# Increased serum soluble interleukin-2 receptor concentrations are linked to high-sensitivity troponin T and disease progression in systemic sclerosis

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## Abstract

### Objective

*To determine serum interleukin-2 receptor (sIL-2R) concentrations as biomarker in systemic sclerosis (SSc) and their association with markers for inflammation (high-sensitivity C-reactive protein, hs-CRP), lymphocyte activation and turnover (beta-2 microglobulin, b2M), and cardiac damage (hs-troponin T, hs-TnT).*

### Methods

*In this longitudinal cross-sectional observational study, serum sIL-2R concentrations were determined in 315 patients with SSc. Clinical data were assessed at baseline and up to 48 months after. Associations were calculated using logistic regression. Clinical deterioration was estimated using the Kaplan-Meier method.*

### Results

*Patients with dcSSc (n=139) displayed increased serum sIL-2R concentrations (p=0.001) compared to lcSSc (n=176). Increase in sIL-2R concentrations was associated with cardiac (p=0.014), pulmonary (p=0.007) and skin involvement (p<0.001) in SSc. Overall, sIL-2R concentrations in SSc correlated with b2M (r=0.6161, p<0.001), hs-CRP (r=0.4091, p<0.001), and hs-TnT (r=0.4548, p<0.001). The serum sIL-2R concentration discriminated normal from pathological range concentrations of hs-TnT (ROC-AUC:0.87; 95%CI, 0.77-0.97; p<0.001; sensitivity 80.0%, specificity 80.1%). In patients with clinical improvement, the concentration of sIL-2R decreased (p=0.004). Using Log-rank test and Mantel-Cox proportional hazard models, we found that a sIL-2R concentration of ≥900 U/ml defined SSc subtypes with increased clinical activity and predicted early disease progression in SSc (HR:2.21, p=0.001).*

### Conclusion

*sIL-2R concentrations reflect disease severity, particularly cardiac damage, and early disease progression, and suggest a potential role for disease and therapy monitoring. Thus, sIL-2R should be further evaluated as a biomarker in SSc in prospective studies.*

**Key words**

systemic sclerosis, biomarker, soluble interleukin-2 receptor, high-sensitivity troponin T, disease progression

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## Introduction

Systemic sclerosis (SSc) is a rare heterogeneous autoimmune connective tissue disease characterised by inflammation, extensive fibrosis and vasculopathy. Depending on the extent of dermal fibrosis, SSc is subclassified into limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc) (1-3). Alterations within the $CD4^+$ T cell compartment such as Th1/Th2 and Th17/Treg imbalances indicate a crucial contribution of T cells in the pathogenesis of SSc (4).

Interleukin-2 (IL-2) is involved in numerous (patho)physiological mechanisms, *e.g.* promotion of T cell proliferation upon IL-2 receptor (IL-2R) engagement. Soluble IL-2 receptor (sIL-2R) is a 45 kDa fragment of the IL-2Rα-chain (CD25), shed into the bloodstream by activated T and NK cells. In clinical practice, sIL-2R is used as a biomarker for T cell activation (1, 5). Interaction of sIL-2R with IL-2 induces various pro-inflammatory effects by extending the half-life of IL-2 and activation of low-affinity dimeric IL-2R. At the same time, binding of IL-2 to IL-2R induces $CD4^+$ T cell differentiation into inducible regulatory T cells facilitating resolution of inflammation (5). sIL-2R is a well-established biomarker for disease activity in several autoimmune inflammatory disorders such as sarcoidosis, rheumatoid arthritis or systemic lupus erythematosus (5, 6). In two earlier studies, circulating sIL-2R concentrations were higher compared to controls and thus, suggested as potential biomarkers in SSc (7, 8). Accordingly, an association between increased serum sIL-2R concentrations and disease progression was shown in a study of 39 SSc patients (8, 9).

In this study, we analysed serum sIL-2R concentrations in SSc patients to determine their association with clinical data and biomarkers of inflammation, lymphocyte activation and turnover and cardiac involvement, and their potential to predict disease progression.

## Methods

*Patients*

All study participants were recruited at the Department of Rheumatology and Clinical Immunology at University Hospital Schleswig-Holstein (UKSH) Lübeck Campus, Germany. Medical records were reviewed, and all patients were examined by a rheumatologist. SSc patients fulfilled the 2013 Classification Criteria for Systemic Sclerosis (10). The study was approved by the regional ethics review board of the University of Lübeck (16-199, 2023-113) and conducted in keeping with the principles of the Declaration of Helsinki. All participants gave their informed consent. Clinical data including sex, age, cutaneous subsets, disease duration, body mass index (BMI), organ involvement, modified Rodnan Skin Score (mRSS), overlap with other rheumatic diseases, malignancies and treatment were assessed at the time of serum sampling and up to 48 months after. Serological markers (high-sensitivity C-reactive protein [hs-CRP], ß2-microglobulin [b2M], N-terminal pro-B-type natriuretic peptide [NT-proBNP], hs-troponin T [hs-TnT], creatine kinase, and absolute neutrophil-, lymphocyte- and platelet counts), autoantibodies (anti-topoisomerase I [Scl-70], anti-RNA polymerase III [subunits RP11 and RP155], anti-centromere protein A [CENP-A], anti-PM-Scl, anti-Ro-52, anti- Ro/SS-A, anti-La/SS-B, anti-U1-RNP), lung function parameters (diffusing capacity for carbon monoxide [DLCO], forced vital capacity [FVC]) and cardiac parameters (left ventricular ejection fraction [LVEF], pulmonary artery pressure [PAP] as determined by echocardiography and pulmonary artery catheter measurement) were collected at baseline and during follow-up visits. The diagnosis of interstitial lung disease (ILD) was supported by demonstration of typical radiological patterns with 1. increased attenuation with ground glass opacity and/or consolidations, 2. reticular pattern with parenchymal distortion (fibrosis), 3. nodules, and/or 4. mosaic patterns and cysts in high-resolution computed tomography (HR-CT) (11). Pulmonary arterial hypertension (PAH) was defined as mean pulmonary artery pressure (PAP)>20 mmHg shown in echocardiography and confirmed by pulmonary artery catheter measurement with a pulmonary artery wedge

pressure (PAWP) of ≤15 mmHg (12). Response to therapy in SSc was defined by the absence of the need for therapy escalation during the follow-up period. In contrast, early disease progression was defined as active SSc-related organ manifestations including cardiac, skin, and/or pulmonary involvement with the need for therapy escalation within 12 months (13). Demographic and clinical findings of the consecutively enrolled patients are described in Table I.

