UNIVERSITE DE LILLE

ECOLE DOCTORALE BIOLOGIE SANTE DE LILLE (n°446)

Année : 2023

THESE DE DOCTORAT D'UNIVERSITE

IMMUNOPATHOLOGIE

Sébastien SANGES

Intérêt de biomarqueurs immunologiques dans l'hypertension artérielle pulmonaire associée à la sclérodermie systémique

Sous la direction du Pr Sylvain DUBUCQUOI

Soutenue publiquement le 21 Novembre 2023 à 09h

JURY

Pr Brigitte GRANEL, PU-PH, Aix-Marseille Université – Présidente
Pr Christian AGARD, PU-PH, Université de Nantes – Rapporteur
Pr Nathalie BARDIN, PU-PH, Aix-Marseille Université – Rapporteuse
Pr Frédéric BATTEUX, PU-PH, Université Paris Cité – Examinateur
Pr David LAUNAY, PU-PH, Université de Lille – Examinateur

Pages liminaires

RESUME

L'hypertension artérielle pulmonaire (HTAP) est désormais la principale cause de mortalité liée à la maladie chez les patients atteints de sclérodermie systémique (SSc). Deux écueils émergent pour améliorer son pronostic :

- d'une part, une physiopathologie mal élucidée. A l'inverse des composantes immunologiques et fibrosantes de la SSc dont la physiopathologie et les interactions ont fait l'objet de plusieurs études, peu de travaux se sont intéressés aux relations entre immunité et vaisseau dans cette pathologie.
- d'autre part, une prise en charge limitée par une importante hétérogénéité clinique, des stratégies diagnostiques sous-optimales et des options thérapeutiques restreintes (reposant principalement sur des traitements vasodilatateurs)

Au cours de ce travail de thèse, notre objectif était d'identifier de nouveaux biomarqueurs associés à l'HTAP-SSc, afin de générer de nouvelles hypothèses physiopathologiques, d'améliorer la caractérisation clinique des patients, et d'ouvrir des pistes thérapeutiques.

Pour cela, nous avons exploré le protéome sérique des patients HTAP-SSc à l'aide d'une approche haut débit (SOMAscan), permettant de doser simultanément 1129 protéines non sélectionnées. Au total, 53 protéines étaient différentiellement exprimées par rapport aux patients SSc sans HTAP. Parmi elles, 2 biomarqueurs candidats étaient explorés plus avant du fait de leur intérêt potentiel en termes de pronostic :

- <u>*Chemerin* et son récepteur CMKLR1</u>

Parmi les 1129 protéines étudiées, les taux de *chemerin* (adipokine impliquée dans différents processus inflammatoires, fibrosants et vasculaires) étaient les seuls corrélés aux résistances vasculaires pulmonaires dans 2 cohortes indépendantes. Une étude en *single cell RNA sequencing* sur poumons HTAP-SSc localisait la production de *chemerin* au niveau des fibroblastes, des cellules musculaires lisses artérielles pulmonaires (CML-AP)/péricytes et des cellules mésothéliales. En immunofluorescence confocale, la présence de *chemerin* n'était pas détectée au niveau pulmonaire ; mais son récepteur CMKLR1 était surexprimé par les CML-AP. Le sérum de patients SSc-HTAP accentuait la prolifération des CML-AP, et cet effet était neutralisé par un inhibiteur de CMKLR1. Ces résultats plaident pour l'implication de l'axe *chemerin*-CMKLR1 dans le remodelage vasculaire associé à l'HTAP-SSc.

- BAFF et le lymphocyte B

Plusieurs protéines associées au lymphocyte B (LB) (telles que β2-microglobuline, CXCL13 et BAFF) étaient différentiellement exprimées entre patients SSc avec et sans HTAP dans l'étude par SOMAscan. Pour compléter, nous avons établi un panel de 14 biomarqueurs LB (comprenant β2-microglobuline, facteur rhumatoïde, immunoglobulines (Ig) G, IgA, IgM, BAFF, APRIL, formes solubles (s) des récepteurs TACI et BCMA, sCD21, sCD23, sCD25, sCD27, et CXCL13), dont les associations entre taux sériques et les caractéristiques cliniques de la SSc étaient étudiées au sein de 2 cohortes indépendantes.

On mettait en évidence plusieurs corrélations entre différents biomarqueurs LB et les paramètres associés à l'HTAP. En particulier, il existait une forte corrélation entre les taux de BAFF, cytokine essentielle à la survie des LB, et les marqueurs de sévérité de l'HTAP. On démontrait enfin la capacité des LB SSc à produire des médiateurs pro-angiogéniques, suggérant ainsi leur implication dans la microangiopathie associée à la maladie.

Les axes *chemerin*-CMKLR1 et BAFF-LB constituent des candidats biomarqueurs robustes de l'HTAP-SSc, présentant un potentiel diagnostique et pronostique, ouvrant la voie à de nouvelles possibilités thérapeutiques et suggérant des interactions physiopathologiques insoupçonnées entre immunité et vaisseau.

MOTS-CLES

Sclérodermie systémique – HTAP – adipokines – endothélium – biomarqueurs – lymphocyte B

SUMMARY

Pulmonary arterial hypertension (PAH) is now the leading cause of disease-related mortality in patients with systemic sclerosis (SSc). Two issues arise to improve its prognosis:

- first, its pathogenesis is poorly elucidated. Interactions between the immunological and fibrosing components of SSc have been extensively investigated, but few studies have focused on the relationship between immunity and the vasculature in this disease;
- second, its management is hindered by clinical heterogeneity, sub-optimal diagnostic strategies and limited therapeutic options (mostly based on vasodilator treatments).

During this PhD project, our objective was to identify new biomarkers associated with SSc-PAH, in order to generate new pathophysiological hypotheses, to improve the clinical characterization of patients, and to open therapeutic avenues.

To do this, we mined the serum proteome of SSc-PAH patients using a high-throughput technique (SOMAscan), allowing the simultaneous measurement of 1129 unselected proteins. Overall, 53 proteins were differentially expressed compared to SSc patients without PAH. Among them, 2 candidate biomarkers were further explored:

- Chemerin and its receptor CMKLR1

Among the 1129 proteins studied, serum levels of chemerin (an adipokine involved in various inflammatory, fibrosing and vascular processes) were the only ones correlated with pulmonary vascular resistance in 2 independent cohorts. A single-cell RNA sequencing study on SSc-PAH lungs localized the production of chemerin within fibroblasts, pulmonary arterial smooth muscle cells (PA-SMC)/pericytes and mesothelial cells. Confocal immunofluorescence did not detect any chemerin staining in the lungs; but its receptor CMKLR1 was overexpressed by PA-SMC. Serum from SSc-PAH patients increased the PASMC proliferation, and this effect was neutralized by a CMKLR1 inhibitor. These results plead for the involvement of the chemerin-CMKLR1 axis in the pathophysiology of SSc-PAH.

- BAFF and B cells

Several B cell-associated proteins were differentially expressed between SSc patients with and without PAH in the SOMAscan dataset. A panel of 14 B-cell biomarkers (including β2-microglobulin, rheumatoid factor, immunoglobulin (Ig) G, IgA, IgM, BAFF, APRIL, soluble forms

(s) of TACI and BCMA receptors, sCD21, sCD23, sCD25, sCD27, and CXCL13) was thus created; and the associations between their serum levels and various SSc characteristics were studied in 2 independent cohorts.

Several correlations were observed between different B-cell biomarkers and parameters associated with PAH. Notably, a strong correlation was identified between the levels of BAFF, a cytokine essential for the survival of B cells, and markers of PAH severity. Finally, we demonstrated the capacity of SSc B cells to produce pro-angiogenic mediators, suggesting their contribution to the microangiopathy associated with the disease.

The chemerin-CMKLR1 and BAFF-B cell axes are robust biomarker candidates of SSc-PAH, presenting diagnostic and prognostic potential, opening the way to new therapeutic possibilities and suggesting unsuspected pathophysiological interactions between immunity and the vasculature.

KEYWORDS

Systemic sclerosis – PAH – adipokines – endothelium – biomarkers – B cells

TABLE DES MATIERES

LISTE DES TABLEAUX

LISTE DES FIGURES

ABREVIATIONS

α-NETA : 2-(alpha-naphthoyl)ethyltrimethylammonium iodide

ALK : *activin receptor-like kinase*

APRIL : *a proliferation-inducing ligand*

BAFF : *B cell activating factor*

BCMA : *B-cell maturation antigen*

BCR : *B cell receptor*

BMPR : *bone morphogenetic protein receptor*

BNP : *brain natriuretic peptide*

CCL : *C-C motif chemokine ligand*

CD : cluster de différentiation

CMKLR1 : *chemokine-like receptor* 1

CML-AP : cellules musculaires lisses artérielles pulmonaires

CVF : capacité vitale fonctionnelle

CXCL : C-*X-C motif chemokine*

DLCO : capacité de diffusion pulmonaire du monoxyde de carbone

HTAP : hypertension artérielle pulmonaire

HTP : hypertension pulmonaire

Ig : immunoglobuline

IL : interleukine

LB : lymphocyte B

MMP : *matrix metallopeptidase*

NT-pro-BNP : *N-terminal prohormone of brain natriuretic peptide*

PDGF : *platelet derived growth factor*

RVP: résistances vasculaires pulmonaires

SSc : sclérodermie systémique

TACI : *transmembrane activator and CAML interactor*

Tfh : T *follicular helper*

Th : T *helper*

TIMP : *tissue inhibitor of metalloproteinases*

VEGFR : *vascular endothelial growth factor receptor*

VITmax : vitesse maximale de la fuite tricuspide

REMERCIEMENTS

Je tiens à adresser mes plus sincères remerciements à :

- **Monsieur le Professeur Sylvain DUBUCCQUOI**
  Professeur des Universités, Praticien Hospitalier
  Immunologie (biologie)
  Institut d'Immunologie
  Centre Biologie Pathologie, CHU de Lille
  Unité INFINITE INSERM U1286
  Université de Lille

Je te remercie sincèrement de ton encadrement bienveillant tout au long de cette thèse. Tu as su me transmettre ta passion pour l'immunologie du lymphocyte B, ainsi que tes connaissances inépuisables sur ce sujet. Tu m'as fait l'honneur d'accepter de diriger ce travail ; sois assuré de ma gratitude et de ma reconnaissance.

- **Monsieur le Professeur David LAUNAY**

  Professeur des Universités, Praticien Hospitalier

  Médecine interne ; gériatrie et biologie du vieillissement

  Département de Médecine Interne et d'Immunologie Clinique

  Hôpital Claude Huriez, CHU de Lille

  Unité INFINITE INSERM U1286

  Université de Lille

Je te remercie de tes conseils, de ta disponibilité, de ton écoute et de ton soutien sans faille, depuis plus d'une dizaine d'années. Tu m'as appris à être rigoureux, curieux, patient et ambitieux dans nos activités de soins et de recherche. Sois assuré de mon estime, mon admiration et ma reconnaissance.

- **Monsieur le Professeur Frédéric BATTEUX**
- **Monsieur le Professeur Christian AGARD**
- **Madame la Professeure Nathalie BARDIN**
- **Madame la Professeure Brigitte GRANEL**

  Professeurs des Universités, Praticiens Hospitaliers

Vous me faites l'honneur d'évaluer ce travail de thèse, et je vous en remercie. Vos regards experts sur nos résultats permettront à coup sûr d'améliorer la qualité de notre travail. Soyez assurés de notre reconnaissance.

Je tiens également à adresser mes remerciements :

- au Dr Thomas Guerrier et à Lucile Guilbert, pour leur aide technique ;
- au Dr Aurore Collet, pour la quantité et la qualité du travail fourni sur nos différents projets ;
- à l'ensemble du groupe FIRE : Pr Vincent Sobanski, Aurélien Chepy, Carine Hauspie, Alexis Largy, Maïté Balden, Manel Jendoubi, Silvia Speca ;
- au Pr Robert Lafyatis et à l'ensemble de l'équipe de la côte Est : Lisa Rice, Julio Mantero, Giuseppina Stifano, Eric Stratton, Anda Bujor, Eleanor Valenzi ;
- au Pr Mark Nicolls, au Dr Amy Tian et à l'ensemble de l'équipe de la côte Ouest : Dongeon Kim, Ryan Vinh, Jason Chang, Shenbiao Gu, Tim Wu, Evan Bao, Peter Kao, Xinguo Jiang, Yu Zhu ;
- aux Pr Marc Humbert, au Pr David Montani, au Dr Christophe Guignabert, au Dr Ly Tu, au Dr Laurent Savale, et à l'ensemble de l'équipe du centre de référence HTAP ;
- au Pr Jean-Luc Cracowski et à l'ensemble de l'équipe de médecine interne grenobloise ;
- au Pr Nicolas Lamblin, au Dr Pascal Degroote, au Dr Marie Fertin et à l'ensemble du service de cardiologie du CHU Lille ;
- au Dr Guillemette Marot et à l'ensemble de la plateforme billile ;
- au Pr Alain Duhamel, Dr Camille Terninck, Dr Michaël Genin et à l'ensemble de la plateforme SEED ;
- au Pr Eric Hachulla et à l'ensemble de l'équipe du service de médecine interne du CHU Lille : Dr Sandrine Morell-Dubois, Dr Louis Terriou, Dr Hélène Maillard, Dr Meryem-Maud Farhat, Dr Emmanuel Ledoult, Pr Marc Lambert, Dr Cécile Yelnik ;
- au Dr Guillaume Lefèvre, au Pr Myriam Labalette, et à l'ensemble de l'équipe du laboratoire d'immunologie du CHU Lille ;
- à Céline Podevin et l'ensemble des équipes de la FHU PRECISE et du CeRAINOM ;
- à mes amis et à ma famille ;
- à Yvette.

LABORATOIRE D'ACCUEIL

Unité INFINITE (*Institute for Translational Research in Inflammation*)

INSERM U1286

Faculté de Médecine – Pôle Recherche, 4ème étage Centre

Place Verdun

59045 Lille Cedex

France

Tel. +33 3 20 62 34 19

Fax +33 3 20 62 35 25

Introduction

La sclérodermie systémique (SSc) est la plus sévère des maladies auto-immunes systémiques (1). A l'heure actuelle, sa physiopathologie repose sur l'interaction entre 3 grands acteurs, qui sous-tendent les principales manifestations cliniques de la maladie (2) : système immunitaire (expliquant notamment la présence d'auto-anticorps), fibroblastes (responsables de complications fibrosantes au niveau de la peau, des poumons, du cœur et du tube digestif) et vaisseaux (microangiopathie s'exprimant notamment au niveau périphérique par le phénomène de Raynaud et les ulcérations digitales, et au niveau pulmonaire par l'hypertension artérielle pulmonaire (HTAP)).

L'hypertension pulmonaire (HTP) est la plus grave des complications vasculaires de la SSc (3). Elle se définit par la mise en évidence d'une élévation de la pression artérielle pulmonaire moyenne $\geq$ 20 mm Hg au cours d'un examen invasif, le cathétérisme cardiaque droit, et peut relever de différents mécanismes. L'HTAP est la principale cause d'HTP observée au cours de la SSc et traduit la diffusion au niveau pulmonaire de la vasculopathie observée au plan systémique. Elle se caractérise par un remodelage vasculaire majeur intéressant les petites artères pulmonaires, et touchant l'intima (dysfonction, prolifération, vasoconstriction et fibrose de l'endothélium), la média (hypertrophiée par prolifération des cellules musculaires lisses et/ou transition endothélio-mésenchymateuse) et l'adventice (infiltration immunitaire et fibrose) (4).

L'HTAP est désormais la principale cause de morbi-mortalité associée à la SSc (5). Deux problématiques majeures contribuent à expliquer ce pronostic sombre :

- d'une part, les mécanismes intimes du remodelage vasculaire observé au cours de l'HTAP-SSc restent mal compris. Des données récentes plaident pour un rôle important de l'immunité dans leur genèse et leur pérennisation, notamment au cours de l'HTAP idiopathique (4). Cependant, les interactions entre systèmes immunitaire et vasculaire n'ont été que très peu étudiées au cours de la SSc, au contraire des relations unissant immunité et fibrose (6). Mieux préciser la nature de ces interactions pourrait permettre d'envisager le recours aux traitements immunosuppresseurs et/ou anti-inflammatoires, potentiellement capables de modifier l'histoire naturelle de la maladie et de prévenir l'apparition de nouvelles lésions ;
- d'autre part, la prise en charge des patients est limitée par leur grande hétérogénéité clinique, des difficultés diagnostiques (notamment en termes de stratégie de dépistage permettant d'orienter la réalisation du cathétérisme cardiaque droit) et des options

thérapeutiques restreintes (reposant essentiellement sur des molécules vasodilatatrices). Il existe donc un besoin insatisfait de mieux stratifier les patients, en termes de probabilité diagnostique, de mécanismes étiologiques, de présentation clinique, de pronostic et de réponse au traitement (notamment immunosuppresseur/anti-inflammatoire).

Un biomarqueur se définit, dans son acception la plus large, comme un marqueur biologique qui se substitue à un évènement clinique ou biologique plus difficile à observer, ou capable de prédire sa survenue future (7). Bien que l'origine du terme remonte aux années 1970, les « approches biomarqueurs » se sont largement déployées dans la fin des années 2000. Les applications cliniques potentielles des biomarqueurs sont vastes et peuvent concerner les aspects diagnostiques (dépistage, diagnostic positif et négatif, classification d'une maladie), pronostiques (prédiction de la survenue d'une maladie, d'une complication ou du décès) et thérapeutiques (prédiction de la réponse au traitement ou de la survenue d'effets secondaires, surveillance sous traitement). La nature biologique du biomarqueur identifié et sa relation avec l'évènement clinique étudié peuvent également ouvrir des pistes physiopathologiques porteuses. Ainsi, il apparaît que l'identification de biomarqueurs robustes et pertinents pourrait contribuer à améliorer la prise en charge des patients présentant une HTAP-SSc.

Plusieurs biomarqueurs ont été découverts pour caractériser le phénotype des patients atteints d'HTAP-SSc et ont ainsi pu être déployés en routine avec succès : on peut ainsi citer les auto-anticorps associés à la maladie (en particulier anti-centromère et anti-U1-RNP) et les peptides natriurétiques (BNP et Nt-pro-BNP) (8). Plusieurs travaux ont depuis tenté d'identifier de nouveaux biomarqueurs permettant de stratifier encore plus précisément les patients (9,10) et ont mis en évidence des associations entre des protéines impliquées dans différents processus biologiques (notamment angiogenèse et inflammation) et la survenue et/ou la sévérité de l'HTAP-SSc. Cependant, la robustesse de ces biomarqueurs reste discutable, la plupart de ces résultats n'étant pas toujours reproduits, ce qui compromet souvent leur transfert en pratique clinique quotidienne et l'investigation de pistes physiopathologiques novatrices.

Dans le cadre de ce travail de thèse, notre objectif était donc d'identifier de nouveaux biomarqueurs associés à l'HTAP-SSc, afin de générer de nouvelles hypothèses

physiopathologiques, d'améliorer la caractérisation clinique des patients, et d'ouvrir des pistes thérapeutiques. Pour cela, nous avons exploré le protéome sérique de patients présentant une HTAP-SSc (**Partie I**) et identifié 2 pistes présentant un intérêt potentiel : l'axe *chemerin*-CMKLR1 (**Partie I**) ; et l'axe BAFF-lymphocyte B (**Partie II**).

L'ensemble de ces résultats ont été publiés ou sont en cours de publication, et sont présentés ci-après.

RÉFÉRENCES

1. Hachulla E, Launay D. Diagnosis and Classification of Systemic Sclerosis. Clinic Rev Allerg Immunol. 1 avr 2011;40(2):78-83.
2. Dumoitier N, Lofek S, Mouthon L. Pathophysiology of systemic sclerosis: State of the art in 2014. La Presse Médicale. oct 2014;43(10):e267-78.
3. Asano Y, Sato S. Vasculopathy in scleroderma. Semin Immunopathol. sept 2015;37(5):489-500.
4. Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, et al. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. Eur Respir J. janv 2019;53(1).
5. Pokeerbux MR, Giovannelli J, Dauchet L, Mouthon L, Agard C, Lega JC, et al. Survival and prognosis factors in systemic sclerosis: data of a French multicenter cohort, systematic review, and meta-analysis of the literature. Arthritis Res Ther. 3 avr 2019;21(1):86.
6. Worrell JC, O'Reilly S. Bi-directional communication: Conversations between fibroblasts and immune cells in systemic sclerosis. Journal of Autoimmunity. 1 sept 2020;113:102526.
7. Aronson JK, Ferner RE. Biomarkers—A General Review. Current Protocols in Pharmacology. 2017;76(1):9.23.1-9.23.17.
8. Hachulla E, Agard C, Allanore Y, Avouac J, Bader-Meunier B, Belot A, et al. French recommendations for the management of systemic sclerosis. Orphanet J Rare Dis. 26 juill 2021;16(Suppl 2):322.
9. Hickey PM, Lawrie A, Condliffe R. Circulating Protein Biomarkers in Systemic Sclerosis Related Pulmonary Arterial Hypertension: A Review of Published Data. Front Med. 6 juin 2018;5:175.
10. Odler B, Foris V, Gungl A, Müller V, Hassoun PM, Kwapiszewska G, et al. Biomarkers for Pulmonary Vascular Remodeling in Systemic Sclerosis: A Pathophysiological Approach. Front Physiol. 19 juin 2018;9:587.

État de l’art

Avant de replacer nos résultats dans leur contexte clinique, nous avons réalisé une revue actualisée de la littérature portant sur l'HTAP-SSc. Afin d'en illustrer l'hétérogénéité clinique et physiopathologique qui a rendu nécessaire notre travail de thèse, le champ de cette revue a été étendu à l'ensemble des groupes d'HTP, sans se limiter aux groupes 1 (HTAP), et à l'ensemble des connectivites.

Ce travail est actuellement en cours de révision dans la *Revue de Médecine Interne*.

RESUME DETAILLE EN FRANÇAIS

L'hypertension pulmonaire (HTP) est une complication rare des connectivites, notamment de la sclérodermie systémique (SSc), du lupus érythémateux systémique et de la connectivite mixte. Elle se définit par une élévation de la pression artérielle pulmonaire moyenne $\geq$ 20 mm Hg documentée par un cathétérisme cardiaque droit. Du fait de leur nature systémique, les connectivites peuvent induire une HTP par différents mécanismes, parfois intriqués : vasculopathie pulmonaire (groupe 1 de la classification de l'HTP) affectant les artérioles (hypertension artérielle pulmonaire, HTAP) et possiblement les veinules (ex-maladie veno-occlusive pulmonaire), cardiopathie gauche (groupe 2), maladie respiratoire chronique (groupe 3) ou maladie thrombo-embolique chronique (groupe 4).

L'existence d'une HTP peut être suspectée par la clinique (dyspnée, asthénie), l'échographie cardiaque (élévation de la vitesse de la fuite tricuspide), une baisse isolée de la DLCO lors des épreuves fonctionnelles respiratoires, et/ou une élévation du BNP/NT-pro-BNP. Son diagnostic nécessite toujours une confirmation hémodynamique par cathétérisme cardiaque droit. Plusieurs stratégies de dépistage ont été étudiées dans l'HTAP associée à la SSc, mais peu de données sont disponibles dans les autres connectivites.

La prise en charge thérapeutique de l'HTP dépend du(es) mécanisme(s) en cause : traitement spécifique en cas d'HTP de groupe 1 (avec discussion d'une immunosuppression en cas de lupus ou de connectivite mixte) ; traitement de la cardiopathie gauche en cas d'HTP de groupe 2 ; traitement de la maladie respiratoire chronique avec discussion d'un traitement par treprostinil inhalé en cas d'HTP du groupe 3 ; anticoagulation, traitement vasodilatateur, endartériectomie et/ou angioplastie pulmonaire en cas d'HTP du groupe 4. Un suivi régulier est indispensable.

Pulmonary hypertension in connective tissue diseases: what every CTD specialist should know – but is afraid to ask!

Sébastien Sanges[1,2,3,4,5,*], MD, MS; Vincent Sobanski[1,2,3,4,5,*], MD, PhD; Nicolas Lamblin, MD, PhD[6,7]; Eric Hachulla[1,2,3,4,5], MD, PhD; Laurent Savale[8,9,10], MD; David Montani[8,9,10,**], MD, PhD; David Launay[1,2,3,4,5,**], MD, PhD.

(1) Univ. Lille, U1286 – INFINITE – Institute for Translational Research in Inflammation, F-59000 Lille, France

(2) INSERM, F-59000 Lille, France

(3) CHU Lille, Département de Médecine Interne et Immunologie Clinique, F-59000 Lille, France

(4) Centre National de Référence Maladies Auto-immunes Systémiques Rares du Nord et Nord-Ouest de France (CeRAINO), F-59000 Lille, France

(5) Health Care Provider of the European Reference Network on Rare Connective Tissue and Musculoskeletal Diseases Network (ReCONNET), F-59000 Lille, France

(6) CHU Lille, Service de Cardiologie, F-59000 Lille, France

(7) Institut Pasteur de Lille, Inserm U1167, F-59000 Lille, France

(8) Université Paris Saclay, School of Medicine, Le Kremlin-Bicêtre, France

(9) AP-HP, Department of Respiratory and Intensive Care Medicine, Hôpital Bicêtre, Le Kremlin-Bicêtre, France

(10) INSERM UMR_S 999, Hôpital Marie-Lannelongue, Le Plessis-Robinson, France

*Both authors contributed equally and share co-first authorship.

**Both authors contributed equally and share co-last authorship.

Corresponding author: Dr Sébastien SANGES, Département de Médecine Interne et Immunologie Clinique, CHU Lille, F-59037 Lille Cedex, France.

Tel: + 33 (0)320445048; Fax: + 33 (0)320445459; E-mail: sebastien.sanges@univ-lille.fr

Pulmonary hypertension (PH) is a possible complication of connective tissue diseases (CTDs), with prevalence and mechanism(s) varying among each condition but often associated with a poor prognosis [1]. In this review, we describe the main pathophysiological and clinical characteristics of PH associated with CTDs, as well as the core principles of its management.

1. DEFINITION AND CLASSIFICATION OF PULMONARY HYPERTENSION

Definition

PH refers to any hemodynamic situation in which the pulmonary circulation operates in a high-pressure regimen. The threshold for elevated mean pulmonary arterial pressure (mPAP) was arbitrarily set at 25 mmHg during the first World Symposium on PH in 1973 [2]. Subsequently, studies showed that normal mPAP revolves around 14±3 mmHg [3]. Therefore, the 2022 European Society of Cardiology (ESC)/European Respiratory Society (ERS) guidelines have adopted a cut-off of 20 mmHg (i.e., 2 standard deviations above normal value) as the new hemodynamic definition of PH [4].

Principles of right heart catheterization

The diagnosis of PH requires the invasive measurement of pulmonary pressures during a right heart catheterization (RHC) [5]. During this exam, a Swan-Ganz catheter is introduced into a peripheral (or central) vein, progresses to the right heart cavities and up to the pulmonary arteries (PA), where the following parameters are evaluated (direct measurement or calculation):

- *right atrial pressure (RAP)* (mmHg);
- *systolic (sPAP), diastolic (dPAP) and mean (mPAP) pulmonary arterial pressure* (mmHg);
- *pulmonary arterial wedge pressure (PAWP)* (mmHg). PAWP is assessed by occluding a small PA branch using an inflated balloon at the tip of the catheter [6]. This creates a static column of blood between the catheter tip and the left atrium (LA), and pressures at both ends of the column equilibrate according to Pascal's law. As such, PAWP allows for an indirect measurement of LA pressure. In the absence of obstruction between LA and the capillary bed (such as venous occlusion), PAWP also provides an estimation of capillary pressure;

- *cardiac output (CO)* (L/min) *and cardiac index (CI)* (L/min/m^2), that can be measured using different methods (thermodilution technique, Fick's equation);
- *mixed venous oxygen saturation (SvO2)* (%);
- *pulmonary vascular resistance (PVR)*: calculated as PVR = (mPAP-PAWP)/CO (mmHg/L/min or Wood units, WU).
- *right ventricular stroke volume index (SVI)* (mL/beats/m^2): calculated as SVI = CI/heart rate. SVI represents the volume of blood ejected from the right ventricle (RV) during each systole, indexed on body surface area.

Of note, *acute vasoreactivity testing* is no longer recommended in CTD-PAH, as long-term response to calcium-channel blockers (CCBs) is exceptional in these patients [7].

Clinical and hemodynamic classifications of PH

Hemodynamic classification of PH is based on whether the process leading to the increased pulmonary pressures is located before the capillary bed (pulmonary arterial diseases, as appreciated by elevated PVR) and/or after the capillary bed (elevated venous pressure due to left heart diseases, as appreciated by elevated PAWP) [4]. Thus, a distinction is made between:

- *pre-capillary PH:* defined by mPAP > 20 mmHg, PAWP ≤ 15 mmHg and PVR > 2 WU;
- *isolated post-capillary PH:* defined by mPAP > 20 mmHg, PAWP > 15 mmHg and PVR ≤ 2 WU;
- *combined pre- and post-capillary PH (cpcPH):* defined by mPAP > 20 mmHg, PAWP > 15 mmHg and PVR > 2 WU.

Clinical classification of PH identifies 5 major groups (**Table 1, Figure 1**) [4]:

- *Group 1: pulmonary arterial hypertension (PAH)*

  PH is caused by increased vascular resistance related to pulmonary arterial damage (parietal remodeling, vasoconstriction, *in situ* thrombosis). PAH can be limited to the arterial side; or also involve lesions on the venous and capillary sides, a situation now called PAH with features of venous/capillary involvement (and previously referred to as pulmonary veno-occlusive disease (PVOD) or pulmonary capillary hemangiomatosis (PCH)).
- *Group 2: PH associated with left heart diseases*

PH is caused by an increased LA pressure, responsible for passive elevation in upstream vascular pressures (pulmonary venous, capillary and arterial pressures).

- *Group 3: PH associated with chronic lung diseases*

  PH is caused by rarefaction of the vascular bed (due to parenchymal destruction), parietal thickening (due to adjacent inflammation and fibrosis), and vasoconstriction reactive to chronic hypoxemia (compensatory mechanism aiming at limiting perfusion in unventilated territories), occurring in the setting of a chronic respiratory condition.

- *Group 4: PH associated with pulmonary artery obstruction*

  PH is caused by chronic PA obstruction (macrovascular component), associated with vascular remodeling induced by chronic blood flow redistribution to perfused territories (microvascular component). This usually occurs in the context of an acute pulmonary embolism (PE) followed by incomplete recanalization of the arterial lumen and/or distal embolizations, a condition called chronic thrombo-embolic PH (CTEPH). CTEPH can also occur in patients without any known history of PE in 50% of cases.

- *Group 5: PH with unclear and/or multifactorial mechanisms*

Discriminating intricated causes of PH

In systemic diseases such as CTDs, several mechanisms may contribute to the emergence of PH in the same patient. For instance, systemic sclerosis (SSc) may cause group 1 (pulmonary vasculopathy causing PAH with or without features of venous involvement), group 2 (due to specific cardiomyopathy), group 3 (in case of extensive interstitial lung disease (ILD)) and/or group 4 (in patients with associated antiphospholipid syndrome (APS)) [8].

Confirming the existence of intricated mechanisms can sometimes be difficult, especially in certain situations:

- *Presence of pulmonary vasculopathy in patients with extensive ILD*

  In patients with CTD and chronic lung diseases, it can be challenging to determine whether PH is exclusively due to the parenchymal condition (group 3-only PH) or if a CTD-associated pulmonary vasculopathy is also participating (group 3+1 PH). The latter must be discussed in case of severe PH, defined as PVR > 5 WU [9].

- *Presence of post-capillary PH due to heart failure with preserved ejection fraction (HFpEF) in patients with PAWP ≤ 15 mmHg*

Post-capillary PH related to HFpEF is volume-dependent and not always associated with a PAWP > 15 mmHg, especially in case of volume depletion. In patients with features of HFpEF and borderline PAWP (13-15 mmHg), a fluid challenge (infusion of 500 mL of isotonic saline by 5-10 min) during RHC can unmask post-capillary PH due to occult diastolic heart failure if PAWP raises above 18 mmHg [6].

- *Presence of venous/capillary involvement (PVOD-like) in patients with PAH*

  In this setting, venous/capillary involvement should be suspected in addition to PAH in case of signs of impaired gas exchange due to capillary alterations (profound hypoxemia, markedly decreased DLCO) and/or signs of fluid overload due to venous obstruction (lymph node enlargement, centrilobular ground-glass opacities, and septal lines on chest CT-scan) [10]. Although there is some degree of post-capillary obstruction, PAWP is normal in PVOD-like (as this is one of the few cases in which PAWP does not accurately reflect capillary pressure; see above) [11].

2. CLINICAL PRESENTATION OF PULMONARY HYPERTENSION ASSOCIATED WITH CONNECTIVE TISSUE DISEASES

Systemic sclerosis (SSc)

SSc is characterized by the triad of vascular (Raynaud's phenomenon (RP), telangiectasias), immunologic (autoantibodies) and fibrosing (skin fibrosis, ILD) manifestations [12]. Over the past decades, lung involvement, and especially PH, has become its leading cause of mortality [13].

As its pathogenesis involves widespread microangiopathy, SSc was identified as the most frequent CTD associated with PAH in Europe and North America [14–16]. SSc-PAH is characterized by vascular remodeling of the small pulmonary arteries (<500 µm) and involves substantial modifications in the intima (endothelial dysfunction, proliferation, vasoconstriction and fibrosis), media (hypertrophy due to smooth muscle cell (SMC) proliferation and/or endothelial-mesenchymal transition, loss of clear intima-media delineation forming a “neo-intima”) and adventitia (immune infiltration and fibrosis) [17–19]. Plexiform lesions and thromboses may rarely be observed [20]. Histological studies suggested that SSc-PAH is frequently associated with venous and capillary involvements, leading to PVOD-like [21]. The exact mechanisms driving endothelial damage and vascular remodeling in

SSc-PAH remain elusive, although recent works have suggested a participation of the immune system in the pathogenesis of the disease [22,23].

Causes of PH can be numerous in SSc due to its systemic nature [8]: PAH with or without features of venous involvement (group 1), specific cardiomyopathy with HFpEF (group 2), extensive ILD (group 3) [24]. A particular subset of SSc-ILD patients, characterized by apical emphysema and basal fibrosis (a condition called combined pulmonary fibrosis and emphysema, CPFE), appeared at higher risk of group 3 PH [25]. SSc patients have an increased incidence of thromboembolic events (even without antiphospholipid antibodies (aPL)) [26], making the occurrence of group 4 PH possible; however, SSc-CTEPH has only been exceptionally reported so far [27,28]. As PH is a heterogeneous complication in SSc, cluster analysis was used on a multicenter US-French cohort to delineate homogeneous subsets of SSc-PH patients: 4 clusters were identified and mainly differed in terms of ILD extent, DLCO, PVR and survival [29]. Careful attention must thus be paid to accurately classify SSc-PH patients in adequate clinical groups.

