Detection of interstitial lung disease in rheumatoid arthritis: diagnostic tests and prognostic value

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PhD Thesis: Detection of interstitial lung disease in rheumatoid arthritis: diagnostic tests and prognostic value

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PhD thesis

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2024

PhD thesis
Detection of interstitial lung disease in rheumatoid arthritis: diagnostic tests and prognostic value

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List of abbreviations

6MWD	6 minute walking distance
ACPA	anti-citrullinated peptide antibodies
ACR20	American College of Rheumatology, at least 20% improvement
ASAS20	Assessment of Spondyloarthritis International Society, at least 20% improvement
BMI	Body mass index
CASP	Critical Appraisal Skills Programme
CD	Crohn's disease
CI	Confidence interval
CID	Chronic inflammatory disease
COPD	Chronic obstructive pulmonary disease
CRP	C-Reactive protein
CT	Computed Tomography
CVD	Cardiovascular disease
DAS28CRP	Disease Activity Score-28 for Rheumatoid Arthritis with CRP
DLCO	Diffusing capacity for carbon monoxide
DMARD	Disease-modifying antirheumatic drugs
DOR	Diagnostic odds ratio
ECM	Extracellular matrix
EQUATOR	Enhancing the Quality and Transparency of Health Research
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
HBI	Harvey Bradshaw Index
HRCT	High-resolution computed tomography
IDT	Interdisciplinary teams
IgM	Immunoglobulin M
ILA	Interstitial lung abnormalities
ILD	Interstitial lung disease
IQR	Interquartile range
IS	Interstitial syndrome
ITT	Intention to treat
LVEF	Left ventricular ejection fraction
MFAP4	Microfibrillar-associated protein 4

MRC	Medical Research Council's dyspnoea scale
NET	Neutrophil extracellular traps
NSAID	Nonsteroidal anti-inflammatory drugs
OR	Odds ratio
OUH	Odense University Hospital
PASI	Psoriasis Area and Severity Index
PASI75	Psoriasis Area and Severity Index, least 75% improvement
PFT	Pulmonary function test
PSO	Psoriasis
QUADAS2	Modified Quality Assessment of Diagnostic Accuracy Studies
RA	Rheumatoid arthritis
RF	Rheumatoid factor
ROC	Receiver operating characteristic
SAP	Statistical analysis plan
SD	Standard deviation
AxSPA	Axial Spondyloarthritis
SSc	Systemic sclerosis
STARD	Standards for Reporting of Diagnostic Accuracy
StdD	Standardised difference
TLC	Total lung capacity
TUS	Thoracic ultrasound
UC	Ulcerative Colitis

List of papers

This PhD thesis is based on the following papers

Paper 1 **Using Thoracic Ultrasound to Detect Interstitial Lung Disease in Patients with Rheumatoid Arthritis: A Protocol for the Diagnostic Test Accuracy AURORA Study**
Bjørk K. Sofíudóttir, Stefan Harders, Philip R. Lage-Hansen, Robin Christensen, Heidi L. Munk, Grith L. Sørensen, Jesper R. Davidsen, and Torkell Ellingsen
Status: Published in *BMJ Open*, December 2022.
DOI: 10.1136/bmjopen-2022-067434

Paper 2 **Detection of Interstitial Lung Disease in Rheumatoid Arthritis by Thoracic Ultrasound: a Diagnostic Test Accuracy Study**
Bjørk K. Sofíudóttir, Stefan Harders, Christian B. Laursen, Philip R. Lage-Hansen, Sabrina M. Nielsen, Robin Christensen, Jesper R. Davidsen, and Torkell Ellingsen
Status: Under peer review

Paper 3 **Detecting respiratory impairment in newly diagnosed rheumatoid arthritis using the MRC dyspnoea score and serum Microfibrillar-associated protein 4 (MFAP4)**
Bjørk K. Sofíudóttir, Robin Christensen, Grith L. Sørensen, Charlotte Hyldgaard, and Torkell Ellingsen
Status: Manuscript ready for submission

Paper 4 **Microfibrillar-associated protein 4 as a predictive biomarker of treatment response in patients with chronic inflammatory diseases initiating biologics: Secondary analyses based on the prospective BELIEVE cohort study**
Bjørk K. Sofíudóttir, Heidi L. Munk, Robin Christensen, Sören Möller, Silja H. Overgaard, Grith L. Sorensen, Karen M. Møllegaard, Jessica Pingel, Anders B. Nexøe, Henning Glerup, Tanja Guldmann, Natalia Pedersen, Jens Frederik Dahlerup, Christian L. Hvas, Karina W. Andersen, Mohammad Jawhara, Ole Haagen Nielsen, Fredrik Olof Bergenheim, Anette Bygum, Jesper R. Davidsen, Signe B. Sørensen, Jacob B. Brodersen, Jens Kjeldsen, Vibeke Andersen, and Torkell Ellingsen
Status: Manuscript ready for submission

English summary

This was a PhD on diagnostic tests and prognostic value in rheumatoid arthritis (RA), the most common autoimmune disease. Treatment options for patients with RA and overall survival have improved over the years. However, there is still increased mortality in RA, largely due to respiratory diseases such as RA-associated interstitial lung disease (RA-ILD). RA-ILD has a 40% mortality rate after 5 years, with a median survival of 7.4 years after diagnosis. It is generally recommended to screen RA for respiratory symptoms to detect ILD. However, this is currently not evidence-based nor routinely applied in clinics. Thoracic ultrasound has also been suggested as a promising tool for the early detection of RA-ILD, but had yet to be tested in a clinical setting.

Treatment options for chronic inflammatory diseases (CIDs) such as RA have increased. It is estimated that about one-third of patients with CIDs who start on biologics will not respond to the treatment. Biological therapy is expensive and can have side effects. There are currently no validated methods of predicting treatment response. Microfibrillar-associated protein 4 (MFAP4) is a promising biomarker of inflammation and fibrotic activity; however, its role in detecting lung disease in RA, as well as predicting treatment response, has not been evaluated.

Papers 1 and 2 investigated the diagnostic accuracy of thoracic ultrasound (TUS) in detecting ILD in RA with pre-defined respiratory symptoms. The results revealed that TUS is a promising tool for detecting ILD, with a high sensitivity and negative predictive value.

Paper 3 investigated the diagnostic accuracy of an established questionnaire on perceived dyspnoea, using the Medical Research Council (MRC) dyspnoea scale, and MFAP4 in detecting respiratory impairment in newly diagnosed and treatment-naïve RA. Overall,

MRC and the crude analysis of MFAP4 showed neither a high sensitivity nor specificity. However, when adjusting for age, sex and smoking status, there was a correlation of MFAP4 ≥ 29.0 U/ml for detecting respiratory impairment.

Paper 4 investigated the prognostic value of high MFAP4 levels on positive treatment outcomes in patients with CIDs (RA, psoriatic arthritis, psoriasis, Axial Spondyloarthritis, Crohn's disease, and ulcerative colitis) who were about to initiate or switch biological therapy. The main results showed that when adjusting for CID, age, sex, smoking and BMI, high MFAP4 had the potential to predict a positive treatment outcome with biological therapy in most CIDs.

Dansk resumé

Denne ph.d. omhandlede diagnostiske og prognostiske undersøgelser ved leddegigt (RA), den mest udbredte af de autoimmune sygdomme. Gennem årene, er behandlingsmuligheder og overlevelsen hos personer med leddegigt forbedret. Der er dog fortsat en overdødelighed, og en stor bidrager til overdødeligheden er lungefibrose (også kaldet RA-ILD). Fem år efter diagnosen RA-ILD er stillet, er 40% af patienterne døde, og gennemsnitsoverlevelsen er 7,4 år. Det anbefales, at man screener RA-patienter for luftvejssymptomer mhp. opsporing af RA-ILD. Der er imidlertid ingen evidens bag anbefalingerne, og screening for luftvejssymptomer er ikke en fast del af den af kliniske vurdering ved RA. Ultralydsskanning af lungerne (LUS) har potentiale til at finde RA-ILD, men det mangler at blive efterprøvet ude i klinikkerne.

Der er tilkommet flere behandlingsmuligheder for kroniske inflammatoriske sygdomme (CID's), så som leddegigt. Omkring 1/3 af CID's opnår ikke en tilstrækkelig positiv behandlingseffekt af biologisk medicin. Biologisk medicin er heller ikke uden bivirkninger, og der findes ingen validerede metoder til at forudsige behandlingsrespons. Microfibrillar-associated protein 4 (MFAP4) er en lovende biomarkør for aktiv inflammation og fibrose aktivitet. MFAP4 er ikke efterprøvet som biomarkør for lungesygdom ved leddegigt og ej heller som biomarkør for at forudsige behandlingsrespons ved opstart af biologisk medicinering.

Studie 1 og 2 undersøgte den diagnostiske præcision af LUS til at opspore lungefibrose hos personer med leddegigt, der har selekterede luftvejssymptomer. LUS havde en god sensitivitet og negativ prædiktiv værdi.

Studie 3 undersøgte den diagnostiske præcision af et velkendt spørgeskema vedrørende oplevet åndenød (MRC) samt biomarkøren MFAP4 til at opspore nedsat lungefunktion hos personer med ny-diagnosticeret leddegigt, som ikke er opstartet i behandling endnu.

Overordnet havde hverken MRC eller den ujusterede MFAP4-analyse en god sensitivitet eller specificitet. Ved en analyse, justeret for alder, biologisk køn og rygerstatus, havde MFAP4 ≥ 29.0 U/ml en association til nedsat lungefunktion.

Studie 4 undersøgte den prognostiske værdi af at have høje MFAP4-niveauer i blodet, for at få et positivt behandlingsrespons hos personer med en kronisk inflammatorisk sygdom (leddegigt, psoriasisgigt, psoriasis, rygsøjlegigt, Chron's sygdom og colitis ulcerosa). Hovedresultaterne viste, at høje MFAP4-niveauer i blodet var associeret med en positiv behandlingsrespons, når der justeres for CID, alder, biologisk køn, rygerstatus og BMI.

Background

Rheumatoid arthritis (RA) is the most common autoimmune disease, afflicting approximately 35,000 Danish citizens [1]. RA is characterised by joint swelling, accompanied by pain as well as irreversible joint damage. Circulating anti-citrullinated peptide antibodies (ACPA) are specific antibodies correlated to RA. Having RA and positive ACPA or immunoglobulin M (IgM) rheumatoid factor (RF) is associated with increased disease severity as well as an increased risk of developing RA-associated interstitial lung disease (RA-ILD) [2-5]. Although about 33%–67% of RA patients have a positive ACPA or IgM RF at diagnosis, few newly diagnosed RA patients have lung disease [5, 6].

The lungs, mucosa and RA

The citrullination process, which underlies the development of ACPA, may occur several years prior to developing arthritis [7]. This mucosa is suspected to play a role in ACPA formation, as the formation of IgA ACPA and IgA RF may be detected years prior to symptoms and diagnosis of RA [8]. In the lungs, higher amounts of ACPA, as well as inflammatory cells (e.g. lymphocytes), are found in the bronchoalveolar lavage of ACPA-positive than in ACPA-negative RA, indicating that the lung is a source of ACPA-related inflammation in patients with early untreated RA [9, 10]. Increased citrullination of proteins in the lungs can occur during disease-induced processes and is also associated with smoking or exposure to smoke or silica particles [11-13]. This is reflected in the RA population, where current or former smokers are highly prevalent [6].

ACPA levels were higher than in the induced sputum of RA patients than in serum, for certain sub-types of ACPA, and positive sputum but negative serum ACPA was found in patients defined as being at risk of RA [14]. The same research group has found associations

with sputum neutrophil extracellular traps (NETs) in subjects at risk of future development of RA (**Figure 1**) [15]. These findings support inflammatory activity in the lungs as the origin of ACPA formation in subgroups of RA. Further supporting this theory is that lung diseases, such as chronic obstructive pulmonary disease (COPD) and ILD, are highly prevalent in RA as well as in patients with RA-associated autoantibodies without arthritis [16-20]. However, citrullination also occurs in other mucosal membranes, such as the gingiva, where periodontitis has been associated with the development of RA, and in the gut, where dysbiosis is suspected to play a role [21].

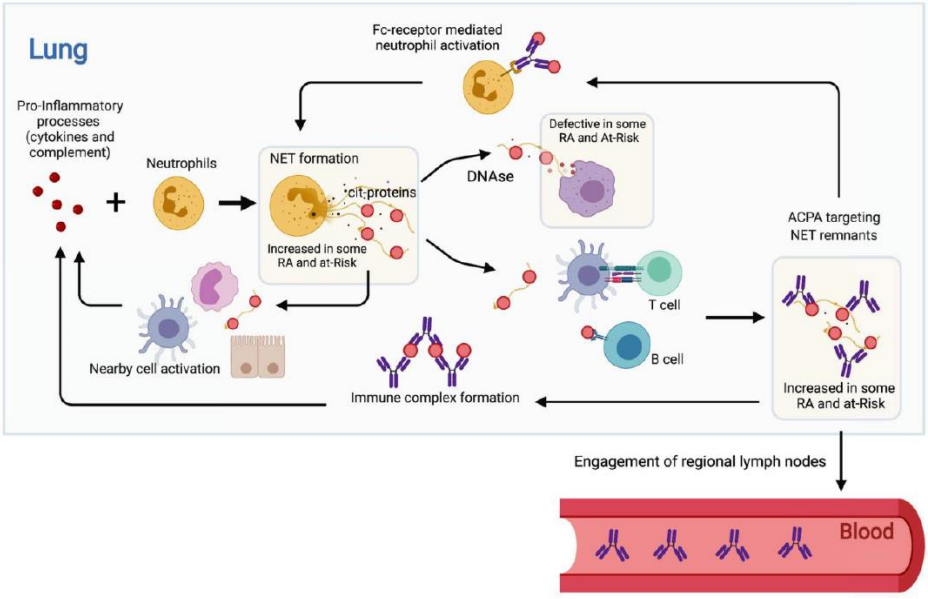


Figure 1: Association of Sputum Neutrophil Extracellular Trap Subsets With IgA Anti-Citrullinated Protein Antibodies in Subjects at Risk for Rheumatoid Arthritis. Arthritis & Rheumatology, DOI: 10.1002/art.41948. With permission from John Wiley and Sons. Original Order Number: 501876625.

RA-ILD prognosis and screening approaches

The survival of patients with RA has improved over the years. However, there is still increased mortality in the RA population, largely due to respiratory diseases [22]. A diagnosis of rheumatoid arthritis-associated interstitial lung disease (RA-ILD) has a 5-year mortality of 40%, and the median survival after diagnosis is 7.4 years [3]. This is equivalent to a cancer diagnosis in Denmark [23].

About 2-10% of patients with RA receive a diagnosis of RA-ILD [1, 24, 25]. Identifying RA-ILD in patients with RA is clinically challenging, and these patients are often diagnosed at the late stages of their lung disease. Early detection and management of the disease is thus crucial to reducing mortality and morbidity in RA-ILD [26]. A diagnosis of ILD is determined by High-resolution computed tomography (HRCT) in combination with interdisciplinary teams (IDTs) discussion between radiologists, pulmonologists, pathologists and rheumatologists [27]. However, triaging which patients with should be referred for an HRCT is also challenging. Several retrospective studies have found ACPA positivity, RF positivity, history of smoking, male sex, older age and longer RA duration, and higher C-reactive protein to be associated with RA-ILD [25, 28, 29]. An expert proposal, based in Delphi methodology, provided an agreed-upon scoring system for risk stratification, with points for age ≥ 60 years, male sex, smoking history, disease activity, RF-positivity level and ACPA positivity level (**Table 1 and Figure 2**) [30]. A recent retrospective study by Kodury et al. largely supports the Delphi study but proposes a more simplified 4-item score for detecting patients with RA who are at high risk of ILD, where a value of ≥ 5 indicates RA-ILD with a sensitivity of 86% and specificity of 58% (**Table 2**) [31]. Further, it is generally recommended that RA with respiratory symptoms be evaluated for ILD, although this approach is not yet evidence-based [26, 32].

Any patient scoring ≥5 points will be considered eligible for screening Set of variables and proposed score for each of the variables for overall calculation	Score
Age ≥ 60 years	2[b]
Male sex	1[b]
History of smoking (active or ex-smoker)	
≤ 20 packs/year: 2 points	2[b]
>20 packs/year: 3 points	3[b]
Disease duration > 5 years	1[c]
Persistent moderate-high disease activity: DAS28-VSG average > 3.2 from diagnosis of the disease in RA onset (time from diagnosis ≤ 12 months) or DAS28-VSG > 3.2 for a minimum of 6 months in established RA	1[c]
Serology (only the criterion with the highest weighting is counted towards the total score)	
RF positive > 3 times above the ULN	1[c]
ACPA positive ≤ 3 times above the ULN	2[c]
ACPA positive > 3 times the ULN	3[c]
Family history of ILD	1[c]

Table 1: Scoring system proposed by Narváez et al. 2023 (Permission for reprint in thesis granted by the publisher and presented in appendix).

The Variables used for RA-ILD probability weightage according to multivariate model			
	0	1	2
Age at RA onset	< 40	40–70	> 70
Smoking	Never	Ex-smoker or current	
RF titre	Negative	Weak positive	Positive
CCP titre	Negative	Weak positive	Positive
DAS 28	DAS 28 > 3.2		

Table 2: Table is adapted from Kodury et al. 2023 [31]. Proposing a 4-item scoring system, where a value of ≥ 5 indicates a high risk of RA-ILD. No changes were made. (Published under Open Access, which permits adaptation and distribution, visit CC BY 4.0 Deed | Attribution 4.0 International | Creative Commons, for more licence details).

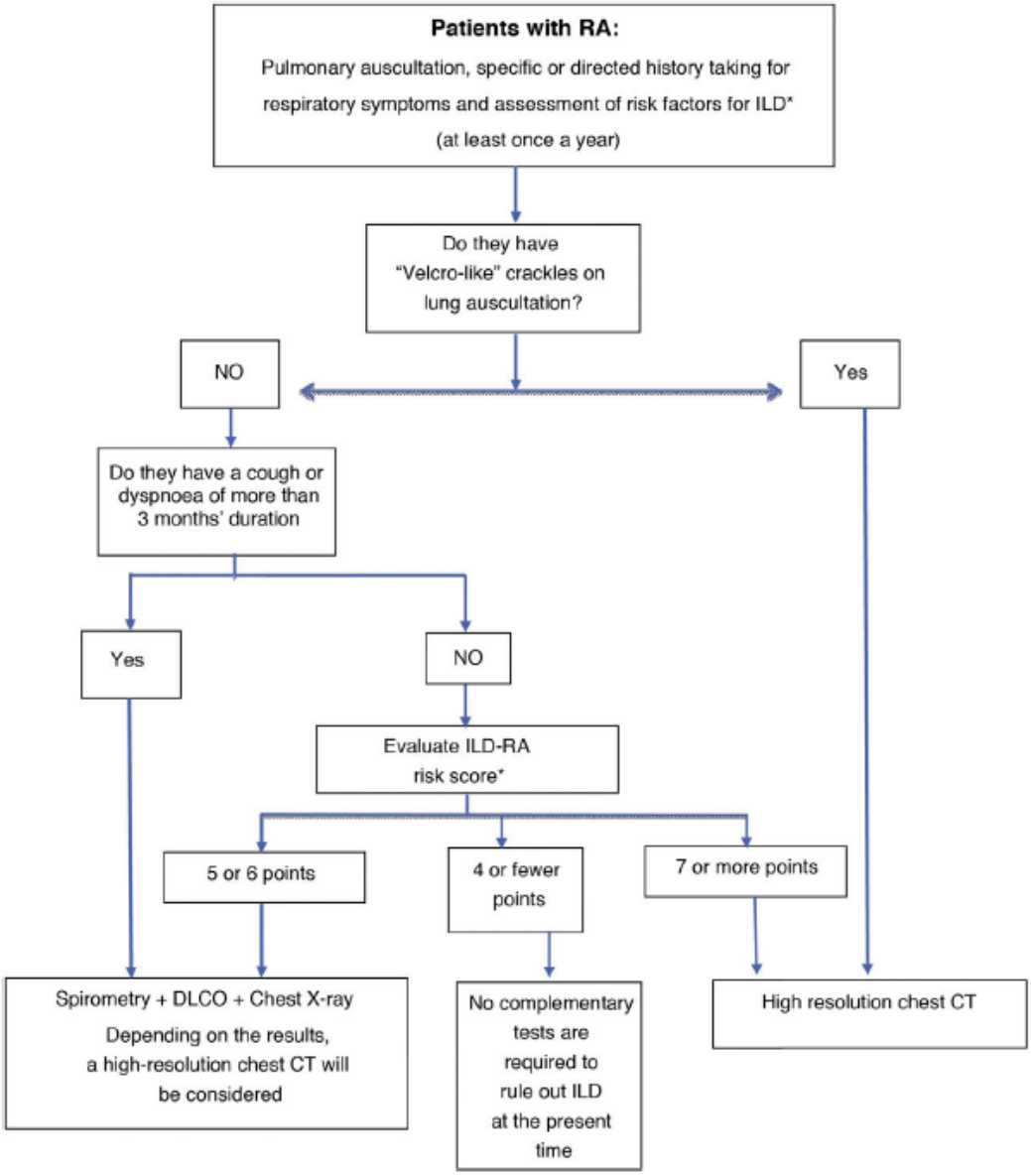


Figure 2: Proposed ILD screening algorithm for patients diagnosed with rheumatoid arthritis (RA). * See risk factors and their score in **Table 1**. If screening tests are negative, screening will be repeated once a year using spirometry + Diffusing capacity for carbon monoxide (DLCO). Narváez et al., 2023 (Permission for reprint in thesis granted by the publisher and presented in appendix).

Respiratory symptoms in RA

There are currently no established questionnaires or universally agreed-upon criteria for assessing respiratory symptoms in RA. The Medical Research Council's dyspnoea scale (MRC) is widely recognized as a simple yet effective tool for assessing the impact of breathlessness on everyday tasks [33, 34] and correlates with performance in walking tests [35]. An MRC grade of one to two is typically deemed as normal. Nevertheless, individuals with chronic obstructive pulmonary disease (COPD) who have an MRC grade of two have reduced exercise tolerance compared to healthy individuals with the same MRC grade [36].

Symptoms of respiratory diseases, such as ILD, are not limited to dyspnoea; another symptom may be a dry cough or rapid breathing (tachypnoea) [37]. Underlying ILD may cause a tendency to get pneumonia or lead to more severe cases of pneumonia that require hospitalisation. However, symptoms of ILD are similar to other lung diseases such as COPD and emphysema, which are three to five times more prevalent in RA [38, 39].

Thoracic ultrasound

Thoracic ultrasound (TUS) is a promising tool for detecting ILD, and has been suggested as a method of detecting ILD in RA [40]. In primarily case-control studies, the B-line artefact is found to be sensitive in detecting systemic sclerosis-associated ILD (SSc-ILD) as well as RA-ILD [41-43]. B lines are defined as hyper-echoic vertical reverberation artefacts that originate from the pleural line and extend uninterrupted to the edge of the screen without fading (previously termed "comet tails") [44]. The exact physiology behind the B-line artefact is still unclear [45]. The fully aerated lung cannot be visualised with TUS. However, a fully de-aerated lung, as is seen with e.g. atelectasis, is visible on TUS. The theory is that partial de-aeration of the lung is the cause of B-lines, as is

seen, e.g. before the formation of a pneumonic consolidation [45, 46]. Pulmonary oedema and interstitial lung disease also cause de-aeration of the lungs, which fits with the more established TUS term, interstitial syndrome (IS). IS is defined as ≥ 3 B lines per intercostal space in two zones in each hemithorax (four zones in total) [44].

When based on the interstitial syndrome definition, B lines have a high predictive value when used in the emergency ward to differentiate pulmonary oedema from other causes of acute dyspnoea, such as COPD [47]. However, potential causes of B lines are numerous and utilising other ultrasonographic findings may increase specificity. The geolocation of B-line distribution in pulmonary oedema follows gravitation and has a caudal/dorsal gradient, depending on the postural position [48]. Further, the pleural line appearance may help determine the aetiology of B lines, with a thickened and fragmented pleural line in ILD, acute lung injury, and acute respiratory distress syndrome, but not in pulmonary oedema [48, 49].

TUS studies on detecting ILD in SSc and RA have found that ≥ 10 B lines is a promising cut-off for ILD in selected populations [42, 50-55]. Other studies have found that a thickened and fragmented visceral pleura was sensitive in sub-pleural fibrosis on HRCT in SSc-ILD and idiopathic pulmonary fibrosis [56, 57]. However, the applicability of TUS to identify clinically relevant and not yet diagnosed RA-ILD is limited and has not yet been validated in a clinical setting [42, 58-60].

Rheumatoid arthritis and treatment

Treatment options and disease remission rates have increased in RA since the first disease-modifying antirheumatic drugs (DMARDs) were introduced between 1970 and 1980. Initially, RA was treated with DMARDs, such as gold salts, which were shown to reduce the progression of joint destruction. However, the treatment had low efficacy and

was discontinued when more promising and currently used synthetic DMARDs were introduced. In the 1980s, many pro-inflammatory mediators and pathways were recognised [61, 62], and in the late 1990s, the first targeted biological therapies were validated as treatment options for RA, as well as other chronic inflammatory diseases [63, 64]. Since then, new drugs and treatment options have increasingly emerged [64-66].

International guidelines all recommend methotrexate as the first choice for treating RA, with differences regarding whether bridging with prednisolone should be included [67-69]. In primary non-responders, biological treatment is recommended, the choice of which is based on the individual patient's comorbidities and an estimate of the cost-effectiveness of the biological agent. However, about one-third of patients with RA are non-responders to biologic therapy [70]. Despite the numerous treatment options, methods have yet to be established for selecting individual treatment, i.e. personalised medicine [71]. The current trial-and-error approach may increase the time to disease remission and exposure to drug side effects.

MFAP4

Microfibrillar-associated protein 4 (MFAP4) is an extracellular matrix (ECM) protein associated with lung elastogenesis [72]. MFAP4 signals have been predominantly detected in elevated levels within affected organs, such as in the lung tissue of mice treated with bleomycin (a model for pulmonary fibrosis) [73]. However, heightened levels of circulating MFAP4 in bleomycin-treated mice have not been found [74] .

Increased circulating MFAP4 levels are strongly associated with liver cirrhosis as well as alcoholic liver disease, with MFAP4 levels correlating to the level of cirrhosis [75, 76]. Molleken et al. suggested that heightened ECM turnover might be the driving factor behind

the increased presence of MFAP4 in liver disease. Moreover, low heritability and basal variation among healthy individuals indicate that elevated levels of MFAP4 could serve as a marker for disease-induced processes [77]. MFAP4 is present in significant amounts in the heart, small intestine, and lungs. Specifically, within the lungs, MFAP4 was identified in the pulmonary arterioles and interalveolar walls [78]. Furthermore, studies indicate a correlation between elevated MFAP4 levels and the worsening of COPD, along with experimental asthma [79, 80]. However, later research could not validate the correlation between MFAP4 and COPD and concluded that the increased MFAP4 levels were due to underlying cardiovascular disease [81]. Increased circulating MFAP4 has been associated with ischemic cardiomyopathy and immune cell activation and migration [82], as well as increased mortality and a decreased risk of vascular occlusion in peripheral artery disease [83]. This could indicate MFAP4 as a potential biomarker of active and possibly reversible inflammation. MFAP4 was increased in early and manifest RA, without association with active synovitis or disease activity and with an inverse correlation to ACPA positivity, where underlying cardiovascular diseases were possible causes of elevated MFAP4 in RA [84].

Diagnostic test reporting guidelines

EQUATOR network (Enhancing the Quality and Transparency of Health Research) has published guidelines on designing and reporting studies based on the study type [85]. According to the EQUATOR network, reporting diagnostic test accuracy and prognostic studies should follow the Standards for Reporting of Diagnostic Accuracy (STARD) guidelines and checklist [86, 87]. As with all clinical studies, there is a risk of bias.

This could be due to methodological issues, such as the appropriate selection of participants, blinding procedures, and appropriate reference standards [88]. The STARD guidelines aim to reduce potential bias by applying an appropriate study reporting framework [87]. The STARD checklist can be used to provide a quick overview of study design and content, whereas the Modified Quality Assessment of Diagnostic Accuracy Studies (QUADAS2) and the Critical Appraisal Skills Programme (CASP) checklists are for guiding critical appraisal of diagnostic test accuracy studies [86, 89, 90]. BMJ Best Practice has developed an evaluation tool utilizing established diagnostic test accuracy checklists and guidelines to provide a more streamlined approach for reviewers and readers assessing diagnostic accuracy studies [91]. Some important aspects of designing the study are ensuring proper blinding procedures and avoiding case-control study designs so that the setting resembles the clinical practice situation. Further, reporting other diseases in the cohort that may affect the index test is relevant and should be considered in the discussion.

Knowledge gaps

Screening for RA-ILD

We searched PubMed before initiating this study and again when writing the manuscript (up to 1 December 2023) for research articles containing the terms “(Rheumatoid Arthritis)” AND “(Respiratory Symptoms)” as well as “(Rheumatoid Arthritis)” AND “(Thoracic Ultrasound or Lung ultrasound or Lung ultrasonography)”, without any date or language restrictions. We also reviewed the reference lists of potentially eligible articles and articles citing potentially relevant articles. Our research did not identify any previous studies using respiratory symptoms in rheumatoid arthritis as inclusion criteria, nor on using thoracic ultrasound to detect interstitial lung disease, where participants were ILD- and HRCT-naïve at inclusion.

Early detection methods are currently being debated, and the newest recommendations include screening for respiratory symptoms, although this is not yet evidence-based [32]. Also under debate is how we should identify RA-ILD at pre-symptomatic stages using a risk stratification approach [31]. Although screening for respiratory symptoms, or ILD in RA, is not yet evidence-based, it has been deemed as highly relevant based on consensus.

Patient-reported outcome measures and biomarkers

We lack an evidence-based method for screening for respiratory symptoms. Part of the solution may lie in already established questionnaires that have been developed for monitoring lung disease, as they may assist in detecting early signs of ILD in RA. Further, no validated biomarker for detecting RA-ILD has been discovered, and MFAP4 seems to reflect inflammatory and fibrotic activity in the liver and possibly even

asthma. This may prove promising in detecting lung disease in RA and predicting treatment response in CIDs.

Contributions of this study

As treatment options for RA-ILD are increasing, methods of screening for ILD in RA, as well as detecting ILD at earlier stages, are warranted [92, 93]. Our approach is a potential solution to this problem. We have tested a precise method of screening for respiratory symptoms, as well as a pre-specified TUS positive definition, for the detection of ILD in RA. Further, circulating MFAP4 has potential as a biomarker of treatment response in patients with chronic inflammatory disease initiating biologic therapy.

Hypotheses

Papers 1 and 2: Thoracic ultrasound can detect interstitial lung disease in rheumatoid arthritis with respiratory symptoms.

Paper 3: MRC scale and/or MFAP4 can detect respiratory impairment in RA.