*sIL-2R detection by chemiluminescent immunometric assay*

Serum sIL-2R concentrations were determined using a commercially available solid-phase, two-site chemiluminescent immunometric assay (CLIA) according to the manufacturer's instructions (IMMULITE 2000 sIL-2R, Siemens Healthcare Diagnostics Products Ltd., Gwynedd, UK). Serum sIL-2R concentrations were analysed at baseline and followed up to 48 months after. Laboratory tests included serum measurements of hs-CRP, b2M, NT-proBNP, hs-TnT, blood cell count and autoantibodies (anti-topoisomerase I [Scl-70], anti-RNA polymerase III [subunits RP11 and RP155], anti-centromere protein A [CENP-A], anti-PM-Scl, anti-Ro-52, anti- Ro/SS-A, anti-La/SS-B, anti-U1-RNP). hs-CRP was determined by particle-enhanced immunoturbidimetric assay, hs-TnT by Electrochemiluminescence immunoassay (ECLIA) and ß2M by immunoturbidimetric assay (all from Roche Diagnostics, Rotkreuz, Switzerland). Autoantibodies were determined using commercial enzyme-linked immunosorbent assay (ELISA) (EUROIMMUNE, Lübeck, Germany).

*Statistical analysis*

The results were analysed with Prism v. 10.1.1 (GraphPad Software) and are depicted as mean±SD. Non-parametric Mann-Whitney U-test and Spearman's rho were used to examine non-categorical, non-normal distributed values. Fisher's exact test was performed to compare categorical values. When two groups of samples were compared for iterating parameters or more than two groups of samples were compared, a one-way ANOVA was performed. Tuk-

**Table I.** Patient characteristics SSc.

| | SSc n=315 | lcSSc n=176 (55.9%) | dcSSc n=139 (44.1%) | *p*-value |
|---|---|---|---|---|
| Female/male | 248/67 | 158/18 | 90/49 | **<0.001** |
| Age (years), mean (range) | 55 (18-84) | 56 (23–83) | 53 (18–84) | 0.313 |
| New-onset diagnosis, n (%) | 36 (11) | 6 (3) | 30 (22) | **<0.001** |
| Disease duration (years), mean (SD)* | 4.7 (6.3) | 4.6 (6.6) | 4.8 (6.0) | 0.951 |
| Body mass index (kg/m$^2$), mean (SD) | 25.14 (5.28) | 25.50 (5.25) | 24.67 (5.28) | 0.357 |
| **SSc manifestations** | | | | |
| ***Skin*** | | | | |
| mRSS, mean (SD) | 7.22 (6.46) | 4.76 (2.88) | 10.35 (8.15) | **<0.001** |
| ***Pulmonary*** | | | | |
| ILD, n (%) | 113 (36) | 35 (20) | 78 (56) | **<0.001** |
| PAH, n (%) | 29 (9) | 14 (8) | 15 (11) | 0.435 |
| ***Cardiac involvement*** | | | | |
| Pericardial effusion, n (%) | 22 (7) | 8 (5) | 14 (10) | 0.074 |
| Cardiac insufficiency, n (%) | 26 (8) | 9 (5) | 17 (12) | **0.037** |
| Peri-/Myo-/Endocarditis, n (%) | 8 (3) | 2 (1) | 6 (4) | 0.145 |
| CHD, n (%) | 21 (7) | 10 (6) | 11 (8) | 0.498 |
| ***Gastrointestinal involvement, n (%)*** | 125 (40) | 69 (39) | 56 (40) | 0.908 |
| ***Renal involvement*** | | | | |
| CKD, n (%) | 47 (15) | 29 (16) | 18 (13) | 0.109 |
| SRC, n (%) | 5 (2) | 2 (1) | 3 (2) | 0.658 |
| **Previous history of malignancy** | | | | |
| Solid tumour, n (%) | 35 (11) | 18 (10) | 17 (12) | 0.593 |
| non-solid tumour, n (%) | 4 (2) | 0 (0) | 4 (3) | **0.037** |
| **Comorbidities** | | | | |
| Rheumatoid arthritis, n (%) | 96 (30) | 44 (25) | 52 (37) | **0.020** |
| Polymyositis / Dermatomyositis, n (%) | 33 (10) | 7 (4) | 26 (19) | **<0.001** |
| Sjögren's syndrome | 32 (10) | 21 (12) | 11 (8) | 0.265 |
| ***Laboratory values*** | | | | |
| sIL-2R (U/ml), mean (SD) | 646.1 (473.6) | 571.7 (361.1) | 765.1 (592.7) | **0.001** |
| hs-CRP (mg/l), mean (SD) | 6.03 (12.54) | 4.37 (9.12) | 8.62 (16.73) | **0.005** |
| NT-proBNP (ng/l), mean (SD) | 450 (1643) | 448 (1997) | 454 (1044) | 0.973 |
| hs-troponin T (ng/l), mean (SD) | 19.56 (37.59) | 9.58 (13.33) | 32.81 (52.53) | **<0.001** |
| ß2-microglobulin (mg/l), mean (SD) | 2.80 (3.69) | 2.83 (3.67) | 2.74 (3.73) | 0.856 |
| Leukocytes (count), mean (SD) | 7.43 (2.53) | 6.85 (1.97) | 8.17 (2.95) | **<0.001** |
| Neutrophils (%), mean (SD) | 66.95 (10.77) | 64.66 (10.09) | 69.91 (10.94) | **<0.001** |
| Lymphocytes (%), mean (SD) | 21.35 (8.38) | 23.46 (8.12) | 18.67 (8.79) | **<0.001** |
| Thrombocytes (count), mean (SD) | 272 (78) | 287 (88) | 260 (67) | **0.003** |
| ***Antinuclear antibody*** | | | | |
| Centromere proteins, n (%) | 125 (40) | 112 (64) | 13 (9) | **<0.001** |
| DNA-topoisomerase I (Scl-70), n (%) | 92 (29) | 19 (11) | 73 (53) | **<0.001** |
| RNA polymerase III, n (%) | 26 (8) | 6 (3) | 20 (14) | **0.001** |
| PM-Scl-100, n (%) | 30 (10) | 9 (5) | 21 (15) | ***0.003*** |
| Ro-52, n (%) | 46 (15) | 25 (14) | 21 (15) | 0.873 |
| Ro/SS-A, n (%) | 26 (8) | 17 (10) | 9 (6) | 0.410 |
| La/SS-B, n (%) | 4 (1) | 4 (2) | 0 (0) | 0.133 |
| U1-RNP, n (%) | 2 (1) | 1 (1) | 1 (1) | >0.999 |
| **Cardiopulmonary parameters, mean (SD)** | | | | |
| FVC, %pred (SD) | 86.99 (18.83) | 93.14 (17.18) | 79.47 (18.24) | 0.074 |
| DLCO, %pred (SD) | 70.39 (23.27) | 74.16 (21.14) | 65.76 (24.84) | **0.003** |
| LVEF, % (SD) | 57.74 (7.42) | 58.46 (7.51) | 56.80 (7.21) | 0.186 |
| **Therapy at time of sampling in %** | | | | |
| Mycophenolate mofetil | 16.67 | 10 | 17.86 | >0.999 |
| Methotrexate | 18.18 | 30 | 16.07 | 0.372 |
| Hydroxychloroquine | 22.73 | 30 | 21.43 | 0.683 |
| Azathioprine | 10.60 | 20 | 8.93 | 0.285 |
| Cyclosporine | 4.55 | 0 | 5.36 | >0.999 |
| Cyclophosphamide | 15.15 | 10 | 16.07 | >0.999 |
| Rituximab | 12.12 | 0 | 13.33 | 0.001 |

