the skin, mouth, liver, lung, and eyes, the oral cavity is one of the most frequently affected organ systems, with a 45–83% prevalence in chronic GVHD patients.^{2–4} The pathogenesis of chronic GVHD basically involves the recognition by

Correspondence to: Dr Jiaan Zhu, E-mail: zhujiiaan@pkuph.edu.cn

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donor T lymphocytes of antigens expressed on recipient cells as foreign, thus triggering an immune reaction accompanied by inflammation that results in damage to multiple organs and tissues.^{5,6} Histopathologic analysis reveals that the oral mucosa and salivary glands are the most frequently involved soft-tissue areas.⁷ The National Institutes of Health (NIH) Consensus Working Group recommended the criteria for diagnosing and scoring the severity of chronic GVHD. The diagnosis of chronic GVHD requires at least one diagnostic manifestation or at least one distinctive manifestation together with a biopsy of GVHD lesions.⁸ However, biopsy is an invasive procedure and there is still no consensus on the application of biopsy in asymptomatic patients taking immunosuppressive medications taking. Moreover, the NIH Consensus Working Group does not approve serial biopsies to be used to evaluate response to treatment. Until recently, the evaluation of disease activity and therapeutic response was largely based on clinical assessment findings. However, clinical experience suggests that the current response criteria lack sensitivity in detecting early curative effect. For the evaluation of treatment efficacy in routine clinical practice, improvement in clinical objective measures often occurs after important qualitative improvement.^{8,9} Therefore, more sensitive methods for depicting disease activity are required to evaluate the oral tissue of patients treated for chronic GVHD.

Ultrasonography is a non-invasive, low cost, and broadly available imaging technique that can be used in the outpatient clinic and repeated as necessary. Buccal soft tissue is convenient for supersonic examination because of its superficial position and absence of bone cover.¹⁰ Although numerous studies focused on the evaluation of therapeutic response in oral chronic GVHD patients, non-invasive imaging modalities are still lacking. Ultrasonography has high resolution and can help detect buccal disease, but its role in characterizing the buccal soft tissue and measuring therapeutic response in these patients has not been investigated yet. Therefore, the aim of this study was to measure the ultrasonography values of buccal soft tissue, including echogenicity, thickness, and power Doppler (PD) in patients with oral chronic GVHD, and compare the ultrasonography findings with clinical data before and after therapy in a 14 day follow-up, and determine the potential of ultrasonography to provide an early and objective indication of a therapeutic response to treatment intervention in oral chronic GVHD.

Methods and materials

This prospective study was approved by the Institutional Ethics Board of Peking University People's Hospital. Informed consent was obtained from all participants.

Patient profile

Between April 2016 and August 2018, 21 patients who had undergone an allogeneic transplantation procedure and had a clinical diagnosis of oral mucosal chronic GVHD were prospectively recruited from our haematology department. The diagnosis of oral chronic GVHD was made according to the method proposed by the NIH Consensus Conference. Other medical conditions related with the differential diagnosis of chronic GVHD were excluded. Diagnostic features of oral chronic GVHD include lichen planus-like changes in the buccal mucosa, characterized by hyperkeratotic white lines and lacy-appearing lesions.⁸ Patient exclusion criteria included the following: (a) under topical treatment for oral lesions of chronic GVHD in the last 3 months; (b) known oral mucosal malignancy and infectious pathogens, such as yeast or herpes virus; (c) radiation therapy to the head and neck region; (d) the habit of chewing gum or cheek sucking and biting; (e) limitation of mouth opening.

All patients were prescribed 5ml of a solution of clobetasol propionate 0.05% and topical tacrolimus ointments (0.1%) at the outpatient clinic. Patients were instructed to use the rinse for 5 min timed by a clock, three times a day, for 14 days.¹¹ One patient with symptomatic ulcerative lesions received intralesional injections of triamcinolone steroids by a stomatologist.

Clinical assessments of GVHD activity and treatment response

Oral clinical assessments of disease activity were performed using the Oral Mucosa Rating Scale (OMRS) both at baseline (*i.e.* before treatment) and at 14 days after treatment.⁸ The OMRS is a scale from 0 to 12, with higher scores indicating more severe findings. Three chronic GVHD manifestations were taken into account for rating: erythema, lichenoid, and ulcers. We defined a “modified” OMRS, which only included the right and left buccal mucosa instead of all areas of the oral cavity.

