

modulation in other cell types that are known to be involved in lung fibrogenesis, including structural (epithelial) and immune (macrophages and T cells) cells, need to be better clarified.

3. ERs are ubiquitously expressed receptors that are involved in physiological and pathological processes, including carcinogenesis, and therefore the side effects of modulating their activity require further consideration. In addition, pulmonary hypertension and embolism have been associated with oral contraceptives through estrogen-induced obliterative and thrombotic vascular lesions, and estrogens exert proinflammatory and oxidative stress effects (1).

Despite the above concerns, this study represents the first attempt to provide a pathogenic linkage between sex hormones and lung collagen deposition, and to explain the female survival bias in lung fibrosis. Linking ER and other hormone analogs with accelerated aging, senescence, and metabolic disorders, which are often seen in patients with fibrotic lung disease, would be an interesting future approach—the best is yet to come. ■

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⊗ The Fibrosis Burden of Systemic Sclerosis

Interstitial lung disease (ILD) tends to occur early in systemic sclerosis (SSc), places a significant burden on the patient, and is a leading cause of death (1–4). Although there is a well-recognized association between SSc and ILD, it is still not clear who will develop ILD, and if ILD does indeed develop, which patients will experience significant progression and over what period that progression might occur. Our current understanding of the

incidence and progression of this disease comes from several well-designed clinical trials, including SLS I (Scleroderma Lung Study I) and SLS II (3–5).

SLS I included 158 patients from 13 centers with symptomatic lung disease and either BAL evidence of alveolitis or any ground-glass opacities on computed tomography (CT), and with FVC between 45% and 85% predicted (3). Approximately 90% of the patients had some ground-glass opacity and 89% had evidence of fibrosis on high-resolution CT of the chest; 73% of the placebo group had worsening of FVC over a 12-month period. In that study, treatment with cyclophosphamide was found to have a modest effect on lung function decline. In the follow-on SLS II study, there was an 11% mortality at 1 year, which was due to progressive ILD, even in patients receiving cyclophosphamide (4).

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The study by Hoffmann-Vold and colleagues (pp. 1258–1266) in the current issue of the *Journal* provides further insight into the natural history of SSc-associated ILD (6). The authors assessed ILD using data from the Norwegian SSc cohort, which included all 815 SSc patients who resided in Norway between 2000 and 2012. This also included all incident cases that occurred during that period and some prevalence cases that occurred before 2000. Because all patients with SSc in Norway are followed in the public system, the dataset likely represents a nearly complete cohort of patients with SSc in the country during this period. This provides a very rich data source that is reflective of a real-world population, as opposed to the more selected cohorts that are recruited into clinical trials. The value of well-characterized population cohorts in understanding the natural history of ILD has been previously documented in the *Journal* (7, 8).

One of the strengths of the study is the robust assessment for ILD at baseline: 80% of the patients had a baseline CT and 86% had a baseline pulmonary function test (PFT). In contrast, a potential weakness is the variable follow-up of these two parameters: 56% of the patients had a follow-up CT after a mean of 3.7 years and 48% had a follow-up PFT after a mean of 6.2 years. Notwithstanding these limitations, the follow-up findings provide significant insight into the behavior of ILD in SSc. Fibrosis was defined as a reticular pattern with a superimposed ground-glass change, and 50% of the patients had radiological evidence of ILD at baseline. The presence of fibrosis on baseline CT correlated with 5- and 10-year survival rates of 69% and 56%, respectively, even with normal baseline FVC, and the extent of fibrosis correlated with the standardized mortality rate. Interestingly, the degree of fibrosis that was present at baseline (either <10% or >10%) was equally associated with decreased overall survival (63% and 62%, compared with 82% if no fibrosis was present at baseline). Therefore, although the extent of fibrosis correlated with the standardized mortality rate, the mere presence of any fibrosis on a baseline CT scan is predictive of decreased survival regardless of any other measurements. Interestingly, the proportion of patients with fibrosis was lower than that reported in previous studies of SSc, and the proportion with ground-glass opacity was very low compared with that observed in the SLS (3). This may be because superimposed ground-glass opacity was defined as being equivalent to fibrosis and therefore may have been underestimated. Alternatively, it may reflect the unselected nature of the cohort as opposed to the randomized controlled trial cohort of SLS I, which was enriched for patients with symptomatic lung disease (3).

The finding that baseline fibrosis is predictive of a decline in lung function is similar to previous observations in a study of patients with idiopathic interstitial pneumonia (IP), where a honeycomb change on CT was shown to be a good surrogate for usual IP (UIP) on biopsy and was associated with a poor prognosis (9). In a similar study, the degree of fibrosis was greater in patients with concordant UIP on CT than in those with either discordant UIP or concordant nonspecific IP findings on biopsy (10). Furthermore, the degree of fibrosis also correlated with survival. Taken together, these findings suggest that a baseline CT that demonstrates fibrosis is a significant predictor of a subsequent decline and poor prognosis. Further support for this comes from the recently published SENSICIS (Safety and Efficacy of Nintedanib in Systemic Sclerosis) trial, which included 576 patients with SSc and >10% fibrosis. In this trial, the annual rate of decline in the placebo group was 93 ml/yr, again indicating that the presence of fibrosis predicts a subsequent decline (11).

