Universidade do Estado do Rio de Janeiro

Centro Biomédico

Faculdade de Ciências Médicas

Bruno Rangel Antunes da Silva

Contribuição do teste de lavagem de nitrogênio e da volumetria de vias aéreas em pacientes com esclerose sistêmica

Rio de Janeiro

2018

Bruno Rangel Antunes da Silva

Contribuição do teste de lavagem de nitrogênio e da volumetria de vias aéreas em pacientes com esclerose sistêmica

Tese apresentada, como requisito parcial para obtenção do título de Doutor, ao Programa de Pós-Graduação em Ciências Médicas, da Universidade do Estado do Rio de Janeiro.

Orientador: Prof. Dr. Agnaldo José Lopes

Rio de Janeiro

2018

CATALOGAÇÃO NA FONTE
UERJ/REDE SIRIUS/BIBLIOTECA CB-A

S586 Silva, Bruno Rangel Antunes da.
Contribuição do teste de lavagem de nitrogênio e da volumetria de vias aéreas em pacientes com esclerose sistêmica / Bruno Rangel Antunes da Silva. – 2018.
147 f.

Orientador: Agnaldo José Lopes.

Tese (Doutorado) – Universidade do Estado do Rio de Janeiro, Faculdade de Ciências Médicas. Pós-graduação em Ciências Médicas.

1. |Escleroderma Sistêmico - Teses. 2. Testes funcionais dos pulmões - Teses. 3. Vias aéreas (Medicina) – Imagem – Teses. 4. Diagnostico por imagem - Teses. I. Lopes, Agnaldo José. II. Universidade do Estado do Rio de Janeiro. Faculdade de Ciências Médicas. III. Título.

CDU 616.24-004

Bibliotecária: Ana Rachel Fonseca de Oliveira
CRB7/6382

_____ _____
Assinatura Data

Br

Bruno Rangel Antunes da Silva

Contribuição do teste de lavagem de nitrogênio e da volumetria de vias aéreas em pacientes com esclerose sistêmica

Tese apresentada, como requisito parcial para obtenção do título de Doutor, ao Programa de Pós-Graduação em Ciências Médicas, da Universidade do Estado do Rio de Janeiro.

Aprovada em 5 de setembro de 2018.

Banca Examinadora: _____

Prof. Dr. Agnaldo José Lopes (Orientador)

Faculdade de Ciências Médicas - UERJ

_____

Prof. Dr. Rogério Lopes Rufino Alves

Faculdade de Ciências Médicas - UERJ

_____

Profa. Dra. Cláudia Henrique da Costa

Faculdade de Ciências Médicas - UERJ

_____

Prof. Dr. Eduardo Pamplona Bethlem

Universidade Federal do Estado do Rio de Janeiro

_____

Prof. Dra. Rosana Souza Rodrigues

Universidade Federal do Rio de Janeiro

Rio de Janeiro

2018

DEDICATÓRIA

Dedico este trabalho às minhas avós, Irene e Maria, grandes entusiastas da minha formação, que já não estão aqui fisicamente presentes para compartilhar comigo a alegria de finalizar esta pesquisa.

AGRADECIMENTOS

Ao meu orientador e amigo, Professor Dr. Agnaldo José Lopes, por ter sido um verdadeiro mestre, mesmo antes de o início desta pesquisa, contribuindo imensamente na minha formação ainda na Residência Médica. Sempre presente e acreditando no meu potencial, me incentivou a chegar até aqui.

Aos meus pais, João e Elenice, por terem se dedicado à minha formação como ser humano, cidadão e profissional, desde o início da minha existência. Aos meus irmãos, Bianca, Beatriz e Anderson, por estarem ao meu lado em todos os momentos. Aos meus tios, Luiz Carlos e Elisabete, pelo incentivo, amor e dedicação que sempre tiveram comigo. Às minhas avós, Irene e Maria, entusiastas que, enquanto em vida, vibravam a cada conquista, agradeço ao amor incondicional. Ao meu padrinho, Leonardo, que mesmo morando distante, sempre esteve tão perto. A meus primos, primas, tios, tias e demais familiares, que sempre torceram pelo meu sucesso.

Ao meu amigo, Dr. Raphael Zenatti, que me apresentou e fez me apaixonar pela Pneumologia.

Aos meus companheiros da equipe de Pneumologia da UERJ, em especial Prof. Dr. Rogério Rufino e Profa. Dra. Cláudia Costa, que me receberam no Serviço para que fosse possível o desenvolvimento deste trabalho e ao Dr. Thiago Mafort, meu supervisor durante a formação na Residência Médica e hoje grande amigo.

À Dra Verônica Vilela, pela gentileza em compartilhar seu conhecimento e me auxiliar como braço no Serviço de Reumatologia da UERJ.

Aos meus amigos de toda uma vida, com quem cresci pessoalmente e profissionalmente.

Aos meus companheiros de Residência Médica na Pneumologia que estiveram comigo ao longo desta jornada.

Aos pacientes e seus familiares, por serem agentes fundamentais para a evolução da ciência e os protagonistas que permitiram a existência deste trabalho.

RESUMO

SILVA, Bruno Rangel Antunes da. *Contribuição do teste de lavagem de nitrogênio e da volumetria de vias aéreas em pacientes com esclerose sistêmica.* 2018. 147f. Tese (Doutorado em Ciências Médicas) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2018.

Esclerose sistêmica (ES) é uma doença crônica de caraterísticas heterogêneas que pode acometer a pele e outros órgãos. Sua fisiopatologia envolve: disfunção dos fibroblastos com consequente fibrose, vasculopatia que leva à hipóxia tecidual e resposta imune alterada com disfunção de linfócitos B e T e produção de autoanticorpos. As manifestações clínicas são diversas, estando o sistema respiratório dentre os mais acometidos, sendo a doença intersticial pulmonar, a principal causa de morbidade e mortalidade. A quase totalidade dos estudos se detém à análise do parênquima pulmonar e pouco se sabe sobre o acometimento das vias aéreas na ES. O objetivo geral deste estudo foi identificar possíveis alterações morfológicas e funcionais nas vias aéreas inferiores através da TC e TLN em pacientes com ES. Os objetivos específicos foram: correlacionar os achados do TLN com outros parâmetros de função pulmonar; determinar o acometimento das vias aéreas inferiores; comparar valores da volumetria de vias aéreas dos pacientes ES com um grupo controle; descrever as alterações traqueais observadas na volumetria de vias aéreas, correlacionando com dados clínicos e parâmetros dos testes de função pulmonar (TFP). Foram realizados dois estudos transversais, em pacientes com ES. Estes pacientes realizaram TC e os seguintes TFP: espirometria, pletismografia de corpo inteiro, capacidade de difusão ao monóxido de carbono (DLCO), força muscular respiratória e teste de lavagem de nitrogênio (TLN). As imagens da TC foram importadas e analisadas, utilizando-se a plataforma MatLab para posterior realização da esqueletonização e volumetria das vias aéreas inferiores. O primeiro estudo envolveu 52 pacientes com ES, que foram submetidos aos TFP e à análise subjetiva da TC de tórax. Os pacientes foram divididos em dois grupos conforme o valor de capacidade vital forçada (CVF) maior ou menor que 70% do predito. Houve diferença estatisticamente significativa nas médias de CVF, volume expiratório forçado em 1 segundo (VEF_1), VEF_1/CVF, DLCO, capacidade pulmonar total (CPT), volume residual (VR), relação VR/CPT, inclinação de fase III e relação volume de fechamento/capacidade vital (VF/CV). Foi encontrada associação com significância estatística entre inclinação de fase III e as seguintes variáveis: CVF, VEF_1, VEF_1/CVF, DLCO, CPT, VR, VR/CPT. A razão VF/CV se correlacionou com CVF, VEF_1, VEF_1/CVF, CPT, VR/CPT e condutância específica de vias aéreas (SGva). O segundo estudo envolveu 28 pacientes com ES e 27 indivíduos controles. O estudo buscou identificar alterações morfológicas na traqueia de pacientes com ES através da técnica de volumetria de vias aéreas em imagens de TC. O grupo ES apresentou maiores valores de área, excentricidade, maior e menor diâmetros e sinuosidade. A área e diâmetro equivalente tiveram correlação negativa com a relação do fluxo expiratório forçado/fluxo inspiratório forçado em 50% da CVF ($FEF_{50\%}/FIF_{50\%}$). A sinuosidade da traqueia se correlacionou negativamente com o pico de fluxo expiratório ($r=-0,51$, $p=0,008$). Como conclusões, os pacientes com ES apresentam alterações funcionais e morfológicas das vias aéreas, que se expressam em exames de imagem e nos TFPs, havendo importantes relações entre estrutura e função nesses indivíduos. Assim, torna-se fundamental incorporar a avaliação das vias aéreas no acompanhamento dessa população de pacientes.

Palavras-chave: Esclerose sistêmica. Vias aéreas. Testes de função pulmonar. Tomografia computadorizada.

ABSTRACT

SILVA, Bruno Rangel Antunes da. *Contribution of nitrogen washout test and airways volumetry in systemic sclerosis patients.* 2018. 147f. Tese (Doutorado em Ciências Médicas) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2018.

Systemic sclerosis (SS) is a chronic disease of heterogeneous characteristics that can affect the skin and other organs. Its pathophisiology involves: fibroblast dysfunction with consequent fibrosis, vasculopathy leading to tissue hipoxya, and impaired immune response with B and T lymphocite dysfunction and autoantibody production. The clinical manifestations are diverse, with the respiratory system among the most affected, being interstitial ling disease, the main cause of morbidity and mortality in the disease. Almost all studies focus on the analysis of the pulmonary parenchyma and little is known about the involvement of the airways in SS. The main objetctive of this study was to identify possible morphological and functional alterations in the lower airways through CT and NWT in SS patients. The specific objectives were: to correlate the NWT findings with other pulmonary function parameters; determine the involvement of the lower airways; compare the airway volume values of SS patients with a control group; and to describe the tracheal changes observed in airways volumetry, correlating with clinical data and pulmonary funcition test (PFT). Two cross-sectional studies were performed with SS patients. This patients performed CT and the following PFT: spirometry, whole body pletismography, carbon monoxide diffusion capacity (DLco), respiratory muscle strength and NWT. CT images were imported and analyzed using the MatLab platform for later squeletonization and lower airways volumetry. The first study involved 52 patients with SS, who performed PFT and a subjective analysis of their chest CT. Patients were allocated into two groups according to the value of their forced vital capacity (FVC) higher or lower than 70% of predicted value. There was a statistically significant difference in mean FVC, forced expiratory volume in 1 second (FEV_1), FEV_1/FVC, DLco, total lung capacity (TLC), residual volume (RV), RV/TLC ratio, phase III slope and closing volume(CV)/ vital capacity (VC) ratio. An association with statistical significance was found between phase III slope and the following variables: FVC, FEV_1, FEV_1/FVC, CMDC, TLC, RV, RV/TLC. The CV/VC ratio correlated with FVC, FEV_1, FEV_1/FVC, TLC, RV/TLC and specific airway conductance (SGaw). The second study involved 28 SS patients and a control group of 27 individuals. The aim of this study was to identify morphological alterations in the trachea of SS patients through the application of airway volumetry technique in CT images. The SS group had higher values of area, eccentricity, greater and smaller diameters and sinuosity. The area and equivalent diameter had a negative correlation with the ratio of forced expiratory flow / forced inpiratory flow in 50% of FVC ($FEF_{50\%}/FIF_{50\%}$). The sinosity of the trachea correlated negatively with peak expiratory flow (PEF) ($r = -0,51$, $p = 0,008$). As conclusion, patients with ES present functional and morphological airways alterations, which are expressed both in imaging tests and in PFT, with important relationships between structure and function in these individuals. Thus, it is essential to incorporate the evaluation of the airways in the monitoring of these patients.

Keywords: Systemic sclerosis. Airways. Pulmonary function tests. Computed tomography.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

ATS	American Thoracic Society
BI	Brônquio intermediário
BIE	Brônquio do lobo inferior esquerdo
BPD	Brônquio principal direito
BPE	Brônquio principal esquerdo
BSD	Brônquio do lobo superior direito
BSE	Brônquio do lobo superior esquerdo
CEP	Comitê de Ética em Pesquisa
CPT	Capacidade pulmonar total
CV	Capacidade vital
CVF	Capacidade vital forçada
DIP	Doença intersticial pulmonar
DLco	Capacidade de difusão ao monóxido de carbono
FCM	Faculdade de Ciências Médicas
HAP	Hipertensão arterial pulmonar
HAQ	Health Assessment Questionnaire
HP	Hipertensão pulmonar
HUPE	Hospital Universitário Pedro Ernesto
HUCFF	Hospital Universitário Clementino Fraga Filho
IMC	Índice de massa corporal
PPC	Policlínica Piquet Carneiro
PEmax	Pressão expiratória máxima
PGCM	Programa de Pós-graduação em Ciências Médicas
PImax	Pressão inspiratória máxima
QV	Qualidade de vida
Rva	Resistência de vias aéreas
SBPT	Sociedade Brasileira de Pneumologia e Tisiologia
SGva	Condutância específica de vias aéreas
SHAQ	Scleroderma Helth Assessment

TC	Tomografia computadorizada
TCAR	Tomografia computadorizada de alta resolução
TCLE	Termo de consentimento livre e esclarecido
TFP	Teste de função pulmonar
TLN	Teste de lavagem do nitrogênio
UERJ	Universidade do Estado do Rio de Janeiro
UFRJ	Universidade Federal do Rio de Janeiro
VA	Volume alveolar
VEF_1	Volume expiratório forçado no primeiro segundo
VEF_1/CV	Índice de Tiffeneau
VF	Volume de fechamento
VR	Volume residual

SUMÁRIO

INTRODUÇÃO

Esclerodermia é uma doença autoimune do tecido conjuntivo, de características heterogêneas, com acometimento multissistêmico. As manifestações principais da doença são a fibrose progressiva da pele e diversos órgãos internos, além das alterações, muitas vezes graves, da microvasculatura (GOH et al., 2008; SCHOENFELD et al., 2015).

Até o momento, a etiologia da esclerodermia não é completamente compreendida. As manifestações clínicas e patológicas são decorrentes de três processos principais: 1) anormalidades no funcionamento do sistema imune inato e adaptativo, levando à produção de autoanticorpos e auto-imunidade mediada por células; 2) alterações na microvasculopatia das células endoteliais e vasculopatia fibroproliferativa de pequenos vasos; e 3) disfunção dos fibroblastos com produção excessiva de colágeno e outros componentes da matriz extracelular, levando ao acúmulo dos mesmos na pele, vasos e demais tecidos do corpo (SCHOENFELD et al., 2015).

A alteração no controle do tônus vascular manifesta-se com o fenômeno de Raynaud. As alterações microvasculares são as manifestações clínicas mais precoces da doença, podendo preceder a fibrose em meses ou, até mesmo, anos. O acometimento vascular leva à isquemia e reperfusão repetidas vezes, reduzindo o fluxo sanguíneo pelos capilares e acarretando hipóxia tecidual. Com sua progressão, a elasticidade dos vasos é perdida, ocorrendo fibrose das camadas média e adventícia e causando oclusão das arteríolas que, em associação com a ativação plaquetária pelo estado inflamatório, propiciam eventos trombóticos em todo o organismo (MANETTI et al., 2010).

A esclerodermia tem incidência estimada entre 50-300 casos por milhão. A doença é mais frequente no sexo feminino, numa relação de 4:1 (BELLANDO-RANDONE et al., 2012). A sobrevida geral dos pacientes varia conforme a agressividade da doença. Naqueles com doença leve, a taxa de sobrevida em 10 anos é estimada em 66%, enquanto naqueles com acometimento orgânico importante essa taxa cai para 38% (BELLANDO-RANDONE et al., 2012). A história familiar positiva eleva o risco relativo em 10-27 vezes nos irmãos de pacientes com esclerodermia. Outros parentes de primeiro grau também têm risco elevado, sendo este estimado em 10-16 vezes para o desenvolvimento da doença. Pressupõe-se que tal aumento do risco

relativo é derivado de fatores genéticos, possivelmente associados a fatores ambientais, ainda que estes últimos tenham pouco suporte científico até o momento (MANETTI et al., 2010).

A heterogeneidade da doença faz com que ela se apresente desde formas mais brandas, com leve acometimento de órgãos internos, até formas agressivas, com extenso acometimento sistêmico. Nestas últimas formas, há acometimento de diversos órgãos, determinando maior prejuízo na qualidade de vida e levando à morte dentro de alguns anos (JIMENEZ, 2013).

Caracteristicamente, apresenta-se com fenômeno de Raynaud, espessamento cutâneo e produção de auto-anticorpos. Já o acometimento orgânico é bastante variável, tendo a doença diferentes apresentações e desfechos (BELLANDO-RANDONE et al., 2012).

Para avaliar a qualidade de vida (QV), se faz necessário o uso de instrumentos específicos. No Brasil, até o momento, não se dispõe de tais ferramentas. Há um questionário especifico para avaliar as repercussões da esclerodermia. Trata-se do *Scleroderma Helth Assessment* (SHAQ), ainda não validado para versão brasileira (MACHADO et al., 2014). O *Health Assessment Questionnaire* (HAQ) é um instrumento especifico de QV, muito utilizado em doenças reumatológicas, validado para avaliação de pacientes com ES. Este é bem semelhante ao SHAQ, porém é limitado por não apresentar uma sessão especifica para estimar o comprometimento da pele. Apesar de sua limitação, o HAQ é um instrumento muito utilizado em pacientes com ES (MACHADO et al., 2014).

A esclerodermia pode ser classificada em três subdivisões principais: esclerose sistêmica (ES), esclerodermia localizada e ES sem escleroderma. A ES é a forma de apresentação mais comumente encontrada. Em sua subclassificação, são levados em conta a extensão do acometimento cutâneo e o comprometimento de órgãos internos pela doença. As formas da ES são: cutânea difusa e cutânea limitada (BERÉZNE et al., 2007).

Os critérios vigentes para diagnóstico da doença formam definidos pelo *American College of Rheumatology* e pela *European League Against Rheumatism*, conforme mostrado no **Quadro 1**. São necessários nove pontos para se firmar o diagnóstico de esclerodermia.

Quadro 1 - Critérios diagnósticos para a esclerodermia

Itens	**Subitens**	**Escore**
Espessamento da pele dos dedos das duas mãos que se estendem proximalmente para as articulações metacarpofalangeanas	-	9
Espessamento da pele dos dedos	Dedos “inchados” Esclerodactilia (metacarpofalangeanas/ interfalangianas)	2 4
Lesões na ponta dos dedos	Úlceras de poupas digitais Cicatrizes nas pontas dos dedos	2 3
Telangiectasias	-	2
Capilares das cutículas anormais	-	2
Hipertensão arterial pulmonar e/ou doença pulmonar intersticial	Hipertensão arterial pulmonar Doença pulmonar intersticial	2 2
Fenômeno de Raynaud	-	3
Auto-anticorpos relacionados com a ES	Anti-centrômeros Anti-topoisomerase I Anti-RNA polimerase III	3

A doença intersticial pulmonar (DIP) clinicamente significativa ocorre em aproximadamente 40% dos pacientes com ES. Contudo, em até 80% dos pacientes é possível encontrar acometimento pela doença em análise histopatológica do tecido pulmonar. A DIP é classicamente associada à forma difusa da doença, apesar de poder estar presente em qualquer das formas, até mesmo na ES sem esclerodermia (SBPT, 2012).

Embora a grande maioria dos pacientes com ES tenha acometimento pulmonar, menos da metade terá doença significativa. Portanto, o tratamento deve ser considerado principalmente para aqueles com acometimento extenso pela doença, caracterizado a partir de achados na tomografia computadorizada de alta resolução (TCAR) do tórax (SBPT, 2012).

A DIP é bastante comum na ES, com achados de alterações intersticiais na TCAR em até 90% dos pacientes. Já 40-75% demonstram alguma alteração nos testes de função pulmonar (TFP) (WOLLEHEIM et al., 2005). A fibrose pulmonar significativa é encontrada em aproximadamente 25% dos pacientes com ES. Porém, este acometimento é heterogêneo devido aos diversos fatores, como o subtipo de doença e a presença de autoanticorpos (WOLLEHEIM et al., 2005).

A forma cutânea difusa é, dentre todas elas, a mais associada à DIP. A presença do auto-anticorpo anti-Scl70, também chamado anti-topoisomerase I, aumenta o risco do paciente com ES desenvolver DIP. Já pacientes com a forma cutânea limitada da ES ou a presença do autoanticorpo anti-centrômero estão sob menor risco de desenvolvimento de DIP. Nestes casos, é mais comum o aparecimento de hipertensão pulmonar (HP) (WOLLEHEIM et al., 2005).

A lesão pulmonar pode ocorrer de duas maneiras distintas: 1) alveolite, levando à DPI; e 2) vasculopatia de pequenos e médios vasos pulmonares associada à HP. Ambas as condições estão presentes, em algum grau, na maioria dos pacientes (KÖNIG et al., 1984).

Dispneia é a queixa mais frequente nos pacientes com acometimento pulmonar pela ES, sendo esta frequentemente associada à tosse seca. A inflamação provocada no pulmão leva a um aumento no número total de células que pode ser detectado no lavado broncoalveolar. Esta inflamação é responsável por provocar alveolite, com consequente DIP e fibrose (SILVER, 1991).

A alveolite provocada pela atividade inflamatória de origem linfocitária pode ser assintomática de início, não causar repercussões clínicas e ser indetectável na radiografia de tórax e mesmo nos TFP. A avaliação do dano pela inflamação alveolar pode ser classificada através de alterações no lavado broncoalveolar, pela contagem diferencial de células, TFP, TC de tórax e biópsia pulmonar, se necessária (HUGLE, 2011).

A vasculopatia na ES também pode apresentar manifestações sistêmicas. Fenômeno de Raynaud, úlceras digitais e alterações de leito ungueal na capilaroscopia são geralmente as alterações mais precoces e importantes no diagnóstico (DE SANTIS et al., 2015). A etiologia do processo é desconhecida. Contudo, é possível que agentes infecciosos, ativação de células T citotóxicas, radicais livres de óxido nítrico e auto-anticorpos contra células endoteliais estejam envolvidos nesse processo (DE SANTIS et al., 2015). A vasculopatia é responsável por levar à HP no paciente com ES. A lesão caracteristicamente encontrada em análise histopatológica de

pulmão desses pacientes evidencia proliferação concêntrica da camada íntima de pequenos vasos, acentuada obstrução da luz, infiltração linfocitária e uma pequena quantidade de lesões plexiformes – proliferação focal das células musculares lisas e do endotélio (DE SANTIS et al., 2015).

A DIP e a HP são as principais causas de morte em pacientes com ES. Nos estágios iniciais, a doença pode ser assintomática, sendo, muitas vezes, o acometimento pulmonar não diagnosticado. Isso pode levar a um retardo no início do tratamento, que deve ser iniciado assim que detectada a alteração na avaliação do órgão (SOLOMON et al., 2013).

O comprometimento do parênquima pulmonar acima de 20% é definido como extenso; abaixo disso, é caracterizado como limitado. Os achados de opacidades em vidro fosco e opacidades reticulares são analisados em cinco níveis predeterminados e a média entre eles determina o nível de comprometimento. Nos casos em que a determinação não é precisa, podem-se usar parâmetros de função pulmonar para auxiliar na caracterização, sendo os mais usados a capacidade vital forçada (CVF) e a capacidade de difusão pulmonar ao monóxido de carbono (DLco). Valores de CVF <70% e DLco <60% indicam acometimento extenso, enquanto CVF >70% e DLco >60%, acometimento limitado (SBPT, 2012).

Os valores obtidos nos resultados dos TFP devem ser interpretados individualmente. Dado o grande intervalo dos limites de referência para a CVF, entre 80 e 120%, os resultados devem ser acompanhados sequencialmente para que alterações significativas sejam identificadas. Uma medida de CVF de 75% pode ser uma alteração mínima no caso de uma CVF prévia de 80%, ou uma substancial queda numa CVF prévia de 120% (GOH et al., 2008).

A espirometria é um dos TFP de maior disponibilidade, sendo, portanto, de grande utilidade para o acompanhamento de uma série de doenças pulmonares. É um exame que fornece informações valiosas em relação a capacidades e volumes, sendo os mais rotineiramente utilizados o VEF_1 e a CVF. Entretanto, no seguimento das doenças intersticiais, o parâmetro da espirometria mais utilizado é a CVF. Este representa o parâmetro mais estudado como desfecho e, consequentemente, o mais utilizado no acompanhamento dos pacientes com ES, apesar de poucas evidências para tal validação (CARON et al., 2018).

A medida da CVF representa o volume de ar assoprado de uma inspiração máxima até uma expiração máxima. Esta medida tem sido aplicada amplamente como parâmetro para o rastreio de acometimento pulmonar, em associação como exame de imagem – TC de tórax – e

seguimento dos pacientes já com acometimento pulmonar diagnosticado. A ampla disponibilidade da espirometria é apontada como grande fator de sustentação para seu uso de maneira sistemática. Contudo, é importante salientar que pode haver acometimento do interstício pulmonar pela ES em uma grande porcentagem de pacientes com CVF > 80%, resultando num grande número de diagnósticos falsos-negativos para DIP associada a ES, quando utilizada como parâmetro de rastreio isolado (HA et al., 2018).

O teste de medida de difusão do monóxido de carbono é utilizado para avaliar a troca gasosa e fornece informações que refletem os possíveis danos provocados na membrana alvéolo-capilar e interstício pela doença. A principal medida obtida através deste teste é a chamada DLco, que representa a capacidade de difusão do gás CO, através da membrana alvéolo-capilar. Os fatores determinantes da difusão são o gradiente de pressão parcial através da membrana alveolocapilar, a espessura da membrana, a área de superfície de troca e a reação do gás com a hemoglobina – que dependem da taxa de reação própria do gás e do volume sanguíneo no capilar (MOTRAM, 2013).

Estudos sugerem que a DLco seja mais sensível para o seguimento da DIP quando comparada com a CVF. Contudo, há algumas desvantagens na utilização da DLco como a maior variabilidade em relação à CVF e a possibilidade de alteração dos seus valores pela presença de hipertensão arterial pulmonar (HAP), que é uma comorbidade muitas vezes presente em pacientes com ES. A HAP promove alterações na hemodinâmica pulmonar e também sistêmica, com consequente prejuízo da troca gasosa, que pode se somar ao dano provocado pela DIP. Isso faz com que a medida da DLco seja mais comprometida naqueles pacientes que possuem ambas as complicações da ES em associação: DIP e HAP (CARON et al.,2018).

A variação de medida da DLco foi o desfecho mais estudado em pacientes com ES até os anos 2010, sendo substituído pela CVF a partir de então, segundo uma recente revisão sistemática. Caron et al. (2018) buscaram os estudos que correlacionaram os parâmetros de TFP como preditores de desfecho nos pacientes com DPI na ES. A maior parte dos estudos utilizou a CVF como parâmetro de desfecho primário no seguimento dos pacientes. Os motivos apontados para a preferência da CVF são sua maior disponibilidade e reprodutibilidade em relação à medida da DLco que, apesar de parecer mais sensível do que a CVF, pode sofrer alterações importantes quando há HAP associada (Tashkin et al, 2016).

A pletismografia de corpo inteiro é uma técnica que permite a medida dos volumes pulmonares estáticos e fornece dados como a capacidade pulmonar total (CPT), volume residual (VR), resistência de vias aéreas (Rva) e a condutância específica de vias aéreas (SGva). Na grande maioria dos estudos, a CPT é avaliada como desfecho secundário em pacientes com DIP relacionada à ES. Além do mais, os estudos não demonstraram superioridade da CPT em relação à CVF na avaliação destes pacientes (TASHKIN et al., 2006; CARON et al., 2018).

As medidas de força muscular inspiratória e expiratória são de grande valor na avaliação do acometimento da ES além da DIP. As variáveis analisadas são a pressão inspiratória máxima (PImáx) e a pressão expiratória máxima (PEmáx), que são obtidas através de manobras de inspiração forçada e expiração forçada, respectivamente. A doença multissistêmica pode levar a fraqueza muscular respiratória e infiltração cutânea com fibrose e rigidez da pele, que parece contribuir para a restrição da mobilização da parede torácica. Este prejuízo na capacidade de expansão da caixa torácica determinada pela constrição cutânea pode levar a alterações na mecânica ventilatória que, por sua vez, podem ser sugeridas através da mensuração das pressões respiratórias máximas (Chausow et al., 1984). A PImáx e a PEmáx são um dos determinantes da CPT e do VR, respectivamente. A parede torácica tem papel fundamental na contribuição para as forças de retração elástica do sistema respiratório na determinação do VR e também com sua complacência na determinação da CPT (MENNA BARRETO & CAVALAZZI, 2002).

O teste de lavagem do nitrogênio (TLN) fornece informações relevantes da ventilação que traduzem o funcionamento das pequenas vias aéreas e a homogeneidade da ventilação pulmonar. Os valores de inclinação de fase III e a relação volume de fechamento/capacidade vital (VF/CV) são as variáveis de maior interesse nesta análise. A inclinação de fase III representa a variação da concentração de N_2 exalado na expiração, tendo seu valor elevado quando há inomogeneidade da ventilação. A relação VF/CV, quando aumentada, sinaliza para o fechamento precoce das pequenas vias aéreas. Tal técnica tem sido utilizada com maior frequência, dado o interesse pelo estudo das pequenas vias aéreas que são consideradas a zona silenciosa do sistema respiratório. Entretanto, até o presente momento, pouca atenção tem sido dada às anormalidades de pequenas vias aéreas em pacientes com DIP, incluindo os portadores de ES.

Além da avaliação das pequenas vias aéreas, o TLN é capaz de fornecer informações valiosas a respeito das propriedades que envolvem a homogeneidade do sistema respiratório. Apesar de ser conhecido há mais de meio século, o TLN tem sido retomado na prática clínica e

utilizado para o diagnóstico precoce e, também, para a avaliação de gravidade em diversas doenças. O teste pode ser realizado de duas maneiras – a técnica de respiração única e a técnica de múltiplas respirações –, cabendo a escolha de qual técnica utilizar a cargo do examinador e de diversos outros fatores técnicos (LOPES, 2015).

A técnica de oscilometria forçada possibilita a avaliação da resistência das vias aéreas. Miranda et al. (2013) avaliou, através da técnica de oscilometria forçada, as vias aéreas de pacientes com ES, comparando os resultados obtidos com o de um grupo controle. Os pacientes do grupo ES apresentaram maiores valores de resistência no intercepto (que está associada com a resistência total do sistema respiratório), resistência média (que está associada com o calibre das vias aéreas mais centrais), resistência em baixas frequências de ressonância (que está associada com as vias aéreas periféricas) e *slope* da resistência (que está associado com a homogeneidade das propriedades resistivas do sistema respiratório). Nesse mesmo estudo, os investigadores observaram que pacientes com ES possuem redução da reatância média (que está associada com a homogeneidade das propriedades reativas do sistema respiratório), complacência dinâmica e complacência alveolar. Os achados deste estudo foram de extrema relevância por ser o primeiro utilizando tal técnica, que permite uma avaliação mais apurada da função de pequenas vias aéreas. O aumento da resistência de vias aéreas, que se correlaciona com a inomogeneidade da ventilação, conseguiu ser demonstrada por esses investigadores.

Entretanto, poucos estudos avaliaram as pequenas vias aéreas em pacientes com ES, e a avaliação através da TLN ainda não foi explorada nesta população. Há algumas décadas, Bjerke (1979) avaliou as pequenas vias aéreas de pacientes com ES e não foi capaz de evidenciar alterações em sua funcionalidade. Mais recentemente o trabalho de Miranda et al. (2013) demonstrou aumento da resistência nas pequenas vias aéreas de pacientes com ES, achado inédito neste grupo até então.

Bjerke et al. (1979) evidenciaram alteração de pequenas vias aéreas em doenças intersticiais difusas caracterizadas por obstrução, em casos de pneumonia em organização criptogênica e sarcoidose. Na DIP, em pacientes com ES, as medidas de TFP não evidenciaram sinais de doença obstrutiva neste estudo.

As imagens de radiografia de tórax e os TFP podem não evidenciar alterações na fase inicial da doença. A TCAR de tórax é o exame não invasivo de escolha para detecção da DIP na ES, sendo capaz de mostrar discretas alterações. Apesar da alta sensibilidade, ela pode ser normal

com alterações detectáveis nos TFP. Pacientes com TCAR normal e alterações no exame físico do tórax desenvolvem alterações nos exames seguintes. Contudo, uma TCAR inicial normal é um bom preditor, tendo o paciente uma pequena chance de desenvolver DIP, com 85% mantendo a TCAR normal em cinco anos (LAUNAY et al., 2006).

A TCAR é um exame importante na rotina de detecção e avaliação da DIP. É mais precisa que a radiografia simples de tórax na detecção e caracterização da doença intersticial, além de ter uma melhor correlação com os parâmetros dos TFP (SALAFFI et al, 2016). A TCAR permite uma avaliação detalhada dos pacientes com DPI associada a ES. Entretanto, seu uso de forma rotineira no seguimento dos pacientes deve ser avaliado cuidadosamente dado o risco de neoplasia por dose acumulada de radiação (NGUYEN-KIM et al., 2018).

O padrão mais comumente encontrado nas TCAR de tórax é a pneumonia intersticial não específica. Neste padrão, há comumente vidro fosco e fibrose, sendo a distribuição tipicamente periférica, bilateral e predominando nas bases pulmonares. As opacidades de vidro fosco são áreas de aumento de atenuação do parênquima pulmonar que podem representar inflamação ativa ou fibrose inicial. O faveolamento é o achado característico da fibrose, sendo outros achados o espessamento reticular do interstício com bronquiectasias de tração (SILVER et al, 2015). O padrão de pneumonia intersticial usual se apresenta na TC de tórax caracteristicamente com redução de volume e faveolamento, além da presença de bronquiectasias de tração e reticulado periférico (SCHOENFELD et al,2015). Os achados de fibrose estão presentes em 55 a 65% dos pacientes com ES e em até 96% daqueles com TFP alterados (SALAFFI et al. 2016).

Em relação às vias aéreas inferiores, poucos estudos avaliaram ou descreveram alterações específicas das vias aéreas nos pacientes com ES através da TCAR. A quase totalidade dos estudos avaliou alterações do parênquima pulmonar e apenas descrevem alterações das vias aéreas como presentes em associação. Em uma revisão que propôs a descrição do estado da arte da imagem em pacientes com doenças do colágeno, Ohno et al. (2015) descreveram as alterações mais comumente encontradas na TC de tórax des pacientes com ES como sendo as alterações intersticiais que levam a consequentes alterações das vias aéreas, expressadas em bronquiectasias e bronquiolectasias.

Não há na literatura, até o momento, estudos que tenham avaliado isoladamente as vias aéreas de paciente com ES.

Com o advento dos computadores de maior desempenho, uma de suas principais funções foi a de realizar o reconhecimento de padrões (FRANÇA et al, 2016). Devido à grande quantidade de informações e dados, foi necessário o surgimento de tecnologias que possibilitassem a compressão destes e que, ao mesmo tempo, conseguissem representar o todo com exatidão (PLOTZE et al, 2004). Isso inclusive torna-se fundamental na área de Radiologia e Diagnóstico por Imagem, em que são formadas milhares de imagens e dados das várias regiões do corpo.

O processo de esqueletonização é a transformação de um componente de imagem digital em um subconjunto do componente original. Existem diferentes categorias de métodos que permitem a realização desta técnica (*skeletonizing algorithm*) (KLETTE, 2002). Frequentemente utilizada, o afinamento é o processo de redução de uma forma para uma versão simplificada que ainda retém as características essenciais do objeto original e passa a ser denominado como esqueleto (PLOTZE et al, 2004).

Quanto à volumetria das áreas de interesse na área médica, surgiram diversos trabalhos que aplicaram este método em várias afecções, inclusive aquelas do sistema respiratório (MADANI et al., 2001; MATSUOKA et al., 2008; STOEL et al., 1999; CARVALHO et al., 2009, DE CASTRO et al., 2014). Nos estudos que dizem respeito às vias aéreas superiores, a técnica foi utilizada para avaliação da faringe por Zinsly et al. (2010) e Rodrigues (2011), corroborando a importância da volumetria através da TC (ZINSLY et al., 2010; RODRIGUES, 2011).

Finalmente, no que tange as vias aéreas inferiores, muitos trabalhos vêm demonstrando a sua importância do ponto de vista prático, como na aplicação para auxílio no diagnóstico de doenças incluindo estenoses traqueais e traqueomalácia, bem como no planejamento cirúrgico, acompanhamento e análise anatômica (PERCHET et al., 2016; GRENIER et al., 1996, FETITA et al., 2004, LIU et al., 2002).

