Published in final edited form as:

Arthritis Rheumatol. 2020 August ; 72(8): 1341–1349. doi:10.1002/art.41265.

The Utility of Plasma Vascular Biomarkers in Systemic Sclerosis: A Prospective Longitudinal Analysis

Christopher A. Mecoli, MD MHS[1], **Ami A. Shah, MD MHS**[1], **Francesco Boin, MD**[2], **Fredrick M. Wigley, MD**[1], **Laura K. Hummers, MD ScM**[1]

[1]Johns Hopkins University School of Medicine, Baltimore, MD

[2]Division of Rheumatology, Department of Medicine, University of California, San Francisco, California

Abstract

Objective: Pulmonary hypertension (PH) and ischemic digital lesions (DL) are two scleroderma vascular outcomes that in cross-sectional studies are associated with abnormalities in biomarkers of angiogenesis. The clinical usefulness of these biomarkers is unknown in part due to lack of data on longitudinal measurement.

Methods: We conducted a prospective cohort study of 300 patients with systemic sclerosis (SSc) who were followed for at least a five-year period, who at enrollment lacked evidence of PH and/or active DL. Levels of hepatocyte growth factor (HGF), soluble Flt-1, soluble endoglin, endostatin, and platelet-derived growth factor (PlGF) were obtained at multiple time points and assessed for their ability to predict the development of PH/DL.

Results: 46 patients (15%) developed PH and 69 (23%) developed a DL. In time-to-event analyses, three biomarkers measured at cohort entry were found to significantly associate with the development of PH: HGF (HR 1.99, 95%CI 1.24–3.17, p=0.004), sFlt1 (HR 3.04, 95%CI 1.29–7.14, p=0.011), and PlGF (HR 2.74, 95%CI 1.32–5.69, p=0.007). As time approaching PH diagnosis decreased, there was no corresponding increase in any biomarker level. Upon converting each continuous vascular biomarker into a binary variable, a dose-response relationship was observed for the number of elevated biomarkers at cohort entry and risk of developing PH: With each additional elevated biomarker at cohort entry, there was a 78% increase in hazard of developing PH (HR 1.78, 95% CI 1.2–2.6, p=0.004).

Conclusion: These data suggest that molecules involved in angiogenesis reflect vascular perturbation and elevations at first encounter can risk-stratify patients.

Keywords

scleroderma; pulmonary hypertension; cohort studies; biomarkers

Corresponding Author and Request for Reprints: Laura K. Hummers, MD ScM, Johns Hopkins University School of Medicine, Division of Rheumatology, 5200 Eastern Ave, MFL Bldg, Center Tower, Suite 4100, Baltimore, MD 21224, lhummers@jhmi.edu, 410-550-6819.

INTRODUCTION

Two major vascular complications in SSc patients associated with substantial morbidity and mortality are ischemic digital lesions (DL) and pulmonary hypertension (PH)[1–7]. Unfortunately, the recognition of both conditions often becomes clinically evident only after advanced vascular injury has occurred. Identifying biomarkers that predict who is at elevated risk to develop PH or DL might allow the opportunity to intervene during the pre-clinical period, or at the very least, identify subjects who need closer monitoring for their development. Several groups, including our own, have studied plasma vascular biomarkers in SSc [8–13], focusing on their ability to predict PH and DL. While promising, the majority of this prior work utilizes cross-sectional data, with biomarker measurements occurring ≤2 time points. Thus, there still exists uncertainty on the trajectory of vascular biomarker measurements over time, and whether they can serve as useful laboratory tests to help guide clinical-decision making. In an effort to provide evidence to answer this question, we describe our experience with a 300 SSc patient prospective cohort with serial plasma vascular biomarker measurement.

MATERIALS AND METHODS

We conducted a prospective cohort study of 300 prevalent SSc patients as previously described [14]. The Johns Hopkins University Institutional Review Board approved of this study (NA_00001412). Briefly, prevalent SSc patients lacking evidence of PH (defined by an estimated right ventricular pressure (RVSP) of <40 mmHg by transthoracic echocardiography) and without current active ischemic digital lesions were recruited during routine outpatient visits. Patients were subsequently followed prospectively with clinical assessments and plasma samples collected at 6–12 month intervals. At each visit the patient was examined for signs and symptoms of development of PH and DL, and history of interval DL was determined at visits and by chart review. Our center's algorithm for screening for PH includes obtaining routine yearly pulmonary function testing and echocardiogram (ECHO). Patients were referred for right heart catheterization (RHC) at the discretion of the treating rheumatologist. Our center's protocol to refer for RHC is based on the following guidelines: A decline in the diffusing capacity of the lungs for carbon monoxide (DLCO) out of proportion to decline in forced vital capacity (FVC) or total lung capacity (TLC), ECHO with estimated right ventricular systolic pressure (RVSP) ≥ 40 mmHg OR increase in RVSP > 10 mmHg from prior study, echocardiogram evidence of right ventricular abnormalities (enlargement, dilatation), dyspnea on exertion not explained by other causes, or clinical signs suggestive of PH (lower extremity edema, increased jugular venous pressure).

