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Outcomes of lung transplantation in patients with telomere-related forms of progressive fibrosing interstitial lung disease pulmonary fibrosis: A systematic review

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KEYWORDS:
lung transplantation;
interstitial lung diseases;
telomere;
genetic;
familial pulmonary fibrosis;
outcomes

BACKGROUND: Lung transplantation (LTX) is the last life-extending option for patients with progressive fibrosing interstitial lung diseases (fILD). Between 12% and 71% of patients with fILD are patients with underlying telomere-dysfunction (trILD) related to pathogenic telomere-related gene (TRG) variants and/or short telomere length. TrILD patients tend to have earlier disease onset, faster progression, and worse prognosis causing them to be referred for LTX more often. Regarding LTX outcomes in trILD, there are contradictory reports on patient and graft survival, as well as numerous other outcomes. There is no consensus on whether trILD is associated with poorer outcomes after LTX and what considerations regarding candidacy are appropriate.

METHODS: We aimed to systematically review LTX outcomes of patients with trILD in comparison to those with non-trILD.

RESULTS: A systematic literature search yielded 13 studies that met the inclusion criteria including 933 LTX, 281 in trILD, and 652 in non-trILD. Despite large heterogeneity in the methodological study

Abbreviations: fILD, fibrotic interstitial lung diseases; ILD, Interstitial lung diseased; trILD, telomere related ILD; IPF, idiopathic pulmonary fibrosis; PPF, progressive pulmonary fibrosis; LTX, lung transplantation; GV, genetic variant; TRG, telomere related genes; TBD, telomere biology disorders; ISHLT, the International Society for Heart and Lung Transplantation; PGD, primary graft dysfunction; ACR, acute cellular rejection; CLAD, chronic lung allograft dysfunction; PRISMA, preferred reporting items for systematic reviews and meta-analyses; TL, Telomere length; HR, hazard ratio; 95% CI, confidence interval of 95%; OR, odds ratio; ECMO, extracorporeal membrane oxygenation; CMV, cytomegalo-virus; WGS, whole genome sequencing; WES, whole exome sequencing; NGS, next generation sequencing

A lung transplantation systematic review highlights patient and graft survival do not seem inferior in patients with interstitial lung disease and telomere-dysfunction deemed eligible for lung transplantation

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https://doi.org/10.1016/j.jhlto.2024.100054

quality and reported outcomes among the studies, patient and graft survival after LTX in trILD did not evidently seem inferior to LTX in non-trILD. However, there may be increased risk of specific complications, such as cytopenias, airway complications, and cytomegalovirus-reactivation.

CONCLUSIONS: In summary, due to large heterogeneity in methodological study quality and reported outcomes, no firm conclusions can be drawn. Patient and graft survival do not seem unequivocally inferior in patients with trILD *deemed eligible* for LTX. On top of limited available high-quality data, specific patient selection and post-transplant management strategies may affect the currently acquired results. As such, differences may exist regarding transplant-related outcomes, which could require special attention and consideration. Further high-quality comparative studies on LTX outcomes in trILD are needed to draw final conclusions and provide recommendations regarding patient selection and post-transplantation management.

JHLT Open 2024;3:100054

Background

Interstitial lung diseases (ILD) comprise a heterogeneous group of conditions affecting pulmonary interstitial tissue, with a variable prognosis. The prognosis is especially poor in idiopathic pulmonary fibrosis (IPF) and other fibrotic ILD manifesting with progressive pulmonary fibrosis (PPF). PPF is defined by the ATS/ERS/JRS/ALAT Clinical Practice Guideline as at least 2 out of 3 criteria of worsening symptoms, increase of fibrotic radiological signs, and physiological decline within the last year.[1]

Among patients with IPF and PPF, it becomes increasingly clear that a significant proportion (10%-20%) demonstrates familial aggregation (2 or more relatives affected from the same family), has a pathogenic variant in telomere-related genes (TRG), and/or demonstrate significant telomere shortening.[2-4] The currently known PPF associated TRG variants, which are mostly germline, are in *TERT*, *TERC*, *RTEL1*, *PARN*, *TINF2*, *DKC1*, *NOP10, NHP2, ACD, NAF1, RPA, POT1*, and *ZCCHC8*.[5-14] Importantly, some rare TRG variants have unknown clinical significance. Pathogenic and likely pathogenic variants in genes regulating telomere maintenance may result in very short telomeres and can lead to a spectrum of illnesses collectively referred to as telomere biology disorders (TBD). For the purpose of this review, we will not discriminate between (likely) pathogenic TRG variants and TRG variants of uncertain significance.[15]

Possible clinical features of TBD range from complex multisystem disorders with onset in childhood, such as dyskeratosis congenita, or to single- or multiorgan manifestations, such as bone marrow failure, liver and/or pulmonary fibrosis, and cancer.

Fibrotic ILD onset in patients with trILD is at relatively younger age and exhibits a faster deterioration than non-trILD patients despite antifibrotic treatment.[16] Therefore, for a large number of patients with trILD, lung transplantation (LTX) becomes their last life-extending available treatment option.[4,17] Indeed, it was recently demonstrated that IPF patients who underwent LTX frequently presented with TBD and a cohort of patients, including all fibrotic ILD referred for LTX presented with a pathogenic or likely pathogenic TRG variant in up to 55% of cases.[18]

Initial studies on LTX in patients with TRG variants[19] and telomere shortening[20,21] reported poor patient and graft survival and increased risk of post-transplantation complications.[22-24] Others, however, do not confirm these findings and suggest similar survival and quality of life as in LTX patients without trILD.[18,25,26] The different studies are highly heterogeneous in size, selected population, and outcomes. This causes current uncertainty how to handle LTX in patients with trILD, and whether trILD should be routinely screened for and whether trILD should be considered a risk factor for inferior outcomes.[4,27-29] To date, no advice is provided by the ISHLT guidelines on criteria for LTX recipient selection, other than that patients with trILD should undergo detailed hematologic evaluation to exclude concurrent hematologic abnormalities.[28]

To gain better insights in the outcomes after LTX for trILD, we aimed to systematically review outcomes of patients with trILD (based on TRG variants or telomere shortening) in comparison to those with non-trILD. Our main outcomes of interest are primary graft dysfunction (PGD), acute cellular rejection (ACR), chronic lung allograft dysfunction (CLAD), and patient survival. Secondary outcomes included all other described post-transplantation outcomes.

Methods

Protocol and registration

This systematic review was conducted following the guidelines for the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[30] The review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO ID: CRD42022340041).

Search strategy

A systematic literature search was be performed in 6 online databases (PubMed, Embase, MEDLINE, Web of Science, Cochrane Library, and Google Scholar) for articles until June 1, 2022. A database-specific search strategy was developed with help of an experienced librarian. The search was performed based on keywords and medical subject

headings terms involving terms related to "Interstitial lung diseases," "pulmonary fibrosis," "telomere," "gene," and "lung transplantation." Furthermore, forward citations search was performed and references of selected papers were assessed for further eligible studies. All search results were collated using EndNote software (Thomson Reuters, New York, NY) and duplicates were removed. The exact search strategy, including the used search terms and syntaxes, is presented in the Supplementary Material.

Inclusion criteria

The titles and abstracts were reviewed independently by 2 authors (J.B.-M. and M.H.) to assess if the records met the predetermined inclusion criteria. For this, we used the following PICOS (population, intervention, comparators, outcomes, and study design).

- Population: Patients with trILD (based on presence of (1) pathogenic or likely pathogenic DNA variants in TRG or (2) telomere shortening)
- Intervention: LTX.
- Comparators: Patients that underwent LTX with non-trILD.
- Outcomes: Description of post-transplantation outcomes, including PGD, ACR, CLAD, survival, or others.
- Study design: Prospective and retrospective observational studies and case series.

