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Diaphragm Ultrasound Identifies Respiratory Pump Dysfunction in Bronchiolitis

Obliterans Syndrome After Hematopoietic Stem Cell Transplantation

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29 diaphragm ultrasound; respiratory pump dysfunction; diaphragm thickening fraction;
30 diaphragm excursion; pulmonary function; air trapping

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32

Abstract

Background. Bronchiolitis obliterans syndrome (BOS) is a life-threatening noninfectious pulmonary complication following hematopoietic stem cell transplantation (HSCT), characterized by progressive airflow obstruction. This study investigated diaphragmatic structure and function using ultrasound and examined their relationship with BOS severity.

Methods. In this cross-sectional study, 41 patients diagnosed with BOS after HSCT and 30 age- and sex-matched healthy controls were enrolled. Diaphragm ultrasound was performed to measure diaphragm thickness at end-expiration and end-inspiration, diaphragm thickening fraction (DTF), and diaphragm excursion during quiet breathing and deep breathing. Pulmonary function tests were collected for BOS patients. Correlation analyses were performed between diaphragm ultrasound parameters and pulmonary function indices. Ordinal logistic regression was used to assess the ability of diaphragm parameters to predict BOS severity.

Results. M-mode ultrasound showed distinct diaphragmatic motion patterns in BOS patients compared with healthy controls. BOS patients had significantly greater diaphragm thickness at both end-expiration and end-inspiration on both sides (all $p < 0.05$), together with higher DTF. In contrast, right diaphragmatic excursion during deep breathing was significantly reduced in BOS patients ($p = 0.010$), and the difference between deep and quiet breathing excursion was markedly decreased ($p < 0.001$). Patients with severe BOS showed greater diaphragm thickening but lower diaphragm excursion than those with mild or moderate BOS. Inspiratory diaphragm thickness and

left DTF were negatively correlated with FEV1% predicted, whereas diaphragm excursion during deep breathing was positively correlated with forced vital capacity and negatively correlated with the residual volume/total lung capacity ratio. An ordinal logistic regression model incorporating left DTF and right diaphragm excursion showed potential in predicting severe BOS.

Conclusions. Diaphragm ultrasound reveals distinct alterations in diaphragmatic structure and function in BOS patients. These findings suggest that respiratory pump dysfunction may contribute to disease severity in BOS and highlight diaphragm ultrasound as a potential non-invasive tool for disease assessment.

Introduction

Bronchiolitis obliterans syndrome (BOS) is a serious pulmonary complication following hematopoietic stem cell transplantation (HSCT). It is characterized by progressive airflow obstruction and is associated with substantial morbidity and mortality¹. The pathological hallmark of BOS involves inflammation, fibrosis, and obliteration of the small airways, ultimately leading to persistent respiratory dysfunction and poor prognosis²⁻⁴.

Current understanding of BOS has primarily focused on structural abnormalities of the lung, including small airway fibrosis, air trapping, and bronchial wall thickening, as identified by high-resolution computed tomography (HRCT), as well as airflow limitation assessed by pulmonary function tests (PFTs)⁵. However, clinical observations indicate that the degree of airflow limitation in BOS does not always fully correspond to structural airway abnormalities⁶. Some patients exhibit marked ventilatory

77 impairment despite relatively modest radiologic changes⁷. These findings suggest that
78 additional physiological factors may contribute to ventilatory dysfunction in BOS.

79 Effective ventilation depends not only on airway patency but also on the balance
80 between respiratory load and the capacity of the respiratory pump⁸. Among the
81 components of the respiratory pump, the diaphragm plays a central role as the primary
82 inspiratory muscle responsible for generating negative intrathoracic pressure. In
83 chronic obstructive airway disease, inspiratory muscles are exposed to increased
84 mechanical loading, while hyperinflation places the diaphragm at a mechanical
85 disadvantage, leading to structural and functional adaptations of the diaphragm⁹.