The definition of partial response (PR) in the cheek was an improvement of 2 or more points in the patient's score from baseline. Disease progression (DP) was defined as a worsening of 2 or more points. Outcomes that did not meet the criteria for PR or DP indicated the absence of change.

Patient-reported measures of GVHD activity and treatment response

Patient self-reported assessments were conducted at baseline and 14 days after treatment. Patients were instructed to report their buccal sensitivity (irritation resulting from normally tolerated spices, foods, liquids, or flavors) rated relative to peak severity during the past week using a 0–10 scale.⁸ We defined a “modified” patient-reported measures, which only included the right and left buccal mucosa instead of all anatomical areas of the oral cavity. Patient-reported measures were classified as response (clinically meaningful improvement)

Figure 1 Intraoral ultrasonic scanning methods of the buccal tissue: a glove finger filled with couplant is placed in the oral cavity, and transducer is longitudinally positioned on the skin between the angulus oris and the earlobe.

and no response (no improvement or worsening), as compared with baseline and 14 day scores.

Ultrasonography images acquisition

At the time of each clinical assessment, two board certified ultrasound physicians with at least 3 years of experience performed ultrasonography examinations in each patient at baseline and at 14 days after treatment. The examinations were performed using ultrasonography units (Aixplorer, Supersonic Imagine, France) equipped with a 20 MHz linear-array transducer and the intraoral scanning method developed in our previous study.¹² Technical parameters remained unchanged during the study. Machine settings were standardized including frequency of 20 MHz and low wall filter. Optimal PD image settings were obtained through the evaluation of blood flow and disappearance of random noise by regulating color gain and Doppler frequency. Ultrasonography examination was performed according to the following procedure. To avoid the transmission of infection and poor-quality images, the transducer was immersed into a couplant-filled glove finger and maintained 0.5 cm away from the mucosa layer. Then, the probe was gently positioned into direct contact with the buccal mucosa and longitudinally between the angulus oris and the earlobe (Figure 1).

Repeated measurements were performed in 10 patients by both ultrasound physicians on two different occasions to evaluate the intraobserver reproducibility and interobserver reliability. The remainder of patients were examined by only one ultrasound physician. The following ultrasonography characteristics of the buccal epithelial layers were evaluated: echogenicity, thickness, and PD.

Epithelial layer measurement and imaging scoring systems

The thickness of buccal mucosa was measured at a standardized anatomical site, between the posterior edge of the orbicularis oris muscle and 1.5 cm posterior. Three sequential measurements of the thickness were recorded in all subjects and the mean was calculated to maintain the maximum intraoperator consistency and greater reliability of measurements.

Imaging scoring systems were proposed to evaluate B-mode (BM) and PD ultrasonography images semi-quantitatively. The scoring system for BM assessment of the echogenicity of the buccal epithelium was developed as follows: if the echogenicity was homogeneously hypoechoic, the grade was 0; if it was partially increased, the grade was 1; if the echogenicity was diffusely increased, the grade was 2 (Figure 2). Semi-quantitative grading of the PD evaluation was as follows: Grade 0, no flow at the epithelium-connective tissue junction; Grade 1, single-vessel signals; Grade 2, flow signals in less than half of the area at the epithelium-connective tissue junction; Grade 3, signals in more than half of the area at the epithelium-connective tissue junction; Grade 4, signals in all areas at the epithelium-connective tissue junction (Figure 3). BM and PD scores were a sum of values on both cheeks, and the total score ranged from 0 to 4 and 0 to 8, respectively. We decided to consider a change in BM and PD scores of at least one point from the baseline value as significant.

For evaluating the intrareader agreement of BM and PD scores, ultrasonography images of buccal soft tissue from 21 patients at baseline were assessed twice by two different readers, who were both blinded to clinical and imaging data.

Figure 2 Semi-quantitative (grade 0–2) greyscale ultrasound score of buccal epithelium.