The prevalence of hemodynamically-proven pre-capillary PH in large SSc cohorts was estimated between 5% and 12% using the former mPAP cut-off of 25 mmHg [30,31]. Recent studies focusing on the 2022 ESC/ERS definition showed that the new criteria reclassified 10-13% of SSc patients with available RHC data [32,33]. In the French multicenter ItinérAIR cohort, the estimated incidence of PAH was 0.61 cases per 100 patient-years [34].

Classical risk factors for PAH occurrence in SSc are longstanding disease (>5 years), older age at onset, limited cutaneous subset, profuse telangiectasias, anti-centromere and anti-U1RNP antibodies [35]. However, PAH occurs within the first 5 years of disease onset in 55% of cases, and in diffuse SSc in 22% of cases, justifying screening for all patients [36].

A meta-analysis including 22 studies (2244 SSc-PH patients) confirmed poor survival rates at 1, 2, and 3 years: 81%, 64%, and 52%, respectively [37]. Survival have been improving in recent years in patients <70 years old, probably due to a more aggressive therapeutic management [38]. Nonetheless, SSc-PAH prognosis remains worse than iPAH, which may be explained by more frequent occurrence of PVOD-like and comorbidities. Prognostic factors associated with survival are age, WHO functional class, six-minute walk distance (6MWD), DLCO, pericardial effusion, and hemodynamics (mPAP, CI, PVR, SvO_2). For patients with group 1 PH specifically, male gender and RAP were also prognostic markers [37,39].

Systemic lupus erythematosus *(SLE)*

While rare in European and Northern American cohorts, SLE is the most frequent CTD associated with PAH in Asian populations [40]. Like SSc, SLE can cause PH through various mechanisms [41], although most patients were classified as group 1 PAH in the French PH registry [42]. As SLE is associated with a higher risk of thromboembolic events and frequent co-occurrence of APS, careful attention should be given to excluding CTEPH [43].

The exact prevalence of PH in SLE remains unknown, with estimates ranging from 0.5% to 17% (depending on echocardiographic cut-offs, RHC confirmation, study populations and care setting), but likely does not exceed 1% in Caucasian patients [41,44,45]. Several risk factors for PH were identified: RP; aPL, anti-U1RNP and anti-SSA/SSB antibodies; and high disease activity [42,45].

SLE-PAH patients have better survival than SSc-PAH patients. A recent meta-analysis including 6 studies (323 patients) estimated 1-, 3- and 5-year survival rates of 88%, 81% and 68%, respectively [46]. In the French PH registry, the 5-year survival of SLE-PAH patients was 84% [42]. Factors associated with mortality were WHO functional class, 6MWD, BNP/NT-proBNP levels, mPAP and PVR [46]. The reasons for this difference in survival are unclear but could be due to a more important role of immunity, making it accessible to immunosuppressive therapy [47–49]. Indeed, complete reversibility of PH has been described in SLE patients treated by immunosuppressants and PAH-approved drugs, with some achieving total withdrawal of pulmonary vasodilators [49].

Mixed connective tissue disease (MCTD)

MCTD is an entity defined by the simultaneous presence of clinical signs usually found in other well-defined CTDs, associated with anti-U1RNP antibodies. Occurrence of group 1 PAH and group 3 PH due to extensive ILD were both described in MCTD patients [50]. In the latter case however, the hemodynamic impairment is usually severe and suggests an underlying pulmonary vasculopathy [50,51]. As they share common clinical features, differential diagnosis between MCTD-PAH and SSc-PAH associated with anti-U1-RNP is often challenging. The prevalence of PH in MCTD patients was historically reported around 19-24% in patients followed in tertiary care centers; but is closer to 2% in unselected cohorts [50]. Its incidence was estimated at 2.1 per million/year [50]. Several risk factors were recognized: pericarditis, polyarthritis, thrombocytopenia, ILD and anti-Sm antibodies [51].

PAH was described as the first cause of death in MCTD patients [52]. The estimated survival rates at 1, 5 and 10 years were 83%, 67% and 56%, respectively [51]. The main prognostic factor in MCTD-PAH is tobacco exposure [51]. Conversely, anti-U1RNP antibodies, which are found in all MCTD patients by definition, were associated with better survival in a study performed in CTD-PAH, even after adjustment on the usual prognostic factors [53].

Primary Sjögren's syndrome (pSS)

PH is a rare occurrence during pSS, although its exact prevalence has not been assessed in large epidemiological studies [54]. Data from the French Health Insurance database however confirmed pSS as a risk factor for PH occurrence (with a hazard ratio of 3.32) [54]. In the majority of reported cases, PH is classified as group 1 (PAH) [55]. Other mechanisms of PH have also been described, more anecdotally [56,57]. The development of PH in patients with pSS seems associated with the presence of extra-glandular signs (RP, cutaneous vasculitis, ILD, pericardial effusion) and immunological anomalies (rheumatoid factor; anti-nuclear, anti-SSA/SSB, anti-RNP antibodies; hypergammaglobulinemia) [55,58,59]. It has a negative impact on prognosis, with an estimated 3-year mortality of 66% in the French PH registry and 88% in a multicenter Chinese cohort [55,58]. CI, PVR and pSS damage index were identified as predictors of death in Chinese patients [59].

Idiopathic inflammatory myopathies (IIM)

The occurrence of PH in patients with IIM seems rare, but robust epidemiological data are lacking [60,61]. Two consecutive studies observed an estimated sPAP >40 mmHg on echocardiography in 16% of patients with polymyositis (PM) or dermatomyositis (DM), but without hemodynamic confirmation [62,63]. Most of the cases described report group 3 PH due to extensive ILD and usually part of an anti-synthetase syndrome (ASS) [64]. Nevertheless, hemodynamics are often too severe to be explained by the chronic respiratory disease alone, which suggest an underlying pulmonary vasculopathy [64]. Several publications also report genuine isolated PAH in these patients [60,61]. Group 2 (cardiac myositis) and group 4 PH have also been described on a more occasional basis [61].

The risk factors for PH occurrence during IIM are not clearly established. In patients with ASS, PH was associated with arthralgias, ILD and low DLCO [64]. In IIM patients without ILD, occurrence of PAH was associated with DM subtype, signs of peripheral microangiopathy, and

anti-SSA antibodies [60]. Prognosis is usually poor, with a 3-year mortality estimated between 42% and 44% [60,61].

Rheumatoid arthritis (RA)

The association between RA and PH is controversial. In the French PH registry, the prevalence of RA was similar to the general population, which pleads for an incidental association (explained by the relative frequency of the disease) [65]. Pharmacovigilance data also suggested an association between treatment by leflunomide and PH occurrence [66], which could account for some cases in RA patients.

In this cohort of 20 patients, PH was classified as group 1 in 50% of cases (including one case of drug-induced PAH and one case of porto-pulmonary hypertension), group 3 in 30% (five cases of pulmonary fibrosis and one case of non-fibrosing ILD), and group 4 in 20% (without APS) [65]. Survival was estimated at 84% at 1 year and 78% at 3 years [65].

3. SCREENING AND RISK ASSESSMENT OF PULMONARY HYPERTENSION ASSOCIATED WITH CONNECTIVE TISSUE DISEASES

A. SCREENING

Several tools are available to screen for PH in CTDs (**Table 2**) but only allow for a PH probability assessment. In case of high suspicion of PH, RHC remains mandatory to formally establish its diagnosis, and to guide etiologic investigations, prognostic evaluation and treatment strategy [4].

Transthoracic echocardiography (TTE)

Resting TTE is the cornerstone of non-invasive PH screening. Several key parameters are measured during this exam [67]:

- *Estimation of sPAP:*

  PA and RV systolic pressures are not directly accessible during TTE. Therefore, sPAP values can only be indirectly estimated by measuring:

  - the *peak tricuspid regurgitation velocity* (TRV), from which the *tricuspid regurgitation pressure gradient* (TRPG) is calculated using Bernouilli's equation ($TRPG = 4xTRV^2$);

- the *diameter and inspiratory collapse of the inferior vena cava (IVC)*, from which *RAP* is roughly approximated;
- with sPAP ≈ TRPG + RAP ≈ 4xTRV^2 + RAP.

Crucially, TTE only gives an imperfect estimate of the true sPAP (risk of over- and underestimation), which explains why hemodynamic confirmation remains essential for the diagnosis of PH.

- *Indirect signs of PH:*

  Aside from the estimation of sPAP, other parameters can further refine the echocardiographic probability of PH. With the recent lowering of the mPAP threshold to 20 mmHg, these have gained a particular importance to allow for an earlier detection of PH. These are indices related to:

  - *heart ventricles*: signs suggestive of RV dilation (RV/LV diameter or area ratio >1, flattening of the interventricular septum) or failure (estimated by the tricuspid annular plan systolic excursion (TAPSE)/sPAP ratio, see below);
  - *pulmonary artery*: signs suggestive of elevated pressure (modifications in the pulse wave of the RV outflow tract, assessed by a shortened acceleration time (AT) and/or a mid-systolic notching; estimation of mPAP by measurement of peak pulmonary regurgitation velocity);
  - *IVC and RA*: signs of IVC dilation (diameter > 21 mm with decreased inspiratory collapse) and/or RA dilation (area > 18 cm^2).

- *Assessment of RV function:*

  RV function can be indirectly appreciated by assessing the movements of the tricuspid annulus during systole, which partially reflect RV contraction. Two main echocardiographic indices are available: tricuspid annular plan systolic excursion (TAPSE) and tissue Doppler-derived tricuspid annulus velocity (S' wave). Recently, the TAPSE/sPAP ratio, an indirect marker of RV-PA coupling (which appreciates RV function in relation to the degree of pressure elevation), was found to have both diagnostic and prognostic values in PH [68].

- *Assessment of LV function and disease*:

  Assessment of LV diastolic and systolic functions, as well as LV conditions (such as mitral or aortic valve diseases), are warranted and may point towards group 2 PH.

Pulmonary function tests (PFTs)

Patients with suspected PH should undergo complete PFTs with forced spirometry, body plethysmography and lung diffusion capacity for carbon monoxide [4]. PH in itself is usually characterized by a mild decrease in DLCO with normal lung volumes, but diffusion can sometimes be normal [69]. A progressive diminution in DLCO values over repeated PFTs during follow-up is predictive of PH occurrence [70]. A profound decrease (< 45%) in DLCO is uncommon in PAH and suggests an associated PVOD-like and/or ILD [71]; in any case, it is associated with poor prognosis [72].

Abnormal pulmonary volumes cannot be attributed to PH and usually indicate an underlying chronic lung disease. In this circumstance, it may be difficult to determine whether the low DLCO is entirely explained by the respiratory disease alone or if an association with PH must be suspected. A decrease in DLCO that is proportionally greater than FVC (FVC(%)/DLCO(%) > 1.6) or alveolar volume (DLCO/VA < 70%) argues for an associated PH [73].

Cardiac biomarkers (BNP and NT-proBNP)

Elevation of natriuretic peptides (BNP and NT-proBNP) is common in PH but mainly reflects RV pressure overload [74]. Thus, peptide levels are not very accurate for PH detection or screening, but are excellent prognostic markers. Another limit is that these anomalies are not specific to PH and may also be explained by left heart diseases or renal failure.

Six-minute walk test (6MWT)

The 6MWT is a submaximal exercise test in which the patient is asked to walk as far as possible during six minutes [75]. A decrease in the walking distance (6MWD) and the occurrence of exercise desaturation can be observed in PH [76]. Interpreting this test in the context of CTDs is delicate, because of other disease manifestations that can impact walking and challenge its interpretation (musculoskeletal involvement, ILD, cardiac disease, etc.) [77]. In all cases, it reflects the functional capacities of the patient, and therefore yields prognostic importance.

Electrocardiogram

The electrocardiogram inconsistently shows right heart signs (P pulmonale, right axis deviation, RV hypertrophy, right bundle branch block), mostly in severe PH [78].

Chest CT scan without and with contrast enhancement

Chest CT can sometimes contribute to the diagnosis of PH by showing dilatation of PA (as assessed by the PA/aorta ratio) and/or right heart chambers. More frequently, it helps in the clinical classification of PH by showing signs of chronic respiratory disease (CTD-ILD), PVOD-like (lymphadenopathy, centrilobular ground-glass opacities, septal lines) and/or CTEPH (filling defects, webs or bands inside Pas, PA retraction/dilatation, mosaic perfusion, and enlarged bronchial arteries) [79].

Cardiac magnetic resonance imaging (MRI)

Cardiac MRI can show features suggestive of PH, such as dilatation of PA or right heart chambers. More importantly, it allows measurements of several prognostic determinants, such as RV ejection fraction, SVI and RV end-systolic volume index (RVESVI) [79].

Ventilation-perfusion (V/Q) lung scan

V/Q lung scan is the gold standard exam to exclude CTEPH and should be part of the initial assessment of CTD-PH patients. In case of CTEPH, it typically shows mismatched perfusion defects persisting after at least 3 months of anticoagulation, with a higher sensitivity but lower specificity than chest angio-CT [79].

Screening strategies and RHC referral

The screening modalities for PH in CTDs differ according to the underlying condition [4]:

- *for SSc (or MCTD with SSc features)*: screening by TTE, PFTs, cardiac biomarkers and 6MWD is recommended annually for asymptomatic patients, and at symptom onset in symptomatic patients [80];
- *for all other CTDs*: screening by TTE, PFTs, cardiac biomarkers and 6MWD should be considered only in symptomatic patients.

At the end of the screening workup, patients with suspected PH should undergo RHC for hemodynamic confirmation. Indications for RHC are poorly codified: several decision algorithms have been proposed and mostly validated in SSc patients [4,81,30,74] (**Table 3**). As of today, the best strategy still remains a area of intensive investigation [82].

Moreover, these algorithms were developed to identify patients that validate the previous hemodynamic definition of PH. Further studies are needed to assess how the new criteria may impact our current screening strategies.

B. INITIAL RISK ASSESSMENT

The 2022 ESC/ERS guidelines provide a 3-strata risk assessment model to estimate the 1-year mortality of PAH patients at diagnosis (**Table 4**) [4]. Several key prognostic factors were identified: clinical presentation, functional evaluation, cardiac biomarkers, TTE parameters (notably RA area and TAPSE/sPAP), cardiac MRI, and hemodynamics (RAP, CI, SVI and SvO_2). Using these prognostic markers, patients are stratified as low risk (estimated 1-year mortality < 5%), intermediate risk (5-20%) or high risk (> 20%).

This stratification drives therapeutic management. However, CTD-PAH patients may have several intricated causes of PH (simultaneously or consecutively), which challenge its interpretation in this setting [8].

C. FOLLOW-UP

Regular follow-up is mandatory in CTD-PH patients and should include clinical assessment (with WHO functional classification), 6MWT, cardiac biomarkers and TTE. A 4-strata risk assessment model was recently developed to refine prognostic evaluation during follow-up and adapt treatment accordingly (**Table 4**) [4]. This model identifies WHO functional class, 6MWT and BNP/Nt-proBNP levels as the main determinants of prognosis, and classifies patients as low risk, intermediate-low risk, intermediate-high risk, and high risk. It has recently been validated in CTD-PAH populations [83].

RHC is not systematically repeated during follow-up but is warranted in patients with clinical worsening and 3-6 months after changes in therapy.

4. TREATMENT STRATEGY IN PULMONARY HYPERTENSION ASSOCIATED WITH CONNECTIVE TISSUE DISEASES

A. GENERAL MEASURES

Several general measures are consistently discussed in all CTD-PH cases [4,84]:

- referral to an *expert center* specialized in the management of pulmonary vascular diseases (for France, see list at: https://respifil.fr/);
- *vaccinations* against influenza, COVID-19 and pneumococcal infections;
- supervised *cardiorespiratory rehabilitation*, after careful safety evaluation;
- anticipation of *high-risk situations*: pregnancy (generally contraindicated), surgery (carefully weighing its risks vs its benefits), anesthesia (avoidance of general anesthesia);
- long-term *oxygen therapy* in case of chronic respiratory failure (PaO_2 < 60 mmHg);
- *diuretics* in case of signs of fluid retention and/or right ventricular failure.

B. MEDICATIONS

The drugs used to treat CTD-PH patients vary according to PH mechanism(s) and the underlying CTD (**Figure 1**).

Group 1: PAH (without features of venous/capillary involvement)

The 2022 ESC/ERS guidelines recommend that treatment of CTD-PAH patients follow the same algorithm as iPAH patients [4]. However, most of the available data regarding CTD-PAH therapy stems from clinical trials that analyzed overall PAH populations, mixing patients with different causes of PAH [84]. Usually, these studies would include about one-third of patients with CTDs; and subgroup analyses are not always available. When they are, they often show that the magnitude of treatment response is lower than for iPAH patients. Furthermore, many trials have used the 6MWD as their primary endpoint, which is not an adequate surrogate for hemodynamics in CTD-PAH, especially in SSc [76].

- Anticoagulation

The relevance of anticoagulation in CTD-PAH is controversial: although scientific evidence (*in situ* thrombosis, pro-coagulant state) suggests a potential interest, clinical data from SSc-PAH patients demonstrated a poor tolerance due to an increased bleeding risk [85,86]. As such, systematic anticoagulation is no longer recommended in CTD-PAH patients, but can be discussed on a case-by-case basis [4]. Anticoagulants used for other indications (such as APS) can be maintained if well tolerated.

- Calcium-channel blockers

Conversely to iPAH, long-term response to CCBs is exceptional in CTD-PAH [7]. As such, acute vasoreactivity testing is no longer recommended in these patients; and CCBs were removed from their therapeutic arsenal [4]. CCBs used for other indications (such as RP) can be maintained if well tolerated.

- Conventional PAH-approved drugs

Conventional drugs approved for PAH try to reverse vasoconstriction and vascular remodeling by limiting the impact of endothelial dysfunction on PA-SMCs. To do so, they target 3 main mediators: endothelin-1 (endothelin receptor antagonists (ERAs)), the NO-cGMP couple (phosphodiesterase 5 inhibitors (PDE5is) and direct soluble guanylate cyclase stimulators (sGCs)), and prostacyclin (prostacyclin analogues (PCA) and prostacyclin receptor agonists (PRA)) [84]. The detailed characteristics of these drugs are provided in **Table 5**. Results from the major studies that focused specifically on their efficacy and safety in CTD-PAH are detailed in **Table 6** [87–98].

As the common feature of these drugs is to induce pulmonary vasodilation, they all expose patients to 2 possible complications [84]:

- *pulmonary edema:* in patients with elevated capillary pressure (as seen in HfpEF or PVOD-like), pulmonary vasodilation will overflow the capillary bed and lead to plasma leakage into the alveolae;
- *worsening of gas exchanges:* in patients with chronic lung diseases (such as CTD-ILD) compensatory vasoconstriction usually occurs in unventilated territories to redistribute blood flow in ventilated segments. Pulmonary vasodilation induced by PAH-specific drugs will alleviate this adaptive mechanism, increasing perfusion in unventilated territories and possibly degrading blood oxygenation.

As such, therapeutic strategy in CTD-PAH now integrates the presence of relevant cardiopulmonary comorbidities, as they condition treatment tolerance [4]. These include cardiovascular risk factors associated with LV diastolic dysfunction (obesity, hypertension, diabetes mellitus, coronary heart disease) and pulmonary conditions (signs of mild parenchymal lung disease, markedly low DLCO as seen in PVOD-like and ILD).

Conventional PAH-approved therapies in CTD-PAH patients are initiated and adapted based on the existence of such comorbidities, as well as on 1-year mortality predicted by risk

assessment models (3-strata at baseline, 4-strata during follow-up) [4]. Treatment objective is to bring and maintain patients in the low-risk category. A detailed therapeutic algorithm is presented in **Figure 2**.

- Immunosuppressants

The place of immune suppression and modulation in the management of CTD-PAH is still a matter of debate [99].

Regarding conventional immunosuppressants, retrospective studies have suggested a potential benefit of corticosteroids combined with cyclophosphamide in SLE- and MCTD-PAH patients, with a response to immunosuppression alone occurring in approximately 50% of patients, especially in case of milder baseline functional limitation and hemodynamic impairment [47,48]. Indications for immunosuppressants in SLE and MCTD-PAH patients should always be discussed with a competence or reference center. Conversely, no response was noted in any of the patients with SSc-PAH [47]. In pSS- and IIM-PAH, response to immunosuppression monotherapy seemed more variable and usually required second-line incrementation by PAH-approved drugs [55,60]. More recently, a small randomized placebo-controlled phase IIa trial demonstrated the safety of tacrolimus in 23 PAH patients (including 5 with CTDs), although no change was noted in the 6MWD, Nt-pro-BNP levels and echocardiographic parameters [100].

Regarding biological therapies, rituximab has recently been evaluated in an NIH-sponsored randomized placebo-controlled trial to treat SSc-PAH patients [101]. The change in 6MWD at week 24, used as the primary outcome measure, did not differ significantly between arms. However, in a secondary analysis using data through week 48, rituximab was superior to placebo. Machine learning identified a subgroup of patients, characterized by low levels of rheumatoid factor, interleukin (IL)-12, and IL-17, that benefited most from treatment. Conversely, a phase II open-label trial of intravenous tocilizumab failed to demonstrate any significant change in PVR in 29 PAH patients (including 10 with CTDs) and was associated with frequent occurrence of moderate/severe adverse effects (20%) [102].

- Sotatercept

Sotatercept, a fusion protein that traps activins involved in PAH pathogenesis, was studied as an add-on therapy in PAH patients, including 18% with CTDs [103]. Treatment with

sotatercept resulted in significant improvement in 6MWD, PVR, Nt-proBNP, WHO functional class and time to clinical worsening in the overall population, although subgroup analysis in CTD-PAH showed a milder effect. Treatment was also associated with an increased incidence of telangiectasias, which warrants careful monitoring in SSc-PAH patients.

- Investigational drugs

Several phase 2 and/or 3 trials are currently investigating the efficacy and safety of novel treatment options in PAH [104]. These includes new drugs that act on "canonical" PAH pathways (vardenafil, an inhaled PDE5i; MK-5475, an inhaled sGCs; ralinepag, an oral PCA); and agents that target original pathways: PDGF signaling (imatinib and seralutinib), serotonin metabolism (rodatristat) or epigenetic regulation (apabetalone).

Group 1: PAH with features of venous/capillary involvement (PVOD-like)

Management of CTD patients with PAH with features of venous/capillary involvement is challenging [11]. Indeed, PAH-specific therapies are usually poorly tolerated in that setting (risk of pulmonary edema; see above). Initial monotherapy with slow dose titration and strict volemia control by diuretics are usually prescribed, but prognosis remains poor.

Group 2

Treatment of group 2 PH mostly consists in the management of the underlying left heart disease [6]. The use of PAH-specific therapies in that setting is controversial, as there are concerns regarding their tolerance (risk of pulmonary edema; see above). However, recent data indicate that PDE5is may be safely used in patients with HFpEF-cpcPH [4].
As such, there is generally no indication for PAH-specific drugs in patients with isolated post-capillary CTD-PH [4]. In patients with cpcPH, the origin of the pre-capillary component should be investigated, as CTD patients may have group 1 and/or group 3 PH due to other disease manifestations. In case a PAH-approved therapy is indicated, PDE5is monotherapy should be preferentially prescribed, with close monitoring of volemia.

Group 3

Similarly, treatment of group 3 PH mostly consists in the management of the underlying chronic lung disease (usually an extensive CTD-ILD) [24]. The use of PAH-specific therapies in

these patients is also an unsettled issue, as these drugs can have detrimental effects on gas exchanges (see above) [105]. Specifically, ambrisentan and riociguat were both associated with increased risk of clinical worsening in patients with idiopathic pulmonary fibrosis-associated PH [4]. Conversely, in a recent randomized controlled trial performed on ILD-PH patients (including 22% with an underlying CTD), inhaled treprostinil was associated with higher 6MWD, lower Nt-proBNP, lower clinical worsening and an excellent tolerance profile [106].

As such, PAH-specific drugs are generally not indicated in most patients with non-severe group 3 CTD-PH [4]. Treatment with inhaled treprostinil (in case of ILD-PH with PVR >3 WU) or PDE5i monotherapy (if PVR >5 WU) can be considered on a case-by-case basis, with close monitoring of blood oxygenation and referral to expert centers.

Group 4

CTEPH is the only potentially curable cause of PH in CTDs [4]. Referral to an expert multidisciplinary team including experienced thoracic surgeons and interventional radiologists is recommended. Treatment takes into consideration the macrovascular (PA obstruction) and microvascular (microangiopathy) components of the disease, and includes a combination of:

- *surgical pulmonary endarterectomy* for accessible lesions;
- *interventional balloon pulmonary angioplasty* and/or *medical therapy with riociguat* for inoperable lesions/patients or PH persistence after surgery.

Prevention of new thromboembolic events by lifelong therapeutic anticoagulation is indicated for all patients.

C. LUNG TRANSPLANTATION

Potentially-eligible CTD-PH patients should be referred early to a transplantation center in case of inadequate response to appropriate therapy (e.g. patients remaining in intermediate-high/high risk or with progressive/worsening disease), treatment by i.v./s.c. prostacyclin analogues or features of venous/capillary involvement [4]. Severe/refractory ILD in patients with group 3 PH can also be an indication in itself.

Exhaustive pre-transplant evaluation is mandatory to identify usual conditions (e.g., chronic/recurrent infections or recent/active neoplasia) as well as CTD-specific manifestations

(especially digital ulcers, active myositis, gastrointestinal disorders, heart conduction/rhythm anomalies, renal involvement) that could act as contraindications [107].

Bilateral lung transplantation is now preferred over heart-lung transplantation in CTD-PH patients. Survival was estimated at 69% at 10 years and is considered similar to (or better than) patients with other transplant indications [108,109].

CONCLUSION

PAH is a rare but severe complication of CTDs. Several progresses have been made over the last decades to improve disease phenotyping, screening, prognosis and treatment. Clinicians managing these patients should be aware of its numerous specificities in terms of clinical features and management.

Table 1. Simplified clinical classification of PH (adapted from [4]).

Group 1. Pulmonary arterial hypertension (PAH)
1.1 Idiopathic PAH
1.2 Heritable PAH
1.3 PAH associated with drugs or toxins
1.4. PAH associated with:
1.4.1. **Connective tissue diseases**
1.4.2. HIV infection
1.4.3. **Portal hypertension**
1.4.4 Congenital heart disease
1.4.5. Schistosomiasis
1.5. **PAH with features of venous/capillary (PVOD/PCH) involvement**
1.6. Persistent PH of the newborn
Group 2. PH associated with left heart disease
2.1. **Heart failure**
2.1.1. with preserved ejection fraction (HFpEF)
2.1.2. with reduced ejection fraction
2.2. Valvular heart disease
2.3. Congenital/acquired cardiovascular conditions leading to post-capillary PH
Group 3. PH associated with lung diseases and/or hypoxia
3.1. **Obstructive lung disease or emphysema**
3.2. **Restrictive lung disease**
3.3. Lung disease with mixed restrictive/obstructive pattern
3.4. Hypoventilation syndromes
3.5. Hypoxia without lung disease (e.g. high altitude)
3.6. Developmental lung disorders
Group 4. PH associated with pulmonary artery obstructions
4.1. **Chronic thrombo-embolic PH**
4.2 Other pulmonary artery obstructions
Group 5. PH with unclear and/or multifactorial mechanisms

Causes of PH that can occur in CTDs are displayed in bold characters.

HIV: human immunodeficiency virus; HFpEF: heart failure with preserved ejection fraction; PCH: pulmonary capillary hemangiomatosis; PH: pulmonary hypertension; PVOD: pulmonary veno-occlusive disease.

Table 2. Exam work-up for CTD patients with suspected/confirmed PH.

	Diagnosis	Causes	Prognosis
TTE	Peak TRV Indirect signs of PH	Left-heart function (group 2 PH) Valves (group 2 PH)	RA area TAPSE/sPAP Pericardial effusion
PFTs and ABGs	DLCO FVC(%)/DLCO(%) DLCO/VA	Lung volumes (group 3 PH) DLCO, PaO_2 (PVOD-like, group 3 PH)	DLCO
BNP/NT-proBNP	Elevation of cardiac biomarkers	/	Elevation of cardiac biomarkers
6MWT	6MWD Exercise desaturation	/	6MWD
ECG	Signs of right heart overload	/	Heart rhythm disorders
Chest CT scan	PA dilation (PA/aorta ratio) Right heart dilation	PVOD-like (group 1 PH) Chronic lung disease (group 3 PH) CTEPH (group 4 PH)	/
Cardiac MRI	PA dilation Right heart dilation	Left-heart function (group 2 PH) Valves (group 2 PH)	RV ejection fraction Stroke volume index RV end-systolic volume index
V/Q lung scintigraphy	/	CTEPH (group 4 PH)	/

6MWD: 6-minute walking distance; 6MWT: 6-minute walking test; ABG: arterial blood gases; BNP: brain natriuretic peptide; CT: computed tomography; CTD: connective tissue disease; CTEPH: chronic thrombo-embolic pulmonary hypertension; DLCO: diffusing capacity of the lung for carbon monoxide; ECG: electrocardiogram; FVC: forced vital capacity; MRI: magnetic resonance imaging; NT-proBNP: N-terminal proBNP; PA: pulmonary artery; PaO2: arterial partial pressure of oxygen; PFT: pulmonary function test; PH: pulmonary hypertension; PVOD: pulmonary veno-occlusive disease; RA: right atrium; RV: right ventricle; sPAP: systolic pulmonary arterial pressure; TAPSE: tricuspid annular plan systolic excursion; TRV: tricuspid regurgitation velocity; TTE: transthoracic echocardiography; V/Q : ventilation/perfusion; VA: alveolar ventilation.

Table 3. RHC referral algorithms in CTD-PH.

2022 ESC/ERS guidelines for PH (adapted from [4])

These recommendations were not specifically developed for CTDs and are based on the assessment of an echocardiographic probability of PH.

Peak TRV (m/s)	Indirect signs of PH	Echocardiographic probability of PH	RHC referral
≤ 2.8 (or unmeasurable)	No	Low	RHC not recommended TTE follow-up
≤ 2.8 (or unmeasurable)	Yes	Intermediate	RHC discussed TTE follow-up
2.9-3.4	No		
2.9-3.4	Yes	Strong	RHC recommended
> 3.4	/		

Khanna et al. 2013 [81]

These recommendations were specifically formulated for CTD-PAH and suggest that RHC should be performed in each of the following situations:

- *TTE criteria:*
  - peak TRV 2.5-2.8 m/s + clinical signs suggestive of PH
  - peak TRV > 2.8 m/s
  - RA (> 53 mm) or RV (> 35 mm) dilation
- *PFT criteria:*
  - FVC(%)/DLCO(%) > 1.6 and/or DLCO < 60% + clinical signs suggestive of PH
  - FVC(%)/DLCO(%) > 1.6 and/or DLCO < 60% + Nt-proBNP > 2xN

DETECT algorithm [30]

The DETECT algorithm was established using data from a prospective study including 466 SSc patients who all received systematic RHC. Patients with disease duration < 3 years or DLCO > 60% were considered at low risk of PH and not included. The developed score has the advantage of a combined multimodal approach (clinical data, labs, PFT and TTE) and proceeds in successive steps:

- *Preliminary step:* eligibility for the DETECT score
  The score can only be used in SSc patients with disease duration > 3 years and DLCO < 60%.
- *Step 1:* collection of six pre-echocardiographic items (telangiectasias, FVC(%)/DLCO(%) ratio, anti-centromere status, NT-proBNP, uricemia, right axial deviation in ECG)
  If the total score exceeds 300 points, the patient should be referred for TTE.
- *Step 2:* collection of two echocardiographic items (peak TRV, RA area)
  If the total score exceeds 35 points, the patient should be referred for RHC.

Its main advantage relies in its excellent sensitivity, better than echocardiography alone. Limitations include its low specificity, the lack of validation in patients with DLCO > 60% and the limited relevance of the pre- echocardiographic step (as TTE is often systematically performed in SSc patients).

ASIG algorithm [74]

The ASIG algorithm was developed in SSc patients and relies on PFTs and Nt-proBNP to shift the burden of PAH screening away from TTE. The screening strategy proceeds as follows:

- *DLCO ≥ 70% with FVC(%)/DLCO(%) < 1.8 AND Nt-proBNP ≤ 210 pg/ml:* PAH unlikely, repeat screening in 12 months
- *DLCO < 70% with FVC(%)/DLCO(%) ≥ 1.8 AND/OR Nt-proBNP > 210 pg/ml:* refer for TTE (± chest CT-scan, V/Q lung scintigraphy, 6MWT) and, if PAH is suspected, to RHC

6MWT: 6-minute walking test; ASIG: Australian Scleroderma Interest Group; CT: computed tomography; CTD: connective tissue disease; DLCO: diffusing capacity of the lung for carbon monoxide; ECG: electrocardiogram; FVC: forced vital capacity; NT-proBNP: N-terminal pro-brain natriuretic peptide; PAH: pulmonary arterial hypertension; PFT: pulmonary function test; PH: pulmonary hypertension; RA: right atrium; RHC: right heart catheterization; RV: right ventricle; SSc: systemic sclerosis; TRV: tricuspid regurgitation velocity; TTE: transthoracic echocardiography; V/Q : ventilation/perfusion

Table 4. Assessment of 1-year mortality in CTD-PAH patients (adapted from Humbert *et al.* 2022 [4]).

Risk assessment at diagnosis (3-strata model)			
	Low risk	**Intermediate risk**	**High risk**
Signs of right heart failure	No	No	Yes
Progression of symptoms	No	Slow	Rapid
Syncope	No	Occasional	Repeated
WHO functional class	I-II	III	IV
6MWD	> 440 m	165-440 m	< 165 m
Cardiopulmonary exercise testing	Peak VO_2 > 15 mL/min/kg (> 65% predicted) VE/VCO_2 slope < 36	Peak VO_2 11-15 mL/min/kg (35-65% predicted) VE/VCO_2 slope 36-44	Peak VO_2 < 11 mL/min/kg (< 35% predicted) VE/VCO_2 slope ≥ 44
Cardiac biomarkers	BNP < 50 ng/L NT-proBNP < 300 ng/L	BNP 50-800 ng/L NT-proBNP 300-1100 ng/L	BNP > 800 ng/L NT-proBNP > 1100 ng/L
Echocardiography	RA area < 18 cm^2 TAPSE/sPAP > 0.32 mm/mmHg No pericardial effusion	RA area 18–26 cm^2 TAPSE/sPAP 0.19–0.32 mm/mmHg Minimal pericardial effusion	RA area >26 cm^2 TAPSE/sPAP <0.19 mm/mmHg Moderate or large pericardial effusion
Cardiac MRI	RVEF >54% SVI >40 mL/m^2 RVESVI <42 mL/m^2	RVEF 37–54% SVI 26–40 mL/m^2 RVESVI 42–54 mL/m^2	RVEF <37% SVI <26 mL/m^2 RVESVI >54 mL/m^2
RHC	RAP <8 mmHg CI ⩾2.5 L/min/m^2 SVI >38 mL/m^2 SvO_2 >65%	RAP 8–14 mmHg CI 2.0–2.4 L/min/m^2 SVI 31–38 mL/m^2 SvO_2 60–65%	RAP >14 mmHg CI <2.0 L/min/m^2 SVI <31 mL/m^2 SvO_2 <60%

Risk assessment at follow-up (4-strata model)				
	Low risk	**Intermediate-low risk**	**Intermediate-high risk**	**High risk**
WHO functional class	I-II	/	III	IV
6MWD	> 440 m	320-440 m	165-319 m	< 165 m
Cardiac biomarkers	BNP < 50 ng/L NT-proBNP < 300 ng/L	BNP 50-199 ng/L NT-proBNP 300-649 ng/L	BNP 200-800 ng/L NT-proBNP 650-1100 ng/L	BNP > 800 ng/L NT-proBNP > 1100 ng/L

6MWD: 6-minute walking distance; BNP: brain natriuretic peptide; CI: cardiac index; MRI: magnetic resonance imaging; NT-proBNP: N-terminal pro-brain natriuretic peptide; PAH: pulmonary arterial hypertension; RA: right atrium; RAP: right atrial pressure; sPAP: systolic pulmonary arterial pressure; SvO_2: mixed venous oxygen saturation; RVESVI: right ventricular end-systolic volume index; RVEF: right ventricular ejection fraction; SVI: stroke volume index; TAPSE: tricuspid annular plane systolic excursion; VE/VCO_2: ventilatory equivalents for carbon dioxide; VO_2: oxygen uptake; WHO-FC: World Health Organization.