Paper 4: High MFAP4 levels predict positive treatment response in patients with CIDs who are about to initiate treatment with biologics.

Methods and main results

Papers 1 and 2

(Study 1: Published manuscript in appendix, Study 2: Full statistical analysis plan (SAP) and manuscript in appendix)

Methods

Paper 1: The aim of paper 1 was to prepare for a diagnostic test accuracy study, following the STARD 2015 guidelines [86, 87], establish consensus within the study group, and assign roles.

Paper 2: The aim of paper 2 was to test the diagnostic accuracy of thoracic ultrasound (TUS) for detecting interstitial lung disease (ILD) in patients with rheumatoid arthritis (RA) with respiratory symptoms who were not already diagnosed with ILD.

Patients with RA, visiting their local outpatient clinic in the Region of Southern Denmark were systematically screened for predefined respiratory symptoms. Patients had to have one of the following symptoms: tendency for dyspnoea (more than others of the same age) > 2 months' duration; cough > 2 months' duration; residual clinical pneumonia (>1 per year); history of hospitalisation due to severe pneumonia; or abnormal chest X-ray. Patients had to be HRCT and ILD naïve before entering the study.

TUS was performed right after participants provided informed consent and before HRCT. TUS clips were evaluated by an expert in TUS who was blinded to HRCT and clinical information. TUS was registered as positive if there were ≥ 10 B lines and/or bilaterally thickened and fragmented pleura. HRCT had to be < 30 days after TUS. An ILD-specialised thorax radiologist, blinded to TUS clips and TUS diagnosis, evaluated all HRCT scans, and IDT was used to diagnose ILD.

To test for variance of variables in the distribution of the patients who were ILD positive and negative, we calculated the Standardised difference (StdD) (also known as Cohen's d) [94]: a StdD of 0.5 units or more indicates a potentially significant imbalance and difference between exposure groups and indicates a potential data-driven confounder. Values from 0.5 indicate a moderate effect size. In contrast, values of 0.8 or more indicate a large effect size of the variables for patient distribution. In randomized trials, StdD can be used to assess for potential confounding variables. For the primary outcomes, we calculated the diagnostic odds ratio (DOR), the sensitivity, specificity, and positive and negative predictive values, all with 95% confidence intervals.

Main results

A total of 77 patients had an HRCT < 30 days after TUS. Twenty-three (30%) received a diagnosis of ILD (**Table 1**). The TUS-positive and TUS-negative categories had the larges effect size on having ILD or not, TUS positive and negative with StdD of 0.8., followed by age (StdD of 0.7) and mean DLCO (StdD of 0.5). All other variables were evenly distributed (StdD < 0.5).

TUS had a sensitivity of 82.6% (95% CI: 61.2 to 95.0) and a specificity of 51.9 (95% CI: 37.8 to 65.7). TUS identified 19 (83%) of the ILD cases correctly with a diagnostic OR of 5.12 (95% CI of 1.5 to 17.0). The positive predictive value was 42.2% (95% CI 27.7 to 57.8%), and the negative predictive value of TUS testing was 87.5% (95% CI 71.0% to 96.5%). TUS reliability (kappa) for verified ILD was 0.27 (95% CI 0.07 to 0.47), i.e. TUS positives with ILD and TUS negatives without ILD.

Of the 45 TUS positive, 19 (42%) had ILD and 10 (22%) had emphysema on HRCT, with interstitial lung abnormalities (ILA),

subpleural and basal bullae, bronchiolitis with surrounding inflammation, and/or oedema of the visceral pleura. Other lung diseases also presented as TUS positive **(Table 2).** Patients who were referred to further diagnostics (as no diagnosis had been made for a specific lung disease i.e., suspected asthma or isolated ILA) were categorised as having lung disease without a specific diagnosis. The patients with ILD who did not have a positive TUS had a BMI of 33 or higher.

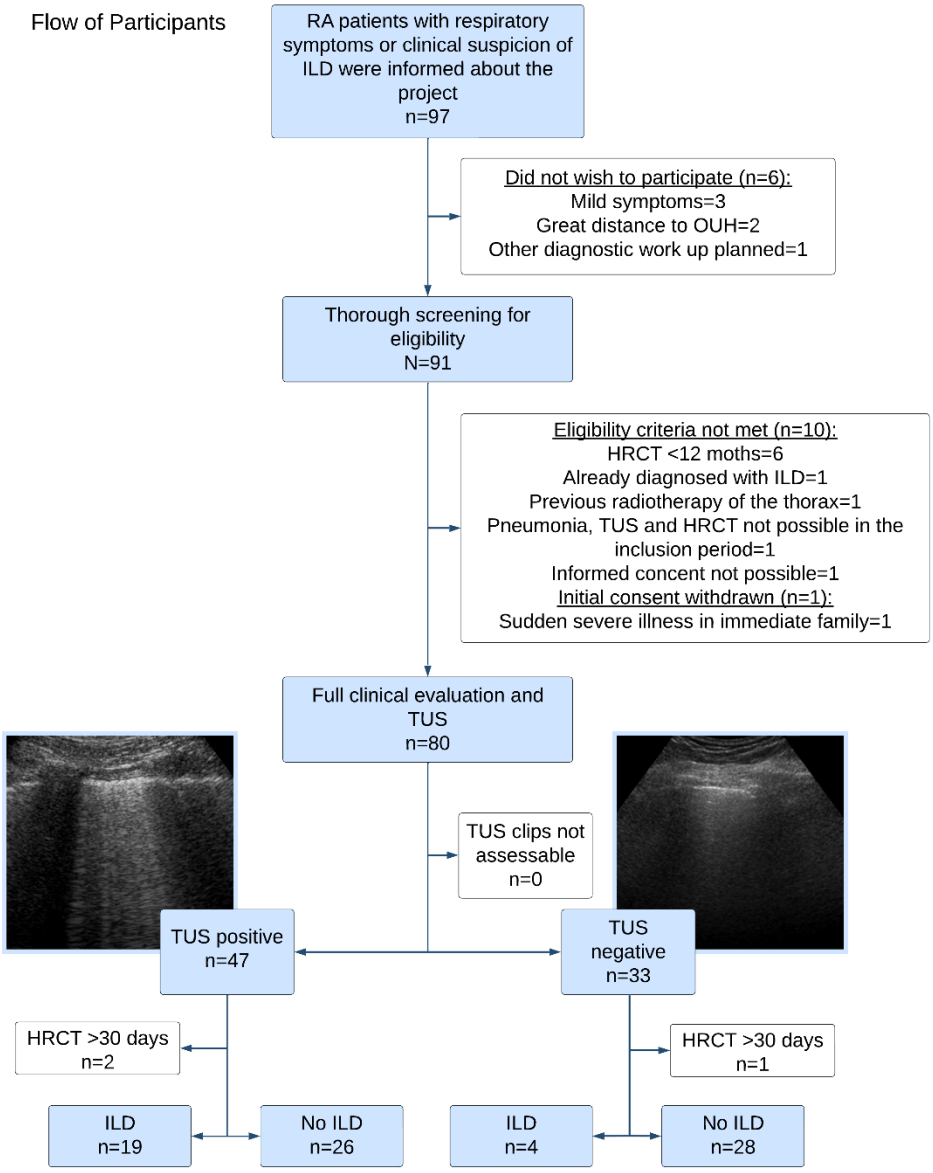


Figure 1 Legend. Participant flow through the project. Abbreviations: RA=Rheumatoid Arthritis, ILD=Interstitial Lung Disease, n=number, OUH=Odense University Hospital, TUS=Thoracic Ultrasound, HRCT=High Resolution CT.

Table 1. Participant Characteristics. Values are no. (%) unless otherwise stated.

	Total n=77	ILD n=23 (30%)	No ILD n=54 (70%)	StdD*
TUS positive	45 (58%)	19 (83%)	26 (48%)	0.8*
TUS negative	32 (42%)	4 (17%)	28 (52%)	-0.8*
Age, years, Mean (SD)	64.6 (11.2)	69.7 (7.6)	62.4 (11.8)	0.7*
Female	52 (68%)	13 (57%)	39 (72%)	-0.3
ACPA positive	46 (66%)	13 (57%)	33 (61%)	0.1
IgM RF positive	49 (72%)	16 (70%)	33 (61%)	0.4
Methotrexat	58 (75%)	18 (78%)	40 (74%)	0.1
Prednisolone	9 (12%)	3 (13%)	6 (11%)	0.1
Biologics	24 (31%)	8 (35%)	16 (30%)	0.1
Pack-years, mean (SD)	16.0 (16.7)	19.3 (19.1)	14.6 (15.7)	0.3
Never smoked	28 (37%)	9 (39%)	19 (35%)	0.1
DAS28CRP, Mean (SD)	2.8 (1.1)	2.9 (1.1)	2.8 (1.1)	0.2
FEV1 % pred., Mean (SD)	89.3 (24.0)	88.2 (24.5)	89.8 (24.1)	-0.1
FVC % pred., Mean (SD)	98.3 (18.9)	93.4 (21.0)	100.4 (17.7)	-0.4
FEV1/FVC %, Mean (SD)	73.6 (15.9)	77.3 (16.1)	72.0 (15.8)	0.3
TLC % pred., Mean (SD)	89.8 (17.3)	85.0 (16.4)	91.8 (17.5)	-0.4
DLCO % pred., Mean (SD)	71.9 (20.1)	64.6 (17.2)	74.8 (20.7)	-0.5*
#6MWD, Mean (SD)	439 (144)	395 (155)	455 (138)	-0.4
#6MWD desat. (Δ %), Median (IQR)	2.0 (0.0; 6.0)	2.0 (1.0; 4.0)	3.0 (0.0; 8.0)	-0.2

Table 1 Legend: ILD=Interstitial lung disease, TUS=Thoracic ultrasound, StdD=Standardised difference, SD=Standard deviation, IQR=Interquartile range, ACPA=Anti-citrullinated protein antibody, IgM RF=IgM Rheumatoid factor, DAS28CRP=Disease Activity Score-28 for Rheumatoid Arthritis with CRP,

FEV1=Forced expiratory volume in 1 second, FVC=Forced vital capacity, TLC=Total lung capacity, DLCO=Diffusing capacity for carbon monoxide, 6MWD=6-min walk distance, #= Data not complete.

Table 2. Pulmonary diagnosis. Values are n (%)

	Total n=77	**TUS positive n=45 (58%)**	**TUS negative n=32 (42%)**	**StdD***
Respiratory disease	52 (68%)	39 (87%)	13 (41%)	1,1*
ILD	23 (30%)	19 (42%)	4 (13%)	0,7*
Airway disease	19 (25%)	15 (33%)	4 (13%)	0,5*
Pleural disease	3 (4%)	2 (4%)	1 (3%)	0,1
Bronchiolitis	3 (4%)	2 (4%)	1 (3%)	0,1
COPD	12 (16%)	9 (20%)	3 (9%)	0,3
Emphysema	13 (17%)	10 (22%)	3 (9%)	0,4
Bronchiectasis	3 (4%)	2 (4%)	1 (3%)	0,1
Cancer	3 (4%)	3 (7%)	0 (0%)	0,4
Lung disease, undergoing diagnostics	6 (8%)	1 (2%)	5 (16%)	-0,5*

Table 2 Legend: n=number, TUS=thoracic ultrasound, StdD=Standardised difference, ILD=Interstitial lung disease, COPD=Chronic obstructive pulmonary disease.

Paper 3

(Full SAP and manuscript in appendix)

Methods

The aim was to test whether the Medical Research Council (MRC) dyspnoea scale or the biomarker MFAP4 could detect respiratory impairment in patients with newly diagnosed, treatment-naïve rheumatoid arthritis.

This study was a secondary analysis of an already established cohort of 150 newly diagnosed and treatment-naïve patients with RA. Inclusion took place in Silkeborg Regional Hospital, Denmark, from 2011 to 2019. We used an adapted approach to the STARD 2015 guidelines for this study. The patients were treatment naïve at the time of blood sample collection and underwent pulmonary function tests within 6 months after inclusion. Patients with pulmonary function tests and available blood samples at baseline were included in the analysis population. Respiratory impairment was defined as diffusion capacity of the lungs for carbon monoxide (DLCO) < 80% predicted or Forced expiratory volume in 1 second over Forced vital capacity (FEV1/FVC) < 70%.

Within this cohort, 97 patients underwent echocardiography, including a determination of left ventricular ejection fraction (LVEF).

We then used receiver operating characteristic (ROC) curves, followed by Youden's Index, to identify the optimal cut-off point of MRC and MFAP4 for detecting respiratory impairment. We also performed logistic regression analysis for MFAP4 to detect respiratory impairment when adjusting for age, sex and smoking status.

Main results

Using only the cut-off point, the crude analysis showed a low correlation between MRC (≥ 2) and respiratory impairment (sensitivity of 39% and specificity of 76). The crude analysis of MFAP4 (≥ 29.0 U/ml) resulted in a sensitivity of 63% and a specificity of 57%. The logistic regression analysis for MFAP4 (≥ 29.0 U/ml) detecting respiratory impairment, adjusted for age, sex and smoking status, resulted in an OR of 3.01 (95% CI: 1.27 to 7.16).

Ninety-seven patients had available LVEF, and patients with respiratory impairment exhibited a trend toward lower LVEF. However, clinically reduced LVEF (< 50%) showed no significant difference between the groups. Additionally, there was no evident correlation between MRC scores, MFAP4 levels, or reduced LVEF.

Figure 1

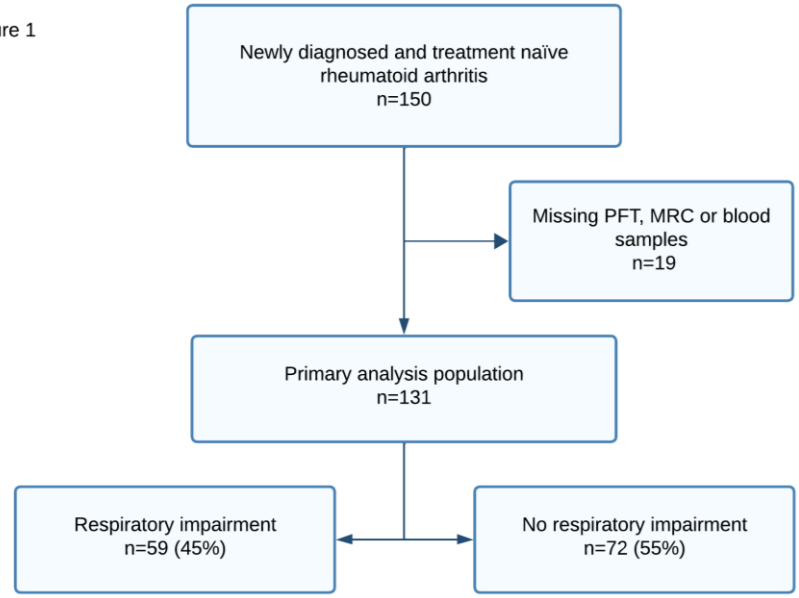


Figure 1 Legend: n=number, PFT=Pulmonary function test, MRC= Medical Research Council dyspnoea scale

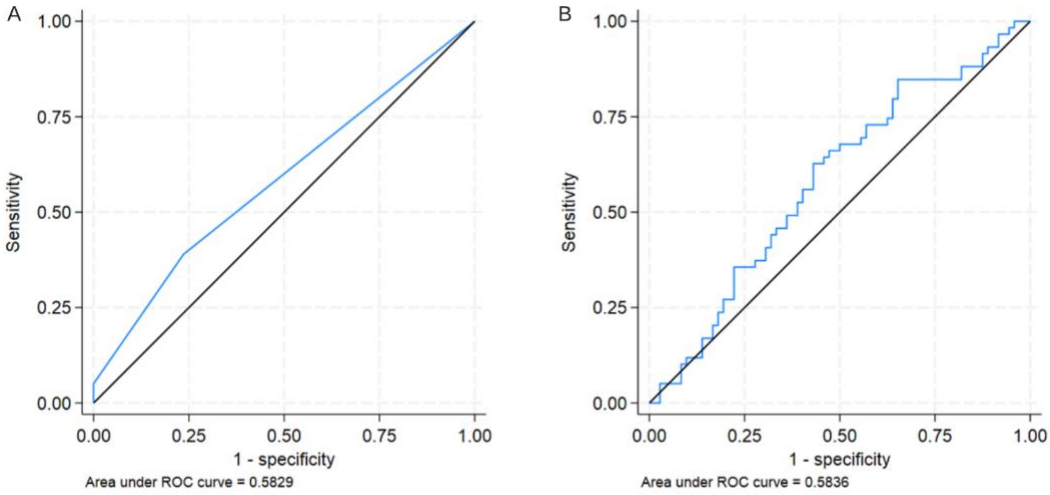
Table 1: Patient characteristics. Values are means (SDs) unless otherwise stated.

	Total (N=131)	Respiratory impairment (n=59)	No respiratory impairment (n=72)	StdD
MRC, median (IQR):	1.0 (1.0; 2.0)	1.0 (1.0; 2.0)	1.0 (1.0; 1.0)	0.4
MFAP4, median (IQR)	30.1 (22.3; 40.8)	32.1 (24.4; 42.1)	27.5 (20.8; 36.6)	0.1
Age, years (SD)	57.7 (10.9)	60.2 (9.8)	55.6 (11.4)	0.4
Female, n (%)	80 (61%)	32 (54%)	48 (67%)	-0.3
BMI (kg/m2) (SD)	26.4 (5.1)	25.9 (4.9)	26.7 (5.3)	-0.2
ACPA, median (IQR)	86.0 (1.0; 340.0)	35.0 (1.0; 250.0)	141.5 (1.0; 340.0)	-0.4
IGM-RF, median (IQR)	14.0 (2.0; 74.0)	11.0 (1.0; 50.0)	18.5 (3.5; 76.0)	-0.1
Packyears, median (IQR)	8.0 (0.0; 25.0)	20.0 (3.0; 36.0)	0.0 (0.0; 14.5)	0.6*
Current smoking status				
Never smoker	55 (42%)	16 (27%)	39 (54%)	-0.6*
Former smoker	42 (32%)	20 (34%)	22 (31%)	0.1
Current smoker	34 (26%)	23 (39%)	11 (15%)	0.6*
CRP, median (IQR)	4.5 (1.6; 12.2)	4.8 (1.6; 10.7)	4.4 (1.6; 13.6)	0.0
DAS28CRP	4.8 (4.1; 5.3)	4.8 (4.2; 5.3)	4.7 (4.1; 5.3)	0.0
FEV1 (% predicted)	99.8 (18.2)	91.5 (16.2)	106.7 (17.0)	N/A
FVC (% predicted)	106.6 (18.3)	102.8 (15.4)	109.7 (20.0)	N/A
FEV1/FVC (%)	77.6 (9.1)	73.1 (10.0)	81.3 (6.1)	N/A
TLC (% predicted)	104.9 (15.3)	105.6 (16.4)	104.4 (14.4)	0.1
DLCO (% predicted)	84.3 (15.8)	72.5 (10.5)	94.0 (12.6)	N/A
**6MWD, meters	616.0 (169.0)	604.4 (180.0)	625.4 (160.2)	-0.1
**6MWD, Δ-Desat.	0.0 (0.0; 1.0)	0.0 (0.0; 2.0)	0.0 (0.0; 1.0)	0.0
**LVEF, % (SD)	56.0 (6.7)	54.4 (6.7)	57.4 (6.4)	-0.5*
**LVEF <50%, n (%)	19 (20%)	12 (26%)	7 (14%)	0.3

Figure 1 Legend: StdD=Standardised difference, SD=Standard deviation, IQR=Interquartile range, BMI=Body mass index, ACPA=Anti-citrullinated protein antibody, IgM RF=IgM Rheumatoid factor, CRP=C-Reactive protein, DAS28CRP=Disease Activity Score-28 for

Rheumatoid Arthritis with CRP, FEV1=Forced expiratory volume in 1 second, FVC= Forced vital capacity, TLC=Total lung capacity, DLCO=Diffusing capacity for carbon monoxide, 6MWD=6-min walk distance, Desat.=Desaturation, LVEF=Left ventricular ejection fraction. *StdD=Moderate effect size, *P-value <0.05, **= Data not complete. **LVEF data on 97 patients

Figure 2. A: ROC curve for MRC detecting lung impairment. **B:** ROC curve for MFAP4 detecting respiratory impairment



Paper 4

(Full SAP and manuscript in appendix)

Methods

This study aimed to test MFAP4 as a biomarker of treatment response in patients with chronic inflammatory diseases (CIDs) initiating or changing to other biologic treatment.

This study is a secondary analysis of the prospective multi-centre cohort (BELIEVE) study of 211 patients about to initiate treatment biologic treatment or change to another biologic agent. The included patients had either rheumatoid arthritis (RA), psoriatic arthritis (PsA), psoriasis (PSO), axial spondyloarthritis (AxSpA), Crohn's disease (CD), or ulcerative colitis (UC). Patients were given a full clinical evaluation, and blood samples were drawn at inclusion; this was repeated 14 to 16 weeks after treatment was initiated to assess whether patients responded to treatment. Patients with available blood samples at baseline were included in the analysis population, i.e. intention to treat (ITT) population.

Serum microfibrillar-associated protein 4 (MFAP4) levels were determined using the AlphaLISA technique [78], and patients were divided into the upper tertile of MFAP4 (High MFAP4) and medium and lower tertile (Other MFAP4). The primary outcome was the proportion of patients with clinical response to biologic therapy after 14–16 weeks.

The criteria for a positive treatment response varied among the different CIDs [95]: RA: clinical response, defined as at least a 20% improvement according to the criteria of the American College of Rheumatology (ACR20)[96]; AxSpa: clinical response, defined as at least a 20% improvement according to the Assessment of Spondyloarthritis International Society (ASAS20) [97]; PsA: clinical response, defined as at least a 20% improvement according to the criteria of ACR20;

PSO: clinical response, defined as at least a 75% improvement in Psoriasis Area and Severity Index (PASI 75). CD: clinical remission, defined as Harvey-Bradshaw Index of 4 or less; UC: clinical remission, defined as Mayo Clinic Score of 2 or less (with no individual sub-score of >1).

The differences in baseline covariates were calculated to compare the distribution of the High MFAP4 and Other MFAP4, using Cohen's d [94]: a standardised difference above 0.5 units indicates a potentially significant imbalance and difference between exposure groups. In cases of missing outcomes, patients were registered as non-responders. Differences in the proportions of responders to biological treatment between groups were analysed using two different logistic regression analysis models: (i) the simple "CID adjusted model" was only adjusted for CID, whereas the adjusted model (ii) was adjusted for CID, sex, age, smoking status (ordinal scale: never, former, occasional, and current), as well as BMI category (ordinal scale: underweight, normal, overweight, and obese). The adjusted variables were a priori considered potential confounding variables (See SAP).

Main results

Of the 211 participants in the ITT population, 110 (52%) had a positive clinical response to biologics. In the High MFAP4 group, 41 (59%) had a clinical response, while 69 (49%) in the Other MFAP4 group had a clinical response (**Table 1**). There was no difference between the groups' treatment responses in the primary CID-adjusted analysis (OR 1.39, 95% CI: 0.77 to 2.53). The adjusted model had an OR of 2.28 (95% CI: 1.07 to 4.85). Among the subgroups, most CIDs favoured the High MFAP4 group in predicting treatment response. As an inconsistency, CD favoured the Other MFAP4 group.

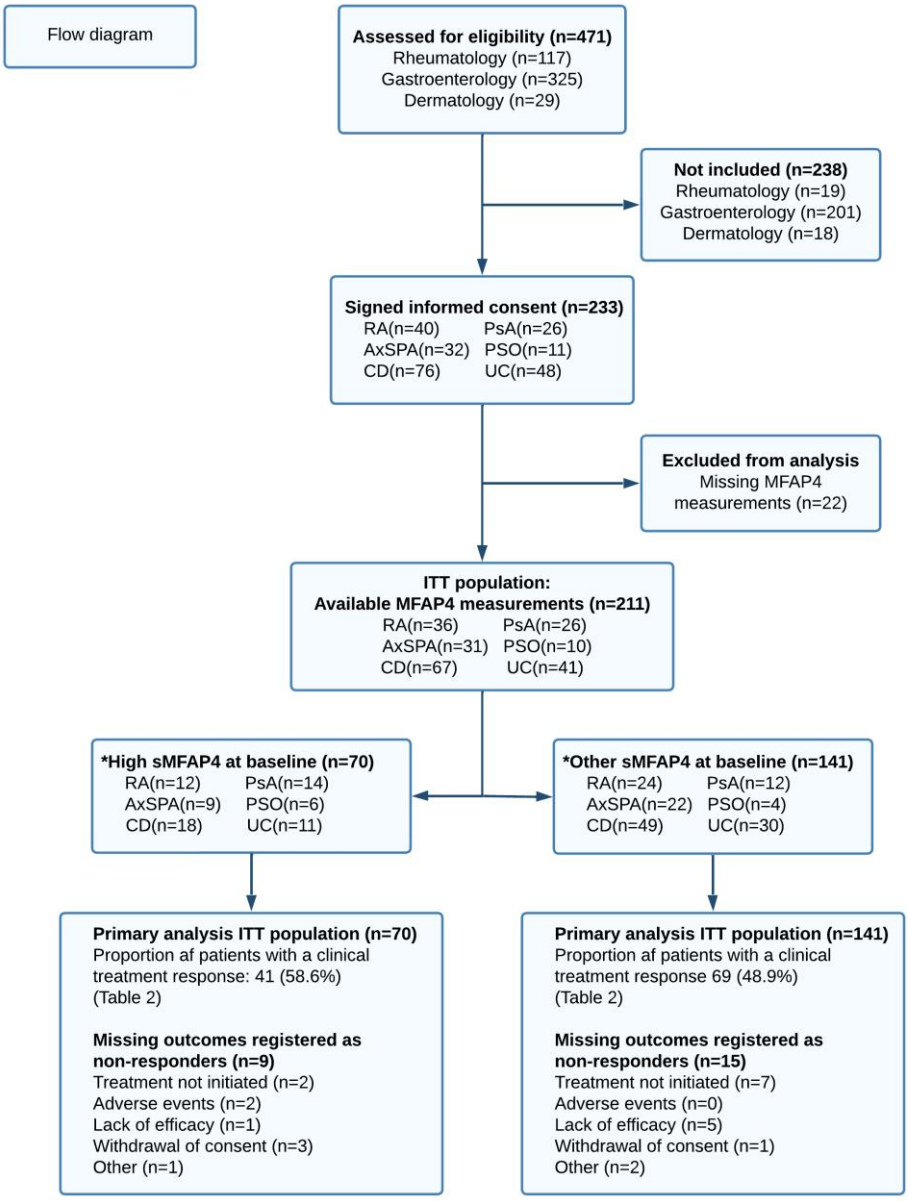


Figure 1 legend: RA; Rheumatoid arthritis, PsA; Psoriatic arthritis, AxSPA; Axial Spondyloarthritis, PSO; Psoriasis, CD; Crohn's disease, UC; Ulcerative Colitis, sMFAP4; serum Microfibrillar-associated protein 4, ITT; Intention to treat. *MFAP4 levels are divided into tertiles; "High sMFAP4" is the upper tertile and "Other sMFAP4" is the medium and lower tertile.

Table 1: Baseline Characteristics in the ITT population				
Characteristic	High MFAP4 n=70	Other MFAP4 n=141	StdD*	P-value
Age (years), mean (SD)	50.5 (14.1)	40.4 (14.0)	0.7*	<0.001*
Female, n (%)	42 (64%)	78 (57%)	0.1	0.446
BMI (kg/m^2), mean (SD)	28.1 (6.9)	26.8 (5.6)	0.2	0.175
Smoking status, n (%):				0.584
Non-smoker	28 (47%)	53 (41%)	0.1	
Former smoker	21 (36%)	48 (38%)	0.0	
Occasionally	2 (3%)	2 (2%)	0.1	
Daily	8 (14%)	25 (20%)	-0.2	
CID diagnosis, n (%):				0.072
Rheumatoid arthritis	12 (17%)	24 (17%)	0.0	
Psoriatic arthritis	14 (20%)	12 (9%)	0.3	
Axial spondylarthritis	9 (13%)	22 (16%)	-0.1	
Psoriasis	6 (9%)	4 (3%)	0.2	
Crohn's disease	18 (26%)	49 (35%)	-0.2	
Ulcerative colitis	11 (16%)	30 (21%)	-0.1	
Disease duration (years)	7.0 (2.5; 13.0)	4.0 (1.0; 10.0)	0.4	0.013*
Naïve to biological treatment, n (%):	54 (78%)	112 (81%)	-0.1	0.712
Medication at inclusion, n (%)				
None	4 (6%)	13 (9%)	-0.1	0.435
NSAID, daily use	8 (14%)	13 (11%)	0.1	0.618
Corticosteroids	22 (31%)	47 (33%)	-0.0	0.876
Immunomodulators	29 (41%)	46 (33%)	0.2	0.224
CRP, mg/L	3.6 (2.1; 14.0)	3.9 (1.6; 12.0)	-0.2	0.668
Exploratory outcome measure:				
MFAP4 (U/mL)	40.9 (34.7; 46.5)	23.1 (17.1; 27.2)	N/A	N/A

Table 1 legend: Numbers are median (IQR), unless stated otherwise. StdD=Standardised difference (Cohen's d), *=Moderate effect size, **=Large effect size. P-value* <0.05. SD=Standard deviation, n=number, NSAID= non-steroidal anti-inflammatory drugs, Immunomodulators=Methotrexate, Azathioprine, or 6-mercaptopurine, CRP=C-Reactive protein

Figure 2: Forest plot; Effect of MFAP4 profile on treatment response (CID adjusted).

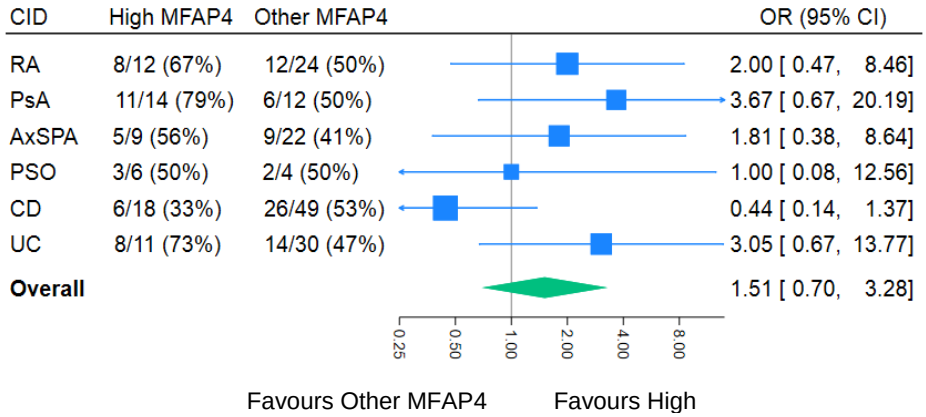


Figure 2 legend: CID=Chronic Inflammatory Disease, OR=Odds ratio, CI=Confidence interval. Values are presented as responder/all and %.