Camilo et al. (2017) compararam as vias aéreas de pacientes com acromegalia com a de indíduos de um grupo controle, incluindo a análise de traqueia e brônquios. Foi utilizada a técnica de volumetria com posterior segmentação e esqueletonização das vias aéreas. Os resultados obtidos evidenciaram que pacientes com acromegalia apresentavam maiores valores de diâmetro equivalente, área e perímetro da traqueia e brônquios-fonte direito e esquerdo, quando

comparados aos pacientes do grupo controle. Os pacientes do grupo acromegalia apresentavam ainda maior tortuosidade da traqueia e a estenose traqueal foi observada em 25% deste grupo.

A traqueia é um órgão cilíndrico que se inicia na laringe e dá origem aos brônquios principais, quando se bifurca para segmentação das vias aéreas. Está localizada no interior do mediastino e tem como principal função servir de passagem para o ar, ligando as vias aéreas superiores às inferiores. Sua parede é composta por aneis cartilagenosos incompletos em suas paredes lateral e anterior, sendo a parede posterior composta por musculatura lisa com fibras dispostas em orientação transversal e longitudinal em relação aos aneis cartilagenosos. As paredes da traqueia são recobertas por camadas de tecido conjuntivo. Permeados ao tecido conjuntivo, existem glândulas responsáveis pela lubrificação da luz traqueal (BRAND-SABERI et al., 2014).

O epitéltio colunar pseudoestratificado cilíndrico ciliado é o que recobre todo o órgão, sendo ligado ao tecido conjuntivo por uma membrana basal. Sua composição se dá por meio de três células: 1) as principais, que são cilíndricas e altas, contendo os cílios; 2) as basais, que são células curtas que não tem altura para alcançar a superfície epitelial, tendo função na renovação celular; e 3) as células caliciformes, que são células cilíndricas em formato de cálice e que secretam muco com função de lubrificação do epitélio. A ação deste epitélio de lubrificação em associação com a ação do batimento ciliar no sentido da ascendente permite a eliminação de dejetos que por ventura tenham adentrado a vias aérea inferior (BRAND-SABERI et al., 2014).

A traqueia representa o local das vias aéreas de maior resistência ao fluxo aéreo, pois, apesar do seu diâmetro de aproxiamdamente 2 cm, a área seccional transversa é muto maior no nível das pequenas vias aéreas. Em condições anatômicas, ela funciona como um tubo semiflexível com eixo centrado, que permite um fluxo aéreo laminar e livre de obstáculos.

As condições que levem a alterações das vias aéreas podem ser bastante sintomáticas e estão diretamente correlacionadas com seu calibre. Alterações na estrutura da traquéia podem provocar alterações no fluxo aéreo e consequentemente de toda a dinâmica da ventilação. Tais alterações podem ser avaliadas através de TFP e também de exames de imagem como a TC (MOTTRAM, 2013).

A TC é o exame de escolha para avaliação da traqueia. Ela é capaz de mostrar a anatomia da via aérea e também avaliar as estruturas adjacentes que podem interferir diretamente em sua arquiterura e funcionamento (SHEPARD et al., 2018). A TC ainda permite avaliação dinâmica do

funcionamento da traqueia, adquirindo imagens em inspiração e expiração, o que é de grande auxílio na investigação diagnóstica e planejamento de exames endoscópicos em envolvem a via aérea (LEE et al., 2010).

Não há registros na literatura da avaliação específica da estrutura traqueal em pacientes com ES.

1 JUSTIFICATIVA

Embora haja diversas alterações na estrutura pulmonar e disfunções respiratórias descritas na ES, poucos estudos avaliaram ou descreveram as alterações nas vias aéreas desses pacientes. Sabe-se que a TC *multislice* é excelente no diagnóstico precoce do acometimento pulmonar em afecções sistêmicas, inclusive naquelas que determinam alterações nas vias aéreas. Quanto à volumetria das vias áreas, surgiram diversos trabalhos que aplicaram este método em várias afecções, inclusive do sistema respiratório, como no auxílio ao diagnóstico de estenoses traqueais e traqueomalácia, bem como no planejamento cirúrgico, acompanhamento e análise anatômica. No entanto, até o momento, nenhum estudo avaliou a volumetria das vias aéreas na ES.

Em relação aos TFP, a maior parte dos estudos em pacientes com ES está relacionada aos métodos tradicionais de avaliação da função pulmonar, incluindo a espirometria e a medida da DLco. Apesar da importância desses TFP tradicionais no manejo do acometimento pulmonar associado à ES, uma parcela significativa dos pacientes apresenta espirometria e medida da DLco dentro da normalidade, mesmo na presença de doença pulmonar diagnosticada pelos métodos de imagem. Nos últimos anos, com a evolução do seu aparato técnico, tem havido um crescente interesse sobre o uso do teste de lavagem de nitrogênio no intuito de medir a homogeneidade na distribuição da ventilação e a função de pequenas vias aéreas em diversas condições clínicas e, assim, diagnosticar precocemente as alterações na função pulmonar. A avaliação de alterações nas pequenas vias aéreas, além da mensuração da inomogeneidade na ventilação pelo método de lavagem de nitrogênio, é inédita em pacientes com ES.

Até o presente momento, não há na literatura nenhum estudo que tenha avaliado o uso da esqueletonização e volumetria das vias aéreas através da TC, nos portadores de ES. Esta pesquisa, em pacientes com ES, avaliou as alterações anatômicas e funcionais das vias aéreas por meio do TLN e da técnica de esqueletonização e volumetria de vias aéreas em imagens de TC e suas correlações com outros TFP de forma a contribuir para o melhor entendimento das complicações do sistema respiratório decorrentes desta condição.

2 **HIPÓTESE**

A TC *multislice* associada à volumetria por processamento de imagens vem ganhando crescente importância na literatura, pois a agregação dos métodos tem permitido o diagnóstico mais acurado de doenças e fornecendo informações adicionais em diversas afecções. O teste de lavagem de nitrogênio é capaz de avaliar alterações nas pequenas vias aéreas, com a medida de volume de fechamento, indicando seu fechamento precoce. Também é possivel analisar da homogeneidade da ventilação, conforme a variação da percentagem de nitrogênio exalado durante a expiração. Estudos anteriores sugeriram haver alteração na resistência das vias aéreas desses pacientes. Nossa hipótese é que pacientes com ES apresentam alterações nas vias aéreas, que podem ser diagnosticas tanto pelos métodos funcionais quanto pelos métodos de imagem.

3 **OBJETIVOS**

3.1 **Objetivo geral**

Avaliar a função e morfologia de vias aéreas de pacientes com ES, através de TFP e TCAR.

3.2 **Objetivos específicos**

a) Avaliar a distribuição da ventilação e a função das pequenas vias aéreas em pacientes com ES pela técnica de lavagem do nitrogênio;
b) Avaliar a volumetria das vias aéreas em pacientes com ES pela técnica de volumetria das vias aéreas;
c) Avaliar a relação entre os achados de função pulmonar e volumetria de vias aéreas da TCAR de tórax em pacientes com ES;
d) Avaliar a correlação entre os achados de lavagem do nitrogênio e outros dados de função pulmonar em pacientes com ES.

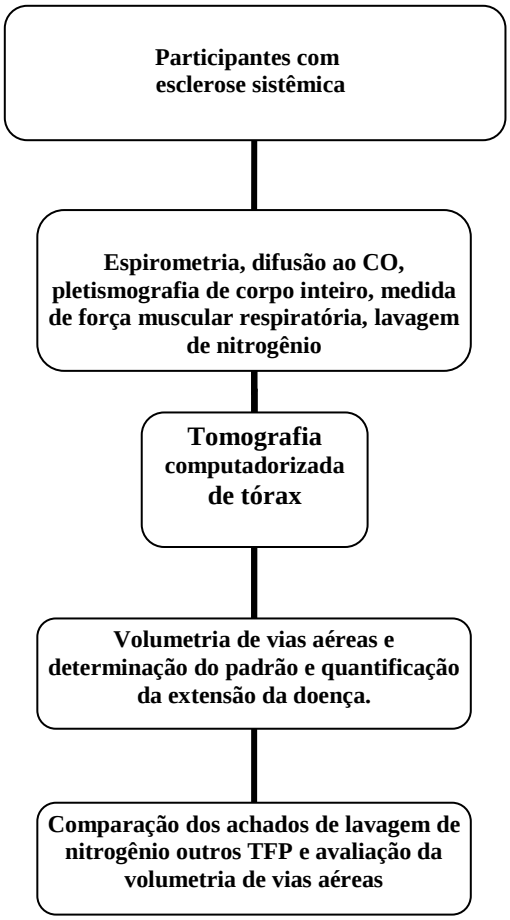
4 DESENHO EXPERIMENTAL

4.1 Desenho do estudo

Através de um estudo transversal e não intervencionista, foram avaliados pacientes com idade acima de 18 anos e diagnóstico de ES segundo os critérios do American College of Rheumatology, sendo este firmado por especialista em Reumatologia e acompanhados no Ambulatório de Doenças Intersticiais da Policlínica Piquet Carneiro (PPC) do Serviço de Pneumologia e Tisiologia da Universidade do Estado do Rio de Janeiro (UERJ). Foram recrutados os pacientes que aceitassem participar da pesquisa, após a leitura e assinatura do termo de compromisso livre e esclarecido (TCLE), que foi previamente aprovado pelo Comitê de Ética em Pesquisa do Hospital Universitário Pedro Ernesto (HUPE) (Anexo 1. Página 119). Os pacientes puderam estar em uso de medicamentos voltados à terapêutica da doença de base ou, ainda, apresentar outras comorbidades que são relacionadas à ES.

Foram feitas as TC em tomógrafo *multislice* de 64 canais, com aquisição inspiratória, expiratória e reconstrução para alta resolução (TCAR) e TFP por meio da espirometria, pletismografia de corpo inteiro, medida da DLco, teste de lavagem de nitrogênio e medida de força muscular respiratória. A TC se faz necessária para avaliar as vias aéreas e o acometimento pulmonar bem como excluir doenças associadas. As imagens tomográficas dos pacientes foram processadas em programa específico. O fluxograma do estudo é mostrado na **Figura 1**.

Figura 1 - Fluxograma do estudo



4.2 **Critérios de inclusão e exclusão**

Critérios de Inclusão

a) Idade acima de 18 e abaixo de 80 anos;

b) Diagnóstico de ES com envolvimento pulmonar segundo os critérios do *American College of Rheumatology* firmado por especialista em Reumatologia;

c) Sintomático respiratório – dispnéia ou tosse.

Critérios de exclusão

a) Diagnóstico de asma, DPOC ou outra pneumopatia crônica;
b) Carga tabágica maior que 10 maços-ano no passado;
c) Gestação ou sem uso de método contraceptivo;
d) Incapacidade de realizar a TC de tórax;
e) Incapacidade de realizar os testes de função pulmonar;
f) Indivíduos com infecção respiratória nos últimos 30 dias;
g) Hipertensão pulmonar;
h) Outras colagenoses associadas.

5 **ANÁLISE MORFOLÓGICA DO PULMÃO E VIAS AÉREAS EM IMAGENS DE TC**

5.1 **Aquisição de imagens**

As TC de tórax foram obtidas em um tomógrafo helicoidal com 64 canais (Philips Medical Systems, Cleveland, OH, USA Brilliance 40, Philips Medical Systems, Cleveland, OH, EUA). O tempo de leitura (*scanning time*) foi ajustado em 4s. A corrente na ampola era modulada. A tensão de 120 kV. Cada aquisição consistiu de um bloco com 250 a 400 cortes transversais de 2 mm de espessura e distância entre os cortes de 1 mm, obtido durante apneia inspiratória e expiratória. As imagens foram representadas por uma matriz quadrada de 768 linhas e 768 colunas. O *gantry* não teveinclinação. Não houveutilização de meio de contraste iodado.

5.2 **Volumetria de Vias Aéreas**

Para realização do presente estudo, foram necessários os seguintes itens;

a) Arquivos .dcm (DICOM) produzidos por tomografias computadorizadas;

b) 3DSlicer 4.4.0 r23774 (https://www.slicer.org/);

c) Extensão para o 3DSlicer, AirwaySegmentation (University College Cork);

d) MatLab 2014a (http://www.mathworks.com/);

e) Programa escrito no Matlab, Airway Processing.

Ainda, houve os módulos adicionais, conforme segue:

a) NRRD Format File Reader (Jeff Mather);

b) Accurate Fast Marching, versão modificada (Dirk-Jan Kroon);

c) Programa escrito em Matlab para processamento estatístico dos dados, Statistic Airway.

O processo de segmentação das vias aéreas foi realizado no 3DSlicer com o auxílio da extensão AirwaySegmentation. Ao final do processamento, um arquivo .nrrd (Nearly Raw Raster Data) foi salvo.

Usando o Airway Processing, dois processos distintos foram executados:

a) O arquivo .nrrd foi lido e transformado em uma matriz binária de dados. Por meio desta, o processo de esqueletonização foi iniciado. Um esqueleto composto por diversos pontos foi então produzido **(Figura 2)**;

b) Através do esqueleto e suas coordenadas nos plano X, Y e Z, foi executado um processamento individual de todos os pontos com o uso do comando *slice*. Os ângulos do processamento foram definidos por uma reta normal entre o ponto em questão e os próximos cinco pontos **(Figuras 3 e 4)**.

Usando o MatLab, as informações obtidas individualmente pelo comando *slice foram* catalogadas com comando *regionprops* e os seguintes dados foram obtidos: 1) área; 2) perímetro; 3) excentricidade (um valor compreendido entre 0 e 1, onde 1 é um segmento de linha e 0 um círculo); 4) diâmetro equivalente; 5) maior diâmetro; e 6) menor diâmetro.

Ao término das etapas supracitadas, o Airway Processing salvou um arquivo .xls com todas as informações processadas. Além do xls, cada ramo encontrado foi salvo em um arquivo de imagem.

Por meio das imagens, os ramos de interesse foram identificados e catalogados. Suas informações foram extraídas do arquivo xls para os devidos processamentos.

Figura 2 - Esqueleto e matriz binária representada por uma superfície

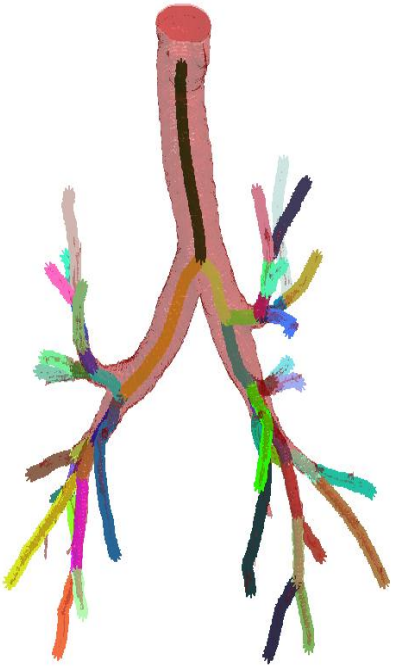


Figura 3 - Processamento de cada ponto (espaçado em 20 pontos para facilitar a visualização)

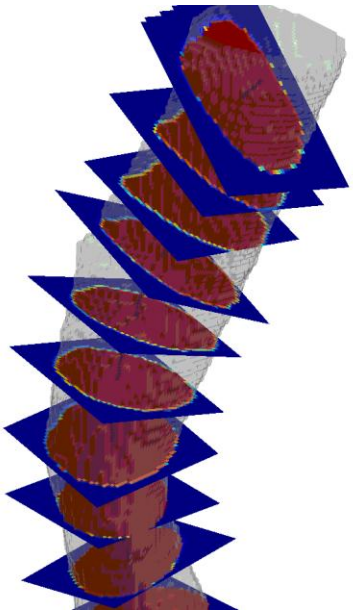
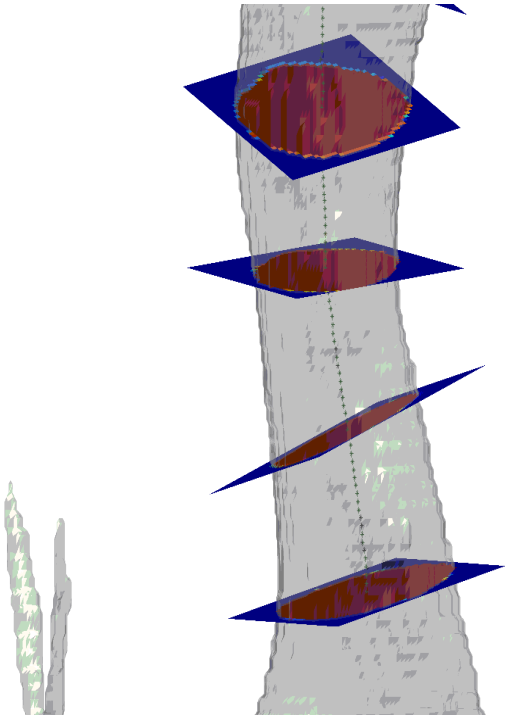


Figura 4 - Processamento de cada ponto numa maior dimensão (espaçado em 20 pontos para facilitar a visualização)



As informações foram catalogadas de acordo com a região de interesse (traqueia, brônquio principal direito (BPD), brônquio principal esquerdo (BPE), brônquio do lobo superior direito (BSD), brônquio do lobo superior esquerdo (BSE), brônquio intermediário (BI), brônquio do lobo inferior esquerdo (BIE) no Statistic Airway **(Tabela 1).**

Tabela 1 - Critérios de organização dos dados

	Área	Perímetro	Excentricidade	Diâmetro equivalente	Maior diâmetro	Menor diâmetro
Traqueia *	Máximo	Máximo	Mediana	Máximo	Máximo	Máximo
BPD	Mediana	Mediana	Mediana	Mediana	Mediana	Mediana
BPE	Mediana	Mediana	Mediana	Mediana	Mediana	Mediana
BSD **	Média	Média	Média	Média	Média	Média
BSE **	Média	Média	Média	Média	Média	Média
BI	Mediana	Mediana	Mediana	Mediana	Mediana	Mediana
BIE	Mediana	Mediana	Mediana	Mediana	Mediana	Mediana

BPD: brônquio principal direito; BPE: brônquio principal esquerdo; BSD: brônquio do lobo superior direito; BSE: brônquio do lobo superior esquerdo; BI: brônquio intermédio; BIE: brônquio do lobo inferior esquerdo.

* O processamento se inicia 1,5 cm acima da região da carina.

** Parâmetros calculados com relação ao menor somatório da área da metade do segmento.

O fluxograma das etapas, assim como o *hardware* utilizado e um exemplo da reconstrução em 3D das vias aéreas inferiores são mostrados nas Figuras 5, 6 e 7, respectivamente.

Figura 5 - Fluxograma das etapas

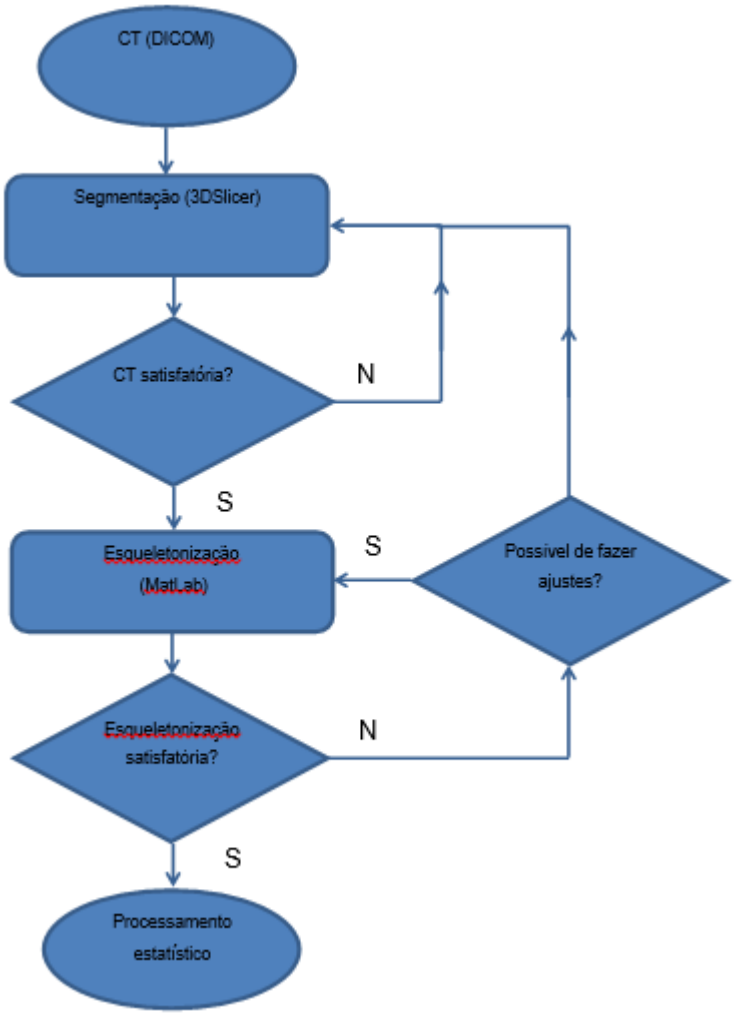
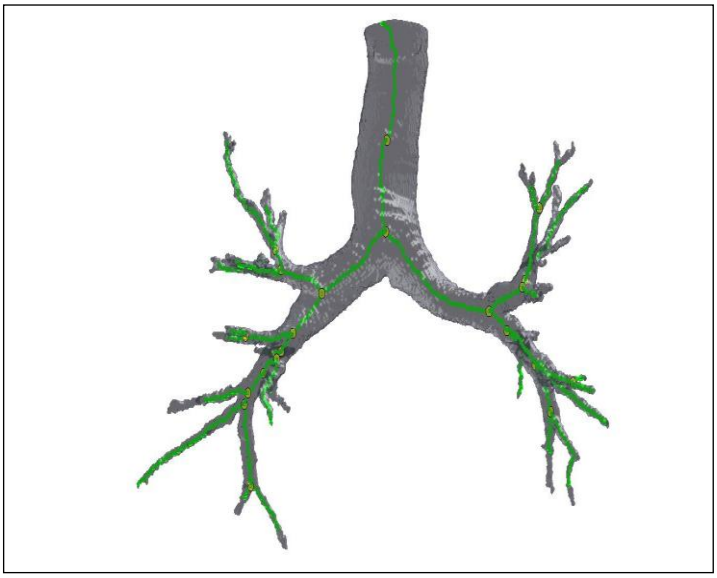


Figura 6 - Hardware utilizado

Nome do Sistema Operacional	Microsoft Windows 7 Professional
Versão	6.1.7601 Service Pack 1 Compilação 7601
Outras Informações sobre o Sistema Operacional	Não disponível
Fabricante do Sistema Operacional	Microsoft Corporation
Nome do sistema	LEP22
Fabricante do sistema	INTEL_
Modelo do sistema	DH87RL__
Tipo do sistema	x64-based PC
Processador	Intel(R) Core(TM) i5-4440 CPU @ 3.10GHz, 3001 Mhz, 4 Núcleo(s), 4 Processador(es) Lógico(s)
Versão/data do BIOS	Intel Corp. RLH8710H.86A.0320.2013.0606.1802, 06/06/2013
Versão do SMBIOS	2.7
Pasta do Windows	C:\Windows
Pasta do sistema	C:\Windows\system32
Dispositivo de inicialização	\Device\HarddiskVolume1
Localidade	Brasil
Camada de Abstração de Hardware	Versão = "6.1.7601.17514"
Nome de usuário	LEP\alan_ranieri
Fuso horário	Hora oficial do Brasil
Memória Física (RAM) Instalada	8,00 GB
Memória física total	7,67 GB
Memória física disponível	5,60 GB
Memória virtual total	15,3 GB
Memória virtual disponível	12,3 GB
Espaço do arquivo de paginação	7,67 GB
Arquivo de paginação	C:\pagefile.sys

Figura 7 - Exemplo da reconstrução em 3D das vias aéreas inferiores



6 AVALIAÇÃO DA FUNÇÃO PULMONAR

6.1 Espirometria

A espirometria é o estudo da função pulmonar, por meio das medidas de fluxo e volumes gerados nos ciclos respiratórios basais e forçados. Exige compreensão e colaboração do paciente, equipamentos exatos e emprego de técnicas padronizadas, aplicadas por profissionais capacitados. Os valores obtidos são comparados a valores previstos adequados para a população avaliada (KNUDSON, 1983; CRAPO et al., 1981, PEREIRA, 2002).

Antes do teste, os pacientes repousam cerca de 5 a 10 minutos. Foram orientados previamente a evitar álcool e café nas últimas 4-6 horas e refeições volumosas 1 hora antes do exame. Previamente ao teste, o técnico explicava claramente a execução das manobras. Durante o teste, os indivíduos permaneceram na posição sentada e com a cabeça em posição neutra, fazendo uso do clipe nasal (PEREIRA, 2002).

O equipamento utilizado para realização deste exame foi o HD CPL (nSpire Health Inc., Longmont, CO, USA), do Setor de Provas de Função Pulmonar do HUPE. No presente trabalho, as equações de referência utilizadas para espirometria foram as de Knudson,1983.(KNUDSON et al., 1983)). Todos os exames seguiram a padronização da American Thoracic Society, 2005 (MILLER et al., 2005).

6.2 Plestismografia de corpo inteiro

O pletismógrafo de corpo inteiro é um aparelho composto de um sistema computadorizado, acoplado a uma cabine hermeticamente fechada e que possui sensores que captam variações de pressão internas com grande sensibilidade. Estas variam de acordo com as mudanças no volume do tórax. As variações de pressão refletem as oscilações de volume

pulmonar (PEREIRA & MOREIRA, 2002). Durante os exames, os pacientes permaneceram em posição sentada dentro da caixa calibrada.

No pletismógrafo de corpo inteiro, foram realizadas pelo menos três manobras de esforços respiratórios rápidos e superficiais (*panting*). O procedimento era previamente explicado e demonstrado ao participante (PEREIRA & MOREIRA, 2002). Neste estudo, foram utilizadas as equações de Neder para o cálculo dos volumes pulmonares estáticos (NEDER et al., 1999).

O equipamento utilizado para realização deste exame foi o HD CPL (nSpire Health Inc., Longmont, CO, USA) do Setor de Provas de Função Pulmonar do HUPE.

6.3 **Capacidade de difusão ao monóxido de carbono (DLco)**

As manobras de difusão foram realizadas após repouso de cinco minutos. Um volume da amostra de 0,5 a 1 L era coletado dentro de quatro segundos, após uma apneia inspiratória de aproximadamente 10 segundos. A média de duas ou mais manobras aceitáveis foi feita. Determinações em duplicata deveriam situar-se dentro de 10% ou 3 ml CO/min/mmHg (PEREIRA, 2002). As equações de referência utilizadas neste estudo foram as de Neder, 1999 (NEDER et al., 1999). O equipamento utilizado para realização deste exame foi o HD CPL (nSpire Health Inc., Longmont, CO, USA) do Setor de Provas de Função Pulmonar do HUPE.

6.4 **Força muscular respiratória**

As mensurações das pressões respiratórias máximas dependem da compreensão das manobras a serem executadas e da colaboração do paciente para a realização de movimentos e esforços respiratórios realmente máximos. Assegurados esses critérios, os valores de pressão inspiratória máxima (PImáx) e de pressão expiratória máxima (PEmáx) dependem não apenas da força dos músculos respiratórios, mas também do volume pulmonar em que forem realizadas as mensurações e do correspondente valor da pressão de retração elástica do sistema respiratório, o

qual resulta da soma algébrica das pressões de retração elástica dos pulmões e da caixa torácica (SOUZA, 2002).

As equações de referência utilizadas neste estudo foram as de Neder, 1999 (NEDER et al., 1999). O equipamento utilizado para realização deste exame foi o HD CPL (nSpire Health Inc., Longmont, CO, USA) do Setor de Provas de Função Pulmonar do HUPE.

6.5 **Teste de lavagem de nitrogênio**

O teste foi realizado no equipamento HD PFR 3000 (NSpire Health, Inc. Longmont, CO, USA). Todos estes testes seguiram as normas estabelecidas pela Sociedade Brasileira de Pneumologia e Tisiologia (SBPT) e pela American Thoracic Society (ATS). O indivíduo, na posição sentada, com as narinas ocluídas por uma pinça e conectado ao espirógrafo por meio de uma peça bucal adaptada a um sistema de válvula de três vias, respira ar ambiente tranquilamente, após realizar ciclos ventilatórios de profundidade superior à habitual. A seguir, efetua uma expiração máxima, alcançando o nível do volume residual; nesse momento, é ligado ao reservatório de O_2 puro e, com uma inspiração lenta e total, inala O_2 até alcançar a capacidade pulmonar total. Inicia-se então uma expiração lenta e total, devendo manter um débito exibido pelo monitor de fluxo de cerca de 500 ml/s. Durante a expiração, o registrador X-Y contrapõe a concentração de N_2 (no eixo de Y) ao volume expirado (no eixo de X), obtendo-se uma curva.

Foram obtidas pelo menos três curvas que eram, à inspeção visual, consideradas aceitáveis. Os valores da capacidade vital obtidos nas diversas curvas não deveriam diferir entre si por mais de 10% da maior delas (ROBINSON et al., 2013). Neste estudo, foram utilizadas as equações de Buist and Ross (1973) para o cálculo das variáveis fornecidas pelo TLN (BUIST & ROSS, 1973).

7 ORDEM PARA REALIZAÇÃO DOS EXAMES

Os testes foram realizados nos Serviços de Pneumologia (Setor de Provas de Função Pulmonar) e Radiologia do HUCFF. Sempre houve um médico para supervisionar a execução de todos os exames, os quais foram realizados na seguinte sequência: primeiro os pacientes eram submetidos aos TFPs - espirometria, medida da DLco, pletismografia de corpo inteiro, medida de força muscular, lavagem de nitrogênio - e, posteriormente, às TCAR em inspiração e expiração. O intervalo entre todos os exames foi de, no máximo, 1 mês.

8 **CÁLCULO DO TAMANHO DA AMOSTRA**

O tamanho da amostra foi calculado através do software MedCalc versão 8.2 (Medcalc Software, Mariakerke, Bélgica). Considerando que o desfecho principal foi a associação das medidas do teste de lavagem de nitrogênio com os dados da TC, estimou-se que uma amostra mínima de 30 pacientes seria necessária para observar uma correlação mínima de 0,30 (fraca ou superior) com um nível de significância de 5% e poder do teste de 80%.

9 **ASPECTOS ÉTICOS**

Todos os pacientes foram previamente informados da pesquisa e consentiram na realização do trabalho por meio da assinatura do termo de consentimento livre e esclarecido (TCLE) (Anexo I), que foi apresentado ao Comitê de Ética em Pesquisa (CEP) do HUPE. O TCLE informa o tipo de pesquisa, seus objetivos e esclarece que a participação do sujeito é voluntária, não prevê qualquer ressarcimento e que tanto sua participação quanto a não concordância em participar do estudo não acarretará prejuízo de qualquer tipo. Nele, também se estabelece um compromisso com a privacidade de cada um e a utilização confidencial e sigilosa dos dados colhidos.

Este estudo foi avaliado e aprovado pelo Comitê de Ética em Pesquisa do HUPE (CEP-HUPE) (CAAE: 50752615.9.0000.5259), sob o número de comprovante 112825/2015, com data final da relatoria de 12/11/2015 (Apêndice I). Não houve conflitos de interesse por parte do autor na realização deste trabalho.

10 **RESULTADOS E DISCUSSÃO**

A seguir, serão apresentados dois artigos produzidos como resultados dos trabalhos desenvolvidos na elaboração da presente tese.

O primeiro foi publicado na Revista Portuguesa de Pneumologia em janeiro de 2017 com base nos dados parciais do estudo. O segundo foi aceito na revista Plos One em julho de 2018 com base nas informações finais obtidas pelo estudo.

10.1 **Artigo 1**

VENTILATION DISTRIBUTION AND SMALL AIRWAY FUNCTION IN PATIENTS WITH SYSTEMIC SCLEROSIS

Running title: Ventilation distribution and small airway in systemic sclerosis

Authors full names and affiliations:

1) Bruno Rangel Antunes da Silva – MSc. Laboratory of Respiratory Physiology, State University of Rio de Janeiro, and Postgraduate Programme in Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil. E-mail: brunocmhfa@gmail.com
   Role in the study: BRA da Silva was responsible for the conception, design, and acquisition of data.
2) Rogério Lopes Rufino Alves – Ph.D. Department of Pulmonology, State University of Rio de Janeiro, and Postgraduate Programme in Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil. E-mail: rrufino.uerj@gmail.com

Role in the study: RLR Alves was responsible for the analysis and interpretation of data.

3) Cláudia Henrique da Costa – Ph.D. Department of Pulmonology, State University of Rio de Janeiro, and Postgraduate Programme in Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil. E-mail: ccosta.uerj@gmail.com

   Role in the study: CH da Costa was responsible for advising it critically for important intellectual content.

4) Veronica Silva Vilela, – MSc. Department of Rheumatology, State University of Rio de Janeiro, and Postgraduate Programme in Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil. E-mail: veronicavilelavs@yahoo.com.br

   Role in the study: VS Virela was responsible for the conception, design, and revising the article.

5) Roger Abramino Levy – MSc. Department of Rheumatology, State University of Rio de Janeiro, and Postgraduate Programme in Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil. E-mail: rogeralevy@gmail.com

   Role in the study: RA Levy was responsible for the final approval of the version of manuscript.

6) Agnaldo José Lopes – Ph.D. Laboratory of Respiratory Physiology, State University of Rio de Janeiro, and Postgraduate Programme in Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil. E-mail: phel.lop@uol.com.br

   Role in the study: AJ Lopes was responsible for the analysis and interpretation of data.

Corresponding author: Agnaldo José Lopes. Rua Araguaia, 1266, bloco 1/405, Freguesia, Jacarepaguá, 22745-271, Rio de Janeiro, RJ, Brazil. Phone and fax numbers: +55 21 21 2576 2030. E-mail: phel.lop@uol.com.br

ABSTRACT

Background: Despite the importance of traditional pulmonary function tests (PFTs) in managing systemic sclerosis (SSc), many patients with pulmonary disease diagnosed by computed tomography (CT) present with normal PFTs.

Objective: To evaluate the efficacy of the nitrogen single-breath washout (N_2SBW) test in diagnosing SSc and to correlate N_2SBW parameters with the PFT indexes used in the follow-up of these patients, clinical data, and CT findings.

Methods: Cross-sectional study in which 52 consecutive SSc patients were subjected to spirometry, body plethysmography, analysis of the diffusing capacity for carbon monoxide (DLco), analysis of respiratory muscle strength, N_2SBW testing, and CT analysis.

Results: Twenty-eight patients had a forced vital capacity (FVC) that was <70% of the predicted value. In the N_2SBW test, 44 patients had a phase III slope (Phase III $slope_{N_2SBW}$) that was >120% of the predicted value, while 15 patients had a closing volume/vital capacity (CV/VC) that was >120% of the predicted value. A significant difference in Phase III $slope_{N_2SBW}$ was observed when the patients with predominant traction bronchiectasis and honeycombing were compared to the patients with other CT patterns ($p<0.0001$). The Phase III $slope_{N_2SBW}$ was correlated with FVC ($r_s=-0.845$, $p<0.0001$) and DLco ($r_s=-0.600$, $p<0.0001$), and the CV/VC was correlated with FVC ($r_s=-0.460$, $p=0.0006$) and residual volume/total lung capacity ($r_s=0.328$, $p=0.017$).

Conclusion: Ventilation heterogeneity is a frequent finding in SSc patients that is associated with restrictive damage, changes in pulmonary diffusion, and CT patterns. In addition, approximately one-third of the patients present with findings that were compatible with small airway disease.

Keywords: systemic sclerosis; respiratory function tests; nitrogen single-breath washout test

INTRODUCTION

Systemic sclerosis (SSc) is a chronic inflammatory disease of the connective tissue that is characterised by cutaneous and visceral fibrosis, self-immunity, and vascular destruction.[1,2] Almost 90% of SSc patients present with some form of lung injury over the evolution of the illness, and interstitial lung diseases associated with the SSc (ILD-SSc) and pulmonary arterial hypertension (PAH) are the most frequent manifestations.[2,3] Among the investigation methods for ILD-SSc, lung biopsy is rarely performed. Therefore, computed tomography (CT) is currently considered the method of choice.[4] Because the frequent use of ionising radiation is a matter of growing concern, CT is rarely used in the follow-up of these patients. Indeed, the severity of the pulmonary involvement of SSc is more frequently quantified using pulmonary function tests (PFTs) in clinical practice.[5]

Among the PFTs used in the diagnosis and follow-up of SSc patients, the most widespread are spirometry and diffusing capacity for carbon monoxide (DLco).[7] Despite the importance of traditional PFTs in the management of pulmonary involvement associated with SSc, a significant proportion of patients present with normal PFT results, even in the presence of ILD-SSc diagnosed by imaging methods.[8] With the evolution of technical equipment in recent years, growing interest has developed in the use of the nitrogen single-breath washout (N_2SBW) test to assess ventilation homogeneity and the role of small airways in several clinical conditions.[9,10] The N_2SBW test is used for the early diagnosis and stratification of patients and to assess the of severity of several lung diseases.[9,11–13] In asthma patients, poor disease control is correlated with both an increase in the closing volume (CV) and the phase III slope of the N_2SBW (Phase III slope$_{N_2SBW}$).[13] In COPD patients, Lopes and Mafort[9] observed that Phase III slope$_{N_2SBW}$ was the only predictor, regardless of the degree of dyspnea and functional capacity for exercise. Mikamo et al.[12] described significant correlations between the Phase III slope$_{N_2SBW}$ and

the measurements of mechanical ventilation and emphysema score evaluated by CT. However, to our knowledge, no studies have previously assessed the use of the N_2SBW test in SSc patients.