Studies were limited to original research studies in adults, peer-reviewed, and in English. Studies were also excluded if it was not possible to extract outcomes for the patients with ILD separately from other diagnoses. Screening of full texts was performed by 2 independent reviewers (J.B.-M. and M.H.) and the reasons for exclusion were recorded. Any discrepancy was resolved by a third reviewer (M.W.).

Data extraction and synthesis

Data extraction was performed by 2 authors independently (J.B.-M. and M.H.) through predesigned forms in Excel (Microsoft, Redmond, WA), divided by studies that looked at TRG variants, telomere shortening, and familial aggregation of ILD. Any discrepancy was resolved by a third reviewer (M.M.-M.). The following data were extracted: author information (name of first author and year of publication), participant features (number of patients described, age, sex, underlying lung disease, and familial aggregation), study design and patient selection, technique and analysis used for genetic variants and measurement technique and type of sample for telomere length (TL), prevalence of genetic variants/telomere shortening, main outcomes and their criteria (PGD, ACR, CLAD, survival, follow-up, and all reported secondary outcomes).

Quality assessment

The methodological quality of the studies was evaluated using Cochrane's "Tool to Assess Risk of Bias in Cohort Studies."[31] This tool consists of 8 questions, of which 2 were excluded because they were not applicable in the setting of outcomes after LTX. The excluded questions were "Can we be confident that the outcome of interest was not present at start of study?" and "Were cointerventions similar between groups?". Regarding the question "*Can we be confident in the assessment of exposure?",* we used this question to assess both the patient selection as the method of genetic testing, as both were relevant. The tool judges studies on a scale from 0 (no risk of bias) to 3 (high risk of bias). To compare the different studies, the percentage of total bias risk was based on the 7 applicable questions (from 0% no bias risk to 100% maximum bias risk). The specific criteria for the application of each question in this study are detailed in Supplementary Material Table 2. Each study was assessed individually by 2 authors (J.B.-M. and M.H.), and discrepancies were resolved by consensus.

Results

A total of 2,230 records were identified searching the 6 databases, including 790 duplicates. One additional record was identified by forward citation searching. Of 1,440 unique papers, title and abstract and 26 full text studies were screened. Of these, 13 studies were included in the current review (11 cohort studies and 2 case-series). A PRISMA flow diagram of the studies' identification, screening, and inclusion is shown in Figure 1.

From the 11 included cohort studies on LTX in trILD, 8 report on TRG variants,[18,19,22,25,26,32-34] 8 on telomere shortening.[18,21,22,26,32,34-36] Of the included papers, 5 report on both TRG variants and telomere shortening.[18,22,26,32,34] Outcomes in a total number of 933 LTX patients were described, 281 in trILD, and 652 in non-trILD. Important to note is that 3 studies have overlap in patients while reporting different complementary outcomes.[18,34,36] Regarding the counts and patient characteristics, the overlapping patients were excluded, and only the largest patient cohort was taken into account.[18]

From the full-read studies, the reasons for exclusion were the following: there were 4 studies without independent data for ILD patients,[20,37-39] 3 concerning familiar pulmonary fibrosis without information regarding TRF or telomere shortening,[40-42] 2 regarding TL of donors, not recipients,[43,44] 1 without outcomes data,[45] and 1 using only previously reported patients and outcomes.[46] Also 5 conference abstracts were excluded.[47-51]

Quality of studies

Scores on applicable questions of "Tool to Assess Risk of Bias in Cohort Studies"[31] are presented in Table 1. Among the 11 cohort studies, 6 were considered to have relatively low bias (< 30%),[18,19,21,26,32,36] but most of the studies were considered to have significant risk of bias. Only 4 studies performed (unbiased) genetic/TL testing on a consecutive sample of patients and related this to outcomes,[18,19,26,32] 1 study only tested patients with short TL (< 25% percentile),[26] whereas others used highly biased patient selection (e.g., inclusion of patients alive > 3 years after LTX[25])[21,22,25,33,35,40] or only describe retrospective inclusion of samples collected without further detail.[34,36]

Table 2 describes the wide range of post-LTX outcomes reported in the studies. Most studies present a multitude of

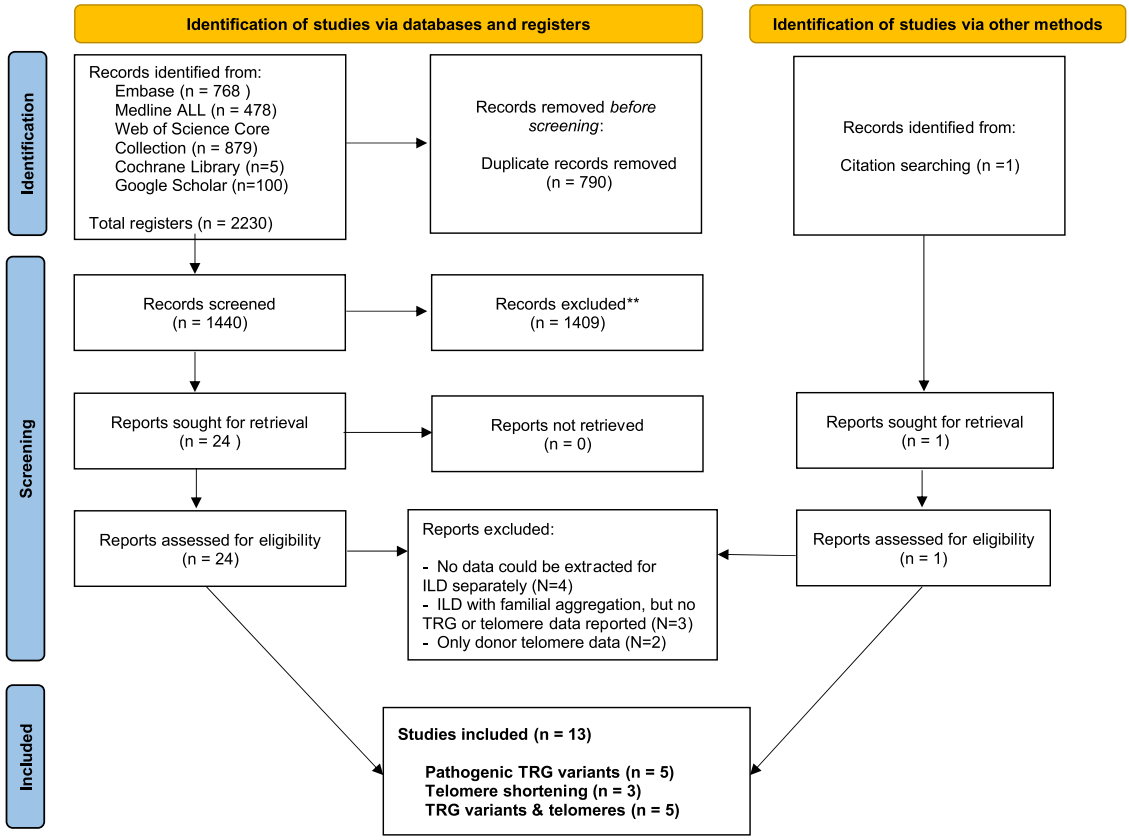

Figure 1 PRISMA flow diagram.

outcomes, primarily PGD, ACR, CLAD, and survival. Less data are available regarding kidney, liver, bone-marrow outcomes, and malignancy. Choi et al[35] focused primarily on airway complications and Popescu et al[32] report specifically on cytomegalovirus (CMV) infection. Owing to the large heterogeneity in study designs, patient selection, diagnostic tests and used cut-offs, definitions of outcomes and comparator groups, as well as the relatively high risk of bias in many studies, a quantitative synthesis through meta-analysis was not performed.