Table 5. Characteristics of the main conventional PAH-approved drugs used in CTD-PAH patients.

	Class	Mode of action	Drugs	Route	Starting dose	Target dose	Adverse effects and precautions	Comments
Endothelin pathway	Endothelin receptor antagonists (ERA)	Prevent binding of ET-1 to its receptor ET_RA (± B) on PA-SMCs, which alleviates ET1-induced vasoconstriction and proliferation	Bosentan (dual antagonist)	PO	62.5mg bid	125mg bid	Drug interactions (IS, PDE5i, HIV drugs, warfarine, contraception) Abnormal liver function tests	Also approved for preventing DU recurrence in SSc Monitor liver function 1/month
			Ambrisentan (selective ET_RA antagonist)	PO	5mg od	10mg od	Peripheral edemas	
			Macitentan (dual antagonist)	PO	10mg od	10mg od	Anemia	
NO-cGMP pathway	Phospho-diesterase-5 inhibitors (PDE5i)	Potentiate NO-induced liberation of cGMP in PA-SMCs by stimulating its producing enzyme (sGCs) or inhibiting its degrading enzyme (PDE5i), which induces vasodilation and antiproliferation	Sildenafil	PO	20 mg tid	20 mg tid	Systemic vasodilation*	Do not combine PDE5is and sGCs together or with nitrates (severe hypotension) Sildenafil can also be used for refractory RP or DU healing in SSc
			Tadalafil	PO	20mg od	40mg od		
	Direct sGC stimulators (sGCs)		Riociguat	PO	1mg tid	2.5mg tid		
Prostacyclin pathway	Prostacyclin analogues (PCA)	Reproduce prostacyclin-induced vasodilation and antiproliferation in PA-SMCs	Epoprostenol	cont. IV	2 ng/kg/min	variable	Systemic vasodilation* Catheter infections or obstructions	Continuous infusion using portable pump (education) Administration over 1 (epo) to 3 (trepro) days Rebound PAH in case of abrupt withdrawal
			Treprostinil	cont. SC	1.25 ng/kg/min	variable	Systemic vasodilation* (rare) Local vasodilation: erythema, induration and pain at infusion site	
	Prostacyclin receptor agonists (PRA)		Selexipag	PO	200 µg bid	maximum tolerated dose, up to 1600 µg bid	Systemic vasodilation*	

Only drugs marketed in Europe are presented (macitentan is not marketed in France).
**Side effects due to systemic vasodilation includes pain (headache, extremity pain, jaw pain), flushing, epistaxis, diarrhea/nausea/vomiting, fatigue, systemic hypotension.*
cGMP: cyclic guanosine monophosphate; cont.: continuous; DU: digital ulcers; epo: epoprostenol; ERA: endothelin receptor antagonist; ET-1: endothelin-1; ETR: endothelin receptor; HIV: human immunodeficiency virus; IS: immunosuppressants; IV: intravenous; NO: nitric oxide; PAH: pulmonary arterial hypertension; PA-SMC: pulmonary arterial smooth muscle cells; PCA: prostacyclin analogue; PDE5i: phosphodiesterase-5 inhibitors; PO: per os; PRA: prostacyclin receptor agonist; RP: Raynaud's phenomenon; SC: sub-cutaneous; sGCs: soluble guanylate cyclase stimulators; SSc: systemic sclerosis; trepro: treprostinil.

Table 6. Therapeutic trials of conventional PAH-approved drugs that specifically focused on CTD-PAH patients.

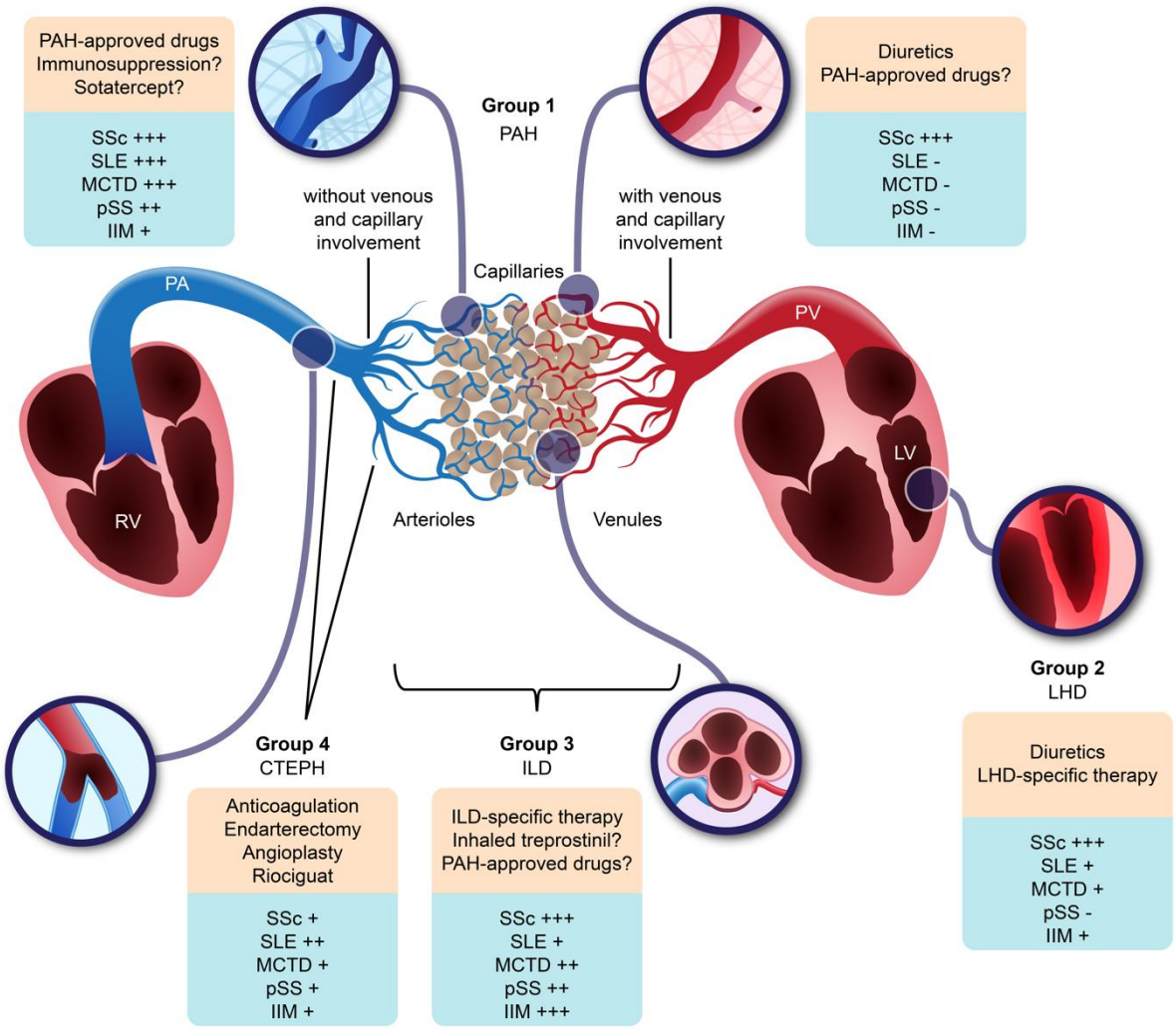
Source	Intervention	Control	Period (weeks)	N	Baseline WHO FC	SSc-PAH (%)	MCTD-PAH (%)	SLE-PAH (%)	Selected outcome measures
Endothelin pathway									
Denton *et al.*, 2006 [87] *Sub-analysis of Study-351 and BREATHE-1*	Bosentan	Placebo	12 or 16	66	III (95%) IV (5%)	79	NA	12	Δ6WMD, TTCW
Launay *et al.*, 2010 [88]	Bosentan	iPAH patients	12 and 52	49	II (12%) III (78%)	100	0	0	Δ6MWD, survival
Badesch *et al.*, 2007 [89] *Sub-analysis of ARIES-1 and -2*	Ambrisentan	Placebo	12	124	NA	NA	NA	NA	Δ6WMD
Badesch *et al.*, 2012 [90] *ARIES-3*	Ambrisentan	Placebo	24	40	II (30%) III (68%)	NA	NA	NA	Δ6WMD
NO-cGMP pathway									
Badesch *et al.*, 2007 [91] *Sub-analysis of SUPER-1*	Sildenafil	Placebo	12	84	II (38%) III (61%)	45	10	22	For 20mg tid: **Δ6WMD +42m** **ΔNYHA ≥ 1 in 29%** **ΔPVR -3.0WU**
Galiè *et al.*, 2009 [92] *Sub-analysis of PHIRST*	Tadalafil	Placebo	16	89	NA	NA	NA	NA	For 40mg od: **Δ6WMD + 49m**
Humbert *et al.*, 2017 [93] *Sub-analysis of PATENT-1 and -2*	Riociguat	Placebo	12	111	II (36%) III (57%)	59	9	16	Δ6WMD, FC, PVR, CI
Combination									
Hassoun *et al.*, 2015 [94]	Combination of ambrisentan and tadalafil	No control	36	24	II (35%) III (65%)	100	0	0	**ΔPVR -55%** **ΔNYHA I/II +26%** **Δ6WMD +52m** (estimated)

Coghlan *et al*., 2017 [95] *Sub-analysis of AMBITION*	Combination of ambrisentan and tadalafil	Ambrisentan or tadalafil monotherapy	NA	187	II (26%) III (74%)	63	12	9	**TTCW reduction of 57%**
Prostacyclin pathway									
Badesch *et al*., 2000 [96]	Epoprostenol IV	Conventional treatment	12	111	III (78%) IV (17%)	100	0	0	**Δ6WMD +63.5m** **ΔPVR -4.58WU**
Oudiz *et al*., 2004 [97] *Sub-analysis of Simonneau et al., 2002*	Treprostinil SC	Placebo	12	90	III (74%) IV (16%)	50	19	28	Δ6WMD
Gaine *et al*., 2017 [98] *Sub-analysis of GRIPHON*	Selexipag	Placebo	NA	334	II (46%) III (53%)	51	11	25	**TTCW reduction of 41%**

Bold characters indicate that the outcome criterion was met. Detailed results are provided only for positive studies; variations are expressed relative to baseline values.

Δ: variation of; 6WMD: 6-minute walking distance; cGMP: cyclic guanosine monophosphate; CI: cardiac index; CTD: connective tissue disease; FC: functional class; i: idiopathic; IV: intravenous; MCTD: mixed connective tissue disease; NA: not available; NO: nitric oxide; PAH: pulmonary arterial hypertension; PVR: pulmonary vascular resistance; RV: right ventricle; SC: sub-cutaneous; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; TTCW: time to clinical worsening; WHO: World Health Organization.

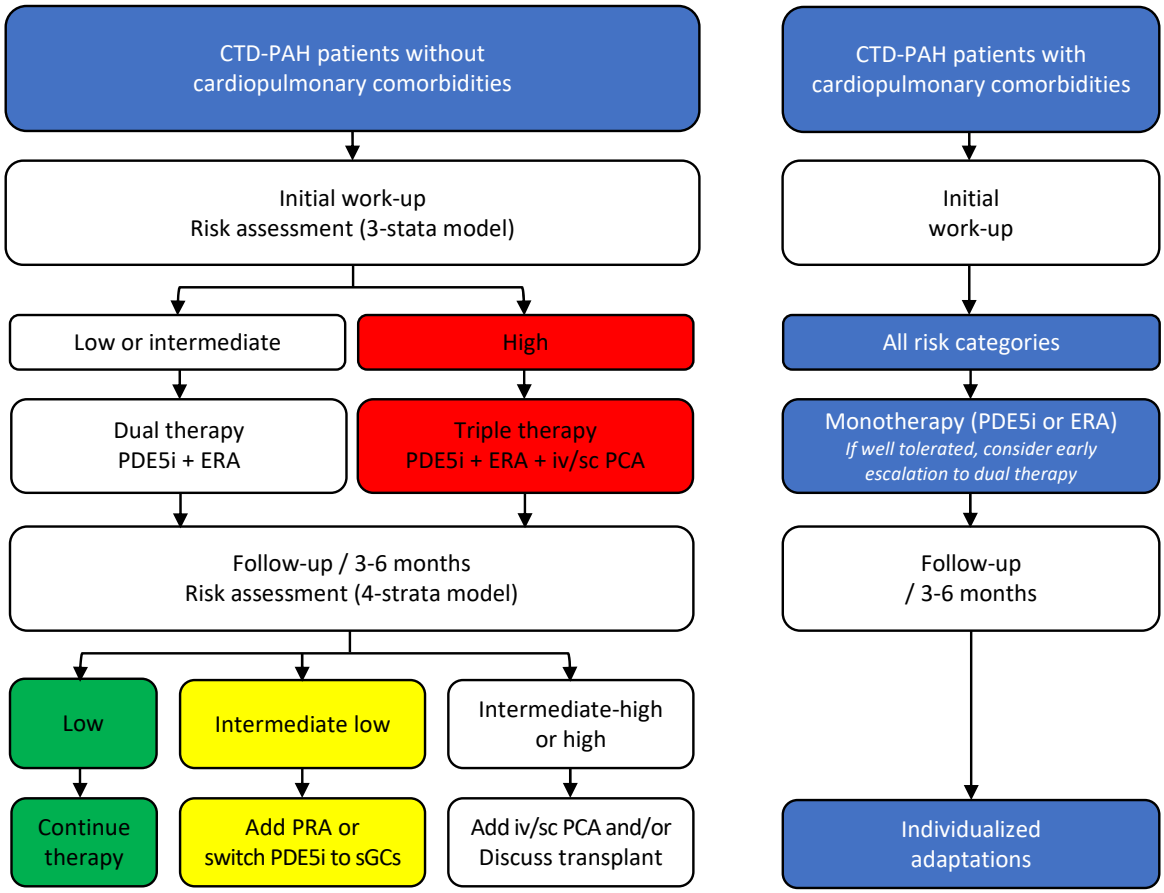
Figure 1. Clinical groups of pulmonary hypertension in connective tissue diseases: pathogenic mechanisms, associated diseases and treatment modalities



Round inserts depict the main pathophysiological lesions responsible for PH in each clinical group.
Yellow squared inserts refer to the main CTDs associated with each PH group. Their relative frequencies are depicted using an arbitrary semi-quantitative scale ranging from - (uncommon association) to +++ (classic association).
Green squared inserts refer to the main therapeutic modalities for each PH group.

CTEPH: chronic thromboembolic PH; IIM: idiopathic inflammatory myopathy; ILD: interstitial lung disease; LHD: left heart disease; LV: left ventricle; MCTD: mixed connective tissue disease; PA: pulmonary artery; PAH: pulmonary arterial hypertension; pSS: primary Sjogren's syndrome; PV: pulmonary vein; RV: right ventricle; SLE: systemic lupus erythematosus; SSc: systemic sclerosis.

Figure 2. Management of conventional PAH-approved therapies in CTD-PAH patients (adapted from Humbert *et al.* 2022 [4])



Relevant cardiopulmonary comorbidities include cardiovascular risk factors associated with LV diastolic dysfunction (obesity, hypertension, diabetes mellitus, coronary heart disease) and pulmonary conditions (signs of mild parenchymal lung disease, DLCO <45%).

Due to the systemic nature of CTDs, treatment adverse effects, and common epidemiology, cardiopulmonary comorbidities are frequent in CTD-PAH. However, it also has a worse prognosis than other forms of PAH and could benefit from early dual therapy. As such, it seems reasonable to escalate treatment to a combination of PDE5i and ERA after a few weeks if the initial monotherapy was well tolerated, and under careful monitoring.

In patients without cardiopulmonary comorbidities, initial triple-combination therapy including an intravenous/subcutaneous prostacyclin analogue should be considered in patients at high risk, or at intermediate risk presenting with major hemodynamic impairment (e.g. PVR ≥12 WU).

CTD: connective tissue disease; ERA: endothelin receptor antagonist; iv: intravenous; PAH: pulmonary arterial hypertension; PCA: prostacyclin analogue; PDE5i: phosphodiesterase-5 inhibitors; PRA: prostacyclin receptor agonist; sc: sub-cutaneous.

REFERENCES

[1] Crestani B. The respiratory system in connective tissue disorders. Allergy 2005;60:715–34. https://doi.org/10.1111/j.1398-9995.2005.00761.x.

[2] Hatano S, Strasser T, World Health Organization. Primary pulmonary hypertension: report on a WHO meeting. Geneva: 1975.

[3] Kovacs G, Olschewski A, Berghold A, Olschewski H. Pulmonary vascular resistances during exercise in normal subjects: a systematic review. Eur Respir J 2012;39:319–28. https://doi.org/10.1183/09031936.00008611.

[4] Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, et al. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Respir J 2022. https://doi.org/10.1183/13993003.00879-2022.

[5] Rosenkranz S, Preston IR. Right heart catheterisation: best practice and pitfalls in pulmonary hypertension. Eur Respir Rev 2015;24:642–52. https://doi.org/10.1183/16000617.0062-2015.

[6] Vachiéry J-L, Tedford RJ, Rosenkranz S, Palazzini M, Lang I, Guazzi M, et al. Pulmonary hypertension due to left heart disease. Eur Respir J 2019;53. https://doi.org/10.1183/13993003.01897-2018.

[7] Montani D, Savale L, Natali D, Jaïs X, Herve P, Garcia G, et al. Long-term response to calcium-channel blockers in non-idiopathic pulmonary arterial hypertension. Eur Heart J 2010;31:1898–907. https://doi.org/10.1093/eurheartj/ehq170.

[8] Launay D, Sobanski V, Hachulla E, Humbert M. Pulmonary hypertension in systemic sclerosis: different phenotypes. Eur Respir Rev 2017;26:170056. https://doi.org/10.1183/16000617.0056-2017.

[9] Olsson KM, Hoeper MM, Pausch C, Grünig E, Huscher D, Pittrow D, et al. Pulmonary vascular resistance predicts mortality in patients with pulmonary hypertension associated with interstitial lung disease: results from the COMPERA registry. Eur Respir J 2021;58. https://doi.org/10.1183/13993003.01483-2021.

[10] Günther S, Jaïs X, Maitre S, Bérezné A, Dorfmüller P, Seferian A, et al. Computed tomography findings of pulmonary venoocclusive disease in scleroderma patients presenting with precapillary pulmonary hypertension. Arthritis Rheum 2012;64:2995–3005. https://doi.org/10.1002/art.34501.

[11] Montani D, Lau EM, Dorfmüller P, Girerd B, Jaïs X, Savale L, et al. Pulmonary veno-occlusive disease. Eur Respir J 2016;47:1518–34. https://doi.org/10.1183/13993003.00026-2016.

[12] Hachulla E, Launay D. Diagnosis and classification of systemic sclerosis. Clin Rev Allergy Immunol 2011;40:78–83. https://doi.org/10.1007/s12016-010-8198-y.

[13] Steen VD, Medsger TA. Changes in causes of death in systemic sclerosis, 1972-2002. Ann Rheum Dis 2007;66:940–4. https://doi.org/10.1136/ard.2006.066068.

[14] Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Pulmonary arterial hypertension in France: results from a national registry. Am J Respir Crit Care Med 2006;173:1023–30.

[15] Condliffe R, Kiely DG, Peacock AJ, Corris PA, Gibbs JSR, Vrapi F, et al. Connective tissue disease-associated pulmonary arterial hypertension in the modern treatment era. Am J Respir Crit Care Med 2009;179:151–7. https://doi.org/10.1164/rccm.200806-953OC.

[16] Chung L, Liu J, Parsons L, Hassoun PM, McGoon M, Badesch DB, et al. Characterization of Connective Tissue Disease-Associated Pulmonary Arterial Hypertension From REVEAL. Chest 2010;138:1383–94. https://doi.org/10.1378/chest.10-0260.

[17] Overbeek MJ, Vonk MC, Boonstra A, Voskuyl AE, Vonk-Noordegraaf A, Smit EF, et al. Pulmonary arterial hypertension in limited cutaneous systemic sclerosis: a distinctive vasculopathy. Eur Respir J 2009;34:371–9. https://doi.org/10.1183/09031936.00106008.

[18] Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, et al. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. Eur Respir J 2019;53:1801887. https://doi.org/10.1183/13993003.01887-2018.

[19] Le Pavec J, Humbert M, Mouthon L, Hassoun PM. Systemic sclerosis-associated pulmonary arterial hypertension. Am J Respir Crit Care Med 2010;181:1285–93. https://doi.org/10.1164/rccm.200909-1331PP.

[20] Cool CD, Kennedy D, Voelkel NF, Tuder RM. Pathogenesis and evolution of plexiform lesions in pulmonary hypertension associated with scleroderma and human immunodeficiency virus infection. Hum Pathol 1997;28:434–42. https://doi.org/10.1016/s0046-8177(97)90032-0.

[21] Dorfmüller P, Humbert M, Perros F, Sanchez O, Simonneau G, Müller K-M, et al. Fibrous remodeling of the pulmonary venous system in pulmonary arterial hypertension associated with connective tissue diseases. Hum Pathol 2007;38:893–902. https://doi.org/10.1016/j.humpath.2006.11.022.

[22] Sanges S, Guerrier T, Duhamel A, Guilbert L, Hauspie C, Largy A, et al. Soluble markers of B cell activation suggest a role of B cells in the pathogenesis of systemic sclerosis-associated pulmonary arterial hypertension.

Front Immunol 2022;13:954007. https://doi.org/10.3389/fimmu.2022.954007.
[23] Chepy A, Bourel L, Koether V, Launay D, Dubucquoi S, Sobanski V. Can Antinuclear Antibodies Have a Pathogenic Role in Systemic Sclerosis? Front Immunol 2022;13.
[24] Launay D, Humbert M, Berezne A, Cottin V, Allanore Y, Couderc L-J, et al. Clinical characteristics and survival in systemic sclerosis-related pulmonary hypertension associated with interstitial lung disease. Chest 2011;140:1016–24. https://doi.org/10.1378/chest.10-2473.
[25] Champtiaux N, Cottin V, Chassagnon G, Chaigne B, Valeyre D, Nunes H, et al. Combined pulmonary fibrosis and emphysema in systemic sclerosis: A syndrome associated with heavy morbidity and mortality. Semin Arthritis Rheum 2019;49:98–104. https://doi.org/10.1016/j.semarthrit.2018.10.011.
[26] Schoenfeld SR, Choi HK, Sayre EC, Aviña-Zubieta JA. The Risk of Pulmonary Embolism and Deep Venous Thrombosis in Systemic Sclerosis: A General Population-Based Study. Arthritis Care Res 2016;68:246–53. https://doi.org/10.1002/acr.22673.
[27] Okyar B, Albayrak F, Torun B, Atilla N, Kızıldağ B, Yıldız F, et al. Experience of chronic thromboembolic pulmonary hypertension (CTEPH) in two cases with scleroderma and immunopathogenesis overview: Case report. J Surg Med 2021;5:188–92. https://doi.org/10.28982/josam.841679.
[28] Matsui T, Kikuchi N, Serizawa N, Hagiwara N. Systemic sclerosis complicated by chronic thromboembolic pulmonary hypertension treated with balloon pulmonary angioplasty: a case report. Eur Heart J Case Rep 2022;6:ytac080. https://doi.org/10.1093/ehjcr/ytac080.
[29] Launay D, Montani D, Hassoun PM, Cottin V, Le Pavec J, Clerson P, et al. Clinical phenotypes and survival of pre-capillary pulmonary hypertension in systemic sclerosis. PloS One 2018;13:e0197112. https://doi.org/10.1371/journal.pone.0197112.
[30] Coghlan JG, Denton CP, Grünig E, Bonderman D, Distler O, Khanna D, et al. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. Ann Rheum Dis 2013:annrheumdis-2013-203301. https://doi.org/10.1136/annrheumdis-2013-203301.
[31] Avouac J, Airò P, Meune C, Beretta L, Dieude P, Caramaschi P, et al. Prevalence of Pulmonary Hypertension in Systemic Sclerosis in European Caucasians and Metaanalysis of 5 Studies. J Rheumatol 2010;37:2290–8. https://doi.org/10.3899/jrheum.100245.
[32] Xanthouli P, Jordan S, Milde N, Marra A, Blank N, Egenlauf B, et al. Haemodynamic phenotypes and survival in patients with systemic sclerosis: the impact of the new definition of pulmonary arterial hypertension. Ann Rheum Dis 2020;79:370–8. https://doi.org/10.1136/annrheumdis-2019-216476.
[33] Puigrenier S, Giovannelli J, Lamblin N, De Groote P, Fertin M, Bervar J-F, et al. Mild pulmonary hemodynamic alterations in patients with systemic sclerosis: relevance of the new 2022 ESC/ERS definition of pulmonary hypertension and impact on mortality. Respir Res 2022;23:284. https://doi.org/10.1186/s12931-022-02205-4.
[34] Hachulla E, de Groote P, Gressin V, Sibilia J, Diot E, Carpentier P, et al. The three-year incidence of pulmonary arterial hypertension associated with systemic sclerosis in a multicenter nationwide longitudinal study in France. Arthritis Rheum 2009;60:1831–9. https://doi.org/10.1002/art.24525.
[35] Jiang Y, Turk MA, Pope JE. Factors associated with pulmonary arterial hypertension (PAH) in systemic sclerosis (SSc). Autoimmun Rev 2020;19:102602. https://doi.org/10.1016/j.autrev.2020.102602.
[36] Hachulla E, Launay D, Mouthon L, Sitbon O, Berezne A, Guillevin L, et al. Is Pulmonary Arterial Hypertension Really a Late Complication of Systemic Sclerosis? Chest 2009;136:1211–9. https://doi.org/10.1378/chest.08-3042.
[37] Lefèvre G, Dauchet L, Hachulla E, Montani D, Sobanski V, Lambert M, et al. Survival and Prognostic Factors in Systemic Sclerosis-Associated Pulmonary Hypertension: A Systematic Review and Meta-Analysis: Survival and Prognosis in SSc-Associated Pulmonary Hypertension. Arthritis Rheum 2013;65:2412–23. https://doi.org/10.1002/art.38029.
[38] Hachulla E, Launay D, Boucly A, Mouthon L, de Groote P, Cottin V, et al. Survival Improved in Patients Aged ≤ 70 Years With Systemic Sclerosis-Associated Pulmonary Arterial Hypertension During the Period 2006 to 2017 in France. Chest 2019:S0012369219342163. https://doi.org/10.1016/j.chest.2019.10.045.
[39] Launay D, Sitbon O, Hachulla E, Mouthon L, Gressin V, Rottat L, et al. Survival in systemic sclerosis-associated pulmonary arterial hypertension in the modern management era. Ann Rheum Dis 2013;72:1940–6. https://doi.org/10.1136/annrheumdis-2012-202489.
[40] Hao Y-J, Jiang X, Zhou W, Wang Y, Gao L, Wang Y, et al. Connective tissue disease-associated pulmonary arterial hypertension in Chinese patients. Eur Respir J 2014;44:963–72. https://doi.org/10.1183/09031936.00182813.
[41] Arnaud L, Agard C, Haroche J, Cacoub P, Piette J-C, Amoura Z. [Pulmonary arterial hypertension in systemic lupus erythematosus]. Rev Med Interne 2011;32:689–97.

https://doi.org/10.1016/j.revmed.2011.01.002.
[42] Hachulla E, Jais X, Cinquetti G, Clerson P, Rottat L, Launay D, et al. Pulmonary Arterial Hypertension Associated With Systemic Lupus Erythematosus: Results From the French Pulmonary Hypertension Registry. Chest 2018;153:143–51. https://doi.org/10.1016/j.chest.2017.08.014.
[43] Jiang X, Du Y, Cheng C-Y, Denas G, Zhou Y-P, Wu T, et al. Antiphospholipid Syndrome in Chronic Thromboembolic Pulmonary Hypertension: A Well-Defined Subgroup of Patients. Thromb Haemost 2019;119:1403–8. https://doi.org/10.1055/s-0039-1692428.
[44] Schreiber BE, Connolly MJ, Coghlan JG. Pulmonary hypertension in systemic lupus erythematosus. Best Pract Res Clin Rheumatol 2013;27:425–34. https://doi.org/10.1016/j.berh.2013.07.011.
[45] Ruiz-Irastorza G, Garmendia M, Villar I, Egurbide M-V, Aguirre C. Pulmonary hypertension in systemic lupus erythematosus: prevalence, predictors and diagnostic strategy. Autoimmun Rev 2013;12:410–5. https://doi.org/10.1016/j.autrev.2012.07.010.
[46] Qian J, Wang Y, Huang C, Yang X, Zhao J, Wang Q, et al. Survival and prognostic factors of systemic lupus erythematosus-associated pulmonary arterial hypertension: A PRISMA-compliant systematic review and meta-analysis. Autoimmun Rev 2016;15:250–7. https://doi.org/10.1016/j.autrev.2015.11.012.
[47] Sanchez O, Sitbon O, Jaïs X, Simonneau G, Humbert M. Immunosuppressive therapy in connective tissue diseases-associated pulmonary arterial hypertension. Chest 2006;130:182–9. https://doi.org/10.1378/chest.130.1.182.
[48] Jais X, Launay D, Yaici A, Le Pavec J, Tchérakian C, Sitbon O, et al. Immunosuppressive therapy in lupus- and mixed connective tissue disease–associated pulmonary arterial hypertension: A retrospective analysis of twenty-three cases. Arthritis Rheum 2008;58:521–31. https://doi.org/10.1002/art.23303.
[49] Sanges S, Savale L, Lamblin N, Rémy-Jardin M, Humbert M, Sobanski V. Efficacy of immunosuppressants with bridge vasodilator therapy in severe lupus erythematosus-associated pulmonary arterial hypertension. ESC Heart Fail 2019;6:1322–5. https://doi.org/10.1002/ehf2.12507.
[50] Gunnarsson R, Andreassen AK, Molberg Ø, Lexberg ÅS, Time K, Dhainaut ASS, et al. Prevalence of pulmonary hypertension in an unselected, mixed connective tissue disease cohort: results of a nationwide, Norwegian cross-sectional multicentre study and review of current literature. Rheumatology 2013;52:1208–13. https://doi.org/10.1093/rheumatology/kes430.
[51] Chaigne B, Chevalier K, Boucly A, Agard C, Baudet A, Bourdin A, et al. In-depth characterization of pulmonary arterial hypertension in mixed connective tissue disease: a French national multicentre study. Rheumatology 2023:kead055. https://doi.org/10.1093/rheumatology/kead055.
[52] Hajas A, Szodoray P, Nakken B, Gaal J, Zöld E, Laczik R, et al. Clinical course, prognosis, and causes of death in mixed connective tissue disease. J Rheumatol 2013;40:1134–42. https://doi.org/10.3899/jrheum.121272.
[53] Sobanski V, Giovannelli J, Lynch BM, Schreiber BE, Nihtyanova SI, Harvey J, et al. Characteristics and Survival of Anti-U1 RNP Antibody-Positive Patients With Connective Tissue Disease-Associated Pulmonary Arterial Hypertension. Arthritis Rheumatol Hoboken NJ 2016;68:484–93. https://doi.org/10.1002/art.39432.
[54] Goulabchand R, Roubille C, Montani D, Fesler P, Bourdin A, Malafaye N, et al. Cardiovascular Events, Sleep Apnoea, and Pulmonary Hypertension in Primary Sjögren's Syndrome: Data from the French Health Insurance Database. J Clin Med 2021;10:5115. https://doi.org/10.3390/jcm10215115.
[55] Launay D, Hachulla E, Hatron P-Y, Jais X, Simonneau G, Humbert M. Pulmonary arterial hypertension: a rare complication of primary Sjögren syndrome: report of 9 new cases and review of the literature. Medicine (Baltimore) 2007;86:299–315.
[56] Yamasaki Y, Suzuki K, Kamijima R, Asari Y, Tsuchida K, Mizushima M, et al. Combined disease with pulmonary arterial hypertension and pulmonary venous hypertension revealed after treatment of heart failure with preserved ejection fraction in a case with primary Sjögren syndrome. Mod Rheumatol 2018;28:193–6. https://doi.org/10.3109/14397595.2015.1059989.
[57] Zeng X, Liu Q, Rathinasabapathy A, Zha L, Liu D, Tang Y, et al. Pulmonary veno-occlusive disease in Sjogren's syndrome: a case report. BMC Pulm Med 2023;23:26. https://doi.org/10.1186/s12890-023-02322-w.
[58] Wang J, Li M, Wang Q, Zhang X, Qian J, Zhao J, et al. Pulmonary arterial hypertension associated with primary Sjögren's syndrome: a multicentre cohort study from China. Eur Respir J 2020;56:1902157. https://doi.org/10.1183/13993003.02157-2019.
[59] Yan S, Li M, Wang H, Yang X, Zhao J, Wang Q, et al. Characteristics and risk factors of pulmonary arterial hypertension in patients with primary Sjögren's syndrome. Int J Rheum Dis 2018;21:1068–75. https://doi.org/10.1111/1756-185X.13290.
[60] Sanges S, Yelnik CM, Sitbon O, Benveniste O, Mariampillai K, Phillips-Houlbracq M, et al. Pulmonary arterial hypertension in idiopathic inflammatory myopathies: Data from the French pulmonary hypertension

