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# BMJ Open Using thoracic ultrasound to detect interstitial lung disease in patients with rheumatoid arthritis: a protocol for the diagnostic test accuracy AURORA study

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## ABSTRACT

**Introduction** Pulmonary diseases are significant contributors to morbidity and mortality in patients with rheumatoid arthritis (RA). RA-associated interstitial lung disease (RA-ILD) may be prevalent in up to 30% and clinically evident in 10% of patients with RA. Feasible methods to detect concomitant ILD in RA are warranted. Our objective is to determine the diagnostic accuracy of thoracic ultrasound (TUS) for ILD in patients with RA with respiratory symptoms, by using chest high-resolution CT (HRCT) as the reference standard. Further, we aim to evaluate the diagnostic accuracy for the promising blood biomarkers surfactant protein-D and microfibrillar-associated protein 4 in the detection of ILD in this group of patients.

**Methods and analysis** By use of a standardised 14 zone protocol patients suspected of having RA-ILD will undergo TUS as index test performed by a junior resident in rheumatology (BKS), who is certified by the European Respiratory Society in performing TUS assessments. Participants form a consecutive series of up to 80 individuals in total. The anonymised TUS images will be stored and scored by the junior resident as well as two senior rheumatologists, who have received training in TUS, and a TUS-experienced pulmonologist. HRCT will be used as the gold standard for ILD diagnosis (reference standard). The two basic measures for quantifying the diagnostic test accuracy of the TUS test are the sensitivity and specificity in comparison to the HRCT.

**Ethics and dissemination** Data will be collected and stored in the Research Electronic Data Capture database. The study is approved by the Committees on Health Research Ethics and the Danish Data Protection Agency. The project is registered at [clinicaltrials.gov](http://clinicaltrials.gov) (NCT05396469, pre-results) and data will be published in peer-reviewed journals.

## INTRODUCTION

Pulmonary diseases are significant contributors to morbidity and mortality in rheumatoid arthritis (RA), and an association between anticitrullinated protein antibody positivity and interstitial lung disease (ILD) has been

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ Minimal time consuming.
- ⇒ Cheap and easy to learn.
- ⇒ Radiation free.
- ⇒ Only suggestive of interstitial lung disease pattern on high-resolution CT.
- ⇒ Examiner dependent.

described.<sup>1–5</sup> The most common pulmonary manifestations in RA are ILD (RA-ILD) and chronic obstructive pulmonary disease (COPD), as previously published by our group and consistent with other RA populations.<sup>6–10</sup> The burden of RA-ILD is increasing, as studies have shown that ILD is prevalent in 33%–44% of patients with RA and is clinically evident in about 10% of the RA population.<sup>4 6 11–14</sup> In our population-based Danish cohort, approximately 2.2% of patients with RA had ILD and there was observed a more than doubled mortality risk in patients with RA-ILD within 30 days after RA diagnosis, when compared with RA without ILD.<sup>15</sup> Moreover, this increased mortality was persistent throughout the 17-year follow-up period with a median survival of 6.6 years after RA-ILD diagnosis. Excess mortality in patients with RA-ILD is observed in other studies as well.<sup>4 14</sup>

## How is ILD currently diagnosed (ie, the reference standard)

In the past two decades, many advances have been made to our understanding of ILD and the way we approach its treatment. Chest high-resolution CT (HRCT) is the most central diagnostic tool of ILD and is regarded as the gold standard for ILD diagnostics.<sup>16</sup> A confident diagnosis can sometimes be made based on HRCT in combination with a clinical context,<sup>17</sup> as for example, in idiopathic



pulmonary fibrosis (IPF).<sup>18</sup> However, achieving a confident ILD diagnosis may necessitate serological as well as histopathological information achieved by transbronchial or surgical lung biopsies and a multidisciplinary discussion team approach is recommended.<sup>17–19</sup> In evaluating patients with suspected ILD, the clinician should confirm the presence of the disease and then try to determine its underlying cause or recognised clinicopathological syndrome. Clues from the medical history along with the clinical context and radiologic findings provide the initial basis for prioritising further diagnostic possibilities for a patient with ILD.<sup>17 19 20</sup>

### Thoracic ultrasound (ie, the primary index test)

Thoracic ultrasound (TUS) has manifested itself as a promising tool in detecting ILD,<sup>21</sup> and has previously been validated for detecting ILD in systemic sclerosis (SSc), where TUS findings  $\geq 3$  B-lines in at least two adjacent scanning sites or a total of  $> 5$  B-lines present, were highly associated with SSc-ILD.<sup>22 23</sup> Similar observations were also found in a recent study of patients with RA with RA-ILD.<sup>24</sup> The European Respiratory Society (ERS) has recently published a statement on TUS, reviewing current research in the field. The statement recommended research in whether TUS can detect early ILD. However, it must be noted that TUS has not been found to have any clinical role in COPD or other cystic ILDs.<sup>25 26</sup>

### Rationale: intended use and clinical role of TUS

RA-ILD is associated with increased mortality compared with RA without ILD; this creates a rationale for a reproducible and radiation-free bedside tool for detection of potential ILD in RA.<sup>27–29</sup> Characteristic TUS signs compatible with ILD have been described in SSc<sup>22 23 30</sup> and in other connective tissue diseases associated with ILD.<sup>31</sup> A recent case-control study, with 71 patients with RA, has found that B-lines in RA may be associated with diffusion capacity of the lung for carbon monoxide (DLCO), APCA-status, inflammatory activity and physical function.<sup>24</sup> However, the applicability of TUS to identify ILD in patients with RA (with manifest ILD on HRCT) is only limited<sup>31 32</sup> and it has not yet been validated as a screening method to identify undiagnosed ILD in patients with RA with, for example, respiratory symptoms.