Table 2: Differences in primary and secondary outcome measures comparing High and Other MFAP4 groups. Values are n (%), unless otherwise stated.

			CID adjusted[1]		Adjusted[2]	
Outcome	High MFAP4 n=70	Other MFAP4 n=141	OR (95%CI)	P-value	OR (95%CI)	P-value
Primary outcome						
Positive treatment response	41 (59%)	69 (49%)	1.39 (0.77 to 2.53)	0.274	2.28 (1.07 to 4.85)	0.033
Sub-components						
RA, ACR20	8 (67%)	12 (50%)	2.00 (0.47 to 8.46)		4.83 (0.27 to 85.75)	
PsA, ACR20	11 (79%)	6 (50%)	3.67 (0.67 to 20.19)		6.07 (0.36 to 101.63)	
AxSPA, ASAS20	5 (56%)	9 (41%)	1.81 (0.38 to 8.64)		16.61 (0.55 to 501.47)	
PSO, PASI75	3 (50%)	2 (50%)	1.00 (0.08 to 12.56)		N/A	
CD, HBI ≤ 4	6 (33%)	26 (53%)	0.44 (0.14 to 1.37)		0.45 (0.10 to 2.09)	
UC, Mayo ≤ 2	8 (73%)	14 (47%)	3.05 (0.67 to 13.77)		8.89 (0.86 to 91.57)	

Table 2 legend: CID=Chronic inflammatory disease, OR=Odds Radio, RA=Rheumatoid Arthritis, ACR20= American College of Rheumatology, atleast 20% improvement, PsA=Psoriatic Arthritis, AxSPA= axial spondyloarthritis, ASAS= Assessment of Spondyloarthritis International Society, at least 20% improvement, PSO=Psoriasis, PASI75= Psoriasis Area and Severity Index, least 75% improvement, CD=Chron's Disease, HBI= Harvey Bradshaw Index, UC=Ulcerative Colitis, Mayo= Mayo Clinic Score of 2 or less.

Ethics

All studies in this PhD thesis comply with the Declaration of Helsinki and were approved by the Regional Ethics Committee (Papers 1 and 2: S-20210154, Paper 3: S20140057, and Paper 4: S-20160124) and the Danish Data Protection Agency (Papers 1 and 2: 22/7044, Paper 3: 2007-58-0010, and Paper 4: 2008-58-035). All participants signed an informed consent form before entering the respective studies. Study participation did not influence the choice of treatment or diagnostic procedures for suspected illness.

Discussion

Papers 1 and 2

This prospective study is the first to evaluate respiratory diagnoses using pre-specified respiratory symptoms in RA as inclusion criteria. It is also the first to evaluate the diagnostic accuracy of predefined TUS-positive criteria for detecting ILD in a cohort of RA patients with respiratory symptoms. Given that RA is associated with many types of lung disease, correctly identifying RA-ILD is a challenge. HRCT, combined with IDT, is the gold standard for diagnosing ILD [27, 98-100]. However, access to HRCT and specialised thoracic radiologists and ILD-specialised respiratory physicians may be limited. Therefore, it is essential to select patients who would benefit from further evaluation with HRCT and to identify those who would not benefit from HRCT. Our screening method for respiratory symptoms proved applicable, as 30% of the cohort had ILD. TUS can identify which patients should be referred to an HRCT, as the pre-defined TUS-positive criteria detected both ILD and emphysema and had a high negative predictive value.

The strengths of this study are the pragmatic and clinical set-up, as well as broad inclusion criteria that reflect the RA population. We did not exclude patients diagnosed with COPD or other airway disease if HRCT was not used in the diagnostic workup. Further, all participants were assessed for ILD and other lung diseases, allowing an overview of pulmonary diagnoses in RA with respiratory diseases.

Other studies have been conducted on respiratory diagnosis in RA patients but without regard to respiratory symptoms. The study by Esposito et al. found that pre-clinical (asymptomatic) emphysema was present in 36% and ILD was present in 15% of RA patients [38]. A recent study by Santos-Moreno et al., published in January 2024, assessed the diagnostic accuracy of TUS in RA with respiratory

symptoms and/or crackles in auscultation (n=192) [101]. Patients with a diagnosis of COPD, a history of COVID-19 pneumonia, atelectasis, or moderate to severe pleural effusion were not included. Of these patients, 55% had respiratory symptoms, while the rest had crackles on auscultation, without symptoms. They found that 117 of the participants had ILD, while 75 did not. They reported a sensitivity of 98.3% and a specificity of 14.7% but did not report whether another lung disease affected their results, as our study did.

In our study, 87% of the participants who were TUS positive had a clinical lung disease. We found that other diseases mimicked ILD on TUS, resulting in the pre-defined TUS criteria not being specific for ILD [102-105]. This was somewhat expected, although we did not know to what extent. We did not expect emphysema to fulfil the TUS-positive criteria, as other studies have not found obstructive or cystic lung disease to appear on TUS [46, 106]. A study by Buda et al. described the presence of a B-line-like artefact (called Am lines) resembling broad B lines in patients with sub-pleural bullous emphysema on HRCT [107]. This could correspond to some of the findings in our study. The TUS-positive emphysema patients were mainly characterised by pulmonary emphysema with predominant basal and sub-pleural distributed bullae, bronchiolitis with peribronchiolar ground glass opacities, interstitial lung abnormalities, and/or oedema of the visceral pleura on their HRCT scans. These HRCT findings may explain our findings, as this can increase the density of the lungs (partially de-aerated lung) and affect the parietal pleura so that it appears thickened and irregular on TUS and presents as ILD on TUS. There were four ILD cases that were TUS negative, all of whom had a BMI ≥ 33. The thickness of adipose tissue, increasing the distance from the ultrasound probe to the targeted organ, and its capacity to absorb ultrasound waves present a challenge

in achieving detailed ultrasound imaging. This may account for the patients not meeting the TUS-positive criteria.

It is currently being discussed that RA-ILD should be detected before it becomes symptomatic, as the symptoms present at late stages of lung disease [32]. This could be proven accurate; however, patients with RA are not routinely screened for respiratory symptoms, and other early detection methods have not yet been validated in prospective settings [30, 40, 108]. In the meantime, we should consider the aspect of over-diagnosis, as it is estimated that about one-half to two-thirds of those with RA-ILD have a non-progressive ILD [3, 98]. Asymptomatic patients may not benefit from receiving a diagnosis of ILD and treatment for ILD, which is not without side effects. However, early symptomatic patients may.

Paper 3

This is the first study to evaluate the ability of MRC to detect respiratory impairment in treatment-naïve patients with RA.

The preliminary and basic analysis showed a higher prevalence of MRC dyspnoea scores ≥ 2 in patients with respiratory impairment. However, the sensitivity and specificity were low at 39 and 76, respectively. Although not statistically significant, patients with lung impairment tended to have elevated MFAP4 levels. Yet, when Youden's index-derived cut-off value (MFAP4 ≥29.0 U/mL) was used, sensitivity was 62.7, and specificity was 56.9, suggesting that MFAP4 might not be an optimal marker for detecting respiratory impairment in newly diagnosed RA, without considering potential confounding variables. When adjusting for the potential confounding factors of age, sex and smoking status, an MFAP4 level ≥ 29.0 U/mL showed an odds ratio of 3.01 for detecting respiratory impairment.

There are no validated questionnaires for detecting potential lung disease, and the study's primary aim did not involve assessing respiratory symptoms. However, participants completed the MRC questionnaire upon inclusion, which enabled us to capture their subjective experiences of dyspnoea. It is important to note that the MRC questionnaire is not specifically designed to identify respiratory diseases, and it lacks inquiry into other respiratory symptoms indicative of lung disease, such as cough or a susceptibility to pneumonia. However, the modest difference in MRC levels between patients with and without respiratory impairment suggests that the MRC dyspnoea score may have potential as a detection method. It was also noted, that the close mean values of the MRC scores between the groups, may be attributed to limited nuance in the questionnaire and the relatively low number of patients in this cohort reporting MRC scores above two.

As RA typically affects smaller joints, the inflamed areas may be limited, potentially causing a less pronounced increase in circulating MFAP4 compared to conditions like liver cirrhosis [109]. In our cohort, with few patients exhibiting clinical lung disease [6], we investigated signs of respiratory impairment based on pulmonary function tests (PFTs). The rationale for this decision was that some patients may have had subclinical lung disease [6]. We also have to keep in mind that there are no validated biomarkers for detecting lung disease. The pre-specified analysis did not reveal a significant increase in MFAP4 among those with respiratory impairment; this could be attributed to tissue remodelling without a substantial release of MFAP4 from the ECM in the lungs into the bloodstream, as MFAP4 is primarily upregulated in the affected tissue [110, 111]. However, it could also suggest that when MFAP4 levels are being assessed, factors such as age, sex, and smoking status need to be taken into consideration, as MFAP4 ≥29.0 U/mL in the adjusted model was associated with respiratory impairment.

Dyspnoea, reduced DLCO, and elevated MFAP4 levels could stem from underlying cardiovascular disease [83, 84]. The 97 patients with available LVEF tended to be in the respiratory impairment group. However, there was no evident correlation between MRC scores, MFAP4 levels, and LVEF < 50%, indicating that LVEF < 50% was not a data-driven confounder for classifying a patient as having respiratory impairment. The patients could have had other underlying cardiovascular diseases that affected MFAP4 levels and physical function, which we were not able to account for in this study [112].

Paper 4

Our primary analysis revealed an association between high levels of MFAP4 and treatment response in this cohort, but only when accounting for type of inflammatory disease (CID), age, sex, smoking status, and BMI. This supports the idea of MFAP4 as a potential biomarker for active inflammation influenced by biological treatments. When examining CID subgroups, we found that having high MFAP4 levels appears to predict a favourable response to biological therapy, although this did not seem to be the case for Crohn's disease, favouring the Other MFAP4 group for a positive treatment response. This observation suggests potential differences in inflammatory mechanisms between Crohn's disease and other CID conditions in our study population. However, we have to consider that a subset of patients with Crohn's disease had received biological treatments before the study, which could have impacted their circulating MFAP4 levels.

The findings of this study suggest that MFAP4 holds promise in predicting treatment response across various CIDs, showing a positive association with RA, psoriatic arthritis, ankylosing spondylitis, and ulcerative colitis, while indicating a potential negative correlation with treatment response in Crohn's disease. As such, MFAP4 has the potential to enhance personalized medicine by helping identify patients who are likely to benefit most from biological treatments, especially when considering factors such as CID, age, sex, smoking status, and BMI.

Notably, age emerged as the sole significant difference between the High MFAP4 and Other MFAP4 groups, aligning with findings from previous studies suggesting an increase in MFAP4 with age [77, 113].

Previous research has indicated that MFAP4 may serve as a marker for fibrotic liver disease and active inflammation in the

lungs and blood vessels, as well as skin diseases [114]. In our subgroup analysis, high MFAP4 levels appeared to be associated with Psoriatic Arthritis (PsA), which affects both joints and skin. This could be attributed to increased MFAP4 release due to active inflammation in the skin and joints. However, the Psoriasis subgroup was too small to yield a clear signal in the analysis, and a significant portion (70%) of the PSO group had prior exposure to biologic treatments, potentially impacting circulating MFAP4 levels.

A strength of this study lies in its inclusion of patients with chronic inflammatory diseases (CIDs) who were either initiating biological therapy or transitioning to a different type of biological therapy, allowing for the examination of predictive markers for treatment outcomes. Additionally, the ROC curve analysis revealed a cut-off value of 34.6, which closely approached the upper tertile of MFAP4 (95% CI 34.7; 46.5), suggesting that this value could serve as an optimal cut-off point.

A limitation is that many patients with Chron's disease were not bio-naïve, which in itself may be a poor prognosis of treatment response [115]. Another limitation of the study is the relatively small number of individual CID diagnoses, which limited the statistical power of subgroup analyses at the disease level, reflected by wide confidence intervals. Additionally, other studies have linked underlying cardiovascular disease (CVD) and/or airway diseases with elevated MFAP4 levels [80, 112, 116, 117]. Unfortunately, this study could not account for these potentially confounding factors.

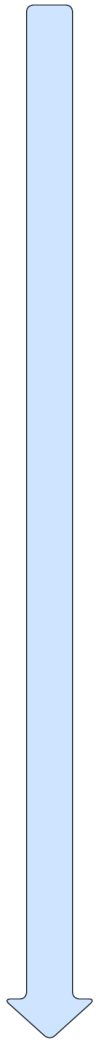
Conclusions

Papers 1 and 2: This study demonstrated that ultrasound examination (TUS) is effective in identifying interstitial lung disease (ILD) in patients with rheumatoid arthritis (RA) who have not previously been diagnosed with ILD but are experiencing respiratory symptoms. TUS can serve as a valuable screening tool to identify individuals who should undergo further diagnostic evaluation for ILD, such as high-resolution computed tomography (HRCT). Moreover, TUS can be feasibly incorporated into outpatient clinics.

Paper 3: In this cohort of early treatment-naïve patients with rheumatoid arthritis, MRC was of limited value for the detection of early respiratory impairment. However, when adjusting for age, sex and smoking status, MFAP4 had potential for detecting early respiratory impairment.

Paper 4: After accounting for confounding variables such as chronic inflammatory diseases (CID), age, sex, smoking status, and BMI, high MFAP4 levels were found to be positively correlated with treatment outcomes across all CIDs except for Crohn's disease. This suggests that high MFAP4 levels could be a potential biomarker for predicting a positive response to biological treatments.

Developments in RA-ILD during the PhD



1'st year of the Phd

RA-ILD is a serious condition, with a high mortality.

No evidence-based methods of treatment or early detection, TUS has a promising potential

2'nd year of the Phd

Anti-fibrotic treatment approved for RA-ILD

No real life evidence-based methods of early detection.
Expert opinions and retrospective studies suggest composite score, respiratory symptoms and TUS

3'rd year of the Phd

Screening at time of diagnosis using PFT has limited clinical value

The AURORA study has validated a screening approact for respiratory symptoms, and clarified TUS's role as a triaging tool for HRCT

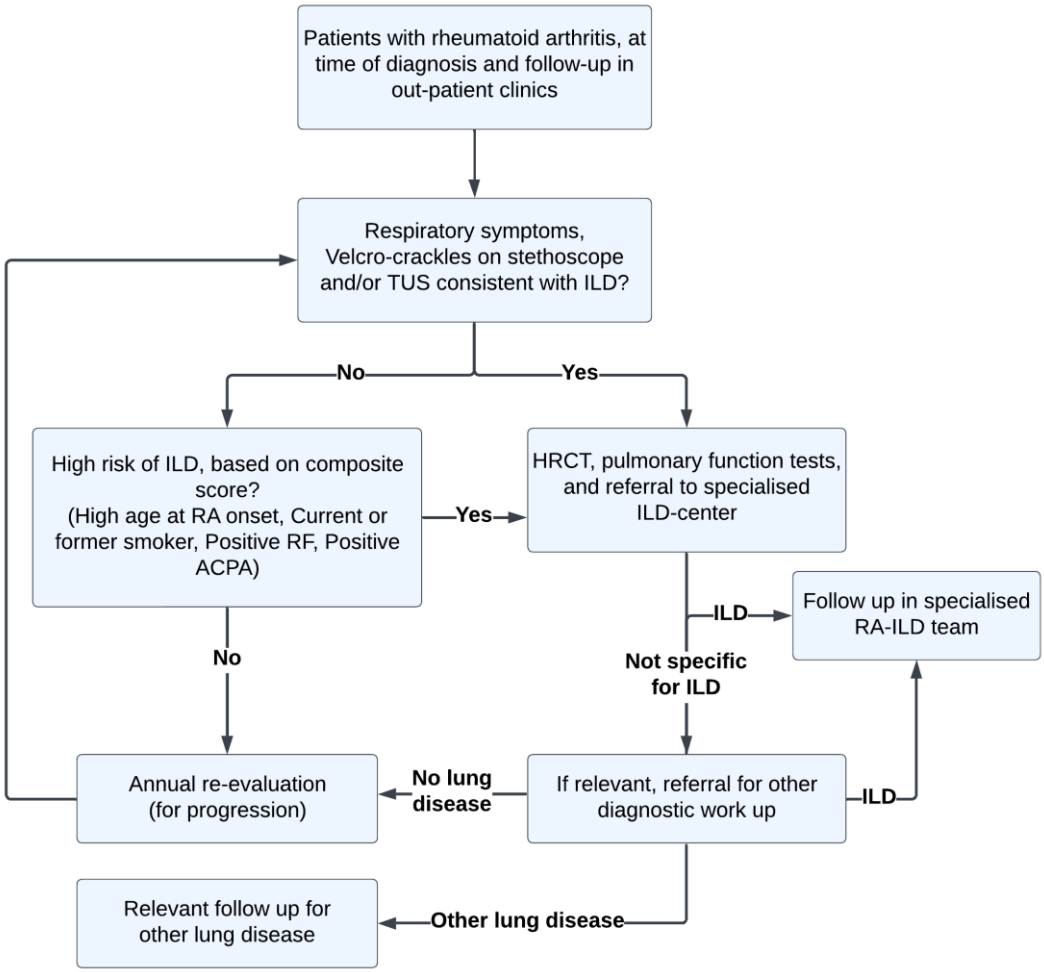
Future Perspectives

As treatment options for RA-ILD are increasing [92], methods of screening for ILD in RA, as well as detecting ILD at earlier stages, are warranted [40]. Our approach is a potential solution to this problem. We have tested a precise method of screening for respiratory symptoms (defined as inclusion criteria) and a pre-specified TUS-positive definition for detecting ILD in RA. For further research, we should test this approach, followed by a composite score, if there are no clinical signs of ILD (Suggested screening flow chart on page 55).

Additionally, longitudinal studies are warranted to assess the predictive value of TUS positivity, particularly in cases where initial HRCT results are negative, to determine whether TUS findings can predict future ILD diagnosis. Establishing the optimal interval for screening for ILD, if initial HRCT was negative for ILD, is also a key area for further investigation.

Other warranted research areas are biomarkers of ILD in RA. MFAP4 may be of value as a fibrotic and inflammatory biomarker [118], and future MFAP4 research could involve larger cohorts where pulmonary diagnosis is established and cardiovascular diagnosis can be accounted for. Further, in regards of MFAP4, future research should involve evaluating MFAP4 levels as a predictive biomarker of treatment response to biologic therapy in larger cohorts of individual CIDs, including RA, psoriatic arthritis, ankylosing spondylitis, psoriasis, Crohn's disease, and ulcerative colitis.

Proposed screening flow chart for RA-ILD*



*Based on the screening proposal by Narváez et al. and Koduri et al. [30, 31] and the data from study 2.

References

1. Hyldgaard, C, Hilberg, O, Pedersen, A B, et al., *A population-based cohort study of rheumatoid arthritis-associated interstitial lung disease: comorbidity and mortality.* Annals of the rheumatic diseases, 2017. **76**(10): p. 1700-1706.
2. Zamora-Legoff, J A, Krause, M L, Crowson, C S, Ryu, J H, and Matteson, E L, *Patterns of interstitial lung disease and mortality in rheumatoid arthritis.* Rheumatology (Oxford, England), 2017. **56**(3): p. 344-350.
3. Hyldgaard, C, Ellingsen, T, Hilberg, O, and Bendstrup, E, *Rheumatoid Arthritis-Associated Interstitial Lung Disease: Clinical Characteristics and Predictors of Mortality.* Respiration; international review of thoracic diseases, 2019: p. 1-6.
4. van der Woude, D, Syversen, S W, van der Voort, E I, et al., *The ACPA isotype profile reflects long-term radiographic progression in rheumatoid arthritis.* Annals of the rheumatic diseases, 2010. **69**(6): p. 1110-6.
5. Svärd, A, Kastbom, A, Reckner-Olsson, A, and Skogh, T, *Presence and utility of IgA-class antibodies to cyclic citrullinated peptides in early rheumatoid arthritis: the Swedish TIRA project.* Arthritis research & therapy, 2008. **10**(4): p. R75.
6. Hyldgaard, C, Harders, S, Blegvad, J, et al., *Clinical and preclinical pulmonary disease in newly diagnosed rheumatoid arthritis: a two-year follow-up study.* Scandinavian journal of rheumatology, 2023: p. 1-8.
7. Nielen, M M, van Schaardenburg, D, Reesink, H W, et al., *Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors.* Arthritis and rheumatism, 2004. **50**(2): p. 380-6.
8. Kokkonen, H, Mullazehi, M, Berglin, E, et al., *Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis.* Arthritis research & therapy, 2011. **13**(1): p. R13.
9. Reynisdottir, G, Olsen, H, Joshua, V, et al., *Signs of immune activation and local inflammation are present in the bronchial tissue of patients with untreated early rheumatoid arthritis.* Annals of the rheumatic diseases, 2016. **75**(9): p. 1722-7.
10. Reynisdottir, G, Karimi, R, Joshua, V, et al., *Structural changes and antibody enrichment in the lungs are early features of anti-citrullinated protein antibody-positive rheumatoid arthritis.* Arthritis & rheumatology (Hoboken, N.J.), 2014. **66**(1): p. 31-9.
11. Klareskog, L, Stolt, P, Lundberg, K, et al., *A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination.* Arthritis and rheumatism, 2006. **54**(1): p. 38-46.

12. van Zanten, A, Arends, S, Roozendaal, C, et al., *Presence of anticitrullinated protein antibodies in a large population-based cohort from the Netherlands.* Annals of the rheumatic diseases, 2017. **76**(7): p. 1184-1190.
13. Hensvold, A H, Magnusson, P K, Joshua, V, et al., *Environmental and genetic factors in the development of anticitrullinated protein antibodies (ACPAs) and ACPA-positive rheumatoid arthritis: an epidemiological investigation in twins.* Annals of the rheumatic diseases, 2015. **74**(2): p. 375-80.
14. Willis, V C, Demoruelle, M K, Derber, L A, et al., *Sputum autoantibodies in patients with established rheumatoid arthritis and subjects at risk of future clinically apparent disease.* Arthritis and rheumatism, 2013. **65**(10): p. 2545-54.
15. Okamoto, Y, Devoe, S, Seto, N, et al., *Association of Sputum Neutrophil Extracellular Trap Subsets With IgA Anti-Citrullinated Protein Antibodies in Subjects at Risk for Rheumatoid Arthritis.* Arthritis & rheumatology (Hoboken, N.J.), 2022. **74**(1): p. 38-48.
16. Esposito, A J, Chu, S G, Madan, R, Doyle, T J, and Dellaripa, P F, *Thoracic Manifestations of Rheumatoid Arthritis.* Clin Chest Med, 2019. **40**(3): p. 545-560.
17. Demoruelle, M K, Weisman, M H, Simonian, P L, et al., *Brief report: airways abnormalities and rheumatoid arthritis-related autoantibodies in subjects without arthritis: early injury or initiating site of autoimmunity?* Arthritis and rheumatism, 2012. **64**(6): p. 1756-61.
18. Rangel-Moreno, J, Hartson, L, Navarro, C, Gaxiola, M, Selman, M, and Randall, T D, *Inducible bronchus-associated lymphoid tissue (iBALT) in patients with pulmonary complications of rheumatoid arthritis.* J Clin Invest, 2006. **116**(12): p. 3183-94.
19. Zhu, J, Zhou, Y, Chen, X, and Li, J, *A metaanalysis of the increased risk of rheumatoid arthritis-related pulmonary disease as a result of serum anticitrullinated protein antibody positivity.* The Journal of rheumatology, 2014. **41**(7): p. 1282-9.
20. Fischer, A, Solomon, J J, du Bois, R M, et al., *Lung disease with anti-CCP antibodies but not rheumatoid arthritis or connective tissue disease.* Respiratory medicine, 2012. **106**(7): p. 1040-7.
21. Holers, V M, Demoruelle, M K, Kuhn, K A, et al., *Rheumatoid arthritis and the mucosal origins hypothesis: protection turns to destruction.* Nat Rev Rheumatol, 2018. **14**(9): p. 542-557.
22. Young, A, Koduri, G, Batley, M, et al., *Mortality in rheumatoid arthritis. Increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis.* Rheumatology (Oxford, England), 2007. **46**(2): p. 350-7.

23. Sundhedsstyrelsen. *Flere overlever kræft i Danmark*. Nyheder/2022 2022 [cited 2022 02-01-2024]; Available from: https://www.sst.dk/da/nyheder/2022/flere-overlever-kraeft-i-danmark.
24. Bongartz, T, Nannini, C, Medina-Velasquez, Y F, et al., *Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study.* Arthritis and rheumatism, 2010. **62**(6): p. 1583-91.
25. Joy, G M, Arbiv, O A, Wong, C K, et al., *Prevalence, imaging patterns and risk factors of interstitial lung disease in connective tissue disease: a systematic review and meta-analysis.* European respiratory review : an official journal of the European Respiratory Society, 2023. **32**(167).
26. Koduri, G and Solomon, J J, *Identification, Monitoring, and Management of Rheumatoid Arthritis-Associated Interstitial Lung Disease.* Arthritis & rheumatology (Hoboken, N.J.), 2023. **75**(12): p. 2067-2077.
27. Raghu, G, Remy-Jardin, M, Myers, J L, et al., *Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline.* American journal of respiratory and critical care medicine, 2018. **198**(5): p. e44-e68.
28. Mori, S, Koga, Y, and Sugimoto, M, *Different risk factors between interstitial lung disease and airway disease in rheumatoid arthritis.* Respiratory medicine, 2012. **106**(11): p. 1591-9.
29. Lake, F and Proudman, S, *Rheumatoid arthritis and lung disease: from mechanisms to a practical approach.* Seminars in respiratory and critical care medicine, 2014. **35**(2): p. 222-38.
30. Narváez, J, Aburto, M, Seoane-Mato, D, et al., *Screening criteria for interstitial lung disease associated to rheumatoid arthritis: Expert proposal based on Delphi methodology.* Reumatol Clin (Engl Ed), 2023. **19**(2): p. 74-81.
31. Koduri, G M, Podlasek, A, Pattapola, S, et al., *Four-factor risk score for the prediction of interstitial lung disease in rheumatoid arthritis.* Rheumatol Int, 2023. **43**(8): p. 1515-1523.
32. Morais, A, Duarte, A C, Fernandes, M O, et al., *Early detection of interstitial lung disease in rheumatic diseases: A joint statement from the Portuguese Pulmonology Society, the Portuguese Rheumatology Society, and the Portuguese Radiology and Nuclear Medicine Society.* Pulmonology, 2023.
33. Fletcher, C, *Standardized questionaires on respiratory symptoms. A statement prepared for, and approved by, the Medical Research Council's Committee on the aetiology of chronic bronchitis.* Br Med J, 1960. **2**(5213): p. 1665.
34. Bestall, J C, Paul, E A, Garrod, R, Garnham, R, Jones, P W, and Wedzicha, J A, *Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease.* Thorax, 1999. **54**(7): p. 581-586.

35. McGavin, C R, Artvinli, M, Naoe, H, and McHardy, G J, *Dyspnoea, disability, and distance walked: comparison of estimates of exercise performance in respiratory disease.* Br Med J, 1978. **2**(6132): p. 241-3.
36. Johnson-Warrington, V, Harrison, S, Mitchell, K, Steiner, M, Morgan, M, and Singh, S, *Exercise capacity and physical activity in patients with COPD and healthy subjects classified as Medical Research Council dyspnea scale grade 2.* J Cardiopulm Rehabil Prev, 2014. **34**(2): p. 150-4.
37. Spagnolo, P, Lee, J S, Sverzellati, N, Rossi, G, and Cottin, V, *The Lung in Rheumatoid Arthritis: Focus on Interstitial Lung Disease.* Arthritis & rheumatology (Hoboken, N.J.), 2018. **70**(10): p. 1544-1554.
38. Esposito, A J, Sparks, J A, Gill, R R, et al., *Screening for Preclinical Parenchymal Lung Disease in Rheumatoid Arthritis.* Rheumatology (Oxford, England), 2021.
39. Hyldgaard, C, Ellingsen, T, and Bendstrup, E, *COPD: an overlooked cause of excess mortality in patients with rheumatoid arthritis.* The Lancet. Respiratory medicine, 2018.
40. Stainer, A, Tonutti, A, De Santis, M, et al., *Unmet needs and perspectives in rheumatoid arthritis-associated interstitial lung disease: A critical review.* Frontiers in medicine, 2023. **10**: p. 1129939.
41. Barskova, T, Gargani, L, Guiducci, S, et al., *Lung ultrasound for the screening of interstitial lung disease in very early systemic sclerosis.* Annals of the rheumatic diseases, 2013. **72**(3): p. 390-5.
42. Xie, H Q, Zhang, W W, Sun, S, et al., *A simplified lung ultrasound for the diagnosis of interstitial lung disease in connective tissue disease: a meta-analysis.* Arthritis research & therapy, 2019. **21**(1): p. 93.
43. Mena-Vázquez, N, Jimenez-Núñez, F G, Godoy-Navarrete, F J, et al., *Utility of pulmonary ultrasound to identify interstitial lung disease in patients with rheumatoid arthritis.* Clinical rheumatology, 2021.
44. Volpicelli, G, Elbarbary, M, Blaivas, M, et al., *International evidence-based recommendations for point-of-care lung ultrasound.* Intensive care medicine, 2012. **38**(4): p. 577-91.
45. Soldati, G, Smargiassi, A, Inchingolo, R, et al., *Lung ultrasonography may provide an indirect estimation of lung porosity and airspace geometry.* Respiration; international review of thoracic diseases, 2014. **88**(6): p. 458-68.
46. Laursen, C B, Rahman, N M, and Volpicelli, G, *Thoracic Ultrasound, ERS Monograph*. ERS Monograph, ed. Bals, R. 2018, www.erspublications.com European Respiratory Society.
47. Gargani, L, Frassi, F, Soldati, G, Tesorio, P, Gheorghiade, M, and Picano, E, *Ultrasound lung comets for the differential diagnosis of acute cardiogenic dyspnoea: a comparison with natriuretic peptides.* Eur J Heart Fail, 2008. **10**(1): p. 70-7.