Telomere-related genetic variants

The characteristics of 10 studies (8 cohorts and 2 case-series) reporting on TRG variants in ILD patients undergoing LTX are summarized in Table 3. Studies were mostly retrospective (9 out of 10)[18,19,22-25,32-34] in nature and included between 7 and 262 LTX recipients with IPF or all types of PPF.

A total of 176 included patients with confirmed TRG variants of unknown significance were reported with a median age of 57.8 (SD 4.5) years and 69.1% being male. Several duplicate patients (N = 10) were also previously reported,[22,23] but could not be analyzed separately. As the cohorts from Hannan and Snyder were derived from the same cohort as Alder but reporting different outcome measures, the outcomes were reported, but numbers of patients were only counted once. Control groups consisted mostly of patients that were tested and did not have a known TRG variant, but in one study the control group did not undergo genetic testing[22] and another study had a second control cohort without genetic testing,[33] thus associated with higher risk of misclassification. There were 2 case-series without control group.[23,24]

Most studies performed genetic tests based on clinical suspicion of a genetic variant,[22,26,33] 3 tested all consecutive patients,[18,19,32] 1 study did not specify selection,[34] 1 tested all consecutive patients that were still alive 3-12 years after LTX,[25] and 1 study only tested patients with confirmed telomere shortening.[26]

A wide spectrum of genetic study techniques and panels were used: whole genome sequencing (WGS),[18,34] whole exome sequencing (WES),[19,26] next-generation sequencing panels (NGS),[18,32,33] and NGS-based hybrid capture method.[25] One study used WGS in 57 patients and NGS in 12 patients.[34]

The main outcomes of the studies assessing disease-associated genetic variants are depicted in Table 4. The prevalence of TRG variants in cohorts that consecutively studied all patients was on average 18% (range 12%-36%),[18,19,32] compared to 60% (range 55%-65%) in cohorts that tested only based on clinical suspicion.[25,33] The tested genetic variants and the TRG variants found (N = 220) in the various studies are shown in Table 5. From TRG variants, *TERT* was the most commonly found mutated gene (47%), followed by *RTEL1* (20%), *TERC* (14%), and *PARN* (11%). However, not all genes were tested in every study, warranting cautious interpretation of these percentages.

Table 1 Bias Assessment in Cohort Studies (Cochrane Tool[30])

Tool to assess risk of bias in cohort studies - Cochrane

	Cohort studies											Case series	
	Alder et al (2022)	Swaminathan et al (2019)	Newton et al (2017)	Snyder et al (2023)	Popescu et al (2018)	Planas-Cerezales et al (2021)	Hannan et al (2023)	Phillips-Houlbracq et al (2022)	Choi et al (2022)	Stark et al (2022)	Borie et al (2015)	Tokman et al (2015)	Silhan et al (2014)
Question 1: Was selection of exposed and nonexposed cohorts draw from the same population? *Were patients included consecutively in both cohorts (*e.g., *no selection of survivors only)?*	0	0	0	2	0	0	2	0	3	3	2	3	3
Question 2A: Can we be confident in the assessment of exposure? *Were patients consecutively sampled (*versus *only upon clinical suspicion)?*	0	0	3	2	1	0	2	2	2	2	3	3	3
Question 2B: Can we be confident in the genetic sampling? *Were the type of sample and their time point of genetic sampling described? Was the sample obtained without the influence of modifying factors (*e.g., *after lung transplantation under immunosuppression treatment)?*	1	0	0	0	0	0	0	1	1	3	11	1	1
Question 3: Can we be confident that the outcome of interest was not present at start of study? *Not applicable in this study*	-	-	-	-	-	-	-	-	-	-	-	-	-
Question 4: Did the study match exposed and unexposed for all variables that are associated with the outcome of interest or did the statistical analysis adjust for these prognostic variables? *Did the statistical analysis take potential confounding factors into account by multivariate analysis?*	0	0	0	0	2	3	0	3	0	3	3	3	3
Question 5: Can we be confident in the assessment of the presence or absence of prognostic factors? *Were patients features (*e.g., *type of ILD and other baseline patient characteristics) described for test and control group?*	0	0	0	0	2	0	0	0	1	1	2	2	2
Question 6: Can we be confident in the assessment of outcome? *Were the outcome criteria and their time point accurately described?*	0	0	0	0	0	3	3	2	0	2	3	0	3

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Table 1 (*Continued*)

Tool to assess risk of bias in cohort studies - Cochrane

	Cohort studies											Case series	
	Alder et al (2022)	Swaminathan et al (2019)	Newton et al (2017)	Snyder et al (2023)	Popescu et al (2018)	Planas-Cerezales et al (2021)	Hannan et al (2023)	Phillips-Houlbracq et al (2022)	Choi et al (2022)	Stark et al (2022)	Borie et al (2015)	Tokman et al (2015)	Silhan et al (2014)
Question 7: Was the follow-up of cohorts adequate? *Was the duration of follow-up < 3 years, < 1 year or not described?*	0	1	0	0	0	0	0	0	3	0	3	0	1
Question 8: Were cointerventions similar between groups? *Not applicable in this study*	-	-	-	-	-	-	-	-	-	-	-	-	-
Total Risk of bias score	5%	5%	14%	19%	24%	29%	33%	38%	48%	67%	81%	57%	76%

The risk score has been calculated as the percentage of the total sum of the individual score for each question (0-3) divided by the maximum possible score.[27] Zero percent is the minimum possible risk and 100% is the maximum risk according to Tool Assess Risk of Bias in Cohort Studies.

Low risk of bias (< 25%)
Probably low risk of bias (25%-50%)
Probably high risk of bias (51%-75%)
High risk of bias (> 75%)

Table 2 Reported Post-Transplantation Outcomes and Sample Sizes per Included Study

Outcome Parameters	Cohort studies										Case series	
	Alder, et al. (2022)	Swaminathan, et al. (2019)	Newton, et al. (2017)	Snyder, et al. (2023)	Popescu, et al. (2018)	Planas-Cerezales, et al. (2021)	Hannan, et al. (2023)	Phillips-Houlbracq, et al. (2022)	Choi, et al. (2022)	Stark, et al. (2022)	Borie, et al. (2015)	Tokman, et al. (2015)	Silhan, et al. (2014)
Genetic mutation	●	●			●	●	●	●		●	●	●	●
Telomere length	●		●	●	●	●	●		●		●		
FPF	●	●	●		●	●	●	●		●	●	●	●
PGF	●		●			●						●	
ACR	●	●	●	●		●		●		●	●	●	●
CLAD	●	●	●			●		●		●		●	
Survival	●	●	●			●		●			●	●	●
Bone marrow complications		●	●	●		●	●	●		●	●	●	●
Kidney problems		●	●			●		●		●	●	●	●
Liver abnormalities		●	●					●			●	●	●
Infections		●	●	●	●	●		●	●		●	●	●
Malignancy		●	●			●		●		●	●	●	
Airway complications									●				●
Thromboembolic complications			●										●
Cutaneous complications								●					
Metabolic complications						●							
Follow-up	●	●	●			●		●		●	●	●	●

Abbrevitions: ACR, acute cellular rejection; CLAD, chronic lung allograft dysfunction; PGD, primary graft dysfunction.

Bullets represent the outcome report and sample size of patients with hereditary pulmonary fibrosis: ● Reported with small sample size (0-10 subjects); ● Reported with medium sample size (11-30 subjects); ● Reported with large sample size (> 30 subjects).