86 Diaphragmatic dysfunction has been associated with dyspnea, reduced exercise
87 tolerance, and worse clinical status in chronic respiratory disease¹⁰. In BOS after HSCT,
88 the disease arises in the setting of systemic chronic graft-versus-host disease rather than
89 being confined to local airway injury alone¹¹. Given this combination of increased
90 ventilatory load and systemic immune-mediated disease, it is plausible that
91 diaphragmatic dysfunction may contribute to ventilatory impairment in BOS beyond
92 what can be explained by airway structural abnormalities alone.

93 However, whether diaphragmatic function is altered in BOS and how it relates to
94 disease severity and pulmonary function remain unclear. Ultrasound has emerged as a
95 non-invasive, bedside tool for assessing diaphragmatic structure and function,
96 including parameters such as diaphragmatic thickness, thickening fraction, and
97 excursion¹². Compared with traditional imaging modalities, diaphragm ultrasound
98 offers real-time dynamic evaluation and has shown promising value in critical care and

chronic respiratory diseases¹³. Nevertheless, its application in BOS, particularly in evaluating disease severity and functional impairment, has not been systematically investigated.

We hypothesized that BOS patients may exhibit diaphragmatic dysfunction related to chronic respiratory loading and systemic inflammation. In this study, we aimed to evaluate diaphragmatic structure and function in patients with BOS using ultrasound and to explore their association with pulmonary function and disease severity. Specifically, we compared diaphragmatic ultrasound parameters between BOS patients and healthy controls, analyzed differences across severity grades, and assessed correlations between diaphragmatic indices and pulmonary function parameters. Our findings may provide novel insights into the pathophysiology of BOS and support the potential role of diaphragm ultrasound as a functional biomarker for disease assessment.

Methods

Study population

This cross-sectional study was performed from July 2025 to December 2025 at Peking University People's Hospital. The study was approved by the Institutional Review Board of Peking University People's Hospital (No. 2025PHB431-001) and was conducted in accordance with the Declaration of Helsinki. The study included 41 patients diagnosed with BOS following HSCT and 30 age- and sex-matched healthy controls. BOS was diagnosed according to established pulmonary function criteria and clinical evaluation guidelines¹⁴. Clinical and biological data were recorded for each patient, including age, sex, height, weight, hematological malignancy, time from HSCT,

time from BOS diagnosis, BOS severity and status, other chronic graft-versus-host disease (cGVHD) manifestations, and pulmonary function parameters. Written informed consent was provided by each subject.

Inclusion and exclusion criteria

(1) Inclusion criteria for healthy controls:

- 1) No history of chronic respiratory diseases;
- 2) No history of neuromuscular disorders;
- 3) No chest wall deformities or respiratory functional impairment;
- 4) Ability to cooperate with and complete ultrasound examination.

(2) Inclusion criteria for BOS group

- 1) $FEV_1/FVC < 0.7$ or below the 5th percentile (lower limit of the 90% confidence interval);
- 2) $FEV_1 < 75\%$ of the predicted value, with a decline of $\geq 10\%$ within 2 years, and a post-bronchodilator response (e.g., salbutamol) showing an increase in $FEV_1 < 200$ mL and $< 12\%$;
- 3) exclusion of respiratory tract infection, based on clinical symptoms and relevant investigations, including high-resolution computed tomography (HRCT) and microbiological testing (nasal swab, throat swab, sputum culture, and bronchoalveolar lavage);
- 4) presence of at least one of the following supporting criteria:
 - (i) manifestations of chronic graft-versus-host disease (cGVHD) in other organs;
 - (ii) evidence on expiratory HRCT of air trapping, small airway wall thickening, or

bronchiectasis;

(iii) pulmonary function evidence of air trapping, defined as $RV > 120\%$ predicted or RV/TLC ratio above the upper limit of the 90% confidence interval.

(3) Exclusion criteria

1) diseases affecting pulmonary function or respiratory muscle function, such as chronic obstructive pulmonary disease (COPD) or asthma;

2) recent thoracic or abdominal surgery that could affect respiratory function;

3) pregnancy;

4) inability to cooperate with examinations;

5) incomplete clinical data.

Diaphragm ultrasound

Diaphragm ultrasound examinations were performed by an experienced sonographer who was blinded to the clinical severity of BOS. All participants were examined in the supine position after a period of quiet rest, in accordance with the recommendations of the EXpert consensus On Diaphragm UltraSonography in the critically ill (EXODUS)¹³.