registry and review of the literature. Medicine (Baltimore) 2016;95:e4911. https://doi.org/10.1097/MD.0000000000004911.
[61] Bhansing KJ, Vonk-Noordegraaf A, Oosterveer FP, van Riel PL, Vonk MC. Pulmonary arterial hypertension, a novelty in idiopathic inflammatory myopathies: insights and first experiences with vasoactive therapy. RMD Open 2017;3:e000331. https://doi.org/10.1136/rmdopen-2016-000331.
[62] Mustafa KN, Dahbour SS. Clinical characteristics and outcomes of patients with idiopathic inflammatory myopathies from Jordan 1996-2009. Clin Rheumatol 2010;29:1381–5. https://doi.org/10.1007/s10067-010-1465-8.
[63] Wang H, Liu T, Cai Y, Luo L, Wang M, Yang M, et al. Pulmonary hypertension in polymyositis. Clin Rheumatol 2015;34:2105–12. https://doi.org/10.1007/s10067-015-3095-7.
[64] Hervier B, Meyer A, Dieval C, Uzunhan Y, Devilliers H, Launay D, et al. Pulmonary hypertension in antisynthetase syndrome: prevalence, aetiology and survival. Eur Respir J 2013;42:1271–82. https://doi.org/10.1183/09031936.00156312.
[65] Montani D, Henry J, O'Connell C, Jaïs X, Cottin V, Launay D, et al. Association between Rheumatoid Arthritis and Pulmonary Hypertension: Data from the French Pulmonary Hypertension Registry. Respir Int Rev Thorac Dis 2018;95:244–50. https://doi.org/10.1159/000485631.
[66] Lacoste-Palasset T, Chaumais M-C, Weatherald J, Savale L, Jaïs X, Price LC, et al. Association between Leflunomide and Pulmonary Hypertension. Ann Am Thorac Soc 2021;18:1306–15. https://doi.org/10.1513/AnnalsATS.202008-913OC.
[67] Augustine DX, Coates-Bradshaw LD, Willis J, Harkness A, Ring L, Grapsa J, et al. Echocardiographic assessment of pulmonary hypertension: a guideline protocol from the British Society of Echocardiography. Echo Res Pract 2018;5:G11–24. https://doi.org/10.1530/ERP-17-0071.
[68] Colalillo A, Hoffmann-Vold A-M, Pellicano C, Romaniello A, Gabrielli A, Hachulla E, et al. The role of TAPSE/sPAP ratio in predicting pulmonary hypertension and mortality in the systemic sclerosis EUSTAR cohort. Autoimmun Rev 2023;22:103290. https://doi.org/10.1016/j.autrev.2023.103290.
[69] Sun X-G, Hansen JE, Oudiz RJ, Wasserman K. Pulmonary function in primary pulmonary hypertension. J Am Coll Cardiol 2003;41:1028–35. https://doi.org/10.1016/S0735-1097(02)02964-9.
[70] Nihtyanova SI, Schreiber BE, Ong VH, Wells AU, Coghlan JG, Denton CP. Dynamic Prediction of Pulmonary Hypertension in Systemic Sclerosis Using Landmark Analysis. Arthritis Rheumatol 2023;75:449–58. https://doi.org/10.1002/art.42349.
[71] Montani D, Achouh L, Dorfmüller P, Le Pavec J, Sztrymf B, Tchérakian C, et al. Pulmonary veno-occlusive disease: clinical, functional, radiologic, and hemodynamic characteristics and outcome of 24 cases confirmed by histology. Medicine (Baltimore) 2008;87:220–33. https://doi.org/10.1097/MD.0b013e31818193bb.
[72] Chandra S, Shah SJ, Thenappan T, Archer SL, Rich S, Gomberg-Maitland M. Carbon monoxide diffusing capacity and mortality in pulmonary arterial hypertension. J Heart Lung Transplant Off Publ Int Soc Heart Transplant 2010;29:181–7. https://doi.org/10.1016/j.healun.2009.07.005.
[73] Donato L, Elisiana CG, Giuseppe G, Pietro S, Michele C, Brunetti ND, et al. Utility of FVC/DLCO ratio to stratify the risk of mortality in unselected subjects with pulmonary hypertension. Intern Emerg Med 2017;12:319–26. https://doi.org/10.1007/s11739-016-1573-9.
[74] Thakkar V, Stevens WM, Prior D, Moore OA, Byron J, Liew D, et al. N-terminal pro-brain natriuretic peptide in a novel screening algorithm for pulmonary arterial hypertension in systemic sclerosis: a case-control study. Arthritis Res Ther 2012;14:R143. https://doi.org/10.1186/ar3876.
[75] ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111–7. https://doi.org/10.1164/ajrccm.166.1.at1102.
[76] Sanges S, Launay D, Rhee RL, Sitbon O, Hachulla É, Mouthon L, et al. A prospective study of the 6 min walk test as a surrogate marker for haemodynamics in two independent cohorts of treatment-naïve systemic sclerosis-associated pulmonary arterial hypertension. Ann Rheum Dis 2015:annrheumdis-2015-207336. https://doi.org/10.1136/annrheumdis-2015-207336.
[77] Sanges S, Giovannelli J, Sobanski V, Morell-Dubois S, Maillard H, Lambert M, et al. Factors associated with the 6-minute walk distance in patients with systemic sclerosis. Arthritis Res Ther 2017;19:279. https://doi.org/10.1186/s13075-017-1489-4.
[78] Henkens IR, Mouchaers KTB, Vonk-Noordegraaf A, Boonstra A, Swenne CA, Maan AC, et al. Improved ECG detection of presence and severity of right ventricular pressure load validated with cardiac magnetic resonance imaging. Am J Physiol Heart Circ Physiol 2008;294:H2150-2157. https://doi.org/10.1152/ajpheart.01312.2007.
[79] Remy-Jardin M, Ryerson CJ, Schiebler ML, Leung ANC, Wild JM, Hoeper MM, et al. Imaging of Pulmonary

Hypertension in Adults: A Position Paper from the Fleischner Society. Radiology 2021;298:531–49. https://doi.org/10.1148/radiol.2020203108.
[80] Hachulla E, Agard C, Allanore Y, Avouac J, Bader-Meunier B, Belot A, et al. French recommendations for the management of systemic sclerosis. Orphanet J Rare Dis 2021;16:322. https://doi.org/10.1186/s13023-021-01844-y.
[81] 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. https://doi.org/10.1002/art.38172.
[82] Launay D, Sobanski V. [Pulmonary hypertension screening in systemic sclerosis: The best strategy remains to be defined]. Rev Med Interne 2017;38:499–501. https://doi.org/10.1016/j.revmed.2017.05.003.
[83] Boucly A, Weatherald J, Savale L, Groote P de, Cottin V, Prévot G, et al. External validation of a refined four-stratum risk assessment score from the French pulmonary hypertension registry. Eur Respir J 2022;59. https://doi.org/10.1183/13993003.02419-2021.
[84] Sobanski V, Launay D, Hachulla E, Humbert M. Current Approaches to the Treatment of Systemic-Sclerosis-Associated Pulmonary Arterial Hypertension (SSc-PAH). Curr Rheumatol Rep 2016;18:10. https://doi.org/10.1007/s11926-015-0560-x.
[85] Preston IR, Roberts KE, Miller DP, Sen GP, Selej M, Benton WW, et al. Effect of Warfarin Treatment on Survival of Patients With Pulmonary Arterial Hypertension (PAH) in the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL). Circulation 2015;132:2403–11. https://doi.org/10.1161/CIRCULATIONAHA.115.018435.
[86] Olsson KM, Delcroix M, Ghofrani HA, Tiede H, Huscher D, Speich R, et al. Anticoagulation and survival in pulmonary arterial hypertension: results from the Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension (COMPERA). Circulation 2014;129:57–65. https://doi.org/10.1161/CIRCULATIONAHA.113.004526.
[87] Denton CP, Humbert M, Rubin L, Black CM. Bosentan treatment for pulmonary arterial hypertension related to connective tissue disease: a subgroup analysis of the pivotal clinical trials and their open-label extensions. Ann Rheum Dis 2006;65:1336–40. https://doi.org/10.1136/ard.2005.048967.
[88] Launay D, Sitbon O, Le Pavec J, Savale L, Tchérakian C, Yaïci A, et al. Long-term outcome of systemic sclerosis-associated pulmonary arterial hypertension treated with bosentan as first-line monotherapy followed or not by the addition of prostanoids or sildenafil. Rheumatol Oxf Engl 2010;49:490–500. https://doi.org/10.1093/rheumatology/kep398.
[89] Badesch DB. Ambrisentan therapy for pulmonary arterial hypertension: a comparison by PAH etiology. Chest 2007;132:488B. https://doi.org/10.1378/chest.132.4_MeetingAbstracts.488b.
[90] Badesch DB, Feldman J, Keogh A, Mathier MA, Oudiz RJ, Shapiro S, et al. ARIES-3: ambrisentan therapy in a diverse population of patients with pulmonary hypertension. Cardiovasc Ther 2012;30:93–9. https://doi.org/10.1111/j.1755-5922.2011.00279.x.
[91] Badesch DB, Hill NS, Burgess G, Rubin LJ, Barst RJ, Galiè N, et al. Sildenafil for pulmonary arterial hypertension associated with connective tissue disease. J Rheumatol 2007;34:2417–22.
[92] Galiè N, Brundage BH, Ghofrani HA, Oudiz RJ, Simonneau G, Safdar Z, et al. Tadalafil therapy for pulmonary arterial hypertension. Circulation 2009;119:2894–903. https://doi.org/10.1161/CIRCULATIONAHA.108.839274.
[93] Humbert M, Coghlan JG, Ghofrani H-A, Grimminger F, He J-G, Riemekasten G, et al. Riociguat for the treatment of pulmonary arterial hypertension associated with connective tissue disease: results from PATENT-1 and PATENT-2. Ann Rheum Dis 2017;76:422–6. https://doi.org/10.1136/annrheumdis-2015-209087.
[94] Hassoun PM, Zamanian RT, Damico R, Lechtzin N, Khair R, Kolb TM, et al. Ambrisentan and Tadalafil Up-front Combination Therapy in Scleroderma-associated Pulmonary Arterial Hypertension. Am J Respir Crit Care Med 2015;192:1102–10. https://doi.org/10.1164/rccm.201507-1398OC.
[95] Coghlan JG, Galiè N, Barberà JA, Frost AE, Ghofrani H-A, Hoeper MM, et al. Initial combination therapy with ambrisentan and tadalafil in connective tissue disease-associated pulmonary arterial hypertension (CTD-PAH): subgroup analysis from the AMBITION trial. Ann Rheum Dis 2017;76:1219–27. https://doi.org/10.1136/annrheumdis-2016-210236.
[96] Badesch DB, Tapson VF, McGoon MD, Brundage BH, Rubin LJ, Wigley FM, et al. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease: a randomized, controlled trial. Ann Intern Med 2000;132:425–34.
[97] Oudiz RJ, Schilz RJ, Barst RJ, Galié N, Rich S, Rubin LJ, et al. Treprostinil, a prostacyclin analogue, in pulmonary arterial hypertension associated with connective tissue disease. Chest 2004;126:420–7. https://doi.org/10.1378/chest.126.2.420.

[98] Gaine S, Chin K, Coghlan G, Channick R, Di Scala L, Galiè N, et al. Selexipag for the treatment of connective tissue disease-associated pulmonary arterial hypertension. Eur Respir J 2017;50:1602493. https://doi.org/10.1183/13993003.02493-2016.
[99] Ding Y, Qian J, Zhang S, Xu D, Leng X, Zhao J, et al. Immunosuppressive therapy in patients with connective tissue disease-associated pulmonary arterial hypertension: A systematic review. Int J Rheum Dis 2022;25:982–90. https://doi.org/10.1111/1756-185X.14368.
[100] Spiekerkoetter E, Sung YK, Sudheendra D, Scott V, Rosario PD, Bill M, et al. Randomised placebo-controlled safety and tolerability trial of FK506 (tacrolimus) for pulmonary arterial hypertension. Eur Respir J 2017;50. https://doi.org/10.1183/13993003.02449-2016.
[101] Zamanian RT, Badesch D, Chung L, Domsic RT, Medsger T, Pinckney A, et al. Safety and Efficacy of B-Cell Depletion with Rituximab for the Treatment of Systemic Sclerosis–associated Pulmonary Arterial Hypertension: A Multicenter, Double-Blind, Randomized, Placebo-controlled Trial. Am J Respir Crit Care Med 2021;204:209–21. https://doi.org/10.1164/rccm.202009-3481OC.
[102] Toshner M, Church C, Harbaum L, Rhodes C, Moreschi SSV, Liley J, et al. Mendelian randomisation and experimental medicine approaches to interleukin-6 as a drug target in pulmonary arterial hypertension. Eur Respir J 2022;59. https://doi.org/10.1183/13993003.02463-2020.
[103] Hoeper MM, Badesch DB, Ghofrani HA, Gibbs JSR, Gomberg-Maitland M, McLaughlin VV, et al. Phase 3 Trial of Sotatercept for Treatment of Pulmonary Arterial Hypertension. N Engl J Med 2023;388:1478–90. https://doi.org/10.1056/NEJMoa2213558.
[104] Weatherald J, Boucly A, Peters A, Montani D, Prasad K, Psotka MA, et al. The evolving landscape of pulmonary arterial hypertension clinical trials. The Lancet 2022;400:1884–98. https://doi.org/10.1016/S0140-6736(22)01601-4.
[105] Le Pavec J, Girgis RE, Lechtzin N, Mathai SC, Launay D, Hummers LK, et al. Systemic sclerosis-related pulmonary hypertension associated with interstitial lung disease: impact of pulmonary arterial hypertension therapies. Arthritis Rheum 2011;63:2456–64. https://doi.org/10.1002/art.30423.
[106] Waxman A, Restrepo-Jaramillo R, Thenappan T, Ravichandran A, Engel P, Bajwa A, et al. Inhaled Treprostinil in Pulmonary Hypertension Due to Interstitial Lung Disease. N Engl J Med 2021;384:325–34. https://doi.org/10.1056/NEJMoa2008470.
[107] Launay D, Savale L, Berezne A, Le Pavec J, Hachulla E, Mouthon L, et al. Lung and heart-lung transplantation for systemic sclerosis patients. A monocentric experience of 13 patients, review of the literature and position paper of a multidisciplinary Working Group. Presse Medicale Paris Fr 1983 2014;43:e345-363. https://doi.org/10.1016/j.lpm.2014.01.020.
[108] de Perrot M, Granton JT, McRae K, Pierre AF, Singer LG, Waddell TK, et al. Outcome of patients with pulmonary arterial hypertension referred for lung transplantation: A 14-year single-center experience. J Thorac Cardiovasc Surg 2012;143:910–8. https://doi.org/10.1016/j.jtcvs.2011.08.055.
[109] Pradère P, Tudorache I, Magnusson J, Savale L, Brugiere O, Douvry B, et al. Lung transplantation for scleroderma lung disease: An international, multicenter, observational cohort study. J Heart Lung Transplant 2018;37:903–11. https://doi.org/10.1016/j.healun.2018.03.003.

PARTIE I

Exploration protéomique et premier candidat biomarqueur : l'axe *chemerin*-CMKLR1

Dans une première étape exploratoire, afin d'identifier des candidats biomarqueurs prometteurs, nous avons exploré sans *a priori* le protéome sérique des patients HTAP-SSc à l'aide une technique de protéomique haut-débit, le SOMAscan.

Afin de renforcer la robustesse et la reproductibilité de nos résultats (et en particulier d'éviter toute interférence liée aux atteintes fibrosantes et aux traitements), nous avons fait le choix d'inclure une cohorte homogène et ciblée de patients présentant une HTP pré-capillaire avec prélèvement de sérum réalisé le jour du cathétérisme cardiaque droit, une SSc cutanée limitée, pas de fibrose pulmonaire extensive, ni de traitement vasodilatateur spécifique. Ce travail a été rendu possible par une collaboration internationale impliquant les équipes de *Boston University*, *University of Pittsburgh*, Le Kremlin-Bicêtre (Paris-Saclay), Grenoble et Lille.

Nous avons ainsi pu identifier un premier candidat biomarqueur, *chemerin*, qui semble présenter un intérêt diagnostique (biomarqueur de substitution des RVP, susceptible de guider la réalisation d'un cathétérisme cardiaque droit), pronostique (association à la sévérité hémodynamique) et physiopathologique (implication de l'axe *chemerin*-CMKLR1 dans le remodelage vasculaire pulmonaire).

Ces résultats ont fait l'objet d'une publication dans *Annals of the Rheumatic Diseases* (Sanges S, *et al.* Biomarkers of haemodynamic severity of systemic sclerosis-associated pulmonary arterial hypertension by serum proteome analysis. *Ann Rheum Dis* 2023;82:365–373. doi:10.1136/ard-2022-223237 ; version PDF accessible sur le lien suivant : https://ard.bmj.com/content/82/3/365.long).

RESUME DETAILLE EN FRANÇAIS

Au cours de ce projet collaboratif international, nous avons étudié le protéome sérique des patients atteints d'HTAP associée à la sclérodermie systémique (SSc) afin d'identifier des biomarqueurs candidats reflétant la sévérité hémodynamique de la maladie. Parmi 1129 protéines évaluées par une approche protéomique à haut débit (SOMAscan), les taux sériques de *chemerin*, une adipokine impliquée dans différents processus inflammatoires, fibrosants et vasculaires, étaient significativement corrélés aux résistances vasculaires pulmonaires dans une cohorte de patients sélectionnée (SSc cutanée limitée, HTP de groupe 1 sans PID extensive, absence de traitement vasodilatateur spécifique), suivie au sein du *Boston University Arthritis Center*. Ces résultats étaient validés dans une cohorte internationale indépendante, confirmant leur robustesse et permettant d'envisager leur transfert en pratique clinique. Les taux sériques de *chemerin* étaient également mesurés dans d'autres phénotypes de patients SSc (notamment atteints de fibrose cutanée diffuse et de pneumopathie interstitielle diffuse extensive) : leur élévation était spécifiquement observée en cas d'HTAP.

Nous avons ensuite exploré le rôle physiopathologique de *chemerin* dans l'HTAP-SSc. Une étude en cellule unique du transcriptome (*single cell RNA sequencing*) réalisée sur poumons de patients HTAP-SSc localisait la production de *chemerin* au niveau des fibroblastes, des cellules musculaires lisses artérielles pulmonaires (CML-AP)/péricytes et des cellules mésothéliales. En immunofluorescence confocale, la présence de *chemerin* n'était pas détectée au niveau pulmonaire ; mais son récepteur CMKLR1 était surexprimé par les CML-AP. Le sérum de patients HTAP-SSc induisait une prolifération plus importante des CML-AP que le sérum de patients SSc sans HTAP ; un effet qui était neutralisé par un inhibiteur de CMKLR1, α-NETA. Ces résultats plaident pour l'implication de l'axe *chemerin*-CMKLR1 dans la physiopathologie de l'HTAP-SSc.

Biomarkers of Hemodynamic Severity of Systemic Sclerosis-Associated Pulmonary Arterial Hypertension by Serum Proteome Analysis

Sébastien Sanges[1,2,3,4,5,6,*], Lisa Rice[1,*], Ly Tu[7,8], Eleanor Valenzi[9], Jean-Luc Cracowski[10], David Montani[7,8,11], Julio Mantero[1], Camille Ternynck[12], Guillemette Marot[12,13,14], Andreea M. Bujor[1], Éric Hachulla[2,3,4,5,6], David Launay[2,3,4,5,6], Marc Humbert[7,8,11], Christophe Guignabert[7,8], Robert Lafyatis[15].

1. Boston University School of Medicine, E5 Arthritis Center, Boston, MA, USA.
2. Univ. Lille, U1286 – INFINITE – Institute for Translational Research in Inflammation, F-59000 Lille, France
3. INSERM, F-59000 Lille, France
4. CHU Lille, Département de Médecine Interne et Immunologie Clinique, F-59000 Lille, France
5. Centre National de Référence Maladies Auto-immunes Systémiques Rares du Nord et Nord-Ouest de France (CeRAINO), F-59000 Lille, France
6. Health Care Provider of the European Reference Network on Rare Connective Tissue and Musculoskeletal Diseases Network (ReCONNET), F-59000 Lille, France
7. Université Paris Saclay, School of Medicine, Le Kremlin-Bicêtre, France
8. INSERM UMR_S 999, Hôpital Marie-Lannelongue, Le Plessis-Robinson, France
9. Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
10. Univ. Grenoble Alpes, INSERM, HP2, F-38000 Grenoble, France.
11. AP-HP, Department of Respiratory and Intensive Care Medicine, Hôpital Bicêtre, Le Kremlin-Bicêtre, France
12. Univ. Lille, CHU Lille, ULR2694 – METRICS: Évaluation des technologies de santé et des pratiques médicales, F-59000 Lille, France
13. Inria ,MODAL: MOdels for Data Analysis and Learning, F-59000 Lille, France
14. Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, UAR 2014 - US 41 -PLBS, bilille, F-59000 Lille, France

15. Division of Rheumatology, Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

* SS and LR contributed equally

Corresponding author: Dr Sébastien SANGES, Département de Médecine Interne et Immunologie Clinique, CHU Lille, F-59037 Lille Cedex, France.

Tel: + 33 (0)320445048; Fax: + 33 (0)320445459; E-mail: sebastien.sanges@univ-lille.fr

INTRODUCTION

Pulmonary arterial hypertension (PAH) is the most devastating complication of systemic sclerosis (SSc) [1]. A common pathological hallmark of PAH is remodeling of precapillary pulmonary arteries, manifested by pulmonary vasoconstriction and medial thickening due to increased expansion of pulmonary artery (PA) smooth muscle cells (SMCs) [2]. This alteration of the small/medium-sized pulmonary arteries contributes to the progressive and rapid increase in pulmonary vascular resistance (PVR) that eventually lead to right ventricular failure [3]. Right heart catheterization (RHC) is required to confirm the diagnosis of PAH, to assess the severity of the hemodynamic impairment and the response to treatment [4].

PAH has become a leading cause of death in SSc, with a standardized mortality ratio of 5.27 and a median survival of 3 years [1,5]. Improving its prognosis is thus a major challenge when managing SSc patients [3], requiring early diagnosis to allow for timely initiation of treatment, as well as rapid detection of treatment failure to allow for immediate adjustment of medications. However, since diagnosis and disease progression in PAH have hemodynamic definitions, this supposes performing iterative RHCs, highlighting the need to identify biomarkers for the accurate and non-invasive prediction of PAH in SSc patients.

Several works have previously tried to address this issue [6–12]. Most of them focused on identifying surrogates for hemodynamic diagnostic parameters, with biomarkers that could accurately discriminate SSc patients with and without PAH [7–10]. Few studies however have tried to find surrogate markers for severity parameters, such as PVR [6,11]. This is a matter of importance, since PVR reflect the ongoing vascular remodeling underlying the disease progression and can be used to guide treatment initiation and assess therapeutic efficacy [11,13].

This work was an exploratory study that aimed to identify proteins that correlate with hemodynamic severity in SSc-PAH patients and to serve as a base on which future hypotheses could be tested. To achieve this, we used a wide-scale approach by investigating alterations in the patients' serum proteome with a high-throughput assay. In a second step, we assessed whether the identified candidate biomarkers were involved in disease pathogenesis.

METHODS

1. Serum proteome signature of SSc-PAH

1.1. Study population

In a first step, in order to identify candidate biomarkers, a discovery cohort was recruited from Boston University Arthritis Centre. Patients were included if they met the following criteria: (1) diagnosis of SSc according to the 2013 ACR/EULAR criteria [14]; (2) limited cutaneous (lc) subset of SSc according to LeRoy's criteria [15]; (3) absence of extensive ILD according to Goh's criteria [16]; (4) absence of PAH-specific therapy. In that first step, patients with diffuse cutaneous (dc)SSc and/or extensive ILD were excluded in order to avoid our biomarker screening to be interfered with by any active organ involvement other than PAH, and our results to be mediated by any pathogenic process other than pulmonary microangiopathy. Additionally, extensive ILD was also considered an exclusion criterion in order to avoid including patients with secondary (group 3) pulmonary hypertension that probably did not have significant pulmonary microangiopathy. Patients fulfilling these inclusion criteria were classified as cases if they had an RHC-proven diagnosis of PAH according to the 2015 ESC/ERS guidelines [4]; and as controls if they had no evidence of PAH. PAH screening modalities, assessment of PAH probability and referral for RHC followed ESC/ERS guidelines in effect at the time of sample collection. For cases, all serum samples were collected on the same day as RHC.

In a second step, in order to confirm the validity of the identified candidate biomarkers, an independent validation cohort was recruited from Boston University, Grenoble University Hospital and Lille national reference centre for SSc. Patients were included if they met the same criteria as the discovery cohort. Cases and controls were also similarly defined. For cases, serum samples were collected on the same day as RHC (except for 4 samples collected within 6 weeks before).

In a third step, in order to determine if our candidate biomarkers were influenced by other SSc manifestations apart from PAH, 3 other patient groups were recruited from Boston University Arthritis Centre. A first group included patients with dcSSc, no extensive ILD, and no evidence of PAH. A second group included patients with lcSSc, extensive ILD, and no evidence of PAH. A third group included healthy controls.

All patients were recruited in a consecutive fashion by screening the databases of participating centres in 2015. Serum samples had been collected between 2004 and 2014.

1.2. SOMAscan assay

The relative expression levels of 1129 serum proteins were assessed in the discovery cohort using the SOMAscan platform (SomaLogic Inc., Boulder, CO, USA), a highly multiplexed aptamer microarray, as previously described [17,18]. Briefly, samples were deposited in a 96-well plate and incubated with a mixture of the 1129 SOMAmer reagents. Two sequential bead-based immobilization and washing steps eliminated unbound proteins, non-specifically bound proteins and unbound SOMAmer reagents. The remaining specifically bound SOMAmer reagents were isolated, and each reagent was quantified simultaneously on a custom Agilent hybridization array. The amount of each SOMAmer measured was quantitatively proportional to the protein concentration in the original sample. Results were expressed in relative fluorescence units (RFU).

1.3. ELISA assays

To confirm result reproducibility and cross-platform consistency, serum concentrations of candidate proteins were measured in duplicate in the validation cohort using commercial ELISA assays (*Quantikine® ELISA Human Chemerin Immunoassay*, R&D systems; *SET Nuclear Oncogene (SET) ELISA Kit (Human)*, cat. #ABIN1152447, antibodies-online.com) at appropriate dilutions (1:100 for chemerin; 1:1 for SET), according to the manufacturer's protocol.

1.4. Statistical analyses

For the description of study populations, quantitative variables were expressed as means ± standard deviation (SD) or medians (interquartile range) for non-normal distributions, and categorical variables were expressed as numbers (percentage). Normality of distributions was assessed using histograms and tested using the Shapiro-Wilk test.

In a first step, data from the discovery cohort were log2 transformed and a differential analysis was performed between cases and controls with the Bioconductor *R* (v3.6.1) package limma (v3.38.3) [19]. Limma uses an empirical Bayesian approach to estimate variances in moderated *t*-tests, which has proven to improve results on standard t-tests, especially when the number of replicates is small. Raw *P*-values were adjusted with the Benjamini Hochberg method [20] and proteins with adjusted *P*-values<0.05 were considered differentially expressed. For cases, the Spearman correlations between PVR and expressions of these

differentially expressed proteins were tested and were considered significant if raw *P*-values were <0.05.

In a second step, differential expression of candidate biomarkers identified in the discovery cohort was assessed between cases and controls from the validation cohort using Mann-Whitney test; and correlations with PVR in cases were analyzed using Spearman test.
In a third step, serum levels of the candidate biomarkers were compared between the 5 patient groups using the Kruskal–Wallis test. Pairwise comparison was conducted by post-hoc Dunn test followed by Bonferroni correction.

As protein production could always be quantified in all datasets, no imputation for missing data was performed. A heatmap was drawn for differentially expressed proteins with the *R* package pheatmap (v1.0.12) after standardization of the expression data. Other figures were created using GraphPad Prism v9.1.0 (GraphPad Software, CA).

2. Chemerin/CMKLR1 axis and pulmonary vascular remodelling

2.1. Study population

Patients were recruited from Le Kremlin-Bicêtre and Pittsburgh reference centres for PAH. Lung specimens were obtained during transplantation in patients diagnosed with SSc-PAH and/or ILD or idiopathic PAH (iPAH) [21], and during lobectomy or pneumonectomy for localized lung cancer in control subjects (non-SSc controls). Lung specimens from controls were collected at a distance from the tumor foci. Preoperative echocardiography was performed in controls to rule out PAH.

2.2. Single-cell RNA sequencing in explanted human lungs

2.2.1. Data generation

Lung samples from 4 SSc-PAH patients and 4 non-SSc controls were transported in Perfadex and processed within 30 minutes of explantation as previously described [22]. Of note, 3 SSc patients had extensive ILD. Following mechanical and enzymatic digestion by Liberase DL and DNase I, resulting single-cell suspensions were loaded into 10x Genomics Chromium instrument (Pleasanton, CA), with scRNA-seq library preparation per manufacturer's protocol. Sequencing was performed on an Illumina NovaSeq 6000 instrument through the UPMC Genome Center.

2.2.2. Data analysis

Data analysis was performed with R (v4.0.4) package Seurat (v4.1.0). Samples were merged with batch correction by Harmony for individual sample, followed by normalization, identification and visualization of cell populations, and differential expression testing. Cell populations were identified by gene markers and visualized by uniform manifold and approximation projection (UMAP) plot.

2.3. Confocal immunofluorescence analyses in explanted human lungs

Immunofluorescent staining for chemerin and chemokine-like receptor 1 (CMKLR1) was performed on lung paraffin sections from SSc-PAH patients, SSc-no PAH patients and non-SSc controls as previously described [23]. Of note, both SSc patient groups had extensive ILD. Briefly, lung sections were deparaffined and incubated with retrieval buffer. Then, sections were saturated with blocking buffer and incubated overnight with specific antibodies (sc-398769, CliniSciences, France), followed by corresponding secondary fluorescent-labelled antibodies (Thermo-Fisher Scientific, France). Nuclei were labelled using 4',6-diamidino-2-phénylindole (DAPI) (Thermo-Fisher Scientific). Mounting was done using ProLong Gold antifade reagent (Thermo-Fisher Scientific). Images were taken using LSM700 confocal microscope (Zeiss, France).

2.4. Role of CMKLR1 in the proliferation of human PA-SMCs

2.4.1. PA-SMC proliferation experiments

PA-SMCs were isolated from distal PA of lung explants from iPAH patients and non-SSc controls, and cultured as previously described [24,25]. The isolated PA-SMCs were strongly positive for α-smooth muscle actin (α-SMA), smooth muscle-specific SM22 protein and calponin, and negative for von Willebrand factor and CD31. Cells were used at passage < 5.
The mRNA expression of CMKLR1 was measured by real-time quantitative PCR using TaqMan gene expression assay (assay ID: Hs01081979_s1) as previously described [23,26]. Relative quantification was calculated by normalizing the Ct (threshold cycle) of the gene of interest to the Ct of 18S in the same sample, according to the comparative CT method (ΔΔCT method).
PA-SMCs were cultured with 5% serum from 5 SSc-PAH and 5 SSc-no PAH patients included in the proteomic validation cohort, in the presence or absence of 2-(anaphthoyl)ethyltrimethylammonium iodide (α-NETA) at 1µM. Proliferation was assessed by

5-bromo-2-deoxyuridine (BrdU) incorporation and direct cell counting.

2.4.2. Descriptive analysis

Figures were created using GraphPad Prism v9.1.0 (GraphPad Software, CA). Differences were assessed visually without statistical analysis due to small sample size.

RESULTS

1. Serum levels of chemerin are elevated and correlate with PVR in SSc-PAH patients

In a first step, serum expression of 1129 biomarkers was assessed by SOMAscan in 15 cases (SSc-PAH patients) and 16 controls (SSc-no PAH patients) from a discovery cohort (***Table 1;*** for additional details, see also ***Supplementary Table 1***). We identified 53 proteins differentially expressed between the 2 groups (***Figure 1***). Among them, 2 analytes showed a significant correlation with PVR in cases: chemerin (ρ=0.62, *P*=0.01), an adipokine (***Figure 2***); and SET nuclear protooncogene (ρ=0.62, *P*=0.01). Similar results were found when removing an outlier patient with markedly high PVR value from the analysis.

In a second step, to confirm these results, serum levels of chemerin and SET were then measured by ELISA in 24 cases and 17 controls from an independent validation cohort (***Table 1***; ***Supplementary Table 1***). Consistently, serum levels of chemerin were significantly higher (*P*=0.01) and correlated significantly with PVR levels (ρ=0.42, *P*=0.04) in cases (***Figure 2***). Serum SET levels were undetectable in both cases and controls when measured by ELISA, consistent with its intracellular nature.

In a third step, in order to determine if other SSc manifestations influenced biomarker concentrations, chemerin serum levels from our discovery cases and controls were compared with other patient groups included in the SOMAscan dataset: patients with dcSSc, no extensive ILD and no PAH (n=11); patients with lcSSc, extensive ILD and no PAH (n=10); and healthy controls (n=11). Circulating concentrations of chemerin were only increased in the PAH group, and similar to healthy controls in all others (***Figure 3***).

Overall, these results could suggest that chemerin seems a potential surrogate biomarker for PVR in SSc-PAH. Since results with SET were not reproduced, and since literature data suggested chemerin to be a more promising pathophysiological lead, we chose to focus our investigations on this latter protein.

2. Expression of chemerin receptor CMKLR1 is increased on PA-SMCs from SSc-PAH patients

Because the binding of chemerin to its receptor CMKLR1 stimulates the proliferation and migration of PA-SMCs [27], we first performed a single cell RNA-sequencing (scRNAseq) analysis in lungs from 4 SSc-PAH and 4 non-SSc controls to obtain a global view of chemerin and CMKLR1 expression patterns. Our scRNAseq data indicate that CMKLR1 was predominantly expressed by a subpopulation of cells expressing α-SMA and clustering with

PA-SMCs/pericytes and myofibroblasts; as well as by endothelial cells and macrophages (***Figure 4***). However, no significant difference between SSc-PAH patients and non-SSc controls was observed in CMKLR1 mRNA expression levels in these different cell populations. In addition, our scRNAseq data indicated that chemerin is mostly expressed by fibroblasts, PA-SMCs/pericytes and mesothelial cells, with a significant increase in SSc-PAH patients as compared to non-SSc controls for this last cell population (***Figure 4***).

To confirm our scRNAseq data, confocal microscopic analyses were next performed on lung specimens dually labelled with CMLKR1 and a specific PA-SMC marker, α-SMA. Examination of CMLKR1 protein expression patterns showed CMLKR1 staining to be more pronounced in the smooth muscle layer in both patients with SSc and SSc-PAH groups relative to non-SSc controls (***Figure 5***). No difference was observed in chemerin staining between groups (***Supplemental Figure S1***).

Taken altogether, these data could suggest an upregulation in the expression pattern of the chemerin/CMRLK1 axis in SSc-PAH pulmonary vessels.