### Potential blood biomarkers (ie, other index tests)

In addition to validation of TUS as a diagnostic test for ILD, there is a need for robust biomarkers that can detect ILD as well as monitor the dynamics of pulmonary involvement in patients with RA.

Surfactant protein-D (SP-D) is a member of the collectin family and is primarily produced in type II pneumocytes.<sup>33 34</sup> Increased SP-D levels are positively associated with smoking status, with higher levels in current smokers and smokers with decreased lung function<sup>35 36</sup> and reflect an increased permeability of SP-D from the lung to the bloodstream, due to significant lung damage.<sup>37</sup> Increased SP-D levels have been found in patients with

severe IPF and reflect disease severity.<sup>38</sup> In patients with SSc, increased SP-D has been associated with decreased diffusion capacity and disease activity due to pulmonary fibrosis development.<sup>39 40</sup> Another study has shown increased SP-D levels in patients with subclinical and clinical RA-ILD.<sup>1</sup> Decreased SP-D levels in early RA may correlate negatively to RA disease activity measures<sup>41 42</sup> and may modulate inflammation in RA.<sup>43</sup>

Increased serum microfibrillar-associated protein 4 (MFAP4) seem to reflect disease-induced processes, due to low heritability and relatively limited basal variation.<sup>44</sup> MFAP4 is found with especially high expression in the heart, small intestine and the lungs. In the lung, MFAP4 were localised in the pulmonary arterioles and interalveolar walls.<sup>45</sup> Molleken *et al* have shown that serum MFAP4 levels were not increased in IPF.<sup>46</sup> Rationale: intended use and clinical role of the biomarkers SP-D and MFAP4 have not been tested as screening/diagnostic biomarkers in patients with RA with suspected ILD.

### Study hypotheses and objectives

First, we hypothesise that rheumatologists can use TUS to detect RA-ILD in patients with RA using chest HRCT as gold standard for ILD diagnosis.<sup>47</sup> Second, we hypothesise that serum SP-D and MFAP4 levels as well as TUS findings are associated with specific HRCT findings and pulmonary function test (PFT) results. Tertiary, we will evaluate the interobserver variability when comparing TUS scores between the TUS-trained rheumatologists and an experienced pulmonologist in the field of TUS and ILD.

## METHODS

### Patient and public involvement

The observational, clinical settings of the study ensure a high external validity. Furthermore, the study is designed with assistance from three Danish patient research partners from the Rheumatology Research Unit (LB, OA and LP). Two with RA-ILD and one with RA, where we discussed their experience on time with respiratory symptoms and until they received their RA-ILD diagnosis. The patient partners warranted focus on respiratory symptoms in patients with RA and methods for earlier detection of ILD. They influenced the patient enrolment and pathway through the project, as well as on the written patient information by giving valuable and critical feedback. The patient partners are not involved in recruitment and conduct of the study. The overall scientific results of our study will either be presented in person, by telephone or via email, depending on the patient partners and participants' preference. This project follows the European League Against Rheumatism recommendations for the inclusion of patient representatives in the contemporary scientific process by adhering to eight important aspects.<sup>48</sup>

### Study design

This is a multicentre, cross-sectional diagnostic test accuracy study of patients with RA and respiratory symptoms,

with TUS performed prior to the HRCT gold (reference) standard.

### Eligibility criteria

Patients eligible for inclusion are consenting adults ( $\geq 18$  years) diagnosed with RA, with the presence of at least one of the following symptoms: unexplained dyspnoea, unexplained cough, residual pneumonia or a chest X-ray indicating ILD. All patients must fulfil the 2010 criteria for RA. A diagnosis of COPD does not exclude the patient from the study.

We will exclude patients with other systemic autoimmune diseases than RA (except secondary Sjögrens syndrome), previous or current cancer treated with chemotherapy and/or radiation therapy of the thorax, lung transplant recipients and patients with known ILD or congenital lung disease, as well as patients who have had an HRCT performed within 12 months prior to the inclusion date. Patients who are unwilling or unable to provide written informed consent will also be excluded (ie, not eligible).