Diaphragm thickness was assessed using a high-frequency linear-array transducer positioned over the zone of apposition between the anterior and mid-axillary lines at the level of the 8th–10th intercostal spaces. B-mode imaging was first used to identify the diaphragm as a three-layered structure, after which M-mode imaging was applied to measure diaphragm thickness at end-expiration (Tdi-exp) and end-inspiration (Tdi-insp). Diaphragmatic excursion was evaluated using M-mode ultrasonography with a low-frequency convex transducer placed below the right costal margin through the liver

165 acoustic window. The ultrasound beam was directed cranially and medially toward the
166 posterior third of the right hemidiaphragm. The location of the diaphragmatic dome was
167 identified according to the anterior transverse scanning technique described by Testa, A
168 et al.¹⁵, which allows reliable visualization of diaphragmatic motion during respiration.
169 Diaphragm excursion was measured during both quiet breathing and maximal deep
170 breathing.

171 Diaphragm thickness was measured at end-expiration (Tdi-exp) and end-inspiration
172 (Tdi-insp) on both sides. Left and right diaphragm thickness at end-expiration were
173 recorded as L-Tdi-exp and R-Tdi-exp, respectively, while thickness at end-inspiration
174 was recorded as L-Tdi-insp and R-Tdi-insp. Diaphragm thickening fraction (DTF), an
175 index of diaphragmatic contractility, was calculated as:

176 $DTF = (Tdi-insp - Tdi-exp) / Tdi-exp \times 100\%$.

177 The corresponding left- and right-sided values were recorded as L-DTF and R-DTF,
178 respectively.

179 Diaphragm excursion (DE) was assessed using M-mode ultrasonography during quiet
180 breathing (QB) and deep breathing (DB). Because patients with diaphragmatic
181 paralysis were excluded from the study and the spleen generally provides a suboptimal
182 acoustic window for reliable assessment of left hemidiaphragm motion, diaphragmatic
183 excursion was measured only on the right side. Right diaphragmatic excursion during
184 quiet breathing and deep breathing were recorded as R-DE during QB and R-DE during
185 DB, respectively.

186 To evaluate the reserve capacity of diaphragmatic motion, the absolute difference

187 between excursion during deep breathing and quiet breathing was calculated as:

188 $DE \text{ diff DB-QB (mm)} = DE \text{ during DB} - DE \text{ during QB}.$

189 In addition, the relative excursion reserve was expressed as:

190 $DE \text{ diff DB-QB (\%)} = (DE \text{ during DB} - DE \text{ during QB}) / DE \text{ during DB} \times 100\%.$

191 These parameters were recorded as DE diff DB-QB (mm) and DE diff DB-QB (%),
192 respectively.

193 **Pulmonary function tests**

194 Pulmonary function tests (PFTs) were performed in all BOS patients according to
195 standard ATS/ERS recommendations. The assessed parameters included forced
196 expiratory volume in one second (FEV1), percentage of predicted FEV1 (FEV1% pred),
197 forced vital capacity (FVC), FEV1/FVC ratio, maximal expiratory flow at 75%, 50%,
198 and 25% of forced vital capacity (MEF75, MEF50, and MEF25), corresponding
199 percentages of predicted values (MEF75% pred, MEF50% pred, and MEF25% pred),
200 single-breath diffusing capacity for carbon monoxide (DLCO-SB), percentage of
201 predicted DLCO-SB (DLCO-SB % pred), total lung capacity (TLC), residual volume
202 (RV), and the residual volume-to-total lung capacity ratio (RV/TLC).

203 FEV1, FEV1% pred, FEV1/FVC, and maximal expiratory flow parameters were used
204 to evaluate the severity of airflow obstruction and small-airway dysfunction, whereas
205 TLC, RV, and RV/TLC were used to assess lung hyperinflation and air trapping. DLCO-
206 SB and DLCO-SB % pred were used to evaluate pulmonary gas transfer function.