48. Gargani, L, *Lung ultrasound: a new tool for the cardiologist.* Cardiovasc Ultrasound, 2011. **9**: p. 6.
49. Copetti, R, Soldati, G, and Copetti, P, *Chest sonography: a useful tool to differentiate acute cardiogenic pulmonary edema from acute respiratory distress syndrome.* Cardiovasc Ultrasound, 2008. **6**: p. 16.
50. Tardella, M, Di Carlo, M, Carotti, M, Filippucci, E, Grassi, W, and Salaffi, F, *Ultrasound B-lines in the evaluation of interstitial lung disease in patients with systemic sclerosis: Cut-off point definition for the presence of significant pulmonary fibrosis.* Medicine (Baltimore), 2018. **97**(18): p. e0566.
51. Cogliati, C, Antivalle, M, Torzillo, D, et al., *Standard and pocket-size lung ultrasound devices can detect interstitial lung disease in rheumatoid arthritis patients.* Rheumatology (Oxford, England), 2014. **53**(8): p. 1497-503.
52. Di Carlo, M, Tardella, M, Filippucci, E, Carotti, M, and Salaffi, F, *Lung ultrasound in patients with rheumatoid arthritis: definition of significant interstitial lung disease.* Clinical and experimental rheumatology, 2021.
53. Gargani, L, Doveri, M, D'Errico, L, et al., *Ultrasound lung comets in systemic sclerosis: a chest sonography hallmark of pulmonary interstitial fibrosis.* Rheumatology (Oxford, England), 2009. **48**(11): p. 1382-7.
54. Wang, Y, Chen, S, Zheng, S, et al., *The role of lung ultrasound B-lines and serum KL-6 in the screening and follow-up of rheumatoid arthritis patients for an identification of interstitial lung disease: review of the literature, proposal for a preliminary algorithm, and clinical application to cases.* Arthritis research & therapy, 2021. **23**(1): p. 212.
55. Hasan, A A and Makhlouf, H A, *B-lines: Transthoracic chest ultrasound signs useful in assessment of interstitial lung diseases.* Annals of thoracic medicine, 2014. **9**(2): p. 99-103.
56. Sperandeo, M, De Cata, A, Molinaro, F, et al., *Ultrasound signs of pulmonary fibrosis in systemic sclerosis as timely indicators for chest computed tomography.* Scandinavian journal of rheumatology, 2015. **44**(5): p. 389-98.
57. Manolescu, D, Oancea, C, Timar, B, et al., *Ultrasound mapping of lung changes in idiopathic pulmonary fibrosis.* Clin Respir J, 2020. **14**(1): p. 54-63.
58. Moazedi-Fuerst, F C, Kielhauser, S M, Scheidl, S, et al., *Ultrasound screening for interstitial lung disease in rheumatoid arthritis.* Clinical and experimental rheumatology, 2014. **32**(2): p. 199-203.
59. Gutierrez, M, Ruta, S, Clavijo-Cornejo, D, Fuentes-Moreno, G, Reyes-Long, S, and Bertolazzi, C, *The emerging role of ultrasound in detecting interstitial lung disease in patients with rheumatoid arthritis.* Joint bone spine, 2022. **89**(6): p. 105407.

60. Laursen, C B, Clive, A, Hallifax, R, et al., *European Respiratory Society Statement on Thoracic Ultrasound.* The European respiratory journal, 2020.
61. Buchan, G, Barrett, K, Turner, M, Chantry, D, Maini, R N, and Feldmann, M, *Interleukin-1 and tumour necrosis factor mRNA expression in rheumatoid arthritis: prolonged production of IL-1 alpha.* Clin Exp Immunol, 1988. **73**(3): p. 449-55.
62. Hirano, T, Matsuda, T, Turner, M, et al., *Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis.* Eur J Immunol, 1988. **18**(11): p. 1797-801.
63. Keating, G M and Perry, C M, *Infliximab: an updated review of its use in Crohn's disease and rheumatoid arthritis.* BioDrugs, 2002. **16**(2): p. 111-48.
64. Maini, R, St Clair, E W, Breedveld, F, et al., *Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group.* Lancet (London, England), 1999. **354**(9194): p. 1932-9.
65. Takeuchi, T, Thorne, C, Karpouzas, G, et al., *Sirukumab for rheumatoid arthritis: the phase III SIRROUND-D study.* Annals of the rheumatic diseases, 2017. **76**(12): p. 2001-2008.
66. Zago, B A, Priyadharshini, A, and Vijayakumar, T M, *Safety and efficacy of newer biologics DMARDs in the management of rheumatoid arthritis: A systematic review.* Osteoarthr Cartil Open, 2020. **2**(4): p. 100116.
67. Smolen, J S, Landewé, R B M, Bergstra, S A, et al., *EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update.* Annals of the rheumatic diseases, 2023. **82**(1): p. 3-18.
68. Fraenkel, L, Bathon, J M, England, B R, et al., *2021 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis.* Arthritis care & research, 2021. **73**(7): p. 924-939.
69. Allen, A, Carville, S, and McKenna, F, *Diagnosis and management of rheumatoid arthritis in adults: summary of updated NICE guidance.* BMJ (Clinical research ed.), 2018. **362**: p. k3015.
70. Aripova, N, Kremer, J M, Pappas, D A, et al., *Anti-citrullinated protein antibody profiles predict changes in disease activity in patients with rheumatoid arthritis initiating biologics.* Rheumatology (Oxford, England), 2023.
71. Brown, P, Pratt, A G, and Hyrich, K L, *Therapeutic advances in rheumatoid arthritis.* BMJ (Clinical research ed.), 2024. **384**: p. e070856.
72. Holm, A T, Wulf-Johansson, H, Hvidsten, S, et al., *Characterization of spontaneous air space enlargement in mice lacking microfibrillar-*

associated protein 4. American journal of physiology. Lung cellular and molecular physiology, 2015. **308**(11): p. L1114-24.
73. Decaris, M L, Gatmaitan, M, FlorCruz, S, et al., *Proteomic analysis of altered extracellular matrix turnover in bleomycin-induced pulmonary fibrosis.* Mol Cell Proteomics, 2014. **13**(7): p. 1741-52.
74. Molleken, C, Poschmann, G, Bonella, F, et al., *MFAP4: a candidate biomarker for hepatic and pulmonary fibrosis?* Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG / World Association of Sarcoidosis and Other Granulomatous Disorders, 2016. **33**(1): p. 41-50.
75. Molleken, C, Sitek, B, Henkel, C, et al., *Detection of novel biomarkers of liver cirrhosis by proteomic analysis.* Hepatology (Baltimore, Md.), 2009. **49**(4): p. 1257-66.
76. Madsen, B S, Thiele, M, Detlefsen, S, et al., *Prediction of liver fibrosis severity in alcoholic liver disease by human microfibrillar-associated protein 4.* Liver Int, 2020. **40**(7): p. 1701-1712.
77. Saekmose, S G, Schlosser, A, Holst, R, et al., *Enzyme-linked immunosorbent assay characterization of basal variation and heritability of systemic microfibrillar-associated protein 4.* PloS one, 2013. **8**(12): p. e82383.
78. Wulf-Johansson, H, Lock Johansson, S, Schlosser, A, et al., *Localization of microfibrillar-associated protein 4 (MFAP4) in human tissues: clinical evaluation of serum MFAP4 and its association with various cardiovascular conditions.* PloS one, 2013. **8**(12): p. e82243.
79. Johansson, S L, Roberts, N B, Schlosser, A, et al., *Microfibrillar-associated protein 4: a potential biomarker of chronic obstructive pulmonary disease.* Respiratory medicine, 2014. **108**(9): p. 1336-44.
80. Pilecki, B, Schlosser, A, Wulf-Johansson, H, et al., *Microfibrillar-associated protein 4 modulates airway smooth muscle cell phenotype in experimental asthma.* Thorax, 2015. **70**(9): p. 862-72.
81. Johansson, S L, Wulf-Johansson, H, Schlosser, A, et al., *Plasma microfibrillar-associated protein 4 is not prognostic of emphysema progression but is associated with cardiovascular disease history and mortality in COPD patients.* ERJ Open Res, 2019. **5**(2).
82. Portokallidou, K, Dovrolis, N, Ragia, G, Atzemian, N, Kolios, G, and Manolopoulos, V G, *Multi-omics integration to identify the genetic expression and protein signature of dilated and ischemic cardiomyopathy.* Front Cardiovasc Med, 2023. **10**: p. 1115623.
83. Hemstra, L E, Schlosser, A, Lindholt, J S, and Sorensen, G L, *Microfibrillar-associated protein 4 variation in symptomatic peripheral artery disease.* Journal of translational medicine, 2018. **16**(1): p. 159.
84. Issa, S F, Lindegaard, H M, Lorenzen, T, et al., *Increased serum levels of microfibrillar-associated protein 4 (MFAP4) are not associated with*

clinical synovitis in rheumatoid arthritis but may reflect underlying cardiovascular comorbidity. Clinical and experimental rheumatology, 2020. **38**(1): p. 122-128.

85. *The EQUATOR Network*. [cited 2024 February]; Available from: https://www.equator-network.org/reporting-guidelines/.
86. *STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies | The EQUATOR Network*. 2021; Available from: https://www.equator-network.org/reporting-guidelines/stard/.
87. Cohen, J F, Korevaar, D A, Altman, D G, et al., *STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration.* BMJ open, 2016. **6**(11): p. e012799.
88. Whiting, P F, Rutjes, A W, Westwood, M E, and Mallett, S, *A systematic review classifies sources of bias and variation in diagnostic test accuracy studies.* J Clin Epidemiol, 2013. **66**(10): p. 1093-104.
89. *QUADAS-2*. 2024 [cited 2024 February]; Available from: https://www.bristol.ac.uk/population-health-sciences/projects/quadas/quadas-2/.
90. (2018)., C A S P. *CASP Checklist: 12 questionsto help you make sense of a Diagnostic Test study*. CASP-UK 2018 [cited 2024 February]; Available from: https://casp-uk.net/checklists/casp-diagnostic-studies-checklist-fillable.pdf.
91. *Diagnostic test studies: assessment and critical appraisal*. Evidence-based medicine (EBM) toolkit 2024 [cited 2024 February]; Available from: https://bestpractice.bmj.com/info/toolkit/learn-ebm/diagnostic-test-studies-assessment-and-critical-appraisal/.
92. Matteson, E L, Kelly, C, Distler, J H W, et al., *Nintedanib in Patients With Autoimmune Disease-Related Progressive Fibrosing Interstitial Lung Diseases: Subgroup Analysis of the INBUILD Trial.* Arthritis & rheumatology (Hoboken, N.J.), 2022. **74**(6): p. 1039-1047.
93. Kadura, S and Raghu, G, *Rheumatoid arthritis-interstitial lung disease: manifestations and current concepts in pathogenesis and management.* European respiratory review : an official journal of the European Respiratory Society, 2021. **30**(160).
94. Austin, P C, *Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples.* Stat Med, 2009. **28**(25): p. 3083-107.
95. Christensen, R, Heitmann, B L, Andersen, K W, et al., *Impact of red and processed meat and fibre intake on treatment outcomes among patients with chronic inflammatory diseases: protocol for a prospective cohort study of prognostic factors and personalised medicine.* BMJ open, 2018. **8**(2): p. e018166.

96. Felson, D T, Anderson, J J, Boers, M, et al., *American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis.* Arthritis and rheumatism, 1995. **38**(6): p. 727-35.
97. Sieper, J, Rudwaleit, M, Baraliakos, X, et al., *The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis.* Annals of the rheumatic diseases, 2009. **68 Suppl 2**: p. ii1-44.
98. Raghu, G, Remy-Jardin, M, Richeldi, L, et al., *Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults: An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline.* American journal of respiratory and critical care medicine, 2022. **205**(9): p. e18-e47.
99. Lynch, D A, Sverzellati, N, Travis, W D, et al., *Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper.* The Lancet. Respiratory medicine, 2018. **6**(2): p. 138-153.
100. Jacob, J and Hansell, D M, *HRCT of fibrosing lung disease.* Respirology (Carlton, Vic.), 2015. **20**(6): p. 859-72.
101. Santos-Moreno, P, Linares-Contreras, M F, Rodríguez-Vargas, G S, et al., *Usefulness of Lung Ultrasound as a Method for Early Diagnosis of Interstitial Lung Disease in Patients with Rheumatoid Arthritis.* Open Access Rheumatol, 2024. **16**: p. 9-20.
102. Marchetti, G, Arondi, S, Baglivo, F, et al., *New insights in the use of pleural ultrasonography for diagnosis and treatment of pleural disease.* Clin Respir J, 2018. **12**(6): p. 1993-2005.
103. Pivetta, E, Goffi, A, Lupia, E, et al., *Lung Ultrasound-Implemented Diagnosis of Acute Decompensated Heart Failure in the ED: A SIMEU Multicenter Study.* Chest, 2015. **148**(1): p. 202-210.
104. Lichtenstein, D A, *BLUE-protocol and FALLS-protocol: two applications of lung ultrasound in the critically ill.* Chest, 2015. **147**(6): p. 1659-1670.
105. Laursen, C B, Sloth, E, Lambrechtsen, J, et al., *Focused sonography of the heart, lungs, and deep veins identifies missed life-threatening conditions in admitted patients with acute respiratory symptoms.* Chest, 2013. **144**(6): p. 1868-1875.
106. Davidsen, J R, Bendstrup, E, Henriksen, D P, Graumann, O, and Laursen, C B, *Lung ultrasound has limited diagnostic value in rare cystic lung diseases: a cross-sectional study.* European clinical respiratory journal, 2017. **4**(1): p. 1330111.
107. Buda, N, Piskunowicz, M, Porzezińska, M, Kosiak, W, and Zdrojewski, Z, *Lung Ultrasonography in the Evaluation of Interstitial Lung Disease in Systemic Connective Tissue Diseases: Criteria and Severity of Pulmonary Fibrosis - Analysis of 52 Patients.* Ultraschall in der Medizin (Stuttgart, Germany : 1980), 2016. **37**(4): p. 379-85.

108. Garrote-Corral, S, Silva-Fernández, L, Seoane-Mato, D, et al., *Screening of interstitial lung disease in patients with rheumatoid arthritis: A systematic review.* Reumatol Clin (Engl Ed), 2022. **18**(10): p. 587-596.
109. Kanaan, R, Yaghi, C, Saade Riachy, C, et al., *Serum MFAP4, a novel potential biomarker for liver cirrhosis screening, correlates with transient elastography in NAFLD patients.* JGH Open, 2023. **7**(3): p. 197-203.
110. Schlosser, A, Thomsen, T, Shipley, J M, et al., *Microfibril-associated protein 4 binds to surfactant protein A (SP-A) and colocalizes with SP-A in the extracellular matrix of the lung.* Scandinavian journal of immunology, 2006. **64**(2): p. 104-16.
111. Lausen, M, Lynch, N, Schlosser, A, et al., *Microfibril-associated protein 4 is present in lung washings and binds to the collagen region of lung surfactant protein D.* J Biol Chem, 1999. **274**(45): p. 32234-40.
112. Meccanici, F, Thijssen, C G E, Dekker, S, et al., *Circulating biomarkers associated with aortic diameter in male and female patients with thoracic aortic disease: a cross-sectional study.* Open Heart, 2023. **10**(1).
113. Blindbaek, S L, Schlosser, A, Green, A, Holmskov, U, Sorensen, G L, and Grauslund, J, *Association between microfibrillar-associated protein 4 (MFAP4) and micro- and macrovascular complications in long-term type 1 diabetes mellitus.* Acta diabetologica, 2017. **54**(4): p. 367-372.
114. Kanaan, R, Medlej-Hashim, M, Jounblat, R, Pilecki, B, and Sorensen, G L, *Microfibrillar-associated protein 4 in health and disease.* Matrix Biol, 2022. **111**: p. 1-25.
115. Clinton, J W and Cross, R K, *Personalized Treatment for Crohn's Disease: Current Approaches and Future Directions.* Clin Exp Gastroenterol, 2023. **16**: p. 249-276.
116. Brandsma, C A, van den Berge, M, Postma, D S, et al., *A large lung gene expression study identifying fibulin-5 as a novel player in tissue repair in COPD.* Thorax, 2015. **70**(1): p. 21-32.
117. Hoffmann-Petersen, B, Suffolk, R, Petersen, J J H, et al., *Microfibrillar-associated protein 4 in serum is associated with asthma in Danish adolescents and young adults.* Immun Inflamm Dis, 2019. **7**(3): p. 150-159.
118. Zhu, L, Gou, W, Ou, L, Liu, B, Liu, M, and Feng, H, *Role and new insights of microfibrillar-associated protein 4 in fibrotic diseases.* APMIS : acta pathologica, microbiologica, et immunologica Scandinavica, 2023.

Appendicies

Paper 1: Published manuscript

Paper 2: Submitted manuscript & Statistical analysis plan

Paper 3: Manuscript & Statistical analysis plan

Paper 4: Manuscript & Statistical analysis plan

STARD 2015 check list for paper 2

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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 (NCT05396469, pre-results) and data will be published in peer-reviewed journals.

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.

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 described.[1–5] 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.[6–10] 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.[4 6 11–14] 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.[15] 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.[4 14]

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.[16] A confident diagnosis can sometimes be made based on HRCT in combination with a clinical context,[17] as for example, in idiopathic

pulmonary fibrosis (IPF).[18] 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.[17–19] 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.[17 19 20]

Thoracic ultrasound (ie, the primary index test)

Thoracic ultrasound (TUS) has manifested itself as a promising tool in detecting ILD,[21]and has previously been validated for detecting ILD in systemic sclerosis (SSc), where TUS findings≥3 B-lines in at least two adjacent scanning sites or a total of>5 B-lines present, were highly associated with SSc-ILD.[22 23] Similar observations were also found in a recent study of patients with RA with RA-ILD.[24] 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.[25 26]

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.[27–29] Characteristic TUS signs compatible with ILD have been described in SSc[22 23 30] and in other connective tissue diseases associated with ILD.[31] 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.[24] However, the applicability of TUS to identify ILD in patients with RA (with manifest ILD on HRCT) is only limited[31 32] 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.[33 34] Increased SP-D levels are positively associated with smoking status, with higher levels in current smokers and smokers with decreased lung function[35 36] and reflect an increased permeability of SP-D from the lung to the bloodstream, due to significant lung damage.[37] Increased SP-D levels have been found in patients with severe IPF and reflect disease severity.[38] In patients with SSc, increased SP-D has been associated with decreased diffusion capacity and disease activity due to pulmonary fibrosis development.[39 40] Another study has shown increased SP-D levels in patients with subclinical and clinical RA-ILD.[1] Decreased SP-D levels in early RA may correlate negatively to RA disease activity measures[41 42] and may modulate inflammation in RA.[43]

Increased serum microfibrillar-associated protein 4 (MFAP4) seem to reflect disease-induced processes, due to low heritability and relatively limited basal variation.[44] 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.[45] Molleken *et al* have shown that serum MFAP4 levels were not increased in IPF.[46] 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.[47] 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.[48]

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 (≥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 Sjøgrens 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).

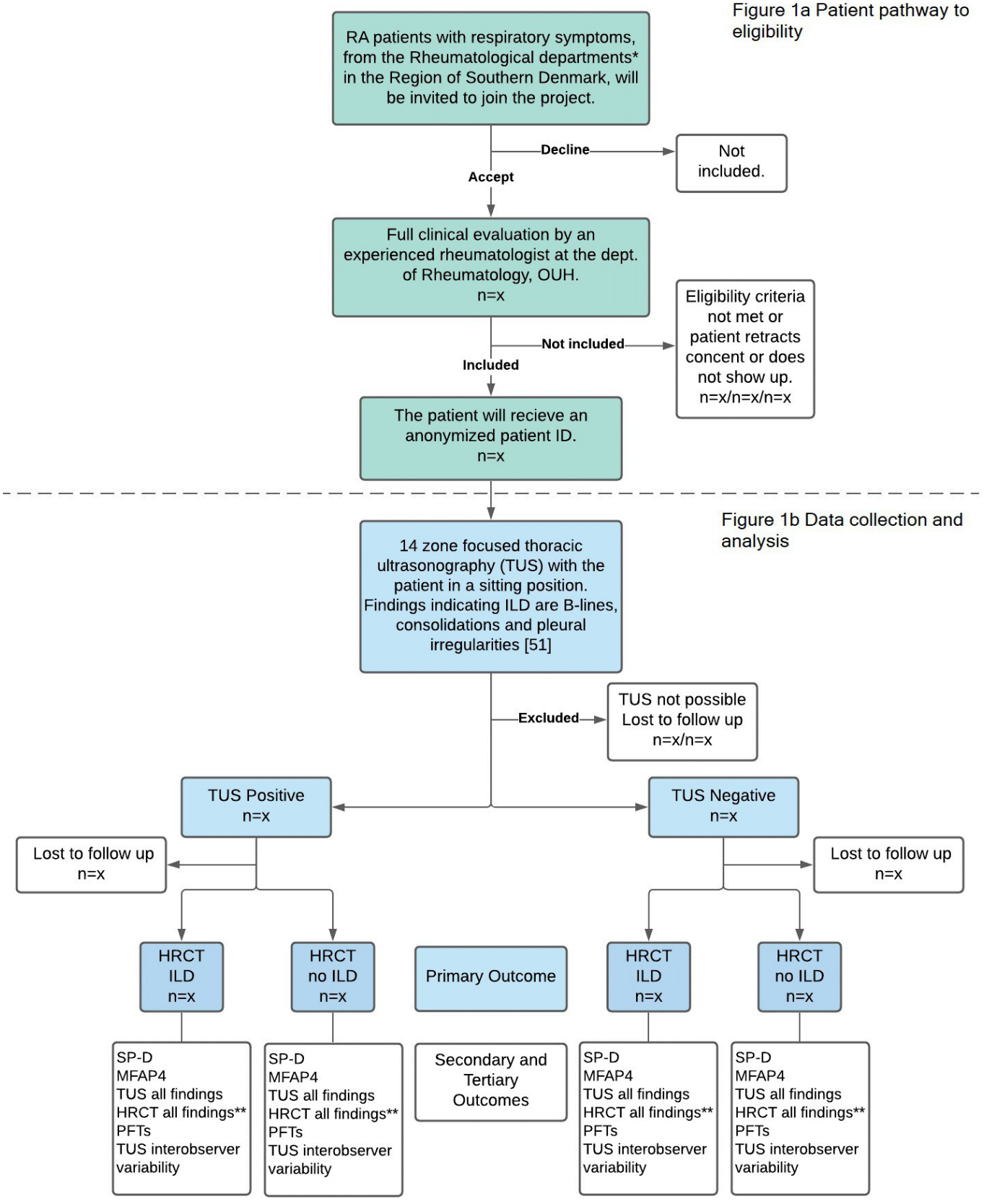
Eligibility will be evaluated by a senior rheumatologist (TE) at the HSU, and if patients are found to be eligible, they will receive an anonymised patient ID and TUS will be performed by the junior rheumatologist (BKS) on the same day as inclusion and always prior to HRCT. Referrals for an HRCT at the 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*[49] 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,[27 50] and also used in previous studies from Davidsens research group.[51 52]

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.[51 52] 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.[21 23 25 27 30 53] We will use the following definitions of TUS findings, as described by Davidsen *et al*[54] : 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').[53] IS: ≥3 B-lines in≥2 anterior or lateral zones on each hemithorax.[25 27] Upper lobe IS: ≥ 3 B-lines in in both zone R/L1 and R/L7. Pleural thickening: pleura thickness>1 mm regardless a normal or



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.[30 55] 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*.[56] Serum MFAP4 levels will be detected using the AlphaLISA technique, as described in Wulf-Johansson *et al*.[45]

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.[57] Current national guidelines recommend that all patients suspected of having ILD, undergo HRCT, as part of their diagnostic workup.[58]

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.[57 59] 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 accessors 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 1 s) and FVC in litres and per cent of predicted (% pred.), and FEV1/FVC ratio.[60] DLCO will be measured as a single-breath diffusion lung capacity. All predicted values will be automatically calculated, following the ERS Official technical standard.[61]

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 in 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.[62] 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.[15] 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.[15 63] 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

		TUS findings				
		>2 B-lines	Interstitial syndrome	Consolidation	Pleural irregularities	Pleural effusion
HRCT findings	**Specific patterns:**					
	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 (%)
	Signs of ILD:					
	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 (%)
	Signs of other airways disease:					
	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 (%)
	Signs of serositis:					
	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 (%)
>2 B-lines$_{(TUS)}$														
All$_{(HRCT)}$														
UIP$_{(HRCT)}$														
NSIP$_{(HRCT)}$														
BO$_{(HRCT)}$														
OP$_{(HRCT)}$														
Interstitial syndrome$_{(TUS)}$														
All$_{(HRCT)}$														
UIP$_{(HRCT)}$														
NSIP$_{(HRCT)}$														
BO$_{(HRCT)}$														
OP$_{(HRCT)}$														
Consolidation$_{(TUS)}$														
All$_{(HRCT)}$														
UIP$_{(HRCT)}$														
NSIP$_{(HRCT)}$														
BO$_{(HRCT)}$														
OP$_{(HRCT)}$														
Pleural irregularities$_{(TUS)}$														
All$_{(HRCT)}$														
UIP$_{(HRCT)}$														
NSIP$_{(HRCT)}$														
BO$_{(HRCT)}$														
OP$_{(HRCT)}$														
Pleural effusion$_{(TUS)}$														
All$_{(HRCT)}$														
UIP$_{(HRCT)}$														
NSIP$_{(HRCT)}$														
BO$_{(HRCT)}$														
OP$_{(HRCT)}$														

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.[64] 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 access 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.[53] 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.[31] 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. Sofíudóttir, 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 (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 in the pre-result stage.

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REFERENCES

1 Doyle TJ, Patel AS, Hatabu H, *et al*. Detection of rheumatoid Arthritis-Interstitial lung disease is enhanced by serum biomarkers. *Am J Respir Crit Care Med* 2015;191:1403–12.
2 Alexiou I, Germenis A, Koutroumpas A, *et al*. Anti-Cyclic citrullinated peptide-2 (CCP2) autoantibodies and extra-articular manifestations in Greek patients with rheumatoid arthritis. *Clin Rheumatol* 2008;27:511–3.
3 Reynisdottir G, Karimi R, Joshua V, *et al*. Structural changes and antibody enrichment in the lungs are early features of anti-citrullinated protein antibody-positive rheumatoid arthritis. *Arthritis Rheumatol* 2014;66:31–9.
4 Bongartz T, Nannini C, Medina-Velasquez YF, *et al*. Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study. *Arthritis Rheum* 2010;62:1583–91.
5 Mori S, Koga Y, Sugimoto M. Different risk factors between interstitial lung disease and airway disease in rheumatoid arthritis. *Respir Med* 2012;106:1591–9.
6 Gabbay E, Tarala R, Will R, *et al*. Interstitial lung disease in recent onset rheumatoid arthritis. *Am J Respir Crit Care Med* 1997;156:528–35.
7 Crestani B. The respiratory system in connective tissue disorders. *Allergy* 2005;60:715–34.
8 Young A, Koduri G, Batley M, *et al*. Mortality in rheumatoid arthritis. increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis. *Rheumatology* 2007;46:350–7.
9 Bieber V, Cohen AD, Freud T, *et al*. Autoimmune smoke and fire--coexisting rheumatoid arthritis and chronic obstructive pulmonary disease: a cross-sectional analysis. *Immunol Res* 2013;56:261–6.
10 Hyldgaard C, Ellingsen T, Bendstrup E. COPD: an overlooked cause of excess mortality in patients with rheumatoid arthritis. *Lancet Respir Med* 2018;6:326–7.
11 Gochuico BR, Avila NA, Chow CK, *et al*. Progressive preclinical interstitial lung disease in rheumatoid arthritis. *Arch Intern Med* 2008;168:159–66.
12 Lake F, Proudman S. Rheumatoid arthritis and lung disease: from mechanisms to a practical approach. *Semin Respir Crit Care Med* 2014;35:222–38.
13 Doyle TJ, Lee JS, Dellaripa PF, *et al*. A roadmap to promote clinical and translational research in rheumatoid arthritis-associated interstitial lung disease. *Chest* 2014;145:454–63.
14 Olson AL, Swigris JJ, Sprunger DB, *et al*. Rheumatoid arthritis-interstitial lung disease-associated mortality. *Am J Respir Crit Care Med* 2011;183:372–8.
15 Hyldgaard C, Hilberg O, Pedersen AB, *et al*. A population-based cohort study of rheumatoid arthritis-associated interstitial lung disease: comorbidity and mortality. *Ann Rheum Dis* 2017;76:1700–6.
16 Raghu G, Remy-Jardin M, Myers JL, *et al*. Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med* 2018;198:e44–68.