### Identification of potentially eligible participants

Eligible patients will be recruited from four departments of rheumatology in the Region of Southern Denmark: The Department of Rheumatology in the Hospital of South West Jutland, Odense University Hospital (OUH)—Svendborg Hospital, Lillebaelt Hospital, and OUH. Patients with a scheduled clinical visit for their RA disease and management will be asked if they have respiratory symptoms. If they do have respiratory symptoms, they will receive oral information about this project and those who are interested in participating will receive written information as well as a signed consent form (not yet to be signed). Subsequently, the patients will be referred to the highly specialised unit (HSU) in the Department of Rheumatology at OUH for an elaborating and undisturbed conversation about the project, where there will be time for questions. At the appointment, the patients will also undergo a full clinical evaluation securing a valid RA diagnosis and screening for eligibility. If eligibility criteria are met, the patient will be asked to sign the informed consent. After consent has been given, the patient will be enrolled and TUS will be performed on the same day. Subsequently, the patients will be referred to an HRCT as well as a full clinical evaluation in the PULmo-REuma (PURE) Clinic located at OUH (See [figure 1](#)). The signed consent will give authority approved researchers collaborating on this project, access to the electronic patient record to obtain relevant clinical information on physical health (for more details, see [table 1](#) template). It is to be noted that if patients do not wish to enrol, they will still receive relevant workup and offered relevant treatment. Inclusion has begun in May 2022 and will continue, till we reach the prespecified (pragmatically defined) sample size of patients we want to recruit; or at the latest 1 of October 2023.

### Consecutive enrolment

This is an inception cohort of patients with RA with suspected ILD where subjects will be enrolled, at their local department of rheumatology in the Region of Southern Denmark (See [figure 1](#)).

Eligibility will be evaluated by a senior rheumatologist (TE) at the HSU, and if patients are found to be eligible, they will receive an anonymised patient ID and TUS will be performed by the junior rheumatologist (BKS) on the same day as inclusion and always prior to HRCT. Referrals for an HRCT at the Department of Radiology, OUH as well as a referral to the PURE Clinic OUH for PFT and clinical evaluation will be made at time of inclusion. Blood samples will be taken in the diagnostic procedure, as well as 100 mL to be stored in a biobank for SP-D and MFAP4 measurements. The blood samples will be taken on the same day as either PFT or HRCT. For patient characteristics, see [table 1](#).

### Test method: TUS (ie, index test #1)

A standardised 14-zone protocol for TUS as described by Davidsen *et al*<sup>49</sup> will be used: patients will be examined in a straight-backed sitting position. The thorax will be systematically scanned according to anterior, lateral and posterior chest wall using an adapted approach of the principles described by Volpicelli and Lichtenstein,<sup>27 50</sup> and also used in previous studies from Davidsens research group.<sup>51 52</sup>

In a vertical and horizontal direction, respectively, the anterior chest wall will be outlined from clavicles to diaphragm, and from sternum to anterior axillary line; lateral chest wall from axilla to diaphragm, and from anterior to posterior axillary line; posterior chest wall from margo superior scapula to diaphragm, and from posterior axillary to paravertebral line. The anterior and lateral chest walls will be divided into an upper (zones 1 and 4); and lower zone (zones 2 and 3), whereas the posterior chest wall will be divided into an upper, middle and lower zone (zones 5–7) equivalent to a total of seven zones for each hemithorax.<sup>51 52</sup> In each zone, the transducer will be systematically placed vertically across an intercostal space corresponding to the centre of the specific zone. Supplementary horizontal views of the intercostal space in a given zone will be performed in case of abnormal findings using the vertical view. In all 14 scanning zones TUS will be performed.

B-lines, interstitial syndrome (IS), and pleural thickening are TUS findings known to be associated with presence of ILD on HRCT.<sup>21 23 25 27 30 53</sup> We will use the following definitions of TUS findings, as described by Davidsen *et al*<sup>54</sup>: number of B-lines: B-lines are defined as vertical reverberation artefacts originating from the pleural line extending uninterrupted to the edge of the screen on the ultrasound machine without fading (previously termed ‘comet-tails’).<sup>53</sup> IS:  $\geq 3$  B-lines in  $\geq 2$  anterior or lateral zones on each hemithorax.<sup>25 27</sup> Upper lobe IS:  $\geq 3$  B-lines in in both zone R/L1 and R/L7. Pleural thickening: pleura thickness  $> 1$  mm regardless a normal or