There is an interesting twist to the findings in the current study: although the presence of any fibrosis at baseline predicted decreased survival, this was not the case for progression of fibrosis on serial CT imaging. Intuitively, one would think that radiological evidence of fibrosis progression would predict further progression. The mean change in extent of lung fibrosis was 3%, whereas previous studies have suggested that the minimal clinically important difference between scans in ILD is about 3.4%, as quantified by a data-driven texture analysis (12). In contrast, evidence of a decline in FVC over serial testing was associated with increased mortality. This may be because the interval was shorter for CT follow-up than for PFT follow-up (3.7 vs. 6.2 yr), or the extent of change in CT fibrosis was at the borderline of the minimal clinically important difference. This suggests that once a baseline CT detects fibrosis, the best follow-up is achieved by means of serial PFTs.

Interestingly, although baseline fibrosis is associated with decreased mortality, not all patients with fibrosis progressed, and a significant proportion were alive at 10 years. In the era of personalized medicine, the challenge therefore is to pick out the patients with fibrosis at baseline who will go on to develop progressive disease. Until we can do so, the search for the holy grail of biomarkers will continue. Furthermore, the current study does not answer the question as to how frequently we should image the patients who have no evidence of fibrosis on baseline CT imaging, despite their good prognosis.

How can the current study influence our practice? From the Norwegian experience, there is now good evidence to recommend a baseline CT in all patients with SSc. Patients who show any evidence of fibrosis on imaging should have close follow-up and serial PFTs, as even mild disease at baseline can have severe disease progression. Remaining questions are, how do we predict which subgroup of patients will have a significant decline, how should we follow patients with no fibrosis at baseline, and who and when do we treat? ■

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Hedgehog and WNT Signaling Hubs in Tracheal Morphogenesis

The trachea provides a direct conduit from the respiratory system to the external environment. In mammals, the trachea is surrounded by cartilaginous rings that perform several duties, including providing structural support and keeping the trachea patent. The development of the trachea has been explored using genetic mouse models that reveal the importance of multiple paracrine signaling pathways, such as the SHH (sonic hedgehog) and WNT (Wingless-related integration site) pathways (1–4). In humans, tracheal defects are common in the pediatric patient population and include defects such as tracheomalacia and the rarer disease, complete tracheal ring deformity (CTRD) (5, 6). In CTRD, the cartilaginous rings surrounding the trachea are complete and lack a dorsal gap filled in with the trachealis muscle. As with other orphan diseases, the pathogenic mechanisms of CTRD have eluded investigators given the paucity of tissue and representative animal models of these diseases. As such, it is frequently unclear whether disease-causing mutations in humans follow molecular pathways similar to those discovered in genetic mouse models. In this issue of the *Journal*, Sinner and colleagues (pp. 1267–1281) uncover multiple human genetic lesions that lead to CTRD, including mutations in genes within the SHH pathway and mutations in multiple genes within the WNT pathway, including *ROR2* (receptor tyrosine kinase–like orphan receptor 2) (7) (Figure 1). This study highlights the close relationship between mouse models of respiratory development and human congenital tracheal abnormalities, and it provides new target genes and pathways that play an important role in tracheal development in humans.

The trachea initially develops from the anterior foregut. Subsequently, mesenchymal–epithelial cross-talk sets up a ventral–dorsal gradient of WNT and BMP (bone morphogenetic protein) signaling to promote the proper specification, patterning, and separation of the trachea and esophagus, respectively (1, 2, 8,

9). Along with WNT and BMP signaling, SHH signaling has been shown to play important roles in separation of the trachea and esophagus, as well as in promoting proper differentiation of mesenchymal derivatives in the trachea and other regions within the respiratory system (3, 4, 10). After separation occurs, the cartilaginous progenitors within the trachea are denoted by expression of the transcription factor SOX9, which is both a marker and functional regulator of cartilage development (11). Defects in the development of these progenitors can lead to failure to form the tracheal cartilaginous rings surrounding the trachea. Sinner and colleagues performed a trio analysis with whole-exome sequencing of patients with CTRD along with their parents to identify novel mutations (7). Remarkably, many of the mutations implicated either the WNT or SHH pathway. These included both heritable and spontaneous mutations. The mutations in the WNT pathway, including *ROR2* and *LRR7* (leucine rich repeat containing 7), involve both β -catenin–dependent (*LRR7*) and β -catenin–independent (*ROR2*) pathways. *De novo* mutations were found in *SHH*, and compound heterozygous mutations were observed in *HSPG2*, an extracellular heparin sulfate proteoglycan that is known to modulate SHH and other paracrine pathways (12).

Previous studies in mice have demonstrated important roles for WNT and SHH in the development of multiple tissues within the respiratory system, including the trachea. Loss of SHH also leads to failure of tracheal cartilage formation (10). As SHH is expressed exclusively within the developing respiratory endoderm, this suggests that SHH acts in a paracrine manner to drive cartilage formation, possibly by regulating the differentiation of SOX9⁺ progenitors. This is consistent with the authors' finding of heterozygous gene variants in the downstream transcriptional effectors of SHH signaling (*GLI1* [GLI family zinc finger 1], *GLI2*, and *GLI3*) in several patients with CTRD. The role of WNT signaling in tracheal cartilage formation was directly tested by the authors through gene deletion of the essential component of WNT ligand secretion, *Wls* (Wntless). Loss of *Wls* in the developing respiratory endoderm resulted in loss of tracheal cartilage formation and a reduction in SOX9⁺ cartilage progenitors. This correlated with a general loss of WNT signaling throughout the

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