3. The SSc-PAH serum-induced PA-SMC proliferation seems inhibited by a chemerin-CMKLR1 inhibitor

To further examine the functional consequences of these changes on PA-SMC proliferation, we next determined the efficacy of the CMKLR1 antagonist α-NETA to attenuate the proliferation of PA-SMCs derived from iPAH patients. First, we confirmed an increased CMKLR1 mRNA production in PA-SMCs from iPAH versus control (***Supplemental Figure S2***). Then, PA-SMCs from iPAH patients were stimulated with serum obtained from 5 SSc-PAH and 5 SSc-no PAH patients in the presence or absence of α-NETA (***Figure 6***). Serum from SSc-PAH cases seemed to induce higher PA-SMC proliferation than serum from SSc-no PAH controls. This difference seemed neutralized in the presence of α-NETA.

Overall, these findings suggest that serum from SSc-PAH patients seems to induce proliferation of PA-SMCs and that inhibition of CMKLR1 activation could partly abolish this phenomenon.

DISCUSSION

To our knowledge, this is the first study that focused on identifying surrogate marker for hemodynamic severity in SSc-PAH using a wide-scale approach. Our results can be summarized as follows: (1) chemerin seems to be a potential surrogate marker for hemodynamic severity in SSc-PAH, as it showed robust correlations with PVR; (2) in lungs, chemerin mRNA was detected in fibroblasts, PA-SMCs/pericytes and mesothelial cell populations; (3) elevated chemerin serum levels and increased expression of its receptor CMKLR1 by PA-SMCs could contribute to SSc-PAH pathogenesis by inducing PA-SMC proliferation.

1. Chemerin as a surrogate marker for hemodynamic severity in SSc-PAH

Since PAH is characterized by progressive pulmonary vascular remodeling leading to increased pulmonary vascular resistance, PVR is thought to reflect the severity of this process [13]. As such, it can be used to monitor treatment efficacy and failure. Developing non-invasive methods to assess PVR is thus a major unmet need in the field of SSc-PAH management, that has only been scarcely investigated so far [6,11]. Although there is some dispersion on the scatterplots that could limit future works, the correlation between PVR and chemerin serum levels was reproducible and can be described as moderate to strong based on Spearman coefficient values. In a recent work [11], Bauer *et al.* measured 313 proteins on sera from the DETECT cohort and identified a panel of 8 analytes capable of accurately discriminating SSc-PAH from SSc-no PAH patients. When testing the ability of these biomarkers to predict PVR, they found moderate correlations for a model comprised of 5 proteins: RAGE, NT-pro-BNP, IGFBP-7, SP-D and VCAM-1. Since this study focused primarily on identifying diagnostic biomarkers, and not PVR surrogates, further comparison with our results is challenging.

Interestingly, the increase in chemerin concentrations appeared restricted to SSc-PAH patients in our study. Previous publications have reported on chemerin serum levels in SSc, albeit with conflicting results [28–30]. Akamata *et al*. found similar values between SSc patients and healthy controls, and no difference between cutaneous subsets of the disease [28]. Chemerin levels correlated with modified Rodnan skin score (mRSS) and disease duration in dcSSc patients, as well as with digital ulcers in the whole cohort; but not with DLCO nor elevated systolic pulmonary arterial pressure (sPAP) on echocardiography [28]. According to

Chighizola *et al.*, chemerin levels were lower in diffuse cutaneous (dc) SSc patients but similar in lcSSc patients when compared to healthy controls; and correlated with disease duration (especially in dcSSc) but not with capillaroscopic pattern, ILD or digital ulcers [29]. Sawicka *et al.* observed higher chemerin values in SSc patients, especially among dcSSc, than in controls [30]. They were also associated with acute phase reactants in the whole cohort and with mRSS in dcSSc patients, but not with disease duration, forced vital capacity nor DLCO [30]. In summary, while no association was identified with ILD, previous studies have reported chemerin levels in dcSSc patients as similar, lower and higher that lcSSc and healthy controls. This discrepancy is challenging to explain. It should be noted however that our work used a different design, as we deliberately constituted homogeneous patient subgroups and compared chemerin levels between them, rather than included a broad heterogenous patient population and assessed correlations. We believe that this allowed us to better ascertain the disease parameter responsible for elevated chemerin levels. Moreover, it is possible that the associations observed in dcSSc patients were in fact mediated by undiagnosed PAH (as chemerin levels were reported to correlate with sPAP among dcSSc patients [30]). Finally, this may also be explained by measurement of different chemerin isoforms [31]. In any case, further studies including well-defined homogeneous patient groups are needed to determine whether abnormal serum levels of chemerin are specific to PAH in SSc.

Of note, serum chemerin levels were also studied in in patients with iPAH and were significantly increased compared to controls, with a satisfactory sensitivity (85.7%) and specificity (100%) at a concentration of 471.76 pg/ml [32].

2. Chemerin as a contributor to the pathogenesis of SSc-PAH

Chemerin is pleiotropic protein that exerts multiple effects (acting to varying degrees as a chemokine, an adipokine and/or a growth factor) on different tissues (notably the immune system, the adipose tissue and the vasculature) [31,33]. In blood vessels, chemerin can act on endothelial cells (regulating angiogenesis, cell adhesion and nitric oxide production) [34–37] as well as SMCs (inducing contraction, migration and proliferation) [38–41]. As such, it has been implicated in several cardiovascular diseases, such as arterial hypertension, atherosclerosis, diabetic microangiopathy and pre-eclampsia [31,33]. Chemerin also yields pro-inflammatory effects by attracting and stimulating cytokine production from macrophages, dendritic cells and natural killer cells [42–44]; and thus has also been involved

in various immunologic conditions, such as rheumatoid arthritis and psoriasis [31].

In our work, chemerin mRNA was expressed primarily by fibroblasts and smooth muscle/pericytes, though not increased compared to non-SSc controls; and its protein was not detected by immunostaining. This discrepancy may reflect difficulties in detecting this secreted protein, limitations of the staining antibody, or lack of mRNA translation, possibly suggesting extra-pulmonary production. In our previous work detailing scRNAseq data in iPAH patients, chemerin was also expressed primarily by fibroblasts and upregulated 2.075-fold [45]. These data suggest that upregulated secretion of chemerin by adventitial pulmonary fibroblasts and/or peripheral tissues may contribute to the pathogenesis of SSc-PAH.

Interestingly, previous studies have suggested a role of chemerin in both SSc and PAH pathogenesis [27,28,46]. In SSc patients, chemerin expression is increased in dermal endothelial cells; and circulating levels of chemerin correlated with occurrence of digital ulcers [28]. Chemerin expression was also decreased in dermal fibroblasts, due to autocrine TGF-b secretion and Fli1 deficiency; and serum chemerin levels correlated with mRSS [28]. Of note, CMKLR1 expression in SSc patients' skin was similar to healthy controls' [28]. In PA from healthy rats, chemerin and CMKLR1 expression is detected in both endothelial cells and SMCs [47]. Exposure to chemerin potentiated vascular responses to vasoconstrictors (phenylephrine, endothelin-1 and serotonin) in rat PA and impaired acetylcholine-induced PA vasodilatation, by mechanisms involving at least partly NO signaling and oxidative stress [47]. In primary cultured PASMCs from healthy rats, exposure to chemerin induced a dose-dependent proliferation and migration (potentiated by combination with endothelin-1) [27,32], and reduced staurosporine-induced apoptosis [27]. Chemerin treatment did not alter gene expression of IL-6, IL-6R and IL-1β, but increased TNF-α at high doses [27]. Healthy rat PASMCs exposed to chemerin up-regulated the ERK1/2 pathways; and treatment by an ERK inhibitor annulled the chemerin-induced proliferation [32]. In the monocrotaline (MCT) rat model of PAH, chemerin-induced contraction of intrapulmonary arteries (IPA) is increased compared to controls [46]. Lungs from MCT rats showed increased expression of CMKLR1 and production of various chemerin isoforms (possibly due to glycosylation) compared to healthy animals [46]. Expression of CMKLR1 in MCT rats was increased in SMCs but decreased expression in endothelial cells compared to controls [32,46]. Finally, in studies performed in other vascular diseases, chemerin was able to induce angiogenesis *in vitro* [48]; and knockdown of chemerin significantly inhibited SMC proliferation and neointimal hyperplasia

in a rat model of vascular injury induced by balloon angioplasty [49].

Overall, these data suggest that the chemerin-CMKLR1 axis could contribute to the pathogenesis of SSc-PAH through vascular effects but could also be involved in the fibrotic and inflammatory events occurring during the disease.

3. Strengths and limitations

Our study draws strength from the sample collection on the same day as RHC (which allowed to perform accurate hemodynamic correlations), its wide-scale proteomic approach, the validation of our results on an independent cohort, and evidences for the pathophysiological relevance of the candidate biomarkers.

It also has limitations. Firstly, we limited our study population to untreated patients with lcSSc and non-extensive ILD, so that our biomarker screening would not be interfered with by an active cutaneous or interstitial lung involvement, nor by the effect of PAH-specific therapy. Although it allowed us to generate reproducible results in this specific subgroup, it also limited the generalizability of our findings. Further studies should try to investigate the relevance of chemerin as a PVR surrogate in other causes of PH (especially group 2 and 3), as well as its PAH-specificity in other patient subgroups (notably dcSSc and extensive ILD). Secondly, although we screened a large number candidate biomarkers, more recent panels now include several thousand proteins, that were not tested here. Thirdly, as we wanted to obtain reliable hits, we used statistical methods that limited the risk of false discoveries. Due to this stringent approach, some other relevant biomarkers may not have reach statistical significance and thus been missed. Fourthly, due to small sample size and missing data, we could not assess the added value of chemerin serum levels when integrated in PAH diagnostic algorithm and/or risk assessment. This should be addressed in future studies by performing multi-level analysis on larger cohorts. Fifthly, due to sample availability considerations, lung experiments included different patient populations, such as iPAH, SSc-ILD and patients with a participation of group 3 pulmonary hypertension. Finally, as our work had a cross-sectional design, we could not assess the capacity of chemerin level variations to reflect changes in PVR during follow-up. Longitudinal studies are warranted to test its value as a surrogate for treatment efficacy and failure.

4. Conclusions

Using a wide-scale proteomic approach, we identified chemerin as a potential surrogate for PVR in SSc-PAH patients, that could be interesting as a non-invasive assessment of hemodynamic severity. Chemerin and its receptor CMKLR1 could contribute to the pathogenesis of the disease through PA-SMC proliferation, but could also be involved in inflammatory and fibrotic processes occurring during SSc. Further studies should investigate the potential of this pathway as a marker of disease progression and as a therapeutic target.

REFERENCES

1 Lefèvre G, Dauchet L, Hachulla E, *et al.* Survival and Prognostic Factors in Systemic Sclerosis-Associated Pulmonary Hypertension: A Systematic Review and Meta-Analysis: Survival and Prognosis in SSc-Associated Pulmonary Hypertension. *Arthritis & Rheumatism* 2013;**65**:2412–23. doi:10.1002/art.38029

2 Humbert M, Guignabert C, Bonnet S, *et al.* Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J* 2019;**53**:1801887. doi:10.1183/13993003.01887-2018

3 Sobanski V, Launay D, Hachulla E, *et al.* Current Approaches to the Treatment of Systemic-Sclerosis-Associated Pulmonary Arterial Hypertension (SSc-PAH). *Curr Rheumatol Rep* 2016;**18**:10. doi:10.1007/s11926-015-0560-x

4 Galiè N, Humbert M, Vachiery J-L, *et al.* 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *European Heart Journal* 2016;**37**:67–119. doi:10.1093/eurheartj/ehv317

5 Pokeerbux MR, Giovannelli J, Dauchet L, *et al.* Survival and prognosis factors in systemic sclerosis: data of a French multicenter cohort, systematic review, and meta-analysis of the literature. *Arthritis Res Ther* 2019;**21**:86. doi:10.1186/s13075-019-1867-1

6 Mathai SC, Bueso M, Hummers LK, *et al.* Disproportionate elevation of N-terminal pro-brain natriuretic peptide in scleroderma-related pulmonary hypertension. *European Respiratory Journal* 2010;**35**:95–104. doi:10.1183/09031936.00074309

7 Affandi AJ, Radstake TRDJ, Marut W. Update on biomarkers in systemic sclerosis: tools for diagnosis and treatment. *Semin Immunopathol* 2015;**37**:475–87. doi:10.1007/s00281-015-0506-4

8 Hickey PM, Lawrie A, Condliffe R. Circulating Protein Biomarkers in Systemic Sclerosis Related Pulmonary Arterial Hypertension: A Review of Published Data. *Front Med* 2018;**5**. doi:10.3389/fmed.2018.00175

9 Odler B, Foris V, Gungl A, *et al.* Biomarkers for Pulmonary Vascular Remodeling in Systemic Sclerosis: A Pathophysiological Approach. *Front Physiol* 2018;**9**. doi:10.3389/fphys.2018.00587

10 Rice LM, Mantero JC, Stratton EA, *et al.* Serum biomarker for diagnostic evaluation of pulmonary arterial hypertension in systemic sclerosis. *Arthritis Research & Therapy* 2018;**20**. doi:10.1186/s13075-018-1679-8

11 Bauer Y, de Bernard S, Hickey P, *et al.* Identifying early pulmonary arterial hypertension biomarkers in systemic sclerosis: Machine learning on proteomics from the DETECT cohort. *Eur Respir J* Published Online First: 17 December 2020. doi:10.1183/13993003.02591-2020

12 Sanges S, Launay D, Rhee RL, *et al.* A prospective study of the 6 min walk test as a surrogate marker for haemodynamics in two independent cohorts of treatment-naïve systemic sclerosis-associated pulmonary arterial hypertension. *Ann Rheum Dis* 2016;**75**:1457–65. doi:10.1136/annrheumdis-2015-207336

13 Chemla D, Castelain V, Hervé P, *et al.* Haemodynamic evaluation of pulmonary hypertension. *European Respiratory Journal* 2002;**20**:1314–31. doi:10.1183/09031936.02.00068002

14 van den Hoogen F, Khanna D, Fransen J, *et al.* Classification Criteria for Systemic Sclerosis: An ACR-EULAR Collaborative Initiative. *Arthritis Rheum* 2013;**65**:2737–47. doi:10.1002/art.38098

15 LeRoy EC, Black C, Fleischmajer R, *et al.* Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;**15**:202–5.

16 Goh NSL, Desai SR, Veeraraghavan S, *et al.* Interstitial lung disease in systemic sclerosis: a simple staging system. *Am J Respir Crit Care Med* 2008;**177**:1248–54. doi:10.1164/rccm.200706-877OC

17 Gold L, Ayers D, Bertino J, *et al.* Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS ONE* 2010;**5**:e15004. doi:10.1371/journal.pone.0015004

18 Rohloff JC, Gelinas AD, Jarvis TC, *et al.* Nucleic Acid Ligands With Protein-like Side Chains: Modified Aptamers and Their Use as Diagnostic and Therapeutic Agents. *Mol Ther Nucleic Acids* 2014;**3**:e201. doi:10.1038/mtna.2014.49

19 Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 2004;**3**:Article3. doi:10.2202/1544-6115.1027

20 Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)* 1995;**57**:289–300. doi:10.1111/j.2517-6161.1995.tb02031.x

21 Authors/Task Force Members:, Galiè N, Humbert M, *et al.* 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS). *Eur Heart J* Published Online First: 29 August 2015. doi:10.1093/eurheartj/ehv317

22 Valenzi E, Bulik M, Tabib T, *et al.* Single-cell analysis reveals fibroblast heterogeneity and myofibroblasts in systemic sclerosis-associated interstitial lung disease. *Ann Rheum Dis* 2019;**78**:1379–87. doi:10.1136/annrheumdis-2018-214865
23 Tu Ly, Desroches-Castan Agnès, Mallet Christine, *et al.* Selective BMP-9 Inhibition Partially Protects Against Experimental Pulmonary Hypertension. *Circulation Research* 2019;**124**:846–55. doi:10.1161/CIRCRESAHA.118.313356
24 Tu L, De Man FS, Girerd B, *et al.* A Critical Role for p130Cas in the Progression of Pulmonary Hypertension in Humans and Rodents. *Am J Respir Crit Care Med* 2012;**186**:666–76. doi:10.1164/rccm.201202-0309OC
25 Huertas A, Tu L, Thuillet R, *et al.* Leptin signalling system as a target for pulmonary arterial hypertension therapy. *European Respiratory Journal* 2015;**45**:1066–80. doi:10.1183/09031936.00193014
26 Bouvard C, Tu L, Rossi M, *et al.* Different cardiovascular and pulmonary phenotypes for single- and double-knock-out mice deficient in BMP9 and BMP10. *Cardiovasc Res* 2021;:cvab187. doi:10.1093/cvr/cvab187
27 Hanthazi A, Jespers P, Vegh G, *et al.* Chemerin Added to Endothelin-1 Promotes Rat Pulmonary Artery Smooth Muscle Cell Proliferation and Migration. *Front Physiol* 2020;**11**:926. doi:10.3389/fphys.2020.00926
28 Akamata K, Asano Y, Taniguchi T, *et al.* Increased expression of chemerin in endothelial cells due to Fli1 deficiency may contribute to the development of digital ulcers in systemic sclerosis. *Rheumatology* 2015;**54**:1308–16. doi:10.1093/rheumatology/keu479
29 Chighizola CB, Raschi E, Privitera D, *et al.* Serum chemerin in systemic sclerosis: a novel marker of early diffuse disease? *Clin Exp Rheumatol* 2017;**35 Suppl 106**:223–4.
30 Sawicka K, Michalska-Jakubus M, Potembska E, *et al.* Visfatin and chemerin levels correspond with inflammation and might reflect the bridge between metabolism, inflammation and fibrosis in patients with systemic sclerosis. *Postepy Dermatol Alergol* 2019;**36**:551–65. doi:10.5114/ada.2018.79104
31 Ferland DJ, Watts SW. Chemerin: A Comprehensive Review Elucidating the Need for Cardiovascular Research. *Pharmacol Res* 2015;**99**:351–61. doi:10.1016/j.phrs.2015.07.018
32 Peng L, Chen Y, Li Y, *et al.* Chemerin Regulates the Proliferation and Migration of Pulmonary Arterial Smooth Muscle Cells via the ERK1/2 Signaling Pathway. *Front Pharmacol* 2022;**13**:767705. doi:10.3389/fphar.2022.767705
33 Ferland DJ, Mullick AE, Watts SW. Chemerin as a Driver of Hypertension: A Consideration. *American Journal of Hypertension* 2020;**33**:975–86. doi:10.1093/ajh/hpaa084
34 Kaur J, Adya R, Tan BK, *et al.* Identification of chemerin receptor (ChemR23) in human endothelial cells: chemerin-induced endothelial angiogenesis. *Biochem Biophys Res Commun* 2010;**391**:1762–8. doi:10.1016/j.bbrc.2009.12.150
35 Bozaoglu K, Curran JE, Stocker CJ, *et al.* Chemerin, a Novel Adipokine in the Regulation of Angiogenesis. *J Clin Endocrinol Metab* 2010;**95**:2476–85. doi:10.1210/jc.2010-0042
36 Neves KB, Lobato NS, Lopes RAM, *et al.* Chemerin reduces vascular nitric oxide/cGMP signalling in rat aorta: a link to vascular dysfunction in obesity? *Clin Sci (Lond)* 2014;**127**:111–22. doi:10.1042/CS20130286
37 Yamawaki H, Kameshima S, Usui T, *et al.* A novel adipocytokine, chemerin exerts anti-inflammatory roles in human vascular endothelial cells. *Biochem Biophys Res Commun* 2012;**423**:152–7. doi:10.1016/j.bbrc.2012.05.103
38 Kunimoto H, Kazama K, Takai M, *et al.* Chemerin promotes the proliferation and migration of vascular smooth muscle and increases mouse blood pressure. *Am J Physiol Heart Circ Physiol* 2015;**309**:H1017-1028. doi:10.1152/ajpheart.00820.2014
39 Kennedy AJ, Yang P, Read C, *et al.* Chemerin Elicits Potent Constrictor Actions via Chemokine-Like Receptor 1 (CMKLR1), not G-Protein-Coupled Receptor 1 (GPR1), in Human and Rat Vasculature. *J Am Heart Assoc* 2016;**5**. doi:10.1161/JAHA.116.004421
40 Ferland DJ, Darios ES, Neubig RR, *et al.* Chemerin-induced arterial contraction is Gi- and calcium-dependent. *Vascul Pharmacol* 2017;**88**:30–41. doi:10.1016/j.vph.2016.11.009
41 Wen J, Wang J, Guo L, *et al.* Chemerin stimulates aortic smooth muscle cell proliferation and migration via activation of autophagy in VSMCs of metabolic hypertension rats. *Am J Transl Res* 2019;**11**:1327–42.
42 Zabel BA, Silverio AM, Butcher EC. Chemokine-like receptor 1 expression and chemerin-directed chemotaxis distinguish plasmacytoid from myeloid dendritic cells in human blood. *J Immunol* 2005;**174**:244–51. doi:10.4049/jimmunol.174.1.244
43 Parolini S, Santoro A, Marcenaro E, *et al.* The role of chemerin in the colocalization of NK and dendritic cell subsets into inflamed tissues. *Blood* 2007;**109**:3625–32. doi:10.1182/blood-2006-08-038844
44 Wittamer V, Franssen J-D, Vulcano M, *et al.* Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. *J Exp Med* 2003;**198**:977–85. doi:10.1084/jem.20030382

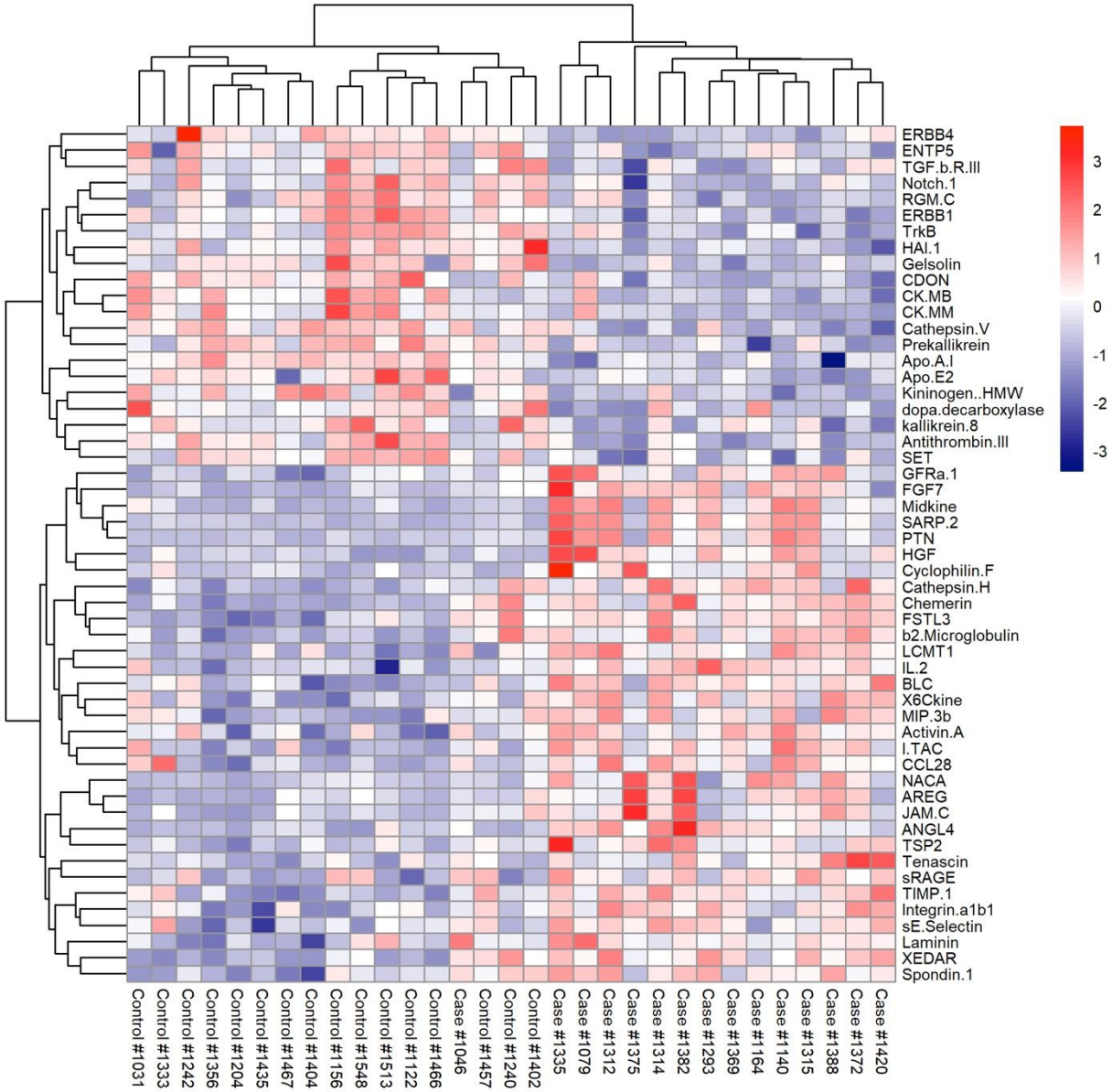
45 Saygin D, Tabib T, Bittar HET, *et al.* Transcriptional profiling of lung cell populations in idiopathic pulmonary arterial hypertension. *Pulm Circ* 2020;**10**:1–15. doi:10.1177/2045894020908782
46 Omori A, Goshima M, Kakuda C, *et al.* Chemerin-9-induced contraction was enhanced through the upregulation of smooth muscle chemokine-like receptor 1 in isolated pulmonary artery of pulmonary arterial hypertensive rats. *Pflugers Arch* 2020;**472**:335–42. doi:10.1007/s00424-019-02345-5
47 Hanthazi A, Jespers P, Vegh G, *et al.* Chemerin influences endothelin- and serotonin-induced pulmonary artery vasoconstriction in rats. *Life Sci* 2019;**231**:116580. doi:10.1016/j.lfs.2019.116580
48 Nakamura N, Naruse K, Kobayashi Y, *et al.* Chemerin promotes angiogenesis in vivo. *Physiological Reports* 2018;**6**:e13962. doi:10.14814/phy2.13962
49 Xiong W, Luo Y, Wu L, *et al.* Chemerin Stimulates Vascular Smooth Muscle Cell Proliferation and Carotid Neointimal Hyperplasia by Activating Mitogen-Activated Protein Kinase Signaling. *PLOS ONE* 2016;**11**:e0165305. doi:10.1371/journal.pone.0165305

Table 1. Characteristics of SSc-PAH patients from the discovery and the validation cohorts

	DISCOVERY COHORT		VALIDATION COHORT	
	N		**N**	
Sex (female)	15	14 (93%)	24	17 (71%)
Age (yo)	15	65 (±7)	24	68 (±9)
RP duration (y)	10	19 (±13)	8	18 (±12)
SSc duration (y)	15	1.25 [0.05 ;6]	9	0.5 [0 ;9]
BMI (kg/m^2)	15	28 (±4)	16	28 (±6)
NYHA functional class	15		18	
- class I		0 (0%)		0 (0%)
- class II		7 (47%)		6 (33%)
- class III		8 (53%)		10 (56%)
- class IV		0 (0%)		2 (11%)
BNP (pg/mL)	12	147 [69 ;505]	14	84 [39 ;264]
or Nt-pro-BNP (pg/mL), if BNP not available	/	/	9	878 [374 ;1124]
eGFR (mL/min)	12	73 (±16)	15	81 (±28)
ANA	13	13 (100%)	17	17 (100%)
- ACA	12	9 (75%)	8	7 (88%)
- ATA	12	0 (0%)	8	0 (0%)
- Anti-U1RNP	12	1 (8%)	8	0 (0%)
LVEF (%)	12	60 (±9)	14	65 (±9)
FVC (% predicted)	13	85 (±10)	20	88 (±20)
TLC (% predicted)	13	90 (±12)	14	87 (±15)
DLCO (% predicted)	15	35 [8;52]	10	41 [33;65]
ILD on chest CT-scan	15	6 (40%)	24	5 (21%)
mPAP (mmHg)	15	44 (±10)	24	37 (±8)
PAWP (mmHg)	15	10 (±2)	24	10 (±3)
CO (L/min)	15	4.9 (±1.6)	24	4.6 (±1.2)
CI (L/min/m^2)	15	2.7 (±1.0)	18	2.5 (±0.5)
PVR (WU)	15	8.5 (±5.0)	24	6.2 (±2.3)

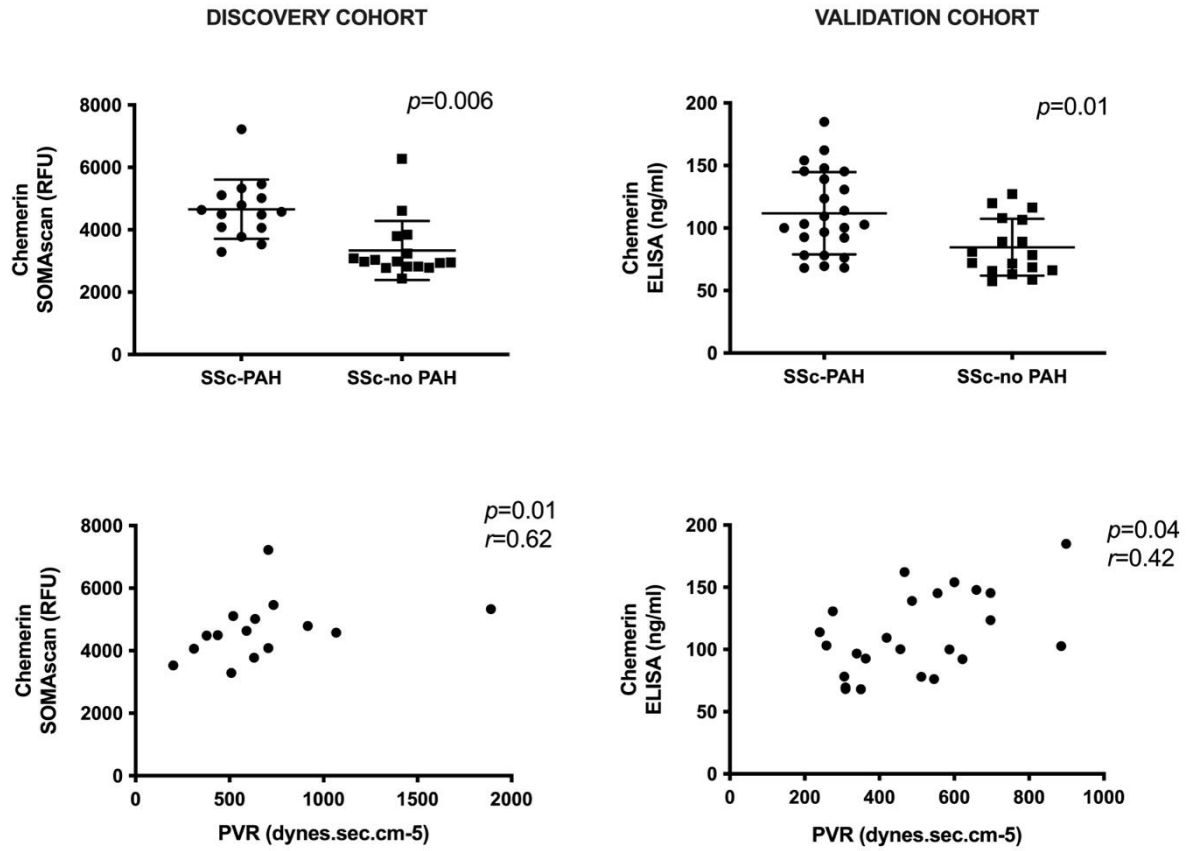
ACA: anti-centromere antibodies; ANA: antinuclear antibodies; ATA: anti-topoisomerase I antibodies; Anti-U1RNP: anti-U1-ribonucleoprotein antibodies; BMI: body-mass index; BNP: brain natriuretic peptide; CI: cardiac index; CO: cardiac output; DLCO: diffusing capacity of the lung for carbon monoxide; eGFR: estimated glomerular filtration rate; FVC: forced vital capacity: ILD: interstitial lung disease: LVEF: left ventricular ejection fraction; mPAP: mean pulmonary arterial pressure; Nt-pro-BNP: N-terminal pro-brain natriuretic peptide; NYHA: New York Heart Association; PAH: pulmonary arterial hypertension; PAWP: pulmonary arterial wedge pressure; PVR: pulmonary vascular resistance; RP: Raynaud phenomenon; SSc: systemic sclerosis; TLC: total lung capacity; WU: Wood unit; y: year; yo: year old.
Values are expressed as the number (%), mean (± standard deviation) or median [interquartile range].

Figure 1. Heatmap depicting the differential expression of the 53 candidate biomarkers identified in the discovery cohort



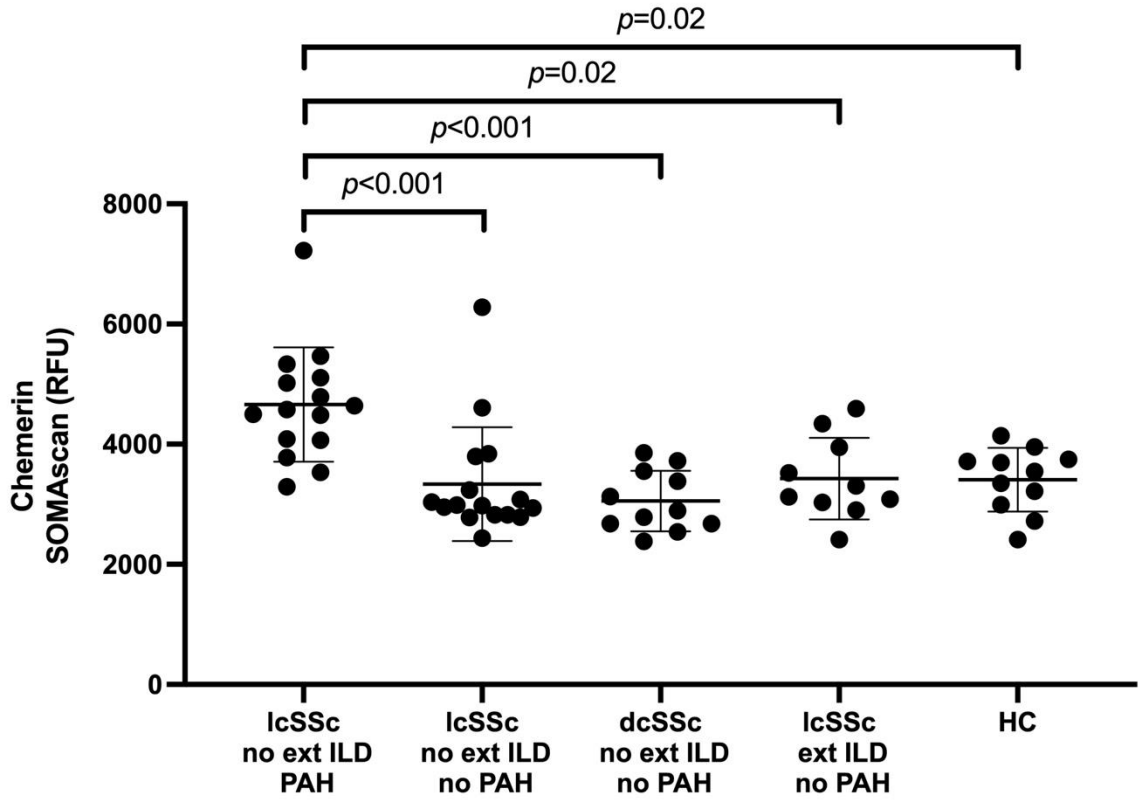
Standardized expression values of the 53 differentially expressed proteins in the discovery cohort. Red values indicate over-expression and blue values under-expression compared to the mean expression level.