SSc: systemic sclerosis; lcSSc: limited cutaneous systemic sclerosis; dcSSc: diffuse cutaneous systemic sclerosis; mRSS: modified Rodnan Skin Score; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension; CHD: coronary heart disease; CKD: chronic kidney disease; SRC: scleroderma renal crisis; sIL-2R: soluble interleukin-2 receptor; hs-CRP: high-sensitivity C-reactive protein; NT-proBNP: N-terminal pro-B-type natriuretic peptide; FVC: forced vital capacity; DLCO: diffusing capacity for carbon monoxide; LVEF: left ventricular ejection fraction.
*disease duration since fulfilment 2013 classification criteria.

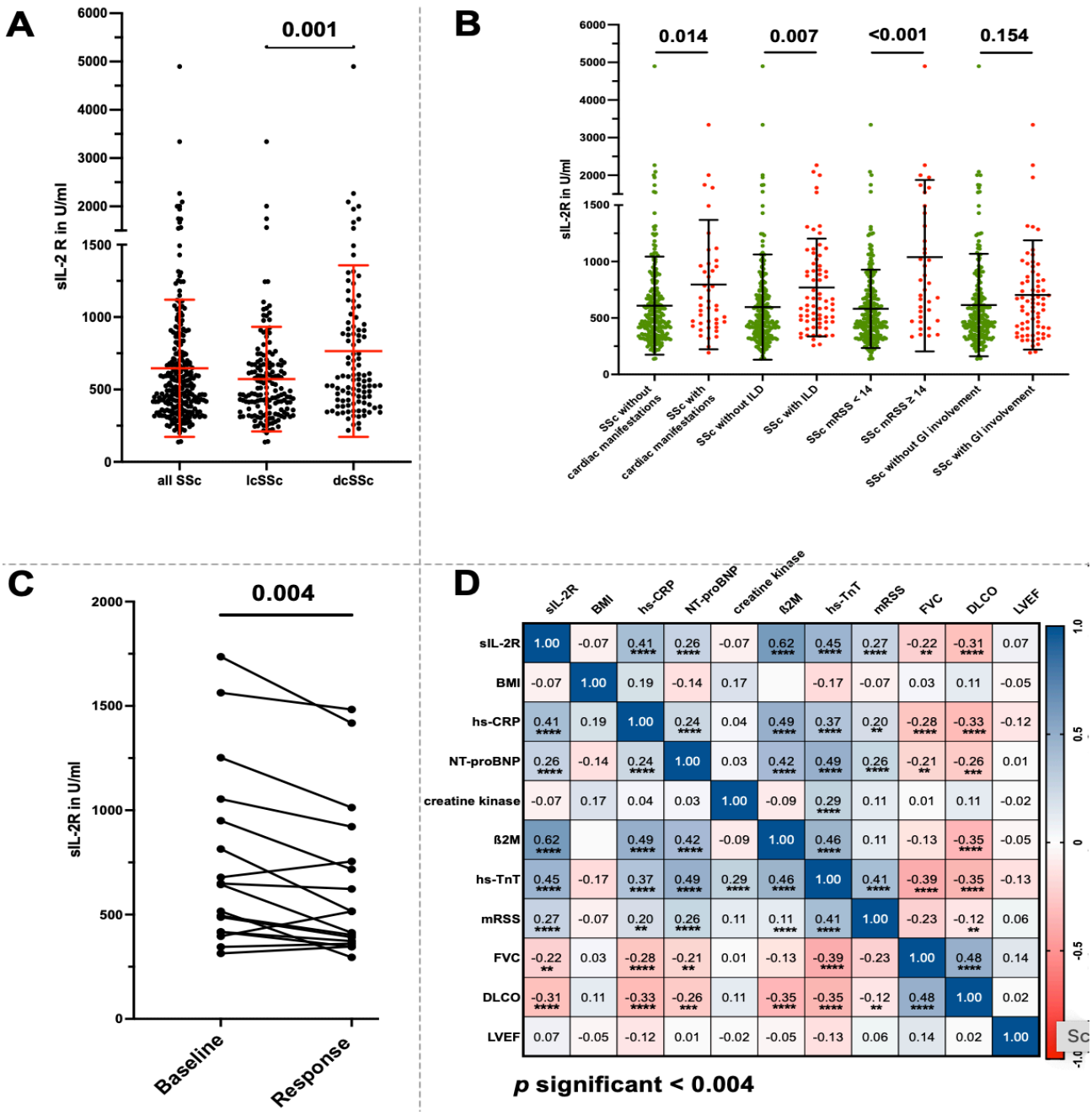


**Fig. 1.** Soluble interleukin-2 receptor (sIL-2R) concentrations in systemic sclerosis (SSc).
**A**: Patients with diffuse cutaneous SSc (dcSSc) had increased concentrations of sIL-2R compared to limited cutaneous SSc (lcSSc). **B**: SSc patients with cardiac, pulmonary (ILD) or extended skin manifestation (mRSS≥14) had increased sIL-2R concentrations, except in patients with gastrointestinal involvement. **C**: Concentrations of sIL-2R in SSc decreased in patients with clinical response after therapy initiation for 6±2 months **D**: In SSc, multivariant analysis showed positive correlations between sIL-2R serum concentrations and hs-CRP, NT-proBNP, b2M, hs-TnT and mRSS, while negative correlations were found for FVC and DLCO.
ILD: interstitial lung disease; mRSS: modified Rodnan Skin Score; BMI: Body Mass Index; hs-CRP: high-sensitivity C-reactive protein; NT-proBNP: N-terminal pro-B-type natriuretic peptide; FVC: forced vital capacity; DLCO: diffusing capacity for carbon monoxide.
*$p$<0.004; **$p$<0.001; ***$p$<0.0001; ****$p$<0.00001.

ey's range test was used as a *post-hoc* analysis of ANOVA. Receiver operating characteristic (ROC) analyses were performed to identify the optimal cut-off points for sIL-2R levels. Clinical deterioration was estimated using the Kaplan-Meier method. Log-rank test and Mantel-Cox proportional hazards model were performed to identify the predictive value of sIL-2R concentration. $p$-values below 0.05 were considered significant. Regarding multivariant analysis, Bonferroni correction was used.

## Results

### *Patient characteristics*

Serum samples from patients with SSc (n=315; lcSSc, n=160; dcSSc, n=137) were examined. The dcSSc cohort had a higher proportion of male patients ($p$<0.001) with new onset disease