Autoantibody data were obtained via the Euroline SSc profile (IgG) (EUROIMMUN, Lübeck, Germany) with a positive result defined as moderately positive (++) or higher per manufacturer. Hepatocyte growth factor (HGF), soluble endoglin (sEng), endostatin, platelet-derived growth factor (PlGF), and soluble fms-like tyrosine kinase 1 (sFlt1) were assayed via Mesoscale Discovery Platform (Rockville, Maryland). The five biomarkers in our study were selected for both scientific and practical reasons; prior research has suggested all five to be alluring candidates in SSc [8–13], but also the ability to multiplex several of these markers simultaneously played a role in their selection. Each biomarker was

run in duplicate from the first two clinic visits for all five biomarkers. For the two biomarkers with strongest association with PH and/or DL, sFlt1 and PlGF, additional time points were analyzed leading up to the development of PH. These additional time points were performed in patients who developed PH as well as matched patients (1:1 for age decile, sex, and race) who did not develop PH.

The primary outcomes were (1) any form of PH defined as a mean pulmonary artery pressure (mPAP)≥21 mm Hg obtained by RHC as recently recommended by the 6th World Symposium on Pulmonary Hypertension [15] and (2) digital ischemic lesions (DL) defined as new onset of severe vascular compromise of fingers as evidenced by development of ischemic digital ulcerations (a lesion on the distal fingertip with loss of surface epithelium), gangrene (the presence of demarcated ischemic territory on a digit which is dark, cool, and painful; the area is clearly demarcated from viable flesh) or digital loss from a scleroderma related vascular event. The clinical importance of so-called “borderline PH” (mPAP 21–24) has been recently described in various populations including SSc and has been the basis of lowering the hemodynamic threshold for PH diagnosis and classification [16–18]. Secondary outcomes included PH subsets defined by recent hemodynamic classification of pulmonary hypertension [15]: pre-capillary PH (mPAP≥21mmHg, pulmonary arterial wedge pressure (PAWP) ≤15mmHg, and PVR≥3 Woods units); isolated post-capillary PH (mPAP≥21mmHg and a PAWP>15mmHg); and combined pre-and post-capillary PH (mPAP≥21mmHg and a PAWP>15mmHg and PVR≥3 Woods units). In addition, we also sought to subgroup PH patients based on the presence of ILD, defined as radiographic evidence of fibrosis on high resolution CT and a FVC<70% predicted performed within one year (± 6 months) from the RHC date that diagnosed PH. Sensitivity analyses using the previous definition of PH defined as mPAP≥25mmHg were also conducted.

Statistical Analysis

Statistical analysis was performed using Stata software Version 14 (College Station, TX). Standard curves for each vascular biomarker were obtained for each plate run and samples were run in duplicate. Samples with a coefficient of variation (CV) ≥25% were excluded from the analysis. For each of the five candidate vascular biomarkers, log transformations were performed given a rightward skew of each distribution. A total of 10 variables (5 biomarkers x 2 time points) were analyzed as four transformations: log(biomarker) at baseline, log(biomarker) at visit 2, the difference between biomarker measurement 2 – biomarker measurement 1, as well as the ratio of biomarker measurement 2/biomarker measurement 1. Spearman correlation was performed to understand the relationship between continuous variables, and student’s t-test was performed for comparisons between groups. Trajectories of biomarker measurements were assessed by the slope of the line obtained from the formula y=mx+b, where m=the slope of the line for each biomarker. Time-to-event analyses were performed using Cox Proportional Hazards models. Entry into the cohort served as time 0, and patients were censored based on the following: death, withdrawal from study, or administratively on January 1 2017, whichever came first. Receiver operating characteristic (ROC) curves were generated using logistic regression models to determine the ability of various models to distinguish patients who developed PH during cohort follow-up versus those who did not. Determination of optimal cut-point for each biomarker was

performed using the Liu method [19], based on maximizing the product of sensitivity and specificity.

RESULTS

Participants

Patient characteristics for the overall cohort (N=300) can be found in Table 1. The majority of patients in the cohort were Caucasian women whose age at cohort entry was 52 ± 12. Twenty-seven (27%) of patients were centromere positive, 23% RNA polymerase III positive, and 23% topoisomerase positive (Scl70). Fifty-eight (58%) of patients had limited cutaneous disease. Patients on average had a diagnosis of SSc 10 ± 8 years from 1st non-RP symptom at study entry. Approximately half of patients were on calcium channel blockers at cohort entry (47%). Other common medications patients were receiving include ACE inhibitors/angiotensin-receptor blockers (ARB) (29%), aspirin (26%), and statins (18%). Common vascular comorbidities in the cohort included hypertension (56%), coronary artery disease (CAD) (11%), diabetes (8%), cerebrovascular disease (7%), and peripheral artery disease (PAD) (6%).

A total of 46 patients (15%) were diagnosed with PH throughout the study, and 69 (23%) developed a DL. The average time from enrollment into the cohort until the development of the outcome was 3.0 ± 2.4 years for PH and 3.5 ± 2.4 years for DL. During the first 5 years of study, 45 patients died (15%) and 41 were lost to follow-up (14%). Sixteen of the 45 patients who died had developed PH. Those lost to follow-up contributed an average of 1.6 ± 0.9 years (0–3.8).