Figure 1a Patient pathway to eligibility

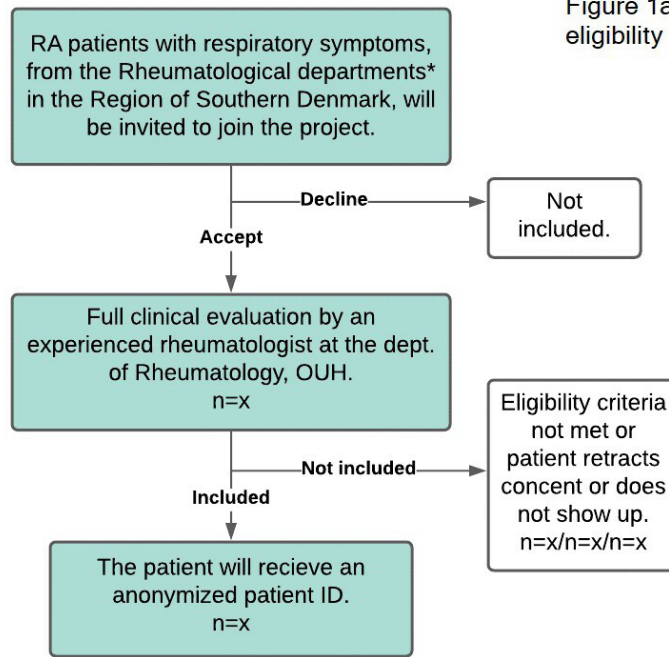
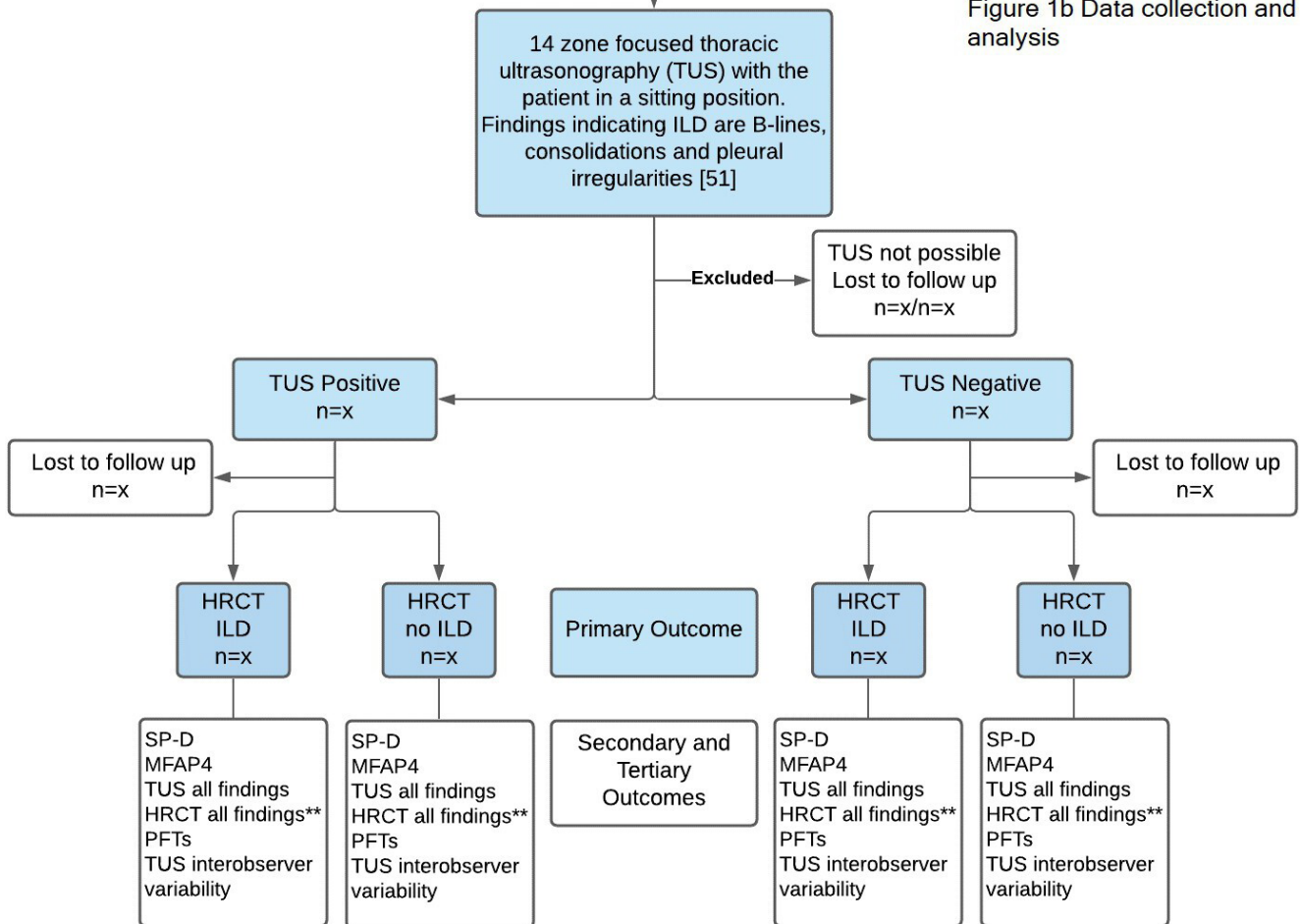


Figure 1b Data collection and analysis



Explanation: \*= Dept of Rheumatology in the Hospital of South West Jutland, Odense University Hospital -Svendborg Hospital, Lillebaelt Hospital and Odense University Hospital. \*\* = UIP, NSIP, BO, irregular pleura, pleural effusion, rheumatic nodules, emfysema, cancer ect.

**Figure 1** Flow diagram of patients in the AURORA study. HRCT, high-resolution CT; ILD, interstitial lung disease; MFAP-4, microfibrillar-associated protein 4; PFT, pulmonary function test; RA, rheumatoid arthritis; SP-D, surfactant protein-D.