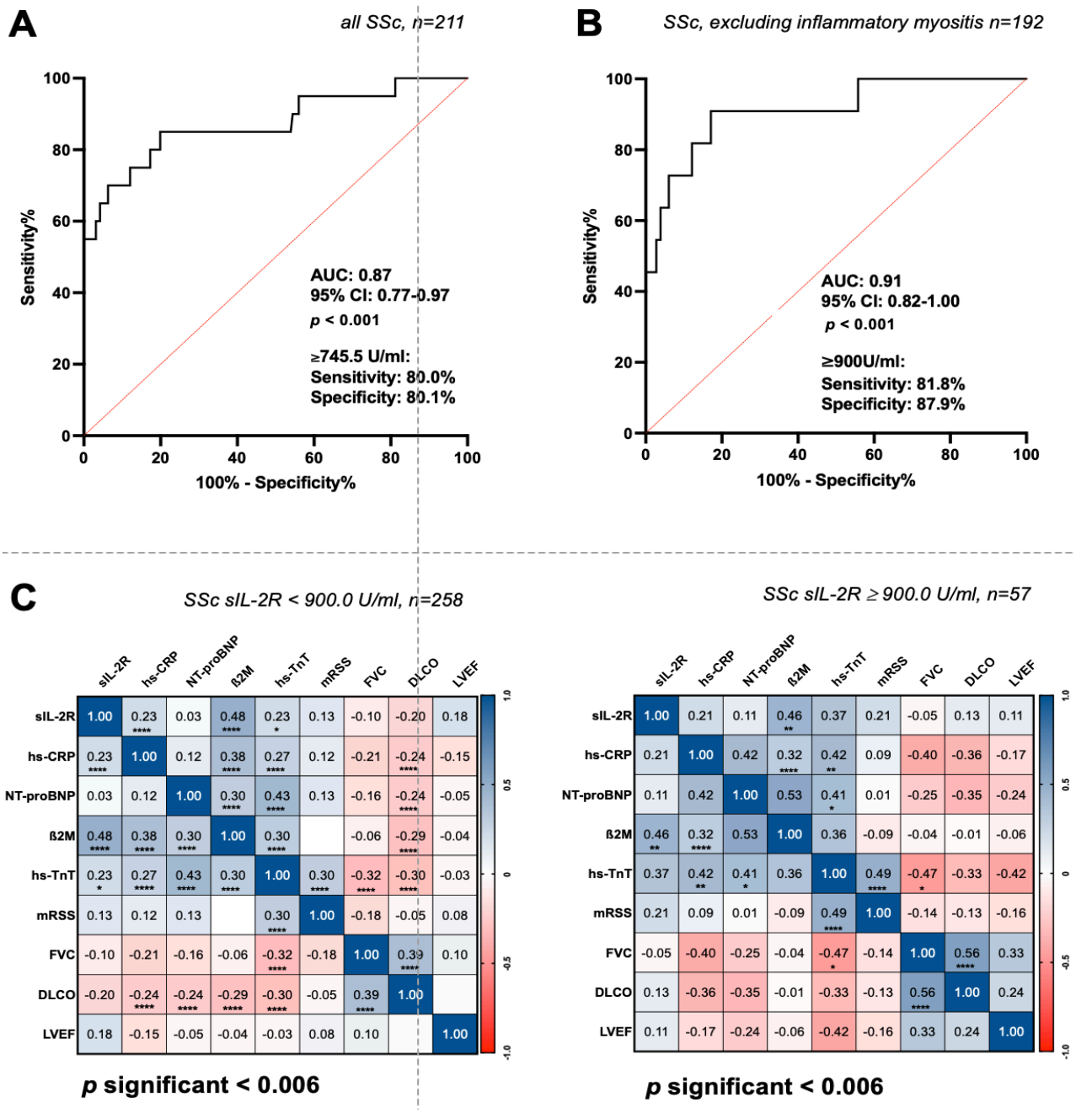


**Fig. 2.** Correlation between soluble interleukin-2 receptor (sIL-2R) and hs-troponin T (hs-TnT) concentrations in systemic sclerosis (SSc).
**A**: Increased concentrations of sIL-2R ≥745.5U/ml correlated with an increase of hs-TnT in SSc (n=221). **B**: Excluding patients with an overlap of inflammatory myositis, sIL-2R ≥900 U/ml discriminated normal from pathological range concentrations of hs-TnT (AUC:0.91, $p$<0.0001; sensitivity 81.8%, specificity 87.9%; n=192). **C**: In contrast to SSc with sIL-2R <900 U/ml, multivariant analyses identified correlations between hs-TnT and organ manifestations (mRSS, FVC, LVEF) in patients with sIL-2R ≥900 U/ml.
AUC: area under the curve; CI: confidence interval; hs-CRP: high-sensitivity C-reactive protein; NT-proBNP: N-terminal pro-B-type natriuretic peptide; mRSS: modified Rodnan Skin Score; FVC: forced vital capacity; DLCO: diffusing capacity for carbon monoxide; LVEF: left ventricular ejection fraction. *$p$<0.006; **$p$<0.001; ***$p$<0.0001; ****$p$<0.00001.

($p$<0.001), comprising increased skin (mRSS, $p$<0.001), pulmonary (ILD, $p$<0.001) and cardiac involvement (cardiac insufficiency, $p$=0.037) in comparison to the lcSSc cohort. In dcSSc, patients displayed increased frequencies of non-solid tumours ($p$=0.037), rheumatoid arthritis ($p$=0.020) and polymyositis/dermatomyositis ($p$<0.001). The use of disease-modifying anti-rheumatic drugs (DMARDs) was comparable in both subgroups, except for rituximab in dcSSc (lcSSc *vs.* dcSSc: 0% *vs.* 13%, $p$=0.001). Demographic and clinical findings of the patients enrolled in this longitudinal cross-sectional observational study are described in Table I.

### *Increased sIL-2R concentrations correlate with other biomarkers and organ manifestations*

Laboratory findings showed increased serum sIL-2R ($p$=0.001; Fig. 1A), hs-