17 Flaherty KR, King TE, Raghu G, *et al*. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? *Am J Respir Crit Care Med* 2004;170:904–10.
18 Raghu G, Remy-Jardin M, Richeldi L, *et al*. Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med* 2022;205:e18–47.
19 Fischer A, Antoniou KM, Brown KK, *et al*. An official European respiratory Society/American thoracic Society research statement: interstitial pneumonia with autoimmune features. *Eur Respir J* 2015;46:976–87.
20 Travis WD, Costabel U, Hansell DM, *et al*. An official American thoracic Society/European respiratory Society statement: update of the International multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013;188:733–48.
21 Hasan AA, Makhlouf HA. B-lines: transthoracic chest ultrasound signs useful in assessment of interstitial lung diseases. *Ann Thorac Med* 2014;9:99–103.
22 Gargani L, Doveri M, D'Errico L, *et al*. Ultrasound lung comets in systemic sclerosis: a chest sonography hallmark of pulmonary interstitial fibrosis. *Rheumatology* 2009;48:1382–7.
23 Barskova T, Gargani L, Guiducci S, *et al*. Lung ultrasound for the screening of interstitial lung disease in very early systemic sclerosis. *Ann Rheum Dis* 2013;72:390–5.
24 Mena-Vázquez N, Jimenez-Núñez FG, Godoy-Navarrete FJ, *et al*. Utility of pulmonary ultrasound to identify interstitial lung disease in patients with rheumatoid arthritis. *Clin Rheumatol* 2021;40:2377–85.
25 Laursen CB, Clive A, Hallifax R, *et al*. European respiratory Society statement on thoracic ultrasound. *Eur Respir J* 2021;57:2001519.
26 Davidsen JR, Bendstrup E, Henriksen DP, *et al*. Lung ultrasound has limited diagnostic value in rare cystic lung diseases: a cross-sectional study. *Eur Clin Respir J* 2017;4:1330111.
27 Volpicelli G, Elbarbary M, Blaivas M, *et al*. International evidence-based recommendations for point-of-care lung ultrasound. *Intensive Care Med* 2012;38:577–91.
28 Lichtenstein DA. BLUE-protocol and FALLS-protocol: two applications of lung ultrasound in the critically ill. *Chest* 2015;147:1659–70.
29 Cervantes-Perez P, Toro-Perez AH, Rodriguez-Jurado P. Pulmonary involvement in rheumatoid arthritis. *JAMA* 1980;243:1715–9.
30 Pinal-Fernandez I, Pallisa-Nuñez E, Selva-O'Callaghan A, *et al*. Pleural irregularity, a new ultrasound sign for the study of interstitial lung disease in systemic sclerosis and antisynthetase syndrome. *Clin Exp Rheumatol* 2015;33:S136–41.
31 Xie HQ, Zhang WW, Sun DS, *et al*. A simplified lung ultrasound for the diagnosis of interstitial lung disease in connective tissue disease: a meta-analysis. *Arthritis Res Ther* 2019;21:93.
32 Moazedi-Fuerst FC, Kielhauser SM, Scheidl S, *et al*. Ultrasound screening for interstitial lung disease in rheumatoid arthritis. *Clin Exp Rheumatol* 2014;32:199–203.
33 Lock-Johansson S, Vestbo J, Sorensen GL. Surfactant protein D, Club cell protein 16, pulmonary and activation-regulated chemokine, C-reactive protein, and fibrinogen biomarker variation in chronic obstructive lung disease. *Respir Res* 2014;15:147.
34 Sorensen GL. Surfactant protein D in respiratory and non-respiratory diseases. *Front Med* 2018;5:18.
35 Lomas DA, Silverman EK, Edwards LD, *et al*. Serum surfactant protein D is steroid sensitive and associated with exacerbations of COPD. *Eur Respir J* 2009;34:95–102.
36 Johansson SL, Tan Q, Holst R, *et al*. Surfactant protein D is a candidate biomarker for subclinical tobacco smoke-induced lung damage. *Am J Physiol Lung Cell Mol Physiol* 2014;306:L887–95.
37 Fakih D, Akiki Z, Junker K, *et al*. Surfactant protein D multimerization and gene polymorphism in COPD and asthma. *Respirology* 2018;23:298–305.
38 Takahashi H, Fujishima T, Koba H, *et al*. Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. *Am J Respir Crit Care Med* 2000;162:1109–14.
39 Hant FN, Ludwicka-Bradley A, Wang H-J, *et al*. Surfactant protein D and KL-6 as serum biomarkers of interstitial lung disease in patients with scleroderma. *J Rheumatol* 2009;36:773–80.
40 Yanaba K, Hasegawa M, Takehara K, *et al*. Comparative study of serum surfactant protein-D and KL-6 concentrations in patients with systemic sclerosis as markers for monitoring the activity of pulmonary fibrosis. *J Rheumatol* 2004;31:1112–20.
41 Hoegh SV, Lindegaard HM, Sorensen GL, *et al*. Circulating surfactant protein D is decreased in early rheumatoid arthritis: a 1-year prospective study. *Scand J Immunol* 2008;67:71–6.
42 Christensen A, Sørensen G, Hørslev-Petersen K, *et al*. Circulating surfactant protein -D is low and correlates negatively with systemic inflammation in early, untreated rheumatoid arthritis. *Arthritis Res Ther* 2010;12:R39.
43 Christensen AF, Sorensen GL, Junker K, *et al*. Localization of surfactant protein-D in the rheumatoid synovial membrane. *APMIS* 2018;126:9–13.
44 Sækmose SG, Schlosser A, Holst R, *et al*. Enzyme-Linked immunosorbent assay characterization of basal variation and heritability of systemic microfibrillar-associated protein 4. *PLoS One* 2013;8:e82383.
45 Wulf-Johansson H, Lock Johansson S, Schlosser A, *et al*. Localization of microfibrillar-associated protein 4 (MFAP4) in human tissues: clinical evaluation of serum MFAP4 and its association with various cardiovascular conditions. *PLoS One* 2013;8:e82243.
46 Mölleken C, Poschmann G, Bonella F, *et al*. MFAP4: a candidate biomarker for hepatic and pulmonary fibrosis? *Sarcoidosis Vasc Diffuse Lung Dis* 2016;33:41–50.
47 Laursen CB, Rahman NM, Volpicelli G. *Thoracic ultrasound*. European Respiratory Society, 2018.
48 de Wit MPT, Berlo SE, Aanerud GJ, *et al*. European League against rheumatism recommendations for the inclusion of patient representatives in scientific projects. *Ann Rheum Dis* 2011;70:722–6.
49 Davidsen JR, Schultz HHL, Henriksen DP, *et al*. Lung ultrasound in the assessment of pulmonary complications after lung transplantation. *Ultraschall Med* 2020;41:148–56.
50 Lichtenstein D, Mézière G, Biderman P, *et al*. The Comet-tail artifact. An ultrasound sign of alveolar-interstitial syndrome. *Am J Respir Crit Care Med* 1997;156:1640–6.
51 Laursen CB, Sloth E, Lassen AT, *et al*. Point-Of-Care ultrasonography in patients admitted with respiratory symptoms: a single-blind, randomised controlled trial. *Lancet Respir Med* 2014;2:638–46.
52 Laursen CB, Sloth E, Lambrechtsen J, *et al*. Focused sonography of the heart, lungs, and deep veins identifies missed life-threatening conditions in admitted patients with acute respiratory symptoms. *Chest* 2013;144:1868–75.
53 Reißig A, Kroegel C. Transthoracic sonography of diffuse parenchymal lung disease. *Journal of Ultrasound in Medicine* 2003;22:173–80.
54 Davidsen JR, Laursen CB, Højlund M, *et al*. Lung ultrasound to phenotype chronic lung allograft dysfunction in lung transplant recipients. A prospective observational study. *J Clin Med* 2021;10. doi:10.3390/jcm10051078. [Epub ahead of print: 05 03 2021].
55 Bittner RC, Schnoy N, Schönfeld N, *et al*. [High-resolution magnetic resonance tomography (HR-MRT) of the pleura and thoracic wall: normal findings and pathological changes]. *Rofo* 1995;162:296–303.
56 Leth-Larsen R, Nordenbaek C, Tornoe I, *et al*. Surfactant protein D (SP-D) serum levels in patients with community-acquired pneumonia. *Clin Immunol* 2003;108:29–37.
57 Jacob J, Hansell DM. HRCT of fibrosing lung disease. *Respirology* 2015;20:859–72.
58 Interstitielle lungesygdomme (ILS) â€“ DLS | Dansk Lungemedicinsk Selskab, 2021. Available: https://lungemedicin.dk/interstitielle-lungesygdomme-ils/
59 Lynch DA, Sverzellati N, Travis WD, *et al*. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society white paper. *Lancet Respir Med* 2018;6:138–53.
60 Miller MR, Hankinson J, Brusasco V, *et al*. Standardisation of spirometry. *Eur Respir J* 2005;26:319–38.
61 Official ERS technical standard: Global Lung Function Initiative reference values for static lung volumes in individuals of European ancestry - 2000289.full.pdf, 2021. Available: https://erj.ersjournals.com/content/erj/57/3/2000289.full.pdf
62 Schwartz A. Diagnostic test calculator, 2021. Available: http://araw.mede.uic.edu/cgi-bin/testcalc.pl
63 Hyldgaard C, Bendstrup E, Pedersen AB, *et al*. Increased mortality among patients with rheumatoid arthritis and COPD: a population-based study. *Respir Med* 2018;140:101–7.
64 Davidsen JR, Lund LC, Laursen CB, *et al*. Dynamics in diagnoses and pharmacotherapy before and after diagnosing idiopathic pulmonary fibrosis. *ERJ Open Res* 2020;6. doi:10.1183/23120541.00479-2020. [Epub ahead of print: 10 11 2020].

Statistical analysis plan (SAP) for the AURORA study

1. Administrative information

1.1 Title, registration and version

Full study title	Using Thoracic ultrasound to detect interstitial lungdisease in patients with rheumatoid arthritis. The diagnostic test accuracy AURORA study.
Acronym	AURORA
Clinicaltrials.gov number	NCT05396469
Ethics Committee number	S-20210154
The Danish Data Protecting Agency number	22/7044
A published protocol is available at BMJ OPEN:	Using thoracic ultrasound to detect interstitial lung disease in patients with rheumatoid arthritis: a protocol for the diagnostic test accuracy AURORA study | BMJ Open
SAP version	1.0
SAP date	2023-06-02

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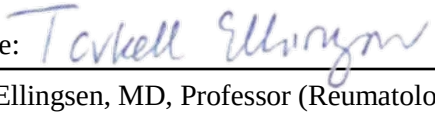
Date: 2023-06-02 Signature: 

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Keywords: Interstitial Lung Disease, Rheumatoid Arthritis, Thoracic Ultrasound, Diagnostic Test Accuracy

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ABSTRACT

Background/Purpose: Interstitial lung disease (ILD) is a severe and common pulmonary manifestation in RA (RA-ILD) and the median survival for patients with manifest RA-ILD is 6.6 years [1, 2]. Feasible methods to detect early RA-ILD are warranted. Our objective was to determine the diagnostic accuracy of thoracic ultrasound (TUS) for ILD in RA patients with respiratory symptoms. Our AURORA study protocol is published in BMJopen [3].

Methods

Eligibility criteria for participants and settings of data collection: Patients eligible for inclusion were consenting adults (≥18 years) diagnosed with RA (according to the 2010 ACR-criteria) with respiratory symptoms in form of: dyspnoea >2 months duration, cough >2 months duration, recurrent pneumonia (≥2 per year) and/or prior severe pneumonia requiring hospitalization or a chest X-ray indicating interstitial abnormalities. Prior to entering the study, the participants were not diagnosed with ILD nor had recent (<12 months) High Resolution Computed Tomography (HRCT) of the lungs. Recruitment took place in the Region of Southern Denmark and the included participants form a consecutive series of 80 individuals.

Description of the index test and reference standard: By use of a standardised 14 zone protocol participants were examined by TUS as index test performed by a junior resident in rheumatology (BKS), who is TUS certified. The anonymised TUS clips were evaluated by a TUS expert (JRD), completely blinded to patient identity and medical history. TUS was registered as positive for ILD, if there were ≥10 B-lines and/or a thickened and fragmented pleura in ≥1 zone bilaterally. HRCT was used as the reference standard for the ILD diagnosis. The diagnostic test accuracy of TUS was quantified by the sensitivity and specificity in comparison to the HRCT, as well as diagnostic odds ratio (OR), positive and negative predictive values and positive and negative likelihood ratios (table 2).

Results: Xx patients were found to have ILD of the xx patients in the target analysis. TUS was able to identify xx% of the ILD cases correctly with a diagnostic OR of xx (95%CI of xx-xx)

Conclusion: This study has reached the conclusion, that TUS *can/ cannot* be used for detecting ILD in RA patients with respiratory symptoms.

Strengths and limitations of this study

- Minimal time consuming
- Cheap and easy to learn
- Radiation free
- Only suggestive of ILD-pattern on HRCT
- Examiner dependent

INTRODUCTION

Pulmonary diseases are significant contributors to morbidity and mortality in RA, and an association between anti-citrullinated protein antibody (ACPA) positivity and interstitial lung disease (ILD) has been described [1, 4-7]. 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 [8-12]. The burden of RA-ILD is increasing, as studies have shown that ILD is prevalent in 33 to 44% of RA patients and is clinically evident in about 10% of the RA population [1, 8, 13-16]. In our population-based Danish cohort, approximately 2.2% of RA patients had ILD and there was observed a more than doubled mortality risk in RA-ILD patients within 30 days after RA diagnosis, when compared to RA without ILD [2]. 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 RA-ILD patients is observed in other studies as well [1, 16]. Screening for lung disease at time of RA diagnosis, did not find many patients with ILD (Hyldgaard 2023). Screening for respiratory symptoms as well as relevant medical history may be a more accurate approach.

How is Interstitial Lung Disease Currently Diagnosed (i.e. the reference standard)

As described in our protocol article for this study (Sofiudottir 2023), chest high-resolution computed tomography (HRCT) is the most central diagnostic tool of ILD and is regarded as the gold standard for ILD diagnostics [17]. A confident diagnosis can sometimes be made based on HRCT in combination with a clinical context, as e.g. in idiopathic pulmonary fibrosis (IPF) [17, 18]. However, achieving a confident ILD diagnosis, may necessitate serologic as well as histopathological information achieved by transbronchial or surgical lung biopsies and a multidisciplinary discussion team approach is recommended [17-19] +20 .

Thoracic ultrasound (i.e. the primary index test)

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) [19]. Thoracic ultrasound (TUS) has manifested itself as a promising tool in detecting ILD [21], and has previously been validated for detecting ILD in systemic sclerosis (SSc), where TUS findings of ≥3 B-lines in at least two adjacent scanning sites or a total of >5 B-lines present, were highly associated with SSc-ILD [22, 23]. Similar observations were also found in a recent study of RA patients with RA-ILD [24]. The European Respiratory Society 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 detecting COPD or other cystic ILDs [25, 26].

Rationale: Intended use and clinical role of TUS

RA-ILD is associated with increased mortality compared to RA without ILD, this creates a rationale for a reproducible and radiation-free bedside tool for detection of potential ILD in RA [27-29]. Characteristic TUS signs compatible with ILD have been described in systemic sclerosis [22, 23, 30] and in other connective tissue diseases associated with ILD (CTD-ILD) [31]. A recent case-control study, with 71 RA patients, 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 [24]. However, the applicability of TUS to identify ILD in RA patients (with manifest ILD on HRCT) is only limited [31, 32] and it has not yet been validated as a screening method to identify undiagnosed ILD in RA patients with e.g. respiratory symptoms.

Study hypotheses and objectives

We hypothesise that TUS can detect ILD in RA patients using chest HRCT as reference standard for ILD diagnosis.

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 RA patients 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 e-mail, depending on the patient partners and participants' preference. This project follows the EULAR recommendations for the inclusion of patient representatives in the contemporary scientific process by adhering to eight important aspects [48].

Study design

This is a multicentre, cross-sectional diagnostic test accuracy study. Recruitment took place in the Region of Southern Denmark and the included participants form a consecutive series of 80 individuals.

Eligibility criteria

Patients eligible for inclusion were consenting adults (≥18 years) diagnosed with RA (according to the 2010 ACR-criteria) with respiratory symptoms in form of: dyspnoea >2 months duration, cough >2 months duration, recurrent pneumonia (≥2 per year) and/or prior severe pneumonia requiring hospitalization or a chest X-ray indicating interstitial abnormalities. Prior to entering the study, the participants were not diagnosed with ILD nor had recent (<12 months) High Resolution Computed Tomography (HRCT) of the lungs. A diagnosis of COPD based on chest X-ray and spirometry did not exclude the patient from the study.

We did not include patients with other systemic autoimmune diseases than RA (except secondary Sjøgrens 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. Patients who had an HRCT performed within 12 months prior to the inclusion date or who are unwilling or unable to provide written informed consent were not eligible for inclusion.

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 – Svendborg Hospital, Lillebaelt Hospital, and Odense University Hospital. Patients with a scheduled clinical visit for their RA disease and management, were asked if they had respiratory symptoms. If they did have respiratory symptoms, they received oral information about this project and those who were interested in participating, were fully screened for eligibility criteria by BKS. Those who were eligible and gave oral consent were given an appointment in the Department of Rheumatology at Odense University Hospital (OUH) for an elaborating and undisturbed conversation about the project, with time for questions and the patient will be asked to sign the informed consent before enrolment. After consent has been given, the patient was enrolled and TUS was be performed on the same day, as well as full clinical evaluation for RA by an RA expert (TE). Subsequently, the patients will be referred to an HRCT as well as a pulmonary function test (See Figure 1). Inclusion has began May 2022 and the last patient was included in april 2023.

Consecutive enrolment

This is an inception cohort of RA patients 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 anonymized 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 the 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: Thoracic ultrasound (i.e. Index test #1)

A standardised 14-zone protocol for TUS as described by Laursen et al was used [20]. Patients were be examined in a straight-backed sitting position. The thorax was be systematically scanned according to anterior, lateral and posterior chest wall using an adapted approach of the principles described by Volpicelli and Lichtenstein [27, 50] and as also used in other studies [49] 51, 52]:

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 [20]. In each zone the transducer was systematically placed vertically across an intercostal space and each zone was swiped to find clearest visualization of the pleura and potential pathology. TUS was performed in all 14 zones.

B-lines, interstitial syndrome (IS), and pleural thickening are TUS findings known to be associated with presence of ILD types with subpleural involvement on HRCT [21, 23, 25, 27, 30, 53]. Based on the current literature on TUS findings in ILD (REF), our team agreed upon the following definition of TUS positive: ≥10 B-lines in total and/or thickened and fragmented pleura in minimum 1 zone bilaterally. [Indsæt ref fra TUS+ dok]

Acceptable window of HRCT in relation to TUS in this study is one month after TUS.

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 anonymized patient. The answers from the experienced pulmonologist (JRD), will be used as reference standard

Reference standard: HRCT

Rationale for choosing the reference standard: HRCT acts as the gold standard for diagnosing ILD [57]. Current national guidelines recommend that all patients suspected of having ILD, undergo HRCT, as part of their diagnostic work up [58].

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 (140-900mA), Noise Index 25, Pitch 0.5, Rotation time 0.35 sec., Asir-V 40%. Images are reconstructed using a 512 x 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 (*MIP*) 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 interstitial lung disease, airways disease and other lung diseases are noted with specific disease patterns as: Usual interstitial pneumonia (UIP), non-specific interstitial pneumonia (NSIP), organizing pneumonia (OP), mosaic attenuation pattern / airways disease. Signs of fibrosis are reticulation and/or traction bronchiectasis and/or honeycombing and/or loss of volume [57, 59]. 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 enlargemement 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 interstitial lung disease. 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 interstitial lung disease.

SH has evaluated all HRCT images for the findings mentioned above and has answered the question "Do the HRCT images indicate ILD?" with either "Yes or No" for each patient, as well as registerd all HRCT findings.

Pulmonary function test

All patients will undergo a pulmonary function test in accordance with the European Respiratory Society (ERS) and American Thoracic Society (ATS) standards including FEV1 (forced expiratory volume in 1 sec) and FVC in litres and percent of predicted (% pred.), and FEV1/FVC ratio [60]. Diffusion capacity of the lung for carbon monoxide (DLCO) will be measured as a single-breath diffusion lung capacity. All predicted values will be automatically calculated, following the European Respiratory Society Official technical standard [61].

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 work up will also be accessible to the non-treating physicians, who are part of this project. TUS, routine blood samples, as well as 100mL 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 pseudo-anonymized and stored in a Research Electronic Data

Capture database (REDCap). Clinical details as well as para-clinical 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 pseudo-anonymized and stored in REDCap.

Training and expertise of the persons executing and reading the tests

A junior resident in rheumatology (BKS), who is TUS certified (TUS EFSUMB level 1), performed TUS and stored the anonymized clips. When inclusion was complete, the anonymized were evaluated by an expert in TUS (JRD) (TUS EFSUMB level 3).

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 was the only physician seeing the project ID and TUS images at inclusion. SH, who described the HRCT scans was blinded to TUS clips or TUS diagnosis. BKS did not attend multidisciplinary meetings regarding possible RA-ILD in the inclusion period, but did have access to the patients' medical journal in order to arrange PFT and collect background information on the patient. After inclusion ended, JRD evaluated the anonymized TUS clips and answered whether the TUS images indicated ILD. JRD was completely blinded to patient identity and medical history. TUS evaluation was done prior to opening HRCT data.

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, i.e. 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 RA patients who do not have the disease i.e., 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 (i.e. correlated) in that as the value of one increases, the value of the other decreases; these measures are dependent on the patient characteristics and the disease spectrum. From these measures, we will calculate the positive Likelihood Ratio (LR+) and the negative Likelihood Ratio (LR-). LR+ is 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 the 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. The LR- is the ratio of (1 – Sensitivity) / Specificity. Furthermore, we will estimate the kappa coefficient as well as the diagnostic odds ratios along with 95% confidence intervals.

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” the images indicateing 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. All participants received the index test at inclusion. If index test cannot be evaluated, this will lead to exclusion of the patient from the study in the target analyses. Furthermore, assuming patients with missing index test have received HRCT and categorized to ILD or no ILD, we will perform sensitivity analyses where we a) assume that all with missing data are TUS positive, and b) assume that all with missing data are TUS negative. Results will be presented in supplementary.

Handling indeterminate and missing reference standard

Possible indeterminate results are not likely, as results are dichotomized into ILD or non-ILD on HRCT. Missing reference standard will lead to exclusion of the patient from the study for the target analysis. Furthermore, we will perform sensitivity analyses where we a) assume that all with

missing data have ILD, and b) assume that all with missing data do not have ILD. Results will be presented in supplementary.

Sample size and power considerations

The study is designed to be able to evaluate the diagnostic test characteristics (sensitivity, specificity, likelihood ratios) and determine the post-test probability of disease given the pre-test probability and test characteristics [62]. 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 likelihood ratio is 3.33 with a 95% confidence interval of 1.81 to 6.13- The posterior probability (odds) is 67% (2.0) with a 95% confidence interval of 52% to 79%, meaning two out of three with a positive TUS have ILD on HRCT. The negative likelihood ratio is 0.42 with a 95% confidence interval of 0.25 to 0.70. The posterior probability (odds) is 20% (0.3) with a 95% confidence interval 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.

Anticipated result outline

Results from the primary analysis will be presented in table 2. Additional descriptive findings will be presented in table 3, where specific TUS findings in relation to specific HRCT findings will be listed.

Figure 1: Flow of participants

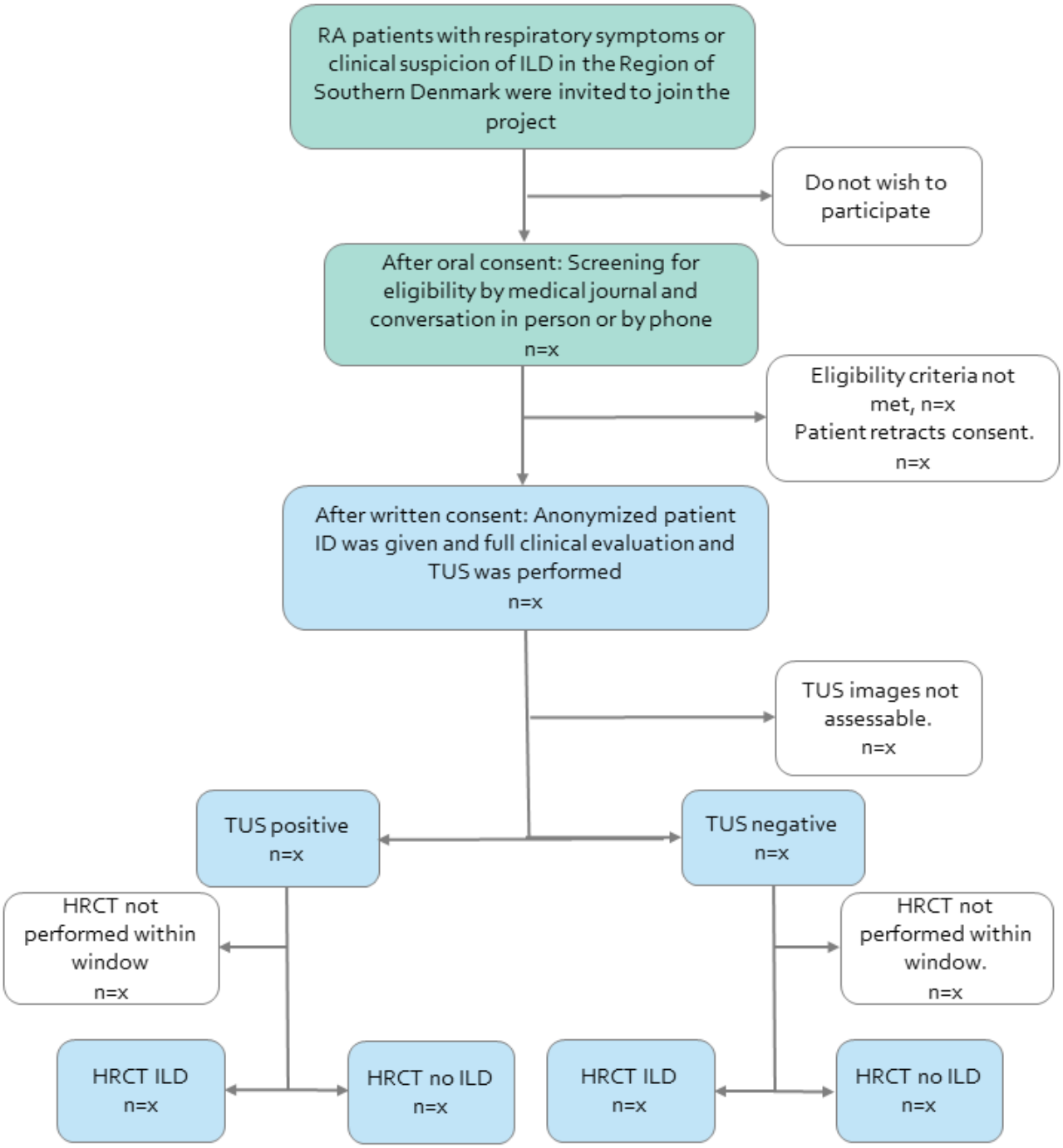

Table 1: Patient characteristics			
	TUS positive (n=x)	TUS negative (n=x)	**STD-diff**
Age, years	x	x	
Female, n (%)	n = x (%)	n = x (%)	
BMI, kg/m²	x	x	
Anti-CCP positive (%)	x	x	
IgM RF positive (%)	x	x	
Time since RA diagnosis (Months)	x	x	
RA treated with:			
MTX, no. (%)	x	x	
Salazopyrin, no. (%)			
Hydroxychlorokin, no. (%)			
Leflonumide, no. (%)	x	x	
Prednisolone, no. (%)	x	x	
JAK-inhibitors, no. (%)	x	x	
Biologics, no. (%)	x	x	
Smoking habits	n = x	n = x	
Pack-years	x	x	
Never smoked, n (%)	x	x	
Current smoker, n (%)	x	x	
Former smoker n (%)	x	x	
Duration of respiratory symptoms (Months)	x	x	
Tender joints 28	x	x	
Swollen joints 28	x	x	
Pain			
Patient Global assessment	x	x	
Physician Global assessment	x	x	
Fatigue	x	x	
HAQ	x	x	
CRP mg/L	x	x	
DAS28CRP	x	x	
FEV1 % pred.	x	x	
FVC % pred.	x	x	
FEV1/FVC %	x	x	
TLC % pred.	x	x	
DLCO % pred.	x	x	
6MWD (meters)	x	x	
6MWD desaturation (△%)	x	x	

Table 1 abbreviattions: TUS: Thoracic ultrasound, STD-diff: Standardized difference, n: number, No.:number, BMI: Body mass index, ACPA: Anti-citrullinated protein antibody, IgM RF: IgM Rheumatid factor, MTX: Methotrexate, JAK-inhibitors: Janus kinase inhibitors, HAQ: Health assessment Questionnaire, CRP: C-Reactive protein, DAS28CRP: Disease Acivity Score-28 for Rheumatoid Arthritis with CRP, FEV1: Forced expiratory volume in 1 second, FVC: Forced vital capacity, TLC: Total lung capacity, DLCO: Diffusing capacity for carbon monoxide, 6MWD: 6-min walk distance

Table 2: Diagnostic accuracy variables

	TUS Positive	TUS Negative	Diagnostic OR 95% CI	Sensitivity %	Specificity %	PPV %	NPV %	LR+	LR-
HRCT									
ILD	x	x	x	x	x	x	x	x	x

Table 2 abbreviations: TUS: Thoracic ultrasound, OR: Odds Ratio, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value, LR+: Positive likelihood ratio, LR-: Negative likelihood ratio, HRCT High resolution computed tomography, ILD: Interstitial lung disease.

Furthermore, TUS reliability (kappa) for ILD will be reported in the text.

Table 3: Descriptive table with TUS findings in relation to HRCT findings

	TUS findings					
	TUS positive no. (%)	≥10 B-lines no. (%)	Pleura Thickened & fragmentet No. (%)	Reduced or no lungsliding No. (%)	Pleural effusion No. (%)	Consolidation No. (%)
Specific HRCT findings						
ILD patterns:						
UIP (n=x)	x	x	x	x	N/A	N/A
NSIP (n=x)	x	x	x	x	N/A	N/A
OP (n=x)	x	x	x	x	N/A	N/A
HP (n=x)	x	x	x	x	N/A	N/A
Descriptive dominant ILD pattern:						
Reticular/fibrotic (n=x)	x	x	x	x	N/A	N/A
Attenuation (n=x)	x	x	x	x	N/A	N/A
Cystic (n=x)	x	x	x	x	N/A	N/A
Nodular (n=x)	x	x	x	x	N/A	N/A
Signs of parietal pleura and pleural cavity involvement:						
Pleural thickening (n=x)	N/A	N/A	x	x	x	x
Pleural effusion (n=x)	N/A	N/A	x	x	x	x
Malignancy:						
Pulmonary malignancy (n=x)	x	x	x	x	x	x
Other malignancy (n=x)	N/A	N/A	N/A	N/A	N/A	N/A

Table 3 abbreviations: TUS: Thoracic ultrasound, HRCT: High resolution computed tomography, ILD: Interstitial lung disease, UIP: Usual interstitial lung disease, NSIP: Non-specific interstitial pneumoia, OP: Organizing pneumonia, HP: Hypersensitivity pneumonitis.

Supplementary material

Supplementary file: This SAP

Supplementary Table 1: Diagnostic accuracy variables for intention-to-infer population, assuming patients with missing data on ILD status from HRCT or TUS have ILD

	TUS Positive	TUS Negative	Diagnostic OR 95% CI	Sensitivity %	Specificity %	PPV %	NPV %	LR+	LR-
HRCT									
ILD	x	X	x	x	x	x	x	x	x

Supplementary Table 2: Diagnostic accuracy variables for intention-to-infer population, assuming patients with missing data on ILD status from HRCT or TUS do **not** have ILD

	TUS Positive	TUS Negative	Diagnostic OR 95% CI	Sensitivity %	Specificity %	PPV %	NPV %	LR+	LR-
HRCT									
ILD	x	X	x	x	x	x	x	x	x

Discussion

RA patients have an increased risk of developing ILD and an increased risk of mortality after ILD diagnosis [15]. 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 RA patients in a national Danish cohort receive medication for chronic obstructive pulmonary disease (COPD) and their increased mortality is comparable to RA-ILD [15, 63]. Smoking is associated with the development of both RA, COPD and ILD. The diagnosis of ILD in RA patients 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 as e.g. IPF [64]. Currently there are no studies on RA patients with unexplained respiratory symptoms, nor any studies on screening for respiratory symptoms in RA. This cohort of RA patients will access the diagnostic accuracy of TUS in detecting ILD in a cohort of RA patients with 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 [53]. TUS has not yet been solidly validated as a screening tool in RA patients with respiratory symptoms, but has been validated in smaller studies, often case-control, with a high pre-test probability of ILD. When joining TUS findings in one recent meta-analysis TUS seems to have its justification as a potential ILD screening tool [31]. TUS is examiner dependent and inter-observer 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. Sofíudóttir, 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 (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.

Data sharing statement

Data from the AURORA study will be stored in the Danish Data Archive (DDA) when data have been analysed and published. Through an agreement with the Danish Data Protection agency, the DDA preserves data materials containing personal identifiers. Data and the personal identifiers will be stored separately and special permits are required for access to the data. Data will be available on request for academic researchers.

Contributorship statement / Acknowledgements

BKS, RC, JRD and TE conceived and developed the idea for the study. TE: Is TUS certified and evaluate all TUS images and will clinically evaluate all participants at inclusion. BKS: is TUS certified and will perform TUS on all the participants as well as collect data, handle logistics, write 1. Draft of the SAP and manuscript and analyse data after inclusion is ended. SH: Will evaluate all HRCT scans and register the findings. JRD and CBL: are TUS expert and has ensured relevant

training and certification for TE, BKS and PLRH and contributed to TUS positive definition in this study, prior to TUS evaluation. JRD has evaluated all TUS clips. CBL has clinically evaluated all patients for respiratory disease (via HRCT and PFTs). RC has contributed with methodological and statistical design of the study. PLRH: Is TUS trained and will evaluate all TUS images. BKS, SH, PLRH, RC, JRD and TE have contributed to the study design, writing of the first draft of the protocol and revision to the protocol paper and will approve the final version of any paper before submission. BKS, TE, PLRH and SAJ: Patient identification and recruitment. All authors (BKS, SH, CBL, PLRH, SMN, SAJ, RC, JRD and TE) are responsible for approval of this SAP and editing and approval of paper before submission. We would like to thank our three Danish patient research partners from the Rheumatology Research Unit (LB, OA and LP), for valuable and critical feedback on research focus, the patient pathways and written patient information.

