Article:
Dumitru, RB, Bissell, LA, Erhayiem, B et al. (11 more authors) (2021) Predictors of subclinical systemic sclerosis primary heart involvement characterised by microvasculopathy and myocardial fibrosis. Rheumatology, 60 (6). pp. 2934-2945. ISSN 1462-0324

https://doi.org/10.1093/rheumatology/keaa742

Title: Predictors of subclinical systemic sclerosis primary heart involvement characterised by microvasculopathy and myocardial fibrosis

Authors: Raluca B. Dumitru[1 2], Lesley-Anne Bissell[1 2], Bara Erhayiem[3], Graham Fent[3], Ananth Kidambi[3], Peter Swoboda[3], Giuseppina Abignano[1 2], Helena Donica[4], Agata Burska[1 2], John P. Greenwood[3], John Biglands[2], Francesco Del Galdo[1 2], Sven Plein[3], Maya H. Buch[1 5 6]

Affiliations:

[1]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, United Kingdom

[2]National Institute for Health Research, Leeds Biomedical Research Centre, United Kingdom

[3]Department of Biomedical Imaging Science, Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, United Kingdom

[4] Department of Biochemical Diagnostics, Medical University of Lublin, Poland

[5]Centre for Musculoskeletal Research, University of Manchester, Manchester, UK

[6]NIHR Manchester Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK

Corresponding author

Professor Maya H Buch

AV Hill, Centre for Musculoskeletal Research, School of Biological Sciences, Faculty of Biology, Medicine & Health, University of Manchester, UK

Email: maya.buch@manchester.ac.uk

Telephone: +44 (0)161 306 0696

Keywords: systemic sclerosis primary heart involvement, cardiovascular magnetic resonance, risk stratification

Key messages:

- Cardiovascular magnetic resonance detects subclinical microvasculopathy, myocardial focal and diffuse fibrosis in systemic sclerosis (SSc)
- Hs-TnI, NT-proBNP, markers of disease severity, complicated peripheral vasculopathy predict subclinical SSc-primary heart involvement (SSc-pHI)
- This largest CMR study provides a basis for risk stratification in SSc-pHI

Abstract

Objectives

Systemic sclerosis primary heart involvement (SSc-pHI) is a significant cause of mortality. We aimed to characterise and identify predictors of subclinical SSc-pHI using cardiovascular magnetic resonance (CMR).

Methods

Eighty-three SSc patients with no history of cardiovascular disease or pulmonary arterial hypertension and 44 healthy controls (HC) underwent 3Tesla contrast-enhanced CMR including T1 mapping and quantitative stress perfusion. High-sensitivity troponin I (Hs-TnI) and N-terminal pro-brain natriuretic peptide (NT-proBNP) were also measured.

Results

CMR revealed lower myocardial perfusion reserve (MPR) compared to HC [median (IQR) 1.9 (1.6, 2.4) vs 3 (2, 3.6), p<0.001]. Late gadolinium enhancement (LGE), indicating focal fibrosis was observed in 17/83 patients but in no HC, with significantly higher extracellular volume (ECV), suggestive of diffuse fibrosis in SSc vs HC [mean (SD) 31 (4) vs 25 (2), p<0.001]. Presence of LGE and higher ECV associated with skin score (OR=1.115, p=0.048; R^2=0.353, p=0.004), and ECV and MPR associated with the presence of digital ulcers at multivariate analysis (R^2=0.353, p<0.001; R^2=0.238, p=0.011). Hs-TnI was significantly higher in patients with LGE, and NT-proBNP associated with ECV (p<0.05).

Conclusion

Subclinical SSc-pHI is characterised by myocardial microvasculopathy, diffuse and focal myocardial fibrosis but preserved myocardial contractile function. This subclinical phenotype of SSc-pHI associates with Hs-TnI, NT-proBNP, SSc disease severity and complicated peripheral vasculopathy.

These data inform on the underlying pathophysiological processes and provide a basis to identify individuals at risk of SSc-pHI.

Introduction

Systemic sclerosis (SSc) is a heterogeneous autoimmune disease characterised by vasculopathy and progressive fibrosis of the skin and internal organs (1, 2). SSc-primary heart involvement (SSc-pHI) (excluding ischaemic heart disease, IHD; and pulmonary arterial hypertension, PAH) develops as a direct consequence of the disease, and is one of the most common causes of death in SSc with a clinical prevalence of between 15% - 35% (3). Its manifestations typically include myocarditis, cardiac failure (systolic or diastolic dysfunction) and arrhythmia (4, 5). Myocardial fibrosis is the pathological hallmark of SSc-pHI and has been postulated to be the consequence of repeated focal ischaemia due to microvasculopathy (6).

Epidemiological datasets have associated poor prognostic factors of SSc including diffuse SSc subtype, anti-topoisomerase antibody (Scl-70), male sex and major internal organ involvement with SSc-pHI (7, 8). However, effective means of risk stratification to guide tailored monitoring and early detection of cardiac involvement in a general, asymptomatic SSc cohort are lacking. Cardiovascular magnetic resonance (CMR) can provide comprehensive assessment of cardiac morphology, function and tissue characterisation and can thus detect subclinical SSc-pHI (6, 9-11); of which a proportion will develop clinical events of SSc-pHI. Late gadolinium enhancement (LGE) CMR can detect focal fibrosis (9, 12, 13), distinctive from IHD where the location of infarction on LGE follows the coronary artery distribution. CMR parametric mapping with estimation of myocardial extracellular volume (ECV) and T1 native provides indicators of diffuse extracellular processes, in particular diffuse fibrosis with studies suggesting good correlation with histological findings of myocardial interstitial fibrosis in various clinical contexts (14-16).

