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Subphenotypes of frailty in lung transplant candidates

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Abstract

Heterogenous frailty pathobiology might explain the inconsistent associations observed between frailty and lung transplant outcomes. Subphenotype analysis could refine frailty measurement. In a three-center pilot cohort study, we measured frailty by the Short Physical Performance Battery, body composition, and serum biomarkers reflecting causes of frailty. We applied latent class modeling these baseline data. We tested class construct validity with disability, waitlist delisting/death, and early post-operative complications. Among 422 lung transplant candidates, 2 class model fit best (p=0.01). Compared to Subphenotype 1 (n=333), Subphenotype 2 (n=89) was characterized by systemic and innate inflammation (higher IL-6, CRP, PTX3, TNF-R1, IL-1RA); mitochondrial stress (higher GDF-15,FGF-21); sarcopenia; malnutrition; lower hemoglobin and

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walk distance. Subphenotype 2 had worse disability and higher risk of waitlist delisting or death (HR 4.0; 95%CI: 1.8–9.1). Of the cohort, , 257 underwent transplant (Subphenotype 1: 196; Subphenotype 2: 61). Subphenotype 2 had higher need for take-back to the operating room (48% vs 28%, p=0.005) and longer post-transplant hospital LOS (21 days [IQR: 14, 33] vs 18 [14, 28]; p=0.04). Subphenotype 2 trended towards fewer ventilator free days, needing more post-operative ECMO and dialysis, and higher need for discharge to rehabilitation facilities (p ≤0.20). In this early phase study, we identified biological frailty subphenotypes in lung transplant candidates. A hyperinflammatory, sarcopenic subphenotype appears to be associated with worse clinical outcomes.

Introduction:

Lung transplantation aims to extend survival, relieve disability, and improve health-related quality of life (HRQL) for adults suffering from end-stage lung disease. Despite advances in the field, morbidity and mortality before and after lung transplantation remains high.[1-4]

We reported that pre-operative physical frailty is both prevalent and portends risk for disability, poor HRQL, and death before and after lung transplantation.[5-7] The associations between frailty and these outcomes, however, are inconsistent.[8]

Frailty is thought to represent a final common pathway resulting from multiple putative biologic mechanisms of aging, chronic illness and behavioral factors such as low activity.[9] There is potential that only some of these heterogeneous pathobiologies would lead to poor transplant outcomes, whereas others might be reversed by correcting end-stage lung disease.

Subphenotype analysis has refined our understanding of the pathobiology and treatment responses in other pulmonary syndromes such as asthma and acute respiratory distress syndrome (ARDS).[10-13] Using latent class analysis, a statistical method used to identify subgroups within a population, two distinct subphenotypes of ARDS with differential mortality risk and intervention responsiveness have been identified and validated.[12,14,15] We hypothesized that applying latent class analysis to frailty may identify distinct subphenotypes in lung transplant candidates and that these subphenotypes may exhibit differential risk for poor outcomes. Distinguishing subphenotypes of frailty could improve our understanding of pathophysiologic drivers of frailty and inform testing subphenotype-specific responses to interventions.

Methods:

(See online supplement for more details)

Study design

The Lung Transplant Body Composition (LTBC) study is an ongoing multicenter prospective cohort study investigating the impact of pre-operative physical frailty and body composition on lung transplant outcomes. Measures representing conceptual physical frailty domains; body composition, and serum-based biomarkers reflecting commonly cited causes of frailty are collected.[16] LTBC centers include UC San Francisco, Columbia University

Medical Center, and University of Pennsylvania. We excluded candidates for multi-organ or redo lung transplantation. For this pilot analysis, we included adult lung transplant candidates enrolled in LTBC between June 2017 and May 2021 who met pre-frail or frail criteria defined by a Short Physical Performance Battery Score (SPPB) <12. The decision to include candidates deemed pre-frail or frail was based on prior work showing that each one unit (point or standard deviation) worsening in frailty below 12 was linearly associated with increased risk of death before or after lung transplant. These data underscore the semi-arbitrary nature of defining frailty, at least in lung transplant candidates, based on binary cutpoints.[5,6,17] Center Institutional Review Boards approved this study and participants provided written informed consent.

Frailty measurement

Assessments were performed during the evaluation process near the time of placement on the waitlist for transplant in all candidates capable of participating, including those in the intensive care unit some of whom were supported by invasive mechanical ventilation and/or extra-corporeal membrane oxygenation (ECMO) . Assessments were not performed if patients were actively infected or deemed so clinically unstable by the treating teams that performing a frailty assessment could trigger clinical deterioration. Assessments were repeated, as possible, every three months while participants were listed. Those assessments most proximal to the time of transplant were used for this analysis. We used the SPPB to define frailty.[18,19]

Candidate variables collected to examine subphenotypes of frailty

Based on widely cited putative causes of physical frailty, we collected additional measures including sarcopenia, adiposity, depressive symptoms, cognitive functioning and banked serum at the time of study visits.[9,20,21]

We quantified skeletal muscle mass (Appendicular Skeletal Muscle Index [ASMI]) and percent body fat by bioelectrical impedance using the InBody S10 (InBody USA, Cerritos, CA). We measured grip strength using a handheld dynamometer (Stoelting, Wood Dale, IL).

We measured depressive symptoms using the Geriatric Depression Scale (GDS) and executive function components of cognitive functioning by the Trails Making B test.[22,23]

Biomarker measurement

We selected biomarkers that could be measured in blood, were associated with frailty in multiple human populations, and ideally were also shown to be relevant in lung transplant. The study of biomarkers of frailty in lung disease is nascent but work in this space also informed our biomarker selection.[20,24] We cross-referenced these data with a recent comprehensive review of 44 candidate biomarkers of frailty that are associated with “hallmarks of aging pathways”.[16] We narrowed this list to 12 final biomarkers representative of key pathophysiologic pathways that are also established or plausible factors in lung disease and transplant. Selected pathways included *systemic inflammation* [Interleukin 6 (IL-6) and C-reactive protein (CRP)]; *innate immune activation* [interferon-inducible protein-10 (IP-10), Tumor Necrosis Factor-α (TNF-α), TNF receptor-1 (TNF-

R1), Interleukin-1 receptor antagonist (IL-1Ra), Long Pentraxin-3 (PTX-3)], *epithelial mesenchymal transition and mitochondrial stress* [Growth differentiation factor 15 (GDF-15), vimentin]; *cytoskeletal hormones/mitochondrial activity* [Fibroblast growth factor 21 (FGF-21) and Fibroblast growth factor-23 (FGF-23)], Insulin like Growth Factor-1 (IGF-1); and *adipokines/exerkines* (leptin, apelin).