In addition to causing poor ventilation distribution, lung interstitium involvement can potentially lead to structural changes in small airways, resulting in a loss of air flow that can reflect increased ventilatory demand.[14] We hypothesised that the structural disarray caused by the excessive secretion of collagen in the respiratory systems of SSc patients may be reflected in the N_2SBW test. Thus, the present study sought to assess the usefulness of the N_2SBW test in SSc patients and to correlate the parameters measured by the N_2SBW test with the PFT indexes classically used in the follow-up of these patients, degree of dyspnea, and CT findings.

METHODS

Patients

This was a cross-sectional study conducted between December 2015 and July 2016 in which 66 consecutive SSc patients were evaluated. These patients were recruited from the Piquet Carneiro Polyclinic of the State University of Rio de Janeiro, Brazil. . Patients ≥18 years of age of both genders who meet the criteria for SSc diagnosis[15] were included in the study. The following exclusion criteria were applied: patients with a previous history of smoking or those who were current smokers; individuals with asthma; evidence of overlap with other connective tissue diseases, except Sjogren's syndrome; reports of infection within the previous four weeks; and inability to perform PFTs. The protocol was approved by the Research Ethics Committee of the Pedro Ernesto University Hospital of the State Universityof Rio de Janeiro under the number CAAE- 50752615.9.0000.5259. All of the patients signed informed consent forms.

Measurements

Dyspnea was assessed by means of the modified Medical Research Council (mMRC) scale.[16]

Spirometry, body plethysmography, measurement of DLco, and measurement of respiratory muscle strength were conducted with Collins Plus Pulmonary Function Testing Systems equipment (Warren E. Collins, Inc., Braintree, MA, USA) using the standardisation of the consensus statement.[17] The Brazilian reference values were used[18–21], and the results are expressed as % predicted.

The N_2SBW test was performed using the HDpft 3000 instrument (nSpire Health, Inc., Longmont, CO, USA). Briefly, individuals exhaled until the residual volume (RV) was reached and then inhaled 100% O_2 until the total lung capacity was reached (TLC). Then, they slowly exhaled at a flow rate of approximately 0.3–0.5 L/s until the RV was reached. The two indexes derived from the procedure are reported as % predicted[22,23] and include the Phase III $slope_{N_2SBW}$, which is the change in the concentration of N_2 between 25%-75% of the exhaled volume, and the closing volume/vital capacity (CV/VC), which is the portion of the VC that is exhaled after the airway begins to close. The N_2SBW test was performed according to the recommendations of the consensus statement.[11]

We also assessed the CT scans of 31 patients that were performed within the last three months prior to recruitment. The CT scans were categorised into three patterns according to the consensus of two radiologists: grade 1 (reticular pattern predominance); grade 2 (ground-glass opacity predominance); and grade 3 (traction bronchiectasis and honeycombing predominance).[4,24]

Statistical analysis

The data analysis was conducted using SAS 6.11 software (SAS Institute, Inc., Cary, NC, USA). The assumption of a normal distribution of the data was evaluated with a Shapiro-Wilk test.

Comparisons of variables between the two groups of patients subdivided according to the FVC or mMRC grades were evaluated by the Mann-Whitney test for numerical variables and by Fisher's exact test for categorical variables. Comparisons of the CT grades according to the different N_2SBW variables were examined using the nonparametric Kruskal-Wallis test followed by Dunn's post-hoc test. Spearman's rank correlation coefficient (r_s) was used to evaluate the associations between the variables. The results are expressed as median values and interquartile ranges or as frequencies (percentages), and statistical significance was considered at $p<0.05$.

RESULTS

Among the 66 patients who were considered for participation in the study, 14 were excluded due to the following reasons: six for reporting a history of smoking, four for presenting with SSc together with other collagenoses, two due to associated asthma, and two due to the inability to perform the PFTs. Thus, the study population consisted of 46 women and six men with a median age of 48 (38.5–56.3) years. Thirty-eight patients had a limited form of the disease, and 14 had the diffuse form. In 36 patients, the mMRC grade was <2 [1.12 (0.64–1.47)], and in 16, it was ≥2 [2.47 (2.31–2.78)]. In the CT analysis, the exams were categorised as grade 1 (n=13), grade 2 (n=10), and grade 3 (n=8).

For the 52 patients who participated in the study, the median values of FVC, DLco, and FVC/DLco were 67 (57–91)% predicted, 62 (44–81.3)% predicted, and 1.18 (0.93–1.52)% of the reference values, respectively. Twenty-eight patients had an FVC <70%, 34 had a DLco <80%, and nine had an FVC/DLco >1.6. Regarding the TLC, the median of the sample was 72.5 (60.3–87.5)% predicted, and this parameter was <80% in 31 patients, which indicated a restrictive disorder. The medians for Phase III$slope_{N_2SBW}$ and CV/VC were 254 (112–450)% predicted and 107 (62–150)% predicted, respectively. In this test, 44 patients had a Phase III$slope_{N_2SBW}$ >120%,

while 15 patients had a CV/VC >120%. When the sample was subdivided into two groups using a cut-off value of FVC <70%, significant differences were observed between the two groups for most of the evaluated functional parameters (Table 1).

Table 1 Demographic data and lung function of patients with systemic sclerosis.

Variable	**Patients with FVC ≥70% (n=24)**	**Patients with FVC <70% (n=28)**	***p*-value**
Demographic data			
Females (%)	22 (91.7)	24 (85.7)	0.67[b]
Age (years)	49.5 (43.3–58.8)	48 (37–53.8)	0.26[a]
Weight (kg)	62.8 (52.2–71)	71.5 (57.3–82)	0.11[a]
Height (cm)	157 (153–162)	160 (156–164)	0.23[a]
BMI (kg/m^2)	25.1 (21.6–28.9)	28.4 (22.1–30.9)	0.15[a]
Lung function			
FVC (% predicted)	93 (80.5–103)	57 (51.3–65)	**<0.0001**[a]
FEV_1 (% predicted)	87.5 (81.3–99)	59.5 (48–65.8)	**<0.0001**[a]
FEV_1/FVC (%)	79.5 (74.5–84.5)	86 (77.3–89.5)	**0.018**[a]
DLco (% predicted)	78 (53.3–95)	50.5 (37.3–65.8)	**0.0004**[a]
FVC/DLco (% of reference values)	1.22 (1.08–1.52)	1.09 (0.89–1.52)	0.23[a]

TLC (% predicted)	89 (79.3–98.8)	61 (55–72)	**<0.0001**[a]
RV (% predicted)	87 (73.3–109)	74 (56.3–85)	**0.047**[a]
RV/TLC (%)	34.5 (28.2–37.2)	40 (34.8–46.3)	**0.007**[a]
Raw (cm H_2O/L/s)	1.66 (1.36–2.43)	1.81 (1.26–3.08)	0.78[a]
SGaw (L/s/cm H_2O/L)	0.205 (0.148–0.268)	0.260 (0.165–0.438)	0.12[a]
MIP (% predicted)	66 (47–87.3)	65 (55.3–92.8)	0.42[a]
MEP (% predicted)	55.5 (32.8–62)	45.5 (34.8–54)	0.40[a]
Nitrogen single-breath washout test			
Phase III $slope_{N_2SBW}$ (% predicted)	130 (88–160)	419 (275–542)	**<0.0001**[a]
CV/VC (%predicted)	71 (56–111)	137 (101–188)	**0.003**[a]

Values are median (interquartile ranges) or number (%).

BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; DLco: diffusing capacity for carbon monoxide; TLC: total lung capacity; RV: residual volume; Raw: airway resistance; SGaw: specific airway conductance; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure; Phase III $slope_{N_2SBW}$: phase III slope of the nitrogen single-breath washout; CV/VC: closing volume/vital capacity. [a] Mann-Whitney test. [b] Fisher exact test.

There was a significant difference between the mMRC grades and N_2SBW variables: Phase III$slope_{N_2SBW}$ [235 (148–287) vs. 352 (240–430), p=0.08] and CV/VC [98 (70–136) vs. 124 (85–161), p=0.13]. Regarding the CT findings, the medians of the Phase III$slope_{N_2SBW}$ values progressively increased from grade 1 to grade 3 [105 (64–138) vs. 185 (147–223) vs. 536 (370–653)%] with significant differences (p<0.0001). The medians of the CV/VC values also progressively increased from grade 1 to grade 3 [53 (32–85) vs. 68 (46–102) vs. 175 (78–217)%]; however, the differences were not significant (p=0.11).

We also assessed the associations between the parameters provided by the N_2SBW test, lung function indices, and CT findings (Table 2 and Figures 1 and 2); the correlation between the Phase III$slope_{N_2SBW}$ and CV/VC was strong and positive (r_s=0.590, p<0.0001).

Table 2 Spearman's correlation coefficients for lung function parameters and nitrogen single-breath washout indexes of patients with systemic sclerosis.

Variable	Phase III$slope_{N_2SBW}$ (%predicted)		CV/VC (%predicted)	
	r_s	*p*-value	r_s	*p*-value
FVC (% predicted)	-0.845	**<0.0001**	-0.460	**0.0006**
FEV_1 (% predicted)	-0.788	**<0.0001**	-0.396	**0.003**
FEV_1/FVC (%)	0.281	**0.044**	-0.282	**0.042**
DLco (% predicted)	-0.600	**<0.0001**	-0.271	0.052
FVC/DLco (% of reference values)	0.088	0.54	0.020	0.89
TLC (% predicted)	-0.708	**<0.0001**	-0.360	**0.008**
RV (% predicted)	-0.354	**0.010**	-0.122	0.39
RV/TLC (%)	0.318	**0.021**	0.328	**0.017**
Raw (cm H_2O/L/s)	0.084	0.55	-0.216	0.12
SGaw (L/s/cm H_2O/L)	0.205	0.15	0.365	**0.007**
MIP (% predicted)	0.123	0.38	0.095	0.50

MEP (% predicted)	-0.080	0.57	-0.163	0.25

Phase III slope$_{N_2SBW}$: phase III slope of the nitrogen single-breath washout; CV/VC: closing volume/vital capacity; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; DLco: diffusing capacity for carbon monoxide; TLC: total lung capacity; RV: residual volume; Raw: airway resistance; SGaw: specific airway conductance; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure.

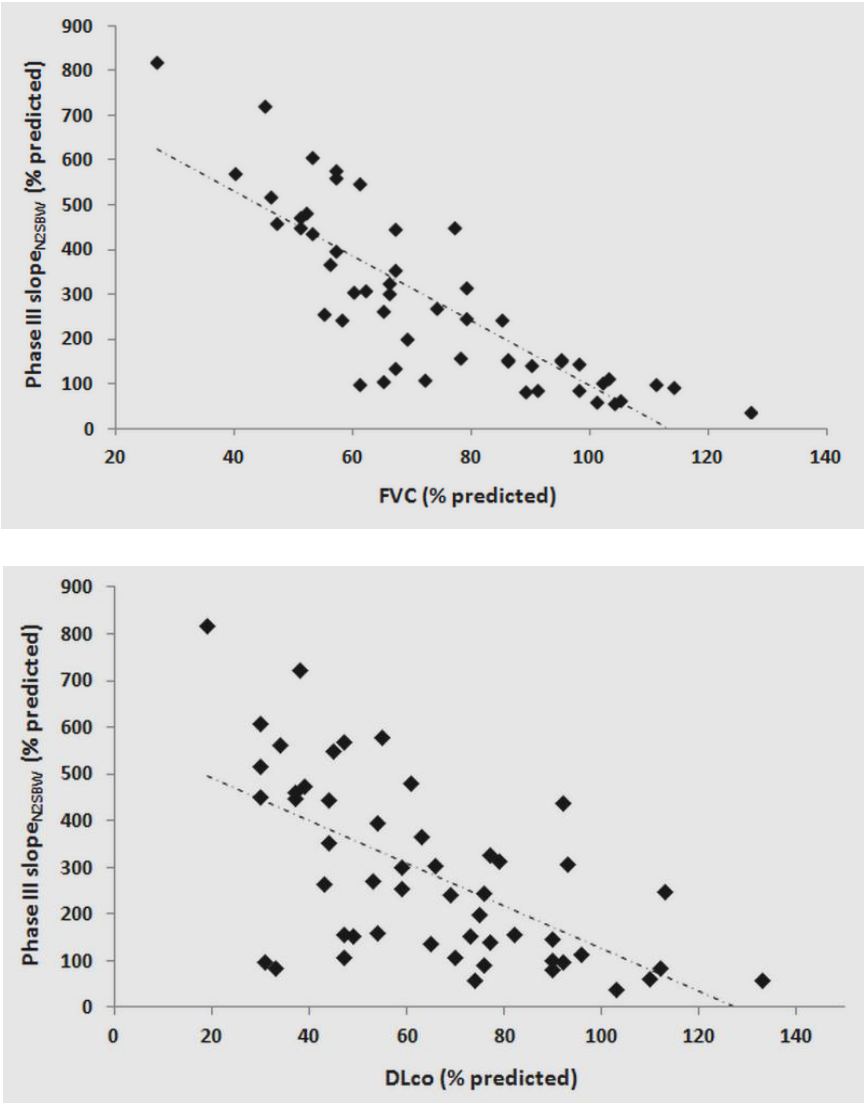


Figure 1 Relationships between the phase III slope of the nitrogen single-breath washout (Phase III $slope_{N2SBW}$) and the forced vital capacity (FVC) (r_s=-0.845, p<0.0001) (A) and diffusing capacity for carbon monoxide (DLco) (r_s=-0.600, p<0.0001) (B).

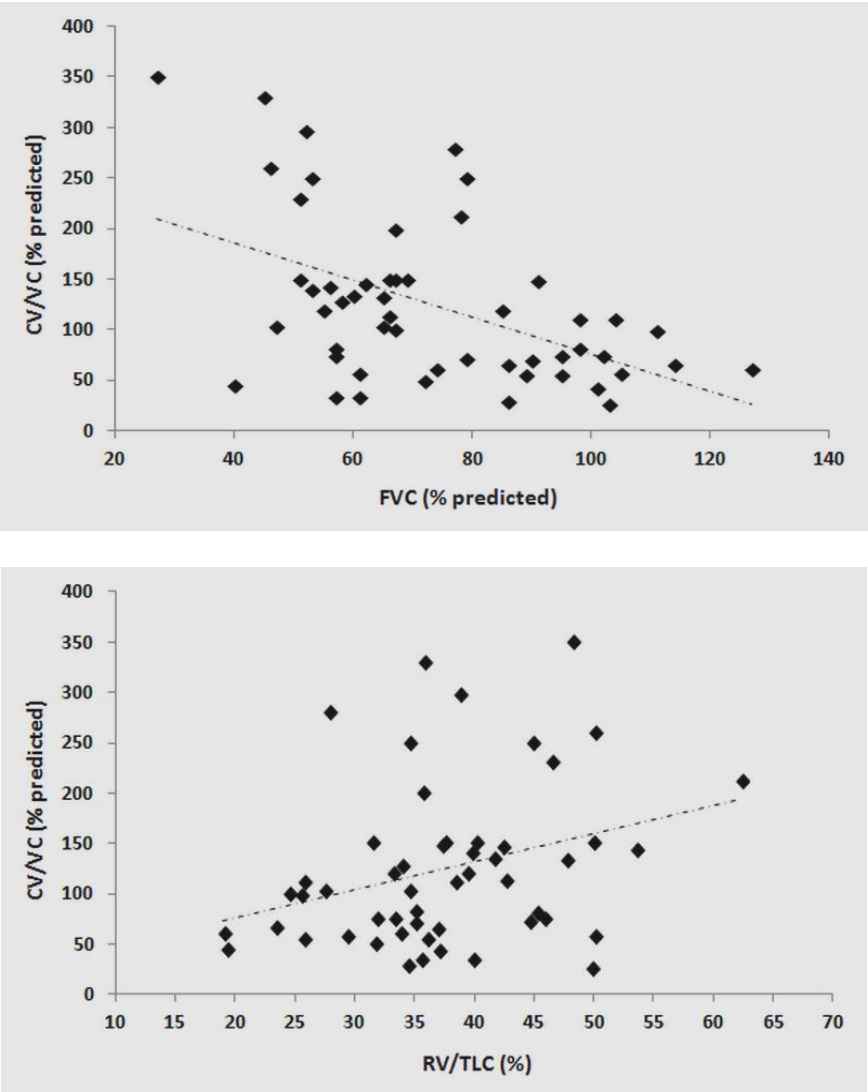


Figure 2 Relationships between the closing volume/vital capacity (CV/VC) and the forced vital capacity (FVC) (r_s=-0.460, p=0.0006) (A) and residual volume/total lung capacity (RV/TLC) (r_s=0.328, p=0.017) (B).

DISCUSSION

The main finding of the present study was that ventilation heterogeneity is the most common abnormality observed in the PFTs of SSc patients, and it occurs even in the absence of restrictive damage. In those patients, the more accentuated the functional or structural pulmonary deterioration is, the worse the ventilation heterogeneity. In addition, small airway disease was also a frequent finding that was related to both air trapping and the loss of lung volume. To our knowledge, this is the first study to assess the potential of the N_2SBW test in SSc patients.

In the present study, the sample was almost exclusively composed of women, which is consistent with the distribution by gender reported by several investigators.[2] In terms of prognosis, functional changes are important markers of the evolution of ILD-SSc, in both the initial and the sequential assessments.[2] In the present study, approximately 60% of the patients had the restrictive syndrome and/or a reduction of the DLco, thereby presenting a sample with substantial lung function involvement. Interestingly, nine patients had an FVC/DLco >1.6, which is the recommended cut-off for study of the right side of the heart in the diagnosis of PAH.[25]

Many patients with pulmonary disease diagnosed by CT present with normal PFTs, which is indicative of insufficient performance of traditional PFTs in tracking pulmonary disease associated with SSc.[8] In a recent study, Suliman et al.[26] evaluated 102 patients with SSc and noted that 63% presented with significant ILD-SSc on CT, while only 26% had an FVC <80%. This finding emphasises the urgent need to develop new lung function parameters to diagnose lung involvement, as well as allow follow-up with patients. In this sense, some tests such as the N_2SBW test, the forced oscillation technique (FOT), and impulse oscillometry can add to our understanding of the

pathophysiology of ILD-SSc and have the potential to be incorporated into the routine assessment of these patients.[27,28]

In the present study, an increase in the Phase III$slope_{N_2SBW}$ was the most frequent lung function abnormality, which was observed in approximately 85% of the cases and indicates the potential of this index as a marker for ILD-SSc. High values are indicative of ventilation inhomogeneity due to regional differences in time constants of the respiratory system, which result from changes in the distensibility or local resistance, thus compromising alveolar emptying.[29] The increase in the Phase III $slope_{N_2SBW}$ in the group of patients with an FVC <70% indicates that the worsening of restrictive functional damage is an important contributor to the ventilation heterogeneity of SSc. We observed a strong association between the increase in Phase III $slope_{N_2SBW}$ and the decay of both the FVC and DLco, which reinforces the routine use of these two functional indexes in the follow-up of patients with SSc in clinical practice. Interestingly, we also observed a strong association between the increase of the Phase III $slope_{N_2SBW}$ and the presence of traction bronchiectasis and honeycombing in CT. Despite the lack of studies correlating the N_2SBW test with CT findings in fibrotic lung diseases, it is noteworthy that some researchers have observed an association between the increase in Phase III $slope_{N_2SBW}$ and structural lung damage in COPD patients.[12,30]

In addition to the Phase III$slope_{N_2SBW}$, the CV/VC ratio is another index provided by the N_2SBW test that has recently been studied .[11,29] In the present study, we observed an increase in the CV/VC ratio in nearly one-third of the patients. A change in the CV/VC ratio has been used as one of the parameters for the diagnosis of small airway disease, and it is functionally characterised by a progressive increase in resistance as the lung is emptied, regional heterogeneity in flow rate and time constants, and premature closing of the airways.[4,11] Using the FOT, Miranda et al.[28] observed

changes in the peripheral resistance of the respiratory system in SSc patients, which were evaluated according to the slope of the resistance as a function of frequency. Similarly, Aronsson et al.[27] observed abnormalities that were compatible with small airway diseases in SSc patients including increases in the R5-R20, which is the difference between the resistance at 5 Hz and the resistance at 20 Hz in impulse oscillometry. Similar to the Phase III $slope_{N_2SBW}$, we observed an increase in the CV/VC in the group of patients with an FVC <70%. Interestingly, we also observed a positive correlation between CV/VC and RV/TLC in the studied sample, suggesting an association between the premature closing of the airways and the presence of air trapping.[31] However, it is worth stressing that an increase in the CV/VC can be observed in patients with restrictive damage in situations where the functional residual capacity is less than the closing volume.[29]

It is noteworthy that there are currently more than 10 measures of ventilation distribution derived from the nitrogen washout (N_2W) tests.[32] The need for maintaining good coordination and cooperation when conducting VC manoeuvres under constant flow is a limiting factor for the routine use of the N_2SBW test.[11] Contrariwise, the multiple breath washout (MBW) test evaluates ventilation distribution during the fixed tidal volume or normal tidal breathing, assessing the release of inert gas in a series of breathing cycles. Thus, MBW shows promise for use in children and adults with difficulties performing forced manoeuvres. However, this test is time consuming, which makes it impractical to use in patients with severe lung disease .[11] It is also noteworthy to highlight the double tracer gas (DTG) single-breath washout, a new N_2W test modality that aims to be more specific to small airways. It distinguishes between convection-dependent and diffusion-convection-dependent ventilation heterogeneities, which occur in the conductive and acinar airways, respectively.[33]

The strength of this study is that it demonstrates the potential of the N_2SBW test for detecting abnormalities in both ventilation and the small airways of SSc patients. Because it is a simple, non-invasive, easy, and fast tool, the N_2SBW test may be incorporated into the clinical assessment of SSc patients in the future. However, our study also has several limitations. First, the sample was small, and the design was cross-sectional. Second, we did not use a control group. However, the absence of a control group in this study was minimised by the use of pulmonary function values as percentages of the predicted values because these are normalised to anthropometric data. Third, the complementary use of DTG single-breath washout could have allowed us to evaluate ventilation heterogeneity in the lung periphery.[33] Despite these limitations, our results provide a perspective for the use of the N_2SBW test in longitudinal studies to verify its prognostic value in SSc patients.

Finally, the present study shows that in patients with SSc, ventilation distribution inhomogeneity is a very frequent finding that is related to restrictive damage, changes in pulmonary diffusion, and CT patterns. In addition, approximately one-third of the patients in this study were compatible with the criteria for small airway disease, which is associated with both the severity of restrictive damage and the presence of air trapping.

ETHICAL RESPONSIBILITIES

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

ACKNOWLEDGEMENTS

This research was supported by the Rio de Janeiro State Research Supporting Foundation (FAPERJ).

REFERENCES

1. Chizzolini C, Brembilla NC, Montanari E, Truchetet ME. Fibrosis and immune dysregulation in systemic sclerosis. Autoimmun Rev. 2011;10:276–81.
2. Sticherling M. Systemic sclerosis-dermatological aspects. Part 1: Pathogenesis, epidemiology, clinical findings. J Dtsch Dermatol Ges. 2012;10:705–18.
3. Tani C, Bellando Randone S, Guiducci S, Della Rossa A. Systemic sclerosis: a critical digest of the recent literature. Clin Exp Rheumatol. 2013;31(Suppl 76):172–9.
4. Lopes AJ, Capone D, Mogami R, Menezes SL, Guimarães FS, Levy RA. Systemic sclerosis-associated interstitial pneumonia: evaluation of pulmonary function over a five-year period. J Bras Pneumol. 2011;37:144–51.

5. Antoniou KM, Margaritopoulos GA, Goh NS, Karagiannis K, Desai SR, Nicholson AG, et al. Combined pulmonary fibrosis and emphysema in scleroderma-related lung disease has a major confounding effect on lung physiology and screening for pulmonary hypertension. Arthritis Rheumatol. 2016;68:1004–12.
6. Silver KC, Silver RM. Management of systemic-sclerosis-associated interstitial lung disease. Rheum Dis Clin North Am. 2015;41:439–57.
7. Hant FN, Herpel LB, Silver RM. Pulmonary manifestations of scleroderma and mixed connective tissue disease. Clin Chest Med. 2010;31:433–49.
8. Hoffmann-Vold AM, Aaløkken TM, Lund MB, Garen T, Midtvedt Ø, Brunborg C, et al. Predictive value of serial high-resolution computed tomography analyses and concurrent lung function tests in systemic sclerosis. Arthritis Rheumatol. 2015;67:2205–12.
9. Lopes AJ, Mafort TT. Correlations between small airway function, ventilation distribution, and functional exercise capacity in COPD patients. Lung. 2014;192:653–9.
10. Timmins SC, Diba C, Farrow CE, Schoeffel RE, Berend N, Salome CM, King GG. The relationship between airflow obstruction, emphysema extent, and small airways function in COPD. Chest. 2012;142:3–319.
11. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. Eur Respir J. 2013;41:507–22.
12. Mikamo M, Shirai T, Mori K, Shishido Y, Akita T, Morita S, et al. Predictors of phase III slope of nitrogen single-breath washout in COPD. Respir Physiol Neurobiol. 2013;189:42–6.

13. Bourdin A, Paganin F, Préfaut C, Kieseler D, Godard P, Chanez P. Nitrogen washout slope in poorly controlled asthma. Allergy. 2006;61:85–9.
14. Chung MP, Rhee CH. Airway obstruction in interstitial lung disease. Curr Opin Pulm Med. 1997;3:332–5.
15. 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. Arthritis Rheum. 2013;65:2737–47.
16. Mahler DA, Weinberg DM, Wells CK, Feinstein AR. The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. Chest. 1984;85:751–8.
17. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardization of spirometry. Eur Respir J. 2005;26:319–38.
18. Pereira CAC, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. J Bras Pneumol. 2007;33:397–406.
19. Neder JA, Andreoni S, Peres C, Nery LE. Reference values for lung function tests. I. Static volumes. Braz J Med Biol Res. 1999;32:703–17.
20. Neder JA, Andreoni S, Peres C, Nery LE. Reference values for lung function tests. III. Carbon monoxide diffusing capacity (transfer factor). Braz J Med Biol Res. 1999;32:729–37.
21. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Braz J Med Biol Res. 1999;32:719–27.
22. Buist AS, Ross BB. Predicted values for closing volumes using a modified single breath nitrogen test. Am Rev Respir Dis. 1973;107:744–52.

23. Buist AS, Ghezzo H, Anthonisen NR, Cherniack RM, Ducic S, Macklem PT, et al. Relationship between the single-breath N test and age, sex, and smoking habit in three North American cities. Am Rev Respir Dis. 1979;120:305–18.

24. Hoffmann-Vold AM, Aaløkken TM, Lund MB, Garen T, Midtvedt Ø, Brunborg C, et al. Predictive value of serial high-resolution computed tomography analyses and concurrent lung function tests in systemic sclerosis. Arthritis Rheumatol. 2015;67:2205–12.

25. Khanna D, Gladue H, Channick R, Chung L, Distler O, Furst DE, et al. Recommendations for screening and detection of connective tissue disease-associated pulmonary arterial hypertension. Arthritis Rheum. 2013;65:3194–201.

26. Suliman YA, Dobrota R, Huscher D, Nguyen-Kim TD, Maurer B, Jordan S, et al. Pulmonary function tests: high rate of false-negative results in the early detection and screening of scleroderma-related interstitial lung disease. Arthritis Rheumatol. 2015;67:3256–61.

27. Aronsson D, Hesselstrand R, Bozovic G, Wuttge DM, Tufvesson E. Airway resistance and reactance are affected in systemic sclerosis. Eur Clin Respir J. 2015;2:28667.

28. Miranda IA, Dias Faria AC, Lopes AJ, Jansen JM, Lopes de Melo P. On the respiratory mechanics measured by forced oscillation technique in patients with systemic sclerosis. PLoS One. 2013;8:e61657.

29. Mottram CD. Ruppel's manual of pulmonary function testing, 10th edn. Elsevier/Mosby: Maryland Heights, 2013.

30. Boeck L, Gensmer A, Nyilas S, Stieltjes B, Re TJ, Tamm M, et al. Single-breath washout tests to assess small airway disease in COPD. Chest. 2016;150:1091–100.

31. Verbanck S. Physiological measurement of the small airways. Respiration. 2012;84:177–88.

32. Latzin P, Thompson B. Double tracer gas single-breath washout: promising for clinics or just a toy for research? Eur Respir J. 2014;44:1113–5.

33. Husemann K, Berg N, Engel J, Port J, Joppek C, Tao Z, et al. Double tracer gas single-breath washout: reproducibility in healthy subjects and COPD. Eur Respir J. 2014;44:1210–22.

10.2 **Artigo 2**

Computed tomography trachea volumetry in patients with scleroderma: Association with clinical and functional findings

Short title: Computed tomography trachea volumetry in scleroderma

Authors:

Bruno Rangel Antunes Silva[1¶], Rosana Souza Rodrigues[2¶], Rogério Rufino[1¶], Cláudia Henrique Costa[1¶], Veronica Silva Vilela[1¶], Roger Abramino Levy[1¶], Alan Ranieri Medeiros Guimarães[3¶], Alysson Roncally Silva Carvalho[34¶], Agnaldo José Lopes[1¶*]

[1] Postgraduate Programme in Medical Sciences, School of Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil

[2] Department of Radiology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

[3] Laboratory of Respiration Physiology, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

[4] Laboratory of Pulmonary Engineering, Biomedical Engineering Programme, Alberto Luiz Coimbra Institute of Post-Graduation and Research in Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

[*] Corresponding author:

E-mail: alopes@souunisuam.com.br (AJL)

¶ These authors contributed equally to this work.

Abstract

Background

In scleroderma, excessive collagen production can alter tracheal geometry, and computed tomography (CT) volumetry of this structure may aid in detecting possible abnormalities. The objectives of this study were to quantify the morphological abnormalities in the tracheas of patients with scleroderma and to correlate these findings with data on clinical and pulmonary function.

Methods

This was a cross-sectional study in which 28 adults with scleroderma and 27 controls matched by age, gender and body mass index underwent chest CT with posterior segmentation and skeletonization of the images. In addition, all participants underwent pulmonary function tests and clinical evaluation, including the modified Rodnan skin score (mRSS).

Results

Most patients (71.4%) had interstitial lung disease on CT. Compared to controls, patients with scleroderma showed higher values in the parameters measured by CT trachea volumetry, including area, eccentricity, major diameter, minor diameter, and tortuosity. The tracheal area and equivalent diameter were negatively correlated with the ratio between forced expiratory flow and forced inspiratory flow at 50% of forced vital capacity ($FEF_{50\%}/FIF_{50\%}$) ($r = -0.44$, $p = 0.03$ and $r = -0.46$, $p = 0.02$, respectively). The tracheal tortuosity was negatively correlated with peak expiratory flow ($r = -0.51$, $p = 0.008$). The mRSS showed a positive correlation with eccentricity ($r = 0.62$, $p < 0.001$)

and tracheal tortuosity (r = 0.51, p = 0.007), while the presence of anti-topoisomerase I antibody (ATA) showed a positive correlation with tracheal tortuosity (r = 0.45, p = 0.03).

Conclusions

In a sample composed predominantly of scleroderma patients with associated interstitial lung disease, there were abnormalities in tracheal geometry, including greater eccentricity, diameter and tortuosity. In these patients, abnormalities in the geometry of the trachea were associated with functional markers of obstruction. In addition, tracheal tortuosity was correlated with cutaneous involvement and the presence of ATA.

Keywords: scleroderma, diagnostic imaging, computed tomography, respiratory function tests

Introduction

Scleroderma or systemic sclerosis (SSc) is a chronic progressive autoimmune disease of connective tissue characterized by microvascular involvement, activation of the immune system, and increased deposition of extracellular matrix in the skin and internal organs by excess collagen fibers, leading to fibrosis [1–4]. Scleroderma is eight times more common in females than in males; its reported prevalence is approximately 10 cases per 100,000 person, and this rate is probably underestimated [5]. The disease can affect several organs and systems; the skin is the most frequently affected site, followed by the lungs, kidneys, musculoskeletal system, cardiovascular system, and gastrointestinal tract. The presence of multiple affected sites worsens the prognosis [1].

In scleroderma, thoracic involvement is observed mainly as diffuse fibrosis or pulmonary hypertension; these conditions are associated with limited (lc-SSc) and

diffuse cutaneous (dc-SSc) forms of scleroderma, respectively [1]. In an autopsy study, parenchymal involvement was seen in up to 100% of patients with scleroderma [2]. Although interstitial lung involvement is subclinical and asymptomatic at early stages in most patients, interstitial lung disease (ILD) associated with scleroderma (ILD-SSc) is observed in approximately 40% of cases and is a major cause of morbidity and mortality [6]. Despite the constellation of thoracic manifestations that occur in patients with scleroderma, little is known about the involvement of the trachea in these patients. Ooi et al. [7] reported that scleroderma affected small and large airways in 45-100% of patients. In this context, the trachea has been a 'forgotten zone' in the study of several diseases because the pathological processes involving this structure have often not received the necessary clinical recognition.

Computed tomography (CT) has become an important part of the detection and evaluation of routine thoracic involvement in scleroderma, and the abnormalities observed by this method are closely correlated with the observed physiological parameters [2]. In the last two decades, various computer tools that can be used to automatically slice the chest using CT images have been developed. These include multiplanar reformation, regional lung attenuation analysis of lung tissue and quantification of anatomical images, including the area and volume of airways and lungs [2,7]. In scleroderma, computer-assisted tomography analysis is highly efficient and, in combination with physiological and patient-centered measurements, may provide a means to accurately assess and monitor lung disease progression and response to therapy [2,8,9].

An understanding of the acquisition, processing and analysis of CT scans and how these processes affect the imaging of the trachea is essential for assessing the accuracy of the measurements and making effective use of newly available tools. In the

study of the trachea, the cross-sectional area and diameter are the most commonly measured dimensions; in the context of assessing possible obstruction, length and caliber are important [11–13]. More recently, the process of skeletonization and volumetry of the airways through CT in normal individuals and in individuals with some clinical conditions has been described [10–15]. In this method, the digital imaging component is transformed into a subset of the original component [10]. After automated segmentation of CT images, the skeletonization algorithm allows the extraction of a tracheal centerline and facilitates the reconstruction of CT data orthogonal to the reduction of the effects of partial volume averages [11]. However, no study has explored the use of this resource in the evaluation of the tracheas of patients with scleroderma.

We hypothesized that the excessive production of collagen that occurs in scleroderma alters the geometry of the trachea and that the skeletonization of this structure would aid in the detection of possible abnormalities. Thus, the present study aimed to identify and quantify the morphological abnormalities in the tracheas of patients with scleroderma and, secondarily, to correlate these findings with data on clinical and pulmonary function.

Methods

Patients

This cross-sectional study was conducted between February 2016 and September 2017 in 43 consecutive patients with scleroderma who were ≥18 years of age, of both sexes, and were seen regularly at the Piquet Carneiro Polyclinic of the State University of Rio de Janeiro, Rio de Janeiro, Brazil. The patients included in the study had been diagnosed with scleroderma by a rheumatologist according to the parameters set forth

by the American College of Rheumatology/European League Against Rheumatism [1]. The following exclusion criteria were used: clinical instability; history of respiratory infection in the last three weeks; history of previous or current smoking; evidence of overlapping of scleroderma with other connective tissue diseases; report of previous tracheal or pleuropulmonary disease not related to scleroderma; and inability to perform pulmonary function tests (PFTs). Regarding cutaneous involvement, patients were classified as having lc-SSc (thickening of the skin distal to the elbows and knees and proximal to the clavicles, including the face) or dc-SSc (thickening of the proximal skin as well as of the skin distal to the elbows and knees and including the trunk and face) [16]. The modified Rodnan skin score (mRSS) was used to assess skin damage in patients with scleroderma. In this system, a score of 0 (no thickening), 1 (light thickening), 2 (moderate thickening) or 3 (severe thickening) is assigned to each area, resulting in a total score ranging from 0 (best) to 51 (worst) [17]. The presence of autoantibodies, including anti-topoisomerase I and anti-centromere, was also investigated.