Competing interests

None of the funders were involved in the process of study design, writing of the protocol or revision to the protocol paper.

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Figure legends

Figure 1: Flow diagram of patients in the AURORA study

References

1. Doyle, T.J., A.S. Patel, H. Hatabu, et al., *Detection of Rheumatoid Arthritis-Interstitial Lung Disease Is Enhanced by Serum Biomarkers.* Am J Respir Crit Care Med, 2015. **191**(12): p. 1403-12.
2. Alexiou, I., A. Germenis, A. Koutroumpas, et al., *Anti-cyclic citrullinated peptide-2 (CCP2) autoantibodies and extra-articular manifestations in Greek patients with rheumatoid arthritis.* Clin Rheumatol, 2008. **27**(4): p. 511-3.
3. Reynisdottir, G., R. Karimi, V. Joshua, et al., *Structural changes and antibody enrichment in the lungs are early features of anti-citrullinated protein antibody-positive rheumatoid arthritis.* Arthritis Rheumatol, 2014. **66**(1): p. 31-9.
4. Bongartz, T., C. Nannini, Y.F. Medina-Velasquez, et al., *Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study.* Arthritis Rheum, 2010. **62**(6): p. 1583-91.
5. Mori, S., Y. Koga and M. Sugimoto, *Different risk factors between interstitial lung disease and airway disease in rheumatoid arthritis.* Respir Med, 2012. **106**(11): p. 1591-9.
6. Gabbay, E., R. Tarala, R. Will, et al., *Interstitial lung disease in recent onset rheumatoid arthritis.* Am J Respir Crit Care Med, 1997. **156**(2 Pt 1): p. 528-35.
7. Crestani, B., *The respiratory system in connective tissue disorders.* Allergy, 2005. **60**(6): p. 715-34.
8. Young, A., G. Koduri, M. Batley, et al., *Mortality in rheumatoid arthritis. Increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis.* Rheumatology (Oxford), 2007. **46**(2): p. 350-7.
9. Bieber, V., A.D. Cohen, T. Freud, et al., *Autoimmune smoke and fire--coexisting rheumatoid arthritis and chronic obstructive pulmonary disease: a cross-sectional analysis.* Immunol Res, 2013. **56**(2-3): p. 261-6.
10. Hyldgaard, C., T. Ellingsen and E. Bendstrup, *COPD: an overlooked cause of excess mortality in patients with rheumatoid arthritis.* Lancet Respir Med, 2018.
11. Gochuico, B.R., N.A. Avila, C.K. Chow, et al., *Progressive preclinical interstitial lung disease in rheumatoid arthritis.* Arch Intern Med, 2008. **168**(2): p. 159-66.
12. Lake, F. and S. Proudman, *Rheumatoid arthritis and lung disease: from mechanisms to a practical approach.* Semin Respir Crit Care Med, 2014. **35**(2): p. 222-38.
13. Doyle, T.J., J.S. Lee, P.F. Dellaripa, et al., *A roadmap to promote clinical and translational research in rheumatoid arthritis-associated interstitial lung disease.* Chest, 2014. **145**(3): p. 454-63.
14. Olson, A.L., J.J. Swigris, D.B. Sprunger, et al., *Rheumatoid arthritis-interstitial lung disease-associated mortality.* Am J Respir Crit Care Med, 2011. **183**(3): p. 372-8.
15. Hyldgaard, C., O. Hilberg, A.B. Pedersen, et al., *A population-based cohort study of rheumatoid arthritis-associated interstitial lung disease: comorbidity and mortality.* Ann Rheum Dis, 2017. **76**(10): p. 1700-1706.
16. Raghu, G., M. Remy-Jardin, J.L. Myers, et al., *Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline.* Am J Respir Crit Care Med, 2018. **198**(5): p. e44-e68.
17. Flaherty, K.R., T.E. King, Jr., G. Raghu, et al., *Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis?* Am J Respir Crit Care Med, 2004. **170**(8): p. 904-10.
18. Raghu, G., M. Remy-Jardin, L. Richeldi, et al., *Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults: An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline.* Am J Respir Crit Care Med, 2022. **205**(9): p. e18-e47.
19. Fischer, A., K.M. Antoniou, K.K. Brown, et al., *An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features.* Eur Respir J, 2015. **46**(4): p. 976-87.
20. Travis, W.D., U. Costabel, D.M. Hansell, et al., *An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias.* Am J Respir Crit Care Med, 2013. **188**(6): p. 733-48.

21. Hasan, A.A. and H.A. Makhlouf, *B-lines: Transthoracic chest ultrasound signs useful in assessment of interstitial lung diseases.* Ann Thorac Med, 2014. **9**(2): p. 99-103.
22. Gargani, L., M. Doveri, L. D'Errico, et al., *Ultrasound lung comets in systemic sclerosis: a chest sonography hallmark of pulmonary interstitial fibrosis.* Rheumatology (Oxford), 2009. **48**(11): p. 1382-7.
23. Barskova, T., L. Gargani, S. Guiducci, et al., *Lung ultrasound for the screening of interstitial lung disease in very early systemic sclerosis.* Ann Rheum Dis, 2013. **72**(3): p. 390-5.
24. Mena-Vázquez, N., F.G. Jimenez-Núñez, F.J. Godoy-Navarrete, et al., *Utility of pulmonary ultrasound to identify interstitial lung disease in patients with rheumatoid arthritis.* Clin Rheumatol, 2021.
25. Laursen, C.B., A. Clive, R. Hallifax, et al., *European Respiratory Society Statement on Thoracic Ultrasound.* Eur Respir J, 2020.
26. Davidsen, J.R., E. Bendstrup, D.P. Henriksen, et al., *Lung ultrasound has limited diagnostic value in rare cystic lung diseases: a cross-sectional study.* Eur Clin Respir J, 2017. **4**(1): p. 1330111.
27. Volpicelli, G., M. Elbarbary, M. Blaivas, et al., *International evidence-based recommendations for point-of-care lung ultrasound.* Intensive Care Med, 2012. **38**(4): p. 577-91.
28. Lichtenstein, D.A., *BLUE-protocol and FALLS-protocol: two applications of lung ultrasound in the critically ill.* Chest, 2015. **147**(6): p. 1659-1670.
29. Cervantes-Perez, P., A.H. Toro-Perez and P. Rodriguez-Jurado, *Pulmonary involvement in rheumatoid arthritis.* Jama, 1980. **243**(17): p. 1715-9.
30. Pinal-Fernandez, I., E. Pallisa-Nunez, A. Selva-O'Callaghan, et al., *Pleural irregularity, a new ultrasound sign for the study of interstitial lung disease in systemic sclerosis and antisynthetase syndrome.* Clin Exp Rheumatol, 2015. **33**(4 Suppl 91): p. S136-41.
31. Xie, H.Q., W.W. Zhang, S. Sun, et al., *A simplified lung ultrasound for the diagnosis of interstitial lung disease in connective tissue disease: a meta-analysis.* Arthritis Res Ther, 2019. **21**(1): p. 93.
32. Moazedi-Fuerst, F.C., S.M. Kielhauser, S. Scheidl, et al., *Ultrasound screening for interstitial lung disease in rheumatoid arthritis.* Clin Exp Rheumatol, 2014. **32**(2): p. 199-203.
47. Laursen, C.B., N.M. Rahman and G. Volpicelli, *Thoracic Ultrasound*. 2018: European Respiratory Society.
48. de Wit, M.P., S.E. Berlo, G.J. Aanerud, et al., *European League Against Rheumatism recommendations for the inclusion of patient representatives in scientific projects.* Ann Rheum Dis, 2011. **70**(5): p. 722-6.
49. Davidsen, J.R., H.H.L. Schultz, D.P. Henriksen, et al., *Lung Ultrasound in the Assessment of Pulmonary Complications After Lung Transplantation.* Ultraschall Med, 2020. **41**(2): p. 148-156.
50. Lichtenstein, D., G. Mézière, P. Biderman, et al., *The comet-tail artifact. An ultrasound sign of alveolar-interstitial syndrome.* Am J Respir Crit Care Med, 1997. **156**(5): p. 1640-6.
51. Laursen, C.B., E. Sloth, A.T. Lassen, et al., *Point-of-care ultrasonography in patients admitted with respiratory symptoms: a single-blind, randomised controlled trial.* Lancet Respir Med, 2014. **2**(8): p. 638-46.
52. Laursen, C.B., E. Sloth, J. Lambrechtsen, et al., *Focused sonography of the heart, lungs, and deep veins identifies missed life-threatening conditions in admitted patients with acute respiratory symptoms.* Chest, 2013. **144**(6): p. 1868-1875.
53. Reißig, A. and C. Kroegel, *Transthoracic Sonography of Diffuse Parenchymal Lung Disease.* Journal of Ultrasound in Medicine, 2003. **22**(2): p. 173-180.
54. Davidsen, J.R., C.B. Laursen, M. Højlund, et al., *Lung Ultrasound to Phenotype Chronic Lung Allograft Dysfunction in Lung Transplant Recipients. A Prospective Observational Study.* J Clin Med, 2021. **10**(5).
55. Bittner, R.C., N. Schnoy, N. Schönfeld, et al., *[High-resolution magnetic resonance tomography (HR-MRT) of the pleura and thoracic wall: normal findings and pathological changes].* Rofo, 1995. **162**(4): p. 296-303.

56. Leth-Larsen, R., C. Nordenbaek, I. Tornoe, et al., *Surfactant protein D (SP-D) serum levels in patients with community-acquired pneumonia.* Clin Immunol, 2003. **108**(1): p. 29-37.
57. Jacob, J. and D.M. Hansell, *HRCT of fibrosing lung disease.* Respirology, 2015. **20**(6): p. 859-72.
58. *Interstitielle lungesygdomme (ILS) â" DLS | Dansk Lungemedicinsk Selskab*. 2021; Available from: https://lungemedicin.dk/interstitielle-lungesygdomme-ils/.
59. Lynch, D.A., N. Sverzellati, W.D. Travis, et al., *Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper.* Lancet Respir Med, 2018. **6**(2): p. 138-153.
60. Miller, M.R., J. Hankinson, V. Brusasco, et al., *Standardisation of spirometry.* Eur Respir J, 2005. **26**(2): p. 319-38.
61. *Official ERS technical standard: Global Lung Function Initiative reference values for static lung volumes in individuals of European ancestry - 2000289.full.pdf*. 2021; Available from: https://erj.ersjournals.com/content/erj/57/3/2000289.full.pdf.
62. Schwartz, A. *Diagnostic Test Calculator*. 2021; Available from: http://araw.mede.uic.edu/cgi-bin/testcalc.pl.
63. Hyldgaard, C., E. Bendstrup, A.B. Pedersen, et al., *Increased mortality among patients with rheumatoid arthritis and COPD: A population-based study.* Respir Med, 2018. **140**: p. 101-107.
64. Davidsen, J.R., L.C. Lund, C.B. Laursen, et al., *Dynamics in diagnoses and pharmacotherapy before and after diagnosing idiopathic pulmonary fibrosis.* ERJ Open Res, 2020. **6**(4).

1. Bongartz, T., et al., *Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study.* Arthritis Rheum, 2010. **62**(6): p. 1583-91.
2. Hyldgaard, C., et al., *A population-based cohort study of rheumatoid arthritis-associated interstitial lung disease: comorbidity and mortality.* Ann Rheum Dis, 2017. **76**(10): p. 1700-1706.
3. Sofíudóttir, B.K., et al., *Using thoracic ultrasound to detect interstitial lung disease in patients with rheumatoid arthritis: a protocol for the diagnostic test accuracy AURORA study.* BMJ Open, 2022. **12**(12): p. e067434.
4. Doyle, T.J., et al., *Detection of Rheumatoid Arthritis-Interstitial Lung Disease Is Enhanced by Serum Biomarkers.* Am J Respir Crit Care Med, 2015. **191**(12): p. 1403-12.
5. Alexiou, I., et al., *Anti-cyclic citrullinated peptide-2 (CCP2) autoantibodies and extra-articular manifestations in Greek patients with rheumatoid arthritis.* Clin Rheumatol, 2008. **27**(4): p. 511-3.
6. Reynisdottir, G., et al., *Structural changes and antibody enrichment in the lungs are early features of anti-citrullinated protein antibody-positive rheumatoid arthritis.* Arthritis Rheumatol, 2014. **66**(1): p. 31-9.
7. Mori, S., Y. Koga, and M. Sugimoto, *Different risk factors between interstitial lung disease and airway disease in rheumatoid arthritis.* Respir Med, 2012. **106**(11): p. 1591-9.
8. Gabbay, E., et al., *Interstitial lung disease in recent onset rheumatoid arthritis.* Am J Respir Crit Care Med, 1997. **156**(2 Pt 1): p. 528-35.
9. Crestani, B., *The respiratory system in connective tissue disorders.* Allergy, 2005. **60**(6): p. 715-34.
10. Bieber, V., et al., *Autoimmune smoke and fire--coexisting rheumatoid arthritis and chronic obstructive pulmonary disease: a cross-sectional analysis.* Immunol Res, 2013. **56**(2-3): p. 261-6.
11. Hyldgaard, C., T. Ellingsen, and E. Bendstrup, *COPD: an overlooked cause of excess mortality in patients with rheumatoid arthritis.* Lancet Respir Med, 2018.

12. Young, A., et al., *Mortality in rheumatoid arthritis. Increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis.* Rheumatology (Oxford), 2007. **46**(2): p. 350-7.
13. Gochuico, B.R., et al., *Progressive preclinical interstitial lung disease in rheumatoid arthritis.* Arch Intern Med, 2008. **168**(2): p. 159-66.
14. Lake, F. and S. Proudman, *Rheumatoid arthritis and lung disease: from mechanisms to a practical approach.* Semin Respir Crit Care Med, 2014. **35**(2): p. 222-38.
15. Doyle, T.J., et al., *A roadmap to promote clinical and translational research in rheumatoid arthritis-associated interstitial lung disease.* Chest, 2014. **145**(3): p. 454-63.
16. Olson, A.L., et al., *Rheumatoid arthritis-interstitial lung disease-associated mortality.* Am J Respir Crit Care Med, 2011. **183**(3): p. 372-8.
17. Raghu, G., et al., *Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline.* Am J Respir Crit Care Med, 2018. **198**(5): p. e44-e68.
18. Flaherty, K.R., et al., *Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis?* Am J Respir Crit Care Med, 2004. **170**(8): p. 904-10.
19. Volpicelli, G., et al., *International evidence-based recommendations for point-of-care lung ultrasound.* Intensive Care Med, 2012. **38**(4): p. 577-91.
20. Laursen, C.B., et al., *Focused sonography of the heart, lungs, and deep veins identifies missed life-threatening conditions in admitted patients with acute respiratory symptoms.* Chest, 2013. **144**(6): p. 1868-1875.

Statistical Analysis Plan (SAP)

1. Administrative information

1.1 Title, registration and version

Full study title	Detecting lung impairment in newly diagnosed rheumatoid arthritis using the MRC dyspnoea score and serum Microfibrillar-associated protein 4 (MFAP4): *A Cross-sectional Diagnostic Test Accuracy Study*
Acronym	ERAS (the Early Rheumatoid Arthritis cohort Study)
Clinicaltrials.gov number	Not registered
Ethics Committee number	S-20140057
The Danish Data Protecting Agency number	15/43686
A published protocol:	
SAP version	1.0
SAP date	2023-12-04

1.2. Roles and responsibility

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1.3. Signatures:

Date: 04.12.2023 Signature: 

Bjørk Khaliqi Sofíudóttir, MD, PhD fellow

Date: 2023 Dec 04 Signature: 

Robin Christensen, MSc, Professor of Biostatistics & Clinical Epidemiology

Date: 2023 dec 4 Signature: Torkell Ellingsen

Torkell Ellingsen, MD, Professor (Rheumatologist)

Detecting lung impairment and reduced left ventricular ejection fraction in newly diagnosed rheumatoid arthritis. *Secondary analyses for the Silkeborg-based Prospective Cohort Study*

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2. Introduction

2.1. Purpose of the Statistical Analysis Plan (SAP)

The purpose of the present SAP is to give a detailed elaboration of the statistical analyses planned to be executed on a cross-sectional sample based on the Early Rheumatoid Arthritis Study (ERAS).[1] This SAP was developed after the first data analyses from this study had been published[2] but before accessing and analysing data regarding Medical Research Council (MRC) dyspnoea scale and serum Microfibrillar-associated protein 4 (MFAP4). Reporting of this diagnostic test accuracy study will follow the STARD 2015-guideline (the *Standards for the Reporting of Diagnostic Accuracy Studies*).[3]

2.2. Background and rationale for this study

Rheumatoid arthritis (RA) is a chronic inflammatory disease and all-cause mortality in RA is highest within the first seven years following the diagnosis.[4] Lung disease is one of the most common causes of death in RA causing 22% of deaths in an inception cohort of patients with early RA with up to 18 years follow-up.[4] This emphasises the importance of early diagnosis and treatment of both primary disease and lung disease in RA. Currently there are no credible patient reported outcome measures (PROMS) or biomarkers for detecting lung disease in RA.

The MRC scale is well known and has been used for several years for grading the effect of breathlessness on daily activities.[5,6] The MRC score is easy to administer and the score reflects the walking test performance.[7] The MRC dyspnoea scale consists of five statements about perceived breathlessness: grade 1, "I only get breathless with strenuous exercise"; grade 2, "I get short of breath when hurrying on the level or up a slight hill"; grade 3, "I walk slower than people of the same age on the level because of breathlessness or have to stop for breath when walking at my own pace on the level"; grade 4, "I stop for breath after walking 100 yards or after a few minutes on the level"; grade 5, " I am too breathless to leave the house". Patients select a grade that applied to them. A MRC grade 1-2 is regarded within the normal range. However, patients with COPD and self-reported MRC grade 2 have lower exercise tolerance than healthy individuals with self-reported MRC grade 2.[8] It has not been tested as a detection tool in a population of RA patients, who have an increased risk of lung disease. The New York Heart Association (NYHA) Classification of Heart Failure dyspnea score (NYHA) is also a well-established questionnaire to establish level of heart failure easily. Patients grade themselves with levels of breathlessness, heart palpitations and chest pain on a scale of one to four, with well defined examples for each score: 1, "Ordinary physical activity does not cause undue fatigue, dyspnea, or palpitations", 2, " Ordinary physical activity causes fatigue, dyspnea, palpitations, or angina.", 3, " Comfortable at rest; less than ordinary physical activity causes fatigue, dyspnea, palpitations, or angina.", 4, "Symptoms occur at rest; any physical activity increases discomfort.". A score of 1 is considered normal, however, the score of 1 and 2 are found to be associated with both normal and mild heart failure and with significant overlap. The score of 3 is associated with moderate hearth failure and the score of 4 is associated with severe heart failure. Symptoms of heart failure and lung disease are very similar.

Increased serum MicroFibrillar-Associated Protein 4 (MFAP4) seem to reflect disease-induced processes, due to low heritability and relatively limited basal variation[9]. MFAP4 is found with especially high expression in the heart, small intestine and the lungs. For the lungs, MFAP4 has been localized in the pulmonary arterioles and interalveolar walls [10]. Increased MFAP4 have been correlated to liver-cirrhosis as well as diabetic neuropathy [11 12]. Further, a study showed a possible correlation between increased MFAP4 levels and exacerbation of chronic obstructive pulmonary disease, while another study found MFAP4 to be a novel contributor to experimental asthma [13 14]. Increased MFAP4 has also been associated with increased 7-year mortality in patients with peripheral artery disease, however, the same study found a decreased risk of vascular occlusion two years after reconstructive surgery in patients with high MFAP4 levels [15]. This indicates that increased MFAP4 may be a marker of active inflammation in the lungs and blood vessels. Issa *et al* (2020) found MFAP4 to be increased in early RA and further increased in manifest RA but not associated to RA disease activity or synovitis. Further, there was an inverse correlation between MFAP4 and APCA positivity and a positive correlation to increased systolic blood pressure,[16] suggesting this as modulating factors.

Rationale: There is a need for a feasible method of screening for lung disease in RA. MRC and NYHA may be an easily implementable triaging tool for detecting symptoms that may reflect lung impairment, while MFAP4 may be a biomarker of importance when assessing the impact of inflammatory lung diseases.

2.3. Aim

The overall aim is to explore the diagnostic accuracy of MRC, NYHA, and the serum MFAP4 in detecting lung impairment in early RA. The lung impairment is defined as a diffusion capacity of the lungs for Carbon monoxide (DLCO) <80% of expected or index (FEV1/FVC) <70%.

Secondary aim, is to evaluate MRC, NYHA and MFAP4 for detection of reduced left ventricular ejection fraction (LVEF), defined as LVEF <50%.

2.4. Hypotheses

The primary hypothesis is that the MRC dyspnoea score and the NYHA score are associated with lung impairment in treatment naïve RA patients. The secondary hypothesis is that serum MFAP4 is associated with the severity of lung impairment in treatment naïve RA patients.

2.5. Objective

To examine if MRC scores, NYHA scores, or levels of MFAP4 can detect lung impairment in RA. Primary objective: To test the diagnostic cuff-off value of MRC scores, NYHA scores, as well as serum MFAP4 in detecting lung impairment, and subsequently assess the diagnostic test accuracy related to these best fit cut-offs.

Secondary objective, To test the diagnostic cuff-off value of MRC scores, NYHA scores, as well as serum MFAP4 in detecting lung impairment, and subsequently assess the diagnostic test accuracy related to these best fit cut-offs.

To examine if MRC scores, NYHA scores, or levels of MFAP4 can detect LVEF <50 in RA.
Tertary objective: To test the diagnostic cuff-off value of MRC scores, NYHA scores, as well as serum MFAP4 in detecting LVED <50, and subsequently assess the diagnostic test accuracy related to these best fit cut-offs.
Quarternary objective, To test the diagnostic cuff-off value of MRC scores, NYHA scores, as well as serum MFAP4 in detecting LVEF <50, and subsequently assess the diagnostic test accuracy related to these best fit cut-offs.

Index test

Patients answered the MRC and NYHA questionnaire within six months after inclusion, at the same day as the pulmonary function tests (PFTs) were performed. Serum MFAP4 levels will be detected using the AlphaLISA technique, as described in Wulf-Johansson, H., et al.[10]

Reference standard

PFTs, including forced expiratory volume in one second (FEV1), forced vital capacity (FVC), total lung capacity, residual volume, diffusing capacity of the lungs for carbon monoxide (DLCO), and Six-Minute Walk Test, were performed according to the European Respiratory Society recommendations.[17-20] All tests were performed within six months after the RA diagnosis and repeated after two years. DLCO measurements were corrected for haemoglobin in case of anaemia. Lung impairment is defined as minimum one of the following findings: DLCO <80% or index (FEV1/FVC) <70%.

As previously described by Logstrup el at.,[21] patients also underwent transthoracic echocardiography (TTE). Left ventricular (LV) dimensions, volumes, ejection fraction (EF) and GLS were measured offline using standard methods. The LV EF was calculated by a modified biplane Simpson's method from apical 4- and 2-chamber views.

Training and expertise of the persons executing and reading the tests

The MRC and NYHA scale are well-established method of grading patient-perceived disability. Patients were asked to choose the grade that applied to them.

The Department of Molecular Medicine, University of Southern Denmark, Denmark is responsible for MFAP4 measurements. They are MFAP4 experts with patented anti-MFAP4 as a new drug for treatment of age-related macular degeneration and diabetic macular oedema.

Blinding of test assessors

As this is a secondary analysis of the ERAS-cohort, pulmonary data have been published. At the time of this SAP, data regarding the correlation of MRC and NYHA to lung impairment and MFAP4 measurements have not been accessed. After the SAP is signed, the MFAP4 data will be added to the ERAS database, thereafter data will be validated and analysed as specified in this SAP.

Statistical Methods

Outcome measures

First, we will explore the best fit for a cut off value for MRC, NYHA as well as for MFAP4 for detection of lung impairment, using receiver operating characteristic (ROC) curves. These plots consists of two parameter estimates: True Positive Rate (sensitivity) and False Positive Rate (1- Specificity), i.e., two basic measures of quantifying the diagnostic accuracy of a test. Sensitivity is defined as the ability of the index tests to detect lung impairment, when it is truly present, i.e., it is the probability of a positive test index test result, given that the patient has lung impairment on PFT. Specificity is the ability of the tests to exclude the condition in the RA patients who do not have the disease i.e., it is the probability of a negative index test result given that the patient does not have the disease (No lung impairment). When reporting the finding from the primary diagnostic test (MRC, NYHA, and MFAP4), both sensitivity and specificity are linked (i.e., correlated) in that as the value of one parameter increases, the value of the other decreases; these measures are dependent on the patient characteristics and the disease spectrum. Finally, we will compare sensitivity and specificity for MRC, HYHA, and MFAP4 compared to each other. 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

Missing MRC score, NYHA score or MFAP4 measurements, will lead to exclusion from the primary analysis.

Handling indeterminate and missing reference standard

Indeterminate results are not likely, as results are dichotomized into DLCO <80% of predicted and index (FEV1/FVC) <70. Missing reference standard (pulmonary function test) will lead to exclusion of the patient from the primary analysis population.

Sample size and power considerations

150 treatment naïve patients with RA were included and underwent PFTs. The PFTs were interpreted by an experienced pulmonologist.

Results: Anticipated outline

Figure 1

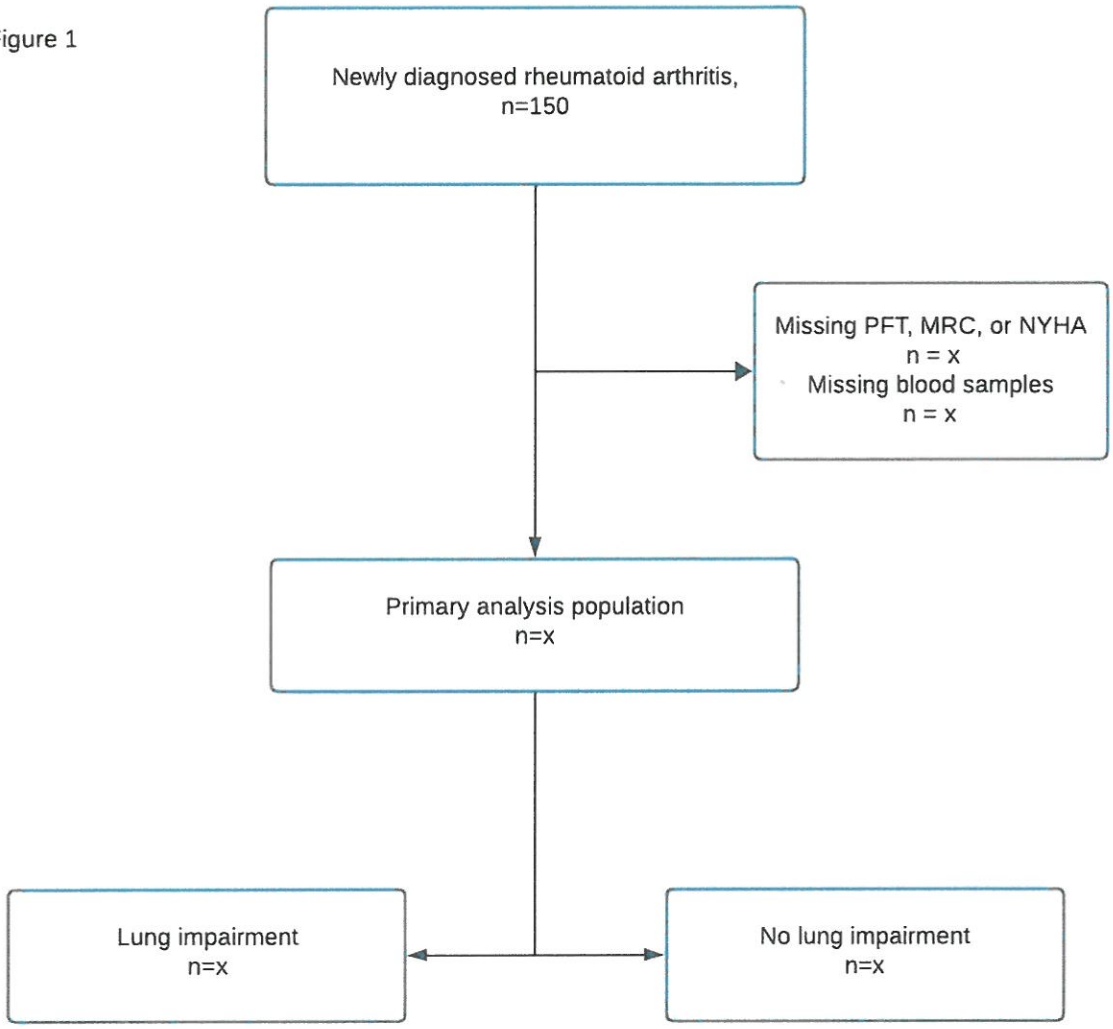

Figure 1 abbreviations: n=number, PFT= Pulmonary function test, MRC= Medical Research Council dyspnoea scale

Table 1. Patient characteristics. Values are Mean (SD), unless otherwise stated				
	Lung impairment (n=x)	No lung impairment (n=x)	Standardized Difference	P-value
MRC score:	x	x	x	x
1, no (%)	x	x	x	x
2, no (%)	x	x	x	x
3, no (%)	x	x	x	x
4, no (%)	x	x	x	x
5, no (%)	x	x	x	x
NYHA score:	x	x	x	x
1, no (%)	x	x	x	x
2, no (%)	x	x	x	x
3, no (%)	x	x	x	x
4, no (%)	x	x	x	x
MFAP4	x	x	x	x
Age (years)	x	x	x	x
Female, n (%)	x	x	x	x
BMI, kg/m^2	x	x	x	x
Anti-CCP positive (%)	x	x	x	x
IgM RF positive (%)	x	x	x	x
Smoking habits				
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
CRP mg/L	x	x	x	x
DAS28CRP	x	x	x	x
FEV1 % pred.	x	x	x	x
FVC % pred.	x	x	x	x
FEV1/FVC %	x	x	x	x
TLC % pred.	x	x	x	x
DLCO % pred.	x	x	x	x
6MWD (meters)	x	x	x	x
6MWD desaturation (Δ%)	x	x	x	x
LVEF	x	x	x	x

Figure 2a: ROC curve for MRC detecting lung impairment (simulated data)

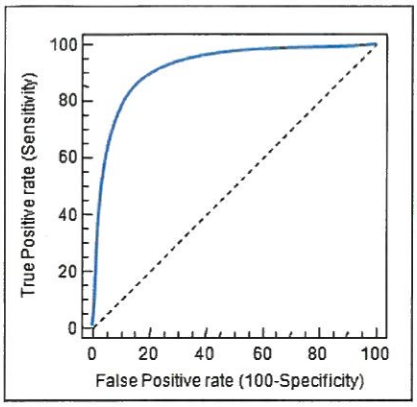

Figure 2b: ROC curve for NYHA detecting lung impairment (simulated data)

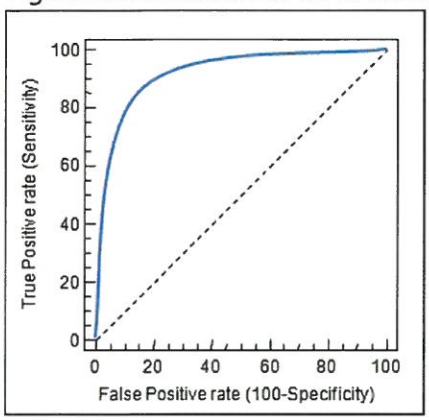

Figure 2c: ROC curve for $MFAP_4$ detecting lung impairment (simulated data)

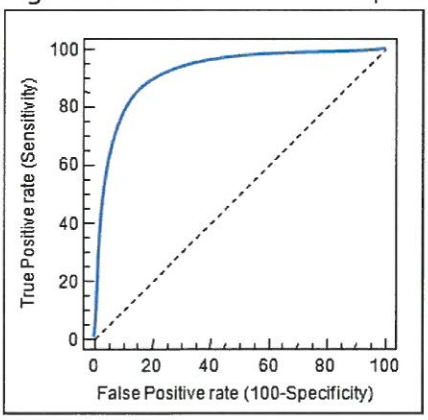

Table 2 Diagnostic outcome measures for lung impairment based on the best cut-offs (see figure 2)

	Lung impairment n=x	No lung impairment n=x	Diagnostic OR (95% CI)	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
MRC >x n = x (%)	x	x	x	x	x	x	x
NYHA n = x (%)	x	x	x	x	x	x	x
MFAP4 >x n=x (%)	x	x	x	x	x	x	x

Table 2 abbreviations: n: Number OR: Odds Ratio, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value, MFAP4: MRC: Medical Research Council Dyspnoea scale, NYHA: New York Heart Association scale, MicroFibrillar-Associated Protein 4. CI: Confidence interval.