Serum concentrations of these biomarkers were determined using the Meso-Scale Discovery (MSD) platform assays (Meso-Scale Diagnostics, LLC, Rockville, MD) with single samples. The lower and upper limits of detection (LLOD, ULOD) for each plate-specific analyte were defined as the concentration 2.5-standard deviations above the background or below the upper plateau of the fitted standard curve, respectively.[25] Vimentin and Apelin were measured in single replicate with commercially available ELISA kits (MyBioSource, Inc., San Diego, CA, catalog #MBS041869, catalog #MBS2883220, respectively). For data below the LLOD or above the ULOD, we imputed their values at the LLOD or ULOD, respectively. Serum albumin (malnutrition/inflammation), hemoglobin (anemia), and creatinine (multiple pathways) were abstracted from the electronic medical record.

Baseline demographics and clinical variables were extracted from the electronic medical record.

Outcome measures:

We selected measures that could plausibly be differentially affected by frailty subphenotypes.

Disability was assessed at the time of frailty assessment with the Lung Transplant Valued Life Activities Scale (LT-VLA).[26] The LT-VLA has a range of 0-3; higher scores reflect worse disability and a difference of 0.3 is considered clinically meaningful.

Delisting or death before transplant was considered as a composite outcome.[5] Time was calculated as the number of days from frailty assessment until the date of delisting or death. Participants were censored for this analysis if they underwent transplantation.

Early post-operative outcomes included ventilator free days[27]; need for post-operative tracheostomy, dialysis, and unplanned take-back to the operating room; hospital length of stay (LOS) after transplant surgery; discharge destination; and unplanned readmission within 30-days. Ventilator free days were estimated by enumerating the number of days within the first 28 post-operative days that participants were alive and free from any mechanical ventilatory support for each full 24 hour period.

Hospital LOS was calculated by the number of days between the dates of transplant surgery and discharge; those who died prior to discharge were not included in this comparison.

Statistical approach

Our goal was to test for unobserved (i.e., “latent”) subgroups of frailty in lung transplant candidates. Of various analytic approaches used to look for homogenous subgroups, we selected latent class analysis (LCA). In contrast to traditional regression-based methods that

tests the association between pre-specified independent variables and a specific outcome, LCA models whether subgroups of subjects defined by a combination of baseline variables exist, without consideration of outcomes. LCA is based on finite mixture modeling, which finds the best fitting model for a set of data based on the hypothesis that the observed distribution is a mixture of two or more “unobserved” distributions, or “latent classes”. Only after latent classes are defined are tests of whether outcomes differ by class performed.

Baseline clinical data, measures of frailty domains, and biomarker concentrations were included as class (e.g., subphenotype)-defining variables. Baseline clinical parameters included age, sex, race/ethnicity, serum creatinine, hemoglobin, and albumin, percent predicted forced vital capacity, and distance walked in six-minutes. Skewed continuous variables were log-transformed and all continuous variables were transformed to a z-scale. Based on contemporary recommendations, we examined the intercorrelation of all variables and removed one of any pair of highly correlated variables with five exeptions.[28] These five pairs included TNF-R1 with GDF15 [r^2 =0.51], IL1-RA [r^2 =0.41]CRP [r^2 =0.44], and with IL-6 [r^2 =0.49];as well as IL-6 with CRP[r^2 =0.57]. Given the consistent association between markers of immune activation and inflammation with frailty in the biomedical literature and that individual biomarkers can reflect distinct pathways within the broad category of inflammation, we allowed them to remain correlated in the model. For variables known to substantively differ by sex, we generated sex-specific z-scales.

We used Mplus (v8.7) to fit models with latent classes ranging from 1-4 classes. To determine the best-fitting model, we examined the Bayesian Information Criteria (BIC) , the Vuong-Lo-Mendell-Rubin (VLMR) test, and the number of participants assigned to the smallest class, where a model with a very small class size would not be meaningful.

Once the number of classes (i.e., subphenotypes) was established, participants were assigned to their most likely subphenotype. We visually inspected the distribution of variables by classes to ensure that they did not reflect scaled groupings (the so called “Salsa Effect) (see online Supplement for more explanation)”[29] We used t-tests, Pearson’s chi-square, or Wilcoxon Rank Sum to test for differences between subphenotypes. We considered differences in variables by subphenotype to be significant if the standardized means differed by 0.5 or greater. We retained both TNF-α and TNF-R1 in our models as TNF-α may drive shedding of TNFR1/TNF-α complexes, which are more stable than TNF-α.[30] As a sensitivity analysis we dropped TNF-α and repeated fitting latent classes. As an additional sensitivity analysis of latent classes, we restricted the cohort to participants with SPPB scores of ≤10.

We analyzed the association between subphenotypes and disability by Wilcoxon Rank Sum and between subphenotypes and waitlist delisting/death by Kaplan Meier methods with Cox proportional hazard modeling with transplantation modeled as a competing risk. Differences in post-operative outcomes by Subphenotype were analyzed by chi-square test for categorical outcomes, and Wilcoxon Rank Sum test for continuous outcomes with skewed distribution.

In a post-hoc analysis, we used mixed-effects models to test whether SPPB scores systematically changed over time on the waitlist among participants who underwent >1 frailty assessment before transplant,. We used random intercepts to account for correlation among serial SPPB measurements within the same participant.