We also evaluated a control group of 27 individuals aged ≥ 18 years of both sexes. The subjects in the control group were asked to perform the PFTs after undergoing chest CT scanning in our service for the following reasons: investigation of contact with tuberculosis patients (n = 9); staging of neoplasms outside the thorax (n = 7); evaluation of trauma (n = 6); and evaluation of fever of unknown origin (n = 5). The following criteria were used to select subjects for the control group: no previous history of smoking or chronic tracheal or pleuropulmonary diseases; chest CT scans without abnormalities; and lung function parameters within the normal range (*i.e.*, no value below the lower limit of normal or above the upper limit of normal in relation to the

predicted value). All CT scans and PFTs for the control group were performed using the same equipment that was used for the scleroderma group.

The protocol was approved by the Research Ethics Committee of the State University of Rio de Janeiro under the number CAAE- 50752615.9.0000.5259, and it complied with the current national and international standards. All individuals signed an informed consent form.

Pulmonary function testing

The PFTs performed were spirometry, body plethysmography, and diffusion capacity for carbon monoxide (DLco). The exams were performed on an HDpft 3000 (nSpire Health, Inc., Longmont, CO, USA) according to the standards set forth by the American Thoracic Society [18]. The Brazilian reference values [19,20] were used, and the results are expressed as percentages of the predicted values. An increase in the ratio between forced expiratory flow and forced inspiratory flow at 50% of forced vital capacity-FVC ($FEF_{50\%}/FIF_{50\%}$) > 1.50 was used as an indicator of extrathoracic airway obstruction [21].

CT scan interpretation and protocol

CT scans were performed on a 64-channel multislice Brilliance 40 scanner (Philips Medical Systems, Cleveland, OH, USA) that was capable of performing volumetric acquisitions with subsequent multiplanar reconstructions. The acquisitions were performed in the axial plane with patients in the supine position using the technical parameters 120 kV and 458 mA (these parameters varied according to the biotype of the patient), slice thickness 2 mm, and pitch 2 mm from the jugular notch to the xiphoid process in maximal inspiration and expiration. After acquisition of the images, a high-

resolution reconstruction with a matrix of 512 × 512 was performed using a high-frequency algorithm, a window width of 1200 HU, and a level centered at -800 HU.

Parenchymal abnormalities on CT were interpreted by two independent readers (R.S.R. and G.B.C.) who were blinded to patient history and physiological results; a consensus opinion was reached in cases in which there was disagreement. CT scans were reviewed at five levels, as follows: 1) origin of major vessels; 2) carina; 3) confluence of pulmonary veins; 4) halfway between the third and the fifth sections; 5) 1 cm above the right hemidiaphragm [22,23]. The total extent of ILD was estimated to the nearest five percent in each of the five levels, with global extent of disease on CT as the mean of the scores [22–24]. The coarseness of pulmonary fibrosis was evaluated as follows: 0, ground-glass opacification alone; 1, fine intralobular fibrosis; 2, microcystic honeycombing comprising air spaces ≤ 4 mm in diameter; and 3, macrocystic honeycombing comprising air spaces > 4 mm in diameter. The total coarseness score for each patient was derived by summing the scores at the five levels (range 0 to 15) [22,23]. For each patient, the total extension of ILD and the coarseness of pulmonary fibrosis were derived by averaging the scores at each level assessed by the two independent readers. Finally, the extent of ILD-SSc was classified as limited (lung parenchyma involvement <20%) or extensive (>20%). For indeterminate cases, ILD-SSc was considered extensive if FVC <70% and limited if FVC >70% [22,25].

Imaging processing

The airways were segmented using 3DSlicer version 4.4.0 (http://slicer.org) [26] with the aid of its AirwaySegmentation extension. At the end of processing, a nearly raw raster data (.nrrd) file was saved. Using AirwayProcessing, two distinct processes were executed: 1) the .nrrd file was read and transformed into a binary matrix of data; in this

way, the process of skeletonization was initiated, and a skeleton composed of several points was then produced; 2) using the skeleton and its coordinates (X, Y and Z axes), cross-sectional planes were generated. Individual processing of all points was performed using the slice command, and the processing angles were defined by a normal line between the point in question and the next five points. The plane of square cross-section was formed by a square grid with 70 pixels on the side. Using MATLAB 2014a (MathWorks Inc., Natick, MA, USA), information obtained individually by the slice command was catalogued using the ‘regionprops’ command.

Figure 1 shows the 3D reconstruction, the tracheal skeleton, the cross-sectional planes and their geometric parameters [9,10]. The scheme show in Fig. 1 was chosen for illustrative purposes and accurately represents the region of the trachea in a 2D environment. In Fig. 1, the axial sections indicated by specific letters represent area (*A), perimeter (*B), eccentricity (*C), equivalent diameter (*D), major diameter (*E) and minor diameter (*F). It is emphasized that the segments shown in red and blue in Fig 1 were enlarged for visualization purposes.

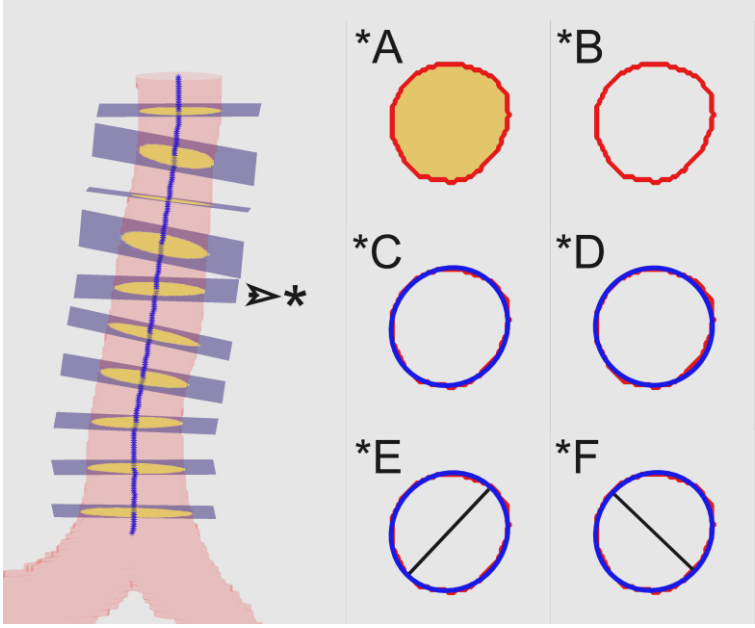


Fig. 1. 3D reconstruction, cross-sectional planes, skeleton and indication of the plane used for representation. The letters A to F and the values for the selected plane indicate, respectively: (*A) area (182.4 mm^2), which is the product of the pixel size and the number of pixels in the region (in this figure, the area is shown in yellow and includes the red line that demarcates the perimeter); (*B) perimeter (47.3 mm), which is the length of the line indicated in red; (*C) eccentricity (0.35), which is indicated by the equivalent ellipse drawn in blue; (*D) equivalent diameter (15.2 mm), which is the circle of area equivalent to the cross-sectional area; (*E) major diameter (15.8 mm), which is the longest segment of the equivalent ellipse (indicated by the inner line); and (*F) minor diameter (14.8 mm), which is the smallest segment (indicated by the inner line).

Thus, the following measures were calculated for each study participant:

1) Area: the measured area of the tracheal lumen intersected by the cross-sectional plane. The area was calculated by multiplying the pixel area value by the number of pixels present, as shown in Eq. 1,

(1)

$$Area = ps * n$$

where *ps* is the individual area of each pixel and *n* is the number of pixels.

2) Perimeter: the length of the outer margin of the tracheal lumen.

3) Eccentricity: a parameter associated with the ellipsoidal shape of a given region. It is the ratio of the focus of the ellipse to its largest diameter. The result is a value between 0 and 1, where 0 is the representation of a circle and 1 is the representation of a line. Fig. 1*C illustrates eccentricity as the equivalent ellipse (shown in blue); the outer edge of the tracheal lumen is shown in red. The eccentricity was calculated according to Eq. 2,

(2)

$$Eccentricity = 2 * \frac{\sqrt{\frac{(MaAL)^2}{2} - \frac{(MiAL)^2}{2}}}{MaAL}$$

where *MaAL* represents the largest diameter and *MiAL* the smallest diameter of the ellipse.

4) Equivalent diameter: a circle of the same area as the region intersected by the cross-sectional plane. In Fig. 1*D, the equivalent diameter is shown as a blue circle with an area equivalent to that of the tracheal lumen in the same region. The equivalent diameter was calculated using Eq. 3,

(3)

$$Equivalent\ diameter = \frac{\sqrt{4 * area}}{\pi}$$

5) Major diameter: the length of the longest segment of the ellipse equivalent to the region. Similarly, the minor diameter represents the length of the smallest segment of the ellipse. Fig. 1*E and Fig. 1*F denote the perimeter of the tracheal lumen (red) along with its ellipse (blue); the center lines indicate the largest and the smallest segment, respectively, of each region.

We also calculated the tortuosity (sinuosity index), which is the deviation of the central axis of the trachea considering its proximal and distal extremities. Mathematically, the tortuosity was determined from the sum of the Euclidean distance of each segment of the trachea, with a distance equivalent to the thickness of each cross-section of the original CT image, divided by the Euclidean distance of the end points of the trachea. The tortuosity may be expressed succinctly using the formula described in Eq. 4,

(4)

$$Tortuosity = \frac{L}{vd}$$

where *L* is the length of the trachea and *vd* is the vectorial distance between the points at the extremities (Fig. 2). The tortuosity is expressed as a value ≥ 1; the higher the index, the greater the tortuosity of the trachea [27].

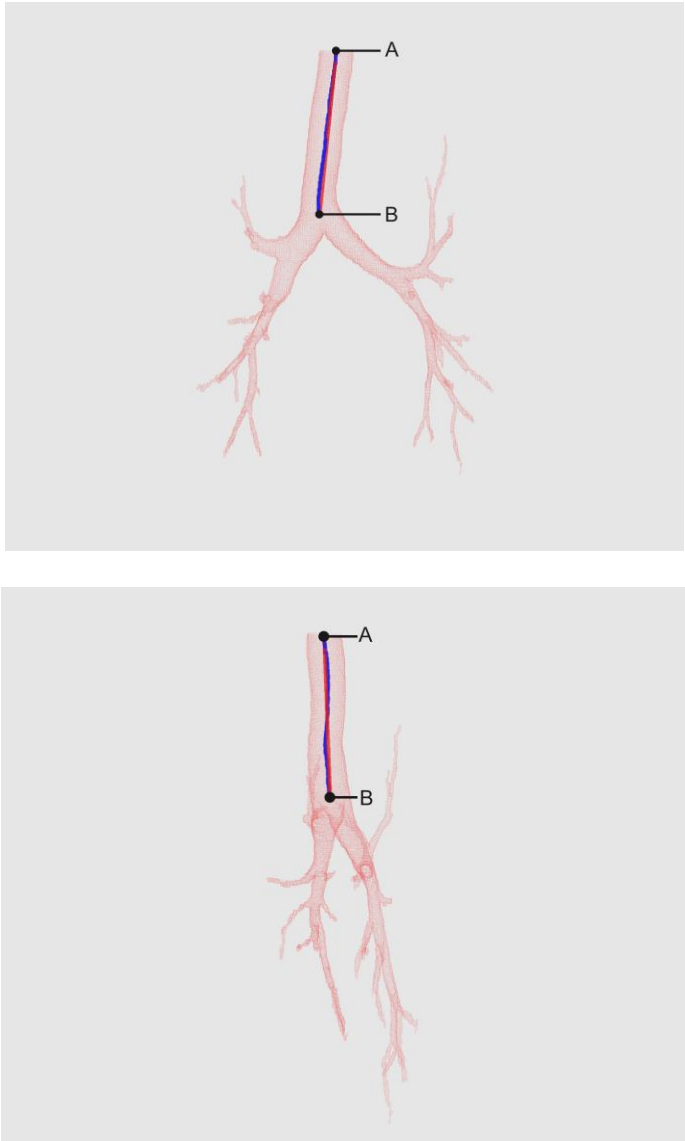


Fig. 2. Measurement of the tortuosity of the trachea. Images in the coronal (A) and sagittal (B) planes are shown. In this scheme, *L* is the length of the trachea (the total length of 'ab', shown in blue) and *vd* is the vectorial distance between the points at the extremities (the length of the shortest possible path between 'a' and 'b', shown in red).

Fig. 3 exemplifies the skeletonization process of the trachea of a study participant. The figure shows the three-dimensional reconstruction images of the trachea in two different planes.

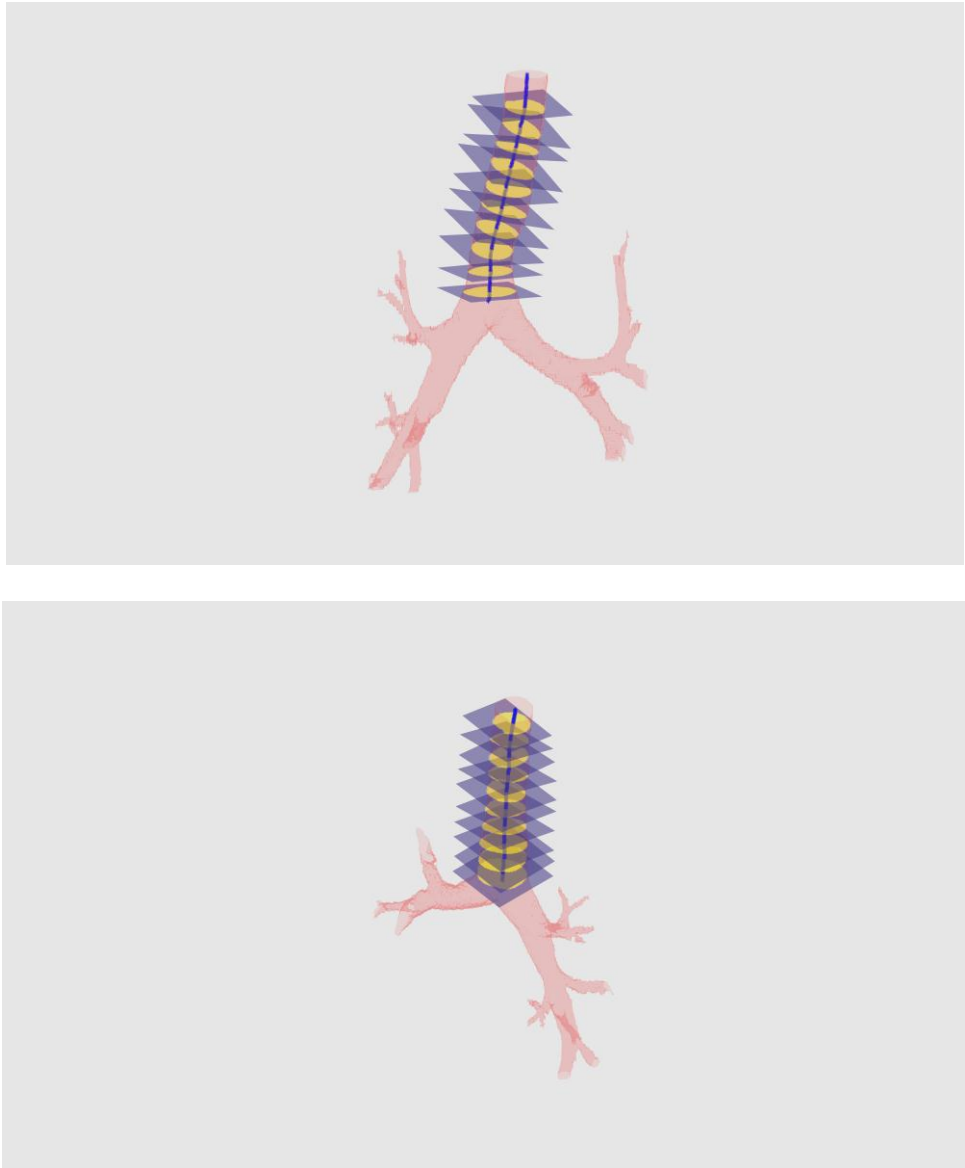


Fig. 3. Skeletonization process of the trachea. Three-dimensional reconstruction of the trachea of one study participant in two planes (A and B). The median values showed in the ten planes of square cross-sections are as follows: area = 184.5 mm^2; perimeter = 47.6 mm; eccentricity = 0.51; equivalent diameter = 15.3 mm; major diameter = 15.9 mm; minor diameter = 14.4 mm; and tortuosity = 1.027.

There is inherent variability in the measures used to determine the quantitative metrics of eccentricity and tortuosity. For eccentricity, there is a value for each section plane of the trachea of a given individual (Fig. 3). We use the median to represent the

value of each individual; therefore, there are *n* medians, where *n* is the number of elements in each group of variables. For tortuosity, only one value was calculated for each trachea; it depends on the extreme values (proximal and distal), as shown in Fig. 2.

Statistical analysis

To better evaluate the effect of the extent of ILD-SSc on the clinical variables, PFTs, and CT trachea volumetric parameters, we divided the participants into three subgroups as follows: 1) a control group; 2) a scleroderma group with no pulmonary involvement/limited ILD; and 3) a scleroderma group with extensive ILD. ANOVA followed by the Bonferroni post hoc test was used to compare the results obtained for the patients in these three groups. Comparison of the clinical variables, PFTs, and CT trachea volumetric parameters of the SSc patients without pulmonary involvement with those of control subjects and with those of SSc patients with no pulmonary involvement/limited ILD-SSc and SSc patients with extensive ILD-SSc was performed using Student's t-test for independent samples in the case of numerical data and using the chi-square or Fisher's exact test in the case of categorical data. To evaluate the associations between the numerical variables of PFTs and CT trachea volumetric parameters, the Pearson correlation coefficient (r) was used. The criterion for determining significance was 5%. Statistical analysis was performed using SAS 6.11 software (SAS Institute, Inc., Cary, NC, USA).

Results

Of the 43 patients included in the study, 15 were excluded for the following reasons: reported being a smoker (n = 8); history of previous pleuropulmonary disease not associated with scleroderma (n = 3); inability to perform PFTs (n = 2); scleroderma-

polymyositis overlap syndrome (n = 1); and scleroderma-rheumatoid arthritis overlap syndrome (n = 1). In the total sample of patients, the mean age of subjects with scleroderma was 52.5 ± 11 years, and 25 (89.3%) were women. The disease duration was 4.21 ± 2.50 years from the onset of non-Raynaud's phenomenon and 9.65 ± 5.13 years from the onset of Raynaud's phenomenon symptoms. Nineteen patients (67.9%) had lc-SSc, and nine (32.1%) had dc-SSc; of the latter, six (21.4%) had a mRSS > 18. Anti-topoisomerase I antibody (ATA), anticentromere antibody and anti-RNA polymerase III were positive in 13 (46.4%), six (21.4%) and three (10.7) patients, respectively; no autoantibodies were identified in six (21.4%) patients. Seven patients (25%) had an $FEF_{50\%}/FIF_{50\%}$ ratio > 1.50. Comparisons of clinical data, pulmonary function parameters and computed tomography scores in the control group, the scleroderma group with no pulmonary involvement/limited ILD, and the scleroderma group with extensive ILD are shown in Table 1. In addition, comparisons of lung function parameters in the control group and in patients without pulmonary involvement on CT did not show any significant difference ($p > 0.05$ for all).

Table 1. Demographic characteristics, clinical data, pulmonary function and computed tomography scores of patients with scleroderma and of patients in the control group.

Variable	Control group (n = 27)	Scleroderma group with no pulmonary involvement/limited ILD (n = 16)	Scleroderma group with extensive ILD (n = 12)	p value
Demographic data				
Females	23 (85.2)	14 (87.5)	11 (91.7)	0.41
Age (years)	49 ± 12.6	51.7 ± 10	52.8 ± 11.2	0.28
BMI (kg/m^2)	27.2 ± 5.87	24.2 ± 5.22	25.1 ± 5.30	0.09
Type of scleroderma				
lc-SSc	-	12 (75)	7 (58.3)	0.34
dl-SSc	-	4 (25)	5 (41.7)	
mRSS	-	10.3 ± 7.44	11.8 ± 7.60	0.12
Type of autoantibody				

Anti-topoisomerase I antibody	-	5 (31.2)	8 (66.7)	0.28
Anticentromere antibody	-	5 (31.2)	1 (8.33)	
Anti-RNA polymerase III	-	2 (12.5)	1 (8.33)	
Autoantibody not identified	-	4 (25)	2 (16.7)	
Lung function				
FVC (L)	3.08 ± 0.81	2.62 ± 0.72*	2.25 ± 0.87*†	**0.008**
FVC (% predicted)	102.4 ± 19.8	85 ± 19.3*	68 ± 21.5*†	**0.005**
FEV_1 (L)	2.51 ± 0.75	2.11 ± 0.63*	1.80 ± 0.59*†	**0.009**
FEV_1 (% predicted)	100.7 ± 18.5	83.1 ± 16*	69.2 ± 17.8*†	**0.006**
FEV_1/FVC (%)	78 ± 12.6	80 ± 12	86 ± 11.3	0.11
PEF (L/s)	7.75 ± 2.14	6.25 ± 2.11	5.26 ± 1.97*	**0.032**
PEF (% predicted)	110 ± 31.7	86 ± 30.5	75.5 ± 29.2*	**0.025**
$FEF_{50\%}/FIF_{50\%}$ (%)	1.04 ± 0.62	1.28 ± 0.72*	1.34 ± 0.70*	**0.046**
DLco (mL/min/mmHg)	20.6 ± 3.44	18 ± 4.22*	9.27 ± 3.23*†	**0.003**
DLco (% predicted)	97 ± 21.6	70.3 ± 18.3*	60 ± 17.8*†	**0.001**

Raw (cm H_2O/L/s)	1.58 ± 0.75	1.78 ± 0.77	1.93 ± 0.78	0.32
SGaw (L/s/cm H_2O/L)	0.210 ± 0.078	0.245 ± 0.089	0.257 ± 0.103	0.09
Computed tomography scores				
ILD-SSc extent (% parenchyma)		9.63 ± 8.80	28.1 ± 10.4	**< 0.0001**
Coarseness of pulmonary fibrosis		2.15 ± 1.44	6.35 ± 4.20	**< 0.0001**

The values shown are means ± SD or number (%). Bold type indicates significant differences. *Significantly different from control group. †Significantly different from scleroderma group with no pulmonary involvement/limited ILD-SSc. ILD-SSc = interstitial lung disease associated with scleroderma. BMI = body mass index; lc-SSc = limited cutaneous form; dc-SSc = diffuse cutaneous form; mRSS = modified Rodnan skin score; FVC = forced vital capacity; FEV_1 = forced expiratory volume in one second; PEF = peak expiratory flow; $FEF_{50\%}/FIF_{50\%}$ = ratio between the forced expiratory flow and forced inspiratory flow at 50% of forced vital capacity; DLco = diffusing capacity for carbon monoxide; Raw: airway resistance; SGaw: specific airway conductance.

On thorax CT, 16 patients (57.1%) were classified as having no pulmonary involvement (n = 8) or limited pulmonary involvement (n = 8), while 12 (42.9%) were classified as having extensive pulmonary involvement. In the measurements obtained through CT trachea volumetry, patients with scleroderma presented higher values for the following parameters: area, eccentricity, major diameter, minor diameter, and tortuosity. Table 2 compares the CT trachea volumetry findings for control subjects, patients in the scleroderma group with no pulmonary involvement/limited ILD, and patients in the scleroderma group with extensive ILD.

We also compared patients without pulmonary involvement on CT (n = 8) with controls. In this evaluation, patients without pulmonary involvement on CT showed higher values, with significant differences in the following CT tracheal volumetric findings: area (210.7 ± 35 vs. 207.6 ± 32.6 mm^2, p = 0.031; eccentricity (0.50 ± 0.05 vs. 0.46 ± 0.04, p = 0.018); major diameter (17.5 ± 1.70 vs. 17.1 ± 2.22 mm, p = 0.039); minor diameter (14.9 ± 1.68 vs. 14.5 ± 2.11 mm, p = 0.044); and tortuosity (1.039 ± 0.017 vs. 1.023 ± 0.014, p = 0.01).

Table 2. Variables of CT trachea volumetry according to group.

Variable	Control group (n = 27)	Scleroderma group with no pulmonary involvement/limited ILD (n = 16)	Scleroderma group with extensive ILD (n = 12)	p value
Area (mm^2)	207.6 ± 32.6	211.3 ± 38.2*	214.6 ± 43.6*	**0.028**
Perimeter (mm)	45.3 ± 4.12	49.2 ± 4.45	51.5 ± 4.67	0.12
Eccentricity	0.46 ± 0.04	0.51 ± 0.06*	0.54 ± 0.06*	**0.013**
Equivalent diameter (mm)	16.1 ± 1.24	16.2 ± 1.55	16.5 ± 1.60	0.82
Major diameter (mm)	17.1 ± 2.22	17.6 ± 1.92*	18.2 ± 2.01*	**0.039**
Minor diameter (mm)	14.5 ± 2.11	15 ± 1.72*	15.3 ± 1.85*	**0.043**
Tortuosity	1.023 ± 0.014	1.039 ± 0.014*	1.058 ± 0.015*†	**0.009**

The values shown are means ± SD or number (%). Bold type indicates significant differences. *Significantly different from control group. †Significantly different from scleroderma group with no pulmonary involvement/limited ILD-SSc. ILD-SSc = interstitial lung disease associated with scleroderma.

We evaluated the correlations between the parameters provided by CT trachea volumetry and the pulmonary function indices (absolute values) (Table 3). The tracheal area was negatively correlated with $FEF_{50\%}/FIF_{50\%}$ (r = -0.44, p = 0.03), while the eccentricity was negatively correlated with FVC (r = -0.57, p = 0.002) (Fig. 4) and forced expiratory volume in one second (r = -0.50, p = 0.009). The equivalent diameter was negatively correlated with $FEF_{50\%}/FIF_{50\%}$ (r = -0.46, p = 0.02), while tortuosity was negatively correlated with peak expiratory flow (PEF) (r = -0.51, p = 0.008) (Fig. 5). A positive correlation of tortuosity with coarseness of pulmonary fibrosis in CT was also found (r = 0.45, p = 0.02).

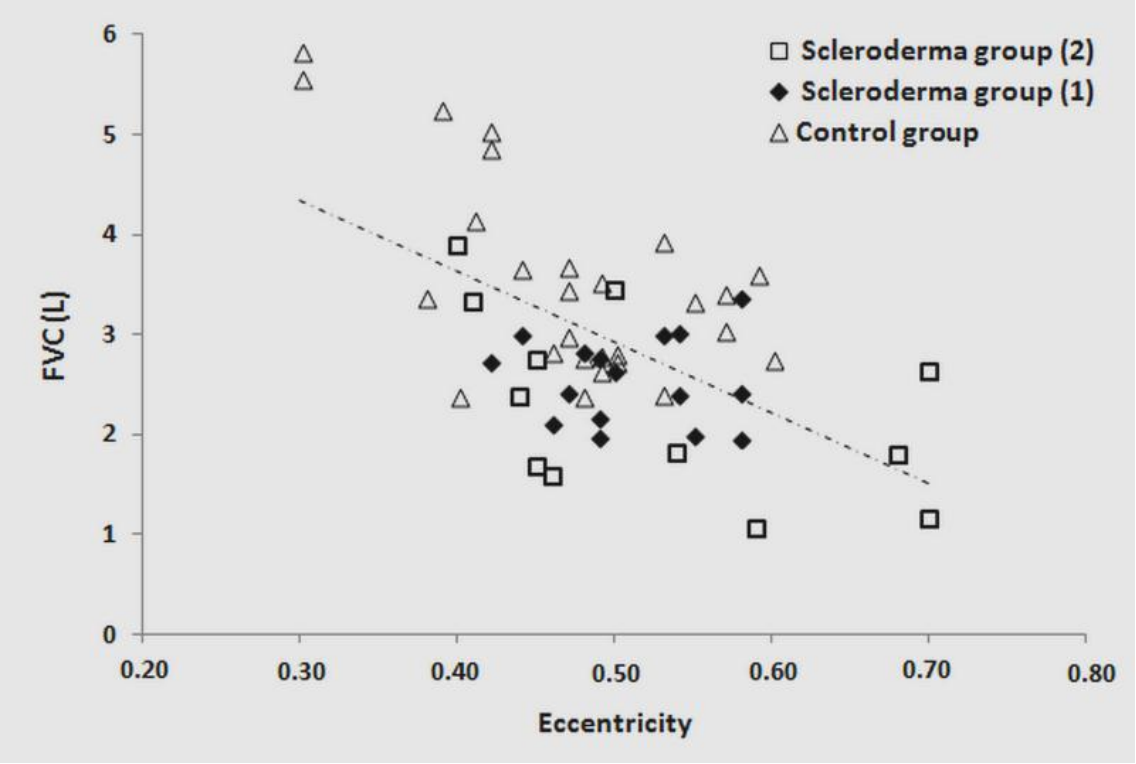


Fig. 4. Relationship between forced vital capacity (FVC) and eccentricity of the trachea (r = -0.57, p = 0.002). Scleroderma group (1) = scleroderma group with no pulmonary involvement/limited interstitial lung disease; scleroderma group (2) = scleroderma group with extensive interstitial lung disease.

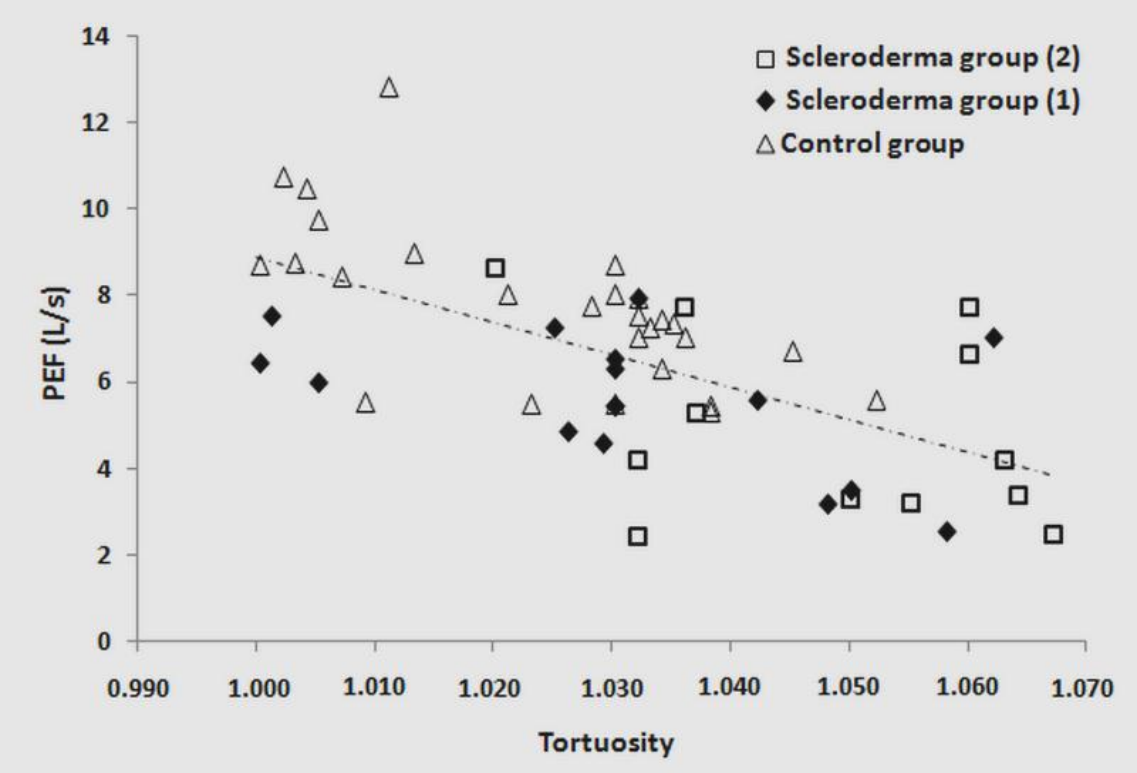


Fig. 5. Relationship between peak expiratory flow (PEF) and tortuosity of the trachea (r = -0.51, p = 0.008). Scleroderma group (1) = scleroderma group with no pulmonary involvement/limited interstitial lung disease; scleroderma group (2) = scleroderma group with extensive interstitial lung disease.

Table 3. Pearson's correlation coefficients for CT trachea volumetry, pulmonary fibrosis and pulmonary function.

	FVC	**FEV_1**	**FEV_1/FVC**	**PEF**	**$FEF_{50\%}/FIF_{50\%}$**	**DLco**	**Raw**	**SGaw**	**Coarseness of fibrosis**
Area	-0.13	-0.11	-0.06	0.24	**-0.44***	-0.24	0.12	-0.19	0.25
Perimeter	-0.09	-0.07	-0.08	0.25	**-0.47**†	-0.18	0.13	-0.21	0.17
Eccentricity	**-0.57**‡	**-0.50**†	-0.12	-0.07	-0.17	-0.35	-0.11	-0.04	0.26
Equivalent diameter	-0.11	-0.10	-0.07	0.25	**-0.46**†	-0.27	0.11	-0.21	0.18
Major diameter	-0.04	-0.06	-0.08	0.25	**-0.42***	-0.16	0.07	-0.20	0.22
Minor diameter	-0.22	-0.19	-0.03	0.21	**-0.47**†	-0.11	0.06	-0.17	0.25
Tortuosity	-0.17	-0.15	-0.26	**-0.51**†	-0.28	-0.12	0.09	-0.15	**0.45***

FVC = forced vital capacity; FEV_1 = forced expiratory volume in one second; PEF = peak expiratory flow; $FEF_{50\%}/FIF_{50\%}$ = ratio between the forced expiratory flow and forced inspiratory flow at 50% of forced vital capacity; DLco = diffusing capacity for carbon monoxide; Raw = airway resistance; SGaw = specific airway conductance. Bold type indicates significant differences.

*$p < 0.05$

†$p < 0.01$

‡$p < 0.005$

We also evaluated the correlations between the clinical findings and the parameters provided by CT trachea volumetry. The mRSS showed a positive correlation with eccentricity ($r = 0.62$, $p < 0.001$) and tortuosity ($r = 0.51$, $p = 0.007$). In turn, the presence of anti-topoisomerase I antibody showed a positive correlation with tortuosity ($r = 0.45$, $p = 0.03$).

Discussion

The main findings of the present study were that in a sample composed predominantly of scleroderma patients with associated ILD, the trachea showed greater area, eccentricity, and tortuosity. In these patients, a greater tracheal tortuosity led to a smaller airflow; in addition, the lower the tracheal diameter, the greater the degree of airway obstruction. Furthermore, tracheal tortuosity was associated with the presence of pulmonary fibrosis, the presence of ATA, and greater mRSS. To our knowledge, this is the first study to show abnormalities in the structure and function of the trachea as well as its correlations with clinical findings in patients with scleroderma.

In recent years, several CT image enhancement techniques have been developed in an attempt to find a method that is both quantitative and reliable and allows more accurate assessment than conventional visual reading. Compared to the traditional visual interpretation of CT findings, computer-based automatic evaluation can improve the objectivity, sensitivity, and repeatability of quantitative chest imaging analyses [2,10]. In this study, we applied a computer-assisted method to evaluate the tracheas of patients with scleroderma in an unprecedented way considering that computerized evaluation has previously only been used in the study of ILD-SSc [2,28]. Our results indicate that CT trachea volumetry and subsequent skeletonization of the images provide a number of interesting measures for the best evaluation of patients with

scleroderma. We observed that patients with scleroderma presented greater area, eccentricity, and tortuosity of the trachea. In scleroderma, myofibroblasts constitutively secrete components of the extracellular matrix and exert excessive cicatrization of the skin and internal organs [3,29]. Thus, we think that the cicatricial changes that occur at the level of the neck can directly impact the geometry of the trachea and alter the measures of diameter and tortuosity of this structure.

In our study, the deviations of the trachea were measured by means of the tortuosity (sinuosity index) after the skeletonization process. The skeletonization process has been used previously by us to obtain the skeletons of specific structures by thinning [10]. Interestingly, we found an association between tracheal tortuosity and the pulmonary fibrosis score obtained by semi-quantitative CT reading. This association between structural alterations of the trachea and the lung, despite being evaluated by different techniques in our study, suggests that the deformity of the trachea in scleroderma may be an extension of the fibrotic disease that occurs at the pulmonary level. However, it is worth mentioning that in our study, patients without pulmonary involvement showed higher values in tracheal volumetry than control subjects. This finding suggests that factors other than pulmonary fibrosis are involved in the tracheal abnormalities that occur in SSc patients. Consistent with this observation, several studies have shown impairment of anatomic structures above the trachea in scleroderma patients; laryngeal dysfunction is relatively common, with pathologic findings demonstrating fibrinoid degeneration and an increase in collagen fibers [30,31]. Considering the promising advent of user-friendly software, evaluation of the trachea in patients with scleroderma may be an interesting approach both in clinical practice and in trials. Thus, precise characterization of the trachea in scleroderma may offer an

additional tool for the follow-up of these patients and may be helpful in the evaluation of clinical treatment.