**Table 1** Patient characteristics

	TUS positive (n=x)		TUS negative (n=x)		Interaction
	HRCT ILD (n=x)	HRCT No ILD (n=x)	HRCT ILD (n=x)	HRCT No ILD (n=x)	P value
Age	x	x	x	x	
Female, n (%)	n=x (%)	n=x (%)	n=x (%)	n=x (%)	
Anti-CCP positive (%)	x	x	x	x	
IgM RF positive (%)	x	x	x	x	
Time since RA diagnosis (months)	x	x	x	x	
RA treated with ...					
Smoking habits	n=x	n=x	n=x	n=x	
Pack years	x	x	x	x	
Never smoked, n (%)	x	x	x	x	
Current smoker, n (%)	x	x	x	x	
Former smoker n (%)	x	x	x	x	
Duration of respiratory symptoms (months)	x	x	x	x	
Swollen joints 28	x	x	x	x	
Tender joints 28	x	x	x	x	
CRP mg/L	x	x	x	x	
HAQ	x	x	x	x	
DAS28CRP	x	x	x	x	
FEV1 % predicted	x	x	x	x	
FVC % predicted	x	x	x	x	
FEV1/FVC %	x	x	x	x	
TLC % predicted	x	x	x	x	
DLCO % predicted	x	x	x	x	
6MWD (metres)	x	x	x	x	
6MWD desaturation (Δ%)	x	x	x	x	
SP-D	x	x	x	x	
MFAP4	x	x	x	x	
ILD patterns on HRCT:					
UIP	n=x (%)	–	n=x (%)	–	
NSIP	n=x (%)	–	n=x (%)	–	
BO	n=x (%)	–	n=x (%)	–	
OP	n=x (%)	–	n=x (%)	–	
TUS kappa value	x	x	x	x	
TUS: B-lines (>2/ICS)	n=x (%)	n=x (%)	n=x (%)	n=x (%)	
TUS: consolidations	n=x (%)	n=x (%)	n=x (%)	n=x (%)	
TUS: pleural irregularities	n=x (%)	n=x (%)	n=x (%)	n=x (%)	
TUS: pleural effusion	n=x (%)	n=x (%)	n=x (%)	n=x (%)	

BO, bronchiolitis obliterans; CCP, cyclic citrullinated peptide; CRP, C reactive protein; DLCO, diffusion capacity of the lung for carbon monoxide; FEV1, forced expiratory volume in 1 s; HAQ, health assessment questionnaire; HRCT, high-resolution CT; ILD, interstitial lung disease; MFAP4, microfibrillar-associated protein 4; 6MWD, 6 minute walking distance; NSIP, non-specific interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; OP, organising pneumonia; RA, rheumatoid arthritis; SP-D, surfactant protein-D; TLC, Total Lung Capacity; TUS, thoracic ultrasound; UIP, usual interstitial pneumonia.

abnormal irregular or fragmented presence of pleura.<sup>30 55</sup> Acceptable window of HRCT in relation to TUS in this study is 1 month after TUS.

### Test method: SP-D (index test #2) and microfibrillar-associated protein (index test #3)

Serum SP-D levels will be detected using a sandwich ELISA technique, as described in Leth-Larsen *et al.*<sup>56</sup> Serum MFAP4 levels will be detected using the AlphaLISA technique, as described in Wulf-Johansson *et al.*<sup>45</sup>

### Test positivity cut-offs: index test

All TUS images will be scored for the findings mentioned in the Method section and the question 'Do the TUS images indicate ILD?' must be answered with a 'Yes or No' for each anonymised patient. In case of disagreement, consensus will be achieved by the experienced pulmonologist (JRD). In both SP-D and MFAP4, there has not been established a normal range in serum yet. We will test whether serum levels of SP-D and MFAP4 differ in patients with RA with and without ILD.

### Reference standard: HRCT

Rationale for choosing the reference standard: HRCT acts as the gold standard for diagnosing ILD.<sup>57</sup> Current national guidelines recommend that all patients suspected of having ILD, undergo HRCT, as part of their diagnostic workup.<sup>58</sup>

All patients will receive a chest HRCT. The initial examination includes a standard radiation dose (*diagnostic*) end-inspiratory scanning and a low radiation dose (*low dose*) end-expiratory scanning. Eventual follow-up examinations always include a diagnostic end-inspiratory scanning, but only patients with suspected small airways disease receive an additional low-dose end-expiratory scanning.

All HRCT scans are performed on a Revolution CT; General Electric Company; Boston, Massachusetts, USA. Acquisition parameters of the diagnostic end-inspiratory scanning are collimation 8 cm, kV 120, SmartmA (140–900 mA), Noise Index 25, Pitch 0.5, Rotation time 0.35 s, Asir-V 40%. Images are reconstructed using a 512×512 matrix and chest algorithm. Slice thickness is axial 0.625 mm, coronal 2 mm and sagittal 2 mm. Image overlap 20%. A maximum intensity projection series is reconstructed using standard algorithm, slice thickness 6 mm. Image overlap 50%. Almost identical acquisition parameters are used for the low-dose end-expiratory scanning. However, noise index is raised to 30. End-expiratory images are reconstructed using chest algorithm. A single axial series is reconstructed. Slice thickness is 2.5 mm. All examinations are assessed on Vue PACS; Koninklijke Philips N.V., Amsterdam, The Netherlands.