Addition of perfusion CMR with vasodilator stress can assess microvascular dysfunction in SSc (9, 17) in the absence of IHD, although few studies have demonstrated global reduction in myocardial blood flow (MBF) and myocardial perfusion reserve (MPR) (18). Standard cardiac biomarkers of cardiac injury, troponin I (TnI) and remodelling, N-terminal pro-brain natriuretic peptide (NT-

proBNP) have been shown to be significantly elevated in SSc compared to healthy control (HC) and both have been associated with the development of cardiovascular (CV) events in SSc (19-22). The value of TnI and NT-proBNP to predict CMR abnormalities in SSc-pHI has however not been explored to date.

The aims of this study were within a general SSc cohort with no known SSc-pHI and no IHD or PAH, firstly, to characterise subclinical heart involvement using a comprehensive CMR protocol that could evaluate for markers of fibrosis and microvasculopathy. Secondly, we evaluated for clinical and standard serum cardiac predictors of such a subgroup with subclinical SSc-pHI at risk of future events.

Methods

Participants

Patients recruited to this study fulfilled the 2013 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for SSc (23) and were classified as limited or diffuse cutaneous SSc (lcSSc or dcSSc respectively) according to LeRoy classification (24). Patients were excluded if they had any prior diagnosis of IHD (or clinically overt SSc-pHI), diabetes or more than two traditional CV risk factors which were defined as current smoker, hypertension, hypercholesterolaemia/ hypertriglyceridemia and family history of premature CV disease (CVD) and/or any evidence of PAH detected on tissue Doppler echocardiography and confirmed by right heart catheterization. All patients had echocardiography performed within one year of the study visit, as part of their routine clinical testing. Patients with any other inflammatory musculoskeletal conditions were also excluded. Forty-four healthy volunteers with no CVD and no CV treatment, frequency matched with the SSc cohort for age and sex were recruited. The research was carried out according to the Declaration of Helsinki and was approved by NRES Committee Yorkshire & The Humber - Leeds East ethics committee. All participants provided written informed consent.

Clinical data

Comprehensive demographic and clinical data were collected including SSc subtype, duration, serology, organ involvement, treatment and nailfold capillaroscopy findings. Patients also had an electrocardiography (ECG) performed. All patients had pulmonary function tests that were undertaken as part of their routine clinical assessment, within one year of the CMR visit. A diagnosis of interstitial lung disease (ILD) was based on HRCT findings.

Serum sample collection

High-sensitivity TnI (Hs-TnI), NT-proBNP and creatine kinase (CK) were measured for all participants. Hs-TnI and CK were tested on a Siemens Advia XPT system (Advia Chemistry XPT and Advia Centaur XPT Immunoassay respectively) and NT-proBNP on Cobas 6000 (immunochemistry module Cobas e601) using appropriate kits supplied by Roche Diagnostics. All patients had antinuclear antibody (ANA) and C reactive protein (CRP) tested as part of standard care.

CMR imaging

Both SSc and HC cohorts underwent CMR on a 3 Tesla Philips Achieva MR system as previously described, (25, 26) including cine imaging for left ventricle (LV) volume estimation, LGE, native and post-contrast T1 mapping for ECV quantification and adenosine stress and rest myocardial perfusion, which enabled quantitative assessment of MBF and MPR (a ratio of maximal stress: resting MBF) (full details in supplementary file). According to the departmental reference ranges, an ECV >29% and native T1 > 1240 (ms) were classed abnormal (27, 28).

Statistical analysis

Descriptive summary statistics were provided for all variables. Continuous variables were reported as mean (SD) or median (IQR) and categorical data reported as percentage. Student's t-test, Mann-Whitney U test or chi square test where appropriate were used to assess for significant between group differences. Bonferroni correction was applied to correct for multiple comparisons when using

multiple t -test. Spearman's rho test was used to assess correlation between CMR indices and clinical/cardiac biomarkers. Linear and logistic regression analyses were used to assess the correlation and predictive value of these biomarkers with CMR measures. For multivariate regression analysis, regressors were eliminated based backwards stepwise regression rule. The model with the highest adjusted R square was selected from the range of models generated by backwards regression. For logistic regression, the presence of CV risk factor was added in the model as this was considered an important factor for the prediction of CMR fibrosis. Receiver-operating characteristic (ROC) curves were built to assess the ability of cardiac biomarkers to identify abnormal CMR measures. Statistical analysis was performed using SPSS (IBM SPSS Statistics 22) and Graph Pad Prism 8.

Results

Baseline characteristics

Eighty-three SSc patients were recruited to the study. Details on patient selection, recruitment and feasibility are described in the supplementary file (Figure S1). Participants had a median (IQR) age of 54 (49, 54) and disease duration (defined as time from first non-Raynaud's phenomenon) of 7 (2,7) years; 84% were females and 34% had dcSSc. Forty percent had known ILD, 24% and 25% a history of digital ulceration (DU) and calcinosis respectively. Seventy-eight (94%) patients were ANA positive of whom 28 (34%) and 24 (29%) were ACA and Scl-70 positive respectively (Table 1). Forty-one (49%) patients were receiving a disease-modifying anti-rheumatic drug (DMARD) at the time of recruitment, the majority with mycophenolate mofetil (35%). Nineteen (23%) participants were receiving iloprost vasodilator treatment, 17 (21%) sildenafil and 4 (5%) bosentan (Table 1), (Supplementary Table S1). Twenty-two (27%) patients had CV risk factors, 17 had one CV risk factor and the remaining 5 had 2 CV risk factors. All SSc patients were in sinus rhythm and none had any signs of IHD on ECG.

None of the patients had evidence of PAH on echocardiography.

CMR assessment in SSc versus HC

Of the 83 patients recruited, complete CMR function/volume assessment were available in all, LGE and native T1 in 80, ECV in 78 and perfusion CMR in 61 patients (Supplementary Figure S1). Of the 44 HC, LV function/volumes, LGE, native T1 and ECV were available in all and perfusion CMR in 36. HC were well matched to patients with a median (IQR) age of 55 (37, 63), 37 (84%) being females.