The presence of obstructive abnormalities in the cervical trachea can be detected by the flow-volume loop of spirometry even when there is no clinical suspicion, with the most used index being the ratio $FEF_{50\%}/FIF_{50\%}$ [32]. In the present study, 25% of our patients had an $FEF_{50\%}/FIF_{50\%} > 1.50$, and we found negative correlations between $FEF_{50\%}/FIF_{50\%}$ and several indices measured by CT trachea volumetry, including area, perimeter, equivalent diameter, major diameter, and minor diameter. Consistent with our findings, Miranda et al. [33] used the forced oscillation technique in patients with scleroderma and found an increase in mean resistance, a parameter that reflects changes in the most central airways. Another study using impulse oscillometry observed an increase in the resistive and reactive properties of the respiratory system in patients with scleroderma, and these alterations were correlated with the findings of fibrosis in CT [34]. Interestingly, we observed an association between tracheal tortuosity and PEF (which reflects flow through large airways), supporting the notion that tracheal deviation is a key contributor to the reduction of airflow in the large airways of patients with scleroderma. However, quantitative measurement of eccentricity was not correlated with $FEF_{50\%}/FIF_{50\%}$. One possible explanation for this finding is that increased tracheal area in scleroderma patients may at least partially counterbalance the effects of reduced inspiratory flow in these patients.

In our study, patients with extensive ILD displayed greater tracheal area than patients without pulmonary involvement and patients with limited ILD; all of these patients, in turn, displayed greater tracheal area than the control subjects. Several recent studies have shown that larger esophageal diameter is associated with higher pulmonary fibrosis and worse lung function in individuals with scleroderma [35,36]. Since

microaspiration secondary to gastro-esophageal reflux is associated with the progression of pulmonary fibrosis in scleroderma [37,38], we think that the tracheal pathology may also contribute to this phenomenon. Longitudinal and controlled studies of larger numbers of patients are required to evaluate whether or not tracheal pathology is involved in the progression of lung fibrosis in patients with scleroderma.

In the present study, the mean value of FVC was higher in the control group and lower in the scleroderma group with extensive ILD and showed an intermediate value in the scleroderma group with no pulmonary involvement (or limited ILD) (102.4 ± 19.8% vs. 85 ± 19.3% vs. 68 ± 21.5% predicted, $p = 0.005$). Although the restrictive pattern in scleroderma is largely explained by the presence of ILD, a reduced compliance of the respiratory system may also be due to chest wall tightening from skin thickening, pleural disease, cardiac involvement, and respiratory muscle weakness [39–41]. Since no significant difference was observed in FVC between the control group and patients without pulmonary involvement in CT, we believe that ILD is the main contributor to the restrictive damage in our sample. However, we observed a significant correlation between FVC and the quantitative measurement of tracheal eccentricity. A possible explanation for this association is that overproduction of collagen and deposition of connective tissue, which are primary pathophysiological mechanisms of SSc, cause both abnormal tracheal geometry and reduced lung volume in these patients.

In scleroderma, cutaneous involvement occurs due to the increased thickness and hardness of the skin, which leads to shrinking of the skin in deeper structures; although cutaneous involvement is usually most prominent on the face and hands, these abnormalities may extend to the upper chest [42,43]. Some studies have shown that subclinical involvement of the upper chest is detectable by high-frequency ultrasound even with normal palpation [44,45]. In our study, the mRSS (a key measure in the

clinical evaluation of patients with scleroderma) was associated with the eccentricity values provided by CT trachea volumetry. Since eccentricity measures the deviation of a conical structure in relation to its circumference [46], we think that skin thickening that occurs at the neck level may negatively impact the geometry of the trachea in patients with scleroderma [47]. Consistent with our findings, Kim et al. [48] found an association between the fibrosis score detected by CT using a computer-assisted method and the mRSS of patients with scleroderma both at baseline and during a 12-month follow-up period. This reinforces the idea that the measure of eccentricity may be a marker of the severity of the process of collagen hyalinization and abnormalities in the elastic tissues that surround the neck and may extend to the intrathoracic structures of patients with scleroderma [42]. Considering that future directions for the management of patients with scleroderma, including epigenetic modulation and antifibrotic or biological therapy, are being discussed [49,50], we think that the parameters provided by the measurement of tracheal geometry can contribute to the evaluation of the outcomes.

The identification of reliable and consistent biomarkers that can be used to predict the course of specific diseases is necessary for better stratification and management of patients and would be of great use in the treatment of a multifaceted disease such as scleroderma [3]. In this context, several attempts have been made in recent years to correlate possible serum biomarkers with clinical features distinct from scleroderma. Several investigators have shown an association between ATA and the risk of development and progression of pulmonary fibrosis in patients with scleroderma [51,52]. A recent study has shown that topoisomerase I peptide-loaded dendritic cells induce not only the autoantibody response but also cutaneous and pulmonary fibrosis [52]. In the present study, we observed a positive correlation between the presence of

ATA and tracheal tortuosity. This significant correlation must be viewed cautiously since we did not evaluate predicting values. However, we think that this finding could serve as a starting point for longitudinal studies designed to evaluate a possible contribution of ATA to the management of tracheal pathology in patients with scleroderma.

A critical analysis of the limitations of the present study is pertinent. First, the sample size is small, and the sample is representative of only one center. Second, the sample is composed predominantly of scleroderma patients with associated ILD. Although we observed significant differences in the abnormalities of trachea volumetry between control subjects and patients without pulmonary involvement on CT, a greater number of patients in this group could allow for more robust conclusions. Third, CT pulmonary densitovolumetry could have aided in the study of correlations between tracheal changes and those observed in the lungs and lower airways. Fourth, an assessment comparing patients with ILD-SSc with patients with ILD due to other causes (e.g., idiopathic pulmonary fibrosis, rheumatoid arthritis-ILD) might better define the role of scleroderma in the development of tracheal abnormalities. Finally, the evaluation of other biomarkers could have helped us better understand the tracheal disease in scleroderma and might have opened new horizons in the field of precision medicine. In fact, new laboratory markers (including TGFβ1, IL-6, sPD-1, sPD-L2, and CXCL4) have been associated with higher mRSS score and more pronounced changes in thoracic CT [3]. Despite these limitations, the quantitative analysis of the trachea through computer software offers a discriminant method that can help produce an objective measure and obtain prognostic information in scleroderma.

In conclusion, the present study shows that, in a sample composed predominantly of scleroderma patients with associated ILD, there were abnormalities in

the geometry of the trachea in eccentricity, diameter, and tortuosity. In these patients, abnormalities in the geometry of the trachea were associated with markers of functional obstruction at the level of the cervical trachea. In addition, the measurement of tracheal tortuosity was correlated with cutaneous involvement, the degree of pulmonary fibrosis, and the presence of ATA. Although the encouraging data presented here require further validation in prospective studies, we believe that skeletonization and CT trachea volumetry may improve the ability of radiologists and rheumatologists to accurately assess the tracheas of patients with scleroderma both in clinical practice and in trials.

Author Contributions

Conceptualization: Bruno Rangel Antunes Silva, Rosana Souza Rodrigues, Rogério Rufino, Cláudia Henrique Costa, Veronica Silva Vilela, Roger Abramino Levy, Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Data curation: Bruno Rangel Antunes Silva, Rosana Souza Rodrigues.

Formal analysis: Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Funding acquisition: Agnaldo José Lopes.

Methodology: Bruno Rangel Antunes Silva, Rogério Rufino, Cláudia Henrique Costa, Veronica Silva Vilela, Roger Abramino Levy, Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Resources: Rogério Rufino, Cláudia Henrique Costa, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Software: Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho.

Supervision: Rosana Souza Rodrigues, Cláudia Henrique Costa, Roger Abramino Levy, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Validation: Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho.

Writing – original draft: Bruno Rangel Antunes Silva, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Writing – review & editing: Bruno Rangel Antunes Silva, Rosana Souza Rodrigues, Rogério Rufino, Cláudia Henrique Costa, Veronica Silva Vilela, Roger Abramino Levy, Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Funding

This research was supported by the Brazilian Council of Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq] and the Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ).

Competing interests

The authors have declared that no competing interests exist.

References

1. 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. Arthritis and Rheumatism. 2013; 65(11):2737–47. https://doi.org/10.1002/art.38098 PMID: 24122180; PubMed Central PMCID: PMC3930146.

2. Salaffi F, Carotti M, Di Donato E, Di Carlo M, Ceccarelli L, Giuseppetti G. Computer-aided tomographic analysis of interstitial lung disease (ILD) in patients with systemic sclerosis (SSc): correlation with pulmonary physiologic tests and patient-centred measures of perceived dyspnea and functional disability. PLoS One. 2016; 11(3):e0149240. https://doi.org/10.1371/journal.pone.0149240 PMID: 26930658; PubMed Central PMCID: PMC4773230.
3. Barsotti S, Bruni C, Orlandi M, Della Rossa A, Marasco E, Codullo V, et al. One year in review 2017: systemic sclerosis. Clinical and Experimental Rheumatology. 2017; 35(Suppl 106 4):3–20. PMID: 29035173.
4. Barnes J, Mayes MD. Epidemiology of systemic sclerosis: incidence, prevalence, survival, risk factors, malignancy, and environmental triggers. Current Opinion in Rheumatology. 2012; 24(2):165–70. https://doi.org/10.1097/BOR.0b013e32834ff2e8 PMID: 22269658.
5. Sticherling M. Systemic sclerosis-dermatological aspects. Part 1: Pathogenesis, epidemiology, clinical findings. Journal der Deutschen Dermatologischen Gesellschaft. 2012; 10(10):705–18. https://doi.org/10.1111/j.1610-0387.2012.07999.x PMID: 22913330.
6. Silva BRA, Rufino R, Costa CH, Vilela VS, Levy RA, Lopes AJ. Ventilation distribution and small airway function in patients with systemic sclerosis. Portuguese Journal of Pulmonology. 2017; 23(3):132–8. https://doi.org/10.1016/j.rppnen.2017.01.004 PMID: 28258938.
7. Ooi GC, Mok MY, Tsang KWT, Wong Y, Khong PL, Fung PCW, et al. Interstitial lung disease in systemic sclerosis: an HRCT-clinical correlative study. Acta Radiologica. 2003; 44(3):258–64.

8. Kim HG, Tashkin DP, Clements PJ, Li G, Brown MS, Elashoff R, et al. A computer-aided diagnosis system for quantitative scoring of extent of lung fibrosis in scleroderma patients. Clinical and Experimental Rheumatology. 2010; 28(5 Suppl 62):S26–35. PMID: 21050542; PubMed Central PMCID: PMC3177564.

9. Ariani A, Carotti M, Gutierrez M, Bichisecchi E, Grassi W, Giuseppetti GM, et al. Utility of an open-source DICOM viewer software (OsiriX) to assess pulmonary fibrosis in systemic sclerosis: preliminary results. Rheumatology International. 2014; 34(4):511–6. https://doi.org/10.1007/s00296-013-2845-6 PMID: 23949623.

10. Camilo GB, Carvalho ARS, Guimarães ARM, Kasuki L, Gadelha MR, Mogami R, et al. Computed tomography airway lumen volumetry in patients with acromegaly: Association with growth hormone levels and lung function. Journal of Medical Imaging and Radiation Oncology. 2017; 61(5):591–9. https://doi.org/10.1111/1754-9485.12598 PMID: 28217888.

11. Williamson JP, James AL, Phillips MJ, Sampson DD, Hillman DR, Eastwood PR. Quantifying tracheobronchial tree dimensions: methods, limitations and emerging techniques. The European Respiratory Journal. 2009; 34(1):42–55. https://doi.org/10.1183/09031936.00020408 PMID: 19567601.

12. Yamashiro T, Tsubakimoto M, Nagatani Y, Moriya H, Sakuma K, Tsukagoshi S, et al. Automated continuous quantitative measurement of proximal airways on dynamic ventilation CT: initial experience using an ex vivo porcine lung phantom. International Journal of Chronic Obstructive Pulmonary Disease. 2015; 10:2045–54. https://doi.org/10.2147/COPD.S87588 PMID: 26445535; PubMed Central PMCID: PMC4590570.

13. Zhang C, Wang H, Cao J, Li C, Mi W, Yang L, et al. Measurement and analysis of the tracheobronchial tree in Chinese population using computed tomography. PLoS One. 2015; 10(6):e0130239. https://doi.org/10.1371/journal.pone.0130239. PMID: 26039717; PubMed Central PMCID: PMC4454718.

14. Sorantin E, Halmai C, Erdohelyi B, Palágyi K, Nyúl LG, Ollé K, et al. 3D cross section of the laryngotracheal tract: a new method for visualization and quantification of tracheal stenosis. Radiologe. 2003; 43(12):1056–68. https://doi.org/10.1007/s00117-003-0990-8 PMID: 14668994.

15. Yamashiro T, Moriya H, Tsubakimoto M, Matsuoka S, Murayama S. Continuous quantitative measurement of the proximal airway dimensions and lung density on four-dimensional dynamic-ventilation CT in smokers. International Journal of Chronic Obstructive Pulmonary Disease. 2016;11:755–64. https://doi.org/10.2147/COPD.S100658 PMID: 27110108; PMCID Central PMC4835141.

16. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. The Journal of Rheumatology. 1988; 15(2):202–5. PMID: 3361530.

17. Clements P, Lachenbruch P, Siebold J, White B, Weiner S, Martin R, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. The Journal of Rheumatology. 1995; 22(7):1281–5. PMID: 7562759.

18. Culver BH, Graham BL, Coates AL, et al. Recommendations for a standardized pulmonary function report. An official American Thoracic Society technical statement. American Journal of Respiratory and Critical Care Medicine. 2017;

196(11):1463–72. https://doi.org/10.1164/rccm.201710-1981ST PMID: 29192835.

19. Pereira CAC, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. Jornal Brasileiro de Pneumologia. 2007; 33(4):397–406. https://doi.org/10.1590/S1806-37132007000400008 PMID: 17982531.
20. Neder JA, Andreoni S, Peres C, Nery LE. Reference values for lung function tests. III. Carbon monoxide diffusing capacity (transfer factor). Brazilian Journal of Medical and Biological Research. 1999; 32(6):729–37. https://doi.org/10.1590/S0100-879X1999000600008 PMID: 10412551.
21. Morewood DJ, Belchetz PE, Evans CC, Whitehouse GH. The extrathoracic airway in acromegaly. Clinical Radiology. 1986; 37(3):243–6. PMID: 3709048.
22. Goh NS, Desai SR, Veeraraghavan S, Hansell DM, Copley SJ, Maher TM, et al. Interstitial lung disease in systemic sclerosis: a simple staging system. American Journal of Respiratory and Critical Care Medicine. 2008; 177(11):1248–54. https://doi.org/10.1164/rccm.200706-877OC7°C PMID: 18369202.
23. Le Gouellec N, Duhamel A, Perez T, Hachulla AL, Sobanski V, Faivre JB, et al. Predictors of lung function test severity and outcome in systemic sclerosis-associated interstitial lung disease. PLoS One. 2017; 12(8):e0181692. https://doi.org/10.1371/journal.pone.0181692 PMID: 28763468; PubMed Central PMCID: PMC5538660.
24. Lopes AJ, Capone D, Mogami R, Lanzillotti RS, Melo PL, Jansen JM. Severity classification for idiopathic pulmonary fibrosis by using fuzzy logic. Clinics (Sao Paulo). 2011; 66(6):1015–9. https://doi.org/10.1590/S1807-59322011000600016 PMID: 21808868; PubMed Central PMCID: PMC3129967.

25. Khanna D, Nagaraja V, Tseng CH, Abtin F, Suh R, Kim G, et al. Predictors of lung function decline in scleroderma-related interstitial lung disease based on high-resolution computed tomography: implications for cohort enrichment in systemic sclerosis-associated interstitial lung disease trials. Arthritis Research and Therapy. 2015; 17:372. https://doi.org/10.1186/s13075-015-0872-2 PMID: 26704522; PubMed Central PMCID: PMC4718035.

26. Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin J-C, Pujol S, et al. 3D Slicer as an image computing platform for the quantitative imaging network. Magnetic Resonance Imaging. 2012; 30(9):1323–41. https://doi.org/10.1016/j.mri.2012.05.001

27. Zámolyi A, Székely B, Draganits E, Timár G. Neotectonic control on river sinuosity at the western margin of the Little Hungarian Plain. Geomorphology 2010; 122: 231–43. https://doi.org/10.1016/j.geomorph.2009.06.028

28. Salaffi F, Carotti M, Bosello S, Ciapetti A, Gutierrez M, Bichisecchi E, et al. Computer-aided quantification of interstitial lung disease from high resolution computed tomography images in systemic sclerosis: correlation with visual reader-based score and physiologic tests. BioMed Research International. 2015; 2015:834262. https://doi.org/10.1155/2015/834262 PMID: 25629053; PubMed Central PMCID: PMC4299560.

29. Bouros D, Wells AU, Nicholson AG, Colby TV, Polychronopoulos V, Pantelidis P, et al. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. American Journal of Respiratory and Critical Care Medicine. 2002; 165(12):1581–6. https://doi.org/10.1164/rccm.2106012 PMID: 12070056.

30. Pepper JP, Kupfer RA, McHugh JB, Hogikyan ND. Histopathologic findings and clinical manifestations in a patient with dysphonia and vocal fold involvement by systemic sclerosis. Archives of Otolaryngology Head & Neck Surgery. 2011; 137(8):816–9. https://doi.org/10.1001/archoto.2011.119 PMID: 21844416.

31. Ramos HV, Pillon J, Kosugi EM, Fujita R, Pontes P. Laryngeal assessment in rheumatic disease patients. Brazilian Journal of Otorhinolaryngology. 2005; 71(4):499–503. https://doi.org/S0034-72992005000400017 PMID: 16446967.

32. Camilo GB, Guimarães FS, Mogami R, Faria AC, Melo PL, Lopes AJ. Functional changes are associated with tracheal structural abnormalities in patients with acromegaly. Archives of Medical Science. 2016; 12(1):78–88. https://doi.org/10.5114/aoms.2016.57582 PMID: 26925121; PubMed Central PMCID: PMC4754368.

33. Miranda IA, Dias Faria AC, Lopes AJ, Jansen JM, Lopes de Melo P. On the respiratory mechanics measured by forced oscillation technique in patients with systemic sclerosis. PLoS One 2013; 8(4):e61657. https://doi.org/10.1371/journal.pone.0061657 PMID: 23637877; PubMed Central PMCID: PMC3637442.

34. Aronsson D, Hesselstrand R, Bozovic G, Wuttge DM, Tufvesson E. Airway resistance and reactance are affected in systemic sclerosis. European Clinical Respiratory Journal. 2015; 2:28667. https://doi.org/10.3402/ecrj.v2.28667 PMID: 26672963; PubMed Central PMCID: PMC4653312.

35. Winstone TA, Hague CJ, Soon J, Sulaiman N, Murphy D, Leipsic J, et al. Oesophageal diameter is associated with severity but not progression of

systemic sclerosis-associated interstitial lung disease. Respirology. 2018. [Epub ahead of print]. https://doi.org/10.1111/resp.13309 PMID: 29641847.

36. Richardson C, Agrawal R, Lee J, Almagor O, Nelson R, Varga J, et al. Esophageal dilatation and interstitial lung disease in systemic sclerosis: a cross-sectional study. Seminars in Arthritis and Rheumatism. 2016; 46(1):109–14. https://doi.org/10.1016/j.semarthrit.2016.02.004 PMID: 27033049; PubMed Central PMCID: PMC5500283.
37. Savarino E, Ghio M, Marabotto E, Zentilin P, Sammito G, Cittadini G, et al. Possible connection between gastroesophageal reflux and interstitial pulmonary fibrosis in patients with systemic sclerosis. Recenti Progressi in Medicina. 2009; 100(11):512–6. PMID: 20066883.
38. Lock G, Pfeifer M, Straub RH, Zeuner M, Lang B, Schölmerich J, et al. ssociation of esophageal dysfunction and pulmonary function impairment in systemic sclerosis. The American Journal of Gastroenterology. 1998; 93(3):341–5. https://doi.org/10.1111/j.1572-0241.1998.00341.x PMID: 9517636.
39. van Laar JM, Stolk J, Tyndall A. Scleroderma lung: pathogenesis, evaluation and current therapy. Drugs. 2007; 67(7):985–96. PMID: 17488144.
40. Farrokh D, Abbasi B, Fallah-Rastegar Y, Mirfeizi Z. The extrapulmonary manifestations of systemic sclerosis on chest high resolution computed tomography. Tanaffos. 2015; 14(3):193–200. PMID: 26858765; PubMed Central PMCID: PMC4745188.
41. Lopes AJ, Justo AC, Ferreira AS, Guimaraes FS. Systemic sclerosis: association between physical function, handgrip strength and pulmonary function. Journal of Bodywork and Movement Therapies. 2017; 21(4): 972–77. https://doi.org/10.1016/j.jbmt.2017.03.018 PMID: 29037654.

42. Hasan O, Jessar M, Ashar M, Noordin S, Ahmad T. Systemic sclerosis: clinical manifestations, anesthetic and orthopedic considerations in a patient. International Journal of Surgery Case Reports. 2017; 42:24–8. https://doi.org/10.1016/j.ijscr.2017.11.051 PMID: 29207307; PubMed Central PMCID: PMC5724744.

43. Steen VD. Clinical manifestations of systemic sclerosis. Seminars in Cutaneous Medicine and Surgery. 1998; 17(1):48–54. PMID: 9512107.

44. Sulli A, Ruaro B, Smith V, Paolino S, Pizzorni C, Pesce G, et al. Subclinical dermal involvement is detectable by high frequency ultrasound even in patients with limited cutaneous systemic sclerosis. Arthritis Research and Therapy. 2017; 19(1): 61. https://doi.org/10.1186/s13075-017-1270-8 PMID: 28320447; PubMed Central PMCID: PMC5360023.

45. Hesselstrand R, Scheja A, Wildt M, Akesson A. High-frequency ultrasound of skin involvement in systemic sclerosis reflects oedema, extension and severity in early disease. Rheumatology. 2008; 47(1):84–7. https://doi.org/10.1093/rheumatology/kem307 PMID: 18077496.

46. Yue Y, Fan Z, Yang W, Pang J, Deng Z, McKenzie E, et al. Geometric validation of self-gating k-space-sorted 4D-MRI vs 4D-CT using a respiratory motion phantom. Medical Physics. 2015; 42(10):5787–97. https://doi.org/10.1118/1.4929552 PMID: 26429253; PubMed Central PMCID: PMC4575318.

47. Adnan ZA. Diagnosis and treatment of scleroderma. Acta Medica Indonesiana. 2008; 40(2):109–12. PMID: 18560030.

48. Kim HJ, Tashkin DP, Gjertson DW, Brown MS, Kleerup E, Chong S, et al. Transitions to different patterns of interstitial lung disease in scleroderma with

and without treatment. Annals of the Rheumatic Diseases. 2016; 75(7):1367–71. https://doi.org/10.1136/annrheumdis-2015-208929 PMID: 26757749.

49. O'Reilly S. Epigenetic modulation as a therapy in systemic sclerosis. Rheumatology. 2018. [Epub ahead of print]. https://doi.org/10.1093/rheumatology/key071. PMID: 29579252.

50. George PM, Wells AU. Disease staging and sub setting of interstitial lung disease associated with systemic sclerosis: impact on therapy. Expert Review of Clinical Immunology. 2018; 14(2):127–135. https://doi.org/10.1080/1744666X.2018.1427064. PMID: 29320306.

51. Hoffmann-Vold AM, Aaløkken TM, Lund MB, Garen T, Midtvedt Ø, Brunborg C, et al. Predictive value of serial high-resolution computed tomography analyses and concurrent lung function tests in systemic sclerosis. Arthritis & Rheumatology. 2015; 67(8):2205–12. https://doi.org/10.1002/art.39166 PMID: 25916462.

52. Mehta H, Goulet PO, Nguyen V, Pérez G, Koenig M, Senécal JL, et al. Topoisomerase I peptide-loaded dendritic cells induce autoantibody response as well as skin and lung fibrosis. Autoimmunity. 2016; 49(8):503–13. https://doi.org/10.1080/08916934.2016.1230848 PMID: 27808577.

CONCLUSÕES

a) O teste de lavagem de nitrogênio monstrou-se mais sensível que os demais na identificação precoce de alterações na função pulmonar;
b) Aproximadamente 85% (44 de 52) dos pacientes possuíam aumento da inclinação de fase III, mesmo com CVF >70%;
c) Os pacientes do grupo ES apresentaram correlação negativa entre a inclinação de fase III medida pelo TLN e os seguintes parâmetros: CVF, VEF_1, CPT, DCLO, VR;
d) Os pacientes do grupo ES apresentaram correlação positiva de inclinação de fase III com VR/CPT;
e) Os pacientes do grupo ES apresentaram correlação negativa de VF/CV com CVF, VEF_1 e CPT;
f) Os pacientes do grupo ES apresentaram correlação positiva entre VF/CV e VR/CPT e Ceva;
g) Os pacientes estudados tinham maiores valores de área, excentricidade e sinuosidade de traqueia que o grupo controle;
h) A excentricidade da traqueia correlacionou negativamente com a CVF e com o VEF_1;
i) O diâmetro equivalente correlacionou negativamente com a relação $FEF_{50\%}/FIF_{50\%}$;
j) A sinuosidade da traqueia correlacionou negativamente com o PFE, e positivamente com a extensão de fibrose pulmonar;
k) A MRSS correlacionou positivamente com a excentricidade e a tortuosidade da traqueia;
l) Anticorpo anti-topoisomerase I correlacionou positivamente com a tortuosidade da traqueia.

Consideções Finais

Inúmeras são as alterações no sistema respiratório dos pacientes com ES. Além das já sabidas alterações intersticiais fibrosantes, os estudos evidenciaram alterações na função das pequenas vias aéreas, além de alterações morfológicas da traqueia, que mostraram possuir também correlação entre várias variáveis estudadas.

O uso das técnicas de esqueletonização e volumetria das vias aéreas possibilitaram a obtenção de novos parâmetros que evidenciam as diversas alterações das vias aéreas dos pacientes com ES até então não descritos. A partir destes achados, pode-se pensar em incluir esta técnica na rotina de avaliação do sistema respiratório neste grupo de pacientes.

O TLN foi capaz de evidenciar alterações na função de pequenas vias aéreas destes pacientes, um dado significativo e até então muito pouco explorado na literatura. Isso aponta na direção de que os TFP ditos “tradicionais” podem, na verdade, não detectar alterações incipientes acerca da homogeneidade do sistema respiratório e da doença de pequenas vias aéreas. Assim, nós pensamos que estudos longitudinais e randomizados possam ser de interesse para o melhor entendimento do acometimento das vias aéreas inferiores em pacientes com ES, inclusive as repercussões a longo prazo das anormalidades traqueais.

Espera-se que os resultados apresentados nesta tese possam contribuir para o aprofundamento do entendimento do acometimento da ES acerca do funcionamento e estrutura do sistema respiratório, em especial, das vias aéreas.

REFERÊNCIAS

Álvarez Puebla MJ, García Río F. Physiology and physiopathology of the distal airways in asthma. Arch Bronconeumol. 2011;47(Suppl 2):10-16.

Arnett FC, Cho M, Chatterjee S, Aguilar MB, Reveille JD, Mayes MD. Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. Arthritis Rheum. 2001;44(6):1359-1362.

Aronsson D, Hesselstrand R, Bozovic G, Wuttge DM, Tufvesson E. Airway resistance and reactance are affected in systemic sclerosis. Eur Clin Respir J. 2015;2:28667.

Bellando-Randone S, Guiducci S, Matucci-Cerinic M. Very early diagnosis of systemic sclerosis. Pol Arch Med Wewn. 2012;122 (Suppl 1):18-23.

Bérezne D, Valeyre D, Ranque B, Guillevin L, Mouthon L. Interstitial lung disease associated with systemic sclerosis: what is the evidence for efficacy of cyclophosphamide? Ann N Y Acad Sci. 2007;1110:271-284.

Bjerke RD, Tashkin DP, Clements PJ, Chopra SK, Gong H Jr, Bein M. Small airways in progressive systemic sclerosis (PSS). Am J Med. 1979;66(2):201-209.

Brand-Saberi BEM, Schäfer T. Trachea: Anatomy and Phisiology. Thorac Surg Clin. 2014; 24(1):1-5.

Buist AS, Ross BB. Predicted values for closing volumes using a modified single breath nitrogen test. Am Rev Respir Dis. 1973;107(5):744-752.

Camilo GB, Carvalho ARS, Guimarães ARM, Kasuki L, Gadelha MR, Mogami R, et al. Computed tomography airway lumen volumetry in patients with acromegaly: association with growth hormone levels and lung function. J Med Imaging Radiat Oncol. 2017;61(5):591-599.

Caron M, Hoa S, Hudson M, Schwartzman K, Steele R. Pulmonary function tests as outcomes for systemic sclerosis interstitial lung disease. Eur Respir Rev. 2018;27(148):pii: 170102.

Carvalho AR, Spieth PM, Pelosi P, Beda A, Lopes AJ, et al. Pressure support ventilation and biphasic positive airway pressure improve oxygenation by redistribution of pulmonary blood flow. Anesth Analg. 2009;109(3):856-865.

Carvalho ARS, Jandre FC, Pino AV, Bozza FA, Salluh J, Rodrigues R, et al. Positive end-expiratory pressure at minimal respiratory elastance represents the best compromise between mechanical stress and lung aeration in oleic acid induced lung injury. Crit Care 2007;11(4):1-13.

Chausow AM, Kane T, Levinson D, Szidon JP. Revertisble hypercapnic respiratory insufficiency in scleroderma caused by respiratory muscle weakness. Am Rev Respir Dis. 1984;130(1):142-144.

Chung MP, Rhee CH. Airway obstruction in interstitial lung disease. Curr Opin Pulm Med. 1997;3(5):332-335.

Crapo RO, Morris AH, Clayton PD, Nixon CR. Lung volumes in healthy nonsmoking adults. Bull Europ Physiopathol Respir. 1982;18(3):419-425.

Crapo RO, Morris AH, Gardner RM. Reference spirometric values using techniques and equipment that meet ATS recommendations. Am Rev Respir Dis. 1981;123(6): 659-664.

de Castro MC, Ferreira AS, Irion KL, Hochhegger B, Lopes AJ, Velarde GC, et al. CT quantification of large opacities and emphysema in silicosis: correlations among clinical, functional, and radiological parameters. Lung 2014;192(4):543-551.

De Santis M, Selmi C. The autoinflammatory side of systemic sclerosis. Isr Med Assoc J. 2015;17(1):47-49.

Diretrizes de Doenças Pulmonares Intersticiais da Sociedade Brasileira de Pneumologia e Tisiologia. J Bras Pneumol. 2002;38(Supl. 2):S1-S133.

Fetita CI, Prêteux F, Beigelman-Aubry C, Grenier P. Pulmonary airways: 3D reconstruction from multislice CT and clinical investigation. IEEE Trans Med Imaging. 2004;23(11):1353-1364.

França JA. Esqueletonização. Disponível em: http://www.uel.br/pessoal/josealexandre/stuff/thinning/. Acessado em 05/02/2016.
Goh NS, Desai SR,Veeraraghavan S, Hansell DM, Copley SJ, Maher T.B., et al. Interstitial lung disease in systemic sclerosis: a simple staging system. Am J Respir Crit Care Med. 2008;177(11):1248-1254.

Grenier P, Beigelman C. Spiral CT of the bronchial tree. In: Medical Radiology: Spiral CT of the Chest. Rémy-Jardin M, Rémy J (Eds.). Berlin, Springer Verlag. 1996. 185-199.

Ha YJ, Lee YJ, Kang EH. Lung Involvements in Rheumatic Diseases: Update on the Epidemiology, Pathogenesis, Clinical Features, and Treatment. Biomed Res Int. 2018 May 8;2018:6930297.

Hoffmann-Vold AM, Aaløkken TM, Lund MB, Garen T, Midtvedt Ø, Brunborg C, et al. Predictive value of serial high-resolution computed tomography analyses and concurrent lung function tests in systemic sclerosis. Arthritis Rheumatol. 2015;67(8):2205-2212.

Hügle T. Immunology of fibrotic lung disease: managing infections whilst preventing autoimmunity? J Inflamm Res. 2011;4:21-27.

Jimenez SA. Role of endothelial to mesenchymal transition in the pathogenesis of the vascular alterations in systemic sclerosis. ISRN Rheumatol. 2013;835948.

Kaminsky DA. What Does Airway Resistance Tell Us About Lung Function? Respir Care. 2012;57(1):99.

Knudson RJ, Lebowitz MD, Holdberg CJ, Burrows B. Changes in normal maximal expiratory flow-volume curve with growth and aging. Am Rev Respir Dis 1983;127(6):725-734.

König G, Luderschmidt C, Hammer C, Adelmann-Grill BC, Braun-Falco O, Fruhmann G. Lung involviment in scleroderma. Chest 1984;85(3):318-324.

Korman BD, Criswell LA. Recent advances in the genetics of systemic sclerosis: toward biological and clinical significance. Curr Rheumatol Rep. 2015;17(3):21.

Lapperre TS, Willems LN, Timens W, Rabe KF, Hiemstra PS, Postma DS, et al. Small airways dysfunction and neutrophilic inflammation in bronchial biopsies and BAL in COPD. Chest 2007;131(1):53-59.

Launay D, Remy-Jardin M, Michon-Pasturel U, Mastora I, Hachulla E, Lambert M, et al. High resolution computed tomography in fibrosing alveolitis associated with systemic sclerosis. J Rheumatol. 2006;33(9):1789-1801.

Lee KS, Boiselle PM. Update on multidetector computed tomography imaging of the airways. J Thorac Imaging. 25(2):112-124.

Liu Y, So RMC, Zhang CH. Modeling the bifurcating flow in human lung airway. J Biomech. 2002;35(4):465-473.

Lopes AJ A retomada do teste do washout do nitrogênio na prática pneumológica. Pulmão RJ 2015;24(1):14-18.

Lopes AJ, Capone D, Mogami R, Menezes SL, Guimarães FS, Levy RA. Systemic sclerosis-associated interstitial pneumonia: evaluation of pulmonary function over a five-year period. J Bras Pneumol. 2011;37:144-151.

Lopes AJ, Mafort TT. Correlations between small airway function, ventilation distribution, and functional exercise capacity in COPD patients. Lung 2014;192(5): 653-659.

Machado RIL, Souto LM, Freire EAM. Tradução, adaptação cultural e validação para a Língua Portuguesa (Brasil) do Systemic Sclerosis Questionnaire (SySQ). Rev Bras Reumatol. 2014;54(2):95-101.

Madani A, Keyzer C, Gevenois PA. Quantitative computed tomography assessment of lung structure and function in pulmonary emphysema. Eur Respir J. 2001;18(4): 720-730.

Manetti M, Guiducci S, Ibba-Manneschi L, Matucci-Cerinic M. Mechanisms in the loss of capillaries in systemic sclerosis:angiogenesis versus vasculogenesis. J Cell Mol Med. 2010;14(6A):1241-1254.

Matsuoka S, Kurihara Y, Yagihashi K, Hoshino M, Watanabe N, Nakajima Y. Quantitative assessment of air trapping in chronic obstructive pulmonary disease using inspiratory and expiratory volumetric MDCT. Am J Roentgenol. 2008; 190(3):762-769.

Melo PL Técnica de oscilações forçadas na prática pneumológica: Princípios e exemplos de potenciais aplicações. Pulmão RJ 2015;24(1):42-48.

Menna Barreto SS, Cavalazzi AC. Métodos de mensuração do volumes pulmonares. Determinação dos volumes pulmonares. J Bras Pneumol. 2002;28(3):95-97.

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-338.

Miranda AL, Faria AC, Lopes AJ, Jansen JM, Melo PL. On the Respiratory Mechanics Measured by Forced Oscillation Technique in Patients with Systemic Sclerosis. Plos One 2013;8(4):1-16.

Mottram CD. Ruppel's manual of pulmonary function testing, 10th edn. Elsevier/Mosby: Maryland Heights, 2013.

Neder AJ, Andreoni S, Castelo-Filho A, Nery LE. Reference values for lung funtion tests. I Static volumes. Braz J Med Biol Res. 1999;32(6):703-717.

Neder AJ, Andreoni S, Lerario MC, Nery LE. Reference values for lung funtion tests. II Maximal respiratory pressures and voluntary ventilation. Braz J Med Biol Res. 1999;32(6):719-727.

Neder AJ, Andreoni S, Peres C, Nery LE. Reference values for lung funtion tests. III Cabon monoxide diffusing capacity (transfer factor). Braz J Med Biol Res. 1999; 32(6):729-737.

Nguyen-Kim TDL, Maurer B, Suliman YA, Morsbach F, Distler O, Frauenfelder T. The impact of slice-reduced computed tomography on histograms based densitometry assessment of lung fibrosis in patients with systemic sclerosis. J Thorac Dis. 2018;10(4):2142-2152.