COVID19-related mandated restrictions on non-essential research activities in 2020 precluded all participants from completing all components of study visits. Each participating institution allowed for the resumption of research activities on different timelines. As a result, we were unable to collect body composition measures and/or research blood samples on a significant number of research participants (Supplemental Table 1). In sensitivity analyses, we tested for differences in biomarker missingness by subphenotype and compared the risk of waitlist delisting/death restricted to participants with complete data.

Results:

(See the online supplement for more details)

Among 700 transplant candidates enrolled, 422 (60%) had SPPB scores <12 and thus composed the cohort for this analysis (Figure 1). Supplemental Table 2 compares demographic characteristics between those with and without complete study visits. Supplemental Table 3 shows demographic characteristics stratified by study center. Overall, the 422 participants had a mean age of 57.7 (SD ±11.9) years, were 49% female and 65% Caucasian. Pulmonary fibrosis was the predominant indication for transplant candidacy. The mean SPPB (SD) score among the prefrail or frail was 8.3 (± 3.1). During the waitlist time period, 42 participants (10%) underwent >1 frailty assessment (range 2-5). Amongst this subgroup, overall frailty scores remained relatively stable (change in SPPB: −0.06, [95% CI: −0.12, −0.00045] per month additional time on the waitlist; p = 0.048). Individual scores, however, varied over time. Of the 42 participants, SPPB scores worsened by ≥1 point in 18 (43%), improved by ≥1 point in 8 (19%), remained stable across assessments in 8 (19%), and, in the remaining 8 all of whom had ≥3 assessments either improved, remained stable, or worsened from one assessment to the next (Supplemental Figure 1). Of the 422 participants, 257 underwent transplant. Amongst these participants, the LAS scores from frailty assessment to transplant changed by a median of 5.3 (IQR: 0.3, 21.1)

Latent-class models suggested that a two subphenotype model provided the optimal fit. The fit statistics including measures of BIC, entropy, and VLMR testing are shown in Table 2. Of the participants, 333 (79%) were assigned to Subphenotype 1 and 89 (21%) were assigned to Subphenotype 2. The mean latent class probabilities for subphenotype assignment were 0.97 for Subphenotype 1 (median 1.0 [IQR: 0.989, 1.0]) and 0.91 for Subphenotype 2 (median 0.99, [IQR:0.903, 1.0]). Amongst this cohort, 196 participants (59%) in Subphenotype 1 and 61 participants (69%) in Subphenotype 2 underwent transplant (p = 0.10).

Clinical and biological features of frailty subphenotypes

We next compared the clinical and biological variables that defined the two subphenotypes. Demographics as well as mean, median, and proportion values of continuous and categorical variables are shown in Table 1; a line plot of the latent profiles of the normalized

continuous variables is shown in Figure 2, Table 4 details the standardized mean values of the continuous variables by subphenotype, and comparison of biomarker and categorical variables is shown in Supplemental Figures 2 and 3. Compared to Subphenotype 1, Subphenotype 2 was younger (median age 56 [Interquartile Range; IQR: 44, 65] compared to 62 [54, 66] years), more likely to be Hispanic (30% vs 14%), and markedly more likely to be hospitalized at the time frailty assessment (71% vs 10%) (all $p \leq 0.01$). Participants in Subphenotype 2 were more likely to have pulmonary fibrosis (72% vs 66%) and less likely to have COPD (10% vs 23%). Overall, SPPB frailty scores were lower in Subphenotype 2 than Subphenotype 1 (mean score 5.8 ±4.0 vs 9.0 ±2.4; $p<0.001$) although the full range of scores (*i.e.,* 0-11) was represented in both groups.

Compared to Subphenotype 1, Subphenotype 2 was characterized by markedly higher systemic inflammation (IL-6, CRP); innate immune activation (PTX-3, TNF-R1, IL-1RA); epithelial mesenchymal transition and mitochondrial stress (GDF-15 and FGF-21); sarcopenia (low muscle mass, grip strength); malnutrition (low albumin); and low adiposity (leptin), hemoglobin (anemia), and shorter 6MWD (Figure 2). A sensitivity analysis dropping TNF-α did not change the fit statistics nor participant class assignment (Supplemental Table 4). There was no difference in the proportion of participants missing biomarker measurements in each Subphenotype (30% in Subphenotype 1, 34% in Subphenotype 2; $p =0.50$).

A sensitivity analysis restricting the test for latent classes to those with SPPB scores ≤10 recapitulated Subphenotypes 1 and 2 ($p = 0.029$; Supplemental Table 5 and Supplemental Figure 4)

Association between Subphenotypes and Clinical Outcomes

Before transplant, compared to Subphenotype 1, Subphenotype 2 had worse physical functioning by LT-VLA (1.76 ±0.72 vs 1.34 ±0.61; $p <0.001$) and a four-fold higher risk of waitlist delisting or death (Hazard Ratio 4.0; 95% CI: 1.8, 9.1; $p< 0.001$, Figure 4). After transplant, Subphenotype 2 had a 3 day longer post-operative hospital LOS compared to Subphenotype 1 (21.0 [interquartile range: 14.0, 33.0] vs 18.0 days [14.0, 28.0] $p=0.04$) (Table 3). This increased length of stay in Subphenotype 2 may have been attributable to a higher need for unplanned take-back to the operating room (32.8% vs 14.8%, $p=0.002$) and *trends* towards fewer ventilator free days and greater need for post-operative ECMO, and dialysis ($p \leq 0.20$) (Table 3). Subphenotype 2 also trended towards higher need for discharge to rehabilitation facilities ($p =0.11$).

Discussion:

In this pilot analysis of the multicenter prospective Lung Transplant Body Composition cohort, we identified two subphenotypes of frailty in lung transplant candidates with distinct clinical and biomarker profiles. Subphenotype 2 was characterized by systemic and innate inflammation; sarcopenia; and metabolic/mitochondrial stress; and a higher likelihood of being hospitalized at the time of assessment. Subphenotype 2 was also characterized by a younger population who were less likely to be Caucasian. The more prevalent Subphenotype 1 was characterized by an older yet more fit group with higher serum albumin and

hemoglobin. The subphenotypes had striking differences in outcomes, with Subphenotype 2 exhibiting worse disability, a 4-fold higher risk of delisting/death prior to transplant, as well as greater need for take-back to the operating room and a three-day longer length of hospital stay after transplant. Although none were statistically significant, patients in Subphenotype 2 exhibited trends towards fewer ventilator free days, greater need for post-operative ECMO, and dialysis, and greater need for discharge to rehabilitation facilities.