Figure 3a: ROC curve for MRC detecting reduced LVEF (simulated data)

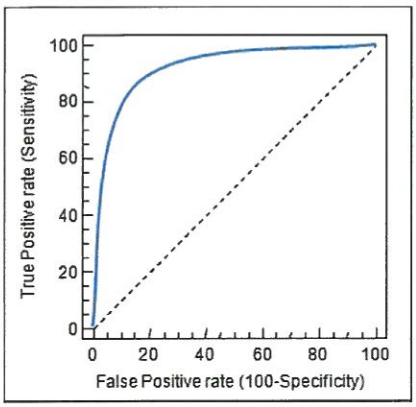

Figure 3b: ROC curve for NYHA detecting reduced LVEF (simulated data)

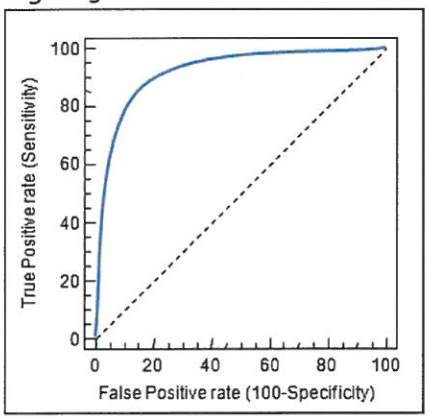

Figure 3c: ROC curve for MFAP4 detecting reduced LVEF (simulated data)

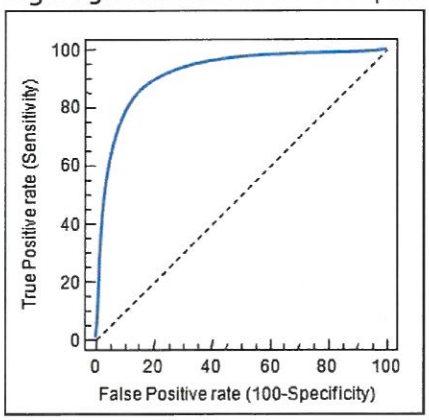

Table 3: Diagnostic outcome measures for reduced LVEF based on the best cut-offs (see figure 2)							
	LVEF ≤50 n=x	LVEF ≥50 n=x	Diagnostic OR (95% CI)	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
MRC >x n = x (%)	x	x	x	x	x	x	x
NYHA n = x (%)	x	x	x	x	x	x	x
MFAP4 >x n=x (%)	x	x	x	x	x	x	x
Table 2 abbreviations: n: Number OR: Odds Ratio, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value, MFAP4: MRC: Medical Research Council Dyspnoea scale, NYHA: New York Heart Association scale, MicroFibrillar-Associated Protein 4. CI: Confidence interval.							

References

1. Gamble C, Krishan A, Stocken D, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. *Jama* 2017;318(23):2337-43. doi: 10.1001/jama.2017.18556 [published Online First: 2017/12/21]
2. Hyldgaard C, Harders S, Blegvad J, et al. Clinical and preclinical pulmonary disease in newly diagnosed rheumatoid arthritis: a two-year follow-up study. *Scandinavian journal of rheumatology* 2023:1-8. doi: 10.1080/03009742.2023.2194105 [published Online First: 2023/04/18]
3. STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies | The EQUATOR Network 2021 [Available from: https://www.equator-network.org/reporting-guidelines/stard/.
4. Young A, Koduri G, Batley M, et al. Mortality in rheumatoid arthritis. Increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis. *Rheumatology (Oxford, England)* 2007;46(2):350-7. doi: 10.1093/rheumatology/kel253 [published Online First: 2006/08/16]
5. Fletcher C. Standardized questionaires on respiratory symptoms. A statement prepared for, and approved by, the Medical Research Council's Committee on the aetiology of chronic bronchitis. *Br Med J* 1960;2(5213):1665.
6. Bestall JC, Paul EA, Garrod R, et al. Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. *Thorax* 1999;54(7):581-86. doi: 10.1136/thx.54.7.581

7. McGavin CR, Artvinli M, Naoe H, et al. Dyspnoea, disability, and distance walked: comparison of estimates of exercise performance in respiratory disease. *Br Med J* 1978;2(6132):241-3. doi: 10.1136/bmj.2.6132.241 [published Online First: 1978/07/22]
8. Johnson-Warrington V, Harrison S, Mitchell K, et al. Exercise capacity and physical activity in patients with COPD and healthy subjects classified as Medical Research Council dyspnea scale grade 2. *J Cardiopulm Rehabil Prev* 2014;34(2):150-4. doi: 10.1097/hcr.0000000000000038 [published Online First: 2014/01/25]
9. Saekmose SG, Schlosser A, Holst R, et al. Enzyme-linked immunosorbent assay characterization of basal variation and heritability of systemic microfibrillar-associated protein 4. *PloS one* 2013;8(12):e82383. doi: 10.1371/journal.pone.0082383 [published Online First: 2013/12/11]
10. Wulf-Johansson H, Lock Johansson S, Schlosser A, et al. Localization of microfibrillar-associated protein 4 (MFAP4) in human tissues: clinical evaluation of serum MFAP4 and its association with various cardiovascular conditions. *PloS one* 2013;8(12):e82243. doi: 10.1371/journal.pone.0082243 [published Online First: 2013/12/19]
11. Blindbaek SL, Schlosser A, Green A, et al. Association between microfibrillar-associated protein 4 (MFAP4) and micro- and macrovascular complications in long-term type 1 diabetes mellitus. *Acta diabetologica* 2017;54(4):367-72. doi: 10.1007/s00592-016-0953-y [published Online First: 2017/01/01]
12. Molleken C, Sitek B, Henkel C, et al. Detection of novel biomarkers of liver cirrhosis by proteomic analysis. *Hepatology (Baltimore, Md)* 2009;49(4):1257-66. doi: 10.1002/hep.22764 [published Online First: 2009/01/30]
13. Johansson SL, Roberts NB, Schlosser A, et al. Microfibrillar-associated protein 4: a potential biomarker of chronic obstructive pulmonary disease. *Respiratory medicine* 2014;108(9):1336-44. doi: 10.1016/j.rmed.2014.06.003 [published Online First: 2014/07/16]
14. Pilecki B, Schlosser A, Wulf-Johansson H, et al. Microfibrillar-associated protein 4 modulates airway smooth muscle cell phenotype in experimental asthma. *Thorax* 2015;70(9):862-72. doi: 10.1136/thoraxjnl-2014-206609 [published Online First: 2015/06/04]
15. Hemstra LE, Schlosser A, Lindholt JS, et al. Microfibrillar-associated protein 4 variation in symptomatic peripheral artery disease. *Journal of translational medicine* 2018;16(1):159. doi: 10.1186/s12967-018-1523-6 [published Online First: 2018/06/10]
16. Issa SF, Lindegaard HM, Lorenzen T, et al. Increased serum levels of microfibrillar-associated protein 4 (MFAP4) are not associated with clinical synovitis in rheumatoid arthritis but may reflect underlying cardiovascular comorbidity. *Clinical and experimental rheumatology* 2020;38(1):122-28. [published Online First: 2019/09/10]
17. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *The European respiratory journal* 2005;26(2):319-38. doi: 10.1183/09031936.05.00034805 [published Online First: 2005/08/02]
18. Macintyre N, Crapo RO, Viegi G, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *The European respiratory journal* 2005;26(4):720-35. doi: 10.1183/09031936.05.00034905 [published Online First: 2005/10/06]
19. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung volumes. *The European respiratory journal* 2005;26(3):511-22. doi: 10.1183/09031936.05.00035005 [published Online First: 2005/09/02]
20. Holland AE, Spruit MA, Troosters T, et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. *The European respiratory journal* 2014;44(6):1428-46. doi: 10.1183/09031936.00150314 [published Online First: 2014/11/02]
21. Logstrup BB, Deibjerg LK, Hedemann-Andersen A, et al. Left ventricular function in treatment-naive early rheumatoid arthritis. *American journal of cardiovascular disease* 2014;4(2):79-86. [published Online First: 2014/07/10]

Statistical analysis plan (SAP)

1. Administrative information

1.1 Title, registration and version

Full study title	Association between serum Microfibrillar-associated protein 4 (MFAP4) and treatment response among patients with chronic inflammatory diseases initiating biological therapy: *Secondary analyses from the prospective BELIEVE cohort study*
Acronym	BELIEVE
Clinicaltrials.gov number	NCT03173144 https://clinicaltrials.gov/ct2/results?cond=&term=NCT03173144
Ethics Committee number	S-20160124
The Danish Data Protecting Agency number	xxxx-xx-xxx
A published protocol is available at BMJ OPEN:	https://bmjopen.bmj.com/content/8/2/e018166.long
SAP version	1.0
SAP date	2022-04-04

1.2. Roles and responsibility

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Statistical advisor Robin Christensen[1,2], MSc, PhD; Professor of Biostatistics & Clinical Epidemiology

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Principle investigator Torkell Ellingsen[1,2], MD, PhD; Professor (Rheumatologist)

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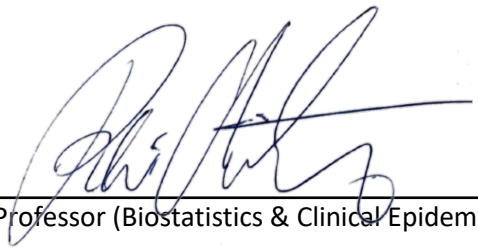
Collaborators/coming manuscript authors: Bjørk K. Sofíudóttir, Heidi L. Munk, Grith L. Sørensen, Jens Kjeldsen, Silja H. Overgaard, Signe B. Sørensen, Robin Christensen, Vibeke Andersen*, Torkell Ellingsen*

* These authors share last authorship.

1.3. Signatures:

Date: 2022-04-04 Signature: 

Bjørk Khaliqi Sofíudóttir, MD, PhD fellow

Date: April 5, 2022 Signature: 

Robin Christensen, MSc, Professor (Biostatistics & Clinical Epidemiology)

Date: April 6, 2022 Signature: 

Torkell Ellingsen, MD, Professor (Reumatologist)

2. Introduction

2.1. Purpose of the Statistical Analysis Plan (SAP)

The purpose of the SAP is to give a detailed elaboration of the statistical analyses planned to be executed on the BELIEVE Cohort study [1]. This SAP was developed after the study protocol was registered and published [2] but before finalizing data collection and conduct of any statistical analyses. Deviations from the pre-specified study protocol are summarized and explained in the SAP (section 4.2.).

Reporting of the study will follow the STROBE-guideline (*Strengthening the Reporting of Observational Studies in Epidemiology*), adhering to the guidelines for reporting of cohort studies [3], as well as the TRIPOD (*Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis*).

2.2. Background and rationale

Chronic inflammatory diseases (CIDs), such as rheumatoid arthritis (RA), psoriatic arthritis (PsA), psoriasis (PSO), Axial Spondyloarthritis (AxSpA), Crohn's disease (CD), and ulcerative colitis (UC) are recurring and lifelong diseases that have a negative impact on the quality of life of those affected. Due to general population growth the burden of the diseases is expected to increase in incidence. As described by Andersen *et al* (2017), CIDs are complex diseases that share genetic and environmental factors but differ in other factors. Drugs targeting the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α), the so-called TNF-α inhibitors, are used for severe CIDs [4-6], and act through targeting and neutralizing the effect of TNF- α, thereby diminishing the downstream effects of TNF [7]. However, these biological agents are not without side effects[8] and about 40% of patients do not respond to the treatment [9]. Currently there are no credible biomarkers enabling an effective prediction of treatment response [10].

Increased serum MicroFibrillar-Associated Protein 4 (sMFAP4) seem to reflect disease-induced processes, due to low heritability and relatively limited basal variation [11]. sMFAP4 is found with especially high expression in the heart, small intestine and the lungs. For the lungs sMFAP4 has been localized in the pulmonary arterioles and interalveolar walls [12]. Increased sMFAP4 have been correlated to liver-cirrhosis as well as diabetic neuropathy [13, 14]. Further, a study showed a possible correlation between increased sMFAP4 levels and exacerbation of chronic obstructive pulmonary disease, while another study found sMFAP4 to be a novel contributor to experimental asthma [15, 16]. Increased sMFAP4 has also been associated with increased 7 year mortality in patients with peripheral artery disease, however, the same study found a decreased risk of vascular occlusion two years after reconstructive surgery in patients with high sMFAP4 levels [17]. This indicates that increased sMFAP4 may be a marker of active inflammation in the lungs and blood vessels. A study by Issa *et al* (2020), has found sMFAP4 to be increased in early RA and further increased in manifest RA but not associated to RA disease activity or synovitis. Further, there was an inverse correlation between sMFAP4 and APCA positivity and a positive correlation to increased systolic blood pressure [18], suggesting this as modulating factors.

2.3. Aim

The overall aim is to explore the prognostic value of sMFAP4 on response to treatment in patients with CIDs initiating treatment with a biological agent.

2.4. Hypotheses

We hypothesize that high sMFAP4 levels at baseline are associated with better treatment outcomes.

2.5. Objective

To examine if treatment outcomes in patients with CID vary with different levels of sMFAP4.

Primary objective: To compare the clinical response rate in patients "exposed" to high sMFAP4 levels (defined by the upper tertile of all individual's' measured sMFAP4 levels), relative to "non-exposed" (the other 66.67% of sMFAP4 measures), on the proportion of individuals achieving a clinical response (defined individually according to their specific condition) after a period of 14-16 weeks, in patients with CID [2].

Key secondary objectives: To compare the changes in generic outcome measures in patients "exposed" to high sMFAP4 levels (defined as above), relative to "non-exposed" (defined as above), on changes in three different measures of health-related quality of life and disability (SF-12 physical component summary [PCS] and mental component summary [MCS], the Short Health Scale [SHS]), as well as physician global assessment, and C-reactive protein (CRP) from baseline to week 14-16, in patients with CID.

Exploratory objectives: To test whether sMFAP4 levels and changes in sMFAP4 (ΔsMFAP4) are associated with changes in the three different measures of health-related quality of life and disability (SF-12 physical component summary [PCS] and mental component summary [MCS], the Short Health Scale [SHS]), as well as physician global assessment, and C-reactive protein (CRP) from baseline to week 14-16, in patients with CID.

3. Study methods

3.1. Study design

The BELIEVE study was designed as a prospective, multi-center cohort study with prospective enrollment of RA, axSpA, PsA, Pso, CD and UC patients initiating treatment with a biologic agent (or switching to another) [2]. Treatment with biologics was assigned to the patients independent of participation in the BELIEVE study. When a patient was found to be a candidate for initiation of biologic treatment or switching to another biologic agent, they were invited to participate in the study. Patients were examined at two timepoints: at baseline and 14-16 weeks after treatment initiation, according to Danish clinical standards. The examination program included questionnaires, clinical assessment and sampling of blood, urine, feces, and intestinal biopsies (the latter only for UC and CD). The inclusion period was between 1st of September 2017 and 31st of March 2020 with follow-up until end of July 2020. The following centers in Denmark participated in inclusion of patients to BELIEVE: Department of Hepatology and Gastroenterology, Aarhus University Hospital; Diagnostic Centre, Silkeborg Regional Hospital; Department of Gastroenterology, Herlev and Gentofte Hospital; Medical Department, Hospital Sønderjylland; Department of Gastroenterology, Hospital of South West Jutland; Department of Medical Gastroenterology, Department of Rheumatology and Department of Dermatology and Allergy Center, Odense University Hospital.

Quantification of serum Microfibrillar-associated protein 4 (MFAP4)

Serum (s) MFAP4 levels will be detected using the AlphaLISA technique, as described in Wulf-Johansson, H., et al[22].

3.2. Sample size and power considerations

Deciding on sample size is a well-known difficulty with exploratory prognostic factor research studies. To obtain an adequate number of outcome events, we applied 'the rule of thumb', whereby 10 outcomes are needed for each independent variable. We planned to enroll 320 patients in total with the anticipation that 50% of these would experience a clinical response within the 14–16 weeks period after initiation of biological therapy. With this in mind and anticipating that we would observe at least 160 events (i.e., clinical response among the 320 patients), the study would be sufficiently powered to explore the impact of as many as 16 independent variables including condition and clinical center. Since using the 'rule of thumb' method to justify sample size is a debated practice, we went one step further and estimated the statistical power to detect differences between two sMFAP4 groups. For the contrast between groups and for a comparison of two independent binomial proportions (those with high sMFAP4 vs. other) using Pearson's χ^2 statistic, with a χ^2 approximation, with a two-sided significance level of 0.05 ($P < 0.05$), a total sample size of 318—assuming an 'allocation ratio' of 1 to 2 (one-third)—has an approximate power of 0.924 (i.e., >90% statistical power) if the anticipated proportions responding to therapy are 60% and 40%, respectively. Inclusion to the BELIEVE cohort was terminated on March 30th, 2020, reaching a sample size of 322 patients in total.

3.3. Statistical interim analyses and stopping guidelines

No statistical interim analyses were planned or executed.

3.4. Timing of final analysis

The final analyses for this study was conducted after completion of the 14-to-16-week follow-up visit of the last patient included in the study. The serum MFAP4 measurements and the statistical analyses described in the present SAP will be conducted after the completion of this SAP has been approved and revised by the research team and collaborators. Biological samples collected in the BELIEVE study are part of this project regarding detection of sMFAP4, as well as other exploratory objectives and the results will be presented in separate coming peer reviewed papers.

3.5. Timing of outcome assessments

As described in the pre-specified protocol [2], patients were examined at two time points; at baseline and 14-16 weeks after treatment initiation, where primary endpoints were evaluated. Taking the COVID-19 pandemic into account, we decided to allow a window of +/- 4 weeks for outcome assessment in order to prevent missing outcomes.

4. Statistical principles

4.1. Statistical significance and confidence intervals

All *P* values and 95% confidence intervals will be two sided. We will not apply explicit adjustments for multiplicity, rather we will interpret the analysis of the secondary objectives according to the Hochberg sequential procedure [19]: The analyses of the secondary outcomes will be performed, and the corresponding *P* values will be ordered from largest to smallest in a list. If the largest *P* value is less than the significance level of 0.05, then all the tests are considered significant. In contrast, if the largest *P* value fails to show statistically significance, then progressively more stringent P values are applied as a significance level (the second largest *P* values: 0.05/2, the third largest: 0.05/3 and so on). When a test succeeds to show a statistically significant difference, then the remaining tests downstream from this are considered significant. Thus, comparisons continue until a test shows a statistical difference or until all comparisons are made. The key secondary statistical tests will be reported with *P* values for hypothesis tests and claims of potential statistical significance. Further, due to potential issues of multiplicity following multiple testing, we will interpret 'statistically significant' findings in the context of whether the 95% confidence interval (CI) excludes outcomes that could be perceived as clinically important. Finally, we will use the Spearman's rank-order correlation (the nonparametric version of the Pearson product-moment correlation) to explore associations with sMFAP4 levels and changes in sMFAP4 (ΔsMFAP4) are associated with the different collected outcome measures; Spearman's correlation coefficient, (ρ, also signified by rs) measures the strength and direction of association between two ranked variables at a time.

4.2. Protocol deviations

Eligibility criteria

Protocol: Eligible patients were patients diagnosed with a CID, including RA, axSpA, PsA, PsO, CD, UC, Hidradenitis Suppurativa (HS) and non-infectious Uveitis (niU) that are initiating biological therapy targeting TNF, who are naïve to biologics.

SAP modifications from the original protocol:

1) There were no patients with Hidradenitis Suppurativa and Non-infectious Uveitis included in the study.
2) Patients initiating all kinds of biological therapy were enrolled instead of only TNFi therapy*.
3) In addition, patients that previously have received biologic treatment were also made eligible*.

**We will apply these new strata in subsequent sensitivity analyses*

Rationale:

1) The Ophthalmology Department, who was expected to enroll patients with non-infectious uveitis did not engage in BELIEVE after all. In the period where The Department of Dermatology and Allergy Centre, OUH were engaged in enrollment of patients, there were too few patients with Hidradenitis Suppurativa initiating biological therapy.

2+3) We decided to broaden the inclusion criteria to increase the study population.

Enrollment period

Protocol: Participant enrollment was expected to run from 1st of April 2017 to 31st of March 2019 or until a minimum of 100 patients with inflammatory bowel disease (CD and UC), 100 patients with RA, and 120 patients with axSpA, PsA, PsO, HS and niU were achieved.

SAP: The enrollment period was between 1st of September 2017 and 31st of March 2020.

Rationale: We extended the enrollment period with a year to increase the study population.

Primary endpoint

Protocol: The disease specific primary endpoint for UC is a Mayo Clinic Index of 2 or less (with no individual subscore >1).

SAP: In cases, where a full Mayo Clinic Index is missing, we will accept a Partial Mayo Clinic Index, which is the Mayo Clinic Index without the endoscopic subscore. However, in these cases, the primary endpoint will be a Partial Mayo Clinic Index of 1 or less [20, 21].

Rationale: Some IBD patients refrained from repeated colonoscopies, hence hindering calculation of a full mayo score for UC patients.

Timing of outcome assessment

Protocol: Patients were examined at two time points; at baseline and 14-16 weeks after treatment initiation, where primary endpoints are evaluated.

SAP: Outcome assessment was scheduled to be 14-16 weeks after treatment initiation but a window of +/- 4 weeks are allowed.

Rationale: Taking the COVID-19 pandemic into account, we decided to allow a window of +/- 4 weeks for outcome assessment at the follow-up visit in order to prevent a large number of missing outcomes.

4.3. Analysis populations

The primary analysis will be conducted on the Intention-to-Treat (ITT) population. According to the ITT principle, all CID participants with available serum samples for MFAP4 measurements at baseline will be included in the analysis regardless of their adherence to the study protocol [22]. A *per-protocol* population will be defined as those who adhered to the biological treatment during the observation period and have complete data used for the analyses and have, at the end of the study, no major protocol violations.

5. Study population

5.1. Screening data

The total number of patients initiating biological therapy screened for eligibility was not registered. An estimate will be calculated based on the usual number of patients initiating biological therapy per month at each clinical center multiplied with the number of months the center has participated in the patient enrolment.

5.2. Eligibility

5.2.1. Inclusion criteria

- Patients with a verified diagnosis of RA, axSpA, PsA, PsO, CD, UC, HS or niU
- Initiation of biological therapy

5.2.2. Exclusion criteria

- Age <18 years
- Unable to read and understand Danish
- Mentally unable to answer the questionnaire

5.2. Recruitment

A flow diagram adapted from http://www.consort-statement.org/ will be used to visualize the flow of participants stratified by baseline "exposure group"; showing the number of people screened, reasons for ineligibility, the number of participants who consented and the number analyzed (summarized by type of exposure, i.e. sMFAP4 levels) (see fig. 1).

5.3. Adherence and reasons for withdrawal

The number of patients adhering to the protocol, i.e., those who continued the biological treatment from baseline to the follow-up visit, will be accounted for in the flowchart. Likewise, the flowchart will also account for the number of patients deviating from the protocol, i.e., they did not initiate biologic treatment after all, they ended treatment due to either adverse events or lack of efficacy, or they withdrew their consent (fig. 1).

5.4. Baseline patient characteristics

Baseline characteristics will be summarized by the defined sMFAP4 exposure groups in table 1 (manuscript outline). The information is extracted from the baseline clinical assessment together with the baseline questionnaire which was sent electronically to the participants prior to treatment initiation. Data will be presented as means with standard deviations (SD) when normally distributed or as medians with interquartile range in case of skewed data. Dichotomous and categorical variables will be presented as absolute numbers and proportions.

The table will include information on age, sex, body mass index (BMI), smoking status, diagnosis, disease duration, medication (current medication and number of previous biological medications used), patient reported outcome measures (short health scale, SF-12 physical component summary [PCS], SF-12 mental component summary [MCS]) as well as physician global assessment and CRP.

6. Analysis

6.1. Outcome definitions, measurement and calculation

6.1.1. Primary outcome

The primary endpoint [2] is the proportion of patients with a clinical response to therapy 14-16 weeks after treatment initiation. Thus, the primary outcome will be a specific dichotomous endpoint depending on the disease specific definitions of clinical response, defined below:

- RA/Rheumatoid arthritis: clinical response, defined as at least a 20% improvement according to the criteria of the American College of Rheumatology (ACR20) [23, 24].
- PsA/Psoriatic arthritis: clinical response, defined as at least a 20% improvement according to the criteria of ACR20 [25].
- AxSpA/Axial spondyloarthritis: clinical response, defined as at least a 20% improvement according to the Assessment of Spondyloarthritis International Society (ASAS20) [26, 27].
- PsO/Psoriasis: clinical response, defined as at least a 75% improvement in the Psoriasis Area and Severity Index (PASI 75) [28].
- CD/Crohn's disease: clinical remission, defined as Harvey-Bradshaw Index (HBI) of 4 or less [29].
- UC/Ulcerative colitis: clinical remission, defined as Mayo Clinic Score of 2 or less (with no individual subscore of >1) [30-32].

6.1.2. Key secondary outcome measures

Predefined key secondary outcomes include changes in generic outcomes from baseline to follow-up (14-16 weeks after treatment initiation) consisting of both clinical and patient reported outcomes (PRO):

- Health-related quality of life (the physical [PCS] and mental component summary measure [MCS] from the Short Form Health Survey [SF-12]
- Physician global assessment (0-100 mm VAS)
- Acute phase reactant (CRP)
- The Short Health Scale [SHS])
- Patient global assessment (0-100 mm VAS)

The 12-item SF-12 is a shorter form of the Short Form 36 (SF-36) which is widely used for measuring Health-related quality of life (HRQoL) and has a range of 0-100 (higher values indicate better health), mean of 50 and a standard deviation of 10. The SF-12 questionnaire comprises questions about physical and social functioning over the past 4 weeks. From the SF-12, two summary measures can be estimated; PCS and MCS [33].

Physician global assessment and patient global assessment* is assessed on 100 mm VAS.

* Patient global assessment is not collected for PsO, CD and UC.

SHS is a four-item questionnaire assessing the patient's subjective experience of how their disease influences four health dimensions: 1) symptom burden, 2) functional status, 3) disease-related burden and 4) general well-being [34]. The four questions are graded on 100 mm VAS and presented individually, where higher scores indicate negative experience. The SHS has been validated for use in UC [34] and CD [35].

CRP: Blood samples as specified in the protocol were collected by a trained laboratory technician and handled according to set procedures. Results from analysis of blood samples will be limited to C-reactive protein (mg/L) in this study and measurements of sMFAP4.

Additionally, a generic outcome, which is not specifically defined in the published protocol [2], is the proportion of patients continuing the biologic treatment (yes/no) after the 14-16 week period.

6.1.3. Other secondary outcomes

Other secondary outcomes are non-generic outcomes specific to a single or two of the CIDs and are listed in the table below (Table A). Unless otherwise specified, the outcomes are changes from baseline to follow-up. Some outcomes are a composite of other outcomes and are grouped together.

6.1.4. Exposure and confounding variables

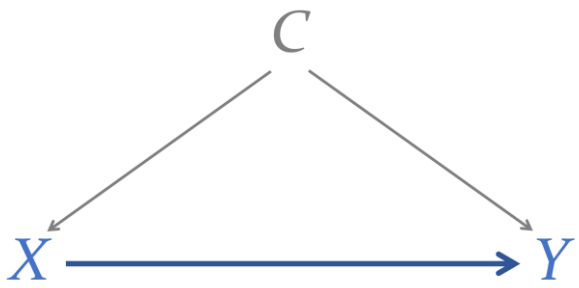
Exposure variables

Participants will be stratified into an exposed and unexposed group based on the serum MFAP4 measured at baseline. The exposed group will be the upper tertile (33.3%) of the cohort based on levels of MFAP4 detected in serum (High MFAP4). The unexposed group will be composite of the medium and lower tertile of the cohort based on MFAP4 detected in serum (66.7%) (Other MFAP4).

Confounding variables

Mandatory (design-related) adjusting variables will include diagnosis (i.e. type of CID). Cohort studies are challenged by non-randomized allocation which can cause selection bias with corresponding confounding variables. Confounding variables are independent variables other than the exposure variable (i.e. the two different sMFAP4 exposure groups) which is associated with the outcome measure. To increase the comparability of our two exposure groups (the balance between groups), we are adjusting observations from each group based on the propensity score.

Propensity score analysis seeks to isolate the treatment as the only difference between our groups. Thus, propensity score methods attempt to correct for the assignment mechanism by providing a "balancing variable that creates" control units similar to treatment units at baseline (i.e. Y_o |High sMFAP4 ≈ Y_o |Other sMFAP4). In order to identify possible confounders and develop propensity scores, we will use the following pragmatic definition adapted from the SAP of the primary analysis of the BELIEVE cohort study (illustrated below) of what potentially makes a confounding variable (*C*):



- The Covariate (*C*) is an ancestor (cause) of the outcome (*Y*)
- The Covariate (*C*) probably causes the exposure (*X*; e.g. group)
- The Covariate (*C*) is not a descendant (effect) of the exposure (*X*) or the outcome (*Y*)

We will test for impact of possible confounding of the association of our primary outcome with, age, sex, smoking status (ordinal scale 0-3 and four groups of smoking frequency) and body mass index (BMI).