Tissue characterisation: Myocardial inflammation and focal and diffuse fibrosis observed in SSc

Seventeen (21%) SSc patients (and none of the HC) had focal LGE fibrosis in a non-ischaemic pattern, with a mean (SD) LGE fibrosis mass of 2.08 (1.74). Sixteen additional patients had evidence of right ventricular insertion point LGE, considered a non-specific finding and thus not included in the LGE analysis (29). ECV, marker of diffuse fibrosis, was significantly higher in SSc compared to HC; mean (SD) 31% (4) vs 25% (2), p<0.001] and remained statistically significant after the Bonferroni correction was applied (p<0.005) (Table 2).

Native T1 was also higher in SSc compared to HC but did not reach statistical significance following Bonferroni correction [mean (SD) 1241ms (76) vs 1209ms (51), p=0.008,>p=0.005]. Mean native ECV was above the normal reference value (>29%) and T1 just above (>1240). Fifty-one (61%) and 43 (52%) SSc patients respectively had native T1 and ECV above normal values.

LGE pattern and distribution

Of the 17 SSc patients with focal fibrosis on LGE CMR, a linear pattern was noted in 9 participants, a focal pattern in 6 and a diffuse pattern in 2 (1 participant had both focal and linear distribution) (Figure 1C). Higher LGE fibrosis mass was noted in those with a focal LGE pattern compared to those with a linear or diffuse pattern [mean (SD) 3.02 (2.3) vs 1.39 (0.45) and 1.5 (1.5) respectively] (Figure 1B). The distribution of LGE was 7 midwall, 6 subepicardial, 2 midwall-subepicardial and 2 transmural/diffuse. The most commonly affected segment was the basal inferolateral segment (n=7)

followed by the basal anteroseptal and inferoseptal segments. Eleven of 17 patients had involvement of more than 1 segment (Figure 1A).

Lower stress MBF and MPR in SSc

None of the HC or SSc patients with perfusion CMR data had regional perfusion defects that would be suggestive of CAD. Quantitative analysis however showed significantly lower global MBF at stress and lower MPR in SSc patients compared to HC [median (IQR) 1.9 (1.4, 2.6) vs 2.6 (2.2, 3.3), p<0.001; median (IQR) 1.9 (1.6, 2.4) vs 3 (2, 3.6), p<0.001 respectively] (Table 2). There was no difference in MBF at rest between the two groups.

Normal functional assessment in SSc

LV volumes and function, including LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), left ventricular stroke volume (LVSV), LV ejection fraction (LVEF) and LV mass were comparable between the HC and SSc participants and the means within normal limits (Table 2). Right ventricular parameters in SSc patients were within normal range (supplementary file).

CMR measures of fibrosis and vasculopathy correlate with SSc disease severity and peripheral vasculopathy

Patients with LGE focal fibrosis had significantly higher modified Rodnan skin score (mRSS) [median (IQR) 4 (2, 9) vs 2 (1,5), p=0.038] (Figure 2A) and higher, albeit modest CRP levels [median (IQR) 5(5,18) vs (5, 5), p=0.038] compared to those without LGE. Logistic regression confirmed an association between mRSS and LGE (OR=1.107, p=0.048) which remained significant after adjusting for the presence of CV risk factors (Table 3). LGE fibrosis mass also moderately correlated with mRSS (rho=0.231, p=0.039).

ECV was significantly higher in patients with DU compared to those without [mean (SD) 34 (4) vs 29 (3) respectively, p<0.001] (Figure 2C). Univariate analysis indicated associations of ECV with DU

(p<0.001), mRSS (p=0.001) (Figure 2B) and DLCO/VA (p=0.040). Multivariate analysis confirmed ECV associated with mRSS and the presence of DU (R^2=0.353, p=0.004, p<0.001 respectively) (Table 3).

Significantly higher MPR values were noted in SSc patients with DU compared to those without [median (IQR) 3(2, 3) vs 2(2.2), p=0.001] (Figure 2D). The presence of DU associated with MPR at both univariate (p=0.013) and multivariate analysis (R^2=0.238, p=0.011) (Table 3). Age was negatively associated with MPR at multivariate analysis (R^2 =0.238, p=0.022).

Neither DMARD nor angiotensin converting enzyme inhibitor (ACEi) treatment associated with the CMR measures of fibrosis and vasculopathy (p>0.05).

The association between native T1 and clinical phenotype is detailed in the supplementary file.

CMR measures of fibrosis and vasculopathy associate with serum cardiac biomarkers

Hs-TnI was significantly higher in patients with LGE focal fibrosis compared to those without [median (IQR) 6.4 (4,36) vs 2.9 (3, 7), p=0.012] (Figure 3A) and correlated with LGE fibrosis mass (rho=0.283; p=0.014). Patients with a focal LGE pattern (also with greater fibrosis mass) had higher hs-TnI levels compared to those with a linear or diffuse pattern [median (IQR) 53 (6, 165) vs 4 (3, 11) vs 6 (5,6) respectively] (Figure 3B).

A positive correlation was observed between NT-proBNP and ECV (rho=224; p=0.048) (Figure 3C) and higher levels of NT-proBNP associated with the presence of LGE fibrosis although this did not reach statistical significance [median (IQR) 105 (49, 377) vs 77 (46,122), p=0.083]. ROC curves were plotted to assess the ability of serum cardiac biomarkers to identify focal or diffuse myocardial fibrosis. AUC (95%CI) of Hs-TnI for identifying LGE was significant at 0.695 (0.55, 0.837), p=0.015. A hs-TnI≥ 5.5 ng/l had a sensitivity of 65% and a specificity of 70% to predict the presence of focal LGE. AUC (95%CI) of NT-proBNP for identifying ECV (ECV<29%; ECV≥29%) was 0.586 (0.447, 0.726),

p=0.213 (Supplementary Figure S2). There was no significant correlation between MPR and cardiac biomarkers (p>0.05) or between CK and CMR measures.