The reason(s) for the markedly higher proportion of participants hospitalized in Subphenotype 2 are unknown but bear considering. One reason may be that Subphenotype 2 is not actually a distinct subphenotype but simply reflects “worse frailty”. This explanation would suggest that severity of expressed physical frailty is determined by the magnitude of the dysregulated drivers and that the underlying biology of frailty is universal. Further, inspection of the LCA line plots (Figure 2) identified clear distinct classes rather than a set of parallel lines that would suggest scaled groupings by frailty severity (the so called “Salsa effect)”[29] which argues against a frailty gradient. Or it may be that hospitalization itself causes a specific phenotype of frailty through reduced physical activity, poorer nutrition, hospital acquired morbidities or other potential mechanisms. Some causes for hospitalization (e.g., pneumonia, sepsis) might trigger changes in the observed biomarkers and “confound” the observed relationship between SPPB and transplant outcomes; however, most transplant candidates are admitted to the hospital for higher levels of oxygen or to attenuate or reverse debilitation and declines in “fitness” for transplant rather than events such as sepsis. Finally, it may be that the multisystem dysregulation observed in Subphenotype 2 may itself contribute to hospitalization. In older adults, frailty is a well-documented risk factor for hospitalization.[18,31] Our data raise the possibility that frailty might be a distinct risk factor for admission. Disentangling these competing theories will require recruiting candidates earlier in the transplant evaluation process and measuring frailty and biomarkers repeatedly over time.

The existence of two subphenotypes may explain some of the inconsistent frailty-attributable risk we and others have previously reported. We previously found that pre-operative physical frailty resolved in over 80% of patients who survived lung transplant surgery.[32] At the time, we considered that heterogenous underlying pathobiology might explain why frailty is strongly associated with risk for death in some yet resolves in others. Our current observation of a high inflammation/dysregulated metabolism and musculoskeletal subtype of frailty suggests a potential mechanism whereby these pathways drive a frail subphenotype particularly susceptible to acute stressors. This subphenotype may reflect more allostatic overload, or the excess physiologic “costs” of maintaining allostasis. In contrast, subphenotype 1 might reflect more “benign” cause of SPPB impairments, such as disuse atrophy, that, to date, have been lumped into the frailty category but may actually identify a biological profile at lower risk for adverse effects. Many of the biological markers defining Subphenotype 2 are associated with frailty and poor outcomes in other populations and some with poor outcomes following lung transplantation. For example, IL-6 is one of the most widely studied potential molecular mediators of frailty. IL-6 is associated with frailty and frailty correlates including sarcopenia, muscle fatigue, metabolic derangements and diabetes, anemia, and age; IL-6 overexpression induces a frailty phenotype in mice.[33,34] In lung transplant, IL-6 and PTX-3 are associated with PGD.[35-37] Further, increased

IL-6 is associated with longer-term outcomes including acute and chronic lung allograft rejection.[38-42] TNF-α is associated with frailty and with impaired diaphragmatic muscle function and allograft dysfunction after lung transplant.[43] Soluble TNF receptor (TNF-R1) may be a better marker of chronic TNF-α-associated inflammation in this context since, given its longer half-life.[30] Sarcopenia is considered a cardinal feature of physical frailty;[44] in lung transplantation, measures of sarcopenia are associated with frailty, waitlist delisting/death, and longer ICU and hospital length of stay after transplant.[45-47] Pre-operative hypoalbuminemia is a strong risk factor for death after lung transplant.[48] Other markers linked to frailty, however, have not previously been described in transplant. In particular, GDF-15, described by some as a mitokine reflecting mitochondrial stress, and FGF-23, involved in the endocrine-bone-kidney axis, have both emerged as promising metabolism and musculoskeletal dysregulation pathways in frailty.[49-55] Our findings have the potential to place some of these prior isolated biomarker studies within the broader context of frailty as well as refine our understanding of why frailty has been such a strong, yet inconsistent, risk factor for poor outcomes in lung transplantation. It remains to be determined, however, whether a dominant pathway such as systemic inflammation *causes* abnormalities seen in other pathways (*e.g.,* sarcopenia or endocrine-bone-kidney axis), whether IL-6 is a sign of systemic inflammation induced through other pathways, or whether multiple dysregulated systems emerge independently.

No single clinical or molecular marker distinguished these frailty subphenotypes. In fact, multiple biomarkers were fundamental to defining our subphenotypes. Of the 15 variables with differences of 0.5 or greater, nine were exploratory biomarkers, two were measures of sarcopenia captured by BIA and grip strength, and only four clinically available lab and performance measures (Table 4 and Figure 2). These findings are consistent with work in geroscience that frames health and aging as a hierarchy of complex dynamical and interrelated systems. Frailty has been proposed as a compromised ‘dysregulated dynamical system’ where multiple physiological abnormalities interact, including the stress-response, metabolism, and musculoskeletal systems.[56] These multiple, interacting systems are likely more important than any one system in isolation.[56] In this framework, changes in cellular function occur first, followed by physiologic changes culminating in the phenotypic frailty profile.