The prevalence of familial aggregation of trILD among patients with TRG variants in cohorts that consecutively studied all patients was 11.5% (range 11.4%-13.4%),[18,19,32] compared with 23.6% (range 13%-27.3%) in cohorts in which TRG variants were tested upon clinical suspicion.[25,33]

Reported outcomes in patients with a TRG variant vs no TRG variant included PGD, ACR, CLAD, and overall survival. Grade 2 to 3 PGD at 72 hours was reported in 8% to 10% of patients with a TRG variant and this was not found to be significantly different in patients without a TRG variant (12%-16%).[18,19] No significant difference in occurrence of ACR in the first year was reported by 3 studies.[18,19,25] Regarding CLAD, Swaminathan et al[19] found a higher risk (adjusted hazard ratio (HR) 2.88 [95% confidence interval (95% CI), 1.42-5.87], $p = 0.004$) and a shorter time to CLAD at 1, 3, and 5 years (0%, 43%, and 62% vs 4%, 16%, and 34%; $p = 0.02$) in patients with a TRG variant (low risk of bias). However, 3 other studies did not identify significant differences in CLAD-free survival between recipients with and without TRG variant (low, medium, and high risk of bias).[18,25,33] Only one study evaluating TRG variants in LTX patients[19] found higher risk of death (HR 1.82 [95% CI 1.07-3.08], $p = 0.03$) and a trend toward a shorter survival ($p = 0.06$) in patients with a TRG variant (low risk of bias), which could not be confirmed by 3 other studies (low, medium, and high risk of bias).[18,22,33]

Secondary outcomes were reported less frequently. Swaminathan et al[19] reported significantly higher risk of post-transplant anemia in patients with TRG variants ($p = 0.03$). In addition to this, Hannan et al report that patients with TRG variants had increased requirements for transfusion and growth factor support.[34] No other significant differences were reported for recipients with TRG variants, infection, kidney or liver function, metabolic complications, thromboembolic complications, and prevalence of malignancy.[18,19,22,25,33]

Telomere shortening

The characteristics of 8 studies analyzing telomere shortening in ILD patients undergoing LTX are presented in Table 6. Studies were mainly retrospective (6 out of

Table 3 Features of Studies Reporting Telomere-Related Genetic Variants

Study	Study type	N	Age at LTX (years)[a]	Sex (male)[a]	Underlying diagnosis	Patient selection	Control group	Genetic analysis sample	Genetic analysis technique	History of familial aggregation
Alder et al (2022)[b]	Single-center cohort	149	Median 60 (Range 37-74)	23/36 (63.9%)	IPF	Retrospective. All consecutive transplant recipients that consented to genetic testing (2000-2019)	Patients without GV	Unspecified	WGS in most, NGS panel in 5	17/149 (11.4%) in total 7/36 (19.4%) in GV
Swaminathan et al (2019)	Single-center cohort	262	Median 63 (IQR 56-68)	23/31 (74.2%)	ILD	Primary lung transplant recipients	Patients without GV	Peripheral blood	WES	35/262 (13.4%) in total 9/31 (29.0%) in GV
Popescu et al (2018)	Multicenter cohort	42	Median 61 (IQR 55.5-69)	8/15 (53.3%)	IPF	Retrospective. Consecutively recruited (2006-2016)	Age matched non-IPF LTX recipients	Peripheral blood lymphocytes	NGS panel	5/15 (33.3%) in GV
Planas-Cerezales et al (2021)	Single-center cohort	20			ILD	Prospective. Genetic testing performed in patients if telomere length < 25th percentile and 1 w/o TS		Oral swab and peripheral blood lymphocytes	WES if telomere length < 25th percentile	
Hannan et al (2023)[b]	Single-center cohort	144	Median 65 (IQR 48-72)	56/72 (77%)	IPF	Retrospective. 72/151 recipients with IPF diagnosis who had peripheral blood mononuclear cells available (2015-2019)	Age matched non-IPF LTX recipients (72 vs 72)	Peripheral blood lymphocytes	Tested in those with TS (N = 72): WGS in 57, NGS panel in 12, 3 no testing	9/72 (12.5%) of total 6/19 (32%) in GV
Phillips-Houlbracq et al (2022)[c]	Multicenter/ multinational cohort	66	Median 53.6 (IQR 45.8-49.3)	26/38 (68%)	ILD	Retrospective, patients referred for LTX with clinical suspicion for GV (2008-2018)	Patients without GV vs patients without genetic testing (Cristal database)	Unspecified	Sanger or NGS panel	18/66 (27.3%) in total 18/38 (47%) in GV
Tokman et al (2015)	Multicenter case-series	14	Median 60.5 (IQR 52.0-62.0)	9/14 (64.3%)	ILD	Retrospective case series (2005-2014)		Unspecified	Direct DNA (Sanger) Sequencing TERT and TERC	12/13 (92.3%) in GV

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Table 3 (*Continued*)

Study	Study type	N	Age at LTX (years)[a]	Sex (male)[a]	Underlying diagnosis	Patient selection	Control group	Genetic analysis sample	Genetic analysis technique	History of familial aggregation
Stark et al (2022)	Single-center cohort	23	Mean 59 (SD 5)	9/9 (100%)	IPF	Patients selected had to be alive at the time of recruitment, 3-12 years after LTX (2007-2015)	Patients without GV or variants non-TRG	Saliva, obtained 3-12 years after LTX	NGS-based hybrid capture method	3/23 (13%) in total (11%) in GV
Silhan et al (2014)	Multicenter case-series	7	Median 44 (IQR 44-53.5)	3/7 (42.9%)	ILD	Retrospective case series (2004-2013)		Peripheral blood lymphocytes	Not reported	5/5 (100%) in GV
Borie et al (2015)	Multicenter cohort	205	Median 48.5 (IQR 42-58)	7/9 (77.8%)	ILD	Retrospective, specific patients with clinical suspicion of GV (2009-2013)	Patients with unknown mutational status	Unspecified. N = 5 before LTX, N = 4 after LTX	Sequencing of TERT and TERC	4/9 (44.4%) in GV

Abbreviations: GV, genetic variant; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; IQR, interquartile range; LTX, lung transplantation; NGS, next generation sequencing; SD, standard deviation; TRG, telomere-related genes; TS, telomere shortening; WES, whole exome sequencing; WGS, whole genome.

[a]Of patients that underwent genetic testing or had a GV, whichever was available.

[b]Contain overlapping patients.

[c]Including 10 patients previously reported by Borie (2016) and Tokman (2015).

8)[18,22,32,34-36] and included between 9 and 127 LTX recipients with ILD. A total of 168 included patients with telomere shortening were reported with a median age of 57.1 (SD 9.6) years and 70% being male.

Control groups consisted mostly of patients without demonstrated telomere shortening and 1 study had a control group that did not undergo TL testing,[35] thus with higher risk of misclassification. There was 1 case-series without a control group.[22]

Patient selection was highly heterogeneous: 3 studies performed TL testing based on clinical suspicion of telomere shortening[21,22,35] (thus with higher risk of misclassification, but perhaps more indicative of TBD syndrome), 2 studies selected those with available data,[34,36] and 3 studies tested all consecutive patients.[18,26,32]

Type of samples tested were not specified in 2 studies,[18,22] consisted of peripheral blood lymphocytes in 4 studies,[21,32,34,36] 1 study used peripheral blood lymphocytes or any other cell lineage,[35] and 1 did both peripheral blood lymphocyte and oral swab sampling in all patients.[26] TL measurement was performed before transplantation in all studies. A wide spectrum of TL more and less reliable tests was used: TL was measured by FlowFISH,[32,34-36] quantitative polymerase chain reaction (qPCR),[21,26] WGS[18] through estimation of TL (using TelSeq software package), and Southern blot.[22] The cut-off for abnormal TL used was predominantly below the 10th percentile for the lymphocyte cell lineage,[18,21,32,34-36] although 1 study considered a length below the 25th percentile as abnormal,[26] and 1 study used a TL in nonlymphocyte cell lineages below the first percentile as cut-off.[35]