Statistical analysis

All statistical data were analyzed with software (SPSS Statistics for Windows, v. 20.0; IBM, Armonk, NY). The buccal epithelial membrane mean thickness data was

Figure 3 Semi-quantitative (grade 0–4) power Doppler ultrasonography score of the buccal soft tissue. Ultrasonographic images of the buccal tissue of oral chronic GVHD patients reveal high flow signals at the epithelium-connective tissue junction on power Doppler imaging suggestive of hyperemia. GVHD, graft-versus-host disease.

tested for normality using the Kolmogorov–Smirnov test. The Wilcoxon rank sum test was used to compare individual values before and after treatment. Comparison between quantitative data was performed using the *t*-test. The association between two quantitative variables was evaluated by means of Spearman's rank correlation coefficient. The intraclass correlation coefficients (ICCs) were used to evaluate the test–retest and interobserver reliabilities. *p* values less than 0.05 were considered to indicate significance.

Results

Subjects characteristics

21 patients who were exclusively Asians and underwent HSCT were analyzed. The clinical and demographic characteristics of patients are shown in Table 1. The median time since transplantation was 7 months (range 3–18 months). Most patients were receiving systemic treatment for chronic GVHD (95.2%) at the time of evaluation. The most commonly affected organs were the skin (55.6%) and liver (22.2%). Seven patients had acute myeloid leukaemia (AML), nine had acute lymphoblastic leukaemia (ALL), four had myelodysplastic syndrome (MDS), and one had non-Hodgkin lymphoma (NHL). The systemic treatment for chronic GVHD consisted of prednisone and cyclosporine in 17 cases, prednisone in 1 case, and cyclosporine in the remaining 2 cases. After the scheduled 14 days of topical treatment, all subjects completed the trial.

Ultrasonography findings

Intraoral ultrasonography depicted the layers starting with (from probe surface to depth) epithelial membrane (innermost hypoechoic layer), the subepithelial connective tissue (the hyperechoic layer) and the buccinator muscle (the hypoechoic layer) (Figure 4). However, the buccal pad of fat, the subcutaneous tissue, and the skin of the cheek were too deep to be detected.

The ultrasonography findings of all patients are presented in Table 2. The ultrasonography findings for oral chronic GVHD in the 21 buccal soft tissues at baseline were as follows: (a) the thickness of the epithelial layers was 0.19–2.25 mm (median thickness, 0.47 mm), (b) the echogenicity of the buccal epithelium was heterogeneously hyperechoic in 18 (85.7%) of 21 patients, and (c) the PD showed flow signals at the epithelium–connective tissue junction in 21 (100%) of the patients. The distribution of BM scores observed in the baseline data were 0 (14.3%), 1 (28.6%), 2 (14.3%), 3 (28.6%), and 4 (14.3%). The distribution of PD scores observed in the baseline cohort were 0 (0%), 1 (4.8%), 2 (9.5%), 3 (4.8%), 4 (14.3%), 5 (9.5%), 6 (14.3%), 7 (19%), and 8 (23.8%). The most frequent BM scores achieved were 1 and 3 (28.6%, equally) and the most frequent PD score was 8 (23.8%). The median buccal epithelial layer thickness after treatment was 0.40 mm (range, 0.22–2.00 mm). The

Table 1 Clinical and demographic characteristics in 21 patients submitted to haematopoietic stem cell transplantation

Clinical characteristics	ALL patients n = 21 (100%)
Gender - n (%)	
Male	14 (66.7%)
Female	7 (33.3%)
Age - (years)	
Median (range)	44 (21–59)
Underlying disease - n (%)	
ALL	9 (42.8%)
AML	7 (33.3%)
MDS	4 (19.0%)
NHL	1 (4.8%)
Type of donor - n (%)	
Related	21 (100%)
Unrelated	-
Cell source - n (%)	
Bone marrow + PBSCs	16 (76.2%)
Bone marrow	2 (9.5%)
PBSCs	3 (14.3%)
History of acute GVHD	
Yes	19 (90.5%)
No	2 (9.5%)
Time post-transplantation - (months)	
Median (range)	7 (3–18)
Systemic treatment for chronic GVHD	
Prednisone	1
Cyclosporine	2
Prednisone and cyclosporine	17
Organs affected by chronic GVHD	
Skin	15 (55.6%)
Liver	6 (22.2%)
Eyes	4 (14.8%)
Gut	1 (3.7%)
Lungs	1 (3.7%)