Vascular Biomarkers and Relationship with Demographics, Disease Characteristics, and Co-morbidities

After excluding samples with CV≥25%, the following numbers of patients were available for analysis: PlGF at time point 1, designated “PlGF1” (N=291), PlGF2 (N=275); sFlt1 (N=291), sFlt2 (N=276); HGF1 (N=274), HGF2 (N=249); endostatin1 (N=276), endostatin2 (N=276); endoglin1 (N=286), and endoglin2 (N=274). To understand the relationship between baseline biomarker levels and demographic/disease characteristics, correlation studies and t-tests were performed. There was no substantial correlation between any biomarker and disease duration, age at enrollment, or age at diagnosis ($r < 0.2$ for all). Furthermore, there was no significant difference in biomarker level based on patient comorbidities (PAD, history of stroke, CAD, diabetes, hypertension, or erectile dysfunction). However, mild positive correlation was found between PlGF and sFlt1 ($r=0.33$, $p<0.001$) and PlGF and HGF ($r=0.38$, $p<0.001$). With regards to SSc disease characteristics, patients with RNApol3 autoantibodies had a significantly higher level of PlGF at baseline (11.8 vs 10.0, $p=0.0026$), whereas patients with CENP or Scl70 had significantly lower values (9.4 vs 10.9, $p=0.0068$ for CENP and 9.7 vs 10.9, $p=0.03$ for Scl70). Similarly, PlGF levels were significantly elevated in patients with diffuse cutaneous skin (11.8 vs 9.6, $p<0.0001$). No significant differences between autoantibody subgroups or cutaneous type were detected for other vascular biomarkers. Lastly, no biomarker was significantly associated with

medication use at cohort entry, including aspirin, ACE inhibitor (ACEi)/Angiotensin receptor blocker (ARB), statins, or calcium channel blockers (CCB).

Baseline Biomarkers Predicting Vascular Outcomes

We first sought to determine the value of baseline biomarker levels for predicting clinical outcomes of interest. All five biomarkers were analyzed in time-to-event analyses for the outcomes PH and DL (Table 2). The median time from when the 1st biomarker was measured to the development of PH was 3.5 years (IQR 1.6–4.5). Three biomarkers measured at cohort entry were found to significantly associate with the development of PH: HGF (HR 1.99, 95% CI 1.24–3.17, p=0.004), sFlt1 (HR 3.04, 95% CI 1.29–7.14, p=0.011), and PlGF (HR 2.74, 95% CI 1.32–5.69, p=0.007). We also sought to measure the association between biomarkers and different classifications of PH (Supp Table 1): For patients with PH-ILD (defined as mPAP>20, FVC<70% and fibrosis on HRCT, n=16): No biomarker was significantly associated, although there was a trend for sFlt1 (HR 4.6, CI 0.99–21.2, p=0.05). For patients with pre-capillary PH (mPAP>20, PAWP≤15, PVR≥3 without ILD, n=14): baseline HGF and PlGF were statistically associated with PH (HR 2.89 95% CI 1.28–6.5, p=0.01, HR 7.59 95%CI 1.9–30.1, p=0.004, respectively). HRs of similar magnitude were observed upon conducting sensitivity analyses defining PH as mPAP ≥ 25 mmHg (Supplemental Table 3). Next, subgroup analyses were performed examining different SSc populations (limited versus diffuse cutaneous subsets, autoantibody subsets). The association between vascular biomarkers was strongest in SSc patients with limited cutaneous disease (Supplemental Table 4). Of the 46 patients with PH, 20 were in patients with limited cutaneous disease. Of these 20, 9 were in Subgroup1B (mPAP>20, PCWP≤15, and PVR≥3) and 2 were in Subgroup1A (mPAP>20 + FVC<70% + fibrosis on HRCT within 6 months from RHC). The other 9 had a mPAP>20 and PVR ranging from 1–3. No clear associations were observed in patients with anti-centromere, Scl70, or RNApol3 antibodies.

No biomarker measured at cohort entry was significantly associated with DL. However, three measurements at timepoint 2 (obtained 2.8 ± 2.5 years prior to DL) were significant: endoglin (HR 1.74 95%CI 1.06–2.86, p=0.028), sFlt1 (HR 1.83 95%CI 1.12–2.99, p=0.015), and PlGF (HR 1.78, 95%CI 1.12–2.83, p=0.014). Given the recently described value of the ratio of sFlt1/PlGF in pre-eclampsia [20], another condition centered on vasculopathy, we also studied this ratio for its ability to associate with and predict vascular outcomes. No significant association was observed between sFlt1/PlGF and DL or PH.

Lastly, we transformed the first two biomarker measurements into two additional variables: the difference between biomarker measurement 2 – biomarker measurement 1, as well as the ratio of biomarker measurement 2/biomarker measurement 1. No significant differences were observed upon analyzing these transformed variables in any analysis.

Longitudinal Assessment of Select Vascular Biomarkers

Given the significant associations of PlGF and sFlt1 measured at the 1st visit with PH, we wanted to determine the trend of these two individual biomarkers over time leading up to the diagnosis of PH. Regarding HGF, given the high number of samples that had a CV≥25%, we elected not to include this biomarker for these additional analyses.

Patients who developed PH had persistently high levels of PlGF and sFlt1 throughout follow-up compared to those who did not develop PH (cumulative mean of 11.8 ± 3.9 vs 10.0 ± 8.6, p=0.01 for PlGF and 111 ± 54 vs 96.9 ± 75, p=0.02 for sFlt1). However, as time approaching the diagnosis of PH decreased, there was no observed change in either biomarker level - the average slope of each biomarker leading up to the diagnosis of PH was 0.97 units PlGF/year and −0.88 units sFlt1/year. Similar results were observed upon conducting sensitivity analyses defining PH as mPAP ≥ 25 mmHg (data available from corresponding author).