207 **Statistical analysis**

208 Continuous variables were expressed as mean $\pm$ standard deviation. Group comparisons

were performed using t-tests or ANOVA as appropriate. Correlations between diaphragm ultrasound parameters and pulmonary function indices were analyzed using Pearson correlation analysis. Ordinal logistic regression was used to evaluate the association between diaphragm parameters and BOS severity.

Results

Baseline characteristics

A total of 41 BOS patients and 30 healthy controls were included in the study. Age and sex distribution were comparable between the two groups, while body mass index was significantly lower in BOS patients. Compared with healthy controls, BOS patients exhibited significantly greater diaphragm thickness at both end-expiration and end-inspiration. Diaphragm thickening fraction was also significantly increased in BOS patients. However, right diaphragm excursion during deep breathing was significantly reduced, indicating impaired diaphragmatic mobility.

Table 1. Baseline Characteristics of BOS vs HC

Variable	BOS (n=41)	HC (n=30)	P value
Sex (Female/Male)	18 (43.9%) / 23 (56.1%)	19 (63.3%) / 11 (36.7%)	0.15
Age (years)	36.51 ± 11.48	32.87 ± 5.20	0.077
BMI (kg/m ²)	19.11 ± 3.58	22.90 ± 3.99	<0.001
L-Tdi-exp (mm)	0.16 ± 0.07	0.13 ± 0.04	0.012
L-Tdi-insp (mm)	0.29 ± 0.14	0.18 ± 0.05	<0.001
L-DTF (%)	76.03 ± 59.01	41.24 ± 28.53	0.002
R-Tdi-exp (mm)	0.17 ± 0.08	0.13 ± 0.04	0.010
R-Tdi-insp (mm)	0.28 ± 0.14	0.18 ± 0.05	<0.001
R-DTF (%)	71.12 ± 49.94	48.81 ± 33.49	0.028
R-DE during DB (mm)	4.30 ± 1.27	5.12 ± 1.21	0.007
R-DE during QB (mm)	2.15 ± 0.67	1.96 ± 0.62	0.214
DE diff DB-QB (mm)	2.15 ± 1.29	3.16 ± 1.04	<0.001

Variable	BOS (n=41)	HC (n=30)	P value
DE diff DB-QB (%)	119.39 ± 109.88	178.21 ± 82.33	0.012

Abbreviations: L-Tdi-exp, left diaphragm thickness at end-expiration; L-Tdi-insp, left diaphragm thickness at end-inspiration; L-DTF, left diaphragm thickening fraction; R-Tdi-exp, right diaphragm thickness at end-expiration; R-Tdi-insp, right diaphragm thickness at end-inspiration; R-DTF, right diaphragm thickening fraction; R-DE during DB, right diaphragm excursion during deep breathing; R-DE during QB, right diaphragm excursion during quiet breathing; DE diff DB-QB, difference between diaphragm excursion during deep breathing and quiet breathing; DE diff DB-QB (%), relative excursion reserve calculated as (DE during DB – DE during QB)/DE during DB × 100%; BMI, body mass index.

Furthermore, we compared diaphragmatic excursion curves during quiet breathing between patients with severe BOS and healthy controls. As shown in Figure 1, the diaphragmatic excursion curve of healthy controls exhibited a sinusoidal-like pattern during tidal breathing. In contrast, patients with severe BOS demonstrated a distinct expiratory profile characterized by an initially steep decline followed by a noticeable inflection point, after which the expiratory velocity gradually decreased. This unique pattern suggests an altered breathing mechanics that differs substantially from that observed in healthy individuals.

Differences among BOS severity groups

When BOS patients were stratified by disease severity, distinct alterations in diaphragm mechanics were observed in Figure 2. Specifically, patients with severe BOS demonstrated significantly increased diaphragm thickening fraction (indicated by

elevated L-Tdi-insp and R-Tdi-insp) compared to those with mild or moderate disease ($p<0.01$). Conversely, these severe cases exhibited markedly reduced diaphragm excursion during deep breathing (reflected by lower R-DE (DB) values), suggesting compromised contractile efficiency. Additionally, parameters related to ventilation distribution, such as DB-QB, showed a significant decline in the severe group ($p<0.05$), further highlighting the deterioration of respiratory function with disease progression.