The following signs of ILD, airways disease and other lung diseases are noted with specific disease patterns as: usual interstitial pneumonia (UIP), non-specific interstitial pneumonia (NSIP), organising pneumonia, mosaic attenuation pattern/airways disease. Signs of fibrosis

are reticulation and/or traction bronchiectasis and/or honeycombing and/or loss of volume.<sup>57 59</sup> Signs of inflammation are areas of ground glass opacity and/or consolidation. Signs of airways disease are mosaic attenuation and/or non-traction bronchiectasis and/or bronchial wall thickening and/or obliterative bronchiolitis and/or exudative bronchiolitis and/or air trapping. Other findings include nodules and/or emphysema as well as subtype and/or pleural or pericardial effusions and/or thickening and/or enlargement of the pulmonary trunk or aorta.

### Test positivity cut-off(s): reference standard

If areas of ground glass opacity, consolidation, reticulation or established fibrosis with traction bronchiectasis, honeycombing or loss of volume are evident on the HRCT, the reference standard is considered positive for ILD. Likewise, indication of airway disease and particularly mosaicism with obliterative bronchiolitis and air trapping will be considered positive for ILD with consensus interpretation by SH, who is an experienced radiologist in the field of HRCT and ILD.

SH will evaluate all HRCT images for the findings mentioned above and after his evaluation and possible multidisciplinary meetings with experienced pulmonologists and rheumatologists, SH will answer the question 'Do the HRCT images indicate ILD?' with either 'Yes or No' for each patient. Other possible findings on HRCT include pleural or pericardial effusion or thickening on the HRCT.

### Data collection process

Patients will receive a full clinical evaluation in the HSU, Departement of Rheumatology, OUH as well as at the HSU, Departement of Respiratory Medicine, OUH. Before signed consent is given, the patients journal will be accessed by the treating physicians at OUH, for clinical evaluation of the diagnosis as well as eligibility. After the informed consent is given, clinical data, PFTs, and radiological workup will also be accessible to the non-treating physicians, who are part of this project. TUS, routine blood samples, as well as 100 mL blood sample for research use, PFTs and HRCT scan of the chest will be performed after informed consent. TUS will be performed immediately after informed consent and always prior to HRCT scans. TUS results will not appear in the patients' journal but will be pseudoanonymised and stored in a Research Electronic Data Capture database (REDCap). Clinical details as well as paraclinical data will be accessed through the patients' medical journal. Relevant information that will be obtained from the journal is listed in [table 1](#). All data in this project will be pseudoanonymised and stored in REDCap.

### Training and expertise of the persons executing and reading the tests

A junior resident in rheumatology (BKS), who is certified by the ERS in performing TUS assessment, will perform

TUS and score the images on site as well as store the anonymised images. When inclusion is complete, the anonymised images will be scored by an experienced pulmonologist in the field of TUS and ILD (JRD) and two experienced rheumatologists (TE and PRLH) in the field of musculoskeletal ultrasonography, who have received training in TUS. In case of disagreement in test assessors on TUS findings, the answer from the experienced pulmonologist (JRD) will be used as consensus.

### Blinding of test assessors

The patients will be evaluated by an experienced rheumatologist. Before referral to HRCT, the TUS examination will be performed by the junior resident (BKS), where the patient will be given a project ID, assigned by and registered in a REDCap database. The junior resident will be the only physician seeing the TUS images at inclusion. The TUS images will be scored on site and stored, labelled with the patient's project ID only, on a secured offline hard drive. Only BKS will see the project ID and the TUS diagnosis. TUS results will not appear in the patients' medical journal but will be saved directly to the REDCap database at inclusion, in a module only visible to the BKS. After TUS images have been saved and scored, the patients Danish personal identifier number (CPR) will be added to the REDCap database in a module only visible to BKS and the radiologist SH. SH will need access to the patients CPR number in order to register HRCT findings in REDCap, as the patients project ID will not appear in the HRCT referral or patient journal. HRCT findings will be registered in a module only visible to SH as long as inclusion is ongoing. BKS will not attend multidisciplinary meetings regarding possible RA-ILD in the inclusion period but will have access to the patients' medical journal in order to register test results. After inclusion has ended, the other TUS assessors (PRLH, TE and JRD) will score the anonymised TUS image and answer whether the TUS images indicate ILD.

### Pulmonary function test

All patients will undergo a PFT in accordance with the ERS and American Thoracic Society standards including FEV1 (forced expiratory volume in 1s) and FVC in litres and per cent of predicted (% pred.), and FEV1/FVC ratio.<sup>60</sup> DLCO will be measured as a single-breath diffusion lung capacity. All predicted values will be automatically calculated, following the ERS Official technical standard.<sup>61</sup>

### Biobank

Blood tissue bank: a blood sample of 100 mL whole blood for serum/plasma (EDTA and Li-Hep plasma) and DNA storage will be obtained at inclusion for analysis of immunological markers. Immunological and early diagnostic markers such as SP-D and MFAP4 will be quantified as potential new biomarkers of lung involvement and severity/subclassification. The blood samples will be stored in a research biobank as long as the project is ongoing and up to 10 years after the project has ended,

so that the project analysis can be revalidated, should this become relevant. Storage will be according to Danish law in the Research unit of Clinical Immunology and in the OPEN research facility at OUH.