Patients who developed DL over the cohort period also had persistently high levels of PlGF throughout follow-up compared to those who did not develop DL (11.3 ± 4.5 vs 9.8 ± 4.1, p<0.001). There was no significant difference for sFlt1 (99 ± 34 vs 96 ± 40, p=0.345) between those who did and did not develop DL. Upon examining patients who **ever** had a history of DL (from time of first non-Raynaud's symptom onwards) compared to those who never had a DL, PlGF was slightly higher (10.3 ± 4.1 vs 9.5 ± 4.5, p=0.024), as was sFlt1 (98 ± 36 vs 91 ± 43, p=0.026).

Model Formation to Predict PH

At cohort entry, we calculated the optimal cut-point for both PlGF and sFlt1 to predict PH using the Liu method [19], which is the cut-point maximizing the product of sensitivity and specificity. For PlGF, the optimal cut-point was 9.89 pg/mL with a corresponding sensitivity, specificity, and AUC of 82%, 56%, and 0.69, respectively. For sFlt1, the optimal cut-point was 93.8 pg/mL corresponding to 71% specificity, 51% sensitivity, and an AUC of 0.61. Using the same Liu method, the optimal cut-point was calculated for the remaining 3 biomarkers at cohort entry, and this cut-point was used to convert each biomarker into a binary variable of "0" (below the cut-point) or "1" (above). This transformation allowed us to assess whether there was a relationship between the number of vascular biomarkers that were elevated at baseline and the likelihood of developing PH (Table 3). For example, with 0 biomarkers elevated above the cut-point upon cohort entry, only 2/25 patients (8%) developed PH during follow-up. Conversely, with all 5 biomarkers elevated upon cohort entry, 12/29 patients (42%) developed PH (Table 3). With each additional elevated biomarker at cohort entry, there was a 78% increase in the hazard of developing PH (HR 1.78 95% CI 1.2–2.6, p=0.004). Similar trends were observed upon conducting sensitivity analyses defining PH as mPAP ≥ 25 mmHg (Supplemental Table 2). For DL, there was no clear dose-responsive relationship between the number of elevated biomarkers at cohort entry and the incidence of DL over follow-up or DL ever from SSc symptom onset (Supplemental Table 5).

We next sought to investigate the added benefit of incorporating baseline levels of different biomarkers for predicting the development of PH in multivariable analyses. We analyzed several clinical variables measured at cohort entry that have been consistently reported to associate with/predict PH (including % predicted DLCO, % predicted FVC, and NT-proBNP level [21–25]) into a logistic regression model for PH. Subsequently, we assessed the impact of including either PlGF or sFlt1 at baseline by comparing the area under the curve (AUC) for each model (Table 5). Of note, in our cohort the baseline median NT-proBNP level did

not differ significantly between patients who developed PH compared to those who did not; however, clinically there was an apparent higher value in the PH group (881 pg/mL vs 377 pg/mL, p=0.18).

Using only the clinical variables of baseline %DLCO, %FVC, and NT-proBNP level, the AUC for predicting PH was 0.72, which did not appreciably increase with the addition of sFlt1 (AUC 0.72), PlGF (AUC 0.75), or both (AUC 0.77). Upon incorporating the number of biomarkers that were elevated at baseline, the AUC increased from 0.72 (clinical variables only) to 0.81.

Of interest, of the 46 patients who developed PH over cohort follow-up, 42 had a measurable DLCO at baseline. Of these 42, 10 (24%) at cohort entry had a DLCO >70%. Of these 10 patients, all had at least 2 biomarkers elevated at cohort entry, and 80% had ≥3. Similarly, 12/46 (26%) patients had a NT-proBNP <155 pg/mL at cohort entry (upper limit of normal for healthy controls per Mesoscale Discovery package insert). Of these 12 patients, all had at least 2 biomarkers elevated at cohort entry, and 75% had ≥3.

DISCUSSION

The goal of this study was to gain insight into how specific vascular biomarkers known to be involved in angiogenesis or vascular disease might be clinically useful to stratify risk for vascular complications in SSc patients. In this prevalent SSc cohort, we found that several biomarkers were significantly associated with the development of PH – HGF, sFlt1, and PlGF. Furthermore, we demonstrate that these biomarkers are elevated years before the diagnosis of PH. Importantly, these biomarkers can be elevated before patients experience a decrease in DLCO or rise in NT-proBNP.

Based on our data, one may state that the clinical utility for serially monitoring these biomarkers appears low; there is no consistent trend of increasing biomarker levels over time as patients approach a diagnosis of PH/DL. However, serial testing may have value in patients with early disease to first detect elevations in biomarkers. Once elevated, the utility of serially monitoring appears low, but the elevated biomarkers may serve to risk stratify SSc patients and inform PH monitoring strategies. Currently, most SSc providers longitudinally monitor several biomarkers for the development of PH (e.g. NT-proBNP, RVSP on ECHO, or DLCO on PFT, etc) with the goal of detecting an abnormality that may reflect the development of early PH. In our study, a quarter of patients have elevations in vascular biomarkers prior to a decrease in DLCO <70%, perhaps providing a more sensitive test to screen for early vascular dysfunction. Once vascular biomarkers are observed to be elevated, the frequency of other screening tests (e.g. NT-proBNP, DLCO) may be increased in a more cost-effective approach.