Pattanaik D, Brown M, Postlethwaite BC, Postlethwaite AE. Pathogenesis of systemic sclerosis. Front Immunol. 2015;6:272.

Ohno Y, Koyama H, Yoshikawa T, Seki S. State-of-the-art imaging of the lung for connective tissue disease (CTD). Curr Rheumatol Rep. 2015;17(12):69

Perchet D, Fetita CI, Vial L, Preteux F, Caillibotte G, Apiou GS, Thiriet M. Virtual investigation of pulmonary airways in volumetric computed tomography. Disponível em: http://www-artemis.it-sudparis.eu/Publications/library/Perchet-CASA04.pdf. Acessado em 05/02/2016.

Pereira CAC, Jansen JM, Menna Barreto SS, Marinho J, Sulmonett N, Dias RM, et al. Espirometria. J Pneumol. 2002;28(3):1-82.

Pereira CAC, Moreira MAF. Pletismografia: resistência das vias aéreas. J Bras Pneumol. 2002;28(3):139-150.

Pereira CAC, Rodrigues SC, Sato T. Novos valores de referência para espirometria forçada em brasileiros adultos de raça branca. J Bras Pneumol. 2007;33(4):397-406.

Plotze RO, Bruno-OM. Estudo e comparação de algoritmos de esqueletonização para imagens binárias. IV Congresso Brasileiro de Computação – CBComp. 2004.

Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. Eur Respir J. 2013;41(3):507-522.

Rodrigues SLF. Avaliação do volume da via aérea superior em indivíduos classe II, submetidos à cirurgia ortognática de avanço maxilomandibular. Dissertação. Universidade Paulista, 2011.

Salaffi F, Carotti M, Di Donato E, Di Carlo M, Ceccarelli L, Giuseppetti G. Computer-Aided Tomographic Analysis of Interstitial Lung Disease (ILD) in Patients with Systemic Sclerosis (SSc). Correlation with pulmonary physiologic tests and patient-centred measures of perceived dyspnea and functional disability. PLoS One 2016;11(3):e0149240.

Schoenfeld SR, Castelino FV. Interstitial lung disease in scleroderma. Rheum Dis Clin North Am. 2015;41(2):237-248.

Shepard JO, Flores EJ, Abbott GF. Imaging of the trachea. Ann Cardiothorac Surg. 2018;7(2):197-209.

Silver RM. Clinical aspects of systemic sclerosis (scleroderma). Ann Rheum Dis. 1991; 50(Supl 4):854-861.

Silver KC, Silver RM. Management of systemic-sclerosis-associated intestitial lung disease. Rheum Dis Clin North Am. 2015;41:439-57.

Sociedade Brasileira de Pneumologia e Tisiologia. Diretrizes de Doenças Pulmonares Intersticiais da Sociedade Brasileira de Pneumologia e Tisiologia. J Bras Pneumol. 2012;38(Supl 2):S1-133.

Solomon JJ, Olson AL, Fischer A, Bull T, Brown KK, Raghu G. Scleroderma lung disease. Eur Respir Rev. 2013;22(127):6-19.

Souza RB. Pressões Respiratórias Estáticas Máximas. J Bras Pneumol. 2002;28(3):155-165.

Souza Junior AS. Tomografia computadorizada de alta resolução (TCAR) nas doenças das pequenas vias aéreas. Capítulo IV. Curso de diagnóstico por imagem do tórax. J Bras Pneumol. 1999:25(4):217-224.

Stoel BC, Vrooman HA, Stolk J, Reiber JHC. Sources of error in lung densitometry with CT. Invest Radiol. 1999;34(4):303-309.

Suliman YA, Dobrota R, Huscher D, Nguyen-Kim TD, Maurer B, Jordan S, et al. Pulmonary function tests: high rate of false-negative results in the early detection and screening of scleroderma-related interstitial lung disease. Arthritis Rheumatol. 2015;67(12):3256-3261.

Tashkin DP, Volkmann ER, Tseng CH, Kim HJ, Goldin J, Clements P, et al. Relationship between quantitative radiographic assessments of interstitial lung disease and physiological and clinical features of systemic sclerosis. Ann Rheum Dis. 2016;75(2):374-381

Tashkin DP1, Elashoff R, Clements PJ, Goldin J, Roth MD, Furst DE, et al. Cyclophosphamide versus placebo in scleroderma lung disease. N Engl J Med. 2006;354(25):2655-2666.

Van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A. 2013 Classification criteria for systemic sclerosis: an American College of Rheumatology/European League Against Rheumatism Collaborative Initiative. Arthritis Rheum. 2013;65(11):2737-2747.

Verbanck S. Physiological measurement of the small airways. Respiration 2012;84(3):177-188.

Vij R, Strek ME. Diagnosis and treatment of connective tissue disease-associated interstitial lung disease. Chest 2013;143(3):814-824.

Wollheim FA. Classification of systemic sclerosis: visions and reality. Rheumatology 2005;44(10):1212-1216.

Zinsly SR, Moraes LC, Moura P, Ursi W Avaliação do espaço aéreo faríngeo por meio da tomografia computadorizada de feixe cônico. Dental Press J Orthod. 2010; 15(5):150-158.

Zompatori M, Poletti V, Rimondi MR, Battaglia M, Carvelli P, Maraldi F. Imaging of small airways disease, with emphasis on high resolution computed tomography. Monaldi Arch Chest Dis. 1997;52(3):242-248.

ANEXO - Parecer consubstanciado do CEP

HOSPITAL UNIVERSITÁRIO
PEDRO ERNESTO/
UNIVERSIDADE DO ESTADO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação da distribuição da ventilação e da função pulmonar em pequenas vias aéreas e correlação com achados da volumetria de vias aéreas em pacientes com esclerose sistêmica

Pesquisador: Bruno Rangel Antunes da Silva

Área Temática:

Versão: 1

CAAE: 50752615.9.0000.5259

Instituição Proponente: Hospital Universitário Pedro Ernesto

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.319.675

Apresentação do Projeto:

A esclerose sistêmica (ES) é uma doença crônica de caraterísticas heterogêneas que pode acometer a pele e outros órgãos do corpo. Sua
fisiopatologia envolve três características principais: disfunção dos fibroblastos com consequente fibrose, vasculopatia que leva à hipóxia tecidual e,
ainda, resposta imune alterada com disfunção de linfócitos B e T e produção de autoanticorpos. As manifestações clínicas são variáveis conforme a
apresentação da doença, tendo a maioria dos pacientes um acometimento cutâneo característico, com enrijecimento da pele, além do acometimento
de outros órgãos. O acometimento pulmonar pela esclerodermia classicamente se apresenta na forma de doença intersticial (DIP). A DIP está
presente com manifestações clínicas em aproximadamente 40% dos pacientes com ES, sendo que aproximadamente 80% dos casos têm
acometimento subclínico. Tal acometimento é a principal causa de morbidade e mortalidade na ES. A DIP pode estar presente em todas as suas
formas, apesar de frequentemente se associar à forma cutânea difusa. O comprometimento clínico significativo pode acometer 25% dos pacientes,

Endereço: Avenida 28 de Setembro 77 - Térreo
Bairro: Vila Isabel **CEP:** 20.551-030
UF: RJ **Município:** RIO DE JANEIRO
Telefone: (21)2868-8253 **Fax:** (21)2264-0853 **E-mail:** cep-hupe@uerj.br

sendo utilizados critérios de imagem de tomografia computadorizada e testes de função pulmonar (TFP) para classificar o acometimento da doença.
O tratamento deve ser considerado, principalmente quando o acometimento é caracterizado como extenso, dado o pior prognóstico desses casos. O
critério mais utilizado para classificar é o que define o acometimento de 20% do parênquima pulmonar e, nos casos de difícil mensuração, a CVF
<70% é utilizada para a sua definição. A DLCO <60% se correlaciona com a CVF <70%. Os TFP são de importância na avaliação diagnóstica e
prognóstica. Tradicionalmente CVF e DLCO são as medidas avaliadas. O teste de lavagem de nitrogênio é uma técnica que permite avaliação da
função das pequenas vias aéreas e a distribuição da ventilação nos pulmões em diversas condições clínicas; porém, até o momento, pouco tem sido
aplicado na avaliação de pacientes com SS. Neste estudo, serão avaliados 40 pacientes adultos, acompanhados no Serviço de Pneumologia da
Universidade do Estado do Rio de Janeiro, com diagnóstico de ES. Será um estudo transversal, com realização dos exames em apenas um
momento e posterior avaliação da correlação dos dados obtidos. Os pacientes realizarão avaliação da função pulmonar, incluindo: espirometria com
prova broncodilatadora, medida de volumes pulmonares estáticos (pelos métodos de diluição com hélio e pletismografia de corpo inteiro), avaliação
da força muscular, difusão do monóxido de carbono e medida da lavagem de nitrogênio. Também será realizada tomografia computadorizada de alta
resolução do tórax (TCAR) no intuito de avaliar a volumetria de vias aéreas.

Objetivo da Pesquisa:
Objetivo Primário:
Avaliar a distribuição da ventilação e a doença de pequenas vias aéreas em portadores de ES.
Objetivo Secundário:
- Avaliar a distribuição da ventilação e a função das pequenas vias aéreas em pacientes com esclerose sistêmica pela técnica de lavagem do
nitrogênio.- Avaliar a relação entre os achados de função pulmonar e volumetria de vias aéreas da TCAR de tórax em pacientes com esclerose
sistêmica.- Avaliar a correlação entre os achados de lavagem do nitrogênio e outros dados de

função pulmonar em pacientes com esclerose
sistêmica

Avaliação dos Riscos e Benefícios:

Riscos:

Este estudo requer que seja feita uma tomografia de tórax que, apesar de ser um exame comum, expõe o paciente à radiação. Contudo, esse
exame é importante para o acompanhamento de doença pulmonar e é rotineiramente solicitado nos casos em que há acometimento pulmonar pela
doença já previamente estabelecido.

Benefícios:

O estudo pode auxiliar na detecção precoce de acometimento pulmonar pela doença, provocando alterações na estrutura das vias aéreas,
determinando fenômeno obstrutivo. A identificação do acometimento pulmonar pode ser um indicativo de progressão e atividade da doença a ser
utilizado como seguimento ou mesmo na determinação de proposta terapêutica.

Comentários e Considerações sobre a Pesquisa:

Estudo relevante e com potencial de impacto na conduta clínica. A pesquisa está bem estruturada e o referencial teórico e metodológico estão explicitados, demonstrando aprofundamento e conhecimento necessários para sua realização. As referências estão adequadas e a pesquisa é exequível.

Considerações sobre os Termos de apresentação obrigatória:

Em conformidade, Foram avaliadas as informações contidas na Plataforma Brasil e as mesmas se encontram dentro das normas vigentes e sem riscos eminentes ao participante de pesquisa envolvido.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

O trabalho pode ser realizado da fora como está apresentado. Diante do exposto e à luz da Resolução CNS nº466/2012, o projeto pode ser enquadrado na categoria – APROVADO. Para ter acesso ao PARECER CONSUBSTANCIADO: Clicar na "LUPA" (DETALHAR) - Ir em "DOCUMENTOS DO PROJETO DE PESQUISA ", clicar na opção da ramificação (pequeno triangulo no entrocamento do

organograma) de pastas chamada – "Apreciação", e depois na Pasta chamada "Pareceres", o Parecer estará nesse local.

Considerações Finais a critério do CEP:

Tendo em vista a legislação vigente, o CEP recomenda ao Pesquisador: 1. Comunicar toda e qualquer alteração do projeto e termo de consentimento livre e esclarecido. Nestas circunstâncias a inclusão de pacientes deve ser temporariamente interrompida até a resposta do Comitê, após análise das mudanças propostas. 2. Os dados individuais de todas as etapas da pesquisa devem ser mantidos em local seguro por 5 anos para possível auditoria dos órgãos competentes. 3. O Comitê de Ética solicita a V. Sª., que encaminhe relatórios parciais e anuais referentes ao andamento da pesquisa ao término da pesquisa encaminhe a esta comissão um sumário dos resultados do projeto.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_579698.pdf	30/10/2015 23:47:18		Aceito
Outros	anuencia_do_servico.pdf	30/10/2015 23:46:34	Bruno Rangel Antunes da Silva	Aceito
Folha de Rosto	folha_de_rosto_assinada.pdf	04/09/2015 19:37:23	Bruno Rangel Antunes da Silva	Aceito
Cronograma	Cronograma.docx	04/09/2015 19:36:20	Bruno Rangel Antunes da Silva	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	04/09/2015 19:34:56	Bruno Rangel Antunes da Silva	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_detalhado.doc	04/09/2015 19:34:26	Bruno Rangel Antunes da Silva	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

HOSPITAL UNIVERSITÁRIO
PEDRO ERNESTO/
UNIVERSIDADE DO ESTADO

Continuação do Parecer: 1.319.675

RIO DE JANEIRO, 12 de Novembro de 2015

Assinado por:
DENIZAR VIANNA ARAÚJO
(Coordenador)

Endereço: Avenida 28 de Setembro 77 - Térreo
Bairro: Vila Isabel **CEP:** 20.551-030
UF: RJ **Município:** RIO DE JANEIRO
Telefone: (21)2868-8253 **Fax:** (21)2264-0853 **E-mail:** cep-hupe@uerj.br

APÊNDICE A - Termo de consentimento livre e esclarecido para pesquisa

(Resolução CNS/MS n° 446/12)

Estudo: "**ANÁLISE DA INFLUÊNCIA HORMONAL SOBRE OS ASPECTOS TOMOGRÁFICOS E DENSITOVOLUMÉTRICOS ATRAVÉS DA VOLUMETRIA PULMONAR E DA VOLUMETRIA DE VIAS AÉREAS EM PACIENTES ACROMEGÁLICOS.**".

Coordenadores: Agnaldo José Lopes

Endereço:
Disciplina de Pneumologia – Boulevard 28 de Setembro 77 – 2º andar – Rio de Janeiro – RJ;
Telefone: 2868-8248

Disciplina de Radiologia – Boulevard 28 de Setembro 77 – Rio de Janeiro – RJ;
Telefone: 2868-8346

INTRODUÇÃO E CONVITE PARA PARTICIPAR:
O(A) Sr.(a) está sendo convidado para participar de uma pesquisa. Antes de concordar em participar desta pesquisa, é importante que o Sr.(a) leia e entenda a explicação. Esta declaração descreve o objetivo, as consultas e exames, benefícios, riscos, desconfortos e cuidados associados com a pesquisa. Ela descreve também o seu direito de sair da pesquisa a qualquer momento.
O(A) Sr.(a) está sendo convidado(a) para participar desta pesquisa para estudar as possíveis alterações dos pulmões da tomografia computadorizada de tórax, provocadas pela acromegalia. Esta doença pode causar aumento do volume pulmonar.

OBJETIVO DO ESTUDO:
O objetivo deste estudo é avaliar o volume pulmonar do ponto de vista tomográfico e funcional respiratório. As imagens da tomografia computadorizada serão avaliadas em um laboratório da Universidade Federal do Rio de Janeiro (UFRJ) utilizando um programa de computador para calcular os volumes do pulmão.

CONSULTAS E EXAMES DO ESTUDO:
Haverá um período de seleção e um período de realização dos exames de tomografia. Caso o senhor (a) já tenha feito um exame de tomografia e/ou teste de função pulmonar no Hospital Universitário Pedro Ernesto (HUPE) nos últimos 6 meses, não haverá necessidade de repeti-los.

Depois que o Sr(a) concordar em participar e assinar este termo de consentimento livre e esclarecido, serão feitos os seguintes exames:
- Exames de rotina, como exame físico e história médica;
- Preenchimento de alguns questionários;
- Teste de função pulmonar (caso não tenha feito nos últimos 6 meses). Neste exame o Sr.(a) será solicitado a realizar inspirações e expirações forçadas (manobras respiratórias) para avaliar como está a função do seu pulmão;
- Tomografia computadorizada de tórax (caso não tenha feito nos últimos 6 meses). Neste exame o Sr.(a) deverá se deitar em uma mesa móvel (parte do aparelho) que se movimentará permitindo a entrada de seu corpo no aparelho que possui um tubo que emite radiação, está radiação será usada para formar as imagens do seu pulmão.

Durante o período de seleção de pacientes, o seu médico assistente lhe orientará quanto aos medicamentos que devem ser utilizados, que consistem naqueles que o sr(a) já habitualmente faz uso. Não será necessário tomar nenhum medicamento adicional para realizar os exames propostos. Não será administrado contraste endovenoso para a realização da Tomografia Computadorizada.
A tomografia computadorizada de tórax e o teste de função pulmonar serão realizados nos Serviços de Radiologia e Pneumologia do Hospital Universitário Pedro Ernesto, conforme marcação.

RISCOS RELACIONADOS AO ESTUDO:
Este estudo requer que seja feita uma tomografia de tórax que apesar de ser um exame comum, expõe o Sr.(a) a radiação. Esse exame é importante para o acompanhamento de doença pulmonar e costuma ser solicitado mesmo sem a participação no estudo.

BENEFÍCIOS:
O Sr.(a) não obterá nenhum benefício direto ao participar desta pesquisa.

RESSARCIMENTO DE DESPESAS:
Não haverá nenhum tipo de compensação financeira para os participantes da pesquisa. Por outro lado todos os exames serão feitos no Hospital Universitário Pedro Ernesto e não serão cobrados.

CONFIDENCIALIDADE:
Seu médico do estudo irá coletar informações a seu respeito. Em todos esses registros um código substituirá seu nome. Todos os dados coletados serão mantidos de forma confidencial e serão usados para avaliação do estudo. Os dados podem ser submetidos às autoridades de saúde, do Comitê de Ética em Pesquisa ou outras pessoas exigidas por lei podem revisar os dados fornecidos. Estes dados podem ser usados em publicações médicas sobre os resultados do estudo. Porém, sua identidade não será revelada em qualquer relatório do estudo ou publicações médicas.

PARTICIPAÇÃO VOLUNTÁRIA/RETIRADA:
Caso queira, o senhor (a) poderá se recusar a participar do estudo, ou retirar seu consentimento a qualquer momento, sem precisar justificar-se, não sofrendo qualquer prejuízo à assistência que recebe.

ANUÊNCIA PARA FAZER PARTE DO ESTUDO:
Assinando este documento você concorda que:
- Você tem chances para fazer perguntas a qualquer momento a respeito do estudo.
- Você é voluntário(a) para participar deste estudo.

EU CONCORDO LIVREMENTE EM PARTICIPAR DESTE ESTUDO

_____ _____
Assinatura do paciente **Data (dia/mês/ano)**

_____
Nome por escrito do paciente

_____ _____
Assinatura da pessoa que explicou **Data (dia/mês/ano)**
O consentimento

_____
Nome e título da pessoa que explicou o consentimento

APÊNDICE B – Publicação: Ventilation distribution and small airway function in patients with systemic sclerosis

Rev Port Pneumol. 2017;23(3):132–138

revista portuguesa de
PNEUMOLOGIA
portuguese journal of pulmonology
www.revportpneumol.org

ORIGINAL ARTICLE

Ventilation distribution and small airway function in patients with systemic sclerosis

B.R.A. Silva[a], R. Rufino[b], C.H. Costa[b], V.S. Vilela[c], R.A. Levy[c], A.J. Lopes[a,*]

[a] *Laboratory of Respiratory Physiology, State University of Rio de Janeiro, and Postgraduate Programme in Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil*
[b] *Department of Pulmonology, State University of Rio de Janeiro, and Postgraduate Programme in Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil*
[c] *Department of Rheumatology, State University of Rio de Janeiro, and Postgraduate Programme in Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil*

Received 8 October 2016; accepted 9 January 2017
Available online 1 March 2017

KEYWORDS
Systemic sclerosis;
Respiratory function tests;
Nitrogen single-breath washout test

Abstract
Background: Despite the importance of traditional pulmonary function tests (PFTs) in managing systemic sclerosis (SSc), many patients with pulmonary disease diagnosed by computed tomography (CT) present with normal PFTs.
Objective: To evaluate the efficacy of the nitrogen single-breath washout (N_2SBW) test in diagnosing SSc and to correlate N_2SBW parameters with the PFT indexes used in the follow-up of these patients, clinical data, and CT findings.
Methods: Cross-sectional study in which 52 consecutive SSc patients were subjected to spirometry, body plethysmography, analysis of the diffusing capacity for carbon monoxide (DLCO), analysis of respiratory muscle strength, N_2SBW testing, and CT analysis.
Results: Twenty-eight patients had a forced vital capacity (FVC) that was <70% of the predicted value. In the N_2SBW test, 44 patients had a phase III slope (Phase III slope$_{N2SBW}$) that was >120% of the predicted value, while 15 patients had a closing volume/vital capacity (CV/VC) that was >120% of the predicted value. A significant difference in Phase III slope$_{N2SBW}$ was observed when the patients with predominant traction bronchiectasis and honeycombing were compared to the patients with other CT patterns ($p < 0.0001$). The Phase III slope$_{N2SBW}$ was correlated with FVC ($r_s = -0.845$, $p < 0.0001$) and DLCO ($r_s = -0.600$, $p < 0.0001$), and the CV/VC was correlated with FVC ($r_s = -0.460$, $p = 0.0006$) and residual volume/total lung capacity ($r_s = 0.328$, $p = 0.017$).

* Corresponding author.
E-mail address: phel.lop@uol.com.br (A.J. Lopes).

http://dx.doi.org/10.1016/j.rppnen.2017.01.004

Conclusion: Ventilation heterogeneity is a frequent finding in SSc patients that is associated with restrictive damage, changes in pulmonary diffusion, and CT patterns. In addition, approximately one-third of the patients presented with findings that were compatible with small airway disease.

Introduction

Systemic sclerosis (SSc) is a chronic inflammatory disease of the connective tissue that is characterised by cutaneous and visceral fibrosis, self-immunity, and vascular destruction.[1,2] Almost 90% of SSc patients present with some form of lung injury over the evolution of the illness, and interstitial lung diseases associated with the SSc (ILD-SSc) and pulmonary arterial hypertension (PAH) are the most frequent manifestations.[2,3] Among the investigation methods for ILD-SSc, lung biopsy is rarely performed. Therefore, computed tomography (CT) is currently considered the method of choice.[4] Because the frequent use of ionising radiation is a matter of growing concern, CT is rarely used in the follow-up of these patients. Indeed, the severity of the pulmonary involvement of SSc is more frequently quantified using pulmonary function tests (PFTs) in clinical practice.[5,6]

Among the PFTs used in the diagnosis and follow-up of SSc patients, the most widespread are spirometry and diffusing capacity for carbon monoxide (DLCO).[7] Despite the importance of traditional PFTs in the management of pulmonary involvement associated with SSc, a significant proportion of patients present with normal PFT results, even in the presence of ILD-SSc diagnosed by imaging methods.[8] With the evolution of technical equipment in recent years, growing interest has developed in the use of the nitrogen single-breath washout (N_2SBW) test to assess ventilation homogeneity and the role of small airways in several clinical conditions.[9,10] The N_2SBW test is used for the early diagnosis and stratification of patients and to assess the severity of several lung diseases.[9,11–13] In asthma patients, poor disease control is correlated with both an increase in the closing volume (CV) and the phase III slope of the N_2SBW (Phase III $slope_{N2SBW}$).[13] In COPD patients, Lopes and Mafort[9] observed that Phase III $slope_{N2SBW}$ was the only predictor, regardless of the degree of dyspnoea and functional capacity for exercise. Mikamo et al.[12] described significant correlations between the Phase III $slope_{N2SBW}$ and the measurements of mechanical ventilation and emphysema score evaluated by CT. However, to the best of our knowledge, no studies have previously assessed the use of the N_2SBW test in SSc patients.

In addition to causing poor ventilation distribution, lung interstitium involvement can potentially lead to structural changes in small airways, resulting in a loss of air flow that can reflect in increased ventilatory demand.[14] We hypothesised that the structural disarray caused by the excessive secretion of collagen in the respiratory systems of SSc patients may be reflected in the N_2SBW test. Thus, the present study sought to assess the usefulness of the N_2SBW test in SSc patients and to correlate the parameters measured by the N_2SBW test with the PFT indexes classically used in the follow-up of these patients, degree of dyspnoea, and CT findings.

Methods

Patients

This was a cross-sectional study conducted between December 2015 and July 2016 in which 66 consecutive SSc patients were evaluated. These patients were recruited from the Piquet Carneiro Polyclinic of the State University of Rio de Janeiro, Rio de Janeiro, Brazil. Patients $\geq$18 years of age of both genders who met the criteria for SSc diagnosis[15] were included in the study. The following exclusion criteria were used: patients with a previous history of smoking or those who were current smokers; individuals with asthma; evidence of overlap with other connective tissue diseases, except Sjogren's syndrome; reports of infection within the previous four weeks; and inability to perform PFTs. The protocol was approved by the Research Ethics Committee of the Pedro Ernesto University Hospital of the State University of Rio de Janeiro, Rio de Janeiro, Brazil under the number CAAE- 50752615.9.0000.5259. All of the patients signed informed consent forms.

Measurements

Dyspnoea was assessed by means of the modified Medical Research Council (mMRC) scale.[16]

Spirometry, body plethysmography, measurement of DLCO, and measurement of respiratory muscle strength were conducted with Collins Plus Pulmonary Function Testing Systems equipment (Warren E. Collins, Inc., Braintree, MA, USA) using the standardisation of the consensus statement.[17] The Brazilian reference values were used,[18–21] and the results are expressed as % predicted.

The N_2SBW test was performed using the HDpft 3000 instrument (nSpire Health, Inc., Longmont, CO, USA). Briefly, individuals exhaled until the residual volume (RV) was reached and then inhaled 100% O_2 until the total lung capacity was reached (TLC). Then, they slowly exhaled at a flow rate of approximately 0.3–0.5 L/s until the RV was reached. The two indexes derived from the procedure are reported as % predicted[22,23] and include the Phase III $slope_{N2SBW}$, which is the change in the concentration of N_2 between 25% and 75% of the exhaled volume, and the closing volume/vital capacity (CV/VC), which is the portion of the VC that is exhaled after the airway begins to close. The N_2SBW test was performed according to the recommendations of the consensus statement.[11]

Table 1 Demographic data and lung function of patients with systemic sclerosis.

Variable	Patients with FVC ≥70% (n = 24)	Patients with FVC <70% (n = 28)	p-value
Demographic data			
Females (%)	22 (91.7)	24 (85.7)	0.67[b]
Age (years)	49.5 (43.3–58.8)	48 (37–53.8)	0.26[a]
Weight (kg)	62.8 (52.2–71)	71.5 (57.3–82)	0.11[a]
Height (cm)	157 (153–162)	160 (156–164)	0.23[a]
BMI (kg/m^2)	25.1 (21.6–28.9)	28.4 (22.1–30.9)	0.15[a]
Lung function			
FVC (% predicted)	93 (80.5–103)	57 (51.3–65)	**<0.0001**[a]
FEV_1 (% predicted)	87.5 (81.3–99)	59.5 (48–65.8)	**<0.0001**[a]
FEV_1/FVC (%)	79.5 (74.5–84.5)	86 (77.3–89.5)	**0.018**[a]
DLCO (% predicted)	78 (53.3–95)	50.5 (37.3–65.8)	**0.0004**[a]
FVC/DLCO (% of reference values)	1.22 (1.08–1.52)	1.09 (0.89–1.52)	0.23[a]
TLC (% predicted)	89 (79.3–98.8)	61 (55–72)	**<0.0001**[a]
RV (% predicted)	87 (73.3–109)	74 (56.3–85)	**0.047**[a]
RV/TLC (%)	34.5 (28.2–37.2)	40 (34.8–46.3)	**0.007**[a]
Raw (cm H_2O/L/s)	1.66 (1.36–2.43)	1.81 (1.26–3.08)	0.78[a]
SGaw (L/s/cm H_2O/L)	0.205 (0.148–0.268)	0.260 (0.165–0.438)	0.12[a]
MIP (% predicted)	66 (47–87.3)	65 (55.3–92.8)	0.42[a]
MEP (% predicted)	55.5 (32.8–62)	45.5 (34.8–54)	0.40[a]
Nitrogen single-breath washout test			
Phase III $slope_{N2SBW}$ (% predicted)	130 (88–160)	419 (275–542)	**<0.0001**[a]
CV/VC (%predicted)	71 (56–111)	137 (101–188)	**0.003**[a]

Values are median (interquartile ranges) or number (%). BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; DLCO: diffusing capacity for carbon monoxide; TLC: total lung capacity; RV: residual volume; Raw: airway resistance; SGaw: specific airway conductance; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure; Phase III $slope_{N2SBW}$: phase III slope of the nitrogen single-breath washout; CV/VC: closing volume/vital capacity.
The values in bold mean statistical significance.
[a] Mann–Whitney test.
[b] Fisher exact test.

We also assessed the CT scans of 31 patients that were performed within the last three months prior to recruitment. The CT scans were categorised into three patterns according to the consensus of two radiologists: grade 1 (reticular pattern predominance); grade 2 (ground-glass opacity predominance); and grade 3 (traction bronchiectasis and honeycombing predominance).[4,8]

Statistical analysis

The data analysis was conducted using SAS 6.11 software (SAS Institute, Inc., Cary, NC, USA). The assumption of a normal distribution of the data was evaluated with a Shapiro–Wilk test. Comparisons of variables between the two groups of patients subdivided according to the FVC or mMRC grades were evaluated by the Mann–Whitney test for numerical variables and by Fisher's exact test for categorical variables. Comparisons of the CT grades according to the different N_2SBW variables were examined using the non-parametric Kruskal–Wallis test followed by Dunn's post hoc test. Spearman's rank correlation coefficient (r_s) was used to evaluate the associations between the variables. The results are expressed as median values and interquartile ranges or as frequencies (percentages), and statistical significance was considered at $p < 0.05$.

Results

Among the 66 patients who were considered for participation in the study, 14 were excluded for the following reasons: six for reporting a history of smoking, four for presenting with SSc together with other collagen diseases, two due to associated asthma, and two due to the inability to perform the PFTs. Thus, the study population consisted of 46 women and six men with a median age of 48 (38.5–56.3) years. Thirty-eight patients had a limited form of the disease, and 14 had the diffuse form. In 36 patients, the mMRC grade was <2 [1.12 (0.64–1.47)], and in 16, it was ≥2 [2.47 (2.31–2.78)]. In the CT analysis, the exams were categorised as grade 1 (n = 13), grade 2 (n = 10), and grade 3 (n = 8).

For the 52 patients who participated in the study, the median values of FVC, DLCO, and FVC/DLCO were 67 (57–91)% predicted, 62 (44–81.3)% predicted, and 1.18 (0.93–1.52)% of the reference values, respectively. Twenty-eight patients had an FVC <70%, 34 had a DLCO <80%, and nine had an FVC/DLCO >1.6. Regarding the TLC, the median of the sample was 72.5 (60.3–87.5)% predicted, and

Table 2 Spearman's correlation coefficients for lung function parameters and nitrogen single-breath washout indexes of patients with systemic sclerosis.

Variable	Phase III slope$_{N2SBW}$ (%predicted)		CV/VC (%predicted)	
	r_s	*p*-value	r_s	*p*-value
FVC (% predicted)	−0.845	**<0.0001**	−0.460	**0.0006**
FEV_1 (% predicted)	−0.788	**<0.0001**	−0.396	**0.003**
FEV_1/FVC (%)	0.281	**0.044**	−0.282	**0.042**
DLCO (% predicted)	−0.600	**<0.0001**	−0.271	0.052
FVC/DLCO (% of reference values)	0.088	0.54	0.020	0.89
TLC (% predicted)	−0.708	**<0.0001**	−0.360	**0.008**
RV (% predicted)	−0.354	**0.010**	−0.122	0.39
RV/TLC (%)	0.318	**0.021**	0.328	**0.017**
Raw (cm H_2O/L/s)	0.084	0.55	−0.216	0.12
SGaw (L/s/cm H_2O/L)	0.205	0.15	0.365	**0.007**
MIP (% predicted)	0.123	0.38	0.095	0.50
MEP (% predicted)	−0.080	0.57	−0.163	0.25

Phase III slope$_{N2SBW}$: phase III slope of the nitrogen single-breath washout; CV/VC: closing volume/vital capacity; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; DLCO: diffusing capacity for carbon monoxide; TLC: total lung capacity; RV: residual volume; Raw: airway resistance; SGaw: specific airway conductance; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure.
The values in bold mean statistical significance.

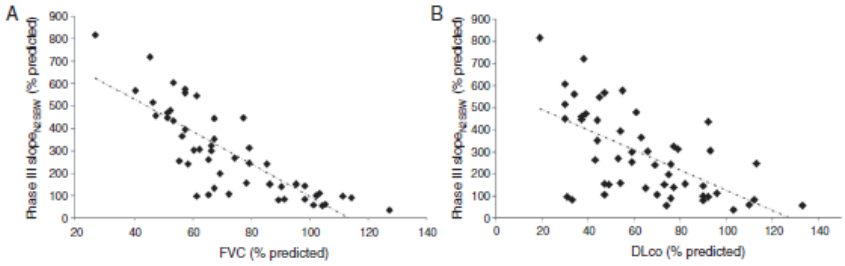


Figure 1 Relationships between the phase III slope of the nitrogen single-breath washout (Phase III slope$_{N2SBW}$) and the forced vital capacity (FVC) ($r_s = -0.845$, $p < 0.0001$) (A) and diffusing capacity for carbon monoxide (DLCO) ($r_s = -0.600$, $p < 0.0001$) (B).

this parameter was <80% in 31 patients, which indicated a restrictive disorder. The medians for Phase III slope$_{N2SBW}$ and CV/VC were 254 (112–450)% predicted and 107 (62–150)% predicted, respectively. In this test, 44 patients had a Phase III slope$_{N2SBW}$ >120%, while 15 patients had a CV/VC >120%. When the sample was subdivided into two groups using a cut-off value of FVC <70%, significant differences were observed between the two groups for most of the evaluated functional parameters (Table 1).

There were no significant differences between the mMRC grades and N_2SBW variables: Phase III slope$_{N2SBW}$ [235 (148–287) vs. 352 (240–430), $p = 0.08$] and CV/VC [98 (70–136) vs. 124 (85–161), $p = 0.13$]. Regarding the CT findings, the medians of the Phase III slope$_{N2SBW}$ values progressively increased from grade 1 to grade 3 [105 (64–138) vs. 185 (147–223) vs. 536 (370–653)%] with significant differences ($p < 0.0001$). The medians of the CV/VC values also progressively increased from grade 1 to grade 3 [53 (32–85) vs. 68 (46–102) vs. 175 (78–217)%]; however, the differences were not significant ($p = 0.11$).

We also assessed the associations between the parameters provided by the N_2SBW test, lung function indices, and CT findings (Table 2 and Figs. 1 and 2); the correlation between the Phase III slope$_{N2SBW}$ and CV/VC was strong and positive ($r_s = 0.590$, $p < 0.0001$).

Discussion

The main finding of the present study was that ventilation heterogeneity is the most common abnormality observed in the PFTs of SSc patients, and it occurs even in the absence of restrictive damage. In those patients, the more accentuated the functional or structural pulmonary deterioration is, the worse the ventilation heterogeneity. In addition, small airway disease was also a frequent finding that was related to

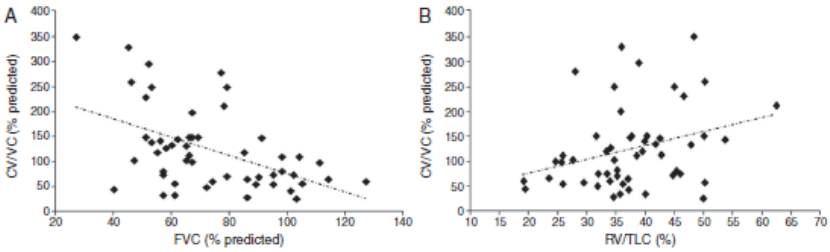


Figure 2 Relationships between the closing volume/vital capacity (CV/VC) and the forced vital capacity (FVC) ($r_s = -0.460$, $p = 0.0006$) (A) and residual volume/total lung capacity (RV/TLC) ($r_s = 0.328$, $p = 0.017$) (B).

both air trapping and the loss of lung volume. To our knowledge, this is the first study to assess the potential of the N_2SBW test in SSc patients.