Figure 2. Differential expression of chemerin and correlation with PVR in the discovery and the validation cohorts



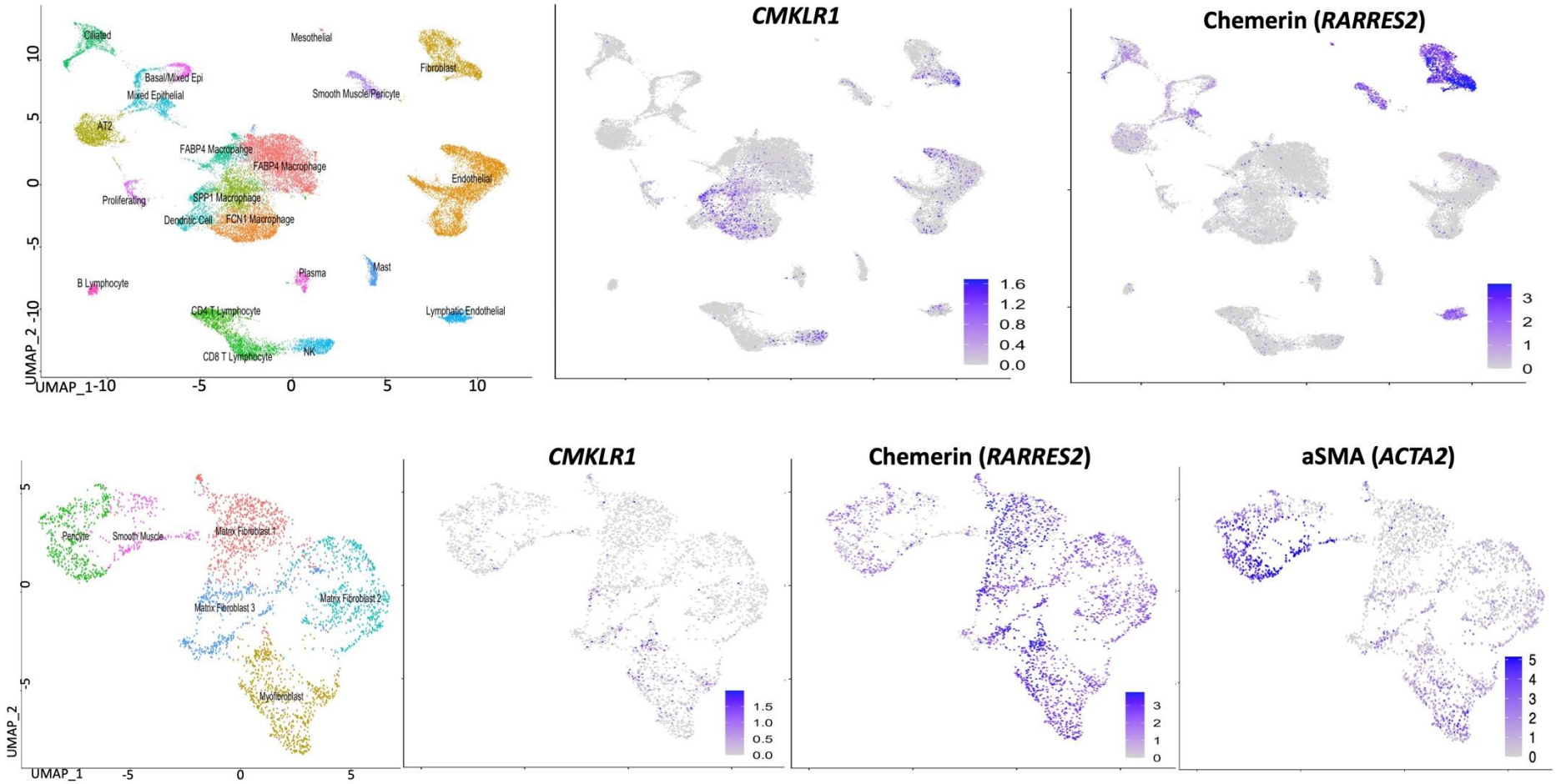
PAH: pulmonary arterial hypertension; PVR: pulmonary vascular resistance; RFU: relative fluorescence units; SSc: systemic sclerosis.

Figure 3. Differential expression of chemerin in different SSc patient groups



dc: diffuse cutaneous; ext ILD: extensive interstitial lung disease; HC: healthy controls; lc: limited cutaneous; PAH: pulmonary arterial hypertension; RFU: relative fluorescence units; SSc: systemic sclerosis.

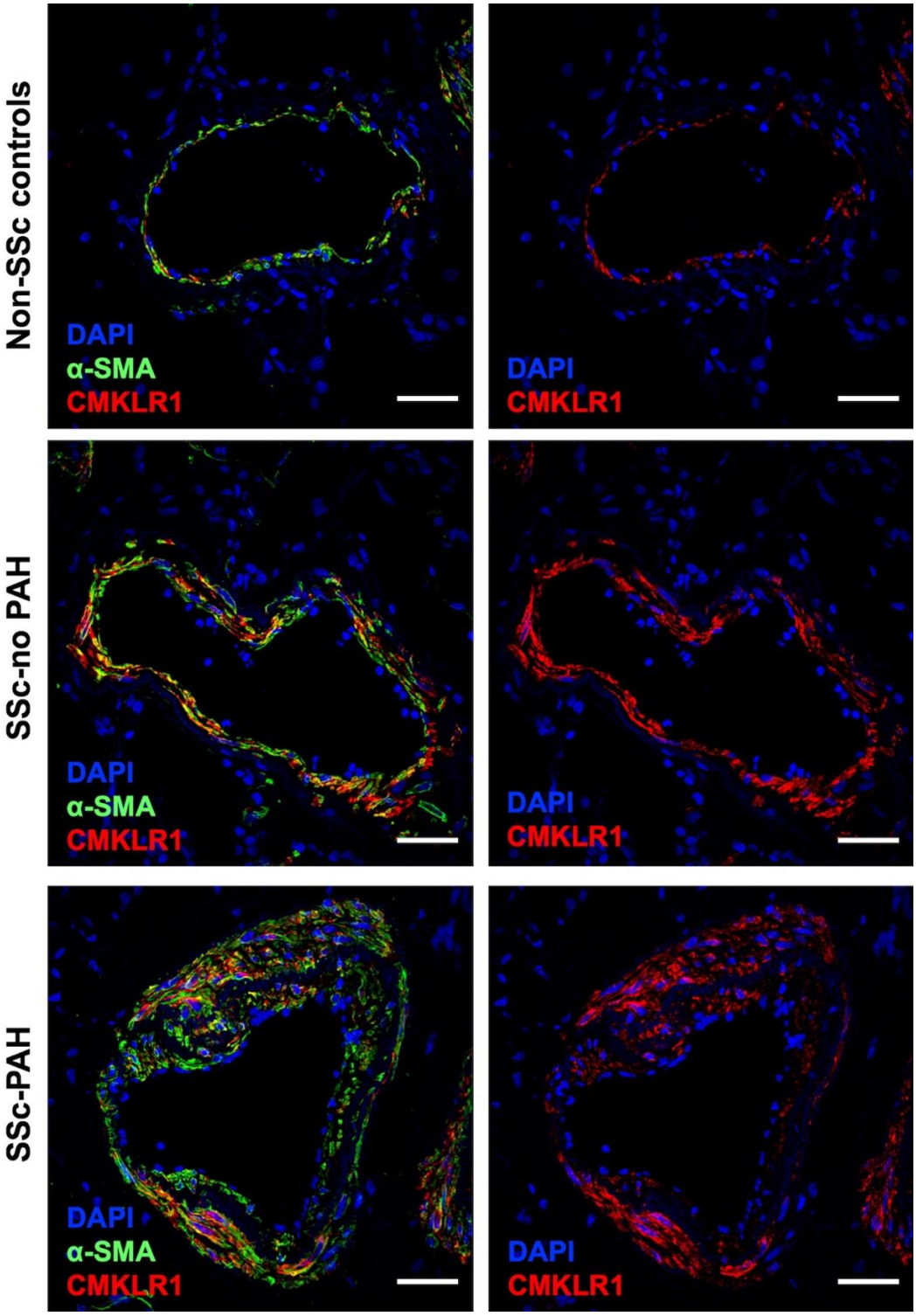
Figure 4. Expression of CMKLR1, chemerin and α-smooth muscle actin mRNA in cell populations from SSc-PAH lungs assessed by single-cell RNA-sequencing



Top panels provide information from all cell populations; Bottom panels provide information on mesenchymal cell populations.

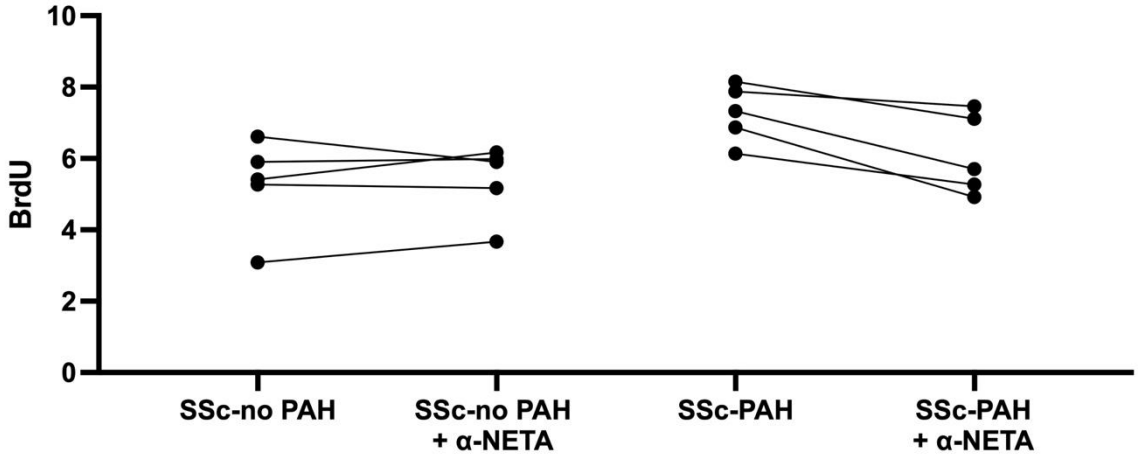
α-SMA: α-smooth muscle actin; CMKLR1: chemokine-Like Receptor 1; PAH: pulmonary arterial hypertension; SSc: systemic sclerosis.

Figure 5. Representative images of lung sections immunostained with DAPI (blue), α-SMA (green) and CMKLR1 (red) from non-SSc controls (top row), SSc-no PAH (middle row) and SSc-PAH (bottom row)



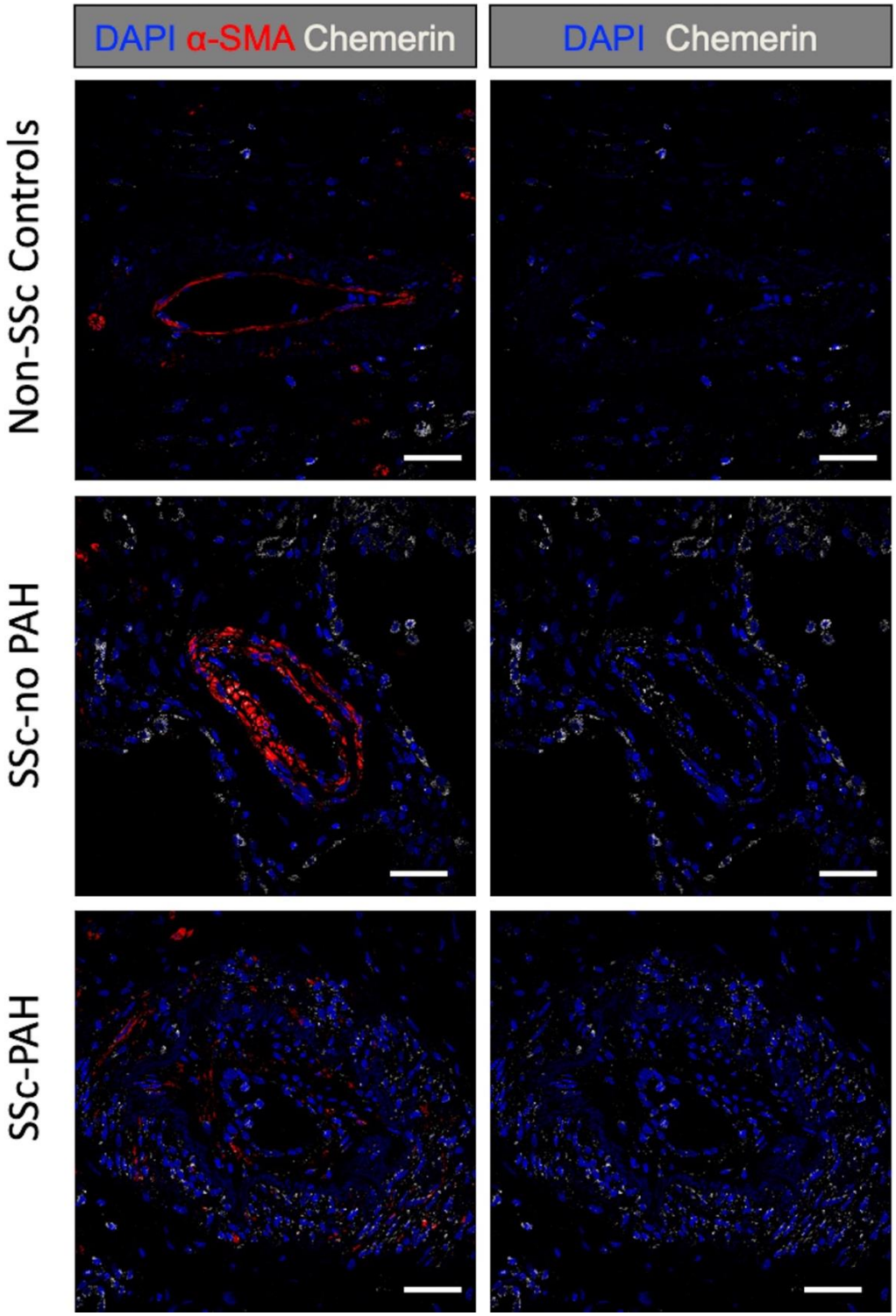
α-SMA: α-smooth muscle actin; CMKLR1: chemokine-Like Receptor 1; DAPI: 4',6-diamidino-2-phenylindole; PAH: pulmonary arterial hypertension; SSc: systemic sclerosis. Scale bar = 50 µm in all sections.

Figure 6. Proliferation of PA-SMCs from idiopathic PAH patients, after stimulation with serum from SSc-PAH and SSc-no PAH patients, in the presence or absence of α-NETA



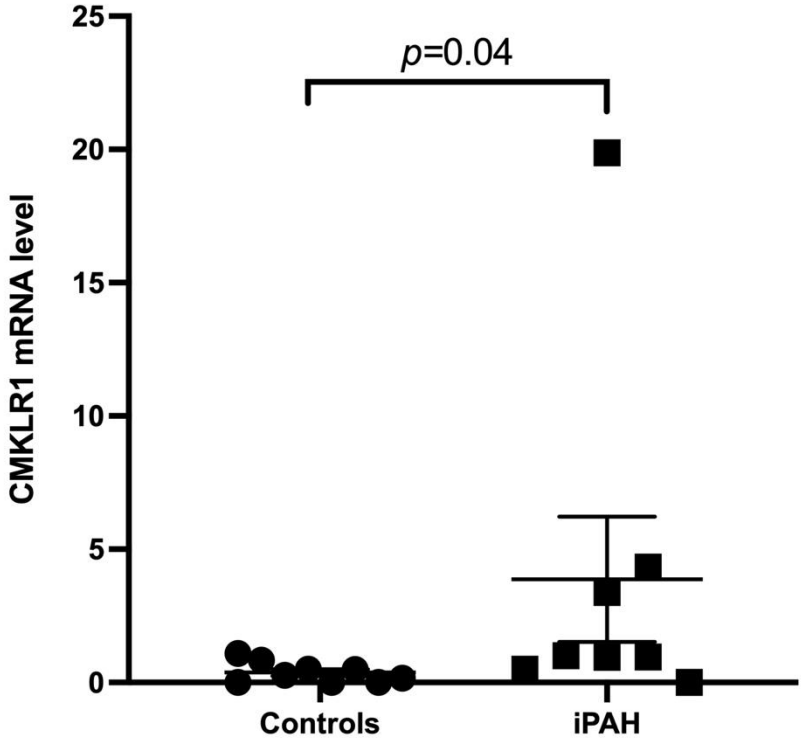
α-NETA: 2-(anaphthoyl)ethyltrimethylammonium iodide; BrdU: 5-bromo-2-deoxyuridine; i: idiopathic; PAH: pulmonary arterial hypertension; PA-SMC: pulmonary arterial smooth muscle cells; SSc: systemic sclerosis.

Supplemental Fig. S1. Representative images of lung sections immunostained with DAPI (blue), α-SMA (green) and chemerin (white) from non-SSc controls (top row), SSc-no PAH (middle row) and SSc-PAH (bottom row)



α-SMA: α-smooth muscle actin; DAPI: 4',6-diamidino-2-phenylindole; PAH: pulmonary arterial hypertension; SSc: systemic sclerosis. Scale bar = 50 µm in all sections.

Supplemental Fig. S2. CMKLR1 mRNA expression in idiopathic PAH patients and controls, assessed by real time quantitative-PCR



CMKLR1: chemokine-like receptor 1; i: idiopathic; mRNA: messenger ribonucleic acid; PAH: pulmonary arterial hypertension; RT-PCR: reverse transcriptase polymerase chain reaction.

PARTIE II

Deuxième candidat biomarqueur :

l'axe BAFF-lymphocyte B

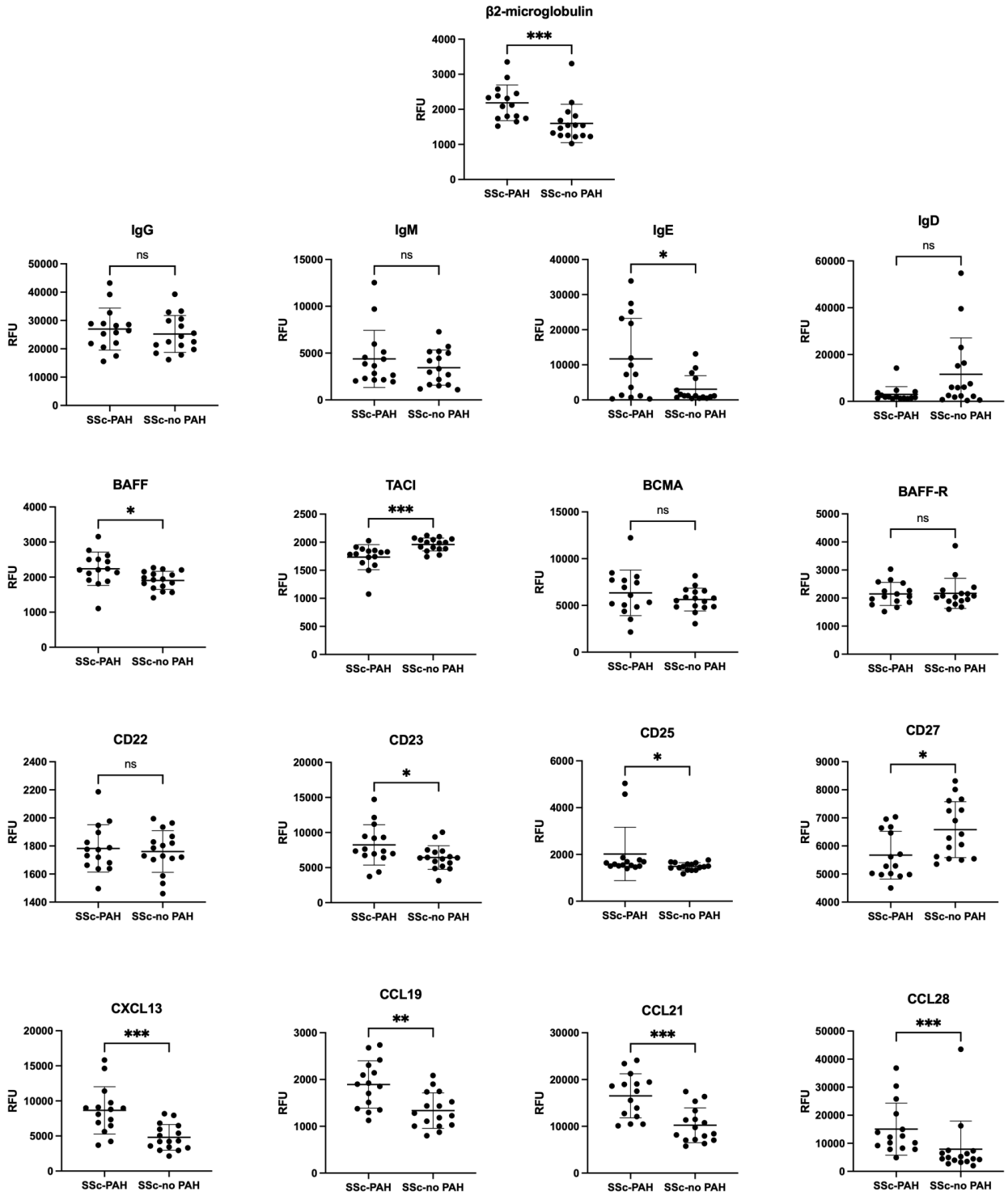
Notre exploration du protéome sérique des patients HTAP-SSc a donc identifié 53 biomarqueurs différentiellement exprimés par rapport aux patients SSc sans HTAP. Parmi eux se trouvaient plusieurs protéines associées au lymphocyte B (LB), notamment la β2-microglobuline et CXCL13 (BLC ; chimiokine majeure des LB), ainsi que CCL19 (MIP-3β), CCL21 (6CKine) et CCL28, chimiokines associées aux LB et LT (cf. *heatmap* présentée en page 81). Après réanalyse de l'ensemble des données SOMAscan, on notait que d'autres biomarqueurs LB étaient également différentiellement exprimés entre cas et témoins, mais n'atteignaient pas la significativité statistique après ajustement sur comparaisons multiples (***Figure 1***). Parmi elles figurait BAFF, cytokine importante pour l'activation, la maturation et la survie des LB, dont on constatait en outre que les taux sériques présentaient une corrélation avec les RVP proche de la significativité statistique (***Figure 2***).

Notre laboratoire s'intéresse de longue date à l'implication du LB dans la physiopathologie de la SSc (voir à ce sujet notre revue [1]), et notamment à l'utilité clinique de biomarqueurs LB (comme les taux circulants de chaînes légères libres d'Ig [2]) dans cette pathologie. Nous avions ainsi déjà pu observer une corrélation significative entre les taux sériques de BAFF et la vitesse maximale de l'insuffisance tricuspide (VITmax, estimation échographique de la pression artérielle pulmonaire systolique) dans une cohorte de patients SSc non sélectionnés (***Figure 3***).

Si l'implication du LB dans les phénomènes fibrosants survenant au cours de la SSc a été avérée (et a permis d'inclure le rituximab, anticorps monoclonal anti-CD20, au sein de l'arsenal thérapeutique des formes cutanées diffuses précoces [3]), leur rôle dans la microangiopathie associée à la maladie, et notamment l'HTAP, n'a été que peu étudié jusqu'ici. Nous avons donc sélectionné un large panel de biomarqueurs LB et cherché à déterminer s'ils pouvaient présenter un intérêt clinique au cours de l'HTAP-SSc et s'ils pouvaient permettre d'identifier de nouveaux mécanismes physiopathologiques sous-tendant la maladie (et en particulier des interactions entre immunité et vaisseaux).

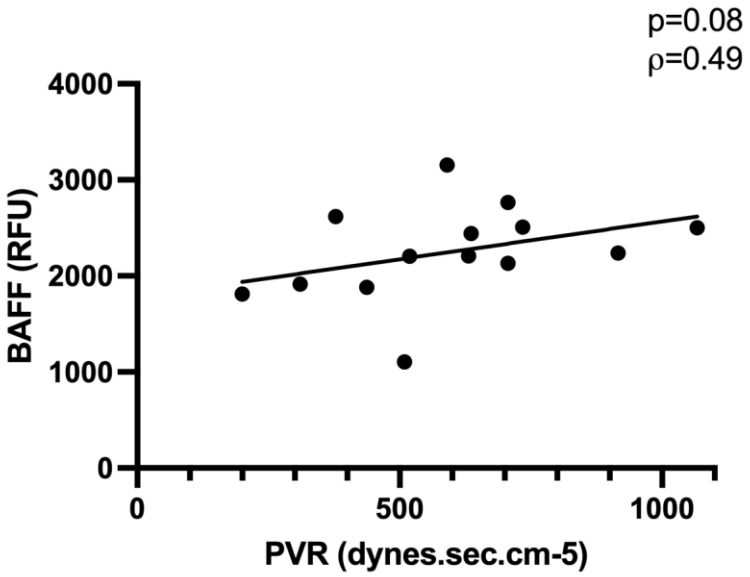
Ce travail fait l'objet d'une publication dans *Frontiers in Immunology* (Sanges S, *et al.* Soluble markers of B cell activation suggest a role of B cells in the pathogenesis of systemic sclerosis-associated pulmonary arterial hypertension. *Front. Immunol.* 13:954007. doi: 10.3389/fimmu.2022.954007 ; version PDF accessible sur le lien suivant : https://www.frontiersin.org/articles/10.3389/fimmu.2022.954007/full).

Figure 1. Taux sériques des biomarqueurs LB mesurés en SOMAscan chez les patients SSc avec et sans HTAP (données non publiées).



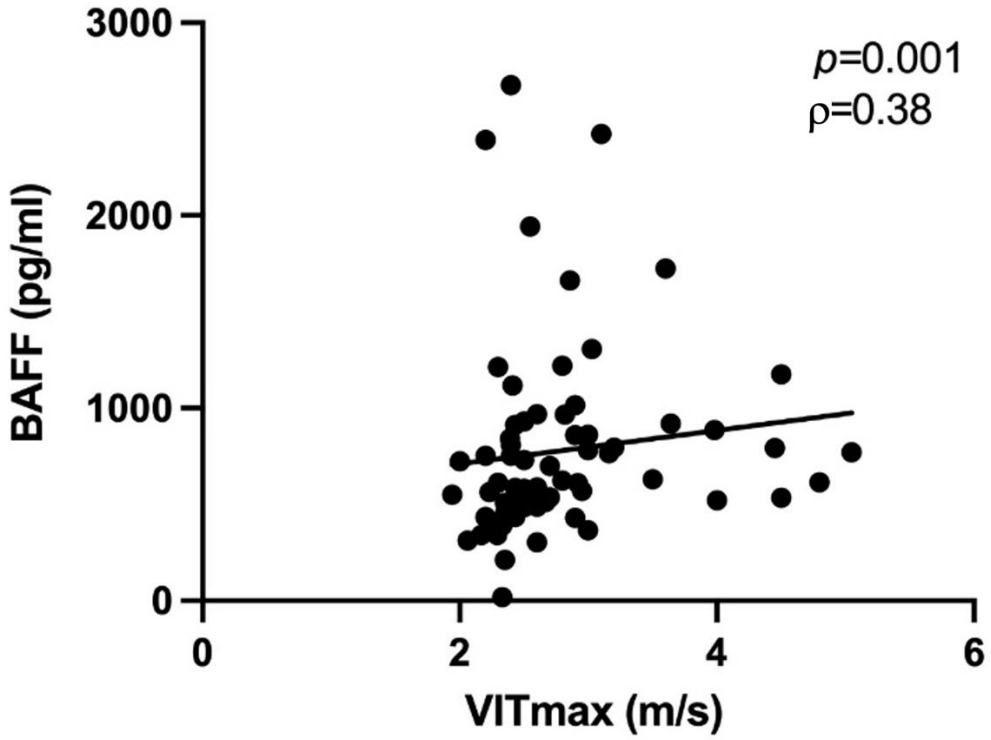
Les comparaisons entre les patients SSc-PAH et SSc-no PAH ont été réalisées à l'aide du test non-paramétrique de Mann-Whitney, sans ajustement sur les comparaisons multiples. Le seuil de significativité statistique a été fixé à p*<0,05. Les valeurs de p sont figurées de la façon suivante : ****** p ≤ 0,001 ; ***** p ≤ 0,01 ; * p < 0,05 ;* ns *pour* p ≥ 0,05.

Figure 2. Association entre les taux sériques de BAFF (mesurés en SOMAscan) et les résistances vasculaires pulmonaires (en cathétérisme cardiaque droit) chez les patients HTAP-SSc (données non publiées).



L'association entre BAFF et les PVR était étudiée à l'aide du test non-paramétrique de Spearman. Le seuil de significativité statistique a été fixé à p*<0,05.*

Figure 3. Association entre les taux sériques de BAFF (mesurés en ELISA) et la vitesse maximale de la fuite tricuspide (en échographie cardiaque) dans une cohorte non sélectionnée de 134 patients SSc (données non publiées).



L'association entre BAFF et la VITmax était étudiée à l'aide du test non-paramétrique de Spearman. Le seuil de significativité statistique a été fixé à p<0,05.

REFERENCES

[1] Sanges S, Guerrier T, Launay D, Lefèvre G, Labalette M, Forestier A, et al. Role of B cells in the pathogenesis of systemic sclerosis. *Rev Médecine Interne* (2017) 38:113–124. doi: 10.1016/j.revmed.2016.02.016

[2] Lanteri A, Sobanski V, Langlois C, Lefèvre G, Hauspie C, Sanges S, et al. Serum free light chains of immunoglobulins as biomarkers for systemic sclerosis characteristics, activity and severity. *Autoimmun Rev* (2014) 13:974–980. doi: 10.1016/j.autrev.2014.07.003

[3] Ebata S, Yoshizaki A, Oba K, Kashiwabra K, Ueda K, Uemura Y, et al. Safety and efficacy of rituximab in systemic sclerosis (DESIRES): a double-blind, investigator-initiated, randomised, placebo-controlled trial. Lancet Rheumatol (2021) 7:E489-7. doi: 10.1016/S2665-9913(21)00107-7.

RESUME DETAILLE EN FRANÇAIS

Les marqueurs solubles associés au lymphocyte B (LB) sont des outils diagnostiques et pronostiques intéressants dans les maladies auto-immunes. Peu de données sont disponibles sur leurs taux sériques au cours de la sclérodermie systémique (SSc) et sur leur association avec les caractéristiques de la maladie.

Nous avons donc mesuré les taux sériques de 14 biomarqueurs LB (β2-microglobuline, facteur rhumatoïde (FR), immunoglobulines (Ig) G, IgA, IgM, BAFF, APRIL, formes solubles (s) des récepteurs TACI et BCMA, sCD21, sCD23, sCD25, sCD27, et CXCL13) dans un panel de 80 patients SSc représentatif de l'hétérogénéité de la maladie, et chez 80 témoins sains. Nous avons observé une positivité plus fréquente du FR et des taux circulants plus élevés de β2-microglobuline, d'IgG et de CXCL13 chez les patients SSc par rapport aux témoins. Nous avons également mis en évidence des associations significatives entre plusieurs biomarqueurs et les caractéristiques de la SSc liées au phénotype, à l'activité et à la sévérité de la maladie. En particulier, les taux sériques d'IgG étaient associés à l'hypertension pulmonaire (HTP) : β2-microglobuline avec Nt-pro-BNP et DLCO ; et BAFF avec vitesse maximale de la fuite tricuspide (VITmax).

Nous avons ensuite exploré cette association dans une cohorte de validation ciblée, composée de 36 patients atteints de SSc cutanée limitée sans fibrose pulmonaire extensive, dont 18 avec HTP de groupe 1 (HTAP). Nous avons observé des taux sériques d'IgG plus faibles et des taux plus élevés de β2-microglobuline, sBCMA, sCD23 et sCD27 chez les patients atteints d'HTAP. Les niveaux de BAFF étaient fortement corrélés aux valeurs de Nt-pro-BNP, au rapport CVF/DLCO et à la VITmax chez les patients HTAP-SSc.

Enfin, nous avons évalué la pertinence physiopathologique de cette association entre biomarqueurs LB et HTAP en étudiant la production de protéines pro- et anti-angiogéniques dans le surnageant de culture de LB circulants provenant de patients SSc. Les LB SSc produisaient plus de facteurs pro-angiogéniques (angiogénine, angiopoïétine-1, VEGFR-1, PDGF-AA, MMP-8, TIMP-1, L-sélectine) et moins de facteurs anti-angiogéniques (angiopoïétine-2) que ceux des témoins sains, indépendamment de la présence d'HTAP.

Soluble markers of B cell activation suggest a role of B cells in the pathogenesis of systemic sclerosis-associated pulmonary arterial hypertension

Sébastien Sanges[1,2,3,4,5,*], Thomas Guerrier[1,2,6,*], Alain Duhamel[7], Lucile Guilbert[1,2,6], Carine Hauspie[1,2,6], Alexis Largy[1,2], Maïté Balden[1,2,6], Céline Podevin[3], Guillaume Lefèvre[1,2,6], Manel Jendoubi[1,2], Silvia Speca[1,2], Éric Hachulla[1,2,3,4,5], Vincent Sobanski[1,2,3,4,5], Sylvain Dubucquoi[1,2,6,**], David Launay[1,2,3,4,5,**]

[1]Univ. Lille, U1286 – INFINITE – Institute for Translational Research in Inflammation, F-59000 Lille, France

[2]INSERM, F-59000 Lille, France

[3]CHU Lille, Département de Médecine Interne et Immunologie Clinique, F-59000 Lille, France

[4]Centre National de Référence Maladies Auto-immunes Systémiques Rares du Nord et Nord-Ouest de France (CeRAINO), F-59000 Lille, France

[5]Health Care Provider of the European Reference Network on Rare Connective Tissue and Musculoskeletal Diseases Network (ReCONNET), F-59000 Lille, France

[6]CHU Lille, Institut d'Immunologie, Lille, France

[7]Univ. Lille, CHU Lille, ULR2694 – METRICS: Évaluation des technologies de santé et des pratiques médicales, F-59000 Lille, France

[*]These authors have contributed equally to this work and share first authorship

[**]These authors have contributed equally to this work and share last authorship

Corresponding author: Dr Sébastien SANGES, Département de Médecine Interne et Immunologie Clinique, CHU Lille, F-59037 Lille Cedex, France.