Our study has several important limitations that, in sum, reflect the preliminary nature of our findings. COVID19 interrupted our ability to collect all measures on all participants. Although the nature of our COVID19-related data missingness was unlikely to differentially impact patient subgroups, the impact of this unusual source of missingness and the resultant smaller sample size is unknown. For example, while a 2 class model optimally fit our data, it is possible that more subtypes exist. We also we did not have a larger pool of more severely frail participants to test whether two subphenotypes were evident across strata of frailty severities, including other cutpoints used to define frailty as a binary state, or in specific lung diseases such as COPD or pulmonary fibrosis. Our sample size may have impacted our ability to definitively determine if a broader range of perioperative complications truly differ between subphenotypes. Larger cohorts may help to resolve the number and nature of frailty subphenotypes and potential differential association with clinical outcomes. Further, pre-operative frailty has been shown to be a risk factor for postoperative complications

and increased length of stay across multiple surgical populations.[57,58] While our work is consistent with this broader body of literature, pre-operative frailty is unlikely to fully explain the range of surgical complications after lung transplantation, some of which cannot be unanticipated. Parsing the relative effects of surgical frailty-attributable and frailty non-attributable surgical complications on short- and longer-term outcomes after transplant remains an unanswered but important question. Although we did not perform frailty assessments on patients when they were deemed actively infected or so clinically unstable by the treating team that performing a frailty assessment could have been life-threatening (rare inpatients), it is possible that subclinical infection or acute changes in steroid and other immunosuppressant dosing not captured by research teams could have impacted the values of some of our measured biomarkers. Further, given frailty assessments were not performed on highly unstable patients, further work is needed to determine whether our findings can be generalized to this small subset of lung transplant candidates. Next, the biomarkers analyzed were chosen based on the extant frailty literature and their association with frailty or other complications in lung disease and transplant.[20] It is possible that other biomarkers representative of key frailty pathways could have been informative.[16] Thus, the biomarkers we selected for this study should not be considered definitive nor comprehensive. We also lacked access to a separate independent cohort with which to externally validate our findings. Further, on an individual level, frailty may be dynamic in the pre-operative period. It is possible that the subphenotype group assignment and associations between subphenotypes and peri- and post-operative outcomes would differ if frailty assessments were repeated following episodes of acute clinical worsening or at the time of transplant. We believe, however, that any “failure” to capture these possible changes in subphenotype group assignments would have biased our findings towards the null. Clinically, waitlisted transplant candidates tend to get sicker rather healthier. Thus, theoretically this bias would have resulted in more participants being misclassified into Subphenotype 1 rather than Subphenotype 2. Work is needed to define the optimal timing and frequency of frailty assessment(s) during the pre-transplant journey and whether these timepoints contribute different information relevant for clinical decision making, risk stratification and prediction, or intervention. Finally, because this study is ongoing and longer term follow up is incomplete, we are underpowered to test for differences in longer term post-operative outcomes leaving our understanding of the overall relevance of these subphenotypes in lung transplantation incomplete. In sum, these limitations argue that our findings should be considered a first and preliminary step in understanding frailty subphenotypes.

Despite these limitations, our study had notable strengths. To our knowledge, this is the first study in lung transplant to demonstrate different subphenotypes of physical frailty. Further, these subphenotypes identify pre-frail/frail lung transplant candidates at differential risk for poor outcomes before and after transplant. This finding, if confirmed, has broad implications for how frailty is measured; how frailty is used for risk stratification; and in designing future interventions to prevent or treat it. A recent NHLBI report stated that identifying responders to disease-specific treatment “requires understanding the disease biology, the likely mechanistic effect of the treatment, and the interaction between the two”.[59] If frailty is the result of a multiplicity of physiological abnormalities, it suggests that a multidimensional measure accounting for subphenotypes may improve our ability to

identify responders to intervention. We also tested the relevance of a broader, intentially selected array of biomarkers recommended by the frailty community.[9,16,56] In doing so, we confirmed previously identified biomarkers (i.e., IL-6, CRP) and identified the relevance of novel biomarkers such as FGF-23 and GDF-15. These findings suggest new areas for investigation into understanding the pathobiology of frailty in advanced lung disease and transplant.

That Subphenotype 2 was generally younger and had a higher prevalence of underrepresented minorities warrants special consideration. The former observation underscores the relevance of considering frailty and other aging-related concepts in advanced lung disease across the age-spectrum. The overrepresentation of underrepresented minorities with advanced lung disease in Subphenotype 2 raises the possibility that incorporation of frailty measurements into clinical practice could introduce or exacerbate disparities in access to lung transplantation.[60-62] Such unintended consequences should be scrupulously guarded against by clinicians, guideline committees, payers and policy makers. Whether social determinants of health contribute to our biomarker and clinical outcome findings is unknown.[63-66]

In sum, we found evidence for two subphenotypes of frailty in lung transplant candidates. These subphenotypes identify patients with different clinical and biological features and risk for poor outcomes before and after lung transplantation. Confirming our findings will have broad implications advancing the field of frailty research in pulmonary medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

6MWD	Six-minute walk distance
ARDS	Acute respiratory distress syndrome
ASMI	Appendicular Skeletal Muscle Index
BIC	Bayesian Information Criteria
CRP	C-reactive protein
FGF-21	Fibroblast growth factor 21

FGF-23	Fibroblast growth factor 23
GDF15	Growth differentiation factor 15
GDS	Geriatric Depression Scale
HRQL	Health-related quality of life
IL-1Ra	Interleukin-1 receptor antagonist
IL6	Interleukin 6
IGF-1	Insulin like Growth Factor-1
IP-10	interferon-inducible protein-10
LCA	latent class anlaysis
LLOD	lower limits of detection
LOS	length of stay
LTBC	Lung Transplant Body Composition
LT-VLA	Lung Transplant Valued Life Activities Scale
MSD	Meso-Scale Discovery
PTX3	Long Pentraxin-3
SPPB	Short Physical Performance Battery Score
TNF-α	Tumor Necrosis Factor-α
TNF-R1	Tumor Necrosis Factor receptor-1
ULOD	upper limits of detection
VLMR	Vuong-Lo-Mendell-Rubin