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; GVHD, graft-versus-host disease; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; PBSC, peripheral blood stem cells.

distribution of BM scores observed in the treatment data were 0 (14.3%), 1 (14.3%), 2 (28.6%), 3 (28.6%), and 4 (14.3%). The distribution of PD scores following treatment was 14.3% (2 (19%), 4 (19%), 5 (23.8%), 6 (9.5%), 7 (4.8%), and 8 (9.5%).

Improvement in BM scores was observed in only 9.5% (2 of 21) of patients in contrast to 66.7% (14 of 21) with no change and 23.8% (5 of 21) with disease progression (Figure 5). Improvement in PD scores was observed in 66.7% (14 of 21), with no change in 9.5% (2 of 21) and worsening in 23.8% (5 of 21) (Figure 6).

Ultrasonography, clinical, and patient-reported assessments after 14-day treatment

After 14 days of treatment, the disease activity as assessed by OMRS and patient self-reports showed a significant decrease (Table 2). The PD scores also decreased significantly, from the median value of 6 to 4 ($p < 0.01$, Wilcoxon rank sum test). In contrast, the ultrasonography evaluation of epithelium thickness and BM scores did not revealed a significant decrease ($p > 0.05$).

Patients were further classified into three categories according to the clinician-assessed responses after 14day treatment (Table 3). The PD scores at baseline and after 14day treatment were compared. A significant decrease in PD scores was observed in buccal soft tissue that responded clinically to the treatment, that is, in patients showing partial clinical response ($p < 0.001$). A significant decrease in PD scores was also observed in buccal soft tissue that remained unchanged, *i.e.* in patients who failed to show clinical improvement ($p < 0.001$). Five patients were separated because the PD scores were significantly increased, with the mean PD scores increasing from 3.8 to 5.2 ($p < 0.05$) after 14day treatment.

Patients were further classified into two categories according to the patient-reported responses after 14day treatment (Table 4). The PD scores at baseline and after 14day treatment were compared. A significant decrease in PD scores was observed in buccal soft tissue that responded to the treatment. No significant decrease in PD scores was observed in buccal soft tissue that

Figure 4 Intraoral ultrasonography images of the buccal tissue. The images shows the buccal epithelial layers and subepithelial connective tissue clearly. E, epithelial membrane; ST, subepithelial connective tissue; M1, buccinator muscle; M2, orbicularis oris muscle; GF, couplant-filled glove finger.

Table 2 Ultrasonography, clinical and patient-reported findings in 21 patients at baseline and 14 days after treatment

Buccal mucosa	Baseline	Day 14	p-value
OMRS			0.011
Median	4	3	
Range	1–12	1–10	
Patient self-report			0.009
Median	6	3	
Range	1–8	1–9	
Epithelial layer thickness (mm)			NS
Median	0.47	0.40	
Range	0.19–2.55	0.22–2.00	
BM scoring			NS
Median	2	2	
Range	0–4	0–4	
PD scoring			0.006
Median	6	4	
Range	1–8	1–8	

BM, B-mode; NS, non-significant; OMRS, Oral Mucosa Rating Scale; PD, power Doppler.

showed no response—*i.e.* no improvement or worsening ($p > 0.05$).

Correlations among changes in ultrasonography, clinical, and patient-reported assessments after 14-day treatment
The change in PD scores correlated significantly with the change in patient-reported measures from baseline ($r = 0.85$, $p < 0.01$). There was a moderate correlation of clinical assessment with PD scores ($r = 0.46$, $p < 0.05$), with patient-reported measures ($r = 0.60$, $p < 0.05$), and with BM-scores ($r = 0.65$, $p < 0.05$) (Table 5).

However, the change in neither BM scores from baseline correlated with a change in clinical and patient-reported parameters.

Reliability
Good intra- and interobserver reliability was observed, with ICC values of 0.844 ($p < 0.05$) and 0.798 ($p < 0.05$) for the measurements of buccal epithelial thickness, respectively.