Discussion

We report on the largest study to date to phenotype subclinical SSc-pHI using CMR markers of myocardial fibrosis and perfusion, and to identify clinical and serum predictors of subclinical SSc-pHI. Myocardial microvasculopathy, as well as focal and diffuse myocardial fibrosis are suggestive of SSc-pHI. In this study, presence of fibrosis associated with raised hs-TnI and NT-proBNP, and both myocardial perfusion impairment and fibrosis associated with SSc disease severity and peripheral vasculopathy. These data inform on the underpinning processes of SSc-pHI and provide a basis to risk stratify a SSc cohort without overt cardiac involvement for CMR-detected subclinical SSc-pHI that would benefit from more focused monitoring.

Cardiac involvement is a significant cause of morbidity and mortality in SSc, but we have limited understanding of the pathophysiological basis and the patients most at risk. In asymptomatic SSc individuals, we demonstrate impaired microvascular perfusion and tissue changes suggestive of focal and diffuse fibrosis, with preservation of ventricular dimensions and function. One quarter of the SSc patients (and no HC) had evidence of focal myocardial fibrosis in a non-coronary LGE pattern. The study did not include patients with IHD, PAH, diabetes, other inflammatory musculoskeletal diseases, with minimum presence of traditional CV risk factors, thus reducing the risk of including cardiac fibrosis associated with IHD or other conditions. Moreover, the study excluded focal LGE localised at the inferior right ventricular insertion point that is considered non-specific and may represent a normal variant (29). Three distinctive patterns of focal fibrosis were observed; linear, diffuse and focal, the latter associated with higher LGE fibrosis mass; although lower compared to fibrosis mass reported in IHD (30, 31). LGE was localised predominantly at the basal anteroseptal and inferoseptal segments and had a midwall or subepicardial distribution, similar to previously reported studies (32, 33). A lateral subepicardial LGE distribution which is more commonly described

in myocarditis (34) was present in nearly half of the SSc patients. This implies that at least in a subgroup of SSc patients, a silent inflammatory process could be the substrate for the development of myocardial fibrosis. The relevance and mechanism for these different LGE patterns needs to be further explored.

By applying T1 mapping, we also demonstrated higher ECV, suggestive of diffuse myocardial fibrosis in SSc compared to HC (15, 35). In non-ischaemic cardiomyopathies, ECV is a poor prognostic marker for CV outcomes (36) with pathophysiological studies suggesting that interstitial fibrosis can progress to replacement fibrosis (seen as LGE on CMR) (37, 38). We recently reported an association between ECV and implantable loop recorder detected arrhythmia in asymptomatic SSc patients (26), underscoring the potential clinical importance of raised ECV in SSc. Further studies are needed to fully establish the prognostic relevance of this finding.

The study also demonstrated significantly lower MPR in SSc patients compared to HC with no regional perfusion defects, indicating myocardial microvascular disease. One previous small study (n=19) also performed quantitative perfusion CMR and demonstrated lower stress MBF in SSc compared to HC (18). In our study, microvascular impairment occurred in SSc patients with normal left ventricular dimensions and function, with and without myocardial fibrosis, suggesting microvascular disease may be the earliest manifestation of heart involvement in SSc. Longitudinal studies are needed to understand whether and how these pathophysiological processes are inter-related and develop over time; and ultimately lead to the clinical manifestations of SSc-pHI.

Of particular value are the analyses identifying possible predictors of subclinical SSc-pHI. Several smaller studies reported an association of ECV with mRSS (15, 39) and longer history of Raynaud's in those with focal fibrosis (12) with another study failing to reveal an association between the CMR measures of fibrosis and SSc disease phenotype (33). Our larger study showed that both LGE and ECV correlated with mRSS in a predictive model. Patients with LGE also had higher CRP levels.

Collectively, these findings support the association of myocardial fibrosis with disease severity and suggest likely concurrent fibrotic process affecting both the skin and myocardium.

Peripheral vasculopathy is considered an early inciting event in the pathogenesis of SSc. In our study, ECV correlated with the presence of DU, indicating co-existing peripheral and myocardial processes. The observed increase in MPR in the presence of DU however appears at first counterintuitive. Patients with DU are commonly treated with vasodilators (80% in our cohort). An increase in resting MBF would have been expected along with a reduction in stress perfusion in the presence of microvascular disease, with consequent reduction, rather than increase, in MPR. However, the coronary microvasculature of SSc patients, and responsiveness to vasodilator treatment compared to a general population may be altered and has not been extensively evaluated. Other studies have also reported increased myocardial perfusion index following vasodilatory therapies (40, 41), whilst one recent study showed a protective role of vasodilator treatment in SSc-pHI (42). Further mechanistic investigation is required to explain these findings and the role of vasodilator therapy on CMR detected SSc-pHI. The results also confirmed a negative association between age and MPR, suggesting a reduction of MPR with age, as documented in the general population (43, 44).

The relevance of modest hs-TnI elevation in the general population is not clear, with renal pathology, inflammation and/or infection possible contributors (45). Our results showed that the moderate hs-TnI levels observed in SSc correlate with the presence of LGE and LGE fibrosis mass indicating that this biomarker is sensitive in detecting myocardial injury in SSc. Moreover, hs-TnI was higher in patients demonstrating a focal pattern of fibrosis, implying a direct relationship between myocardial fibrosis mass (that we observed is increased in focal pattern of fibrosis) and hs-TnI. This is of particular interest as extent of LGE has been associated with worse CV outcomes in both ischaemic and non-ischaemic cardiomyopathies (46, 47). NT-proBNP, released by ventricular myocytes in response to increased wall tension associated with ECV, which might be explained by interstitial expansion and remodelling of the myocardium. Higher levels of NT-proBNP also

associated with LGE, although the results did not reach statistical significance. Both hs-TnI and NT-proBNP thus appear to be sensitive tools for detecting (focal and diffuse) fibrosis. Whilst ROC curve analysis showed a moderate diagnostic performance of hs-TnI for identifying LGE, the diagnostic performance of NT-proBNP for identifying ECV was poor. This is likely due to a relatively modest sample size despite this study being the largest CMR study in SSc to look at the association between cardiac biomarkers and CMR measures.