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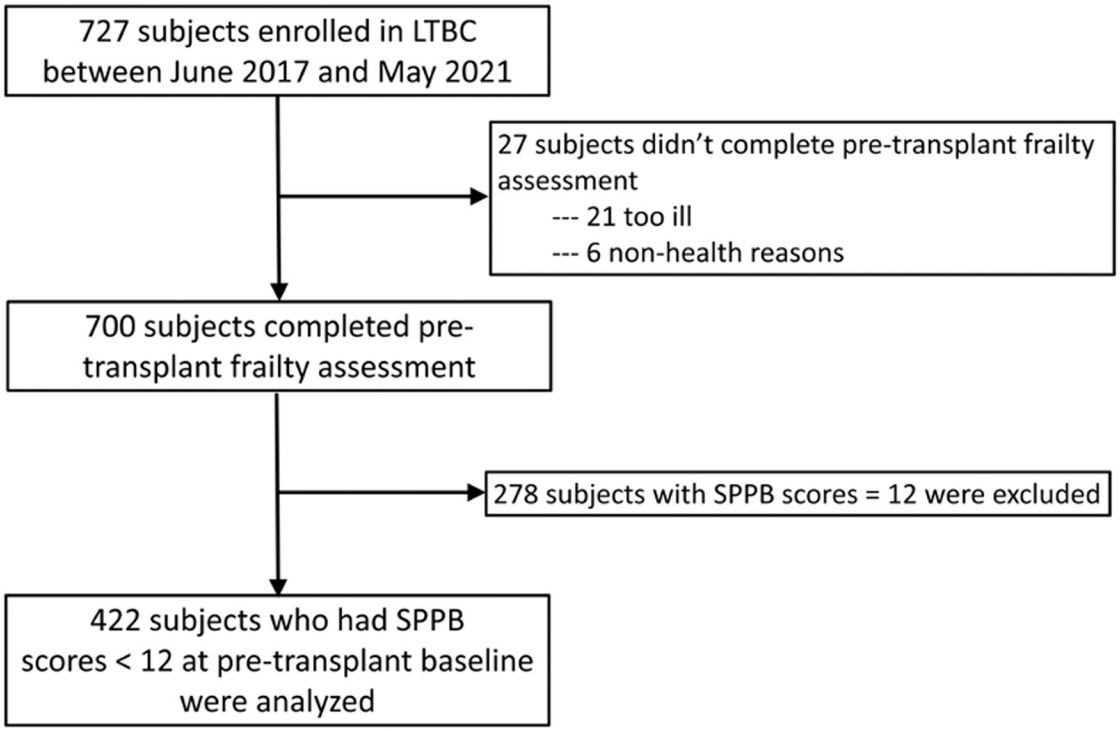

Figure 1:
Study Flow

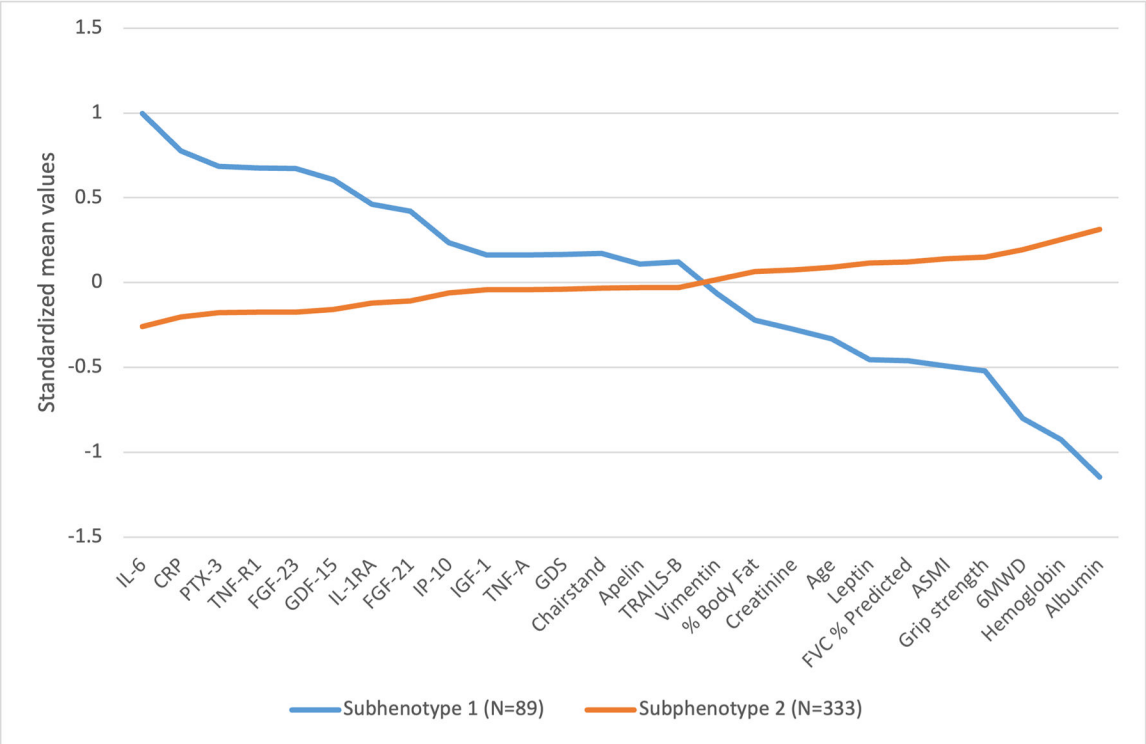

Figure 2:
Differences in standardized mean values of variables by frailty subphenotype