Tel: + 33 (0)320445048; Fax: + 33 (0)320445459; E-mail: sebastien.sanges@univ-lille.fr

INTRODUCTION

Systemic sclerosis (SSc) is one of the most severe systemic autoimmune diseases (1). It is characterized by a clinical triad that combines immunological anomalies (autoantibodies, hypergammaglobulinemia, elevated acute phase reactants), fibrosing manifestations (in the skin, lungs and digestive tract) and vascular complications (such as pulmonary hypertension (PH), Raynaud phenomenon and digital ulcers) (2). SSc is a heterogeneous condition, whose burden on patient quality of life is variable and can range from mild symptoms to life-threatening situations (3–5). As such, there is an unmet need to develop tools that can better predict and diagnose severe organ involvements, as well as accurately assess disease activity and severity.

The pathophysiology of SSc reflects this clinical triad since it is currently seen as the result of the interactions between 3 major players: the immune system (aberrant activation of innate and adaptive immunity), fibroblasts (activation and acquisition of a myofibroblast phenotype) and the vasculature (obliterative microangiopathy with endothelial dysfunction) (6). Among the different immunity actors involved in SSc, the almost-constant presence of autoantibodies has long suggested a potential implication of B cells in the pathogenesis of the disease (7). Recent works have confirmed that B cells are activated in SSc, especially in early active forms of the disease, and contribute to fibrosis and vascular damage through production of pathogenic autoantibodies directed against endothelial cells and fibroblasts, pro-inflammatory and pro-fibrotic cytokines (8–10).

Interestingly, in other conditions involving B cells, several circulating proteins reflecting B cell activation have proved to be valuable diagnostic and prognostic markers (11). In patients with systemic *lupus erythematosus* (SLE), serum levels of BAFF (B-cell-activating factor) and APRIL (a proliferation-inducing ligand), 2 cytokines involved in the maturation and survival of B cells, as well as the soluble fraction of their receptors TACI (transmembrane activator and CAML interactor) and BCMA (B-cell maturation antigen), are elevated and correlate with disease activity (12–17). APRIL levels are also associated with the occurrence of lupus nephritis and may predict response to immunosuppressants (18). In patients with various types of B-cell lymphomas, serum concentrations of soluble CD23 (sCD23), sCD27 and sCD30 are elevated several years before diagnosis (19,20). Circulating levels of CXCL13 (C-X-C motif chemokine 13), a chemokine known to attract B cells, are elevated, correlate with disease activity, severity and treatment response in patients with SLE, Sjögren's syndrome, rheumatoid arthritis and ANCA-associated vasculitides (21–26). However, in the

field of SSc, the relevance of such markers has not been extensively studied; and this was mostly done in ancient studies with small sample sizes (10,11,27–56).

To address this issue, we assessed the serum levels of several markers of B cell activation in a large and well-phenotyped SSc population, and tested their correlations with various disease characteristics including organs involvement, activity and severity. We discovered an association between B cell biomarkers and pulmonary arterial hypertension (PAH); and demonstrated that B cells can produce angiogenic mediators in SSc patients.

PATIENTS AND METHODS

Study population

All patients included were followed in the Lille National Referral Center for SSc and met the 2013 ACR/EULAR classification criteria for SSc (57). The cutaneous subset of SSc was defined as limited (lc) or diffuse (dc) according to LeRoy's criteria (58). Early dcSSc was defined as dcSSc with disease duration < 3 years at the time of inclusion. Interstitial lung disease (ILD) was diagnosed on high-resolution CT-scan (HRCT); and staged as limited (if HRCT extent < 20% and forced vital capacity (FVC) ≥ 70%) or extensive (if HRCT extent > 20% or FVC < 70%) according to Goh's system (59). PH was diagnosed by right-heart catheterization (RHC) using the 6th World Symposium hemodynamic definitions and classified into causative groups according to the updated clinical classification of PH (60). Specifically, patients with PAH (= group 1 PH) fulfilled the hemodynamic definition for pre-capillary PH: mean pulmonary arterial pressure (mPAP) > 20 mmHg with pulmonary arterial wedge pressure (PAWP) ⩽ 15 mmHg and pulmonary vascular resistance (PVR) ⩾ 3 Wood units (WU).

In our first exploratory step, we designed our discovery cohort to be enriched in the most severe SSc complications, in order to unmask associations with biomarkers that could have been missed otherwise. To do so, we selected a panel of 80 patients from our clinical database, aiming at including approximately an equal proportion of patients with limited and diffuse cutaneous subsets, an equal proportion of patients with no / limited / extensive ILD, and about a quarter of patients with PH. For this population, there were no exclusion criteria.

In our second validation step focusing on PAH, we constituted an independent validation cohort, consisting of SSc patients with limited cutaneous subset, no extensive ILD, no treatment by immunosuppressants or corticosteroids; and either a diagnosis of group 1 PH (PAH) or no evidence of PH on screening exams. These criteria were chosen so that our biomarker assessment would not be biased by any active skin or lung involvement, nor by the effect of immunosuppressive drugs. For this population, patients with dcSSc, extensive ILD and/or treatment by immunosuppressants or corticosteroids were excluded.

Data and samples collection

Data were collected systematically by a physician in a standardized case-report form for all patients referred to our day-patient clinic. All of them underwent a comprehensive evaluation performed within the same day. Data collected included patient and SSc characteristics, clinical

parameters (including modified Rodnan skin score (mRSS) and New York Heart Association (NYHA) functional class), biological results (including C-reactive protein (CRP) and N-terminal prohormone of brain natriuretic peptide (Nt-pro-BNP) levels), transthoracic echocardiography (TTE) (including peak tricuspid regurgitation velocity (TRV) and left ventricle ejection fraction (LVEF)), pulmonary function tests (PFT) (including forced vital capacity (FVC), total lung capacity (TLC) and diffusing capacity of the lung for carbon monoxide (DLCO)), composite scores (European Scleroderma Study Group Activity Index (EScSG-AI) (61), Medsger severity score (62), scleroderma Health Assessment Questionnaire (sHAQ) (63)) and treatments. For patients with PAH, hemodynamic data at PAH diagnosis as well as from the last available RHC evaluation were also collected.

Whole blood samples were collected during routine venipuncture on the same day as clinical evaluation and were processed immediately after collection. Serum samples were obtained after clotting and centrifugation and were immediately stored at -80°C. Peripheral blood mononuclear cells (PBMCs) were isolated with density gradient centrifugation using Ficoll medium (Eurobio©), freezed in dimethyl sulfoxide-containing cryopreservative medium and stored in liquid nitrogen.

Serum levels of soluble markers of B cell activation

A panel of circulating proteins reflecting immune activation, especially associated with B cells, was selected based on data from the literature. Serum concentrations of β2-microglobulin were measured using commercially available assays on the fully automated *Optilite®* turbidimetric analyzer (The Binding Site Group Ltd). Serum concentrations of rheumatoid factor (RF) were measured using commercially available fluorimetric assays (*Phadia 250®*, Thermo Fischer Scientific). Serum concentrations of IgA, IgG and IgM were measured using commercially available nephelemetric assays (Siemens). Normal range values were defined according to the manufacturer's specifications. Serum concentrations of sBCMA and sCD21 were measured in duplicate using commercial ELISA assays (*Human BCMA/TNFRSF17 DuoSet ELISA*, cat. #DY193; *Human CD21 DuoSet ELISA*, cat. #DY4909-05). Serum concentrations of APRIL, BAFF, sTACI, CXCL13, sCD23, sCD25 and sCD27 were measured in duplicate using commercial customized pre-mixed multiplex assays (*Luminex Assay*, R&D Systems). All experiments were conducted according to the manufacturer's protocol.

B cell isolation and culture

PBMCs were thawed and washed in complete medium (Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% heat-inactivated fetal calf serum (FCS), 100 UI/mL penicillin, 100 µg/mL streptomycin, 2 mM Glutamax®, and 1 mM pyruvate). B cells were isolated from PBMCs using a negative magnetic bead-assisted sorting assay (*EasySep Human Pan-B Cell Enrichment Kit*, cat. #19554, StemCell Technologies) according to the manufacturer's protocol. Expression of a B cell marker (CD19) was analyzed by flow cytometry to assess the purity of sorted B cells. Purity (*i.e* proportion of CD19+ cells) was higher than 93% in all samples (*Supplemental Figure 1*).

Immediately after sorting, purified B cells were seeded on a 96-well U-bottom plate (200 000 B cells/mL, i.e. 40 000/well) within complete medium. They were cultured during 48 hours at 37°C in humidified atmosphere with 5% CO2; and either stimulated with anti-B cell receptor (BCR) 10 µg/mL (cat. #109-006-064, Jackson Immuno Research), CpG 10µg/mL (cat. #tlrl-2006-1, Invivogen), CD40L-his 50ng/mL (cat. #2706-CL, R&D Systems) with anti-his crosslinking antibody 5µg/mL (cat. #MAB050, R&D Systems), and BAFF 100 ng/mL (cat. #7537-BF, R&D Systems); or left without stimulation. After culture, supernatants were collected and immediately stored at -80°C.

B cell production of angiogenic mediators

A panel of proteins associated with angiogenesis was selected based on data from the literature, and included angiogenin, angiopoietin 1, angiopoietin 2, angiopoietin-like protein 6 (ANGPTL-6), Tie-2, endostatin, endothelin-1, vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR)-1, VEGFR-2, VEGFR-3, platelet derived growth factor (PDGF)-AA, PDGF-AB, PDGF-BB, L-selectin, matrix metallopeptidase (MMP)-3, MMP-8, MMP-9, tissue inhibitor of metalloproteinases (TIMP)-1, bone morphogenetic protein (BMP)-9, thrombospondin 2, uPAR, and neuropilin 1. B cell production of these angiogenic mediators was assessed in culture supernatants in duplicate using commercial customized pre-mixed multiplex assays (*Luminex Assay*, R&D Systems), according to the manufacturer's protocol.

Statistical analyses

Description of the populations

Quantitative variables were expressed as means (± standard deviation, SD) in the case of normal distribution or medians (first quartile, Q1; third quartile, Q3) otherwise. Normality of distributions

was assessed using histograms and the Shapiro-Wilk test. Categorical variables were expressed as numbers (percentage).

B cell biomarkers associations in the discovery population

In a first discovery step, associations between preselected quantitative B cell biomarkers and the presence of SSc were tested using analysis of covariance with an adjustment for age and gender (after log-transformation for normalization of distribution). The Cohen *d* (standardized adjusted difference between the 2 groups "SSc patients" and "healthy controls") was computed as effect size with 95% confidence interval (CI). For this analysis, RF was considered as binary biomarker (positive/negative); and we used a multivariable logistic regression with RF biomarker as dependent variable and the status ("SSc patient"/"healthy control (HC)"), age and gender as independent variables. The effect size was assessed by the odds ratio (OR) with 95% CI for the risk of positive RF (with the status "HC" as reference value). All *p*-values were corrected for multiplicity using the Benjamini Hochberg procedure (False Discovery Rate) with a cut-off of 5%.

Exploratory analyses were performed among the "SSc patients" group to investigate the potential associations between B cell biomarkers and 13 prespecified disease characteristics. All analyses were adjusted for age, gender and immunosuppressive treatments. Given the nature exploratory of these analyses, no correction for multiplicity were performed. For the quantitative biomarkers, we used non-parametric correlation analysis or analysis of covariance, depending on the type of disease characteristics (quantitative or categorical). Effect sizes were assessed by the partial Spearman correlation coefficient ρ for quantitative characteristics, the adjusted Cohen *d* for binary characteristics and partial η^2 statistics for categorical characteristics (64). The partial η^2 is the proportion of variation accounted for by the characteristic being tested, after adjustment for all others. For the binary biomarker (RF), we used multivariable logistic regressions; and OR with 95% CI were computed as effect sizes.

Effect sizes were interpreted as follows. For Cohen *d*, values between 0.20-0.49 represent a small change, 0.50-0.79 a medium change, and ≥ 0.80 a large change. For the partial correlation coefficient ρ, values between 0.00-0.19 are very weak, 0.20-0.39 weak, 0.40-0.59 moderate, 0.60-0.79 strong, and ≥ 0.80 very strong. For partial η^2, values of 0.01 are small, 0.06 medium, 0.14 large.

B cell biomarkers associations in the validation population

In a second validation step, we compared serum levels of B cell biomarkers between SSc patients with (n=18) and without (n=18) PAH using Mann-Whitney tests (given the small sample sizes) and computed the Cohen *d* effect sizes with 95% CI. Associations between B cell biomarkers and 3 prespecified disease characteristics (Nt-pro-BNP levels, peak TRV and FVC/DLCO ratio) were investigated among SSc-PAH patients using non-parametric correlation analysis with adjustment for age. Effect sizes were assessed by partial Spearman correlation coefficients ρ with 95% CI. For these analyses, RF was considered a quantitative variable. Given the nature exploratory of these analyses, no correction for multiplicity were performed.

B cell production of angiogenic mediators

In a third mechanistic step, we compared levels of several angiogenic mediators in B cell culture supernatants from HC (n=9) and SSc patients (n=18) using Mann-Whitney tests (given the small sample sizes) and computed the Cohen *d* effect sizes with 95% CI. Angiogenic mediators that were significantly different at the p=0.05 level between HC and SSc patients were compared between SSc patients with (n=9) and without (n=9) PAH. Given the nature exploratory of these analyses, no correction for multiplicity were performed.

Softwares

All statistical analyses were performed with the SAS software v9.4 (SAS Institute Inc.). Figures were created using GraphPad Prism v9.3.1 (GraphPad Software).

RESULTS

Soluble markers of B cell activation are differentially expressed in SSc patients and correlate with several disease characteristics, including pulmonary hypertension

In a first exploratory step, we have determined the clinical and pathophysiological relevance of 14 soluble markers of B cell activation (RF, β2-microglobulin, IgG, IgA, IgM, BAFF, APRIL, sBCMA, sTACI, sCD21, sCD23, sCD25, sCD27, CXCL13) in SSc. We have selected a discovery panel of 80 SSc patients encompassing the whole spectrum of SSc clinical manifestations, measured the serum concentrations of these 14 biomarkers in this population, and compared them to those of 80 healthy blood donors (female 70%, mean age 43 ± 3 years).

The main characteristics of our discovery population are displayed in *Table 1* and can be summarized as follows: female 81%, mean age 56 ± 13 years; 41 lcSSc and 15 early dcSSc; 29 extensive and 25 limited ILD; and 19 PH among which 13 were group 1 PH (PAH). Conditions associated with RF positivity in the 44 RF-positive SSc patients were detailed in *Supplemental Table 1.* Overlap syndrome with another connective tissue disease was noted in 32% of cases, which seemed higher than previously reported (65–68): this could be explained by the enriched proportion of SSc-ILD patients in our population (67), a complication associated with overlap syndrome, and by systematic CTD screenings routinely performed during patient follow-up in our center. Among the 80 SSc patients, 7 had been treated by rituximab (RTX); and 3 of them received their last infusion less than 12 months before inclusion (in others, treatment was stopped several years prior). Detailed information regarding these 7 patients and their RTX regimens are provided in *Supplemental Table 2*.

After adjustment on age, gender and multiplicity, we observed a significantly increased proportion of RF positivity (55% *vs* 10%, $p<0.0001$) and higher median levels of β2-microglobulin (2.09 [1.76;2.64] *vs* 1.55 [1.34;1.74] mg/L, $p<0.0001$), IgG (9.66 [8.30;12.04] *vs* 9.30 [7.96;10.31] g/L, $p=0.001$) and CXCL13 (81.73 [46.58;120.5] *vs* 36.95 [24.46;55.77] pg/mL, $p<0.0001$) in SSc patients compared with HC (*Table 2*, *Figure 1*). There was also a trend for an increase in IgA concentrations (2.03 [1.64;2.91] *vs* 1.82 [1.31;2.45] g/L, $p=0.06$) in SSc patients. There was no difference in serum levels of IgM, BAFF, APRIL, sBCMA, sTACI, sCD21, sCD23, sCD25 and sCD27 between the 2 groups. A sensitivity analysis excluding the 7 patients that received RTX yielded similar results, except that the increase in serum IgA levels in the SSc patient group reached statistical significance ($p=0.04$) (*Supplemental Table 3*).

As previous studies reported elevated serum BAFF levels in SSc patients (10,29,31,33–35,69), we tried to further investigate the association between BAFF concentrations and disease status. In univariate analysis, the difference in circulating BAFF levels between the 2 groups reached statistical significance (620 [478;864] *vs* 534 [446;624] pg/mL in SSc patients and HC respectively, p=0.04). However, this was no longer the case after adjustment on age and gender (p=0.90): indeed, there was a positive association between age and BAFF concentrations in our population (r=0.17, p=0.03). As none of the HC were under immunosuppressants, we also wondered immunosuppressive therapy in SSc patients could contribute explain our result; however we did not observe any difference in the serum BAFF levels of HC when compared to SSc patients with (534 [446;624] *vs* 630 [434;887] pg/mL respectively, p=0.80) or without (534 [446;624] *vs* 586 [487;859] pg/mL respectively, p=0.53) immunosuppressants.

Next, we assessed the associations between serum levels of soluble markers of B cell activation and various disease characteristics in the SSc patient group (*Table 3*). After adjustment on age, gender and immunosuppressive drugs, significant associations were identified between B cell biomarkers and SSc parameters related to:

- disease phenotype: SSc subtype with RF positivity (p=0.004) and sCD23 (p=0.03); autoantibody profile with IgG (p<0.001) and APRIL (p=0.02);
- global disease activity and severity: CRP with β2-microglobulin (p=0.03), BAFF (p=0.001), APRIL (p=0.002), sCD21 (p=0.02) and sCD25 (p=0.03); EScSG-AI score with sCD21 (p=0.02); total Medsger score with β2-microglobulin (p=0.01);
- ILD: history of ILD with APRIL (p=0.02) and sBCMA (p=0.02); FVC with IgA (p=0.002) and with IgG (p=0.03);
- PH (*Figure 2*): history of PH with RF positivity (p=0.03) and IgG (p=0.01); Nt-pro-BNP with β2-microglobulin (p=0.01); peak TRV with RF positivity (p=0.004) and BAFF (p=0.002); DLCO with RF positivity (p=0.01) and β2-microglobulin (p=0.02). There was also a trend for associations between Nt-pro-BNP and sBCMA (p=0.07), DLCO and BAFF (p=0.09) and DLCO and sCD23 levels (p=0.07).

Overall, these data suggest that soluble markers of B cell activation are differentially expressed in SSc patients and correlate with several disease characteristics, especially PH.

Circulating levels of β2-microglobulin, IgG, sBCMA, sCD23 and sCD27 are differentially expressed in SSc patients with and without PAH; and serum BAFF levels correlate with clinical markers of PH in SSc-PAH patients

Since involvement of B cells in skin fibrosis, SSc-ILD and disease activity has already been reported (7), we chose to further focus on the association between B cell activation and SSc-PAH observed in our discovery population. We assessed the serum levels of the same B cell biomarkers in an independent validation cohort, consisting of SSc patients with limited cutaneous subset, no extensive ILD, no treatment by immunosuppressants or corticosteroids; and either a diagnosis of group 1 PH (PAH) confirmed by RHC (n=18) or no evidence of PH on screening exams (n=18) (*Table 4*). These inclusion criteria were chosen so that our biomarker assessment would not be biased by any active skin or lung involvement, nor by the effect of immunosuppressive drugs. Groups were matched on age (± 5 years) and gender.

In SSc patients with PAH, we observed significantly higher median levels of β2-microglobulin (3.15 [2.76;3.49] *vs* 2.46 [2.14;3.15] mg/L, *p*=0.02), sBCMA (36699 [32360;45190] *vs* 32,068 [27656;35352] pg/mL, *p*=0.03), sCD23 (4609 [3469;7628] *vs* 3094 [2554;4200] pg/mL, *p*=0.04), and sCD27 (8859 [6889;10667] *vs* 6282 [5825;7645], *p*=0.05), and lower median levels of IgG (8.40 [7.10;10.40] *vs* 10.45 [9.10;11.80] g/L, *p*=0.02), compared to those without PAH (*Table 5*, *Figure 3*). There was no difference in serum levels of RF, IgA, IgM, BAFF, APRIL, sTACI, sCD25 and CXCL13 between the 2 groups.

Next, we assessed the associations between serum levels of soluble markers of B cell activation and clinical markers of PH in the SSc-PAH group (*Table 6*). We identified moderate to strong associations of BAFF with Nt-pro-BNP levels (*p*=0.01, ρ=0.62), FVC/DLCO ratio (ρ=0.60, *p*=0.01) and peak TRV (ρ=0.45, *p*=0.09) (*Figure 4*). Peak TRV also correlated moderately with IgM (ρ=-0.59, *p*=0.02); and FVC/DLCO ratio with β2-microglobulin (ρ=0.43, *p*=0.08) and IgG (ρ=-0.41, *p*=0.10).

Overall, these data suggest that soluble markers of B cell activation are differentially expressed in SSc-PAH patients and correlate with clinical markers of PH, especially for BAFF.

B cells from SSc patients showed differential production of various pro- and anti-angiogenic mediators compared to HC

As our previous results suggested a link between B cell activation and SSc-PAH, we next wondered if B cells could be involved in the pathogenesis of SSc microangiopathy. In order to investigate this hypothesis, we tried to determine whether SSc B cells can produce proteins involved in angiogenic

processes. As pathogenic antibodies against endothelial antigens have already been described in SSc patients (70–81), we chose to focus on non-Ig angiogenic mediators.

Circulating B cells from HC (n=9), and SSc patients with (n=9) and without (n=9) PAH from our validation cohort were cultured for 48h with and without stimulation. Our *in vitro* stimulation of B cells was designed to mimic an antigen- and T cell-dependent stimulation in presence of Toll-like receptor ligand and BAFF, as it is believed to occur in SSc patients. Groups were matched on age (± 5 years) and gender. Concentrations of several angiogenic mediators were assessed in culture supernatants (*Table 7*, *Figure 5*).

We observed higher production of angiogenin (p=0.004 and p=0.008), angiopoietin 1 (p=0.03 and p=0.02), PDGF-AA (p=0.09 and p=0.04) and TIMP-1 (p=0.03 and p=0.05) in B cell culture supernatants from SSc patients compared to HC, both with and without stimulation (respectively). We also found lower levels of angiopoietin 2 (p=0.05) as well as higher levels of VEGFR-1 (p=0.04) and MMP-8 (p=0.04) in supernatants of SSc B cells only after stimulation. Concentrations of ANGPTL-6 and MMP-9 were similar in supernatants from SSc patients and HC, both with and without stimulation. There was no detectable or negligeable B cell production of any of the other selected angiogenic mediators in any groups and regardless of stimulation.

When comparing supernatant levels of SSc patients with and without PAH, we observed no significant difference in B cell secretion of any of these angiogenic factors, in both stimulation conditions.

Overall, these results suggest that B cell produce angiogenic mediators in SSc patients, although with no significant difference in case of PAH.

DISCUSSION

To our knowledge, this is the first study that assessed a wide panel of soluble markers of B cell activation in 2 large and well-characterized SSc patient cohorts. Our results can be summarized as follows: 1/ in a discovery cohort of SSc patients, we identified associations of B cell biomarkers with SSc phenotype, disease activity and severity, ILD and PH; 2/ in this discovery population as well as in an independent validation cohort enriched with SSc-PAH patients, serum IgG, β2-microglobulin, BAFF, sBCMA and sCD23 levels were associated with PH status and/or clinical markers (*Table 8*); 3/ B cells from SSc patients showed differential production of various pro- and anti-angiogenic mediators compared to HC.

Soluble markers of B cell activation in systemic sclerosis

Previous publications that studied B cell biomarkers in SSc reported various associations with disease characteristics, especially with cutaneous and interstitial lung involvements. RF positivity in SSc patients varied considerably between studies, ranging from 12 to 71%, with no clear relationship with articular involvement (39–45). In line with our results, circulating concentrations of β2-microglobulin were found increased in SSc patients, with no difference between cutaneous subsets, and correlated with erythrocyte sedimentation rate (30). Conversely, serum IgG levels were previously reported as similar in SSc patients and HC, although significant differences were found among IgG subclasses (38).

Associations of BAFF with SSc characteristics have been more thoroughly documented, yet with conflicting results that challenge comparisons with our work. Conversely to our study, previous publications have consistently observed elevated serum BAFF levels in SSc patients (10,31,33–35,69). Our result could be at least partly explained by a positive correlation between age and BAFF concentrations in our cohort; an unexpected result since BAFF levels have been described as negatively associated with age in HC (82) and SLE patients (83). Although we do not have a satisfying explanation for this phenomenon, it should be noted that these studies included patients with a very wide spectrum of ages; and it is not sure that this negative association observed over several decades of life remains true when focusing on shorter age ranges.

We did not observe any association of BAFF with skin involvement, ILD or autoantibody profile. Previous studies reported highly contradictory results: some observed significant associations with cutaneous subset and mRSS (35,69) while others did not (31,33,34); serum BAFF levels were found

positively (69), negatively (31) or not (33,34) correlated with ILD; and association with autoantibody profile have been observed in one study (31) but not others (33,34,69). BAFF concentrations were also associated with CRP and EScSG-AI score (although not reaching statistical significance for the latter in our work), which was not observed in other publications (33,35,69). Overall, these discrepancies could be due to heterogeneities between study populations, especially since our discovery cohort was selected to include a wide range of SSc manifestations; and probably reflect a differential implication of B cells between patients, organ involvements, and disease stages.

Serum APRIL levels have been described as either similar (10) or increased (28,29) compared to HC. Contrary to our work, no correlation was found with CRP levels or autoantibody profile; and associations with ILD were inconsistently observed (28,29). CXCL13 levels were increased in SSc (10,33,84) and correlated with EScSG-AI score (an association that we also observed in our work, although not reaching statistical significance). Associations with cutaneous subset and ILD were inconsistently reported (33,84).

Levels of sCD21 were found lower in SSc than in HC, especially in case of lcSSc and ILD, but not associated with autoantibody profile (46). Concentrations of sCD23 in SSc were higher than in HC in a single study (47). Previous publications regarding sCD25 levels in SSc found discordant results: sCD25 concentrations have been reported as both higher than (51,52,54–56) and similar to (49,50) HC; and correlations with skin fibrosis (53,54,56) and ILD (48,53,54) were not consistently observed. However, associations with disease activity seemed more systematically reported (49,52,56,85), a result that we also observed in our study.

We could not find previous reports of sCD27, sTACI and sBCMA levels in SSc patients.

Overall, these data suggest that soluble markers of B cell activation could be interesting tools to assess organ involvement, disease activity and severity in SSc patients. Future studies should try to further validate their relevance as diagnostic and prognostic biomarkers in this disease.

B cell activation in SSc-PAH

A further original finding of our work is the identification of associations between several markers of B cell activation (IgG, β2-microglobulin, BAFF, sBCMA and sCD23) and PAH status and/or clinical markers in 2 independent populations. Few publications have focused on B cell biomarkers and PAH in SSc patients: serum BAFF, APRIL and CXCL13 levels were found similar between patients with and without PH (31,33,34,84); but apart from one study that reported an almost-significant correlation

between CXCL13 and TTE-estimated systolic pulmonary arterial pressure (84), associations with clinical markers of PH were not studied. Although some results overlap between our discovery and validation cohorts, B cell biomarkers associations with PH status and clinical markers were not exactly similar in the 2 populations (*Table 8*). This could be explained by differences in inclusion criteria between cohorts (and notably the presence of patients with group 2 and group 3 PH in the discovery cohort), but could also suggest heterogeneities in B cell activation among SSc patients with PH.

Aside from SSc, B cell biomarkers have also been studied in other causes of PH. For instance, serum concentrations of CXCL13 were increased in patients with idiopathic PAH, connective tissue diseases-associated PAH and chronic thromboembolic PH (86,87) with weak correlations with hemodynamic parameters in this latter subgroup (87). In patients with Sjögren's syndrome, occurrence of PAH was associated with higher RF titers and hypergammaglobulinemia (88,89). Interestingly, a novel mutation of *TNFRSF13B*, the gene coding for TACI, was identified as responsible for a familial form of PAH in Japanese patients (90).

Several evidence pleads for a participation of B cell activation in the pathogenesis of SSc-PAH. Firstly, recent works have observed abnormal B cell homeostasis in SSc-PAH patients, with lower total B cell counts, expanded IgD^+ naïve subset and decreased memory B cells (91). SSc-PAH B cells display features of activation, with membrane over-expression of CD25 and increased susceptibility to apoptosis (92); and B-cell related genes were amongst the most differentially expressed between SSc patients with and without PAH (93). Moreover, a recently described B cell population, characterized by a low membrane expression of CD21, appeared particularly associated with vascular events in SSc patients: indeed, the proportion of circulating $CD21^{low}$ B cells was associated with sPAP, DLCO and FVC/DLCO ratio in SSc patients (94).

Secondly, there is also evidence of functional anomalies in SSc B cells that can contribute to pulmonary vasculopathy. Indeed, SSc-PAH B cells display anomalies in their antibody repertoire, with an over-representation of specific VDJ rearrangements and somatic hypermutations, suggesting small clonal selections and expansions (91). Furthermore, several autoantibodies have been identified in the serum of SSc patients, that seem to have a direct pathogenic effect on the endothelium: indeed, antibodies directed against endothelial cells, endothelin 1 receptor and angiotensin II receptor can induce activation and apoptosis of endothelial cells and are associated with PAH onset (70–81).

Finally, a recent randomized placebo-controlled trial tested the efficacy of rituximab to treat SSc-PAH patients (95). In the primary analysis based on longitudinal data through week 24, the adjusted

mean change in the six-minute walk distance (6MWD) from baseline to 24 weeks did not differ significantly between arms. However, in a secondary analysis using 6MWD data through week 48, the rituximab arm was superior to placebo. Interestingly, machine learning analysis identified low levels of RF, as well as IL-12 and IL-17, as robust predictors to response to rituximab. The authors suggested that this phenomenon may be explained by a reduction of complement-dependent cytotoxicity due to the binding of RF to the Fc portion of rituximab.

Overall, these data further suggest the occurrence of B cell activation in SSc-PAH patients.

B cells as producers of angiogenic mediators: a new player in SSc microangiopathy?

Another original finding of our study is the demonstration that SSc B cells can produce angiogenic factors, which to our knowledge has never been reported before in this disease. B cells have been shown to secrete various pro- and anti-angiogenic mediators (including angiopoietins, VEGFs and their receptors, PDGFs, MMPs and their inhibitors TIMPs) in other conditions, but essentially in the context of B cell neoplasias (96–105).

Angiogenesis is a highly regulated process that leads to the formation of newly formed capillaries from preexisting vessels (106). It is initiated by the release of proteolytic enzymes, such as matrix metalloproteinases (MMPs), resulting in the degradation of the endothelial cell basement membrane and the perivascular extracellular matrix (106). Following matrix degradation, endothelial cells activate, proliferate and migrate into the surrounding area, forming vessel sprouts, under the effect of specific proangiogenic factors such as angiogenin, angiopoietin 1, VEGFs and PDGFs (106). Proteins that inhibit matrix degradation (like TIMPs) or endothelial cell mobilization (such as angiopoietin 2, a natural antagonist of angiopoietin 1) thus have angiostatic properties (106). During SSc, chronic vascular injury induces hypoxia and tissue ischemia, which are classical triggers of angiogenesis, but eventually leads to loss of capillaries and avascular areas: as such, angiogenesis is generally considered to be dysregulated in SSc and insufficient to compensate for the loss of vasculature occurring during the disease (107,108).

Dysregulation of several pro- and anti-angiogenic factors have been documented in SSc patients (109). Circulating angiogenin concentrations are elevated in SSc patients, with no correlation with Raynaud phenomenon or disease duration (110). SSc patients also displays an imbalance in angiopoietin production, with decreased serum levels of angiopoietin 1 and increased serum levels of angiopoietin 2 (associated with digital ulcers) (111). VEGF is strongly upregulated in the skin and

serum of SSc patients, including its anti-angiogenic VEGF(165)b isoform. Studies on VEGFR-1 and -2 expression by skin endothelial cells have yielded conflicting results (112). PDGF levels are increased in the serum, skin and bronchoalveolar lavage of SSc patients (113); and stimulatory autoantibodies directed against PDGF receptors have also been documented (114). Dysregulated production of various MMPs and TIMPs was observed in the skin and serum of SSc patients (115,116).

Although the balance between pro- and anti-angiogenic mediator dysregulation clearly favors the former, this does not seem sufficient to induce compensatory angiogenesis in SSc, which suggests that more complex mechanisms are at work to account for SSc vasculopathy (109). Uncontrolled chronic over-expression of proangiogenic mediators throughout various disease stages might contribute to disturbed vessel morphology and endothelial dysfunction rather than to promote new vessel formation (109). As such, it is difficult to predict how the production of angiogenic mediators by B cells would contribute to SSc microangiopathy. Further mechanistic works are warranted to elucidate the pathophysiological relevance of this finding.

Strengths and limitations

Our study draws strength from its large and well-phenotyped study populations, the wide panel of biomarkers screened, the sample collection on the same day as clinical evaluation (which allowed to perform accurate correlations), the validation of our results on an independent cohort, and the confirmation of the pathophysiological relevance of our observations.

It also has limitations. Firstly, we included 2 selected SSc populations, which may limit the generalization of our findings. Secondly, as this was an exploratory, hypothesis-generating study, statistical analyses were not systematically corrected for multiplicity, which could have increased the a-risk score. Thirdly, most of the associations observed between B cell biomarkers and SSc characteristics have low to medium effect sizes, which could limit the scope of our findings. Fourthly, patients from the discovery cohort were treated by drugs targeting the immune system (corticosteroids, hydroxychloroquine, immunosuppressants), which could have influenced the serum levels of our B cell biomarkers, or biased the associations that were studied. However, we have tried to take their effect into account in our analyses by adjusting our associations on these treatments in our discovery population, and excluding patients treated with such drugs from our validation population. Fifthly, some patients in the discovery cohort had received rituximab infusions, although treatment was discontinued several years before inclusion for most of them. Finally, SSc-PAH patients

were treated by PAH-specific drugs, which may have biased the associations between B cell biomarkers and PH clinical markers, and possibly the B cell production of angiogenic factors.

Conclusion

Soluble markers of B cell activation could be relevant tools to assess organ involvements, disease activity and severity in SSc patients. Further studies with larger samples and longitudinal design are warranted to test their capacity to predict the occurrence of severe organ involvements. Notably, some of them showed interesting associations with PAH, which can plead for a role of B cell activation in the pathogenesis of the pulmonary microangiopathy. B cells could contribute to the SSc vasculopathy through the production of non-Ig angiogenic mediators.

Table 1. Characteristics of SSc patients from the discovery population.