There are some limitations to this study. Time of occurrence of SSc-pHI and whether treatment can alter the course of SSc-pHI are important clinical questions that would ideally require recruitment of an inception cohort. Such studies in a rare disease are challenging. Atherosclerotic disease is also difficult to exclude fully, however, we minimised this risk by excluding patients with CVD, diabetes and more than two CV risk factors. None of the patients had evidence of IHD on ECG or myocardial perfusion defects indicative of flow limiting IHD and no evidence of ischaemic fibrosis on CMR, making inadvertent inclusion of secondary IHD in our cohort unlikely. Whilst myocardial stress perfusion imaging provides important information on the pathophysiological process of SSc-pHI, there is not enough evidence currently to support its use in routine clinical practice. T2-weighted imaging was not included in the CMR protocol, however, CMR mapping techniques which have shown superior accuracy for detecting myocardial oedema and fibrosis compared with T2-weighted imaging were instead used (48, 49). The use of a more specific investigative tool for assessing peripheral vasculopathy would have provided more insights into the association between peripheral and myocardial vascular involvement in SSc.

In conclusion, we report on the pathophysiological basis of subclinical SSc-pHI in the largest CMR study in SSc to employ quantitative myocardial perfusion, quantitative fibrosis mass and T1 mapping. In asymptomatic SSc patients, microvasculopathy and myocardial focal and diffuse fibrosis but preserved myocardial contractile function characterise subclinical SSc-pHI. Hs-TnI, NT-proBNP, markers of SSc disease severity and complicated peripheral vasculopathy may predict subclinical SSc-

pHI. Collectively, these data provide an initial basis to risk stratify for subclinical SSc-pHI and the opportunity for more tailored monitoring and intervention of those liable to develop clinically overt SSc-pHI.

Funding: The study was partly supported by Scleroderma and Raynaud's UK. Dr Dumitru and Bissell have been funded by the ACORN charity. Dr Dumitru has been also funded by the Charitable Foundation Fellowship, Leeds Teaching Hospital. Dr Biglands is funded by a National Institute of Health Research (NIHR) Clinical Lecturship (ICA-CL-2016-02-017). Dr Fent was funded by a NIHR grant (number: 11/117/27). Prof Plein is funded by a British Heart Foundation Personal Chair (CH/16/2/32089).

Acknowledgments: We wish to thank all patients participating in the study. We also thank Petra Bijsterveld for helping with the coordination of the CMR visit, to Margaret Saysell, Lisa Lewis and Gavin Bainbridge, radiographers who performed the CMR studies and to Dr Arka Das who helped with the CMR image preparation.

The research is supported by the National Institute for Health Research (NIHR) infrastructure at Leeds. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Conflicts of interest: The authors have no competing interests to declare

Ethical approval: REC: RR10/9608, Yorkshire & The Humber - Leeds East Research Ethics Committee

References

1. Ferri C, Valentini G, Cozzi F, Sebastiani M, Michelassi C, La Montagna G, et al. Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients. Medicine. 2002;81(2):139-53.
2. Deswal A, Follansbee WP. Cardiac involvement in scleroderma. Rheum Dis Clin North Am. 1996;22(4):841-60.
3. Tyndall AJ, Bannert B, Vonk M, Airo P, Cozzi F, Carreira PE, et al. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. Ann Rheum Dis. 2010;69(10):1809-15.
4. Lambova S. Cardiac manifestations in systemic sclerosis. World journal of cardiology. 2014;6(9):993-1005.
5. Medsger TA, Jr., Masi AT. Survival with scleroderma. II. A life-table analysis of clinical and demographic factors in 358 male U.S. veteran patients. Journal of chronic diseases. 1973;26(10):647-60.
6. Follansbee WP, Miller TR, Curtiss EI, Orie JE, Bernstein RL, Kiernan JM, et al. A controlled clinicopathologic study of myocardial fibrosis in systemic sclerosis (scleroderma). J Rheumatol. 1990;17(5):656-62.
7. Steen VD, Medsger TA, Jr. Severe organ involvement in systemic sclerosis with diffuse scleroderma. Arthritis Rheum. 2000;43(11):2437-44.
8. Allanore Y, Meune C, Vonk MC, Airo P, Hachulla E, Caramaschi P, et al. Prevalence and factors associated with left ventricular dysfunction in the EULAR Scleroderma Trial and Research group (EUSTAR) database of patients with systemic sclerosis. Ann Rheum Dis. 2010;69(1):218-21.
9. Rodriguez-Reyna TS, Morelos-Guzman M, Hernandez-Reyes P, Montero-Duarte K, Martinez-Reyes C, Reyes-Utrera C, et al. Assessment of myocardial fibrosis and microvascular damage in systemic sclerosis by magnetic resonance imaging and coronary angiotomography. Rheumatology (Oxford). 2015;54(4):647-54.
10. Mueller KA, Mueller, II, Eppler D, Zuern CS, Seizer P, Kramer U, et al. Clinical and histopathological features of patients with systemic sclerosis undergoing endomyocardial biopsy. PLoS One. 2015;10(5):e0126707.
11. Mavrogeni SI, Bratis K, Karabela G, Spiliotis G, Wijk K, Hautemann D, et al. Cardiovascular Magnetic Resonance Imaging clarifies cardiac pathophysiology in early, asymptomatic diffuse systemic sclerosis. Inflammation & allergy drug targets. 2015;14(1):29-36.
12. Tzelepis GE, Kelekis NL, Plastiras SC, Mitseas P, Economopoulos N, Kampolis C, et al. Pattern and distribution of myocardial fibrosis in systemic sclerosis: a delayed enhanced magnetic resonance imaging study. Arthritis and rheumatism. 2007;56(11):3827-36.
13. Hachulla AL, Launay D, Gaxotte V, de Groote P, Lamblin N, Devos P, et al. Cardiac magnetic resonance imaging in systemic sclerosis: a cross-sectional observational study of 52 patients. Ann Rheum Dis. 2008.
14. Barison A, Gargani L, De Marchi D, Aquaro GD, Guiducci S, Picano E, et al. Early myocardial and skeletal muscle interstitial remodelling in systemic sclerosis: insights from extracellular volume quantification using cardiovascular magnetic resonance. Eur Heart J Cardiovasc Imaging. 2015;16(1):74-80.