## STATISTICAL METHODS

### Comparison of measures of diagnostic accuracy

The two basic measures of quantifying the diagnostic accuracy of a test will be the sensitivity and specificity measures. Sensitivity is defined as the ability of the TUS index test to detect the RA-ILD condition when it is truly present, that is, it is the probability of a positive test (TUS positive) result given that the patient has the disease (HRCT positive). Specificity is the ability of the TUS test to exclude the condition in the patients with RA who do not have the disease that is, it is the probability of a negative test (TUS negative) result given that the patient does not have the disease (HRCT negative). When reporting the finding from the primary diagnostic test (TUS index test), both sensitivity and specificity are linked (ie, correlated) in that as the value of one increases, the value of the other decreases; these measures dependent on the patient characteristics and the disease spectrum. From these measures we will calculate the likelihood ratio (LR), defined as the ratio of the probability of the index test result among patients who truly have RA-ILD to the probability of the same test among patients who do not have RA-ILD. The LR is the ratio of Sensitivity/(1 - Specificity); the LR is independent of prevalence of the RA-ILD in our sample. The magnitude of the LR will inform us about the certainty of a positive diagnosis: a value of LR=1 indicates that the TUS index test result is equally likely in patients with and without the RA-ILD, while values of LR>1 indicate that the TUS index test result is more likely positive in patients with the RA-ILD and values of LR<1 indicate that the TUS index test result is more likely in patients without RA-ILD.

Finally, we will also compare sensitivity and specificity for TUS compared with the secondary index tests. Since all diagnostic tests will be performed on each patient, then paired data result and methods that account for the correlated binary outcomes are necessary (McNemar's test).

### Handling indeterminate and missing index test

Possible indeterminate TUS results: will be unlikely, given the nature of this study, where test assessors must answer 'Yes' or 'No' to if the images indicate ILD. However, if the quality of the images is poor, the answer 'No' is more likely to occur, and this may lead to false negative results. Missing index test will lead to exclusion of the patient from the study in the primary analyses.

### Handling indeterminate and missing reference standard

Possible indeterminate results are not likely, as results are dichotomised into ILD or non-ILD on HRCT. Missing



**Table 2** 2x2 table TUS/HRCT

HRCT ILD	True positives n=a	False negative n=b	= a + b
HRCT no ILD	False positive n=c	True negatives n=d	= c + d
Total	= a + c	= b + d	= a + b + c + d

Positive predicted value (%) =  $a/(a+c)$ .  
 Negative predicted value (%) =  $d/(b+d)$ .  
 Sensitivity (%) =  $a/(a+b)$ .  
 Specificity (%) =  $d/(c+d)$ .  
 HRCT, high-resolution CT.

reference standard will lead to exclusion of the patient from the study.

### Sample size and power considerations

The study is designed to be able to evaluate the diagnostic test characteristics (sensitivity, specificity, LRs) and determine the post-test probability of disease given the pretest probability and test characteristics.<sup>62</sup> Given the sample size n=80 (and *guestimated* proportionate distributions), the following will be enabled:

Corresponding to disease prevalence, test sensitivity, and test specificity (based on the suggested sample size): given a prevalence of 0.375, a sensitivity of 0.667, a specificity of 0.800 in a sample size of 80, the prior probability (odds) is 38% (0.6). The positive LR is 3.33 with a 95% CI of 1.81 to 6.13—the posterior probability (odds) is 67% (2.0) with a 95% CI of 52% to 79%, meaning two out of three with a positive TUS have ILD on HRCT. The

negative LR is 0.42 with a 95% CI of 0.25 to 0.70. The posterior probability (odds) is 20% (0.3) with a 95% CI of 13% to 30%, meaning 10 of 13 with a negative TUS to not have ILD on HRCT. Odds=probability/(1–probability). Positive likelihood ratio (LR+) = sensitivity/(1–specificity). Negative likelihood ratio (LR–) = (1–sensitivity)/specificity. Posterior odds=prior odds × LR.

### RESULTS

Results from the primary analysis will be presented in [table 2](#). Additional observational findings will be presented in [table 3](#) and [table 4](#), where specific TUS findings in relation to specific HRCT findings will be listed.

### DISCUSSION

Patients with RA have an increased risk of developing ILD and an increased risk of mortality after ILD diagnosis.<sup>15</sup> The increased mortality may be due to ILD diagnosis at late stages of their lung disease. As treatment options are increasing, we should do more to detect and treat RA-ILD at earlier stages. About 10% of patients with RA in a national Danish cohort receive medication for COPD and their increased mortality is comparable to RA-ILD.<sup>15 63</sup> Smoking is associated with the development of both RA, COPD and ILD. The diagnosis of ILD in patients with RA may be masked, as symptoms of ILD are compatible with COPD (dyspnoea, cough, recurrent clinical pneumonia). ILD may easily be mistaken for COPD and vice versa which has previously been pointed out for other ILD subtypes