CRP ($p$=0.005), and hs-TnT ($p$<0.001) concentrations in dcSSc compared to lcSSc. In particular, SSc patients with cardiac involvement ($p$=0.014), ILD ($p$=0.007), and extended skin sclerosis (mRSS ≥14, $p$<0.001) presented elevated concentrations of sIL-2R compared to patients without these manifestations. In contrast, SSc patients with gastrointestinal involvement did not show elevated sIL-2R concentrations (Fig. 1B). In patients with clinical improvement in response to therapy after an average of 6(±2) months (median [range]), the concentration of sIL-2R decreased (baseline *vs*. response: 748.7±428.5 *vs*. 640.6±370.3, $p$=0.004), whereas SSc patients with clinical progress showed no changes ($p$=0.399) (Supplementary Fig. S1). At baseline, serum concentrations of sIL-2R positively correlated with hs-CRP ($p$<0.001, r=0.41), NT-proBNP ($p$<0.001, r=0.26), b2M ($p$<0.001, r=0.62), hs-TnT ($p$<0.001, r=0.45), and mRSS ($p$<0.001, r=0.26), but showed no correlations with the BMI (Fig. 1D). Of note, FVC ($p$=0.001, r=-0.22) and DLCO ($p$<0.001, r=- 0.31) correlated negatively with sIL-2R (Fig. 1D). Subgroup analysis showed no association between sIL-2R concentrations and other serological findings (*e.g*. antinuclear antibody) or therapy. Further subgroup analysis showed no therapeutic effects of a reduced sIL-2R concentration in SSc patients following rituximab therapy ($p$=0.278) (Suppl. Fig. S2).

### *sIL-2R concentrations ≥900 U/ml discriminated normal from pathological range concentrations of hs-TnT*

ROC analyses (Youden's index) were performed, identifying a cut-off value of sIL-2R in relation to other biomarkers and organ involvement. Weak associations were found between sIL-2R and pathological values of b2M (AUC: 0.84, 95%CI: 0.79-0.89, $p$<0.001, cut-off sIL-2R ≥522.0 U/ml, sensitivity 81.4%, specificity 72.1%, (Suppl. Fig. S3A), increased hs-CRP concentrations (AUC: 0.71, 95%CI: 0.64–0.78, $p$<0.001, sIL-2R ≥607.5 U/ml, sensitivity 64.6%, specificity 73.8%) (Suppl. Fig. S3B), and pathological NT-proBNP levels (AUC: 0.68, 95%CI: 0.61–

**Table II.** Patient characteristics subgrouped according to sIL-2R concentration with a cut-off below or above 900 U/ml.