15. Ntusi NA, Piechnik SK, Francis JM, Ferreira VM, Rai AB, Matthews PM, et al. Subclinical myocardial inflammation and diffuse fibrosis are common in systemic sclerosis--a clinical study using myocardial T1-mapping and extracellular volume quantification. J Cardiovasc Magn Reson. 2014;16:21.
16. Diao K-y, Yang Z-g, Xu H-y, Liu X, Zhang Q, Shi K, et al. Histologic validation of myocardial fibrosis measured by T1 mapping: a systematic review and meta-analysis.
17. Moroncini G, Schicchi N, Pomponio G, Dziadzio M, della Costanza OP, Pierfederici A, et al. Myocardial perfusion defects in scleroderma detected by contrast-enhanced cardiovascular magnetic resonance. La Radiologia medica. 2014;119(12):885-94.
18. Gyllenhammar T, Kanski M, Engblom H, Wuttge DM, Carlsson M, Hesselstrand R, et al. Decreased global myocardial perfusion at adenosine stress as a potential new biomarker for microvascular disease in systemic sclerosis: a magnetic resonance study. BMC cardiovascular disorders. 2018;18(1):16.
19. Bosello S, De Luca G, Berardi G, Canestrari G, de Waure C, Gabrielli FA, et al. Cardiac troponin T and NT-proBNP as diagnostic and prognostic biomarkers of primary cardiac involvement and disease severity in systemic sclerosis: A prospective study. Eur J Intern Med. 2018.
20. Avouac J, Meune C, Chenevier-Gobeaux C, Borderie D, Lefevre G, Kahan A, et al. Cardiac Biomarkers in Systemic Sclerosis: Contribution of High-Sensitivity Cardiac Troponin in Addition to N-Terminal Pro-Brain Natriuretic Peptide. Arthritis Care & Research. 2015;67(7):1022-30.
21. Allanore Y, Wahbi K, Borderie D, Weber S, Kahan A, Meune C. N-terminal pro-brain natriuretic peptide in systemic sclerosis: a new cornerstone of cardiovascular assessment? Ann Rheum Dis. 2009;68(12):1885-9.
22. Nordin A, Svenungsson E, Bjornadal L, Elvin K, Larsson A, Jensen-Urstad K. Troponin I and echocardiography in patients with systemic sclerosis and matched population controls. Scand J Rheumatol. 2016:1-10.
23. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. Ann Rheum Dis. 2013;72(11):1747-55.
24. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA, Jr., et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol. 1988;15(2):202-5.
25. Erhayiem B, Pavitt S, Baxter P, Andrews J, Greenwood JP, Buch MH, et al. Coronary Artery Disease Evaluation in Rheumatoid Arthritis (CADERA): study protocol for a randomized controlled trial. Trials. 2014;15.
26. Bissell LA, Dumitru RB, Erhayiem B, Abignano G, Fent G, Kidambi A, et al. Incidental significant arrhythmia in scleroderma associates with cardiac magnetic resonance measure of fibrosis and hs-TnI and NT-proBNP. Rheumatology (Oxford). 2019.
27. Dabir D, Child N, Kalra A, Rogers T, Gebker R, Jabbour A, et al. Reference values for healthy human myocardium using a T1 mapping methodology: results from the International T1 Multicenter cardiovascular magnetic resonance study. J Cardiovasc Magn Reson. 2014;16:69.
28. McDiarmid AK, Swoboda PP, Erhayiem B, Lancaster RE, Lyall GK, Broadbent DA, et al. Athletic Cardiac Adaptation in Males Is a Consequence of Elevated Myocyte Mass. Circ Cardiovasc Imaging. 2016;9(4):e003579.

29. Yi JE, Park J, Lee HJ, Shin DG, Kim Y, Kim M, et al. Prognostic implications of late gadolinium enhancement at the right ventricular insertion point in patients with non-ischemic dilated cardiomyopathy: A multicenter retrospective cohort study. PLoS One. 2018;13(11):e0208100.
30. Plein S, Younger JF, Sparrow P, Ridgway JP, Ball SG, Greenwood JP. Cardiovascular magnetic resonance of scar and ischemia burden early after acute ST elevation and non-ST elevation myocardial infarction. J Cardiovasc Magn Reson. 2008;10(1):47.
31. Alexandre J, Saloux E, Dugué AE, Lebon A, Lemaitre A, Roule V, et al. Scar extent evaluated by late gadolinium enhancement CMR: a powerful predictor of long term appropriate ICD therapy in patients with coronary artery disease. J Cardiovasc Magn Reson. 2013;15(1):12.
32. Krumm P, Mueller KA, Klingel K, Kramer U, Horger MS, Zitzelsberger T, et al. Cardiovascular magnetic resonance patterns of biopsy proven cardiac involvement in systemic sclerosis. J Cardiovasc Magn Reson. 2016;18(1):70.
33. Hachulla AL, Launay D, Gaxotte V, de Groote P, Lamblin N, Devos P, et al. Cardiac magnetic resonance imaging in systemic sclerosis: a cross-sectional observational study of 52 patients. Ann Rheum Dis. 2009;68(12):1878-84.
34. Friedrich MG, Sechtem U, Schulz-Menger J, Holmvang G, Alakija P, Cooper LT, et al. Cardiovascular magnetic resonance in myocarditis: A JACC White Paper. Journal of the American College of Cardiology. 2009;53(17):1475-87.
35. Thuny F, Lovric D, Schnell F, Bergerot C, Ernande L, Cottin V, et al. Quantification of myocardial extracellular volume fraction with cardiac MR imaging for early detection of left ventricle involvement in systemic sclerosis. Radiology. 2014;271(2):373-80.
36. Wong TC, Piehler K, Meier CG, Testa SM, Klock AM, Aneizi AA, et al. Association between extracellular matrix expansion quantified by cardiovascular magnetic resonance and short-term mortality. Circulation. 2012;126(10):1206-16.
37. Martos R, Baugh J, Ledwidge M, O'Loughlin C, Conlon C, Patle A, et al. Diastolic heart failure: evidence of increased myocardial collagen turnover linked to diastolic dysfunction. Circulation. 2007;115(7):888-95.
38. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. Circulation. 1991;83(6):1849-65.
39. Lee DC, Hinchcliff ME, Sarnari R, Stark MM, Lee J, Koloms K, et al. Diffuse cardiac fibrosis quantification in early systemic sclerosis by magnetic resonance imaging and correlation with skin fibrosis. Journal of scleroderma and related disorders. 2018;3(2):159-69.
40. Vignaux O, Allanore Y, Meune C, Pascal O, Duboc D, Weber S, et al. Evaluation of the effect of nifedipine upon myocardial perfusion and contractility using cardiac magnetic resonance imaging and tissue Doppler echocardiography in systemic sclerosis. Ann Rheum Dis. 2005;64(9):1268-73.
41. Allanore Y, Meune C, Vignaux O, Weber S, Legmann P, Kahan A. Bosentan increases myocardial perfusion and function in systemic sclerosis: a magnetic resonance imaging and Tissue-Doppler echography study. J Rheumatol. 2006;33(12):2464-9.
42. Valentini G, Huscher D, Riccardi A, Fasano S, Irace R, Messiniti V, et al. Vasodilators and low-dose acetylsalicylic acid are associated with a lower incidence of distinct primary myocardial disease manifestations in systemic sclerosis: results of the DeSScipher inception cohort study. Ann Rheum Dis. 2019;78(11):1576-82.