The main outcomes of the studies assessing TL are depicted in Table 7. The prevalence of telomere shortening according to different cut-off in cohorts consecutively studied was 39% (range 25%-71%),[18,26,32] whereas in the cohorts studied based on clinical suspicion the prevalence was 34% (range 32%-56%).[21,22] The prevalence of familial aggregation among patients that underwent systematic testing of telomere shortening was on average 16% (range 13%-40%),[18,26] compared to 27% in a cohort studied based on clinical suspicion.[32] Whereas the prevalence of familial aggregation among telomere shortening patients was on average 15% (range 19%-42%) among those systematically studied,[18,26] compared to 31% in the cohort tested based on clinical suspicion.[21]

Reported outcomes in patients with clinically relevant telomere shortening vs no telomere shortening included PGD, ARC, CLAD, and overall survival. Grade 3 PGD at 72 hours was significantly increased up to 28% in patients with telomere shortening in 1 study (high quality),[21] whereas 2 studies did not find significant differences (both quality).[18,26] No significant difference in ACR in the first year was reported by multiple studies (all 4 high quality).[18,21,26,36] One found a significant decline in ACR with increasing age, only in patients with significant telomere shortening.[36] Regarding CLAD, Newton et al[21] found higher (adjusted HR 6.3 [95% CI 2.0-20.0], $p = 0.002$) and a shorter time to CLAD at 2, 4, and 6 years (57.7%, 84.6%, and 92.3% vs 39.3%, 71.4%, and 89.3%; $p = 0.005$) in

Table 4 Outcomes of Studies Reporting Telomere-Related Genetic Variants

				Primary graft dysfunction (PGD)			Acute cellular rejection (ACR)			Chronic lung allograft dysfunction (CLAD)			Survival			
Study	N (included)	Genetic variant	No genetic variant	Criteria	Outcome	Statistics	Criteria	Outcome	Statistics	Criteria	Outcome	Statistics	Outcome	Statistics	Follow-up (years)	Risk of bias (0-3)
Alder et al (2022)[a]	149	36 (24.2%)	113 (75.8%)	T72 grade 3 PGD	7.7% in GV vs 16.2% in no GV	NS ($p = 0.502$)	ACR score (< 1 year)	0.42 (IQR 0.11-0.89) in GV vs 0.57 (IQR 0.10-0.85) in no GV	NS ($p = 0.661$)	Beyond 5 years	Time to CLAD not reached in GV vs 6.3 years in no GV	Time to CLAD in GV NS (adj. HR 1.10 [95% CI 0.54-2.24], $p = 0.788$)	5.2 years in GV vs 6 years in no GV	NS higher risk of death in GM (adj. HR 1.55 [95% CI: 0.79-3.06], $p = 0.203$)	Median 3.8	5%
Swaminathan et al (2019)[b]	262	31 (11.8%)	231 (88.2%)	T72 grade 3 PGD	10% in GV vs 12% in no GV	NS (OR 0.81 [95% CI 0.19-2.49], $p = 0.74$)	ACR (< 1 year); grade A rejection scores (> grade A/ gradable TBB < 1 year)	64% of GV vs 77% of no GV. 0.19 (0-0.53) in GV vs 0.33 (0.14-0.57) in no GV	NS (ACR $p = 0.15$; ACR grade $p = 0.23$)	Survival > 90 days and > 5 PFT to be included in analysis (11 excluded)	CLAD at 1, 3, and 5 years, respectively, 0%, 43%, and 62% in GV vs 4%, 16%, and 34% in no GV	Higher risk of CLAD in GV (adj. HR 2.88 [95% CI,1.42-5.87], $p = 0.004$), shorter time to CLAD ($p = 0.02$)	Survival at 1, 3, and 5 years, respectively, 84%, 59%, and 42% in GV vs 85%, 68%, and 57% in no GV	Higher risk of death in GM (adj. HR 1.82 [95% CI 1.07-3.08], $p = 0.03$), shorter survival ($p = 0.06$)	Median 2.8 (IQR 1.2-5.1)	5%
Popescu et al (2018)	42	15 (35.7%)	27 (64.3%)												Median 3.5	24%
Planas-Cerezales et al (2021)	13	11 (84.6%)	2 (15.4%)													29%
Hannan et al (2023)[a]	69	19 (28%)	50 (72%)													33%
Phillips-Houlbracq et al (2022)[c]	66	38 (57.6%)	28 (42.4%)				ACR; grade A rejection scores (> grade A/ gradable TBB)	13 (34%) in GV, not reported in no GV		ISHLT stage ≥1 (of > 1 year survivors)	7/29 (24%) in GV. CLAD prevalence 2, 3, and 4 years was 21%, 26%, and 29%, respectively, in GM. CLAD-free survival 2.2 years (IQR (1.0-NA) in GM		Survival 3.75 years (IQR 1.8 NA) in GV vs 3.0 years (IQR 1.4-NA) in no GV	NS (in GV $p = 0.36$)	Median 3.3 (IQR 1.3-3.9)	38%

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Table 4 (*Continued*)

Study	N (included)	Genetic variant	No genetic variant	Primary graft dysfunction (PGD)			Acute cellular rejection (ACR)			Chronic lung allograft dysfunction (CLAD)			Survival		Follow-up (years)	Risk of bias (0-3)
				Criteria	Outcome	Statistics	Criteria	Outcome	Statistics	Criteria	Outcome	Statistics	Outcome	Statistics		
Tokman et al (2015)		14		T72 grade 2-3 PGD	1 (7.1%)		Not described	A1 7 (50%), no >A1 0 (0%)		ISHLT criteria	4/12 (33.3%); time to BOS 3.1 years (IQR 1.0-6.0)	No control group	13/14 (92.9%)	No control group	Mean 3.2 (SD 2.9)	57%
Stark et al (2022)	23	15 (65.2%)	8 (34.8%)				ACR in TBB (< 1 year)	33% in GV vs 57% in no GV; time to ACR 59 days (SD 15) in GV vs 73 days (SD 49) in no GV	NS (ACR $p = 0.40$; time to ACR $p = 0.63$)	ISHLT criteria (< 1 year)	33% in GV vs 43% in no GV; time to CLAD 4.0 years (SD 0.9) in GV vs 3.3 years (SD 1.5) in no GV	NS (CLAD $p = 0.99$; time to CLAD $p = 0.42$)			Mean 8.0 (SD 2.2)	67%
Silhan et al (2014)		7					Not described	3 (33.3%)	No control group				6/7 (85.7%)	No control group	Median 1.9 (IQR 0.88-3.45)	76%
Borie et al (2015)	205	9 (4.4%)	196 (95.6%)				TBB upon clinical suspicion	3 (33.3%)	No comparison with control group				0.56 years in GV vs 3.56 years in no GV	NS ($p = 0.8$-5)	Median 0.56 (IQR 0.23-4.68)	81%

Abbreviations: 95% CI, 95% confidence interval; ACR, acute cellular rejection; adj., adjusted; CLAD, chronic lung allograft dysfunction; GV, genetic variant; ISHLT: International Society for Heart and Lung Transplantation; NS, not significant; OR, odds ratio; PFT, pulmonary function test; PGD, primary graft dysfunction; TBB, transbronchial biopsy.

Low risk of bias (< 25%): 0.

Probably low risk of bias (25%-50%): 1.

Probably high risk of bias (51%-75%): 2.

High risk of bias (> 75%): 3.

[a]Contain overlapping patients.

[b]Including patients previously reported by Petrovski et al AJRCC (2017).

[c]Including 10 patients previously reported by Borie (2016) and Tokman (2015).