A major observation from this study is a dose-response effect of the number of vascular biomarkers elevated at cohort entry and the subsequent risk of developing PH. Interestingly, this dose-response relationship is not seen for DL. This finding provides insight into the vascular dysfunction that characterizes SSc patients with PH. Intuitively our data make sense, in that a higher number of elevated biomarkers relating to vascular dysfunction would

correspond to a higher risk of PH. The fact that the same relationship is not seen with DL is perhaps consistent with epidemiologic studies demonstrating a lack of association between DL and PH [26–28]. Clearly there is a generalized vasculopathy in SSc patients, but perhaps more than one mechanism exists as to organ-specific manifestations (scleroderma renal crisis, PH, and DL). In the case of DL, these mechanisms may include microclotting, trauma, or paroxysmal exposure to cold.

The five biomarkers in our study were selected for both scientific and practical reasons. However, we acknowledge there are several other putative vascular biomarkers that may have relevance in risk-stratifying SSc patients for vascular outcomes. Much of our understanding of these molecules stems from their pathogenic role in preeclampsia [20], and to a lesser extent, retinal vascular disease [29] and cancer [30]. Whereas PlGF and HGF are proangiogenic molecules, sFlt1, endostatin and sEng have anti-angiogenic properties. All of them have been shown to be impacted by hypoxemia in animal or *in vitro* models [29, 31–34]. The observation that all five molecules are elevated in patients who go on to develop PH suggests defects in vascular homeostasis, possibly a feedback loop where proangiogenic molecules beget antiangiogenic beget proangiogenic, and so on. In support of our data, both pro- and anti-angiogenic molecules have been found to be elevated in SSc patients in multiple studies [8–13].

While it is notable that no biomarker met statistical significance in the PH-ILD group (Subgroup 1A in Supplemental Table 1), several HRs were above 2, indicating a subgroup of PH-ILD patients have elevations in these biomarkers. This suggests a subgroup of PH-ILD may have PH due to both ILD as well as innate scleroderma pulmonary vascular disease. These data may explain the varied outcome of treating PH-ILD patients with vasoactive medications [35–37], wherein a subgroup of this population may respond to therapy. Further study is warranted to determine whether subgroups as defined by elevated vascular biomarkers respond better to PAH therapies.

While these biomarkers hold promise in the risk stratification of SSc patients, many more vascular molecules exist which may have similar or greater value. Questions remain regarding at what point do these biomarkers first become elevated in SSc patients; whether they are elevated at SSc diagnosis, symptom onset, or even years before (as is the case for autoantibodies) would offer further insight into preclinical vascular abnormalities. There are a number of limitations in this study. Our center does not use a strict protocol for referring patients for RHC; rather, this is done based on the physicians' clinical judgment. Furthermore, part of our exclusion for PH at cohort entry required a RVSP on ECHO <40, which by itself may have initially missed some patients with PH. Together these factors may have resulted in a misclassification of PH. Notably, our cohort was not an inception cohort but a prevalence cohort; the average disease duration for patients upon entry was 10 ± 8 years. This was intentional to maximize the number of PH cases diagnosed over the cohort period. However, as a result of this design we may have missed patients who developed PH early in their disease course, which may have different predictors of PH. Lastly, it is possible DL events were missed in between clinic visits. However, given most patients with DL have recurrent lesions, thus increasing the likelihood of recording the event, we were less likely to have misclassified patients.

These data suggest that molecules involved in angiogenesis reflect vascular perturbation in scleroderma in a dose-responsive relationship with PH, and elevations at first encounter may be used to risk-stratify patients and aid in clinical phenotyping. Measures of specific biomarkers of angiogenesis may also help to identify a population of PH-ILD that may be more responsive to conventional PAH therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS:

We thank Adrianne Woods and Margaret Sampedro for their assistance in database querying, quality control, and aliquoting of biospecimens for this study. We also thank the Soloski Lab and Marcia Villegas de Flores for sample processing.

Funding:

Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number T32AR048522. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. C.M. is a recipient of the Clinician Scientist Award from The Johns Hopkins School of Medicine and a Jerome L. Greene Foundation Scholar and the Foundation has provided support for his work. This work was also supported by NIH grants 1K23AR075898, 5K23AR52742-5, R01 AR-073208 and P30-AR-070254 from NIAMS, and P50-HL084946-01 from NHLBI. This work was also supported by the Scleroderma Research Foundation, Martha McCrory professorship, The Manugian Fund, The John Staurulakis Endowed Scholar in Rheumatology, and Chresanthe Staurulakis Memorial Fund for Scleroderma Research.