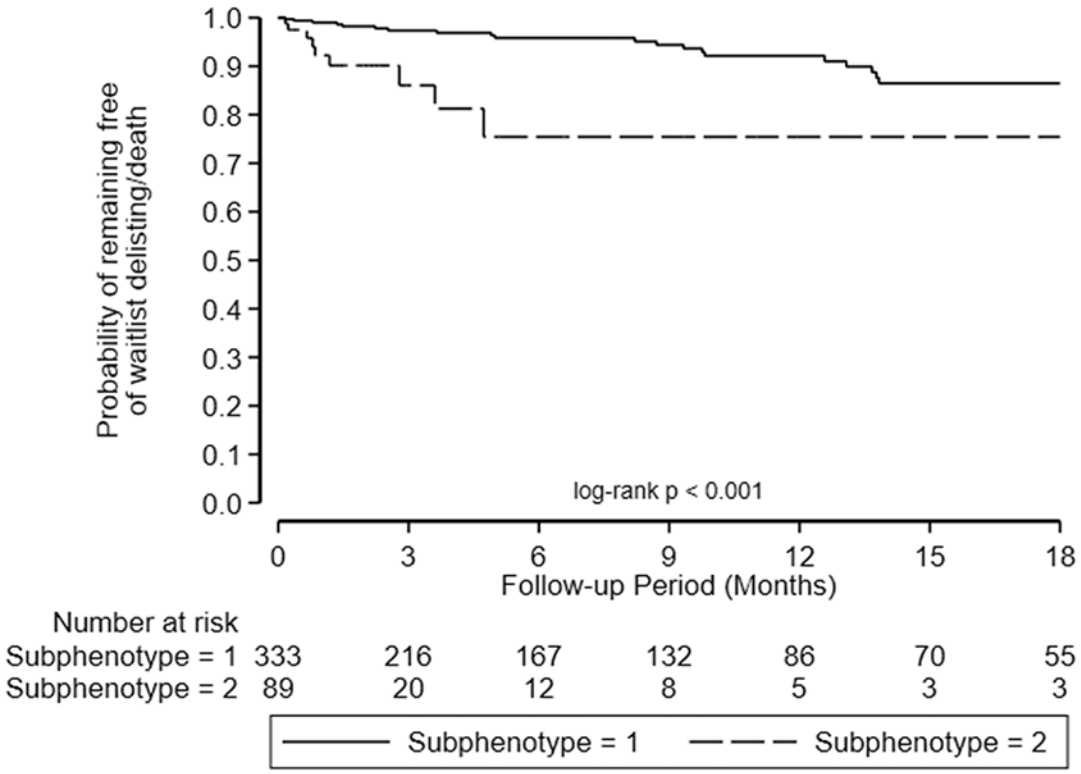

Figure 3:
Time to waitilist delisting/death by frailty Subphenotype

Table 1:

Baseline demographics of cohort

Variable	Subphenotype 1 (n=333)	Subphenotype 2 (n=89)	p-value
Age, years	62 [54, 66]	56 [44, 65]	0.002
Race			
Non-Hispanic White	233 (70.0)	42 (47.2)	0.001
Asian	21 (6.3)	7 (7.9)	
Black	29 (8.7)	11 (12.4)	
Hispanic	46 (13.8)	27 (30.3)	
Other	4 (1.2)	2 (2.3)	
Female	161 (48.4)	47 (52.8)	0.455
Diagnosis			
Group A (COPD)	78 (23.4)	9 (10.1)	<0.001
Group B (PH)	25 (7.5)	4 (4.5)	
Group C (CF)	12 (3.6)	12 (13.5)	
Group D (ILD)	218 (65.5)	64 (71.9)	
SPPB	9.0 ± 2.4	5.8 ± 4.0	<0.001
Hemoglobin (g/dL)	13.0 ± 1.7	10.7 ± 2.1	<0.001
Creatinine (mg/dL)	0.8 [0.7, 0.9]	0.7 [0.6, 0.8]	<0.001
Albumin (g/dL)	4.1 [3.8, 4.4]	3.3 [2.8, 3.6]	<0.001
FEV_1 (L)	1.3 ± 0.6	1.2 ± 0.5	0.051
FEV_1 %	43.3 ± 19.0	37.8 ± 15.5	0.015
FVC (L)	2.0 ± 0.8	1.6 ± 0.7	<0.001
FVC %	51.8 ± 17.1	41.2 ± 15.0	<0.001
Six-min walk distance (meters)	265.2 [182.0, 335.3]	118.9 [74.4, 201.2]	<0.001
Hospitalized at the time of frailty assessment	33 (9.9)	63 (70.8)	<0.001
LAS at the time of frailty assessment	42.5 ± 12.8	57.8 ± 19.9	<0.001
Grip strength, female (kg)	21.6 ± 8.4	15.4 ± 7.4	<0.001
Grip strength, male (kg)	34.9 ± 10.6	26.5 ± 13.2	<0.001
Percent body fat, female	38.2 ± 8.8	31.7 ± 11.8	<0.001
Percent body fat, male	28.0 ± 8.3	28.1 ± 9.3	0.962
ASMI, female	6.7 ± 1.0	6.0 ± 1.2	0.001
ASMI, male	8.3 ± 1.4	7.2 ± 1.1	<0.001
BMI (kg/m2)	27.1 ± 4.4	24.1 ± 4.6	<0.001
Trails making B test (seconds)	96.9 [71.0, 120.3]	94.5 [77.4, 139.4]	0.467
GDS depression score	5.0 [3.0, 7.0]	6.0 [3.5, 8.5]	0.080
Frailty biomarkers			
IL-6 (pg/ml)	2.0 [1.4, 3.4]	6.2 [3.5, 13.0]	<0.001
TNF-alpha (pg/ml)	1.4 [1.1, 2.0]	1.8 [1.4, 2.3]	0.002

Variable	Subphenotype 1 (n=333)	Subphenotype 2 (n=89)	p-value
TNF-receptor 1 (pg/ml)	1385.4 [1112.3, 1772.8]	1853.4 [1408.8, 2738.6]	<0.001
Long pentraxin 3 (pg/ml)	2428.2 [1556.3, 3676.2]	4247.5 [3100.0, 6692.4]	<0.001
CRP (mg/l)	7.8 [3.5, 18.5]	39.0 [15.7, 77.6]	<0.001
IL-1 RA (pg/ml)	321.2 [234.9, 472.1]	484.9 [300.3, 684.0]	<0.001
IP-10 (pg/ml)	381.6 [251.3, 579.4]	412.7 [324.8, 706.8]	0.062
GDF-15 (pg/ml)	2294.3 [1389.5, 3382.4]	3370.2 [2268.2, 5102.9]	<0.001
FGF-21 (pg/ml)	928.9 [495.4, 1676.9]	1297.9 [711.6, 3106.3]	0.001
FGF-23 (pg/ml)	44.2 [31.9, 75.2]	96.9 [50.1, 230.6]	<0.001
IGF-1 (pg/ml)	27.2 [2.7, 340.2]	60.7 [2.9, 890.6]	0.129
Vimentin (pg/ml)	1730 [1240, 2160]	1520 [940, 2160]	0.339
Leptin (pg/ml)	14475.0 [5999.9, 30871.7]	5166.7 [1790.0, 15782.3]	<0.001
Apelin (pg/ml)	478.1 [308.5, 714.6]	462.4 [346.0, 763.3]	0.716