6.2. Analysis methods

The baseline characteristics of the participants (**Table 1**) will be summarized for each dietary exposure profile (High sMFAP4 and other sMFAP4) using descriptive statistics. Data will be presented as either means and standard deviations (SDs), medians and interquartile ranges (IQRs), or numbers and percentages for binary outcomes.

The primary outcome is the proportion of patients with a clinical response to therapy 14-16 weeks after treatment initiation. Response is defined for each CID (disease-specific endpoints; see section 6.1.1.). The number and proportion of participants who achieved treatment response will be summarized based on levels of sMFAP4 (Fig. 2 and Table 2). We will investigate the association between sMFAP4 levels at baseline (High sMFAP4 *vs*. other sMFAP4) and treatment response (yes *vs.* no) based on the ITT population with a logistic regression analysis. A simple (unadjusted) logistic regression model and an adjusted model (adjusted for type of age, sex, smoking status and BMI) will be presented in Table 2. The Odds ratio (OR) of achieving clinical response at week 14-16 for CID patients in the High sMFAP4 group (the upper 33.3 % of the study population based on the baseline serum levels of MFAP4) *versus* the Other sMFAP4 group (the lower 66.7 %) will be reported along with the 95 % confidence interval (CI) and two-sided p-value (fig. 2 and table 2 templates).

Secondary analyses will include repeating the logistic regression models for secondary treatment outcomes using the predictors of interest; the upper 33.3% (high sMFAP4) vs. the lower 66.6% (other sMFAP4) measured at baseline, in relation to predicting improvement in both clinical and patient reported outcomes (table 2 template).

Exploratory analysis will include Spearman Rank order correlation of sMFAP4 levels as well as changes in sMFAP4 levels, in clinical and patient reported outcomes.

The results from the primary and secondary objectives will be presented in table 2, while exploratory analyses will be presented in table 3. Results from secondary explorative analyses will be presented in Table S1 among the Appendix files.

6.2.1. Sensitivity and subgroup analysis

Sensitivity

For the purpose of sensitivity, multiple sensitivity analyses will be performed to assess the robustness of the primary analyses. Sensitivity analyses will include analyses based on 1) the 'non-responder-imputation' 2) the per protocol population, 3) the population meeting the unmodified eligibility criteria (exclusion of patients initiating treatment with biologics targeting other agents than TNF and patients previously treated with biologics). Results of the sensitivity analyses are presented in the Appendix, table 2.

Non-responder imputation will be the approach used for missing data in the primary analyses. It represents a simplistic 'null response imputation' and represents a conservative base case and is potentially valid even if data is '*missing not at random*' (MNAR) [22] as it assumes and implies that patients have not improved or have worsened after entering the study independent of their baseline characteristics.

6.3. Missing data

Every effort will be made to minimize missing outcome data. A simplistic 'non-responder imputation' will be used to account for participants who have MFAP4 measured at baseline but are missing the clinical outcome(s) at follow-up.

6.4. Statistical software

Statistical programming will be done using the software STATA version 16.0 with transparent reporting of the source code used to analyze the data.

7. References

1. Gamble, C., et al., *Guidelines for the Content of Statistical Analysis Plans in Clinical Trials.* Jama, 2017. **318**(23): p. 2337-2343.
2. Christensen, R., et al., *Impact of red and processed meat and fibre intake on treatment outcomes among patients with chronic inflammatory diseases: protocol for a prospective cohort study of prognostic factors and personalised medicine.* BMJ Open, 2018. **8**(2): p. e018166.

3. Vandenbroucke, J.P., et al., *Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration.* Ann Intern Med, 2007. **147**(8): p. W163-94.
4. Acosta-Colman, I., et al., *GWAS replication study confirms the association of PDE3A-SLCO1C1 with anti-TNF therapy response in rheumatoid arthritis.* Pharmacogenomics, 2013. **14**(7): p. 727-34.
5. Geiler, J., M. Buch, and M.F. McDermott, *Anti-TNF treatment in rheumatoid arthritis.* Curr Pharm Des, 2011. **17**(29): p. 3141-54.
6. Leso, V., et al., *Role of the tumor necrosis factor antagonists in the treatment of inflammatory bowel disease: an update.* Eur J Gastroenterol Hepatol, 2010. **22**(7): p. 779-86.
7. Andersen, V., et al., *A Proposal for a Study on Treatment Selection and Lifestyle Recommendations in Chronic Inflammatory Diseases: A Danish Multidisciplinary Collaboration on Prognostic Factors and Personalised Medicine.* Nutrients, 2017. **9**(5).
8. Singh, J.A., et al., *Adverse effects of biologics: a network meta-analysis and Cochrane overview.* Cochrane Database Syst Rev, 2011. **2011**(2): p. Cd008794.
9. Hanauer, S.B., et al., *Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial.* Lancet, 2002. **359**(9317): p. 1541-9.
10. Smolen, J.S. and D. Aletaha, *Forget personalised medicine and focus on abating disease activity.* Ann Rheum Dis, 2013. **72**(1): p. 3-6.
11. Saekmose, S.G., et al., *Enzyme-linked immunosorbent assay characterization of basal variation and heritability of systemic microfibrillar-associated protein 4.* PLoS One, 2013. **8**(12): p. e82383.
12. Wulf-Johansson, H., et al., *Localization of microfibrillar-associated protein 4 (MFAP4) in human tissues: clinical evaluation of serum MFAP4 and its association with various cardiovascular conditions.* PLoS One, 2013. **8**(12): p. e82243.
13. Blindbaek, S.L., et al., *Association between microfibrillar-associated protein 4 (MFAP4) and micro- and macrovascular complications in long-term type 1 diabetes mellitus.* Acta Diabetol, 2017. **54**(4): p. 367-372.
14. Molleken, C., et al., *Detection of novel biomarkers of liver cirrhosis by proteomic analysis.* Hepatology, 2009. **49**(4): p. 1257-66.
15. Johansson, S.L., et al., *Microfibrillar-associated protein 4: a potential biomarker of chronic obstructive pulmonary disease.* Respir Med, 2014. **108**(9): p. 1336-44.
16. Pilecki, B., et al., *Microfibrillar-associated protein 4 modulates airway smooth muscle cell phenotype in experimental asthma.* Thorax, 2015. **70**(9): p. 862-72.
17. Hemstra, L.E., et al., *Microfibrillar-associated protein 4 variation in symptomatic peripheral artery disease.* J Transl Med, 2018. **16**(1): p. 159.
18. Issa, S.F., et al., *Increased serum levels of microfibrillar-associated protein 4 (MFAP4) are not associated with clinical synovitis in rheumatoid arthritis but may reflect underlying cardiovascular comorbidity.* Clin Exp Rheumatol, 2020. **38**(1): p. 122-128.
19. Cao, J. and S. Zhang, *Multiple comparison procedures.* Jama, 2014. **312**(5): p. 543-4.
20. Walsh, A.J., R.V. Bryant, and S.P. Travis, *Current best practice for disease activity assessment in IBD.* Nat Rev Gastroenterol Hepatol, 2016. **13**(10): p. 567-79.
21. Turner, D., et al., *A systematic prospective comparison of noninvasive disease activity indices in ulcerative colitis.* Clin Gastroenterol Hepatol, 2009. **7**(10): p. 1081-8.
22. White, I.R., et al., *Strategy for intention to treat analysis in randomised trials with missing outcome data.* Bmj, 2011. **342**: p. d40.
23. Taylor, P.C., et al., *Baricitinib versus Placebo or Adalimumab in Rheumatoid Arthritis.* N Engl J Med, 2017. **376**(7): p. 652-662.
24. Felson, D.T., et al., *American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis.* Arthritis Rheum, 1995. **38**(6): p. 727-35.
25. Mease, P.J., et al., *Secukinumab Inhibition of Interleukin-17A in Patients with Psoriatic Arthritis.* N Engl J Med, 2015. **373**(14): p. 1329-39.
26. Baeten, D., et al., *Secukinumab, an Interleukin-17A Inhibitor, in Ankylosing Spondylitis.* N Engl J Med, 2015. **373**(26): p. 2534-48.
27. Sieper, J., et al., *The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis.* Ann Rheum Dis, 2009. **68 Suppl 2**: p. ii1-44.
28. Lebwohl, M., et al., *Phase 3 Studies Comparing Brodalumab with Ustekinumab in Psoriasis.* N Engl J Med, 2015. **373**(14): p. 1318-28.
29. Khanna, R., et al., *Early combined immunosuppression for the management of Crohn's disease (REACT): a cluster randomised controlled trial.* Lancet, 2015. **386**(10006): p. 1825-34.
30. Vermeire, S., et al., *Etrolizumab as induction therapy for ulcerative colitis: a randomised, controlled, phase 2 trial.* Lancet, 2014. **384**(9940): p. 309-18.

31. Harvey, R.F. and J.M. Bradshaw, *A simple index of Crohn's-disease activity.* Lancet, 1980. **1**(8167): p. 514.
32. Schroeder, K.W., W.J. Tremaine, and D.M. Ilstrup, *Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study.* N Engl J Med, 1987. **317**(26): p. 1625-9.
33. Gandek, B., et al., *Cross-validation of item selection and scoring for the SF-12 Health Survey in nine countries: results from the IQOLA Project. International Quality of Life Assessment.* J Clin Epidemiol, 1998. **51**(11): p. 1171-8.
34. Hjortswang, H., et al., *The Short Health Scale: a valid measure of subjective health in ulcerative colitis.* Scand J Gastroenterol, 2006. **41**(10): p. 1196-203.
35. Stjernman, H., et al., *Short health scale: a valid, reliable, and responsive instrument for subjective health assessment in Crohn's disease.* Inflamm Bowel Dis, 2008. **14**(1): p. 47-52.

8. Manuscript outline

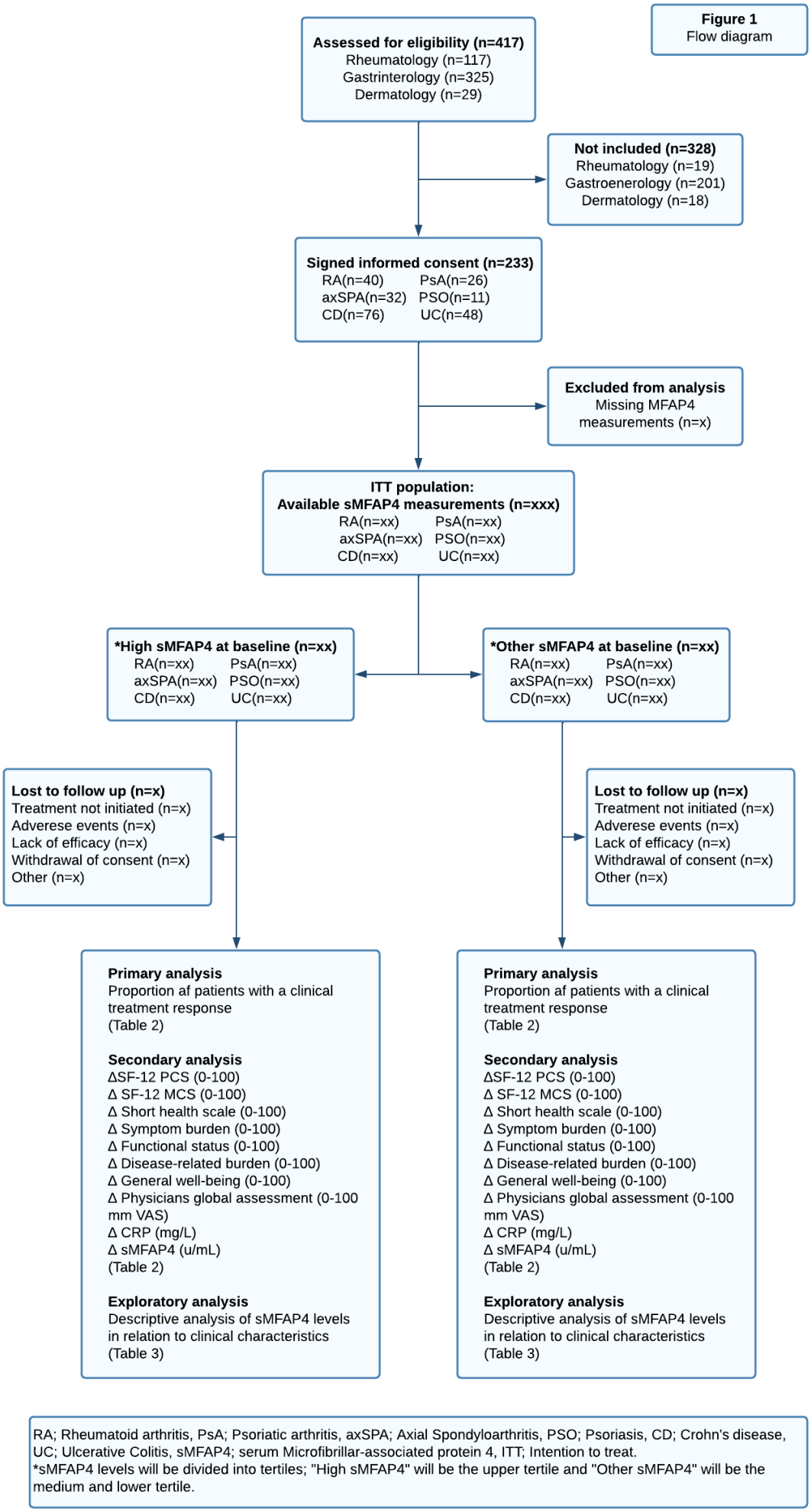
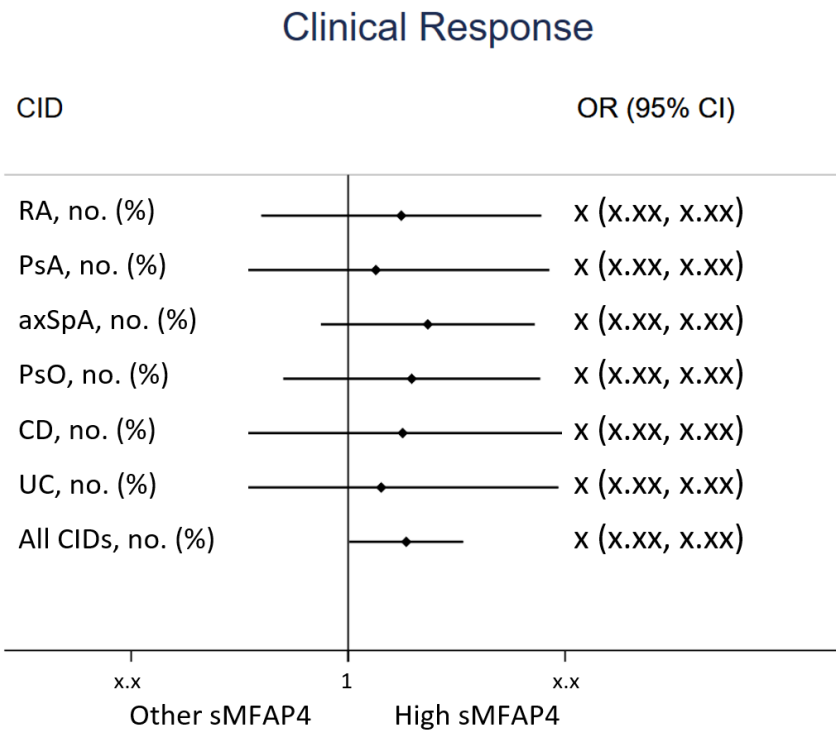


Table 1. Baseline Characteristics in the ITT population

Characteristic	Total (n=xxx)	High sMFAP4	N (High sMFAP4)	Other sMFAP4	N (Other sMFAP4)	SMD*	P-value
Age (years), mean (SD)							
Female, n (%)							
BMI (kg/m²), mean (SD)							
Smoking status, no. (%):							
Non-smoker							
Former smoker							
Occasionally							
Daily							
Smoking category (score: 0-4)							
CID diagnosis, no. (%):							
Rheumatoid arthritis							
Psoriasis arthritis							
Axial Spondylarthropathy							
Psoriasis							
Crohn's disease							
Colitis ulcerosa							
Disease duration (years), median (IQR)							
Naïve to biological treatment, no. (%):							
Medication, no. (%)							
None							
NSAID, daily use							
Corticosteroids							
Immunomodulators							
5-ASA/SASP							
Leflunomide							
Hydroxycloroquin							
Antibiotics							
Secondary outcome measures							
SF-12 PCS (0-100)							
SF-12 MCS (0-100)							
Short health scale (0-100)							
Symptom burden (0-100)							
Functional status (0-100)							
Disease-related burden (0-100)							
General well-being (0-100)							
Phys. Global assessment (0-100)							
CRP, mg/L							
Exploratory outcome measure:							
sMFAP4 (U/mL)							

*A standardized difference (StandDiff) between High sMFAP4 and other sMFAP4 levels above 0.5 SD-units will be evaluated as a potential (data driven) confounding variable, High sMFAP4; upper tertile of serum Microfibrillar-associated protein 4 (sMFAP4) measurements, Other sMFAP4; medium and lower tertile of sMFAP4, CID; Chronic inflammatory disease, SD; Standard deviation, IQR; Interquartile range, NSAID; Non-steroidal Anti-inflammatory Drugs, 5-ASA/SASP; 5-aminosalicyclic acid/sulfasalazine, SF-12; 12-item short form survey, PCS; physical component summary, MCS; mental component summary, Phys.; physician, VAS; visual analog scale, CRP; C-reactive protein.

Figure 1. Forest plot; Effect of MFAP4 profile on treatment response. Data are simulated for the purpose of visualization.



Explanation: CID; Chronic Inflammatory Disease, OR; Odds ratio, CI; Confidence interval, CD; Crohn's disease, UC; Ulcerative Collitis, RA; Rheumatoid Arthritis, axSpA; axial Spondyloarthritis, PsA; Psoriatic arthritis, Pso; Psoriasis, sMFAP4; High sMFAP4 (the exposed group: the upper 33.3 % of the study sample based on the levels of sMFAP4 measurements), Other sMFAP4 (the unexposed group; the lower 66.7 % of the study sample based on the level of sMFAP4 measurements)

Table 2: Primary and key secondary outcomes. Values are numbers (percentages) with odds ratios (95% CI) for dichotomous outcomes and least squares means with differences (95% CI) for the continuous variables.

	Crude model[1]				Adjusted model[2]			
Outcome	High sMFAP4	Other sMFAP4	Contrast (95%CI)	*P*-value*	High sMFAP4	Other sMFAP4	Contrast (95%CI)	*P*-value*
Primary outcome (composite)								
Clinical responders, no. (%)								
Sub-components, no. (%)								
Rheumatoid arthritis, ACR20								
Psoriatic arthritis, ACR20								
Axial Spondyloarthritis, ASAS 20								
Psoriasis, PASI75								
Crohn's Disease, HBI ≤ 4								
Ulcerative Colitis, Mayo ≤ 2								
Key secondary outcomes								
Health-related quality of life:								
Δ SF-12 PCS (0-100)								
Δ SF-12 MCS (0-100)								
Δ Short health scale (0-100)								
Δ Symptom burden (0-100)								
Δ Functional status (0-100)								
Δ Disease-related burden (0-100)								
Δ General well-being (0-100)								
Δ Physicians global assessment (0-100 mm VAS)								
Δ CRP (mg/L)								
Δ sMFAP4 (u/mL)								
Safety/harms, no. (%)								
Continuation of treatment, no. (%)								
Withdrawals								
Discontinuation due to adverse events								
Serious adverse events (SAEs)								
Deaths								

[1]The crude model is adjusted for CID condition as the only default factor
[2]The adjusted model is adjusted for CID, age, sex, smoking status and BMI

Figure 3: ROC curve various levels of sMAP4 in predicting treatmet response in all CIDs

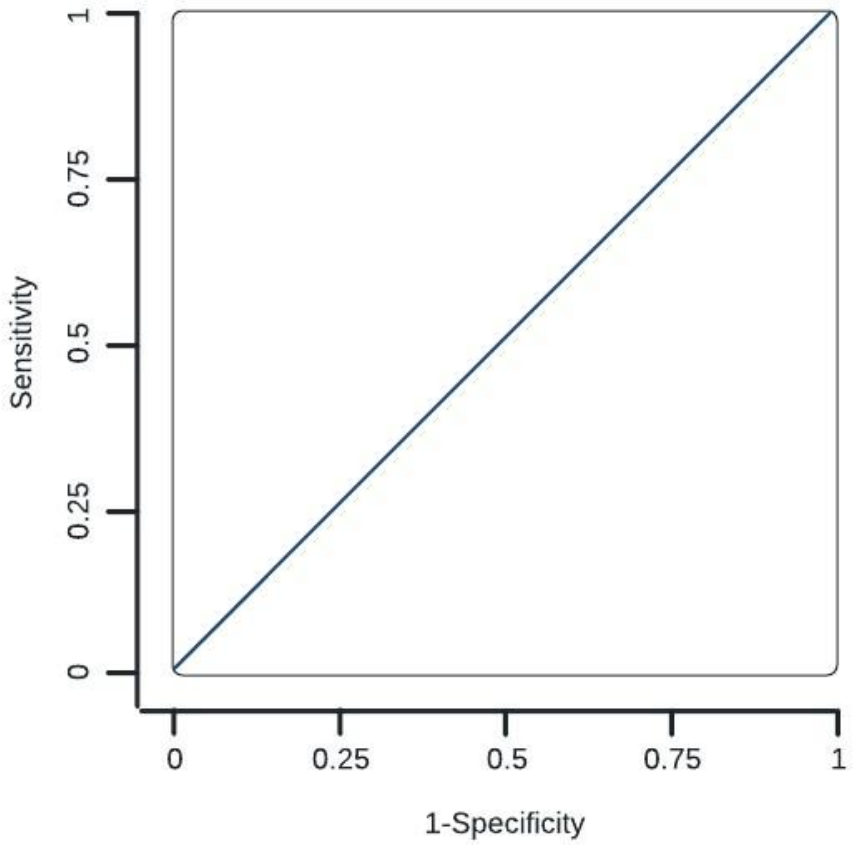


Table 3: Exploratory analyses. Descriptive analysis of sMFAP4 levels in relation to clinical characteristics and change in sMFAP4 (Δ MFAP4) based on Spearman's Rank-Order Correlation.

	Baseline sMFAP4 (U/mL), mean (SD)	Δ sMFAP4
Age	r =	r =
	p =	p =
Female	r =	r =
	p =	p =
BMI	r =	r =
	p =	p =
Current smoker	r =	r =
	p =	p =
Baseline CRP (mg/L)	r =	r =
	p =	p =
Change from baseline:		
Δ SF-12 PCS (0-100)	r =	r =
	p =	p =
Δ SF-12 MCS (0-100)	r =	r =
	p =	p =
Δ Short health scale (0-100)	r =	r =
	p =	p =
Δ Symptom burden (0-100)	r =	r =
	p =	p =
Δ Functional status (0-100)	r =	r =
	p =	p =
Δ Disease-related burden (0-100)	r =	r =
	p =	p =
Δ General well-being (0-100)	r =	r =
	p =	p =
Δ CRP (mg/L)	r =	r =
	p =	p =
Δ Physicians global assessment (0-100 mm VAS)	r =	r =
	p =	p =

Appendix table S1: Changes from baseline in all outcome measures stratified by CID

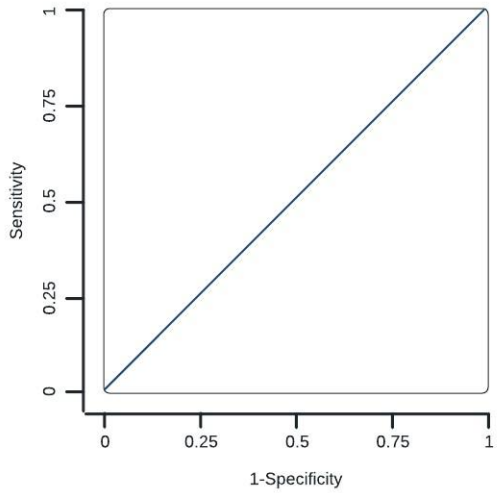
	RA	PsA	axSpA	PsO	CD	UC	All CIDs
Δ SF-12 PCS (0-100)							
Δ SF-12 MCS (0-100)							
Δ Short health scale (0-100)							
Δ Symptom burden (0-100)							
Δ Functional status (0-100)							
Δ Disease-related burden (0-100)							
Δ General well-being (0-100)							
Δ sMFAP4 (U/mL)							
Δ CRP (mg/L)							
Δ Physicians global assessment (0-100 mm VAS)							
Continuation of treatment, no. (%)							

*Values are medians (IQR), unless otherwise stated. IQR; Interquartile range.

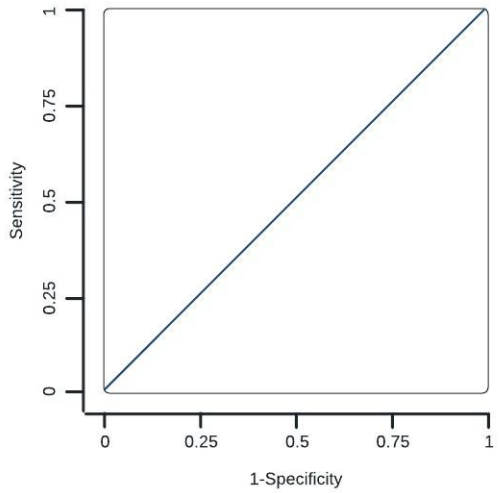
Appendix

Figure S1 ROC-curves for all of the six CIDs (A,B,C,D,E,F)

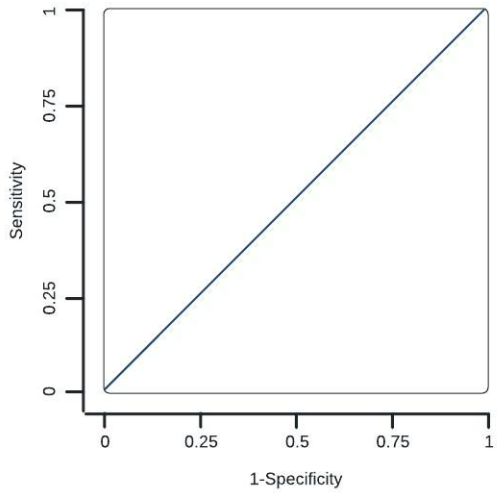
A) High sMFAP4 in treatment response in RA



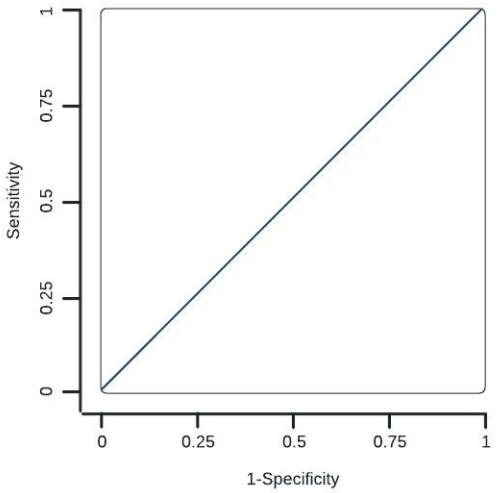
B) High sMFAP4 in treatment response in PsA



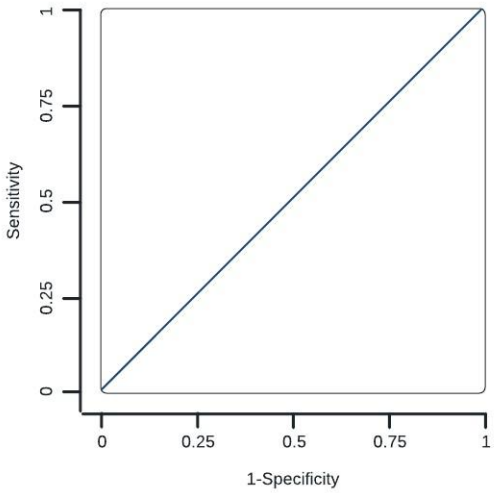
C) High sMFAP4 in treatment response in AxSpA



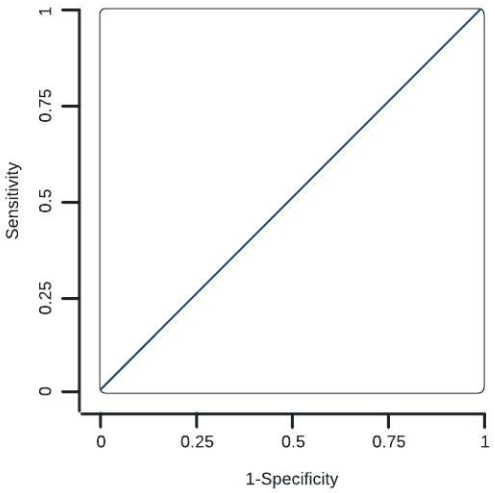
D) High sMFAP4 in treatment response in PsO



E) High sMFAP4 in treatment response in CD



F) High sMFAP4 in treatment response in UC



Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2(Abstract)
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2 (Abstract follows AC&R guidelines)
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4
	4	Study objectives and hypotheses	4
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	4-5
Participants	**6**	Eligibility criteria	5
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5
	9	Whether participants formed a consecutive, random or convenience series	5
Test methods	**10a**	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	7
	11	Rationale for choosing the reference standard (if alternatives exist)	7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	7
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	8
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	8
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8-9
	15	How indeterminate index test or reference standard results were handled	8 + 11
	16	How missing data on the index test and reference standard were handled	8
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	8
	18	Intended sample size and how it was determined	9
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 1
	20	Baseline demographic and clinical characteristics of participants	10 (+Table 1)
	21a	Distribution of severity of disease in those with the target condition	N/A
	21b	Distribution of alternative diagnoses in those without the target condition	11-12
	22	Time interval and any clinical interventions between index test and reference standard	8
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Table1
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	10 + Table 2
	25	Any adverse events from performing the index test or the reference standard	12
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	15
	27	Implications for practice, including the intended use and clinical role of the index test	16
OTHER INFORMATION			
	28	Registration number and name of registry	19
	29	Where the full study protocol can be accessed	19
	30	Sources of funding and other support; role of funders	18

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

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Best
Laura

On Thu, 8 Feb 2024 at 08:46, Bjørk K. Sofíudóttir <Bjork.K.Sofiudottir@rsyd.dk> wrote:

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>
> Sofíudóttir BK, Harders SMW, Lage-Hansen PR, *et al*
>
> Using thoracic ultrasound to detect interstitial lung disease in patients with rheumatoid arthritis: a protocol for the diagnostic test accuracy AURORA study
>
> *BMJ Open* 2022;**12:**e067434. doi: 10.1136/bmjopen-2022-067434
>
> Sincerely,
>
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