REFERENCES

1. Mukerjee D, St George D, Coleiro B, Knight C, Denton CP, Davar J, et al. Prevalence and outcome in SSc associated pulmonary arterial hypertension: Application of a registry approach. Ann Rheum Dis 2007;62:1088–93.
2. Steen VD, and Medsger TA. Changes in causes of death in SSc, 1972–2002. Ann Rheum Dis 2007;66:940–4. [PubMed: 17329309]
3. Hachulla E, Clerson P, Launay D, Lambert M, Morell-Dubois S, Queyrel V et al. Natural history of ischemic digital ulcers in SSc: Single-center retrospective longitudinal study. J Rheumatol 2007;34:2423–30. [PubMed: 17985402]
4. Lefevre G, Dauchet L, Hachulla E, Montani D, Sobanski V, Lambert M, et al. Survival and prognostic factors in SSc-associated pulmonary hypertension: a systematic review and meta-analysis. Arthritis Rheum 2013;65:2412–2423. [PubMed: 23740572]
5. Walker UA, Tyndall A, Czirjak L, Denton C, Farge-Bancel D, Kowal-Bielecka O, et al. Clinical risk assessment of organ manifestations in SSc: A report from the EULAR scleroderma trials and research group database. Ann Rheum Dis 2007;66:754–63. [PubMed: 17234652]
6. Steen V, Denton CP, Pope JE, and Matucci-Cerinic M. Digital ulcers: Overt vascular disease in SSc. Rheumatology (Oxford). 2009;48:19–24.
7. Nihtyanova SI, Brough GM, Black CM, Denton CP. Clinical burden of digital vasculopathy in limited and diffuse cutaneous SSc. Ann Rheum Dis 2008;67:120–123. [PubMed: 17660220]
8. Avouac J, Meune C, Ruiz B, Couraud PO, Uzan G, Boileau C et al. Angiogenic biomarkers predict the occurrence of digital ulcers in SSc. Ann Rheum Dis 2012;71:394–9. [PubMed: 22085793]
9. Hamaguchi Y, Hasegawa M, Tanaka C, Kumada S, Sato S, Takehara K et al. Elevated serum placenta growth factor (PlGF) levels in patients with SSc: a possible role in the development of skin but not lung fibrosis. J Dermatol Sci 2010;58:229–31. [PubMed: 20451352]
10. Hummers LK, Hall A, Wigley FM, Simons M. Abnormalities in the regulators of angiogenesis in patients with scleroderma. J Rheumatol 2009;36:576–82. [PubMed: 19228661]

11. McMahan Z, Schoenhoff F, Van Eyk JE, Wigley FM, Hummers LK. Biomarkers of pulmonary hypertension in patients with scleroderma: a case-control study. Arthritis Res Ther 2015;17:201. [PubMed: 26245195]
12. Chora I, Guiducci S, Manetti M, Romano E, Mazzotta C, Bellando-Randone S et al. Vascular biomarkers and correlation with peripheral vasculopathy in SSc. Autoimmun Rev 2015;14:314–22. [PubMed: 25485941]
13. Avouac J, Meune C, Ruiz B, Couraud PO, Uzan G, Boileau C, et al. Angiogenic biomarkers predict the occurrence of digital ulcers in SSc. Ann Rheum Dis 2012;71:394–399. [PubMed: 22085793]
14. Mecoli CA, Shah AA, Boin F, Wigley FM, Hummers LK. Vascular complications in SSc: a prospective cohort study. Clin Rheumatol 2018;37:2429–2437. [PubMed: 29804150]
15. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J 2019;24:53.
16. Maron BA, Hess E, Maddox TM, et al.: Association of borderline pulmonary hypertension with mortality and hospitalization in a large patient cohort: insights from the Veterans Affairs Clinical Assessment, Reporting and Tracking program. Circulation 2016;133:1240–1248 [PubMed: 26873944]
17. Assad TR, Maron BA, Robbins IM, et al.: Prognostic effect and longitudinal hemodynamic assessment of borderline pulmonary hypertension. JAMA Cardiol 2017;2:1361–1368. [PubMed: 29071338]
18. Kovacs G, Maier R, Aberer E, et al.: Borderline pulmonary arterial pressure is associated with decreased exercise capacity in scleroderma. Am J Respir Crit Care Med 2009;180:881–886. [PubMed: 19679693]
19. Liu X. Classification accuracy and cut point selection. Stat Med 2012;31:2676–86. [PubMed: 22307964]
20. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M et al. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. N Engl J Med 2016;374:13–22. [PubMed: 26735990]
21. Allanore Y, Borderie D, Avouac J, Zerkak D, Meune C, Hachulla E et al. High N-terminal pro-brain natriuretic peptide levels and low diffusing capacity for carbon monoxide as independent predictors of the occurrence of precapillary pulmonary arterial hypertension in patients with SSc. Arthritis Rheumatol 2008;58:284–91.
22. Shah AA, Chung SE, Wigley FM, Wise RA, Hummers LK. Changes in estimated right ventricular systolic pressure predict mortality and pulmonary hypertension in a cohort of scleroderma patients. Ann Rheum Dis 2013;72:1136–1140. [PubMed: 22887850]
23. Meune C, Avouac J, Airo P, Beretta L, Dieude P, Wahbi K, et al. Prediction of pulmonary hypertension related to SSc by an index based on simple clinical observations. Arthritis Rheum 2011;63:2790–2796. [PubMed: 21547892]
24. Chung L, Fairchild RM, Furst DE, Li S, Alkassab F, Bolster MB, et al. Utility of B-type natriuretic peptides in the assessment of patients with SSc-associated pulmonary hypertension in the PHAROS registry. Clin Exp Rheumatol 2016;106:106–113.
25. Allanore Y, Borderie D, Meune C, Cabanes L, Weber S, Ekindjian OG et al. N-terminal pro-brain natriuretic peptide as a diagnostic marker of early pulmonary artery hypertension in patients with SSc and effects of calcium-channel blockers. Arthritis Rheumatol 2003;48:3505–3508.
26. Allanore Y, Distler O, Matucci-Cerinic M, Denton CP. Review: Defining a Unified Vascular Phenotype in Systemic Sclerosis. Arthritis Rheumatol 2018;70:162–170. [PubMed: 29145709]
27. Tolosa-Vilella C, Morera-Morales ML, Simeón-Aznar CP, Marí-Alfonso B, Colunga-Arguelles D, Callejas Rubio JL et al. Digital ulcers and cutaneous subsets of systemic sclerosis: Clinical, immunological, nailfold capillaroscopy, and survival differences in the Spanish RESCLE Registry. Semin Arthritis Rheum 2016;46:200–208. [PubMed: 27312381]
28. Khimdas S, Harding S, Bonner A, Zummer B, Baron M, Pope J et al. Associations with digital ulcers in a large cohort of systemic sclerosis: results from the Canadian Scleroderma Research Group registry. Arthritis Care Res (Hoboken). 2011;63:142–9. [PubMed: 20740608]