| | sIL-2R<900 U/ml n=258 (81.90%) | sIL-2R≥900 U/ml n=57 (18.10%) | $p$-value |
|---|---|---|---|
| Female/male | 212/46 | 48/9 | 0.848 |
| lcSSc/dcSSc | 156/102 | 20/37 | **0.001** |
| Age (years), mean (range) | 52 (18–81) | 56 (24-84) | 0.316 |
| Disease duration (years), mean (SD) * | 4.9 (6.5) | 3.9 (5.4) | 0.271 |
| Body mass index (kg/m$^2$), mean (SD) | 25.05 (5.08) | 25.51 (6.20) | 0.572 |
| **SSc manifestations** | | | |
| ***Skin*** | | | |
| mRSS, mean (SD) | 6.50 (5.55) | 10.79 (8.91) | **<0.001** |
| ***Pulmonary*** | | | |
| ILD, n (%) | 77 (30) | 36 (63) | **<0.001** |
| PAH, n (%) | 23 (9) | 6 (11) | 0.800 |
| ***Cardiac involvement*** | | | |
| Pericardial effusion, n (%) | 17 (7) | 5 (9) | 0.567 |
| Cardiac insufficiency, n (%) | 17 (7) | 10 (18) | **0.015** |
| Peri-/Myo-/Endocarditis, n (%) | 6 (2) | 2 (4) | 0.639 |
| CHD, n (%) | 15 (6) | 6 (11) | 0.236 |
| ***Gastrointestinal involvement, n (%)*** | 94 (36) | 30 (53) | **0.026** |
| ***Renal involvement*** | | | |
| CKD, n (%) | 31 (12) | 16 (28) | **0.004** |
| SRC, n (%) | 2 (1) | 3 (5) | **0.043** |
| **Previous history of malignancy** | | | |
| Solid tumour, n (%) | 26 (10) | 9 (16) | 0.243 |
| non-solid tumour, n (%) | 2 (1) | 2 (4) | 0.151 |
| **Comorbidities** | | | |
| Rheumatic arthritis, n (%) | 79 (31) | 17 (30) | **0.111** |
| Polymyositis/dermatomyositis, n (%) | 22 (9) | 11 (19) | **0.028** |
| Sjögren's syndrome | 30 (12) | 2 (4) | 0.088 |
| ***Laboratory values*** | | | |
| sIL-2R (U/ml), mean (SD) | 488.2 (172.60) | 1368 (692.20) | **<0.001** |
| hs-CRP (mg/l), mean (SD) | 3.87 (8.05) | 17.30 (23.24) | **<0.001** |
| NT-proBNP (ng/l), mean (SD) | 259 (617.0) | 1314 (3523.0) | **<0.001** |
| hs-troponin T (ng/l), mean (SD) | 11.38 (14.95) | 54.12 (71.02) | **<0.001** |
| ß2-microglobulin (mg/l), mean (SD) | 2.22 (1.40) | 6.03 (8.19) | **<0.001** |
| Leukocytes (count), mean (SD) | 7.20 (2.16) | 8.74 (3.63) | **0.001** |
| Neutrophils (%), mean (SD) | 65.91 (10.17) | 71.87 (12.19) | **0.001** |
| Lymphocytes (%), mean (SD) | 22.45 (8.19) | 16.33 (9.48) | **<0.001** |
| Thrombocytes (count), mean (SD) | 269.4 (67.60) | 283.7 (112.80) | 0.213 |
| ***Antinuclear antibody*** | | | |
| Centromere proteins, n (%) | 107 (41) | 19 (33) | 0.296 |
| DNA-topoisomerase I (Scl-70), n (%) | 73 (28) | 19 (33) | 0.525 |
| RNA polymerase III, n (%) | 20 (8) | 6 (11) | 0.438 |
| PM-Scl-100, n (%) | 24 (9) | 7 (12) | 0.468 |
| Ro-52, n (%) | 38 (15) | 11 (19) | 0.420 |
| Ro/SS-A, n (%) | 23 (9) | 4 (7) | 0.797 |
| La/SS-B, n (%) | 4 (2) | 0 (0) | >0.999 |
| U1-RNP, n (%) | 1 (0.39) | 1 (2) | 0.330 |
| **Cardiopulmonary parameters, mean (SD)** | | | |
| FVC, %pred (SD) | 88.94 (18.62) | 77.15 (18.17) | **<0.001** |
| DLCO, %pred (SD) | 73.46 (22.21) | 60.69 (24.46) | **0.001** |
| LVEF, % (SD) | 57.92 (6.70) | 56.82 (9.82) | 0.385 |
| **Therapy at time of sampling, in %** | | | |
| Mycophenolate mofetil | 13.95 | 17.39 | 0.730 |
| Methotrexate | 20.93 | 17.39 | >0.999 |
| Hydroxychloroquine | 23.26 | 21.74 | 0.559 |
| Azathioprine | 11.63 | 8.70 | >0.999 |
| Cyclosporine | 2.33 | 8.70 | 0.276 |
| Cyclophosphamide | 9.30 | 26.09 | 0.085 |
| Rituximab | 18.60 | 0 | **0.043** |

SSc: systemic sclerosis; lcSSc: limited cutaneous systemic sclerosis; dcSSc: diffuse cutaneous systemic sclerosis; mRSS: modified Rodnan Skin Score; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension; CHD: coronary heart disease; CKD: chronic kidney disease; SRC: scleroderma renal crisis; sIL-2R: soluble interleukin-2 receptor; hs-CRP: high-sensitivity C-reactive protein; NT-proBNP: N-terminal pro-B-type natriuretic peptide; FVC: forced vital capacity; DLCO: diffusing capacity for carbon monoxide; LVEF: left ventricular ejection fraction.
*disease duration starting at fulfilment of 2013 classification criteria.