Figure 5 Mode ultrasonography of buccal soft tissue of one patient. The images at baseline (a) and after 14 day treatment (b) shows no change in B-mode scores: from 0 to 0 point.

Figure 6 Power Doppler ultrasonography images of buccal soft tissue of one patient. The images at baseline (a) and after 14 day treatment (b) show reduction in the Doppler signal (red area): from 4 to 3 points.

Table 3 Ultrasonography PD scoring modulation according to baseline and 14 day clinical assessments

OMRS at baseline and day 14a	PD scoring (N = 16)b	
	Baseline	day 14
Partial response (n = 6)	6.5 ± 1.87	3.7 ± 2.34c
Disease progression (n = 0)	-	-
Unchanged (n = 10)	5.8 ± 2.04	3.9 ± 2.18c

OMRS, Oral Mucosa Rating Scale; PD, power Doppler.

aThe first clinical status of the buccal mucosa is that at baseline, and the second clinical status is that 14 days after treatment.

bWith exception of 5 patients for PD scoring values which was increased after 14 day treatment, values are expressed as means ± SDs.

c

Discussion

Results of the present study of the buccal mucosa in 21 patients with oral chronic GVHD treated for 14 days show that ultrasonography findings can be used as an outcome measure of response to therapy. To the best of our knowledge, this is the first evaluation of ultrasonography appearances as a measure of the therapeutic response in patients with oral chronic GVHD. Moreover, ultrasonography offered several advantages compared with simultaneously performed clinical assessments. In buccal mucosa that clinically responded to treatment, ultrasonography allowed visualization of the reduction of vascularity at the epithelium–connective tissue junction, which is a sign of improvement. Our study data show that in response to 14 day treatment, PD scores for the buccal mucosa improved as the disease activity decreased.

Various imaging modalities can be used to evaluate soft tissue. Contrast-enhanced MRI and diffusion-weighted MRI are useful non-invasive imaging modalities for differentiating between malignant and benign soft tissue tumors, but they have the drawbacks of long acquisition times and high examination costs, which limited their routine use.¹³ Ultrasonography is a portable, non-invasive, real-time assessment and low-cost imaging system for detection of superficial tissue. Color and power Doppler can be used to assess blood flow in soft-tissue masses. Its main drawback is the dependence on operators.^{14,15}

According to the published literature on ultrasonic examination of buccal tissue, ultrasonography is an

effective imaging method for the buccal layer structure, and previous study clearly identified the buccal mucosa surface, the submucosa, and the buccinators.¹⁰ The buccal mucosa membrane has two layers: a stratified squamous epithelium which is relatively thick and avascular, and the slightly vascular fibrous connective tissue layer underlying the epithelium (*i.e.* lamina propria).¹⁶ Although other group has demonstrated that the buccal mucosa was a hyperechoic line and the submucosa was a hypoechoic area,¹⁷ our results revealed that the mucosal epithelial membrane was hypoechoic and the lamina propria could not be differentiated from the submucosa, with a hyperechoic zone lateral to the epithelium. There was a clear distinct differentiation between the mucosa epithelium and subepithelial connective tissue. The reason is that our study acquired better grayscale resolution owing to the higher frequency. Other studies also detected the mucosa by using intraoral ultrasonography, which is consistent with our assessment of the echogenicity of the epithelium and subepithelial connective tissue.¹⁸

Oral chronic GVHD is believed to be common after HSCT and results in loss of quality of life in long-term survivors.¹⁹ Quantitative research tools are needed to measure short-term responses to therapy to accelerate development and approval of new drugs for chronic GVHD.⁹ Several studies, to date, have evaluated the activity and treatment response of oral chronic GVHD. Bassim *et al* demonstrated that the NIH OMS was a favorable comprehensive construct validity and was intended to evaluate therapeutic response to treatment.²⁰ But the major weakness of this study arise from its cross-sectional design. In contrast, our study was prospective and longitudinal. Our results agree with

Table 4 Ultrasonography PD scoring modulation according to baseline and 14 day patient-reported measures