29. Ogura S, Kurata K, Hattori Y, Takase H, Ishiguro-Oonuma T, Hwang Y et al. Sustained inflammation after pericyte depletion induces irreversible blood-retina barrier breakdown. JCI Insight 2017;2:e90905. [PubMed: 28194443]

30. Li T, Kang G, Wang T, Huang H. Tumor angiogenesis and anti-angiogenic gene therapy for cancer. Oncol Lett 2018;16:687–702. [PubMed: 29963134]

31. Raevens S, Geerts A, Paridaens A, Lefere S, Verhelst X, Hoorens A et al. Placental growth factor inhibition targets pulmonary angiogenesis and represents a therapy for hepatopulmonary syndrome in mice. Hepatology. 2018;68:634–651. [PubMed: 29023811]

32. Kelaidi C, Kattamis A, Apostolakou F, Poziopoulos C, Lazaropoulou C, Delaporta P et al. PlGF and sFlt-1 levels in patients with non-transfusion-dependent thalassemia: Correlations with markers of iron burden and endothelial dysfunction. Eur J Haematol 2018;100:630–635. [PubMed: 29543340]

33. Xiang L, Varshney R, Rashdan NA, Shaw JH, Lloyd PG. Placenta growth factor and vascular endothelial growth factor a have differential, cell-type specific patterns of expression in vascular cells. Microcirculation. 2014;21:368–79. [PubMed: 24410720]

34. Hayashi S, Morishita R, Nakamura S, Yamamoto K, Moriguchi A, Nagano T et al. Potential role of hepatocyte growth factor, a novel angiogenic growth factor, in peripheral arterial disease: downregulation of HGF in response to hypoxia in vascular cells. Circulation. 1999;100:II301–8. [PubMed: 10567320]

35. Volkmann ER, Saggar R, Khanna D, Torres B, Flora A, Yoder L, Clements PJ, Elashoff RM, Ross DJ, Agrawal H, Borazan N, Furst DE, Saggar R. Improved transplant-free survival in patients with systemic sclerosis-associated pulmonary hypertension and interstitial lung disease. Arthritis Rheumatol 2014;66:1900–8. [PubMed: 24729406]

36. Le Pavec J, Girgis RE, Lechtzin N, Mathai SC, Launay D, Hummers LK, Zaiman A, Sitbon O, Simonneau G, Humbert M, Hassoun PM. Systemic sclerosis-related pulmonary hypertension associated with interstitial lung disease: impact of pulmonary arterial hypertension therapies. Arthritis Rheum 2011;63:2456–64. [PubMed: 21538327]

37. Furuya Y, Kuwana M. Effect of Bosentan on systemic sclerosis-associated interstitial lung disease ineligible for cyclophosphamide therapy: a prospective open-label study. J Rheumatol 2011;38:2186–92. [PubMed: 21885489]

Table 1.

Baseline Characteristics of Cohort at Study Entry.

Demographics	**N=300 (%)**
Female sex	260 (87)
Race	238 Caucasian (79)
	43 African American (14)
	19 Other (7)
Disease Characteristics	
Classification of SSc	280 (93) ACR, 20 (7) CREST
Type	175 (58) Limited
	124 (41) Diffuse
Antibody	66 (23) RNApol3
	81 (27) CENP
	66 (23) Scl70
Age at entry into cohort	52±12
Time from RP to cohort entry (year ± SD)	12±11
Time from 1st non-RP symptom to cohort entry (year ± SD)	10±8
Treatment at Cohort Entry	
Vasoactive medications	
calcium channel blockers	143 (47)
phophodiesterase inhibitors	5 (2)
prostacyclin (for digital ischemia)	1 (0.3)
Disease Modifying agents	
corticosteroids	55 (18)
mycophenolate	37 (12)
methotrexate	30 (10)
cyclophosphamide	6 (2)
intravenous immunoglobulin (IVIG)	2 (1)
Other	
aspirin	78 (26)
statin	54 (18)
ACE inhibitor/ARB	87 (29)
Comorbidity	
Peripheral artery disease	16 (6)
Coronary artery disease	28 (11)
Cerebrovascular disease	18 (7)
Hypertension	140 (56)
Erectile Dysfunction	9 (5)
Diabetes	23 (8)

Demographics	**N=300 (%)**
Outcome During Study Follow-up	
Digital Lesions	69 (23)
Pulmonary hypertension (All) mPAP>20	46 (15)
A. Subgroup accounting for ILD:	
Subgroup 1: Patients with PH-ILD (Group 3), defined as mPAP>20 AND FVC<70% and ILD on HRCT	16 (5)
Subgroup 2: Pre-capillary PH (mPAP>20, PAWP</=15, PVR>/=3) *without ILD*	14 (5)
Subgroup 3: Isolated post-cap PH (mPAP>20, PAWP>15, PVR< 3) *without ILD*	3 (1)
Subgroup 4: Combined pre and post PH (mPAP>20, PAWP>15 PVR>/=3) *without ILD*	2 (1)
B: Subgroup independent of ILD Status:	
Subgroup 1: Pre-capillary PH (mPAP>20, PAWP</=15, PVR>/=3) *independent of ILD*	26 (8)
Subgroup 2: Isolated post-cap PH (mPAP>20, PAWP>15, PVR< 3) *independent of ILD*	4 (2)
Subgroup 3: Combined pre and post PH (mPAP>20, PAWP>15 PVR>/=3) *independent of ILD*	3 (1)