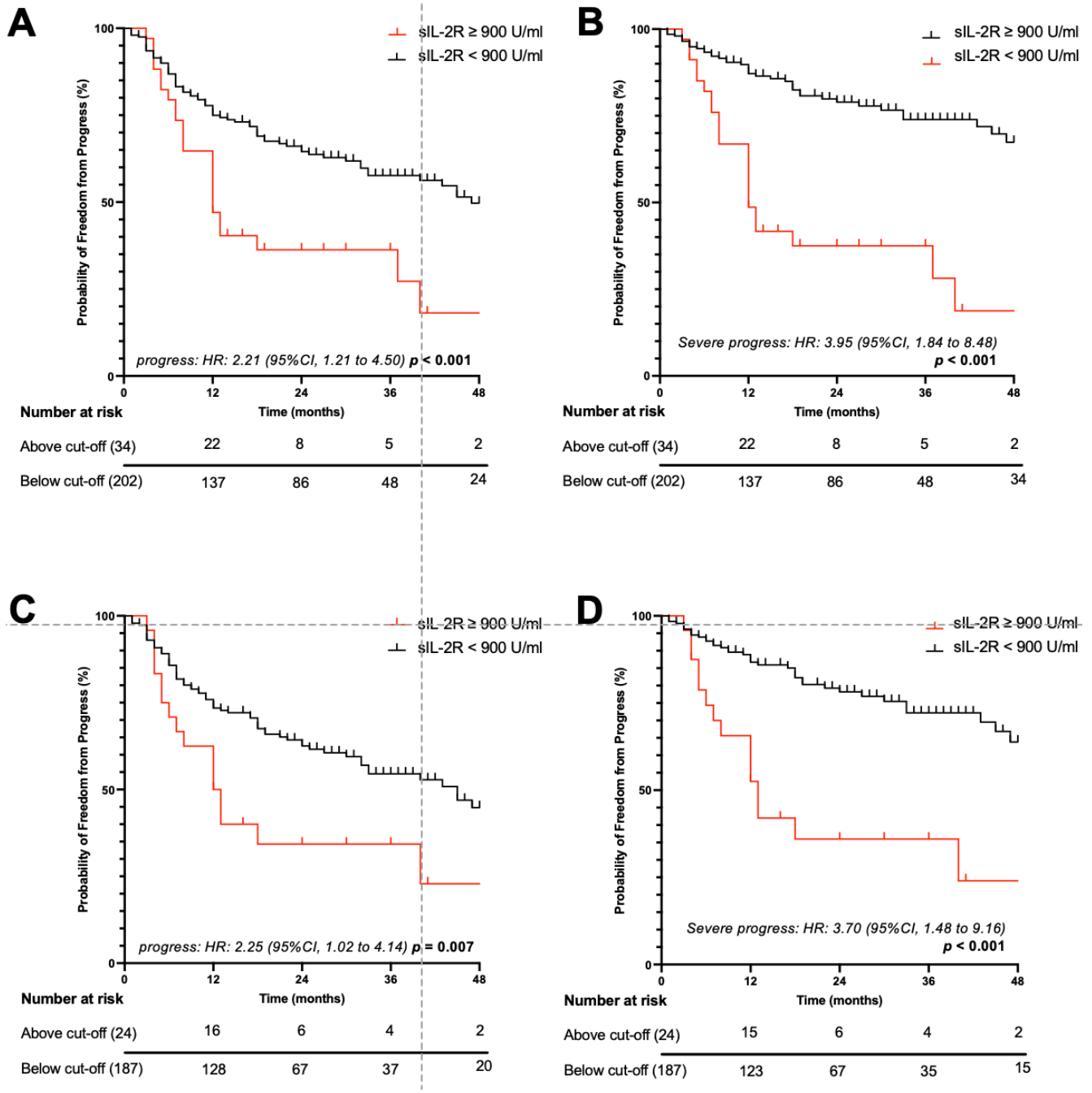


**Fig. 3.** Kaplan-Meier survival analyses for the whole SSc group (**A, B**), and the subgroup excluding inflammatory myositis patients (**C, B**). Soluble Interleukin-2 receptor (sIL-2R) concentrations ≥900 U/ml were associated with disease progression within 12 months and severe organ involvement with pulmonary and cardiac involvement, respectively (**C, D**).

0.75, $p<0.001$, sIL-2R ≥543.0 U/ml, sensitivity 60.0%, specificity 65.2%, (Suppl. Fig. S3C). In all SSc patients, sIL-2R concentrations ≥745.5 U/ml were associated with pathological range concentrations of hs-TnT (>99$^{th}$ percentile upper reference limit; AUC: 0.87, 95%CI: 0.77–0.97, $p<0.001$, sensitivity 80.00%, specificity 80.10%) (Fig. 2A). To evaluate the impact of myositis as a potential non-cardiac source for the increase in hs-TnT concentrations (14), we performed a subgroup analysis based on the presence or absence of myositis overlap showing that in SSc patients without myositis overlap, the AUC was higher compared to those with myositis overlap (sIL-2R ≥900 U/ml: AUC: 0.91, 95%CI: 0.82–1.00, $p<0.001$, sensitivity 81.8%, specificity 87.9% *vs.* sIL-2R ≥673 U/ml: AUC: 0.84, 95%CI: 0.66–1.00, $p=0.007$, sensitivity 80.0%, specificity 75.0%) (Fig. 2B and Suppl. Fig. S4).