Table 5 Genetic Variants Found in the Studies Reporting Telomere-Related Genetic Variants

Study	Total N	Variant	No variant found	TERT	TERC (TR)	RTEL1	PARN	TINF2	NAF1	DCKC1	HPS1	EDN3	SFTPA2	Tested - other variant demonstrated
Alder et al (2022)[a]	149	39 (26.2%)[b,c]	110 (73.8%)[c,d]	9 (6.0%)[c]	5 (3.4%)[d]	12 (8.1%)	9 (6.0%)	0 (0%)	4 (2.7%)	2 (1.3%)				
Swaminathan et al (2019)	262	31 (11.8%)	231 (88.2%)	13 (0.5%)		10 (0.4%)	8 (0.3%)							
Popescu et al (2018)	42	15 (35.7%)	27 (64.3%)	1 (2.4%)	0 (0%)	5 (11.9%)	4 (9.5%)	2 (4.8%)	2 (4.8%)	1 (2.4%)				SFTPC
Planas-Cerezales et al (2021)	13	11 (84.6%)[d]	2 (15.4%)	3 (23.1%)	0 (0%)	5 (38.5%)	1 (7.7%)	0 (0%)		2 (15.4%)				TERF1
Hannan et al (2023)[a]	69	19 (26%)	50 (74%)	2 (10.5%)	1 (5.3%)	6 (31.6%)	6 (31.6%)	1 (5.3%)	1 (5.3%)	2 (10.5%)				
Phillips-Houlbracq et al (2022)[e]	66	38 (57.6%)	28 (42.4%)	23 (34.8%)	9 (13.6%)	6 (9.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				NHP2, NOP10, ZCCHC8
Choi et al (2022)	63	45 (71.4%)	18 (28.6%)	28 (44.4%)	10 (15.9%)	7 (11.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Stark et al (2022)	23	12 (52.2%)	11 (47.8%)	5 (21.7%)			2 (8.7%)	1 (4.3%)		1 (4.3%)	1 (4.3%)	1 (4.3%)	1 (4.3%)	
Tokman et al (2015)	NA	11	NA	10 (90.9%)	1 (9.1)									
Silhan et al (2014)[f]	NA	7	NA	5 (71.4%)	2 (28.6%)									
Borie et al (2015)	NA	9	NA	6 (66.7%)	3 (33.3%)									

Abbreviations: N, Number; NA, not available.

Note: Surfactant genes are not the focus of this review.

[a]Contain overlapping patients.

[b]One TERT variant occurred in an individual with RTEL1 variant.

[c]One TERC variant occurred in an individual with PARN variant.

[d]Genetic testing was performed in patients with telomere length < 10th percentile (*n* 12) and 1 without TS (*n* 1);

[e]Including 10 patients previously reported by Borie (2016) and Tokman (2015).

[f]There are 8 patients reported, but 7 underwent genetic testing.

Table 6 Features of Studies Reporting on Telomere Length

Study	Study type	N	Age at LTX[a]	Sex (male)[a]	Underlying diagnosis	Patient selection	Control group	Telomere analysis sample	Telomere length analysis technique	TS cut-off	History of familial aggregation
Alder et al (2022)[a]	Single-center cohort	127	Median 60 (range 37-74)[b]	24/32 (75%)	IPF	Retrospective. All transplant recipients consented to testing. Measurements not reliable in 22 LTX recipients (2000-2019)	Patients without TS	Unspecified	WGS estimation of telomere length (using TelSeq software package)	< 10th percentile	16/127 (13%) of total 6/32 (18.8%) of TS
Newton et al (2017)	Single-center cohort	82	Mean 59 (SD 9.0)	21/26 (80.8%)	ILD	Prospective, enrolled before lung transplant (2007-2017). Sequencing of telomere-related genes was not systematically performed	Patients without TS	Peripheral blood lymphocytes	qPCR	< 10th percentile	22/82 (27%) of total 8/26 (31%) of TS
Snyder et al (2023)[a]	Single-center cohort	106	Median 61 (IQR 43-76)	82/106 (77%)	IPF	Retrospective. Patients with IPF who underwent LTX and had telomere length tested	Patients without TS	Peripheral blood lymphocytes	FlowFISH	< 10th percentile	
Popescu et al (2018)	Multicenter cohort	42			IPF	Retrospective. Consecutively recruited (2006-2016)	Age-matched non-IPF LTX recipients	Peripheral blood lymphocytes	FlowFISH	< 10th percentile	
Planas-Cerezales et al (2021)	Single-center cohort	20	Mean 51.8 (SD 9.18)	9/12 (75%)	ILD	Prospective, enrolled recipients of LTX for ILD (2013-2018)	Patients without TS	Oral swab and peripheral blood lymphocytes	qPCR (confirmed by telomere restriction fragment)	< 25th percentile	8/20 (40%) of total 5/12 (41.6%) of TS
Hannan et al (2023)[a]	Single-center cohort	72	Median 65 (IQR 48-72)	56/72 (77%)	IPF	Retrospective. All transplant recipients with IPF diagnosis who had peripheral blood mononuclear cells available (72/151) (2015-2019)	Age-matched non-IPF LTX recipients (72 of 378)	Peripheral blood lymphocytes (and granulocyte)	FlowFISH (and 57 also by WGS using TelSeq software package)	< 10th percentile	9/72 (12.5%) of total

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Table 6 (*Continued*)

Study	Study type	N	Age at LTX[a]	Sex (male)[a]	Underlying diagnosis	Patient selection	Control group	Telomere analysis sample	Telomere length analysis technique	TS cut-off	History of familial aggregation
Choi et al (2022)	Multicenter cohort	63	Median 57 (IQR 51-63)	40/63 (63.5%)	ILD	Retrospective. No systematic telomere study, tested patients based on clinical suspicion (2009-2019)	Patients without telomere testing	Lymphocyte lineage or any other cell lineage	FlowFISH (and qPCR in several cases)	< 10th and < 1st percentile in lymphocytes or other lineages, respectively	
Borie et al (2015)	Multicenter cohort	9	Median 42.5 (range 40-52)	3/5 (60%)	PF	Retrospective TRG case series (2009-2013), tested patients based on clinical suspicion		Unspecified	Southern blot	TL shorter than control subjects	

Abbreviations: FISH, fluorescence in situ hybridization; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; LT, lung transplant; PF, pulmonary fibrosis; qPCR, quantitative polymerase chain reaction; TS, telomere shortening; WGS, whole genome sequencing.

[a]Overlapping patients.

[b]At time of diagnosis.

patients with telomere shortening (high quality). However, 2 other studies did not identify significant differences in and CLAD-free survival between recipients with and without telomere shortening (both high quality).[18,26] Likewise, Newton et al[21] found higher risk of death (adjusted HR 10.9 [95% CI 2.7-44.8], $p = 0.001$) and a shorter survival ($p = 0.019$) in patients with telomere shortening (high quality), but this was not confirmed in another larger and high-quality study.[18]

Regarding secondary outcomes, Popescu et al[32] identified a high prevalence of relapsing CMV viremia in recipients with telomere shortening compared with nonshortening (69% vs 31%, odds ratio (OR) 4.98 [95% CI 1.95-12.5], $p < 0.001$) and shorter time to CMV viremia ($p < 0.001$). However, these differences were not confirmed in 2 other but smaller studies that report this as a secondary outcome.[18,26] Additionally, Choi et al[35] identified higher risk of airway complications post-transplant in patients with telomere shortening, with higher odds of dehiscence (OR 8.24 [95% CI 3.34-20.29], $p < 0.001$) and stenosis (OR 4.63 [95% CI 2.21-9.69], $p < 0.01$). Planas-Cerezales et al[26] described potential higher rate of early post-transplant complications in patients with telomere shortening with more frequent need of extracorporeal membrane oxygenation, hematological complications (cytopenias), longer stay in intensive care unit and higher number of long-term hospital admissions, although not statistically significant. Hannan et al report patients with telomere shortening were more likely to have immunosuppression agents discontinued due to cytopenias and bone marrow dysfunction[34] and Snyder et al report (in the same patients) signs of premature "aging" of their circulating T cell compartment post-transplantation.[36] No differences were reported in patients with telomere shortening vs without telomere shortening regarding kidney or liver function, metabolic complications, thromboembolic complications, and malignancy.[18,21,22,26,32,35]

Discussion

In this systematic review, we summarize outcomes after LTX for trILD compared to non-trILD. Overall, the methodological heterogeneity of studies was very large and study quality ranged from very high quality to very high risk of bias.