In the present study, the sample was almost exclusively composed of women, which is consistent with the distribution by gender reported by several investigators.[2] In terms of prognosis, functional changes are important markers of the evolution of ILD-SSc, in both the initial and the sequential assessments.[2] In the present study, approximately 60% of the patients had the restrictive syndrome and/or a reduction of the DLCO, thereby presenting a sample with substantial lung function involvement. Interestingly, nine patients had an FVC/DLCO >1.6, which is the recommended cut-off for study of the right side of the heart in the diagnosis of PAH.[24]

Many patients with pulmonary disease diagnosed by CT present with normal PFTs, which is indicative of insufficient performance of traditional PFTs in tracking pulmonary disease associated with SSc.[8] In a recent study, Suliman et al.[25] evaluated 102 patients with SSc and noted that 63% presented with significant ILD-SSc on CT, while only 26% had an FVC <80%. This finding emphasises the urgent need to develop new lung function parameters to diagnose lung involvement, as well as allow follow-up with patients. In this sense, some tests such as the N_2SBW test, the forced oscillation technique (FOT), and impulse oscillometry can add to our understanding of the pathophysiology of ILD-SSc and can potentially be incorporated into the routine assessment of these patients.[26,27]

In the present study, an increase in the Phase III slope$_{N2SBW}$ was the most frequent lung function abnormality, which was observed in approximately 85% of the cases and indicates the potential of this index as a marker for ILD-SSc. High values are indicative of ventilation inhomogeneity due to regional differences in time constants of the respiratory system, which result from changes in the distensibility or local resistance, thus compromising alveolar emptying.[28] The increase in the Phase III slope$_{N2SBW}$ in the group of patients with an FVC <70% indicates that the worsening of restrictive functional damage is an important contributor to the ventilation heterogeneity of SSc. We observed a strong association between the increase in Phase III slope$_{N2SBW}$ and the decay of both the FVC and DLCO, which reinforces the routine use of these two functional indexes in the follow-up of patients with SSc in clinical practice. Interestingly, we also observed a strong association between the increase of the Phase III slope$_{N2SBW}$ and the presence of traction bronchiectasis and honeycombing in CT. Despite the lack of studies correlating the N_2SBW test with CT findings in fibrotic lung diseases, it is noteworthy that some researchers have observed an association between the increase in Phase III slope$_{N2SBW}$ and structural lung damage in COPD patients.[12,29]

In addition to the Phase III slope$_{N2SBW}$, the CV/VC ratio is another index provided by the N_2SBW test that has recently been studied.[11,28] In the present study, we observed an increase in the CV/VC ratio in nearly one-third of the patients. A change in the CV/VC ratio has been used as one of the parameters for the diagnosis of small airway disease, and it is functionally characterised by a progressive increase in resistance as the lung is emptied, regional heterogeneity in flow rate and time constants, and premature closing of the airways.[4,11] Using the FOT, Miranda et al.[27] observed changes in the peripheral resistance of the respiratory system in SSc patients, which were evaluated according to the slope of the resistance as a function of frequency. Similarly, Aronsson et al.[26] observed abnormalities that were compatible with small airway diseases in SSc patients including increases in the R5-R20, which is the difference between the resistance at 5 Hz and the resistance at 20 Hz in impulse oscillometry. Similar to the Phase III slope$_{N2SBW}$, we observed an increase in the CV/VC in the group of patients with an FVC <70%. Interestingly, we also observed a positive correlation between CV/VC and RV/TLC in the studied sample, suggesting an association between the premature closing of the airways and the presence of air trapping.[30] However, it is worth emphasising that an increase in the CV/VC can be observed in patients with restrictive damage in situations where the functional residual capacity is less than the closing volume.[28]

It is noteworthy that there are currently more than 10 measures of ventilation distribution derived from the nitrogen washout (N_2W) tests.[31] The need for maintaining good coordination and cooperation when conducting VC manoeuvres under constant flow is a limiting factor for the routine use of the N_2SBW test.[11] Contrariwise, the multiple breath washout (MBW) test evaluates ventilation distribution during the fixed tidal volume or normal tidal breathing, assessing the release of inert gas in a series of breathing cycles. Thus, MBW shows promise for use in children and adults with difficulties performing forced manoeuvres. However, this test is time consuming, which makes it impractical

to use in patients with severe lung disease.[11] It is also noteworthy to highlight the double tracer gas (DTG) single-breath washout, a new N_2W test modality that aims to be more specific to small airways. It distinguishes between convection-dependent and diffusion-convection-dependent ventilation heterogeneities, which occur in the conductive and acinar airways, respectively.[32]

The strength of this study is that it demonstrates the potential of the N_2SBW test for detecting abnormalities in both ventilation and the small airways of SSc patients. Because it is a simple, non-invasive, easy and fast tool, the N_2SBW test may be incorporated into the clinical assessment of SSc patients in the future. However, our study also has several limitations. First, the sample was small, and the design was cross-sectional. Second, we did not use a control group. However, the absence of a control group in this study was minimised by the use of pulmonary function values as percentages of the predicted values because these are normalised to anthropometric data. Third, the complementary use of DTG single-breath washout could have allowed us to evaluate ventilation heterogeneity in the lung periphery.[32] Despite these limitations, our results provide a perspective for the use of the N_2SBW test in longitudinal studies to verify its prognostic value in SSc patients.

Finally, the present study shows that in patients with SSc, ventilation distribution inhomogeneity is a very frequent finding that is related to restrictive damage, changes in pulmonary diffusion, and CT patterns. In addition, approximately one-third of the patients in this study were compatible with the criteria for small airway disease, which is associated with both the severity of restrictive damage and the presence of air trapping.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgements

This research was supported by the Rio de Janeiro State Research Supporting Foundation (FAPERJ).

References

1. Chizzolini C, Brembilla NC, Montanari E, Truchetet ME. Fibrosis and immune dysregulation in systemic sclerosis. Autoimmun Rev. 2011;10:276–81.
2. Sticherling M. Systemic sclerosis-dermatological aspects. Part 1: pathogenesis, epidemiology, clinical findings. J Dtsch Dermatol Ges. 2012;10:705–18.
3. Tani C, Bellando Randone S, Guiducci S, Della Rossa A. Systemic sclerosis: a critical digest of the recent literature. Clin Exp Rheumatol. 2013;31 Suppl. 76:172–9.
4. Lopes AJ, Capone D, Mogami R, Menezes SL, Guimarães FS, Levy RA. Systemic sclerosis-associated interstitial pneumonia: evaluation of pulmonary function over a five-year period. J Bras Pneumol. 2011;37:144–51.
5. Antoniou KM, Margaritopoulos GA, Goh NS, Karagiannis K, Desai SR, Nicholson AG, et al. Combined pulmonary fibrosis and emphysema in scleroderma-related lung disease has a major confounding effect on lung physiology and screening for pulmonary hypertension. Arthritis Rheumatol. 2016;68:1004–12.
6. Silver KC, Silver RM. Management of systemic-sclerosis-associated interstitial lung disease. Rheum Dis Clin North Am. 2015;41:439–57.
7. Hant FN, Herpel LB, Silver RM. Pulmonary manifestations of scleroderma and mixed connective tissue disease. Clin Chest Med. 2010;31:433–49.
8. Hoffmann-Vold AM, Aaløkken TM, Lund MB, Garen T, Midtvedt Ø, Brunborg C, et al. Predictive value of serial high-resolution computed tomography analyses and concurrent lung function tests in systemic sclerosis. Arthritis Rheumatol. 2015;67:2205–12.
9. Lopes AJ, Mafort TT. Correlations between small airway function, ventilation distribution, and functional exercise capacity in COPD patients. Lung. 2014;192:653–9.
10. Timmins SC, Diba C, Farrow CE, Schoeffel RE, Berend N, Salome CM, et al. The relationship between airflow obstruction, emphysema extent, and small airways function in COPD. Chest. 2012;142:3–319.
11. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single-breath tests. Eur Respir J. 2013;41:507–22.
12. Mikamo M, Shirai T, Mori K, Shishido Y, Akita T, Morita S, et al. Predictors of phase III slope of nitrogen single-breath washout in COPD. Respir Physiol Neurobiol. 2013;189:42–6.
13. Bourdin A, Paganin F, Préfaut C, Kieseler D, Godard P, Chanez P. Nitrogen washout slope in poorly controlled asthma. Allergy. 2006;61:85–9.
14. Chung MP, Rhee CH. Airway obstruction in interstitial lung disease. Curr Opin Pulm Med. 1997;3:332–5.
15. 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. Arthritis Rheum. 2013;65:2737–47.
16. Mahler DA, Weinberg DM, Wells CK, Feinstein AR. The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. Chest. 1984;85:751–8.
17. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardization of spirometry. Eur Respir J. 2005;26:319–38.
18. Pereira CAC, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. J Bras Pneumol. 2007;33:397–406.
19. Neder JA, Andreoni S, Peres C, Nery LE. Reference values for lung function tests. I. Static volumes. Braz J Med Biol Res. 1999;32:703–17.

20. Neder JA, Andreoni S, Peres C, Nery LE. Reference values for lung function tests. III. Carbon monoxide diffusing capacity (transfer factor). Braz J Med Biol Res. 1999;32:729–37.
21. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Braz J Med Biol Res. 1999;32:719–27.
22. Buist AS, Ross BB. Predicted values for closing volumes using a modified single breath nitrogen test. Am Rev Respir Dis. 1973;107:744–52.
23. Buist AS, Ghezzo H, Anthonisen NR, Cherniack RM, Ducic S, Macklem PT, et al. Relationship between the single-breath N test and age, sex, and smoking habit in three North American cities. Am Rev Respir Dis. 1979;120:305–18.
24. Khanna D, Gladue H, Channick R, Chung L, Distler O, Furst DE, et al. Recommendations for screening and detection of connective tissue disease-associated pulmonary arterial hypertension. Arthritis Rheum. 2013;65:3194–201.
25. Suliman YA, Dobrota R, Huscher D, Nguyen-Kim TD, Maurer B, Jordan S, et al. Pulmonary function tests: high rate of false-negative results in the early detection and screening of scleroderma-related interstitial lung disease. Arthritis Rheumatol. 2015;67:3256–61.
26. Aronsson D, Hesselstrand R, Bozovic G, Wuttge DM, Tufvesson E. Airway resistance and reactance are affected in systemic sclerosis. Eur Clin Respir J. 2015;2:28667.
27. Miranda IA, Dias Faria AC, Lopes AJ, Jansen JM, Lopes de Melo P. On the respiratory mechanics measured by forced oscillation technique in patients with systemic sclerosis. PLoS One. 2013;8:e61657.
28. Mottram CD. Ruppel's manual of pulmonary function testing. 10th ed. Maryland Heights: Elsevier/Mosby; 2013.
29. Boeck L, Gensmer A, Nyilas S, Stieltjes B, Re TJ, Tamm M, et al. Single-breath washout tests to assess small airway disease in COPD. Chest. 2016;150:1091–100.
30. Verbanck S. Physiological measurement of the small airways. Respiration. 2012;84:177–88.
31. Latzin P, Thompson B. Double tracer gas single-breath washout: promising for clinics or just a toy for research? Eur Respir J. 2014;44:1113–5.
32. Husemann K, Berg N, Engel J, Port J, Joppek C, Tao Z, et al. Double tracer gas single-breath washout: reproducibility in healthy subjects and COPD. Eur Respir J. 2014;44:1210–22.

APÊNDICE C - Publicação: Computed tomography trachea volumetry in patients with scleroderma: Association with clinical and functional findings

PLOS ONE

RESEARCH ARTICLE

Computed tomography trachea volumetry in patients with scleroderma: Association with clinical and functional findings

Bruno Rangel Antunes Silva[1☯], Rosana Souza Rodrigues[2☯], Rogério Rufino[1☯], Cláudia Henrique Costa[1☯], Veronica Silva Vilela[1☯], Roger Abramino Levy[1☯], Alan Ranieri Medeiros Guimarães[3☯], Alysson Roncally Silva Carvalho[3,4☯], Agnaldo José Lopes[1☯*]

1 Postgraduate Programme in Medical Sciences, School of Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil, **2** Department of Radiology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, **3** Laboratory of Respiration Physiology, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, **4** Laboratory of Pulmonary Engineering, Biomedical Engineering Programme, Alberto Luiz Coimbra Institute of Post-Graduation and Research in Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

☯ These authors contributed equally to this work.
* alopes@souunisuam.com.br

OPEN ACCESS

Citation: Silva BRA, Rodrigues RS, Rufino R, Costa CH, Vilela VS, Levy RA, et al. (2018) Computed tomography trachea volumetry in patients with scleroderma: Association with clinical and functional findings. PLoS ONE 13(8): e0200754. https://doi.org/10.1371/journal.pone.0200754

Editor: Masataka Kuwana, Keio University, JAPAN

Received: February 23, 2018

Accepted: July 1, 2018

Published: August 1, 2018

Data Availability Statement: All data are available from the Open Science Framework (https://osf.io/ue7mn/).

Funding: This research was supported by the Brazilian Council of Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq) and the Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ). The funders had no role in study design, data

Abstract

Background

In scleroderma, excessive collagen production can alter tracheal geometry, and computed tomography (CT) volumetry of this structure may aid in detecting possible abnormalities. The objectives of this study were to quantify the morphological abnormalities in the tracheas of patients with scleroderma and to correlate these findings with data on clinical and pulmonary function.

Methods

This was a cross-sectional study in which 28 adults with scleroderma and 27 controls matched by age, gender and body mass index underwent chest CT with posterior segmentation and skeletonization of the images. In addition, all participants underwent pulmonary function tests and clinical evaluation, including the modified Rodnan skin score (mRSS).

Results

Most patients (71.4%) had interstitial lung disease on CT. Compared to controls, patients with scleroderma showed higher values in the parameters measured by CT trachea volumetry, including area, eccentricity, major diameter, minor diameter, and tortuosity. The tracheal area and equivalent diameter were negatively correlated with the ratio between forced expiratory flow and forced inspiratory flow at 50% of forced vital capacity ($FEF_{50\%}/FIF_{50\%}$) ($r = -0.44$, $p = 0.03$ and $r = -0.46$, $p = 0.02$, respectively). The tracheal tortuosity was negatively correlated with peak expiratory flow ($r = -0.51$, $p = 0.008$). The mRSS showed a positive correlation with eccentricity ($r = 0.62$, $p < 0.001$) and tracheal tortuosity ($r = 0.51$, $p = 0.007$),

collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

while the presence of anti-topoisomerase I antibody (ATA) showed a positive correlation with tracheal tortuosity ($r = 0.45$, $p = 0.03$).

Conclusions

In a sample composed predominantly of scleroderma patients with associated interstitial lung disease, there were abnormalities in tracheal geometry, including greater eccentricity, diameter and tortuosity. In these patients, abnormalities in the geometry of the trachea were associated with functional markers of obstruction. In addition, tracheal tortuosity was correlated with cutaneous involvement and the presence of ATA.

Introduction

Scleroderma or systemic sclerosis (SSc) is a chronic progressive autoimmune disease of connective tissue characterized by microvascular involvement, activation of the immune system, and increased deposition of extracellular matrix in the skin and internal organs by excess collagen fibers, leading to fibrosis [1–4]. Scleroderma is eight times more common in females than in males; its reported prevalence is approximately 10 cases per 100,000 person, and this rate is probably underestimated [5]. The disease can affect several organs and systems; the skin is the most frequently affected site, followed by the lungs, kidneys, musculoskeletal system, cardiovascular system, and gastrointestinal tract. The presence of multiple affected sites worsens the prognosis [1].

In scleroderma, thoracic involvement is observed mainly as diffuse fibrosis or pulmonary hypertension; these conditions are associated with limited (lc-SSc) and diffuse cutaneous (dc-SSc) forms of scleroderma, respectively [1]. In an autopsy study, parenchymal involvement was seen in up to 100% of patients with scleroderma [2]. Although interstitial lung involvement is subclinical and asymptomatic at early stages in most patients, interstitial lung disease (ILD) associated with scleroderma (ILD-SSc) is observed in approximately 40% of cases and is a major cause of morbidity and mortality [6]. Despite the constellation of thoracic manifestations that occur in patients with scleroderma, little is known about the involvement of the trachea in these patients. Ooi et al. [7] reported that scleroderma affected small and large airways in 45–100% of patients. In this context, the trachea has been a 'forgotten zone' in the study of several diseases because the pathological processes involving this structure have often not received the necessary clinical recognition.

Computed tomography (CT) has become an important part of the detection and evaluation of routine thoracic involvement in scleroderma, and the abnormalities observed by this method are closely correlated with the observed physiological parameters [2]. In the last two decades, various computer tools that can be used to automatically slice the chest using CT images have been developed. These include multiplanar reformation, regional lung attenuation analysis of lung tissue and quantification of anatomical images, including the area and volume of airways and lungs [2,7]. In scleroderma, computer-assisted tomography analysis is highly efficient and, in combination with physiological and patient-centered measurements, may provide a means to accurately assess and monitor lung disease progression and response to therapy [2,8,9].

An understanding of the acquisition, processing and analysis of CT scans and how these processes affect the imaging of the trachea is essential for assessing the accuracy of the measurements and making effective use of newly available tools. In the study of the trachea, the cross-sectional area and diameter are the most commonly measured dimensions; in the

context of assessing possible obstruction, length and caliber are important [10–13]. More recently, the process of skeletonization and volumetry of the airways through CT in normal individuals and in individuals with some clinical conditions has been described [10–15]. In this method, the digital imaging component is transformed into a subset of the original component [10]. After automated segmentation of CT images, the skeletonization algorithm allows the extraction of a tracheal centerline and facilitates the reconstruction of CT data orthogonal to the reduction of the effects of partial volume averages [11]. However, no study has explored the use of this resource in the evaluation of the tracheas of patients with scleroderma.

We hypothesized that the excessive production of collagen that occurs in scleroderma alters the geometry of the trachea and that the skeletonization of this structure would aid in the detection of possible abnormalities. Thus, the present study aimed to identify and quantify the morphological abnormalities in the tracheas of patients with scleroderma and, secondarily, to correlate these findings with data on clinical and pulmonary function.

Methods

Patients

This cross-sectional study was conducted between February 2016 and September 2017 in 43 consecutive patients with scleroderma who were ≥18 years of age, of both sexes, and were seen regularly at the Piquet Carneiro Polyclinic of the State University of Rio de Janeiro, Rio de Janeiro, Brazil. The patients included in the study had been diagnosed with scleroderma by a rheumatologist according to the parameters set forth by the American College of Rheumatology/European League Against Rheumatism [1]. The following exclusion criteria were used: clinical instability; history of respiratory infection in the last three weeks; history of previous or current smoking; evidence of overlapping of scleroderma with other connective tissue diseases; report of previous tracheal or pleuropulmonary disease not related to scleroderma; and inability to perform pulmonary function tests (PFTs). Regarding cutaneous involvement, patients were classified as having lc-SSc (thickening of the skin distal to the elbows and knees and proximal to the clavicles, including the face) or dc-SSc (thickening of the proximal skin as well as of the skin distal to the elbows and knees and including the trunk and face) [16]. The modified Rodnan skin score (mRSS) was used to assess skin damage in patients with scleroderma. In this system, a score of 0 (no thickening), 1 (light thickening), 2 (moderate thickening) or 3 (severe thickening) is assigned to each area, resulting in a total score ranging from 0 (best) to 51 (worst) [17]. The presence of autoantibodies, including anti-topoisomerase I and anti-centromere, was also investigated.

We also evaluated a control group of 27 individuals aged ≥ 18 years of both sexes. The subjects in the control group were asked to perform the PFTs after undergoing chest CT scanning in our service for the following reasons: investigation of contact with tuberculosis patients (n = 9); staging of neoplasms outside the thorax (n = 7); evaluation of trauma (n = 6); and evaluation of fever of unknown origin (n = 5). The following criteria were used to select subjects for the control group: no previous history of smoking or chronic tracheal or pleuropulmonary diseases; chest CT scans without abnormalities; and lung function parameters within the normal range (*i.e.*, no value below the lower limit of normal or above the upper limit of normal in relation to the predicted value). All CT scans and PFTs for the control group were performed using the same equipment that was used for the scleroderma group.

The protocol was approved by the Research Ethics Committee of the State University of Rio de Janeiro under the number CAAE- 50752615.9.0000.5259, and it complied with the current national and international standards. All individuals signed an informed consent form.

Pulmonary function testing

The PFTs performed were spirometry, body plethysmography, and diffusion capacity for carbon monoxide (DLco). The exams were performed on an HDpft 3000 (nSpire Health, Inc., Longmont, CO, USA) according to the standards set forth by the American Thoracic Society [18]. The Brazilian reference values [19,20] were used, and the results are expressed as percentages of the predicted values. An increase in the ratio between forced expiratory flow and forced inspiratory flow at 50% of forced vital capacity-FVC ($FEF_{50\%}/FIF_{50\%}$) > 1.50 was used as an indicator of extrathoracic airway obstruction [21].

CT scan interpretation and protocol

CT scans were performed on a 64-channel multislice Brilliance 40 scanner (Philips Medical Systems, Cleveland, OH, USA) that was capable of performing volumetric acquisitions with subsequent multiplanar reconstructions. The acquisitions were performed in the axial plane with patients in the supine position using the technical parameters 120 kV and 458 mA (these parameters varied according to the biotype of the patient), slice thickness 2 mm, and pitch 2 mm from the jugular notch to the xiphoid process in maximal inspiration and expiration. After acquisition of the images, a high-resolution reconstruction with a matrix of 512 × 512 was performed using a high-frequency algorithm, a window width of 1200 HU, and a level centered at -800 HU.

Parenchymal abnormalities on CT were interpreted by two independent readers (R.S.R. and G.B.C.) who were blinded to patient history and physiological results; a consensus opinion was reached in cases in which there was disagreement. CT scans were reviewed at five levels, as follows: 1) origin of major vessels; 2) carina; 3) confluence of pulmonary veins; 4) halfway between the third and the fifth sections; 5) 1 cm above the right hemidiaphragm [22,23]. The total extent of ILD was estimated to the nearest five percent in each of the five levels, with global extent of disease on CT as the mean of the scores [22–24]. The coarseness of pulmonary fibrosis was evaluated as follows: 0, ground-glass opacification alone; 1, fine intralobular fibrosis; 2, microcystic honeycombing comprising air spaces ≤ 4 mm in diameter; and 3, macrocystic honeycombing comprising air spaces > 4 mm in diameter. The total coarseness score for each patient was derived by summing the scores at the five levels (range 0 to 15) [22,23]. For each patient, the total extension of ILD and the coarseness of pulmonary fibrosis were derived by averaging the scores at each level assessed by the two independent readers. Finally, the extent of ILD-SSc was classified as limited (lung parenchyma involvement <20%) or extensive (>20%). For indeterminate cases, ILD-SSc was considered extensive if FVC <70% and limited if FVC >70% [22,25].

Imaging processing

The airways were segmented using 3DSlicer version 4.4.0 (http://slicer.org) [26] with the aid of its AirwaySegmentation extension. At the end of processing, a nearly raw raster data (.nrrd) file was saved. Using AirwayProcessing, two distinct processes were executed: 1) the .nrrd file was read and transformed into a binary matrix of data; in this way, the process of skeletonization was initiated, and a skeleton composed of several points was then produced; 2) using the skeleton and its coordinates (X, Y and Z axes), cross-sectional planes were generated. Individual processing of all points was performed using the slice command, and the processing angles were defined by a normal line between the point in question and the next five points. The plane of square cross-section was formed by a square grid with 70 pixels on the side. Using MATLAB 2014a (MathWorks Inc., Natick, MA, USA), information obtained individually by the slice command was catalogued using the 'regionprops' command.

Fig 1 shows the 3D reconstruction, the tracheal skeleton, the cross-sectional planes and their geometric parameters [9,10]. The scheme show in Fig 1 was chosen for illustrative purposes and accurately represents the region of the trachea in a 2D environment. In Fig 1, the axial sections indicated by specific letters represent area (*A), perimeter (*B), eccentricity (*C), equivalent diameter (*D), major diameter (*E) and minor diameter (*F). It is emphasized that the segments shown in red and blue in Fig 1 were enlarged for visualization purposes.

Thus, the following measures were calculated for each study participant:

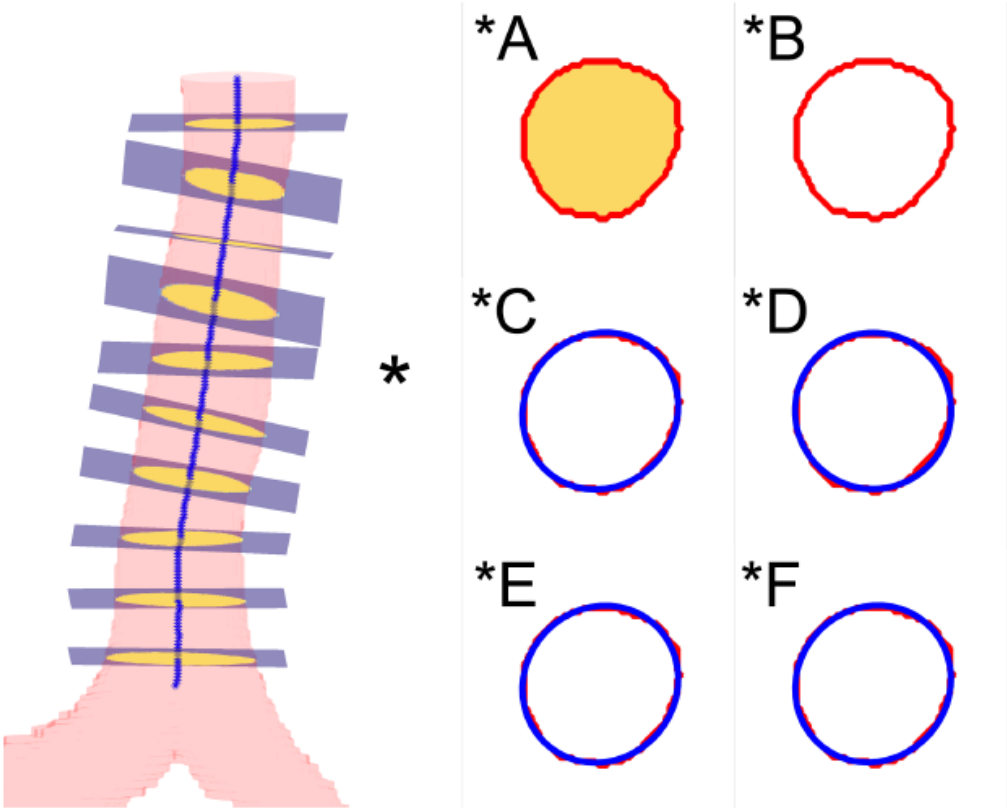


Fig 1. 3D reconstruction, cross-sectional planes, skeleton and indication of the plane used for representation. The letters A to F and the values for the selected plane indicate, respectively: (*A) area (182.4 mm^2), which is the product of the pixel size and the number of pixels in the region (in this figure, the area is shown in yellow and includes the red line that demarcates the perimeter); (*B) perimeter (47.3 mm), which is the length of the line indicated in red; (*C) eccentricity (0.35), which is indicated by the equivalent ellipse drawn in blue; (*D) equivalent diameter (15.2 mm), which is the circle of area equivalent to the cross-sectional area; (*E) major diameter (15.8 mm), which is the longest segment of the equivalent ellipse (indicated by the inner line); and (*F) minor diameter (14.8 mm), which is the smallest segment (indicated by the inner line).

https://doi.org/10.1371/journal.pone.0200754.g001

1) Area: the measured area of the tracheal lumen intersected by the cross-sectional plane. The area was calculated by multiplying the pixel area value by the number of pixels present, as shown in Eq 1,

$$Area = ps * n \tag{1}$$

where ps is the individual area of each pixel and n is the number of pixels.

2) Perimeter: the length of the outer margin of the tracheal lumen.

3) Eccentricity: a parameter associated with the ellipsoidal shape of a given region. It is the ratio of the focus of the ellipse to its largest diameter. The result is a value between 0 and 1, where 0 is the representation of a circle and 1 is the representation of a line. Fig 1*C illustrates eccentricity as the equivalent ellipse (shown in blue); the outer edge of the tracheal lumen is shown in red. The eccentricity was calculated according to Eq 2,

$$Eccentricity = 2 * \frac{\sqrt{\frac{(MaAL)^2}{2} - \frac{(MiAL)^2}{2}}}{MaAL} \tag{2}$$

where $MaAL$ represents the largest diameter and $MiAL$ the smallest diameter of the ellipse.

4) Equivalent diameter: a circle of the same area as the region intersected by the cross-sectional plane. In Fig 1*D, the equivalent diameter is shown as a blue circle with an area equivalent to that of the tracheal lumen in the same region. The equivalent diameter was calculated using Eq 3,

$$Equivalent\ diameter = \frac{\sqrt{4 * area}}{\pi} \tag{3}$$

5) Major diameter: the length of the longest segment of the ellipse equivalent to the region. Similarly, the minor diameter represents the length of the smallest segment of the ellipse. Fig 1*E and 1*F denote the perimeter of the tracheal lumen (red) along with its ellipse (blue); the center lines indicate the largest and the smallest segment, respectively, of each region.

We also calculated the tortuosity (sinuosity index), which is the deviation of the central axis of the trachea considering its proximal and distal extremities. Mathematically, the tortuosity was determined from the sum of the Euclidean distance of each segment of the trachea, with a distance equivalent to the thickness of each cross-section of the original CT image, divided by the Euclidean distance of the end points of the trachea. The tortuosity may be expressed succinctly using the formula described in Eq 4,

$$Tortuosity = \frac{L}{vd} \tag{4}$$

where L is the length of the trachea and vd is the vectorial distance between the points at the extremities (Fig 2). The tortuosity is expressed as a value ≥ 1; the higher the index, the greater the tortuosity of the trachea [27].

Fig 3 exemplifies the skeletonization process of the trachea of a study participant. The figure shows the three-dimensional reconstruction images of the trachea in two different planes.

There is inherent variability in the measures used to determine the quantitative metrics of eccentricity and tortuosity. For eccentricity, there is a value for each section plane of the trachea of a given individual (Fig 3). We use the median to represent the value of each individual; therefore, there are n medians, where n is the number of elements in each group of variables.

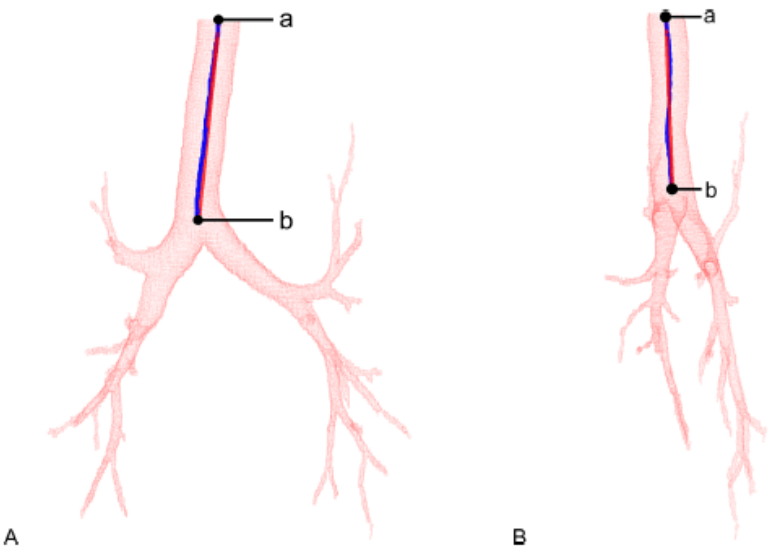


Fig 2. Measurement of the tortuosity of the trachea. Images in the coronal (A) and sagittal (B) planes are shown. In this scheme, *L* is the length of the trachea (the total length of 'ab', shown in blue) and *vd* is the vectorial distance between the points at the extremities (the length of the shortest possible path between 'a' and 'b', shown in red).

https://doi.org/10.1371/journal.pone.0200754.g002

For tortuosity, only one value was calculated for each trachea; it depends on the extreme values (proximal and distal), as shown in Fig 2.

Statistical analysis

To better evaluate the effect of the extent of ILD-SSc on the clinical variables, PFTs, and CT trachea volumetric parameters, we divided the participants into three subgroups as follows: 1) a control group; 2) a scleroderma group with no pulmonary involvement/limited ILD; and 3) a scleroderma group with extensive ILD. ANOVA followed by the Bonferroni post hoc test was used to compare the results obtained for the patients in these three groups. Comparison of the clinical variables, PFTs, and CT trachea volumetric parameters of the SSc patients without pulmonary involvement with those of control subjects and with those of SSc patients with no pulmonary involvement/limited ILD-SSc and SSc patients with extensive ILD-SSc was performed using Student's t-test for independent samples in the case of numerical data and using the chi-square or Fisher's exact test in the case of categorical data. To evaluate the associations between the numerical variables of PFTs and CT trachea volumetric parameters, the Pearson correlation coefficient (r) was used. The criterion for determining significance was 5%. Statistical analysis was performed using SAS 6.11 software (SAS Institute, Inc., Cary, NC, USA).

Results

Of the 43 patients included in the study, 15 were excluded for the following reasons: reported being a smoker (n = 8); history of previous pleuropulmonary disease not associated with

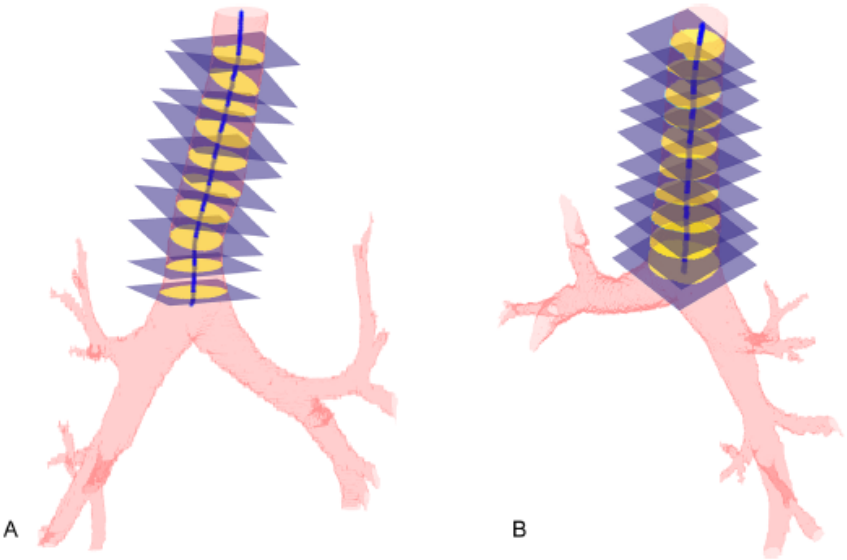


Fig 3. Skeletonization process of the trachea. Three-dimensional reconstruction of the trachea of one study participant in two planes (A and B). The median values showed in the ten planes of square cross-sections are as follows: area = 184.5 mm^2; perimeter = 47.6 mm; eccentricity = 0.51; equivalent diameter = 15.3 mm; major diameter = 15.9 mm; minor diameter = 14.4 mm; and tortuosity = 1.027.

https://doi.org/10.1371/journal.pone.0200754.g003

scleroderma (n = 3); inability to perform PFTs (n = 2); scleroderma-polymyositis overlap syndrome (n = 1); and scleroderma-rheumatoid arthritis overlap syndrome (n = 1). In the total sample of patients, the mean age of subjects with scleroderma was 52.5 ± 11 years, and 25 (89.3%) were women. The disease duration was 4.21 ± 2.50 years from the onset of non-Raynaud's phenomenon and 9.65 ± 5.13 years from the onset of Raynaud's phenomenon symptoms. Nineteen patients (67.9%) had lc-SSc, and nine (32.1%) had dc-SSc; of the latter, six (21.4%) had a mRSS > 18. Anti-topoisomerase I antibody (ATA), anticentromere antibody and anti-RNA polymerase III were positive in 13 (46.4%), six (21.4%) and three (10.7) patients, respectively; no autoantibodies were identified in six (21.4%) patients. Seven patients (25%) had an $FEF_{50\%}/FIF_{50\%}$ ratio > 1.50. Comparisons of clinical data, pulmonary function parameters and computed tomography scores in the control group, the scleroderma group with no pulmonary involvement/limited ILD, and the scleroderma group with extensive ILD are shown in Table 1. In addition, comparisons of lung function parameters in the control group and in patients without pulmonary involvement on CT did not show any significant difference ($p > 0.05$ for all).

On thorax CT, 16 patients (57.1%) were classified as having no pulmonary involvement (n = 8) or limited pulmonary involvement (n = 8), while 12 (42.9%) were classified as having extensive pulmonary involvement. In the measurements obtained through CT trachea volumetry, patients with scleroderma presented higher values for the following parameters: area, eccentricity, major diameter, minor diameter, and tortuosity. Table 2 compares the CT trachea volumetry findings for control subjects, patients in the scleroderma group with no pulmonary involvement/limited ILD, and patients in the scleroderma group with extensive ILD.

Table 1. Demographic characteristics, clinical data, pulmonary function and computed tomography scores of patients with scleroderma and of patients in the control group.