### *sIL-2R concentrations ≥900 U/ml defined different clinical subtypes of SSc*

Subgroup analyses of SSc patients based on serum sIL-2R concentrations below or above a cut-off level of 900 U/ml identified different clinical subtypes. In contrast to the SSc subgroup with a sIL-

2R concentration <900 U/ml, the SSc subgroup with sIL-2R concentrations ≥900 U/ml displayed a higher proportion of patients with dcSSc ($p$=0.001) and more severe organ involvement with a higher mRSS ($p$<0.001), ILD ($p$<0.001) and increased prevalence of cardiac insufficiency ($p$=0.015), gastrointestinal involvement ($p$=0.026), chronic kidney disease ($p$=0.004), reduced FVC ($p$<0.001), and reduced DLCO ($p$=0.001) (Table II). In the subgroup of SSc patients with a sIL-2R concentration ≥900 U/ml, multivariant analyses showed correlations between hs-TnT and clinical markers of skin sclerosis (mRSS, r=0.49), pulmonary restriction (FVC, r=-0.47) and cardiac function (LVEF, r=-0.42) (Fig. 2C). Interestingly, both subgroups (sIL-2R <900U/ml and sIL-2R ≥900 U/ml, respectively) did not differ with regard to immunosuppressive therapy, with the exception of rituximab. Here, RTX-treated SSc patients more frequently revealed sIL2-R concentrations <900 U/ml ($p$=0.043) (Table II).

*Increased sIL-2R concentrations are associated with early disease progression*

The possible association between sIL-2R concentrations and early disease progression was analysed during a 48-month follow-up period. Early disease progression was defined as active SSc-related organ manifestations including cardiac, skin, and/or pulmonary involvement with the need for therapy escalation within 12 months. More than 50% of the patients with SSc displaying a sIL-2R concentration ≥900 U/ml showed disease progression within 12 months (Fig. 3A, HR: 2.21, $p$<0.001) including severe manifestations with pulmonary and cardiac involvement (Fig. 3B, HR: 3.95, $p$<0.001). By contrast, only 25% of the patients with sIL-2R concentrations <900 U/ml showed disease progression within 12 months.

When SSc patients with myositis overlap were excluded, the association between early disease progression and increased sIL-2R concentrations remained demonstrable (Fig. 3C-D; HR: 2.25, $p$=0.007; severe progression HR: 3.70, $p$<0.001, respectively). To evaluate therapeutic effects, especially of RTX, SSc patients were divided according to the induced specific therapy. Kaplan-Meier analysis confirmed no superiority of RTX-based therapy compared to other DMARDs in this retrospective SSc cohort (Suppl. Fig. S5)

## Discussion

Recent cohort studies of SSc reporting an increased risk of mortality emphasise an urgent need of biomarkers which are suited to detect early disease progression in SSc (1-5, 15-17). Increased serum sIL-2R concentrations suggestive of immunological perturbation were previously described as indicator of disease progression and severity in small cohorts of SSc patients (6, 8). Herein, applying a larger monocentric cohort including those with dcSSc, we confirm and extend those findings by showing increased serum sIL-2R concentrations in SSc and a positive correlation between sIL-2R and hs-TnT, a serological marker of cardiac involvement in SSc suggestive of early cardiac damage (16, 18). Increased sIL-2R concentrations were predictive for pathological range hs-TnT concentrations suggestive of a link between immunological perturbations and cardiac damage. The pathogenesis of cardiac involvement in SSc is multifactorial, including heart inflammation and inflammation-driven fibrosis (19). Increased sIL-2R concentration due to IL-2R shedding is found during T cell differentiation and activation, inducing IL-6-mediated inflammation and fibrosis in SSc (1-4). In murine models of SSc, T cell-derived IL-6 induces cardiac fibrosis by angiotensin-II through TGF-b/Smad activation (19). A recent study in non-SSc patients with acute myocardial infarction suggested sIL-2R in combination with IL-8 as biomarkers of an increased risk for major cardiovascular events (MACE) (20). In SSc, increased NT-proBNP concentrations are regarded as a biomarker of pulmonary arterial hypertension (PAH) (21). However, in the present study, there was no association between increased NT-proBNP concentrations and PAH analysed by echocardiography and pulmonary artery catheter measurement, suggesting early PAH stages in this cohort. Consistent with the assumption that sIL-2R is a marker of early disease, a weak association was found between sIL-2R and pathological range concentrations of NT-proBNP.

The increased mortality risk in SSc is mainly caused by cardiac and pulmonary involvement (1-4, 15-17). In addition to the strong association between sIL-2R and hs-TnT, we found a moderate negative correlation between sIL-2R and markers of pulmonary function (FVC, DLCO). Especially in the group of patients with sIL-2R ≥900 U/ml at baseline, the majority displayed radiological features of ILD. Moreover, our study shows an increased risk for early disease progression at sIL-2R concentrations of ≥900 U/ml. SSc patients displaying sIL-2R ≥900 U/ml at baseline presented with more severe organ involvement. Thus, sIL-2R may be useful as a biomarker for the identification of SSc patients at risk of severe organ involvement and disease progression. These data are in support of high sIL-2R concentrations as marker of SSc disease severity. In addition, low sIL-2R concentrations in patients who respond to therapy and higher sIL-2R concentrations in SSc patients with clinical progression and lack of response to therapy suggest a potential role for sIL-2R in monitoring responses to therapy. Here, patients treated with rituximab, mostly in combination with other drugs, showed low sIL-2R concentrations at baseline. However, compared to other therapies, rituximab therapy did not demonstrate a clear follow-up reduction in sIL-2R concentration or disease activity. While the single-centre DESIRES cohort study demonstrated clinical follow-up improvements in skin and lung fibrosis in patients with SSc treated with rituximab (22), this was not initially shown in our study. This could be due to the retrospective design and focus on sIL-2R. However, the performance of sIL-2R as a marker for disease and therapy monitoring needs to be validated in further studies. While the strength of our study lies in the comparatively large size of the SSc cohort and its longitudinal observation period, it is limited by its observational design and monocentric character.

In conclusion, the present study identified increased concentrations of sIL-2R as a risk factor for severe organ manifestations and early disease progression in SSc, and possibly as a biomarker for disease and therapy monitoring. sIL-2R levels disclosed a previously unknown link with cardiac involvement. The results of this study have to be supported by future prospective multicentric studies to confirm the prognostic value of sIL-2R and hs-TnT levels as biomarkers for disease progression, response to therapy, and cardiac involvement in SSc.

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