43. Uren NG, Camici PG, Melin JA, Bol A, de Bruyne B, Radvan J, et al. Effect of aging on myocardial perfusion reserve. J Nucl Med. 1995;36(11):2032-6.
44. Dandekar VK, Bauml MA, Ertel AW, Dickens C, Gonzalez RC, Farzaneh-Far A. Assessment of global myocardial perfusion reserve using cardiovascular magnetic resonance of coronary sinus flow at 3 Tesla. J Cardiovasc Magn Reson. 2014;16:24.
45. Tanindi A, Cemri M. Troponin elevation in conditions other than acute coronary syndromes. Vascular health and risk management. 2011;7:597-603.
46. Neilan TG, Farhad H, Mayrhofer T, Shah RV, Dodson JA, Abbasi SA, et al. Late gadolinium enhancement among survivors of sudden cardiac arrest. JACC Cardiovascular imaging. 2015;8(4):414-23.
47. Alexandre J, Saloux E, Dugue AE, Lebon A, Lemaitre A, Roule V, et al. Scar extent evaluated by late gadolinium enhancement CMR: a powerful predictor of long term appropriate ICD therapy in patients with coronary artery disease. J Cardiovasc Magn Reson. 2013;15:12.
48. Ferreira VM, Piechnik SK, Dall'Armellina E, Karamitsos TD, Francis JM, Ntusi N, et al. T(1) mapping for the diagnosis of acute myocarditis using CMR: comparison to T2-weighted and late gadolinium enhanced imaging. JACC Cardiovasc Imaging. 2013;6(10):1048-58.
49. Nazir SA, Shetye A, Khan JN, Vanezis AP, Singh A, Kanagala P, et al. Comparison of T1-mapping and T2-weighted imaging for diagnostic oedema assessment in ST-segment elevation myocardial infarction.

Figure Legends

Figure 1. LGE fibrosis in SSc patients (A) Number of patients with LGE fibrosis as per each cardiac segment (left figure). 17 segment model (right figure) 1: basal anterior; 2: basal anteroseptal; 3: basal inferoseptal; 4: basal inferior; 5: basal inferolateral; 6: basal anterolateral; 7: mid anterior; 8: mid anteroseptal; 9: mid inferoseptal; 10: mid inferior; 11: mid inferolateral; 12: mid anterolateral; 13: apical anterior; 14: apical septal; 15: apical inferior; 16: apical lateral; 17: apex. (B) LGE fibrosis mass in those with focal, linear and diffuse pattern. (C) LGE patterns: a- focal; b-linear; c-diffuse

LGE late gadolinium enhancement.

Figure 2. Disease phenotype and CMR parameters: (A) Presence or absence of LGE fibrosis and median (IQR) mRSS. (B) Association between ECV and mRSS. (C) Presence or absence of DU and ECV. (D) Presence or absence of DU and MPR.

DU digital ulcers; ECV extracellular volume; LGE late gadolinium enhancement; MPR myocardial perfusion reserve; mRSS modified Rodnan skin score.

Figure 3. Association between cardiac biomarkers and CMR variables: (A) Presence or absence of LGE and hs-TnI. (B) Hs-TnI in focal, linear or diffuse LGE pattern. (C) Association between ECV and NT-proBNP.

ECV extracellular volume; hs-TnI high sensitivity troponin I; LGE late gadolinium enhancement; NT-proBNP N-terminal pro-brain natriuretic peptide.