Variable	Control group (n = 27)	Scleroderma group with no pulmonary involvement/limited ILD (n = 16)	Scleroderma group with extensive ILD (n = 12)	p value
Demographic data				
Females	23 (85.2)	14 (87.5)	11 (91.7)	0.41
Age (years)	49 ± 12.6	51.7 ± 10	52.8 ± 11.2	0.28
BMI (kg/m^2)	27.2 ± 5.87	24.2 ± 5.22	25.1 ± 5.30	0.09
Type of scleroderma				
lc-SSc	-	12 (75)	7 (58.3)	0.34
dl-SSc	-	4 (25)	5 (41.7)	
mRSS	-	10.3 ± 7.44	11.8 ± 7.60	0.12
Type of autoantibody				
Anti-topoisomerase I antibody	-	5 (31.2)	8 (66.7)	0.28
Anticentromere antibody	-	5 (31.2)	1 (8.33)	
Anti-RNA polymerase III	-	2 (12.5)	1 (8.33)	
Autoantibody not identified	-	4 (25)	2 (16.7)	
Lung function				
FVC (L)	3.08 ± 0.81	2.62 ± 0.72*	2.25 ± 0.87*†	**0.008**
FVC (% predicted)	102.4 ± 19.8	85 ± 19.3*	68 ± 21.5*†	**0.005**
FEV_1 (L)	2.51 ± 0.75	2.11 ± 0.63*	1.80 ± 0.59*†	**0.009**
FEV_1 (% predicted)	100.7 ± 18.5	83.1 ± 16*	69.2 ± 17.8*†	**0.006**
FEV_1/FVC (%)	78 ± 12.6	80 ± 12	86 ± 11.3	0.11
PEF (L/s)	7.75 ± 2.14	6.25 ± 2.11	5.26 ± 1.97*	**0.032**
PEF (% predicted)	110 ± 31.7	86 ± 30.5	75.5 ± 29.2*	**0.025**
$FEF_{50\%}/FIF_{50\%}$ (%)	1.04 ± 0.62	1.28 ± 0.72*	1.34 ± 0.70*	**0.046**
DLco (mL/min/mmHg)	20.6 ± 3.44	18 ± 4.22*	9.27 ± 3.23*†	**0.003**
DLco (% predicted)	97 ± 21.6	70.3 ± 18.3*	60 ± 17.8*†	**0.001**
Raw (cm H_2O/L/s)	1.58 ± 0.75	1.78 ± 0.77	1.93 ± 0.78	0.32
SGaw (L/s/cm H_2O/L)	0.210 ± 0.078	0.245 ± 0.089	0.257 ± 0.103	0.09
Computed tomography scores				
ILD-SSc extent (% parenchyma)		9.63 ± 8.80	28.1 ± 10.4	**< 0.0001**
Coarseness of pulmonary fibrosis		2.15 ± 1.44	6.35 ± 4.20	**< 0.0001**

The values shown are means ± SD or number (%). Bold type indicates significant differences.

*Significantly different from control group.

†Significantly different from scleroderma group with no pulmonary involvement/limited ILD-SSc. ILD-SSc = interstitial lung disease associated with scleroderma. BMI = body mass index; lc-SSc = limited cutaneous form; dc-SSc = diffuse cutaneous form; mRSS = modified Rodnan skin score; FVC = forced vital capacity; FEV_1 = forced expiratory volume in one second; PEF = peak expiratory flow; $FEF_{50\%}/FIF_{50\%}$ = ratio between the forced expiratory flow and forced inspiratory flow at 50% of forced vital capacity; DLco = diffusing capacity for carbon monoxide; Raw: airway resistance; SGaw: specific airway conductance.

https://doi.org/10.1371/journal.pone.0200754.t001

We also compared patients without pulmonary involvement on CT (n = 8) with controls. In this evaluation, patients without pulmonary involvement on CT showed higher values, with significant differences in the following CT tracheal volumetric findings: area (210.7 ± 35 vs. 207.6 ± 32.6 mm^2, p = 0.031; eccentricity (0.50 ± 0.05 vs. 0.46 ± 0.04, p = 0.018); major diameter (17.5 ± 1.70 vs. 17.1 ± 2.22 mm, p = 0.039); minor diameter (14.9 ± 1.68 vs. 14.5 ± 2.11 mm, p = 0.044); and tortuosity (1.039 ± 0.017 vs. 1.023 ± 0.014, p = 0.01).

Table 2. Variables of CT trachea volumetry according to group.

Variable	Control group (n = 27)	Scleroderma group with no pulmonary involvement/limited ILD (n = 16)	Scleroderma group with extensive ILD (n = 12)	p value
Area (mm^2)	207.6 ± 32.6	211.3 ± 38.2*	214.6 ± 43.6*	**0.028**
Perimeter (mm)	45.3 ± 4.12	49.2 ± 4.45	51.5 ± 4.67	0.12
Eccentricity	0.46 ± 0.04	0.51 ± 0.06*	0.54 ± 0.06*	**0.013**
Equivalent diameter (mm)	16.1 ± 1.24	16.2 ± 1.55	16.5 ± 1.60	0.82
Major diameter (mm)	17.1 ± 2.22	17.6 ± 1.92*	18.2 ± 2.01*	**0.039**
Minor diameter (mm)	14.5 ± 2.11	15 ± 1.72*	15.3 ± 1.85*	**0.043**
Tortuosity	1.023 ± 0.014	1.039 ± 0.014*	1.058 ± 0.015*†	**0.009**

The values shown are means ± SD or number (%). Bold type indicates significant differences.

*Significantly different from control group.

†Significantly different from scleroderma group with no pulmonary involvement/limited ILD-SSc. ILD-SSc = interstitial lung disease associated with scleroderma.

https://doi.org/10.1371/journal.pone.0200754.t002

We evaluated the correlations between the parameters provided by CT trachea volumetry and the pulmonary function indices (absolute values) (Table 3). The tracheal area was negatively correlated with $FEF_{50\%}/FIF_{50\%}$ ($r = -0.44$, $p = 0.03$), while the eccentricity was negatively correlated with FVC ($r = -0.57$, $p = 0.002$) (Fig 4) and forced expiratory volume in one second ($r = -0.50$, $p = 0.009$). The equivalent diameter was negatively correlated with $FEF_{50\%}/FIF_{50\%}$ ($r = -0.46$, $p = 0.02$), while tortuosity was negatively correlated with peak expiratory flow (PEF) ($r = -0.51$, $p = 0.008$) (Fig 5). A positive correlation of tortuosity with coarseness of pulmonary fibrosis in CT was also found ($r = 0.45$, $p = 0.02$).

We also evaluated the correlations between the clinical findings and the parameters provided by CT trachea volumetry. The mRSS showed a positive correlation with eccentricity ($r = 0.62$, $p < 0.001$) and tortuosity ($r = 0.51$, $p = 0.007$). In turn, the presence of anti-topoisomerase I antibody showed a positive correlation with tortuosity ($r = 0.45$, $p = 0.03$).

Discussion

The main findings of the present study were that in a sample composed predominantly of scleroderma patients with associated ILD, the trachea showed greater area, eccentricity, and

Table 3. Pearson's correlation coefficients for CT trachea volumetry, pulmonary fibrosis and pulmonary function.

	FVC	FEV_1	FEV_1/FVC	PEF	$FEF_{50\%}/FIF_{50\%}$	DLco	Raw	SGaw	Coarseness of fibrosis
Area	-0.13	-0.11	-0.06	0.24	**-0.44***	-0.24	0.12	-0.19	0.25
Perimeter	-0.09	-0.07	-0.08	0.25	**-0.47†**	-0.18	0.13	-0.21	0.17
Eccentricity	**-0.57‡**	**-0.50†**	-0.12	-0.07	-0.17	-0.35	-0.11	-0.04	0.26
Equivalent diameter	-0.11	-0.10	-0.07	0.25	**-0.46†**	-0.27	0.11	-0.21	0.18
Major diameter	-0.04	-0.06	-0.08	0.25	**-0.42***	-0.16	0.07	-0.20	0.22
Minor diameter	-0.22	-0.19	-0.03	0.21	**-0.47†**	-0.11	0.06	-0.17	0.25
Tortuosity	-0.17	-0.15	-0.26	**-0.51†**	-0.28	-0.12	0.09	-0.15	**0.45***

FVC = forced vital capacity; FEV_1 = forced expiratory volume in one second; PEF = peak expiratory flow; $FEF_{50\%}/FIF_{50\%}$ = ratio between the forced expiratory flow and forced inspiratory flow at 50% of forced vital capacity; DLco = diffusing capacity for carbon monoxide; Raw = airway resistance; SGaw = specific airway conductance. Bold type indicates significant differences.

*$p < 0.05$

†$p < 0.01$

‡$p < 0.005$

https://doi.org/10.1371/journal.pone.0200754.t003

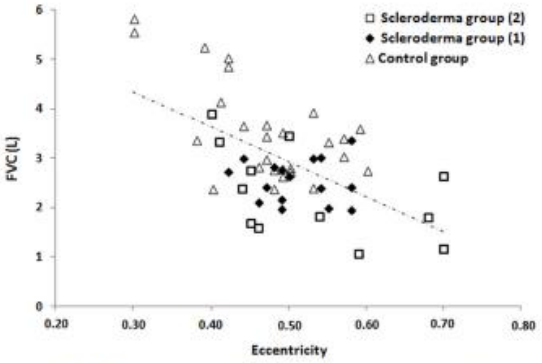


Fig 4. Relationship between forced vital capacity (FVC) and eccentricity of the trachea (r = -0.57, p = 0.002). Scleroderma group (1) = scleroderma group with no pulmonary involvement/limited interstitial lung disease; scleroderma group (2) = scleroderma group with extensive interstitial lung disease.

https://doi.org/10.1371/journal.pone.0200754.g004

tortuosity. In these patients, a greater tracheal tortuosity led to a smaller airflow; in addition, the lower the tracheal diameter, the greater the degree of airway obstruction. Furthermore, tracheal tortuosity was associated with the presence of pulmonary fibrosis, the presence of ATA, and greater mRSS. To our knowledge, this is the first study to show abnormalities in the structure and function of the trachea as well as its correlations with clinical findings in patients with scleroderma.

In recent years, several CT image enhancement techniques have been developed in an attempt to find a method that is both quantitative and reliable and allows more accurate assessment than conventional visual reading. Compared to the traditional visual interpretation of

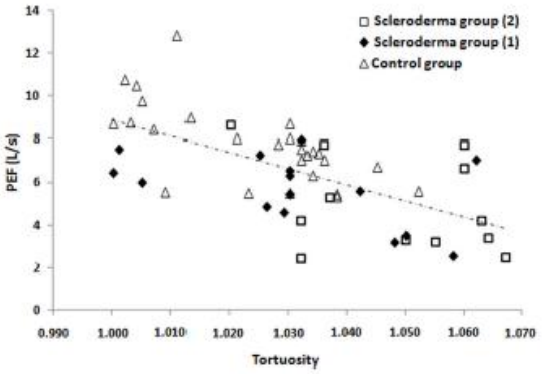


Fig 5. Relationship between peak expiratory flow (PEF) and tortuosity of the trachea (r = -0.51, p = 0.008). Scleroderma group (1) = scleroderma group with no pulmonary involvement/limited interstitial lung disease; scleroderma group (2) = scleroderma group with extensive interstitial lung disease.

https://doi.org/10.1371/journal.pone.0200754.g005

CT findings, computer-based automatic evaluation can improve the objectivity, sensitivity, and repeatability of quantitative chest imaging analyses [2,10]. In this study, we applied a computer-assisted method to evaluate the tracheas of patients with scleroderma in an unprecedented way considering that computerized evaluation has previously only been used in the study of ILD-SSc [2,28]. Our results indicate that CT trachea volumetry and subsequent skeletonization of the images provide a number of interesting measures for the best evaluation of patients with scleroderma. We observed that patients with scleroderma presented greater area, eccentricity, and tortuosity of the trachea. In scleroderma, myofibroblasts constitutively secrete components of the extracellular matrix and exert excessive cicatrization of the skin and internal organs [3,29]. Thus, we think that the cicatricial changes that occur at the level of the neck can directly impact the geometry of the trachea and alter the measures of diameter and tortuosity of this structure.

In our study, the deviations of the trachea were measured by means of the tortuosity (sinuosity index) after the skeletonization process. The skeletonization process has been used previously by us to obtain the skeletons of specific structures by thinning [10]. Interestingly, we found an association between tracheal tortuosity and the pulmonary fibrosis score obtained by semi-quantitative CT reading. This association between structural alterations of the trachea and the lung, despite being evaluated by different techniques in our study, suggests that the deformity of the trachea in scleroderma may be an extension of the fibrotic disease that occurs at the pulmonary level. However, it is worth mentioning that in our study, patients without pulmonary involvement showed higher values in tracheal volumetry than control subjects. This finding suggests that factors other than pulmonary fibrosis are involved in the tracheal abnormalities that occur in SSc patients. Consistent with this observation, several studies have shown impairment of anatomic structures above the trachea in scleroderma patients; laryngeal dysfunction is relatively common, with pathologic findings demonstrating fibrinoid degeneration and an increase in collagen fibers [30,31]. Considering the promising advent of user-friendly software, evaluation of the trachea in patients with scleroderma may be an interesting approach both in clinical practice and in trials. Thus, precise characterization of the trachea in scleroderma may offer an additional tool for the follow-up of these patients and may be helpful in the evaluation of clinical treatment.

The presence of obstructive abnormalities in the cervical trachea can be detected by the flow-volume loop of spirometry even when there is no clinical suspicion, with the most used index being the ratio $FEF_{50\%}/FIF_{50\%}$ [32]. In the present study, 25% of our patients had an $FEF_{50\%}/FIF_{50\%} > 1.50$, and we found negative correlations between $FEF_{50\%}/FIF_{50\%}$ and several indices measured by CT trachea volumetry, including area, perimeter, equivalent diameter, major diameter, and minor diameter. Consistent with our findings, Miranda et al. [33] used the forced oscillation technique in patients with scleroderma and found an increase in mean resistance, a parameter that reflects changes in the most central airways. Another study using impulse oscillometry observed an increase in the resistive and reactive properties of the respiratory system in patients with scleroderma, and these alterations were correlated with the findings of fibrosis in CT [34]. Interestingly, we observed an association between tracheal tortuosity and PEF (which reflects flow through large airways), supporting the notion that tracheal deviation is a key contributor to the reduction of airflow in the large airways of patients with scleroderma. However, quantitative measurement of eccentricity was not correlated with $FEF_{50\%}/FIF_{50\%}$. One possible explanation for this finding is that increased tracheal area in scleroderma patients may at least partially counterbalance the effects of reduced inspiratory flow in these patients.

In our study, patients with extensive ILD displayed greater tracheal area than patients without pulmonary involvement and patients with limited ILD; all of these patients, in turn, displayed greater tracheal area than the control subjects. Several recent studies have shown that larger esophageal diameter is associated with higher pulmonary fibrosis and worse lung function in

individuals with scleroderma [35,36]. Since microaspiration secondary to gastro-esophageal reflux is associated with the progression of pulmonary fibrosis in scleroderma [37,38], we think that the tracheal pathology may also contribute to this phenomenon. Longitudinal and controlled studies of larger numbers of patients are required to evaluate whether or not tracheal pathology is involved in the progression of lung fibrosis in patients with scleroderma.

In the present study, the mean value of FVC was higher in the control group and lower in the scleroderma group with extensive ILD and showed an intermediate value in the scleroderma group with no pulmonary involvement (or limited ILD) (102.4 ± 19.8% vs. 85 ± 19.3% vs. 68 ± 21.5% predicted, $p = 0.005$). Although the restrictive pattern in scleroderma is largely explained by the presence of ILD, a reduced compliance of the respiratory system may also be due to chest wall tightening from skin thickening, pleural disease, cardiac involvement, and respiratory muscle weakness [39–41]. Since no significant difference was observed in FVC between the control group and patients without pulmonary involvement in CT, we believe that ILD is the main contributor to the restrictive damage in our sample. However, we observed a significant correlation between FVC and the quantitative measurement of tracheal eccentricity. A possible explanation for this association is that overproduction of collagen and deposition of connective tissue, which are primary pathophysiological mechanisms of SSc, cause both abnormal tracheal geometry and reduced lung volume in these patients.

In scleroderma, cutaneous involvement occurs due to the increased thickness and hardness of the skin, which leads to shrinking of the skin in deeper structures; although cutaneous involvement is usually most prominent on the face and hands, these abnormalities may extend to the upper chest [42,43]. Some studies have shown that subclinical involvement of the upper chest is detectable by high-frequency ultrasound even with normal palpation [44,45]. In our study, the mRSS (a key measure in the clinical evaluation of patients with scleroderma) was associated with the eccentricity values provided by CT trachea volumetry. Since eccentricity measures the deviation of a conical structure in relation to its circumference [46], we think that skin thickening that occurs at the neck level may negatively impact the geometry of the trachea in patients with scleroderma [47]. Consistent with our findings, Kim et al. [48] found an association between the fibrosis score detected by CT using a computer-assisted method and the mRSS of patients with scleroderma both at baseline and during a 12-month follow-up period. This reinforces the idea that the measure of eccentricity may be a marker of the severity of the process of collagen hyalinization and abnormalities in the elastic tissues that surround the neck and may extend to the intrathoracic structures of patients with scleroderma [42]. Considering that future directions for the management of patients with scleroderma, including epigenetic modulation and antifibrotic or biological therapy, are being discussed [49,50], we think that the parameters provided by the measurement of tracheal geometry can contribute to the evaluation of the outcomes.

The identification of reliable and consistent biomarkers that can be used to predict the course of specific diseases is necessary for better stratification and management of patients and would be of great use in the treatment of a multifaceted disease such as scleroderma [3]. In this context, several attempts have been made in recent years to correlate possible serum biomarkers with clinical features distinct from scleroderma. Several investigators have shown an association between ATA and the risk of development and progression of pulmonary fibrosis in patients with scleroderma [51,52]. A recent study has shown that topoisomerase I peptide-loaded dendritic cells induce not only the autoantibody response but also cutaneous and pulmonary fibrosis [52]. In the present study, we observed a positive correlation between the presence of ATA and tracheal tortuosity. This significant correlation must be viewed cautiously since we did not evaluate predicting values. However, we think that this finding could serve as a starting point for longitudinal studies designed to evaluate a possible contribution of ATA to the management of tracheal pathology in patients with scleroderma.

A critical analysis of the limitations of the present study is pertinent. First, the sample size is small, and the sample is representative of only one center. Second, the sample is composed predominantly of scleroderma patients with associated ILD. Although we observed significant differences in the abnormalities of trachea volumetry between control subjects and patients without pulmonary involvement on CT, a greater number of patients in this group could allow for more robust conclusions. Third, CT pulmonary densitovolumetry could have aided in the study of correlations between tracheal changes and those observed in the lungs and lower airways. Fourth, an assessment comparing patients with ILD-SSc with patients with ILD due to other causes (e.g., idiopathic pulmonary fibrosis, rheumatoid arthritis-ILD) might better define the role of scleroderma in the development of tracheal abnormalities. Finally, the evaluation of other biomarkers could have helped us better understand the tracheal disease in scleroderma and might have opened new horizons in the field of precision medicine. In fact, new laboratory markers (including TGFβ1, IL-6, sPD-1, sPD-L2, and CXCL4) have been associated with higher mRSS score and more pronounced changes in thoracic CT [3]. Despite these limitations, the quantitative analysis of the trachea through computer software offers a discriminant method that can help produce an objective measure and obtain prognostic information in scleroderma.

In conclusion, the present study shows that, in a sample composed predominantly of scleroderma patients with associated ILD, there were abnormalities in the geometry of the trachea in eccentricity, diameter, and tortuosity. In these patients, abnormalities in the geometry of the trachea were associated with markers of functional obstruction at the level of the cervical trachea. In addition, the measurement of tracheal tortuosity was correlated with cutaneous involvement, the degree of pulmonary fibrosis, and the presence of ATA. Although the encouraging data presented here require further validation in prospective studies, we believe that skeletonization and CT trachea volumetry may improve the ability of radiologists and rheumatologists to accurately assess the tracheas of patients with scleroderma both in clinical practice and in trials.

Author Contributions

Conceptualization: Bruno Rangel Antunes Silva, Rosana Souza Rodrigues, Rogério Rufino, Cláudia Henrique Costa, Veronica Silva Vilela, Roger Abramino Levy, Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Data curation: Bruno Rangel Antunes Silva.

Formal analysis: Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Funding acquisition: Agnaldo José Lopes.

Methodology: Bruno Rangel Antunes Silva, Rogério Rufino, Cláudia Henrique Costa, Veronica Silva Vilela, Roger Abramino Levy, Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Resources: Rogério Rufino, Cláudia Henrique Costa, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Software: Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho.

Supervision: Rosana Souza Rodrigues, Cláudia Henrique Costa, Roger Abramino Levy, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Validation: Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho.

Writing – original draft: Bruno Rangel Antunes Silva, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

Writing – review & editing: Bruno Rangel Antunes Silva, Rosana Souza Rodrigues, Rogério Rufino, Cláudia Henrique Costa, Veronica Silva Vilela, Roger Abramino Levy, Alan Ranieri Medeiros Guimarães, Alysson Roncally Silva Carvalho, Agnaldo José Lopes.

References

1. 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. Arthritis and Rheumatism. 2013; 65(11):2737–47. https://doi.org/10.1002/art.38098 PMID: 24122180; PubMed Central PMCID: PMC3930146.
2. Salaffi F, Carotti M, Di Donato E, Di Carlo M, Ceccarelli L, Giuseppetti G. Computer-aided tomographic analysis of interstitial lung disease (ILD) in patients with systemic sclerosis (SSc): correlation with pulmonary physiologic tests and patient-centred measures of perceived dyspnea and functional disability. PLoS One. 2016; 11(3):e0149240. https://doi.org/10.1371/journal.pone.0149240 PMID: 26930658; PubMed Central PMCID: PMC4773230.
3. Barsotti S, Bruni C, Orlandi M, Della Rossa A, Marasco E, Codullo V, et al. One year in review 2017: systemic sclerosis. Clinical and Experimental Rheumatology. 2017; 35(Suppl 106 4):3–20. PMID: 29035173.
4. Barnes J, Mayes MD. Epidemiology of systemic sclerosis: incidence, prevalence, survival, risk factors, malignancy, and environmental triggers. Current Opinion in Rheumatology. 2012; 24(2):165–70. https://doi.org/10.1097/BOR.0b013e32834ff2e8 PMID: 22269658.
5. Sticherling M. Systemic sclerosis-dermatological aspects. Part 1: Pathogenesis, epidemiology, clinical findings. Journal der Deutschen Dermatologischen Gesellschaft. 2012; 10(10):705–18. https://doi.org/10.1111/j.1610-0387.2012.07999.x PMID: 22913330.
6. Silva BRA, Rufino R, Costa CH, Vilela VS, Levy RA, Lopes AJ. Ventilation distribution and small airway function in patients with systemic sclerosis. Portuguese Journal of Pulmonology. 2017; 23(3):132–8. https://doi.org/10.1016/j.rppnen.2017.01.004 PMID: 28258938.
7. Ooi GC, Mok MY, Tsang KWT, Wong Y, Khong PL, Fung PCW, et al. Interstitial lung disease in systemic sclerosis: an HRCT-clinical correlative study. Acta Radiologica. 2003; 44(3):258–64. PMID: 12751995
8. Kim HG, Tashkin DP, Clements PJ, Li G, Brown MS, Elashoff R, et al. A computer-aided diagnosis system for quantitative scoring of extent of lung fibrosis in scleroderma patients. Clinical and Experimental Rheumatology. 2010; 28(5 Suppl 62):S26–35. PMID: 21050542; PubMed Central PMCID: PMC3177564.
9. Ariani A, Carotti M, Gutierrez M, Bichisecchi E, Grassi W, Giuseppetti GM, et al. Utility of an open-source DICOM viewer software (OsiriX) to assess pulmonary fibrosis in systemic sclerosis: preliminary results. Rheumatology International. 2014; 34(4):511–6. https://doi.org/10.1007/s00296-013-2845-6 PMID: 23949623.
10. Camilo GB, Carvalho ARS, Guimarães ARM, Kasuki L, Gadelha MR, Mogami R, et al. Computed tomography airway lumen volumetry in patients with acromegaly: Association with growth hormone levels and lung function. Journal of Medical Imaging and Radiation Oncology. 2017; 61(5):591–9. https://doi.org/10.1111/1754-9485.12598 PMID: 28217888.
11. Williamson JP, James AL, Phillips MJ, Sampson DD, Hillman DR, Eastwood PR. Quantifying tracheobronchial tree dimensions: methods, limitations and emerging techniques. The European Respiratory Journal. 2009; 34(1):42–55. https://doi.org/10.1183/09031936.00020408 PMID: 19567601.
12. Yamashiro T, Tsubakimoto M, Nagatani Y, Moriya H, Sakuma K, Tsukagoshi S, et al. Automated continuous quantitative measurement of proximal airways on dynamic ventilation CT: initial experience using an ex vivo porcine lung phantom. International Journal of Chronic Obstructive Pulmonary Disease. 2015; 10:2045–54. https://doi.org/10.2147/COPD.S87588 PMID: 26445535; PubMed Central PMCID: PMC4590570.
13. Zhang C, Wang H, Cao J, Li C, Mi W, Yang L, et al. Measurement and analysis of the tracheobronchial tree in Chinese population using computed tomography. PLoS One. 2015; 10(6):e0130239. https://doi.org/10.1007/s00117-003-0990-8
14. Sorantin E, Halmai C, Erdohelyi B, Palágyi K, Nyúl LG, Ollé K, et al. 3D cross section of the laryngotracheal tract: a new method for visualization and quantification of tracheal stenosis. Radiologe. 2003; 43 (12):1056–68. https://doi.org/10.1007/s00117-003-0990-8 PMID: 14668994.
15. Yamashiro T, Moriya H, Tsubakimoto M, Matsuoka S, Murayama S. Continuous quantitative measurement of the proximal airway dimensions and lung density on four-dimensional dynamic-ventilation CT in smokers. International Journal of Chronic Obstructive Pulmonary Disease. 2016; 11:755–64. https://doi.org/10.2147/COPD.S100658 PMID: 27110108; PMCID Central PMC4835141.

16. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. The Journal of Rheumatology. 1988; 15(2):202–5. PMID: 3361530.

17. Clements P, Lachenbruch P, Siebold J, White B, Weiner S, Martin R, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. The Journal of Rheumatology. 1995; 22(7):1281–5. PMID: 7562759.

18. Culver BH, Graham BL, Coates AL, et al. Recommendations for a standardized pulmonary function report. An official American Thoracic Society technical statement. American Journal of Respiratory and Critical Care Medicine. 2017; 196(11):1463–72. https://doi.org/10.1164/rccm.201710-1981ST PMID: 29192835.

19. Pereira CAC, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. Jornal Brasileiro de Pneumologia. 2007; 33(4):397–406. https://doi.org/10.1164/rccm.200706-877OC

20. Neder JA, Andreoni S, Peres C, Nery LE. Reference values for lung function tests. III. Carbon monoxide diffusing capacity (transfer factor). Brazilian Journal of Medical and Biological Research. 1999; 32 (6):729–37. https://doi.org/10.1590/S0100-879X1999000600008 PMID: 10412551.

21. Morewood DJ, Belchetz PE, Evans CC, Whitehouse GH. The extrathoracic airway in acromegaly. Clinical Radiology. 1986; 37(3):243–6. PMID: 3709048.

22. Goh NS, Desai SR, Veeraraghavan S, Hansell DM, Copley SJ, Maher TM, et al. Interstitial lung disease in systemic sclerosis: a simple staging system. American Journal of Respiratory and Critical Care Medicine. 2008; 177(11):1248–54. https://doi.org/10.1164/rccm.200706-877OC PMID: 18369202.

23. Le Gouellec N, Duhamel A, Perez T, Hachulla AL, Sobanski V, Faivre JB, et al. Predictors of lung function test severity and outcome in systemic sclerosis-associated interstitial lung disease. PLoS One. 2017; 12(8):e0181692. https://doi.org/10.1371/journal.pone.0181692 PMID: 28763468; PubMed Central PMCID: PMC5538660.

24. Lopes AJ, Capone D, Mogami R, Lanzillotti RS, Melo PL, Jansen JM. Severity classification for idiopathic pulmonary fibrosis by using fuzzy logic. Clinics (Sao Paulo). 2011; 66(6):1015–9. https://doi.org/10.1590/S1807-59322011000600016 PMID: 21808868; PubMed Central PMCID: PMC3129967.

25. Khanna D, Nagaraja V, Tseng CH, Abtin F, Suh R, Kim G, et al. Predictors of lung function decline in scleroderma-related interstitial lung disease based on high-resolution computed tomography: implications for cohort enrichment in systemic sclerosis-associated interstitial lung disease trials. Arthritis Research and Therapy. 2015; 17:372. https://doi.org/10.1186/s13075-015-0872-2 PMID: 26704522; PubMed Central PMCID: PMC4718035.

26. Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin J-C, Pujol S, et al. 3D Slicer as an image computing platform for the quantitative imaging network. Magnetic Resonance Imaging. 2012; 30(9):1323–41. https://doi.org/10.1016/j.mri.2012.05.001 PMID: 22770690

27. Zámolyi A, Székely B, Draganits E, Timár G. Neotectonic control on river sinuosity at the western margin of the Little Hungarian Plain. Geomorphology 2010; 122: 231–43. https://doi.org/10.1155/2015/834262; PubMed Central PMCID: PMC4299560.

28. Salaffi F, Carotti M, Boselio S, Ciapetti A, Gutierrez M, Bichisecchi E, et al. Computer-aided quantification of interstitial lung disease from high resolution computed tomography images in systemic sclerosis: correlation with visual reader-based score and physiologic tests. BioMed Research International. 2015; 2015:834262. https://doi.org/10.1155/2015/834262 PMID: 25629053; PubMed Central PMCID: PMC4299560.

29. Bouros D, Wells AU, Nicholson AG, Colby TV, Polychronopoulos V, Pantelidis P, et al. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. American Journal of Respiratory and Critical Care Medicine. 2002; 165(12):1581–6. https://doi.org/10.1164/rccm.2106012 PMID: 12070056.

30. Pepper JP, Kupfer RA, McHugh JB, Hogikyan ND. Histopathologic findings and clinical manifestations in a patient with dysphonia and vocal fold involvement by systemic sclerosis. Archives of Otolaryngology Head & Neck Surgery. 2011; 137(8):816–9. https://doi.org/10.5114/aoms.2016.57582; PubMed Central PMCID: PMC4754368.

31. Ramos HV, Pillon J, Kosugi EM, Fujita R, Pontes P. Laryngeal assessment in rheumatic disease patients. Brazilian Journal of Otorhinolaryngology. 2005; 71(4):499–503. S0034-72992005000400017 PMID: 16446967.

32. Camilo GB, Guimarães FS, Mogami R, Faria AC, Melo PL, Lopes AJ. Functional changes are associated with tracheal structural abnormalities in patients with acromegaly. Archives of Medical Science. 2016; 12(1):78–88. https://doi.org/10.5114/aoms.2016.57582 PMID: 26925121; PubMed Central PMCID: PMC4754368.

33. Miranda IA, Dias Faria AC, Lopes AJ, Jansen JM, Lopes de Melo P. On the respiratory mechanics measured by forced oscillation technique in patients with systemic sclerosis. PLoS One 2013; 8(4):e61657. https://doi.org/10.1371/journal.pone.0061657 PMID: 23637877; PubMed Central PMCID: PMC3637442.

34. Aronsson D, Hesselstrand R, Bozovic G, Wuttge DM, Tufvesson E. Airway resistance and reactance are affected in systemic sclerosis. European Clinical Respiratory Journal. 2015; 2:28667. https://doi.org/10.3402/ecrj.v2.28667 PMID: 26672963; PubMed Central PMCID: PMC4653312.

35. Winstone TA, Hague CJ, Soon J, Sulaiman N, Murphy D, Leipsic J, et al. Oesophageal diameter is associated with severity but not progression of systemic sclerosis-associated interstitial lung disease. Respirology. 2018. [Epub ahead of print]. https://doi.org/10.1111/resp.13309 PMID: 29641847.

36. Richardson C, Agrawal R, Lee J, Almagor O, Nelson R, Varga J, et al. Esophageal dilatation and interstitial lung disease in systemic sclerosis: a cross-sectional study. Seminars in Arthritis and Rheumatism. 2016; 46(1):109–14. https://doi.org/10.1016/j.semarthrit.2016.02.004 PMID: 27033049; PubMed Central PMCID: PMC5500283.

37. Savarino E, Ghio M, Marabotto E, Zentilin P, Sammito G, Cittadini G, et al. Possible connection between gastroesophageal reflux and interstitial pulmonary fibrosis in patients with systemic sclerosis. Recenti Progressi in Medicina. 2009; 100(11):512–6. PMID: 20066883.

38. Lock G, Pfeifer M, Straub RH, Zeuner M, Lang B, Schölmerich J, et al. ssociation of esophageal dysfunction and pulmonary function impairment in systemic sclerosis. The American Journal of Gastroenterology. 1998; 93(3):341–5. https://doi.org/10.1111/j.1572-0241.1998.00341.x PMID: 9517636.

39. van Laar JM, Stolk J, Tyndall A. Scleroderma lung: pathogenesis, evaluation and current therapy. Drugs. 2007; 67(7):985–96. PMID: 17488144.

40. Farrokh D, Abbasi B, Fallah-Rastegar Y, Mirfeizi Z. The extrapulmonary manifestations of systemic sclerosis on chest high resolution computed tomography. Tanaffos. 2015; 14(3):193–200. PMID: 26858765; PubMed Central PMCID: PMC4745188.

41. Lopes AJ, Justo AC, Ferreira AS, Guimaraes FS. Systemic sclerosis: association between physical function, handgrip strength and pulmonary function. Journal of Bodywork and Movement Therapies. 2017; 21(4): 972–77. https://doi.org/10.1016/j.jbmt.2017.03.018 PMID: 29037654.

42. Hasan O, Jessar M, Ashar M, Noordin S, Ahmad T. Systemic sclerosis: clinical manifestations, anesthetic and orthopedic considerations in a patient. International Journal of Surgery Case Reports. 2017; 42:24–8. https://doi.org/10.1016/j.ijscr.2017.11.051 PMID: 29207307; PubMed Central PMCID: PMC5724744.

43. Steen VD. Clinical manifestations of systemic sclerosis. Seminars in Cutaneous Medicine and Surgery. 1998; 17(1):48–54. PMID: 9512107.

44. Sulli A, Ruaro B, Smith V, Paolino S, Pizzorni C, Pesce G, et al. Subclinical dermal involvement is detectable by high frequency ultrasound even in patients with limited cutaneous systemic sclerosis. Arthritis Research and Therapy. 2017; 19(1): 61. https://doi.org/10.1186/s13075-017-1270-8 PMID: 28320447; PubMed Central PMCID: PMC5360023.

45. Hesselstrand R, Scheja A, Wildt M, Akesson A. High-frequency ultrasound of skin involvement in systemic sclerosis reflects oedema, extension and severity in early disease. Rheumatology. 2008; 47 (1):84–7. https://doi.org/10.1093/rheumatology/kem307 PMID: 18077496.

46. Yue Y, Fan Z, Yang W, Pang J, Deng Z, McKenzie E, et al. Geometric validation of self-gating k-space-sorted 4D-MRI vs 4D-CT using a respiratory motion phantom. Medical Physics. 2015; 42(10):5787–97. https://doi.org/10.1118/1.4929552 PMID: 26429253; PubMed Central PMCID: PMC4575318.

47. Adnan ZA. Diagnosis and treatment of scleroderma. Acta Medica Indonesiana. 2008; 40(2):109–12. PMID: 18560030.

48. Kim HJ, Tashkin DP, Gjertson DW, Brown MS, Kleerup E, Chong S, et al. Transitions to different patterns of interstitial lung disease in scleroderma with and without treatment. Annals of the Rheumatic Diseases. 2016; 75(7):1367–71. https://doi.org/10.1136/annrheumdis-2015-208929 PMID: 26757749.

49. O'Reilly S. Epigenetic modulation as a therapy in systemic sclerosis. Rheumatology. 2018. [Epub ahead of print]. https://doi.org/10.1093/rheumatology/key071 PMID: 29579252.

50. George PM, Wells AU. Disease staging and sub setting of interstitial lung disease associated with systemic sclerosis: impact on therapy. Expert Review of Clinical Immunology. 2018; 14(2):127–135. https://doi.org/10.1002/art.39166

51. Hoffmann-Vold AM, Aalokken TM, Lund MB, Garen T, Midtvedt Ø, Brunborg C, et al. Predictive value of serial high-resolution computed tomography analyses and concurrent lung function tests in systemic sclerosis. Arthritis & Rheumatology. 2015; 67(8):2205–12. https://doi.org/10.1002/art.39166 PMID: 25916462.

52. Mehta H, Goulet PO, Nguyen V, Pérez G, Koenig M, Senécal JL, et al. Topoisomerase I peptide-loaded dendritic cells induce autoantibody response as well as skin and lung fibrosis. Autoimmunity. 2016; 49 (8):503–13. https://doi.org/10.1080/08916934.2016.1230848 PMID: 27808577.