Correlation with pulmonary function

We made a correlation analysis between diaphragm ultrasound parameters and pulmonary function. As is revealed in Figure 3, left inspiratory diaphragm thickness ($r=-0.66$, $p<0.001$) and left diaphragm thickening fraction (L-DTF) ($r=-0.57$, $p<0.01$) were negatively correlated with FEV1% predicted, a key indicator of BOS severity. In contrast, diaphragm excursion during deep breathing showed a positive correlation with forced vital capacity ($r=0.7$, $p<0.001$) and a negative correlation ($r=-0.65$, $p<0.001$) with RV/TLC, suggesting a relationship with lung reserve and air trapping.

Association between diaphragmatic ultrasound parameters and BOS severity

Among all candidate diaphragmatic ultrasound parameters, LASSO regression identified L-DTF and right diaphragmatic excursion during deep breathing (R-DE-DB) as the most informative variables associated with BOS severity (Table 2). As shown in Table 3, in the primary ordinal logistic regression model performed in the complete-case cohort ($n = 41$), L-DTF was significantly associated with BOS severity ($\beta = 0.026$, $p = 0.004$), whereas R-DE-DB demonstrated a borderline association ($\beta = -0.547$, $p = 0.052$). After additional adjustment for age and BMI, both L-DTF ($\beta = 0.027$, $p = 0.004$)

and R-DE-DB ($\beta = -0.595, p = 0.043$) were independently associated with BOS severity. Further adjustment for sex yielded nearly identical effect estimates (L-DTF: $\beta = 0.028, p = 0.004$; R-DE-DB: $\beta = -0.612, p = 0.040$). The effect estimates for the two diaphragmatic parameters changed by less than 10% across all models, indicating little evidence of confounding by demographic or anthropometric characteristics. Age, BMI, and sex were not independently associated with BOS severity. Predicted probability analyses demonstrated a clear dose-response relationship between reduced diaphragmatic contractility and increasing risk of severe BOS (Figure 4A). Stratified analyses further showed that reduced diaphragmatic excursion consistently shifted the risk curves upward across all levels of L-DTF (Figure 4B). No significant interaction was observed between L-DTF and R-DE-DB (P for interaction = 0.65), which was supported by largely parallel marginal effect curves.

Table 2. LASSO regression for variable selection among diaphragmatic ultrasound parameters

Variable	LASSO coefficient
L-DTF (%)	0.335
R-DE-DB (cm)	-0.223
R-DTF (%)	0.144
R-DE-QB (cm)	-0.082
DB-QB (%)	0.000

Table 3. Ordinal logistic regression models for BOS severity

Variable	Model 1 β (P)	Model 2 β (P)	Model 3 β (P)
L-DTF (%)	0.026 (0.004)	0.027 (0.004)	0.028 (0.004)
R-DE-DB (cm)	-0.547 (0.052)	-0.595 (0.043)	-0.612 (0.040)
Age	—	-0.009 (0.746)	-0.008 (0.763)
BMI	—	0.048 (0.595)	0.037 (0.702)
Sex	—	—	0.292 (0.687)

Variable	Model 1 β (P)	Model 2 β (P)	Model 3 β (P)
AIC	77.34	80.93	82.76

Discussion

This study identifies a respiratory pump phenotype in BOS after HSCT and extends current understanding of BOS beyond small-airway obstruction alone. The principal findings were that BOS patients had increased diaphragm thickness and thickening fraction compared with healthy controls, reduced diaphragmatic excursion during deep breathing, altered M-mode motion patterns in severe disease, and significant associations between diaphragm ultrasound parameters and pulmonary function impairment. Together, these findings suggest that BOS is accompanied by a dissociation between diaphragmatic effort and mechanical output, supporting respiratory pump dysfunction as an underrecognized component of BOS pathophysiology.

The central physiological finding was the coexistence of increased diaphragmatic thickening and reduced diaphragmatic excursion. Increased thickening should not be interpreted as improved diaphragm function in this setting. Rather, it may reflect greater inspiratory effort in response to increased respiratory load, while reduced excursion indicates that this effort generates less effective mechanical displacement. One plausible explanation is hyperinflation-related impairment of diaphragm mechanics¹⁶.