Scleroderma = SSc, RP = Raynaud's Phenomenon, ACR = American College of Rheumatology 1980 or 2013 Criteria, CREST = 3 out of 5 of the following criteria: calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias, PH = pulmonary hypertension, mPAP=mean pulmonary arterial pressure, ILD=interstitial lung disease, PAWP=pulmonary artery wedge pressure, PVR=pulmonary vascular resistance, FVC=forced vital capacity, RNApol3 = RNA polymerase 3, Scl-70 = topoisomerase, CENP = centromere, ARB=angiotensin receptor blocker, Peripheral artery disease = history of ankle-brachial index <0.9, history of claudication, amputation/ulceration due to peripheral macrovascular disease. Coronary artery disease = history of angina, abnormal exercise test, history of myocardial infarction or coronary revascularization; cerebrovascular disease = prior transient ischemic attack or thrombotic/embolic stroke.

Table 2:

Univariable Cox proportional hazard models for 5 vascular biomarkers for the outcomes of PH and DL. Each biomarker was log-transformed; (1) indicates baseline visit (2) indicates second visit during cohort follow-up.

Variable	Univariable HR for PH (all, n=46)	95% CI	p-value	Variable	Univariable HR for DL (n=69)	95% CI	p-value
log_endoglin 1	1.42	0.75–2.66	0.270	log_endoglin 1	1.62	0.98–2.67	0.058
log_endoglin 2	1.42	0.75–2.69	0.277	**log_endoglin 2**	**1.74**	**1.06–2.86**	**0.028**
log_endostatin 1	1.83	0.85–3.92	0.118	log_endostatin 1	0.68	0.38–1.20	0.186
log_endostatin 2	2.13	0.97–4.66	0.058	log_endostatin 2	0.80	0.45–1.41	0.445
log_hgf 1	**1.99**	**1.24–3.17**	**0.004**	log_hgf 1	0.98	0.65–1.46	0.935
log_hgf 2	1.33	0.86–2.05	0.188	log_hgf 2	1.01	0.70–1.44	0.964
log_sflt1 (1)	**3.04**	**1.29–7.14**	**0.011**	log_sflt1 (1)	1.68	0.87–3.21	0.116
log_sflt1 (2)	1.46	0.77–2.76	0.236	**log_sflt1 (2)**	**1.83**	**1.12–2.99**	**0.015**
log_plgf 1	**2.74**	**1.32–5.69**	**0.007**	log_plgf 1	1.36	0.77–2.40	0.277
log_plgf 2	1.57	0.87–2.84	0.134	**log_plgf 2**	**1.78**	**1.12–2.83**	**0.014**

HGF=hepatocyte growth factor, sFlt1=soluble Flt-1, PlGF=platelet derived growth factor. All=46 patients with PH, 69 with DL out of cohort of 300.

Table 3.

Incidence of PH stratified by number of elevated vascular biomarkers measured at cohort entry. Increased number of elevated biomarkers associates with higher incidence of PH during follow-up (HR 1.78). With each increased in the number of biomarkers elevated at cohort entry, the risk of PH increases by 78%.

	PH Defined as mPAP≥21		
Number of Biomarkers at Baseline > Optimal Cutoff	**No (%)**	**Yes (%)**	**Total**
0	23 (92)	2 (8)	25
1	59 (97)	2 (3)	61
2	73 (90)	8 (10)	81
3	53 (80)	13 (20)	66
4	29 (76)	9 (24)	38
5	17 (58)	12 (42)	29
Total	254	46	300

Table 4.

Different clinical and laboratory variables and their relationship with the development of PH in logistic regression models. While both sFlt1 and PlGF are statistically associated with the development of PH, the addition of either sFlt1 or PLGF does not appreciably increase the area under the curve (AUC) for predicting PH. Upon incorporating the number of biomarkers that were elevated at baseline, the AUC increased from 0.72 (clinical variables only) to 0.81.

Variable at Cohort Entry	OR (95% CI)	p-value	AUC
Univariable Analysis			
DLCO	0.96 (0.95–0.97)	<0.0001	0.72
FVC	0.96 (0.95–0.97)	<0.0001	0.68
BNP Level	1.1 (1.0–1.2)	0.041	0.57
sFlt1	2.5 (1.6–3.7)	<0.0001	0.61
PLGF	3.23 (2.2–4.7)	<0.0001	0.65
Multivariable			
DLCO, FVC, BNP	N/A	N/A	0.72
DLCO, FVC, BNP + *sFlt1*	*1.8 (1.2–2.9)*	0.009	0.72
DLCO, FVC, BNP + *PLGF*	*2.7 (1.8–4.0)*	<0.0001	0.75
DLCO, FVC, BNP + *sFlt1 + PLGF*	N/A	N/A	0.77
DLCO, FVC, BNP + *# Elevated Biomarkers*	*1.8 (1.4–2.4)*	<0.0001	0.81