We have summarized short- and long-term patient and graft outcomes, such as PDG, ACR, CLAD, and survival, and found that results on all outcomes are rather contradictory, with no convincing evidence that patients that undergo LTX for trILD have inferior outcomes. Although several high-quality studies find such signals, other large and high-quality studies do not. Based on these findings, we cannot unambiguously conclude that LTX for trILD—*in the selected group of trILD deemed candidates for LTX*—leads to inferior primary patient and graft outcomes. Regarding secondary outcomes, such as CMV infection, cytopenias, malignancy, and anastomotic

Table 7 Outcomes of Studies Reporting on Telomere Length

				Primary graft dysfunction (PGD)			Acute cellular rejection (ACR)			Chronic lung allograft dysfunction (CLAD)			Survival			
Study	N (included)	Telomere shortening	No telomere shortening	Criteria	Outcome	Statistics	Criteria	Outcome	Statistics	Criteria	Outcome	Statistics	Outcome	Statistics	Follow-up (y)	Risk of bias (0-3)
Alder et al (2022)[a]	127	32 (25.2%)	95 (74.8%)	T72 grade 3 PGD	2/25 (8.0%) in TS vs 11/69 (15.9%) in no TS	NS ($p = 0.502$)	Grade A rejection scores (> grade A/ gradable TBB < 1 year)	0.5 (0.7-1.1) in TS vs 0.6 (0.1-0.84) in no TS	NS ($p = 0.664$)	CLAD ISHLT criteria	Time to CLAD 6.5 year in TS vs 6.1 year in no TS	Time to CLAD NS (adj. HR 1.10 [95% IC 0.55-2.21], $p = 0.779$)	6.6 years in TS vs 5.2 years in no TS	Risk of death NS (adj. HR 1.02 [95% CI: 0.48-2.15], $p = 0.961$)	Median 3.8	5%
Newton et al (2017)	82	26 (31.7%)	56 (68.3%)	T24 and T72 grade 3 PGD	5 ($n = 18$) (27.8%) in TS vs 3 ($n = 56$) (6.5%) in no TS	Higher risk in TS ($p = 0.034$)	Grade A rejection scores (> grade A/ gradable TBB)	0.27 (IQR 0-0.4) in TS vs 0.37 (IQR 0-0.57) in no TS	NS ($p = 0.099$)	CLAD ISHLT criteria	13 (50%) in TS vs 13 (23.2%) in no TS; time to CLAD 2.7 years (95%CI 1.5-) in TS vs NR in no TS	Higher risk of CLAD in TS (adj. HR 6.3 [95%CI 2.0-20.0], $p = 0.002$-), shorter time to CLAD ($p = 0.00$-5)	45.2% in TS vs 82.1% in no TS; survival 6.2 years (95% CI 2.3-) in TS vs NR in no TS	Higher risk of death in TS (adj. HR 10.9 [95% CI 2.7-44.8], $p = 0.001$-), shorter survival ($p = 0.01$-9)	Mean 5.0 (SD 2.5)	14%
Snyder et al (2023)[a]	106	57 (54%)	49 (46%)				Grade A rejection scores (> grade A/ gradable TBB)		Lower ACR score for those with TS ($n = 57$, $p < 0.05$) < 1 year, > 1 year NS							19%
Popescu et al (2018)	42	30 (71.4%)	12 (28.6%)												Median 3.5	24%

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Table 7 (*Continued*)

Study	N (included)	Telomere shortening	No telomere shortening	Primary graft dysfunction (PGD)			Acute cellular rejection (ACR)			Chronic lung allograft dysfunction (CLAD)			Survival		Follow-up (y)	Risk of bias (0-3)
				Criteria	Outcome	Statistics	Criteria	Outcome	Statistics	Criteria	Outcome	Statistics	Outcome	Statistics		
Planas-Cerezales et al (2021)	20	12 (60.0%)	8 (40.0%)	T24 PGD (grade NA)	2/12 (16.7%) in TS vs 1/8 (12.5%) in no TS	NS ($p = 0.495$)	ACR ISHLT criteria	3/12 (27.3%) in TS vs 5/8 (62.5) in no TS	NS ($p = 0.18$)	CLAD ISHLT criteria	1/12 (33.3%) in TS vs 0/8 (0%) in no TS	NS	10/12 (83.3%) in TS vs 7/8 (87.5%) in no TS	NS	Median 3.25 (IQR 2.25-4.16)	29%
Hannan et al (2023)[a]	72	49 (68%)	23 (32%)													33%
Choi et al (2022)		63														48%
Borie et al (2015)	9	5 (56%)	4 (44%)										2 (40%) in TS vs 1 (25%) in no TS	NS ($p = 0.85$)	Median 0.38 (range 0.23-2.05)	81%

Abbreviations: 95% CI, 95% confidence interval; ACR, acute cellular rejection; CLAD, chronic lung allograft dysfunction; ISHLT, The international Society of Heart and Lung Transplantation; NR, not reached; NS, no significant; PGD, primary graft dysfunction; PTS, patients with telomere shortening; TS, telomere shortening.

Low risk of bias (< 25%): 0.

Probably low risk of bias (25%-50%): 1.

Probably high risk of bias (51%-75%): 2.

High risk of bias (> 75%): 3.

[a]Contain overlapping patients.

complications, the summarized studies suggest that more complications may occur in patients with LTX for trILD compared to non-trILD, but there is considerable under-reporting of reliable outcomes, and many studies lack details on immunosuppression modification that will have affected the outcomes.

It is important to note that this systematic review only summarizes the patients that were selected and found eligible to undergo LTX. This in itself represents a selected population, as this only entails patients that were deemed fit for transplantation by their transplant center. Given the high risk of co-occurrence of liver problems, bone marrow failure, myelodysplastic syndrome, and malignancies, a subset of perhaps the most affected patients with trILD does not qualify for transplantation in the first place.

This preselection is not reported on and may have affected the studies' results. As limited guidance is currently available on how to handle patients with trILD, apart from the advice that patients with telomeropathy should "undergo detailed evaluation,"[28] it will differ between transplant centers how the results of such evaluation are weighed, affecting patient selection and therewith transplantation outcomes. The review article by Kapnadak[17] tries to fill in this gap and suggests to integrate TL testing into the routine pretransplant evaluation, for better risk-stratification and prediction of post-transplant complications, mostly based on the various reports on inferior LTX outcomes in trILD.

Regarding the evaluation and clinical management of trILD, no firm recommendations can be given. In general, in patients with telomere-related disease apart from their ILD have increased risk of liver and bone-marrow failure and increased risk of malignancy. As such, in patient selection, additional assessment of mostly liver (fibro-scan) and bone-marrow examination could be considered in the evaluation, as well as intensified screening for incident malignancy at time of screening and after transplantation.