**Table 3** TUS findings in relation to HRCT findings

HRCT findings	Specific patterns:	TUS findings				
		>2 B-lines	Interstitial syndrome	Consolidation	Pleural irregularities	Pleural effusion
	UIP (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	NSIP (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	OP (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	Mosaic pattern/BO and air trapping (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	<b>Signs of ILD:</b>					
	GGO (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	Consolidation (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	Reticulation (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	Traction bronchiectasis (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	Honeycombing (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	<b>Signs of other airways disease:</b>					
	Bronchiectasis (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	Bronchial wall thickening (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	Bronchiolitis obliterative/exudative (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	<b>Signs of serositis:</b>					
	Pleural thickening or effusion (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)
	Pericardial thickening or effusion (N=x)	N (%)	N (%)	N (%)	N (%)	N (%)

HRCT, high-resolution CT; ILD, interstitial lung disease; NSIP, non-specific interstitial pneumonia; OP, organising pneumonia; TUS, thoracic ultrasound; UIP, usual interstitial pneumonia.

**Table 4** Specific TUS findings in TUS scanning zones in relation to ILD pattern on HRCT

	TUS zones (L1–7 and R1–7)													
	L1	L2	L3	L4	L5	L6	L7	R1	R2	R3	R4	R5	R6	R7
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
<b>&gt;2 B-lines</b> <sub>(TUS)</sub>														
All <sub>(HRCT)</sub>														
UIP <sub>(HRCT)</sub>														
NSIP <sub>(HRCT)</sub>														
BO <sub>(HRCT)</sub>														
OP <sub>(HRCT)</sub>														
<b>Interstitial syndrome</b> <sub>(TUS)</sub>														
All <sub>(HRCT)</sub>														
UIP <sub>(HRCT)</sub>														
NSIP <sub>(HRCT)</sub>														
BO <sub>(HRCT)</sub>														
OP <sub>(HRCT)</sub>														
<b>Consolidation</b> <sub>(TUS)</sub>														
All <sub>(HRCT)</sub>														
UIP <sub>(HRCT)</sub>														
NSIP <sub>(HRCT)</sub>														
BO <sub>(HRCT)</sub>														
OP <sub>(HRCT)</sub>														
<b>Pleural irregularities</b>														
<sub>(TUS)</sub>														
All <sub>(HRCT)</sub>														
UIP <sub>(HRCT)</sub>														
NSIP <sub>(HRCT)</sub>														
BO <sub>(HRCT)</sub>														
OP <sub>(HRCT)</sub>														
<b>Pleural effusion</b> <sub>(TUS)</sub>														
All <sub>(HRCT)</sub>														
UIP <sub>(HRCT)</sub>														
NSIP <sub>(HRCT)</sub>														
BO <sub>(HRCT)</sub>														
OP <sub>(HRCT)</sub>														

HRCT, high-resolution CT; ILD, interstitial lung disease; NSIP, non-specific interstitial pneumonia; OP, organising pneumonia; TUS, thoracic ultrasound; UIP, usual interstitial pneumonia.

as, for example, IPF.<sup>64</sup> Currently, there are no studies on patients with RA with unexplained respiratory symptoms, nor any studies on screening for respiratory symptoms in RA. This cohort of patients with RA will assess the diagnostic accuracy of TUS in detecting ILD in a cohort of patients with RA with unexplained respiratory symptoms. Further, we will identify all clinically relevant pulmonary diagnosis in this cohort of patients.

The strengths of this study is that TUS is minimal time consuming, cheap and radiation free and has shown to be a promising tool in ILD detection.<sup>53</sup> TUS has not yet

been solidly validated as a screening tool in patients with RA with respiratory symptoms, but has been validated in smaller studies, often case control, with a high pretest probability of ILD. When joining TUS findings in one recent meta-analysis, TUS seems to have its justification as a potential ILD screening tool.<sup>31</sup> TUS is examiner dependent and interobserver variability may vary. To test for variability, four clinicians trained in TUS will score the same images and evaluate whether the images indicate ILD. The senior physicians will all be blinded to the patients' identity and data, when scoring the images. The

junior rheumatologist will know the patients clinical background and will therefore score the TUS images before HRCT is performed.

## ETHICS AND DISSEMINATION

### Ethics and registration number and name of registry

This study is initiated by Bjørk K. Sofiudóttir, Robin Christensen, Jesper R. Davidsen and Torkell J. Ellingsen. This study is approved by the Regional Committees on Health Research Ethics for Southern Denmark (S-20210154) and by the Danish Data Protection Agency (22/7044). The project is registered at [clinicaltrials.gov](https://clinicaltrials.gov) (NCT05396469).

### Ethical aspects

Patients with clinically relevant findings on HRCT and/or PFT will all receive relevant diagnostic follow-up and guideline treatment.

RA-ILD is a serious condition and this study may lead to a simple and radiation free method of early detection. Patients will not receive more radiation when entering the study, than in the usual clinical setting when ILD is suspected.

### Publication

The aim is to publish all results derived from this project in peer-reviewed journals. This will be done with positive, negative and inconclusive results. The project is registered at [clinicaltrials.gov](https://clinicaltrials.gov) in the pre-result stage.

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