In obstructive lung disease, air trapping increases operating lung volume, flattens the diaphragm, shortens the zone of apposition, and places muscle fibers at a less favorable length-tension relationship¹⁷. This mechanism has been well described in COPD, where hyperinflation reduces diaphragmatic mobility and mechanical efficiency despite preserved or increased neural activation^{16,18}. The pattern observed in BOS therefore

suggests neuromechanical inefficiency: greater diaphragmatic activation is required, but the mechanical output of each contraction is reduced.

BOS may also differ from COPD because the respiratory pump is exposed to immune-mediated systemic disease as well as mechanical loading. COPD is primarily driven by smoking-related airway injury, whereas BOS after HSCT results from alloimmune-mediated small-airway fibrosis and often occurs in the context of chronic graft-versus-host disease. The combination of increased respiratory load, air trapping, and systemic inflammatory or immune-mediated injury may promote structural adaptation or remodeling of respiratory muscle. Previous studies have shown that inflammatory cytokines, including TNF- α , IL-6, and TGF- β ¹⁹, can influence skeletal muscle remodeling and contractile behavior²⁰. Although the present study cannot establish a direct inflammatory mechanism, the coexistence of increased diaphragm thickness and reduced excursion is compatible with a combined mechanical and systemic pathophysiological process rather than a simple compensatory response.

The associations between diaphragm ultrasound parameters and pulmonary function support the physiological validity of this respiratory pump phenotype. FEV1 remains central to BOS diagnosis and staging, but it primarily quantifies airflow obstruction. In this study, inspiratory diaphragm thickness and DTF were negatively correlated with FEV1% predicted, suggesting that worsening obstruction is accompanied by greater diaphragmatic thickening or effort. Conversely, diaphragm excursion during deep breathing was positively associated with FVC and negatively associated with RV/TLC, indicating that reduced excursion is linked to lower ventilatory reserve and greater air

trapping. These relationships suggest that diaphragm ultrasound captures the downstream mechanical consequences of BOS that are not directly measured by spirometry, particularly the interaction between hyperinflation and respiratory muscle performance.

The abnormal M-mode waveform observed in severe BOS provides an additional dynamic view of disease physiology. Healthy controls showed a sinusoidal-like motion pattern during quiet breathing, whereas severe BOS patients demonstrated an initially rapid expiratory decline followed by an inflection point and slower terminal expiratory motion. Although this observation requires validation, it is physiologically plausible in severe obstructive disease. Expiration may begin with elastic recoil, but progressive airway narrowing, dynamic airway compression, and delayed lung emptying can slow terminal expiratory flow²¹. Standard diaphragm ultrasound parameters such as thickness, DTF, and excursion summarize selected points in the respiratory cycle, whereas M-mode recordings preserve temporal information on respiratory motion²². Recent studies have emphasized that M-mode ultrasound contains valuable information regarding the kinetics and temporal characteristics of diaphragmatic motion beyond simple excursion measurements²³. The waveform inflection observed here may therefore represent a novel sonographic marker of altered expiratory mechanics in severe BOS.

The ordinal logistic regression findings further emphasize that diaphragm thickening and excursion reflect complementary dimensions of BOS severity. L-DTF and R-DE-DB emerged as the most informative ultrasound-derived variables, and their

associations with severity persisted after adjustment for age, BMI, and sex. DTF reflects diaphragmatic thickening during inspiration and can be interpreted as an index of contractile effort, whereas excursion reflects the mechanical displacement produced by that effort. The simultaneous association of higher L-DTF and lower R-DE-DB with more severe BOS supports the concept of impaired neuromechanical efficiency. In clinical terms, patients with advanced BOS may need to generate greater diaphragmatic effort to achieve progressively smaller mechanical displacement. This pattern is consistent with the effects of airflow obstruction, air trapping, and hyperinflation on respiratory pump performance^{18,25}.