Patients can be counseled regarding their potential increased risk for a wide range of complications, requiring more-intensive follow-up for liver and bone-marrow toxicity and additional post-transplantation screening for malignancy. Therapeutic drug monitoring of bone-marrow toxic medication with narrow therapeutic width (such as ganciclovir, mycophenolate mofetil) could be used, to prevent cytopenias. It may be considered to prevent CMV mismatches if applicable, to avoid hard to manage primary CMV infections and associated drug toxicity.

Negative outcomes associated with LTX in trILD are driven by 3 cohorts. Newton et al[21] reported increased risk of PDG, CLAD, and death, but not ACR in patients with telomere shortening. Courtwright et al[37] assessed post-LTX TS, which was associated with CLAD and leucopenia. Swaminathan et al reported higher risk of CLAD and death, but not PDG or ACR or cytopenias in patients with telomere-related genetic variant.

From the reports, it is very hard to identify what makes these cohorts "different" from the cohort that did not find such differences in primary outcomes, and whether type of TL testing, patient selection, and post-transplantation management played a role in their results.

The impact of post-transplant management may be very relevant as several studies increased risk of cytopenias requiring intervention (medication cessation, bone-marrow assessment, transfusion, or growth factor support) are being described,[19,34] but it is very likely that not all studies will have assessed, such outcomes and the reports are underrepresentative of this problem. Overall, many questions remain despite the increased attention for this topic.

What does become increasingly clear is that in patients with ILD that are being referred to LTX, trILD is over-represented. In all studies that consecutively tested all transplanted ILD patients for TRG variants or telomere shortening, the proportion of trILD was on average 23% (range 12%-71%),[18,19,26,32] with prevalence of a TRG variant ranging from 12% to 55% and the prevalence of telomere shortening ranging from 25% to 71%. This relates to the relatively younger age of onset, faster progression, and reduced response to therapy that has been noted in trILD.[18,19,32,18,26,32] The large difference between these studies can be explained by differences in patient selection between various centers, but also relate to the various techniques used to assess TRG variants and TL. Regarding the genetic analyses NGS panels are most commonly used, but depending on the study the panels differ in what variants were being tested for. Several studies for example only looked at the TERT and TERC variant that are the known most prevalent TRG variants[22-24] or a somewhat larger panel of TRG variants.[18,26] Other studies performed more extensive testing, also performing WES that is substantially more complete.[19] In the summary of all studies, TERT was by far the most commonly found variant, followed by RTEL1, TERC, and PARN.

Importantly, most papers do not make a distinction between pathogenic TRG variants and those of unknown significance, that may impact the studies' results. Given this lack of information, in this review, we were also unable to make this distinction. Although more rare, lung fibrosis can be caused by underlying surfactant-gene variants, rather than being telomere related. Given the nature of the included studies, these results could also not be separated. As it pertains small numbers of cases, this will likely not affect our results.

Regarding the techniques used to measure telomeres; TL measurement by FlowFISH is widely validated and proven to be reliable in TBD diagnostics.[52] It is essential to have a sufficiently large cohort of healthy controls with a sufficiently wide age range to compare patients with and to report absolute TL/TL percentile. qPCR is also commonly used. Controversy exists as to how this method compares to FlowFISH. Again, for this method, it is also essential to have a large control-cohort of healthy controls. There is no universal consensus on the best telomere-length cut-off, and usually not only TL is used to classify disease. In the telomere biology field, the term "telomere biology disorder" is used for patients with proven pathogenic variants in a telomere biology gene plus short telomeres < 1st or < 10th percentile plus clinical symptoms. In pulmonary medicine, ILD in combination with short telomeres (< 10th) would currently suffice to diagnose trILD, although in the telomere biology field this would be insufficient to be classified

as a TBD. TL < 25th percentile definitely should not be used to diagnose a patient with telomere disease/trILD.

Given the conclusions drawn in the included studies, results are not universally reliably based on the fact that frequently no TRG variants are reported in relation to short telomeres, TLs are not always short, control cohorts are not always healthy controls, and not all studies used techniques validated for diagnostic purposes.

Regarding methodological quality of the included studies, there also was considerable heterogeneity, which limits their interpretation and complicates comparison. Not all studies consecutively tested all patients, nor did all studies test all patients in the control group, but used all patients without *known* trILD as the control group, despite the inherent risk of misclassification bias.

Although several studies in this field have used increasingly more rigid methodology, many remains uncertain on this topic and more rigid research will be needed to reach final answers. Prospective studies with consecutive inclusion with systematic high-standard testing for both TL and TRG variants (WES), preferentially also including patients evaluated and not deemed eligible for transplantation, are needed to understand the real prevalence of trILD in patients assessed for LTX. Pretransplant sample collection and structured assessment of outcomes, including relevant potential immunosuppression modifications would be recommended.

Conclusions

About one-third of LTX recipients with progressive fibrosing ILD have trILD, based on TRG variants and/or significant telomere shortening. This systematic review suggests that LTX in patients with trILD that are *deemed eligible* for LTX, this is not unequivocally associated with poorer short- and mid-term patient and graft outcomes compared to LTX in patients with non-trILD. In itself, trILD should thus not preclude these patients to undergo LTX. Patient selection, method of TRG variant/TL testing, and post-transplantation management may all play a role in the mixed results on outcomes of LTX for trILD reported to date. Nonetheless, there are signs that the rate of post-transplantation complications, such as CMV, cytopenias, and anastomic complications, may be increased in trILD, and medical management may be more complicated but no firm conclusions can be drawn at this point. Future well-developed prospective studies are needed to give final insights on outcomes in trILD and from there to develop consensus on whether TRG variant and TL testing should be incorporated in screening and how these patients should be optimally treated.

Disclosure statement

J.B.-M. has been funded by Instituto de Salud Carlos III through the grant MV21/00043 and CM20/00093 (cofunded by European Social Fund. ESF investing in your future), SEPAR grant 2022 “Ayuda para estancias en otros centros,” SOCAP grant 2022 “Ajuts per a estades a l’extranger,” and Boehringer Ingelheim support for attending meetings unrelated with this work. J.M. has received consulting fees from Boehringer Ingelheim; lecture fees from Boehringer Ingelheim, F. Hoffmann-La Roche, Novartis. M.W. has received grants or contracts from Hoffman la Roche, The Netherlands Organisation for Health Research and Development, The Dutch Lung Foundation, and The Dutch Pulmonary Fibrosis; has received consulting fees from Bristol Myers Squibb, Boehringer Ingelheim, Galapagos, Galecto, Hoffman la Roche, Horizon therapeutics, Kinevant Sciences, Molecure, Nerre Therapeutics, Novartis, PureTech Health, and Respivant; honoraria for lectures from Boehringer Ingelheim, CSL Behring, Hoffman la Roche, Novartis, unrelated to this work; support for attending meetings from Boehringer Ingelheim, Hoffman la Roche, and Galapagos; participation on a data safety monitoring board or advisory board in Savara and Galapagos; and is Chair of the Idiopathic Interstitial Pneumonia group of the European Respiratory Society, Member of the board of the Netherlands Respiratory Society, Member of the scientific advisory board of the European Idiopathic Pulmonary Fibrosis and related disorders federation, Chair of the educational committee of the European Reference Network for rare Lung Diseases, Advisory board of the Dutch Lung fibrosis, and Sarcoidosis patient associations. M.M.-M. has been funded by Instituto de Salud Carlos III and received consulting fees from Boehringer-Ingelheim, Roche, Esteve-Tijin, and Ferrer, unrelated to this work. M.H. has received honoraria for lectures from Pfizer and Boehringer Ingelheim, unrelated to this work. The following authors B.J.M, R.-J.H.G., L.S.S., A.M.A., M.H.G.P.R. have nothing to disclose.

The authors wish to thank Dr Maarten (M.F.M.) Engel from the Erasmus MC Medical Library for developing and updating the search strategies.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhlto.2024.100054.

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