Data presented as mean ± standard deviation or median [interquartile range]. Diagnostic indication for transplantation was categorized by the A (COPD), B (PAH), C (Cystic Fibrosis), D (ILD) groupings in the Lung Allocation Score.[67] Data presented as mean ± SD, n (%), or median [interquartile range]. IL-6 = Interleukin 6; CRP = C-reactive protein; PTX-3 = Long Pentraxin-3; TNF-R1 = Tumour Necrosis Factor- Receptor 1; FGF-23 = Fibroblast growth factor-23; GDF-15 = Growth differentiation factor 15, IL-1RA = Interleukin-1 receptor antagonist; FGF-21 = Fibroblast growth factor-21; IP-10 = Interferon-Inducible Protein 10 (also called CXCL10), TNF-a = Tumor Necrosis Factor-alpha; GDS = Geriatric Depression Scale; TRAILS-B = Trail Making Test Part B; FEV1 = Forced Expriatory Volume in 1 second; FVC = Forced Vital Capacity; ASMI = Appendicular Skeletal Muscle Index; 6MWD = six minute walk distance

Table 2:

Fit statistics

Classes	BIC	Entropy	N1	N2	N3	N4	p-value
1	28047.9		422				
2	27670.6	.86	333	89			.0158
3	27656.3	.78	214	163	45		.5855
4 *	27637.4.	.82	187	182	43	10	.2820

BIC = Bayesian information criteria. *no full replication of likelihood, untrustworthy standard errors

Table 3.
Early post-operative outcomes by Subphenotype

	Subphenotype 1 (n=196)	Subphenotype 2 (n=61)	p-value
Ventilator free days*	21.1 ± 7.7	19.2 ± 9.1	0.102
Need for post-operative tracheostomy	35 (17.9)	15 (24.6)	0.246
Acute kidney injury requiring dialysis	15 (7.7)	8 (13.1)	0.192
Need for post-operative ECMO	51 (26.0)	21 (34.4)	0.202
Unplanned take-back to the Operating Room within 30-days (with reasons detailed below)	29 (14.8)	20 (32.8)	0.002
Bleeding/hemothorax/washout	20 (66.7)	17 (85.0)	N/A
Chest closure unrelated to ECMO	1 (3.3)	1 (5.0)	
Other#	8 (26.7)	2 (10.0)	
Hospital Length of Stay, days	18.0 [14.0, 28.0]	21.0 [14.0, 33.0]	0.037
Discharge destination			0.119
Home	151 (80.7)	42 (71.2)	
Acute rehabilitation/Skilled Nursing	36 (19.3)	17 (28.8)	
Unplanned readmission within 30 days (with reasons detailed below)	44 (22.4)	15 (24.6)	0.728
Infection	9 (20.5)	5 (33.3)	N/A
Allograft dysfunction	10 (22.7)	1 (6.7)	
Surgical complication	3 (6.8)	0 (0)	
Arrythmia	1 (2.3)	1 (6.7)	
Gastrointestinal	3 (6.8)	2 (13.3)	
**Other	18 (40.9)	6 (40.0)	

*Ventilator free days = days alive and free from invasive mechanical ventilation within the first 28 post-operative days.

#Reasons for “other” listed in Supplemental Table 6

Table 4.
Standardized mean values by subphenotype.

Bold denotes variables standardized mean difference was 0.5 or greater between subphenotypes

Variable	Subhenotype 1 (N=333)	Subphenotype 2 (N=89)	Difference
IL-6	−0.258	0.997	**−1.255**
CRP	−0.201	0.776	**−0.977**
PTX-3	−0.177	0.686	**−0.863**
TNF-R1	−0.175	0.675	**−0.85**
FGF-23	−0.174	0.673	**−0.847**
GDF-15	−0.157	0.606	**−0.763**
IL-1RA	−0.119	0.462	**−0.581**
FGF-21	−0.109	0.421	**−0.53**
IP-10	−0.061	0.237	−0.298
IGF-1	−0.043	0.164	−0.207
TNF-A	−0.042	0.162	−0.204
GDS	−0.04	0.165	−0.205
Chairstand	−0.031	0.171	−0.202
Apelin	−0.028	0.109	−0.137
TRAILS-B	−0.028	0.122	−0.15
Vimentin	0.017	−0.065	0.082
% Body Fat	0.064	−0.221	0.285
Creatinine	0.075	−0.274	0.349
Age	0.09	−0.33	0.42
Leptin	0.117	−0.454	**0.571**
FVC % Predicted	0.122	−0.459	**0.581**
ASMI	0.142	−0.491	**0.633**
Grip strength	0.15	−0.521	**0.671**
6MWD	0.196	−0.799	**0.995**
Hemoglobin	0.253	−0.928	**1.181**
Albumin	0.313	−1.146	**1.459**

IL-6 = Interleukin 6; CRP = C-reactive protein; PTX-3 = Long Pentraxin-3; TNF-R1 = Tumour Necrosis Factor- Receptor 1; FGF-23 = Fibroblast growth factor-23; GDF-15 = Growth differentiation factor 15, IL-1RA = Interleukin-1 receptor antagonist; FGF-21 = Fibroblast growth factor-21; IP-10 = Interferon-Inducible Protein 10 (also called CXCL10), TNF-a = Tumor Necrosis Factor-alpha; GDS = Geriatric Depression Scale; TRAILS-B = Trail Making Test Part B; FEV1 = Forced Expriatory Volume in 1 second; FVC = Forced Vital Capacity; ASMI = Appendicular Skeletal Muscle Index; 6MWD = six minute walk distance