These findings have several potential clinical implications. First, diaphragm ultrasound may complement PFTs by adding a bedside assessment of respiratory pump performance to conventional measures of airflow limitation and air trapping. Second, ultrasound-derived measures such as DTF and excursion reserve may help identify patients in whom respiratory symptoms reflect not only airway obstruction but also impaired respiratory muscle mechanics. Third, if confirmed longitudinally, diaphragm ultrasound could be explored as a tool for monitoring disease progression, evaluating response to therapy, and selecting patients for respiratory rehabilitation strategies aimed at improving ventilatory efficiency. Several limitations should be acknowledged. This was a single-center cross-sectional study with a relatively small sample size, limiting generalizability and preventing causal inference. Direct measurements of respiratory muscle strength, such as maximal inspiratory pressure or transdiaphragmatic pressure, were not available. Longitudinal follow-up was not performed, so whether diaphragm

ultrasound predicts future BOS progression or clinical outcomes remains unknown.

Larger multicenter prospective studies are needed to validate these findings and define the prognostic and therapeutic relevance of diaphragm ultrasound in BOS.

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Authors' contributions

Draft structure: WB and TB contributed to conceptualization, data curation, investigation, formal analysis, and writing-original draft. YY, XM, KW, YZ, CL, PG, and RL contributed to data collection, clinical interpretation, and manuscript review. JB, YH, and ZG contributed to conceptualization, supervision, methodology, and writing-review and editing.

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Competing interests

The authors declare that they have no competing interests.

388 **Availability of data and materials**

389 The datasets generated and/or analysed during the current study are not publicly
390 available due to patient privacy and ethical restrictions but are available from the
391 corresponding author on reasonable request and with appropriate institutional approval.

392 **Consent for publication**

393 Not applicable.

394 **Ethics approval and consent to participate**

395 This study was approved by the Institutional Review Board of Peking University
396 People's Hospital (No. 2025PHB431-001) and was conducted in accordance with the
397 Declaration of Helsinki. Written informed consent was provided by each subject.

398 **List of abbreviations**

399 BOS, bronchiolitis obliterans syndrome; cGVHD, chronic graft-versus-host disease;
400 DB, deep breathing; DE, diaphragm excursion; DLCO-SB, single-breath diffusing
401 capacity for carbon monoxide; DTF, diaphragm thickening fraction; FEV1, forced
402 expiratory volume in one second; FVC, forced vital capacity; HC, healthy controls;
403 HRCT, high-resolution computed tomography; HSCT, hematopoietic stem cell
404 transplantation; LASSO, least absolute shrinkage and selection operator; L-DTF, left
405 diaphragm thickening fraction; L-Tdi-exp, left diaphragm thickness at end-expiration;
406 L-Tdi-insp, left diaphragm thickness at end-inspiration; MEF, maximal expiratory flow;
407 PFT, pulmonary function test; QB, quiet breathing; R-DE-DB, right diaphragmatic
408 excursion during deep breathing; R-DE-QB, right diaphragmatic excursion during quiet

409 breathing; R-DTF, right diaphragm thickening fraction; R-Tdi-exp, right diaphragm
410 thickness at end-expiration; R-Tdi-insp, right diaphragm thickness at end-inspiration;
411 RV, residual volume; TLC, total lung capacity.

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Figure legends

Figure 1. Diaphragmatic excursion during quiet breathing in a healthy control and a patient with severe BOS. A: M-mode ultrasound image of diaphragmatic excursion during quiet breathing in a healthy control. B: M-mode ultrasound image of diaphragmatic excursion during quiet breathing in a patient with BOS.

Figure 2. Comparison of diaphragmatic ultrasound parameters in BOS patients with different severity grades.

Figure 3. Correlation heatmap of diaphragm ultrasound parameters and pulmonary function in BOS.

Figure 4. Predicted probability of severe BOS according to diaphragmatic ultrasound parameters. A: Predicted probability of severe BOS according to left diaphragmatic thickening fraction (L-DTF), derived from an ordinal logistic regression model including L-DTF and right diaphragmatic excursion during deep breathing (R-DE-DB). R-DE-DB was fixed at its median value. B: Predicted probability of severe BOS according to L-DTF stratified by the 25th, 50th, and 75th percentiles of R-DE-DB. Reduced diaphragmatic excursion shifted the risk curves upward across all levels of diaphragmatic contractility, indicating an additive contribution of diaphragmatic

497 excursion impairment to BOS severity.