Patient-reported measures at baseline and day 14a	PD scoring (N = 21)b	
	Baseline	day 14
Response (n = 13)	6.1 ± 2.02	3.5 ± 2.07c
No response (n = 8)	4.6 ± 2.39	5.3 ± 2.05d

PD, power Doppler; SD, standard deviation.

aThe first clinical status of the buccal mucosa is that at baseline, and the second clinical status is that 14 days after treatment.

bValues are expressed as means ± SDs.

c

d

Table 5 Correlations among changes in ultrasonography, clinical and patient-reported variables of the treatment response in 21 patients

	OMRS	Patient-reported measures
Epithelial layer thickness	0.65 ^a	0.29
BM scoring	−0.03	0.31
PD scoring	0.46 ^a	0.85 ^b
OMRS	-	0.60 ^a

OMRS, Oral Mucosa Rating Scale, BM B-mode, PD power Doppler

^ap < 0.05

^bp < 0.01

results of previous study that clinical measurement alone may not be the most accurate representation of oral chronic GVHD disease ‘status’.²¹ While PD and clinical assessments are related, they do not strictly convey the same information. When PD and clinical assessments are considered simultaneously, response to treatment is greater in the buccal mucosa evaluated using PD than in buccal mucosa evaluated using clinical visual assessment. A significant reduction in PD scores was observed in buccal mucosa that was classified as clinical “unchanged” after 14 day treatment. These data further illustrate that, although clinical assessment is the current standard of reference, it is not a “gold-standard” and the possibility of combining clinical and ultrasonographic assessments should be further investigated. As shown in the present study, PD scoring and patient-reported measures are markedly related. Ultrasonography depicted a significant decrease in the mean PD scoring of the epithelium–connective tissue junction that were thought to be patient-reported “response” and a no significant decrease in the mean PD scoring that were classified as patient-reported “no response”. Epithelium thickness and BM scores did not show any changes in this study, probably owing to the short follow-up period after treatment resulting in no changes in their manifestations pathologically.

Given that the ultrasonography yields form, structure, and blood flow signals data for the evaluation of buccal soft tissue, we introduced a novel set of imaging methods including BM and PD, which are illustrated in [Figures 2 and 3](#). Pathological changes in oral chronic GVHD patients are considered to be intralobular or periductal diffuse lymphocytic infiltration in labial salivary glands, subepithelial lymphocytic infiltration, and basal cell degeneration in the buccal mucosa.⁵ Lichen planus-like changes are the diagnostic characteristics of oral chronic GVHD and lesions that resemble oral lichen planus clinically and histologically with the microscopic features of hyperparakeratosis, hyperorthokeratosis and basal cell hydropic.^{22,23} These pathological changes might explain the elevated echogenicity in the buccal epithelium and the increased PD scores at the epithelium–connective tissue junction that we observed.

We performed measurements at standardized anatomical points, from the posterior edge of the

orbicularis oris muscle to a centimeter posterior to ensure consistent location for measurements, because the thickness of the epithelium becomes thinner near the corner of the mouth. This led to good intra- and interobserver agreements.

There are many limitations to this work. Epithelium and subepithelial layers as assessed by intraoral ultrasonography only represent the middle and front part of the cheek, and this transoral approach does not facilitate direct contact with the probe when examining the mucobuccal folds and the retromolar region. Other physiological factors, such as age and sex, associating with buccal epithelial morphology, may be confounding factors in this study and need to be explored in future research. Moreover, the sample size of this clinical study was small. A larger, long-term trial is necessary to confirm the results of this pilot study and is already underway in our clinic.

Conclusion

In conclusion, our study addressed the feasibility of assessing buccal soft tissue in oral chronic GVHD patients based on ultrasonography examination and evaluate the value of grayscale and PD ultrasound in buccal epithelium and subepithelial connective tissues. Response to therapy was measurable through clinical and patient-reported parameters as well as by ultrasonography. PD ultrasound was able to detect the improvements in disease activity before clinical objective measures themselves improved, making PD ultrasound helpful in evaluating disease activity and making earlier therapy decisions. The PD semi-quantitative scoring systems proposed in our study are expected to become an evaluation tool for the activity of oral chronic GVHD and clinical treatment efficacy in clinical practice.

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