Table 1. Disease characteristics of SSc patients

Baseline characteristic	**SSc cohort, n=83**
Age, median (IQR)	54 (49,63)
Female, n (%)	70 (84)
Disease subtype, n (%)	
lcSSc	55 (66)
dcSSc	28 (34)
Disease duration (years), median (IQR)	7 (2,7)
History of, n (%)	
Digital ulceration	20 (24)
Calcinosis	21 (25)
Myositis	3 (4)
GORD	73 (88)
Interstitial lung disease	33 (40)
NSIP	31 (37)
UIP	2 (2)
Current use of DMARD, n (%)	49 (59)
Name of current DMARD, n (%)	
Mycophenolate	29 (35)
Methotrexate	8 (10)
Hydroxychloroquine	1 (5)
Sulfasalazine	1 (1)
Cyclophosphamide	3 (4)
Rituximab	1 (1)

Previous use of cyclophosphamide, n (%)	23 (28)
Current use of prednisolone, n (%)	18 (22)
Current treatment with, n (%)	
Iloprost	19 (23)
Sildenafil	17 (21)
Bosentan	4 (5)
ACE inhibitor	31 (37)
Calcium channel blocker	48 (58)
Statin use	5 (6)
Clinical profile	
Total modified Rodnan skin score, median (IQR)	2 (1,6)
Presence of, n (%)	
Digital pitting scars	27 (33)
Digital ulceration	14 (17)
Tendon friction rubs	3 (4)
Calcinosis	17 (21)
Joint contractures	13 (16)
Serology & acute phase	
Antibody positive, n (%)	
ANA	78 (94)
ACA	28 (34)
Scl70	24 (29)
Anti-RNA polymerase III	3 (4)
CRP (mg/L), median (IQR)	5 (5,5.1)
Cardiovascular risk profile, n%	

Dyslipidaemia	3 (4)
Hypertension	8 (10)
Smoking	7 (8)
Family history of CVD	8 (10)
Patients with any CV risk factors, n%	22 (27%)

N % presented unless stated otherwise

ACA, anti-centromere antibody; ACE, angiotensin converting enzyme; ANA, antinuclear antibodies; CRP, C-reactive protein; dcSSc, diffuse cutaneous systemic sclerosis; CV, cardiovascular; CVD, cardiovascular disease; DMARD, disease modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; GORD, gastro-oesophageal reflux disease; IQR, interquartile range; lcSSc, limited cutaneous systemic sclerosis; RP, Raynaud's Phenomenon; Scl70, anti-topoisomerase antibody; SD, standard deviation; SSc, Systemic Sclerosis.

Table 2. CMR parameters in SSc patients versus HC

CMR variable	**HC (n=44)**	**SSc patients (n=83)**	**p value**	**Bonferroni**
	Mean (SD)	**Mean (SD)**		**correction**
Fibrosis				
ECV (%)	25 (2)	31 (3)	<0.001**	<0.001
Native T1 (ms)	1209 (51)	1241 (76)	0.008*	NS
LGE, n%	0	17/80 (21%)		
LGE fibrosis mass (g)	0	2.08 (1.74)		
Myocardial perfusion, median (IQR)				
Stress MBF (ml/g/min)	2.6 (2.2,3.3)	1.9 (1.4, 2.6)	<0.001**	<0.001
Rest MBF (ml/g/min)	0.9 (0.7, 1.2)	0.9 (0.6, 1.2)	0.734	NS
MPR	3 (2,3.6)	1.9 (1.6, 2.4)	<0.001**	<0.001
LV Function/volume				
LVEDV/BSA (ml/m^2)	77 (14)	77 (15)	0.513	NS
LVESV/BSA (ml/m^2)	30 (9)	30 (9)	0.898	NS
LVSV/BSA (ml/m^2)	47 (9)	48 (7)	0.553	NS
LVEF (%)	61 (7)	61 (7)	0.565	NS
LV mass/BSA (g/m^2)	45 (10)	43 (10)	0.415	NS

* p<0.05; ** p<0.001

Mean (SD) unless stated otherwise. Bonferroni correction was applied for all t tests (n=10), with a p<0.005 indicating statistical significance.

BSA, body surface area; CMR, cardiovascular magnetic resonance; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume LGE, late gadolinium enhancement; LV, left ventricular; EF, ejection fraction; MBF, myocardial blood flow; MPR, myocardial perfusion reserve; NS, non-significant; SV, stroke volume.

Table 3. Logistic and linear regression to predict the relationship between SSc clinical phenotype and CMR variables LGE, ECV, MPR

	LGE presence (n=17)/absence (n=63)				**ECV**				**MPR**			
Variable	Logistic regression - Univariate analysis		Logistic regression - Multivariate analysis		Linear univariate analysis		Multivariate analysis, R^2 =0.353		Linear univariate analysis		Multivariate analysis, R^2 =0.238	
	OR	p value	OR	p value	Beta	p value	Beta	p value	Beta	p value	Beta	p value
Male sex	2.11	0.275			-0.05	0.966			-0.195	0.133	-0.180	0.167
Age	0.986	0.541			0.019	0.869			-0.231	0.073	-0.295	0.022*
Presence of CV risk factors	1.106	0.875	1.401	0.630	0.209	0.066	0.113	0.277	-0.139	0.287	-0.071	0.568
Disease duration	0.969	0.411			-0.216	0.059	-0.157	0.137	0.028	0.831		
Presence of ILD	1.444	0.504			-0.12	0.296	-0.085	0.425	0.031	0.810		
DLCO/VA	0.893	1.003			-0.238	0.040*			-0.112	0.356		
MRSS	1.107	0.048*	1.115	0.048*	0.369	0.001*	0.329	0.004*	-0.145	0.264	-0.159	0.210
DcSSc	1.621	0.392			0.185	0.105			0.009	0.945		

Digital ulcers	0.222	0.154	0.395	<0.001**	0.388	<0.001**	0.299	0.013*	0.319	0.011*
CRP	1.064	0.067	-0.056	0.624	-0.154	0.135	-0.075	0.567		
DMARD treatment	0.402	0.148	0.151	0.186			-0.003	0.984		
ACE inhibitor treatment	1.428	0.447	-0.087	0.451			-0.04	0.978		

* p<0.05; ** p<0.001

ACE, angiotensin converting enzyme; CV, cardiovascular; CRP, C-reactive protein; dcSSc, diffuse cutaneous systemic sclerosis; DLCO/VA, DLCO adjusted for volume; DMARD, disease modifying antirheumatic drugs; ECV, extracellular volume; ILD, interstitial lung disease; LGE, late gadolinium enhancement; MPR, myocardial perfusion reserve; MRSS, modified Rodnan skin score; OR odds ratio