	N	Value
Demographic data		
Females, n (%)	80	65 (81)
Age at inclusion (years), mean ± SD	80	56 ± 13
BMI (kg/m^2), mean ± SD	80	24 ± 5
Diagnosis of SSc		
SSc subtype	80	
Early dcSSc, n (%)		15 (19)
Late dcSSc, n (%)		24 (30)
lcSSc, n (%)		41 (51)
Immunological profile	80	
Anti-centromere antibodies, n (%)		20 (25)
Anti-topoisomerase I antibodies, n (%)		21 (26)
Anti-RNA polymerase III antibodies, n (%)		7 (9)
Other antibody specificities, n (%)		17 (21)
Positive ANA without antibody specificity, n (%)		6 (8)
Negative ANA		9 (11)
Overlap with another connective tissue disease	79	25 (32)
Systemic *lupus erythematosus*, n (%)		4 (5)
Sjogren's syndrome, n (%)		12 (15)
Inflammatory myopathy, n (%)		4 (5)
Rheumatoid arthritis, n (%)		2 (3)
Lymphoma (current or previous history), n (%)	80	0 (0)
Disease duration at inclusion		
Since diagnosis (years), mean ± SD	80	10 ± 10
Since first non-Raynaud symptom (years), mean ± SD	78	11 ± 10
Since Raynaud phenomenon onset (years), mean ± SD	76	14 ± 13
History of organ involvements		
Interstitial lung disease	80	
No ILD, n (%)		26 (33)
Limited ILD, n (%)		25 (31)
Extensive ILD, n (%)		29 (36)
ILD duration at inclusion (years), mean ± SD	54	7 ± 7
Pulmonary hypertension, n (%)	80	19 (24)
Group 1, n (%)		13 (17)
Group 1', n (%)		1 (1)
Group 2, n (%)		6 (8)
Group 3, n (%)		3 (4)
PH duration at inclusion (years), mean ± SD	19	4 ± 4
Scleroderma renal crisis, n (%)	80	0 (0)
History of digital ulcers, n (%)	80	49 (61)
Clinical evaluation at inclusion		
Modified Rodnan skin score, mean ± SD	79	7.6 ± 6.5
in dcSSc patients, mean ± SD	41	10.8 ± 6.8
NYHA functional class	80	
Class I, n (%)		29 (36)
Class II, n (%)		21 (26)
Class III, n (%)		23 (29)
Class IV, n (%)		7 (9)
6-minute walk distance (m), mean ± SD	74	401 ± 117

6-minute walk distance (% predicted), mean ± SD	74	72 ± 18
Active Raynaud attacks at inclusion, n (%)	76	35 (46)
Active digital ulcers at inclusion, n (%)	80	16 (20)
Telangiectasias, n (%)	79	59 (75)
Calcinosis cutis, n (%)	74	12 (16)
Biological data		
ESR (mm/h), mean ± SD	74	17 ± 19
CRP (mg/L), mean ± SD	80	6.9 ± 8.9
Creatinin (mg/L), mean ± SD	80	7.9 ± 2.4
Estimated GFR (mL/min/1,73m^2), mean ± SD	80	94 ± 27
Nt-pro-BNP (pg/mL), mean ± SD	80	578 ± 1452
Ferritin (ng/mL), mean ± SD	79	112 ± 121
Uric acid (mg/L), mean ± SD	80	47 ± 15
Complement activation, n (%)	80	1 (1)
Transthoracic echocardiography		
Left ventricular ejection fraction (%), mean ± SD	79	64 ± 7
Left ventricular diastolic dysfunction, n (%)	78	62 (80)
Peak TRV (m/s), mean ± SD	69	2.78 ± 0.68
RA area (cm^2), mean ± SD	67	15 ± 5
Pulmonary function tests		
TLC (% predicted), mean ± SD	78	83 ± 19
FVC (% predicted), mean ± SD	80	88 ± 23
DLCO (% predicted), mean ± SD	77	57 ± 20
KCO (% predicted), mean ± SD	78	73 ± 21
Composite scores		
EScSG-AI score, mean ± SD	78	1.58 ± 1.31
in lcSSc patients, mean ± SD	41	1.46 ±1.22
in dcSSc patients, mean ± SD	37	1.72 ±1.40
Medsger severity score, mean ± SD	77	5.90 ± 2.86
sHAQ score, mean ± SD	72	0.90 ± 0.63
Treatments at inclusion		
Corticosteroids, n (%)	80	47 (59)
Corticosteroid dosage (mg prednisone equivalent/day), mean ± SD	47	8 ± 3
Immunosuppressants, n (%)	80	43 (54)
Cyclophosphamide, n (%)		0 (0)
Mycophenolate mofetil, n (%)		31 (39)
Methotrexate, n (%)		3 (4)
Azathioprine, n (%)		2 (3)
Rituximab (current or previous), n (%)		7 (9)
Current treatment by rituximab (last infusion < 12 months)		3 (4)
Hydroxychloroquine, n (%)	80	7 (8.8)
PAH specific drugs*, n (%)	80	21 (26)

ANA: antinuclear antibodies; BMI: body mass index; CRP: C-reactive protein; dc: diffuse cutaneous; DLCO: diffusing capacity of the lung for carbon monoxide; EScSG-AI: European Scleroderma Study Group Activity Index; ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; GFR: glomerular filtration rate; sHAQ: scleroderma Health Assessment Questionnaire; ILD: interstitial lung disease; KCO: diffusing coefficient for carbon monoxide; lc: limited cutaneous; Nt-pro-

BNP: N-terminal prohormone of brain natriuretic peptide; NYHA: New York Heart Association; PAH: pulmonary arterial hypertension; RA: right atrium; SSc: systemic sclerosis; TLC: total lung capacity; TRV: tricuspid regurgitation velocity.

**PAH specific drugs included phosphodiesterase 5 inhibitors, endothelin receptor antagonists and prostacyclin analogues. These could have been prescribed for PAH, digital ulcers and/or refractory Raynaud phenomenon.*

Table 2. Serum levels of soluble markers of B cell activation in the SSc patients and healthy controls (discovery cohort).

Biomarkers	**Healthy controls** (N=80)	**SSc patients** (N=80)	**Effect size**[1]	***p*-values**[2]	***p*-values adjusted for FDR**[3]
Positive RF, n (%)	8 (10%)	44 (55%)	12.68 (4.78 ; 33.61)	<0.0001	**<0.0001**
β2-microglobulin (mg/L), median (Q1;Q3)	1.55 (1.34 ; 1.74)	2.09 (1.76 ; 2.64)	0.61 (0.35 ; 0.87)	<0.0001	**<0.0001**
IgA (g/L), median (Q1;Q3)	1.82 (1.31 ; 2.45)	2.03 (1.64 ; 2.91)	0.36 (0.05 ; 0.68)	0.02	0.06
IgG (g/L), median (Q1;Q3)	9.30 (7.96 ; 10.31)	9.66 (8.30 ; 12.04)	0.55 (0.25 ; 0.86)	0.0004	**0.001**
IgM (g/L), median (Q1;Q3)	0.81 (0.61 ; 1.20)	1.01 (0.62 ; 1.57)	0.12 (-0.17 ; 0.41)	0.42	0.53
BAFF (pg/ml), median (Q1;Q3)	534 (446 ; 624)	620 (478 ; 864)	0.02 (-0.28 ; 0.32)	0.90	0.90
APRIL (pg/ml), median (Q1;Q3)	1911 (1619 ; 2236)	1958 (1425 ; 2313)	0.03 (-0.28 ; 0.34)	0.83	0.90
sBCMA (pg/ml), median (Q1;Q3)	37344 (29070 ; 47014)	44019 (24470 ; 59028)	0.24 (-0.07 ; 0.55)	0.13	0.23
sTACI (pg/ml), median (Q1;Q3)	3.79 (1.64 ; 7.18)	5.05 (1.79 ; 10.62)	0.24 (-0.07 ; 0.55)	0.14	0.90
sCD21 (pg/ml), median (Q1;Q3)	51516 (39990 ; 64006)	45520 (30635 ; 58414)	-0.17 (-0.47 ; 0.13)	0.27	0.42
sCD23 (pg/ml), median (Q1;Q3)	1952 (1405 ; 3168)	1803 (984.8 ; 3144)	-0.13 (-0.44 ; 0.18)	0.40	0.53
sCD25 (pg/ml), median (Q1;Q3)	305 (246 ; 391)	327.7 (248 ; 569)	0.24 (-0.07 ; 0.55)	0.13	0.23
sCD27 (pg/ml), median (Q1;Q3)	4440 (3615 ; 5658)	4906 (4191 ; 7395)	0.28 (-0.02 ; 0.59)	0.07	0.16
CXCL13 (pg/ml), median (Q1;Q3)	36.95 (24.46 ; 55.77)	81.73 (46.58 ; 120.5)	1.01 (0.69 ; 1.33)	<0.0001	**<0.0001**

APRIL: a proliferation-inducing ligand; BAFF: B-cell-activating factor; BCMA: B-cell maturation antigen; CD: cluster of differentiation; CXCL13: C-X-C motif chemokine 13; FDR: false discovery rate; Ig: immunoglobulin; Q: quartile; RF: rheumatoid factor; s: soluble; SSc: systemic sclerosis; TACI: transmembrane activator and CAML interactor.

Results are expressed as median (first quartile; third quartile) for quantitative biomarkers and as frequency (percentage) otherwise.

All analyses were adjusted for age and gender.

[1] For quantitative biomarkers, effect sizes were calculated on log transformed variables using the Cohen d. Absolute values of 0.20–0.49 represent a small change; values of 0.50–0.79 a medium change; and values of ³ 0.80 a large change. For the binary biomarker, effect size is the odds ratio of the status for the risk of positive RF with the status control as reference value.

[2] p-values calculated on log-transformed variables for quantitative biomarkers.

[3] p-values corrected for multiplicity using the False Discovery Rate (FDR) method (Benjamini Hochberg procedure).

Table 3. Associations between serum levels of soluble markers of B cell activation and disease characteristics assessed among the 80 SSc patients from the discovery cohort.

	Positive RF[1]	β2m[5] (mg/L)	IgA[5] (g/L)	IgG[5] (g/L)	IgM[5] (g/L)	BAFF[5] (pg/mL)	APRIL[5] (pg/mL)	sBCMA[5] (pg/mL)	sTACI[5] (pg/mL)	sCD21[5] (pg/mL)	sCD23[5] (pg/mL)	sCD25[5] (pg/mL)	sCD27[5] (pg/mL)	CXCL13[5] (pg/mL)
SSc subtype lc *vs* dc	**p=0.004** OR=5.18 (1.72;15.63)	p=0.72 d=0.07 (-0.31;0.44)	p=0.53 d=0.14 (-0.30;0.58)	p=0.98 d=0.01 (-0.40;0.42)	p=0.73 d=-0.07 (-0.45;0.31)	p=0.91 d=-0.02 (-0.45;0.40)	p=0.79 d=0.06 (-0.37;0.48)	p=0.84 d=-0.04 (-0.46;0.37)	p=0.17 d=0.30 (-0.13;0.74)	p=0.15 d=0.30 (-0.11;0.72)	**p=0.03** d=0.46 (0.04;0.89)	p=0.23 d=-0.27 (-0.71;0.17)	p=0.08 d=-0.39 (-0.83;0.04)	p=0.22 d=0.27 (-0.16;0.71)
mRSS[2]	p=0.29 OR=0.75 (0.44;1.27)	p=0.50 ρ=-0.08 (-0.30;0.15)	p=0.67 ρ=-0.05 (-0.27;0.18)	p=0.21 ρ=-0.14 (-0.36;0.09)	p=0.51 ρ=0.08 (-0.15;0.30)	p=0.12 ρ=0.18 (-0.05;0.39)	p=0.16 ρ=0.16 (-0.06;0.37)	p=0.56 ρ=-0.07 (-0.29;0.16)	p=0.79 ρ=-0.03 (-0.25;0.20)	p=0.66 ρ=-0.05 (-0.27;0.18)	p=0.36 ρ=-0.11 (-0.32;0.12)	p=0.65 ρ=0.05 (-0.18;0.27)	p=0.98 ρ=-0.003 (-0.23;0.22)	p=0.87 ρ=-0.02 (-0.24;0.21)
Disease duration[2] (years)	p=0.90 OR=1.03 (0.65;1.64)	p=0.62 ρ=-0.06 (-0.28;0.17)	p=0.70 ρ=-0.04 (-0.27;0.18)	p=0.57 ρ=-0.07 (-0.28;0.16)	p=0.85 ρ=0.02 (-0.20;0.24)	p=0.42 ρ=0.09 (-0.14;0.31)	p=0.33 ρ=-0.11 (-0.33;0.11)	p=0.78 ρ=-0.03 (-0.25;0.19)	p=0.44 ρ=-0.09 (-0.31;0.14)	p=0.78 ρ=-0.03 (-0.25;0.19)	p=0.71 ρ=0.04 (-0.18;0.26)	p=0.88 ρ=-0.02 (-0.24;0.21)	p=0.90 ρ=-0.01 (-0.24;0.21)	p=0.41 ρ=-0.10 (-0.31;0.13)
Auto-Ab ATA *vs* ACA ARA *vs* ACA other *vs* ACA	p=0.46 OR 1.90 (0.42;8.53) OR 5.12 (0.68;38.46) OR 2.11 (0.48;9.25)	p=0.63 η^2=0.03 (0.00;0.10)	p=0.23 η^2=0.06 (0.00;0.16)	**p<0.0001** η^2=0.29 (0.09;0.42)	p=0.82 η^2=0.01 (0.00;0.06)	p=0.64 η^2=0.03 (0.00;0.09)	**p=0.02** η^2=0.14 (0.00;0.26)	p=0.40 η^2=0.04 (0.00;0.13)	p=0.62 η^2=0.03 (0.00;0.10)	p=0.51 η^2=0.03 (0.00;0.11)	p=0.40 η^2=0.04 (0.00;0.13)	p=0.64 η^2=0.03 (0.00;0.09)	p=0.19 η^2=0.07 (0.00;0.17)	p=0.24 η^2=0.06 (0.00;0.16)
ILD lim *vs* none ext *vs* none	p=0.18 OR 0.94 (0.28;3.14) OR 0.34 (0.09;1.23)	p=0.70 η^2=0.01 (0.00;0.07)	p=0.10 η^2=0.06 (0.00;0.17)	p=0.36 η^2=0.03 (0.00;0.11)	p=0.96 η^2=0.001 (0.00;0.01)	p=0.16 η^2=0.05 (0.00;0.15)	**p=0.02** η^2=0.10 (0.00;0.22)	**p=0.02** η^2=0.10 (0.00;0.22)	p=0.76 η^2=0.01 (0.00;0.06)	p=0.17 η^2=0.05 (0.00;0.15)	p=0.89 η^2=0.003 (0.00;0.04)	p=0.20 η^2=0.04 (0.00;0.14)	p=0.11 η^2=0.06 (0.00;0.16)	p=0.36 η^2=0.03 (0.00;0.11)
FVC[4] (%)	p=0.38 OR=0.39 (0.05;3.15)	p=0.05 ρ=-0.22 (-0.42;0.004)	**p=0.002** ρ=-0.35 (-0.53;-0.14)	**p=0.03** ρ=-0.25 (-0.45;-0.03)	p=0.59 ρ=-0.06 (-0.28;0.16)	p=0.99 ρ=-0.001 (-0.22;0.22)	p=0.94 ρ=-0.01 (-0.23;0.22)	p=0.56 ρ=-0.07 (-0.29;0.16)	p=0.16 ρ=-0.16 (-0.37;0.06)	p=0.67 ρ=0.05 (-0.18;0.27)	p=0.79 ρ=-0.03 (-0.25;0.20)	p=0.47 ρ=0.08 (-0.14;0.30)	p=0.66 ρ=0.05 (-0.18;0.27)	p=0.06 ρ=-0.22 (-0.42;0.01)
PH *vs* no PH	**p=0.03** OR=4.41 (1.15;16.92)	p=0.07 d=0.41 (-0.03;0.85)	p=0.97 d=-0.01 (-0.52;0.50)	**p=0.01** d=-0.68 (-1.17;-0.19)	p=0.87 d=-0.04 (-0.48;0.41)	p=0.82 d=0.06 (-0.44;0.56)	p=0.34 d=-0.24 (-0.74;0.25)	p=0.47 d=0.18 (-0.31;0.67)	p=0.90 d=-0.03 (-0.54;0.47)	p=0.07 d=0.46 (-0.03;0.94)	p=0.17 d=0.34 (-0.15;0.84)	p=0.36 d=-0.24 (-0.75;0.27)	p=0.14 d=-0.39 (-0.89;0.12)	p=0.65 d=0.12 (-0.39;0.63)
Nt-pro-BNP[3] (pg/mL)	p=0.36 OR=1.20 (0.82;1.75)	**p=0.01** ρ=0.31 (0.09;0.50)	p=0.83 ρ=0.03 (-0.20;0.25)	p=0.96 ρ=0.01 (-0.22;0.23)	p=0.50 ρ=-0.08 (-0.30;0.15)	p=0.85 ρ=-0.02 (-0.24;0.20)	p=0.83 ρ=-0.02 (-0.25;0.20)	p=0.07 ρ=0.21 (-0.02;0.41)	p=0.46 ρ=0.09 (-0.14;0.30)	p=0.35 ρ=0.11 (-0.12;0.32)	p=0.28 ρ=0.13 (-0.10;0.34)	p=0.92 ρ=0.01 (-0.21;0.24)	p=0.85 ρ=-0.02 (-0.24;0.20)	p=0.29 ρ=0.12 (-0.11;0.34)
Peak TRV[3] (m/s)	**p=0.004** OR=12.23 (2.23;66.90)	p=0.26 ρ=0.14 (-0.11;0.37)	p=0.48 ρ=-0.09 (-0.32;0.16)	p=0.27 ρ=-0.14 (-0.37;0.11)	p=0.22 ρ=0.15 (-0.09;0.38)	**p=0.002** ρ=0.37 (0.13;0.56)	p=0.40 ρ=0.10 (-0.14;0.34)	p=0.80 ρ=-0.03 (-0.27;0.21)	p=0.28 ρ=-0.14 (-0.36;0.11)	p=0.23 ρ=-0.15 (-0.38;0.10)	p=0.62 ρ=0.06 (-0.18;0.30)	p=0.98 ρ=-0.003 (-0.24;0.24)	p=0.31 ρ=-0.13 (-0.36;0.12)	p=0.95 ρ=-0.01 (-0.25;0.23)
DLCO[4] (%)	**p=0.01** OR=0.03 (0.002;0.43)	**p=0.02** ρ=-0.27 (-0.47;-0.05)	p=0.21 ρ=-0.15 (-0.36;0.08)	p=0.74 ρ=0.04 (-0.19;0.26)	p=0.83 ρ=0.03 (-0.20;0.25)	p=0.09 ρ=-0.20 (-0.41;0.03)	p=0.90 ρ=-0.01 (-0.24;0.21)	p=0.96 ρ=-0.01 (-0.23;0.22)	p=0.87 ρ=0.02 (-0.21;0.25)	p=0.84 ρ=0.02 (-0.21;0.25)	p=0.07 ρ=-0.21 (-0.42;0.02)	p=0.44 ρ=0.09 (-0.14;0.31)	p=0.36 ρ=0.11 (-0.12;0.33)	p=0.15 ρ=-0.17 (-0.38;0.06)
CRP[2] (mg/L)	p=0.68 OR=1.15 (0.59;2.28)	**p=0.03** ρ=0.25 (0.02;0.45)	p=0.51 ρ=-0.08 (-0.29;0.15)	p=0.76 ρ=0.03 (-0.19;0.26)	p=0.20 ρ=0.15 (-0.08;0.36)	**p=0.001** ρ=0.37 (0.15;0.54)	**p=0.002** ρ=0.35 (0.13;0.53)	p=0.67 ρ=-0.05 (-0.27;0.18)	p=0.81 ρ=0.03 (-0.20;0.25)	**p=0.02** ρ=-0.26 (-0.46;-0.04)	p=0.99 ρ=0.001 (-0.22;0.23)	**p=0.03** ρ=0.24 (0.02;0.44)	p=0.92 ρ=0.01 (-0.21;0.23)	p=0.85 ρ=0.02 (-0.20;0.25)
EScSG-AI score[2]	p=0.15 OR=2.12 (0.76;5.92)	p=0.12 ρ=0.18 (-0.05;0.39)	p=0.34 ρ=0.11 (-0.12;0.33)	p=0.85 ρ=-0.02 (-0.25;0.21)	p=0.30 ρ=0.12 (-0.11;0.34)	p=0.08 ρ=0.20 (-0.03;0.41)	p=0.38 ρ=0.10 (-0.13;0.32)	p=0.48 ρ=-0.08 (-0.30;0.15)	p=0.45 ρ=0.09 (-0.14;0.31)	**p=0.02** ρ=-0.28 (-0.47;-0.05)	p=0.84 ρ=-0.02 (-0.25;0.20)	p=0.17 ρ=0.16 (-0.07;0.37)	p=0.74 ρ=0.04 (-0.19;0.26)	p=0.07 ρ=0.21 (-0.02;0.42)
Medsger score[2]	p=0.97 OR=1.00 (0.85;1.18)	**p=0.01** ρ=0.29 (0.06;0.48)	p=0.57 ρ=0.07 (-0.16;0.29)	p=0.87 ρ=0.02 (-0.21;0.25)	p=0.74 ρ=0.04 (-0.19;0.26)	p=0.06 ρ=0.22 (-0.01;0.43)	p=0.40 ρ=0.10 (-0.13;0.32)	p=0.90 ρ=-0.01 (-0.24;0.21)	p=0.26 ρ=0.13 (-0.10;0.35)	p=0.64 ρ=0.06 (-0.18;0.28)	p=0.80 ρ=0.03 (-0.20;0.26)	p=0.91 ρ=0.01 (-0.22;0.24)	p=0.74 ρ=0.04 (-0.19;0.26)	p=0.20 ρ=0.15 (-0.08;0.37)

ACA: anti-centromere antibodies; ARA: anti-RNA polymerase III antibodies; ATA: anti-topoisomerase antibodies; APRIL: a proliferation-inducing ligand; BAFF: B-cell-activating factor; BCMA: B-cell maturation antigen; CD: cluster of differentiation; CRP: C-reactive protein; CXCL13: C-X-C motif chemokine 13; dc: diffuse cutaneous; DLCO: diffusing capacity of the lung for carbon monoxide; EScSG-AI: European Scleroderma Study Group Activity Index; ext: extensive; FVC: forced vital capacity; Ig: immunoglobulin; lc: limited cutaneous; lim: limited; mRSS: modified Rodnan skin score; Nt-pro-BNP: N-terminal prohormone of brain natriuretic peptide; PH: pulmonary hypertension; RF: rheumatoid factor; s: soluble; SD: standard deviation; SSc: systemic sclerosis; TACI: transmembrane activator and CAML interactor; TRV: tricuspid regurgitation velocity; b2m: β2-microglobulin.

All analyses are adjusted for age, gender and immunosuppressive treatments. Given the exploratory nature of this analysis, p-values were not corrected for multiplicity.

[1] For associations between binary biomarkers (RF) and disease characteristics, effect sizes are expressed as odds ratio (OR) for the risk of positive RF (with 95% confidence interval).

[2] OR per increasing of one unit in the log transformed variable.

[3] OR per increasing of one unit in the original variable.

[4] OR per increasing of 100 units in the original variables.

[5] For associations between quantitative biomarkers and quantitative disease characteristics, effect sizes are expressed as the partial Spearman correlation coefficient r (with 95% confidence interval). The usual interpretation is that an absolute value of correlation between 0.00 to 0.19 is very weak; 0.20 to 0.39 weak; 0.40 to 0.59 moderate; 0.60 to 0.79 strong; and greater than 0.80 very strong.

For associations between quantitative biomarkers and binary disease characteristics, effect sizes are expressed as the Cohen d. Values of 0.20–0.49 represent a small change; values of 0.50–0.79 a medium change; and values of ³ 0.80 a large change.

For associations between quantitative biomarkers and nominal disease characteristics, effect sizes are expressed by the partial h^2 statistics proposed by Cohen. It is the partial proportion of variation accounted for by the effect being tested. Values of 0.01 are small; 0.06 medium; and 0.14 large.

Table 4. Characteristics of SSc-PAH and SSc-no PAH patients from the validation population.

	SSc-no PAH		SSc-PAH	
	N	**Value**	**N**	**Value**
Demographic data				
Females, n (%)	18	17 (94)	18	17 (94)
Age at inclusion (years), mean ± SD	18	70 ± 10	18	69 ± 9.3
BMI (kg/m^2), mean ± SD	18	26 ± 5	18	25 ± 4.8
Diagnosis of SSc				
SSc subtype	18		18	
lcSSc, n (%)		18 (100)		18 (100)
dcSSc, n (%)		0 (0)		0 (0)
Immunological profile	18		18	
Anti-centromere antibodies, n (%)		18 (100)		17 (94)
Anti-topoisomerase I antibodies, n (%)		0 (0)		0 (0)
Anti-RNA polymerase III antibodies, n (%)		0 (0)		0 (0)
Anti-fibrillarin, n (%)		0 (0)		1 (6)
Overlap with another connective tissue disease	18	6 (33)	18	6 (33)
Systemic *lupus erythematosus*, n (%)		0 (0)		0 (0)
Sjogren's syndrome, n (%)		6 (33)		6 (33)
Inflammatory myopathy, n (%)		0 (0)		0 (0)
Lymphoma (current or previous history), n (%)	18	0 (0)	18	0 (0)
Disease duration at inclusion				
Since diagnosis (years), mean ± SD	18	14 ± 9	18	17 ± 9.8
Since first non-Raynaud symptom (years), mean ± SD	16	16 ± 7	17	18 ± 8.1
Since Raynaud phenomenon onset (years), mean ± SD	18	23 ± 11	18	29 ± 15
History of organ involvements				
Interstitial lung disease	18		18	
No ILD, n (%)		14 (73)		14 (73)
Limited ILD, n (%)		4 (22)		4 (22)
Extensive ILD, n (%)		0 (0)		0 (0)
ILD duration at inclusion (years), mean ± SD	4	7 ± 4	4	10 ± 11
Pulmonary hypertension, n (%)	18	0 (0)	18	18 (100)
Group 1, n (%)		0 (0)		18 (100)
Group 1', n (%)		0 (0)		2 (15)
Group 2, n (%)		0 (0)		0 (0)
Group 3, n (%)		0 (0)		0 (0)
PH duration at inclusion (years), mean ± SD			18	3 ± 2.3
Scleroderma renal crisis, n (%)	18	0 (0)	18	0 (0)
History of digital ulcers, n (%)	18	11 (61)	18	12 (67)
Clinical evaluation at inclusion				
Modified Rodnan skin score, mean ± SD	18	4.1 ± 3.8	18	7.1 ± 6.1
NYHA functional class	18		18	
Class I, n (%)		8 (44)		4 (22)
Class II, n (%)		8 (44)		3 (17)
Class III, n (%)		1 (6)		7 (39)
Class IV, n (%)		1 (6)		4 (22)
6-minute walk distance (m), mean ± SD	14	383 ± 86	17	298 ± 125
6-minute walk distance (% predicted), mean ± SD	14	78 ± 20	17	59 ± 21
Active Raynaud attacks at inclusion, n (%)	18	10 (56)	14	4 (29)
Active digital ulcers at inclusion, n (%)	18	5 (28)	18	2 (11)
Telangiectasias, n (%)	18	16 (89)	18	16 (89)

Calcinosis cutis, n (%)	18	4 (22)	15	6 (40)
Biological data				
ESR (mm/h), mean ± SD	17	16 ± 8.6	16	19 ± 13
CRP (mg/L), mean ± SD	18	3.8 ± 1.8	18	6.6 ± 5.2
Creatinin (mg/L), mean ± SD	18	7.1 ± 1.6	18	8.7 ± 1.8
Estimated GFR (mL/min/1,73m^2), mean ± SD	18	92 ± 29	18	73 ± 20
Nt-pro-BNP (pg/mL), mean ± SD	18	150 ± 123	18	950 ± 1241
Ferritin (ng/mL), mean ± SD	18	81 ± 73	18	58 ± 44
Uric acid (mg/L), mean ± SD	18	46 ± 11	17	61 ± 24
Complement activation, n (%)	18	0 (0)	17	0 (0)
Transthoracic echocardiography				
Left ventricular ejection fraction (%), mean ± SD	16	64 ± 8.2	16	66 ± 6.6
Left ventricular diastolic dysfunction, n (%)	16	4 (25)	17	7 (41)
Peak TRV (m/s), mean ± SD	14	2.46 ± 0.37	16	3.63 ± 0.65
RA area (cm^2), mean ± SD	17	14 ± 4.8	18	21 ± 7.7
Pulmonary function tests				
TLC (% predicted), mean ± SD	17	104 ± 12	16	92 ± 9.3
FVC (% predicted), mean ± SD	18	117 ± 16	18	102 ± 17
DLCO (% predicted), mean ± SD	18	70 ± 13	18	38 ± 11
KCO (% predicted), mean ± SD	18	76 ± 13	18	45 ± 11
FVC (% predicted)/DLCO (% predicted) ratio, mean ± SD	18	1.73 ± 0.35	18	2.86 ± 0.76
Right heart catheterization				
At PAH diagnosis				
Time between RHC and inclusion (months), mean ± SD			18	34 ± 26
RAP (mmHg), mean ± SD			18	5.7 ± 3.2
sPAP (mmHg), mean ± SD			18	60 ± 16
dPAP (mmHg), mean ± SD			18	21 ± 6.5
mPAP (mmHg), mean ± SD			18	36 ± 10
PAWP (mmHg), mean ± SD			18	7.9 ± 2.3
Cardiac output (L/min), mean ± SD			18	4.7 ± 1.0
Cardiac index (L/min/m²), mean ± SD			18	2.8 ± 0.6
SvO_2 (%), mean ± SD			16	69 ± 6
PVR (Wood units), mean ± SD			18	6.13 ± 2.38
Latest available RHC data				
Time between RHC and inclusion (months), mean ± SD			18	12 ± 14
RAP (mmHg), mean ± SD			17	5.5 ± 3.4
sPAP (mmHg), mean ± SD			17	57 ± 16
dPAP (mmHg), mean ± SD			17	26 ± 24
mPAP (mmHg), mean ± SD			18	35 ± 9.3
PAWP (mmHg), mean ± SD			18	9.1 ± 3.2
Cardiac output (L/min), mean ± SD			18	5.1 ± 1.3
Cardiac index (L/min/m²), mean ± SD			18	3.0 ± 0.6
SvO_2 (%), mean ± SD			16	66 ± 9.6
PVR (Wood units), mean ± SD			18	5.6 ± 2.9
Composite scores				
EScSG-AI score, mean ± SD	18	1.17 ± 1.32	18	1.44 ± 1.21
Medsger severity score, mean ± SD	18	0.78 ± 1.48	16	1.26 ± 1.93
sHAQ score, mean ± SD	13	0.87 ± 0.67	15	1.01 ± 0.54
Treatments at inclusion				

Corticosteroids, n (%)	18	0 (0)	18	0 (0)
Immunosuppressants, n (%)	18	0 (0)	18	0 (0)
Hydroxychloroquine, n (%)	18	0 (0)	18	0 (0)
PAH specific drugs*, n (%)	18	1 (6)	18	12 (67)
Phosphodiesterase 5 inhibitors, n (%)		1 (6)		12 (67)
Endothelin receptor antagonists, n (%)		0 (0)		8 (44)
Prostacyclin analogues, n (%)		0 (0)		1 (6)
Oxygen, n (%)	18	0 (0)	18	4 (22)

ANA: antinuclear antibodies; BMI: body mass index; CRP: C-reactive protein; dc: diffuse cutaneous; DLCO: diffusing capacity of the lung for carbon monoxide; dPAP: diastolic pulmonary arterial pressure; EScSG-AI: European Scleroderma Study Group Activity Index; ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; GFR: glomerular filtration rate; sHAQ: scleroderma Health Assessment Questionnaire; ILD: interstitial lung disease; KCO: diffusing coefficient for carbon monoxide; lc: limited cutaneous; mPAP: mean systolic pulmonary arterial pressure; Nt-pro-BNP: N-terminal prohormone of brain natriuretic peptide; NYHA: New York Heart Association; PAH: pulmonary arterial hypertension; PAWP: pulmonary arterial wedge pressure; PVR: pulmonary vascular resistance; RA: right atrium; RAP: right atrial pressure; sPAP: systolic pulmonary arterial pressure; SSc: systemic sclerosis; SvO_2: venous saturation in oxygen; TLC: total lung capacity; TRV: tricuspid regurgitation velocity.

**In SSc-no PAH patients, these drugs were prescribed for digital ulcers and/or refractory Raynaud phenomenon.*

Table 5. Serum levels of soluble markers of B cell activation in SSc patients with and without PAH (validation cohort).

Biomarkers	**SSc-no PAH** (N=18)	**SSc-PAH** (N=18)	**Effect size**	***p*-values**
RF (IU/mL), median (Q1;Q3)	7.35 (2.90 ; 37.00)	10.25 (4.60 ; 28.00)	0.1 (-0.55 ; 0.75)	0.78
β**2-microglobulin** (mg/L), median (Q1;Q3)	2.46 (2.14 ; 3.15)	3.15 (2.76 ; 3.49)	0.89 (0.2 ; 1.57)	**0.02**
IgA (g/L), median (Q1;Q3)	2.18 (1.61 ; 2.59)	1.78 (1.33 ; 2.44)	-0.28 (-0.94 ; 0.37)	0.40
IgG (g/L), median (Q1;Q3)	10.45 (9.10 ; 11.80)	8.40 (7.10 ; 10.40)	-0.85 (-1.53 ; -0.17)	**0.02**
IgM (g/L), median (Q1;Q3)	1.26 (0.98 ; 1.90)	1.55 (1.00 ; 1.93)	0.15 (-0.51 ; 0.8)	0.67
BAFF (pg/mL), median (Q1;Q3)	727.5 (649.1 ; 897.9)	731.5 (602.3 ; 925.3)	-0.09 (-0.75 ; 0.56)	0.79
APRIL (pg/mL), median (Q1;Q3)	1907 (1532 ; 2358)	1804 (1384 ; 2169)	-0.29 (-0.95 ; 0.36)	0.38
sBCMA (pg/mL), median (Q1;Q3)	32068 (27656 ; 35352)	36699 (32360 ; 45190)	0.76 (0.08 ; 1.44)	**0.03**
sTACI (pg/mL), median (Q1;Q3)	15.35 (9.88 ; 20.67)	18.61 (9.39 ; 22.49)	0.24 (-0.42 ; 0.89)	0.49
sCD23 (pg/mL), median (Q1;Q3)	3094 (2554 ; 4200)	4609 (3469 ; 7628)	0.72 (0.05 ; 1.39)	**0.04**
sCD25 (pg/mL), median (Q1;Q3)	552.6 (448.2 ; 686.0)	584.2 (471.6 ; 756.6)	0.33 (-0.33 ; 0.98)	0.33
sCD27 (pg/mL), median (Q1;Q3)	6282 (5825 ; 7645)	8859 (6889 ; 10667)	0.71 (0.03 ; 1.38)	**0.05**
CXCL13 (pg/mL), median (Q1;Q3)	83.82 (59.15 ; 142.3)	87.49 (64.77 ; 158.4)	0.14 (-0.51 ; 0.79)	0.68

APRIL: a proliferation-inducing ligand; BAFF: B-cell-activating factor; BCMA: B-cell maturation antigen; CD: cluster of differentiation; CXCL13: C-X-C motif chemokine 13; FDR: false discovery rate; Ig: immunoglobulin; IU: international units; PAH: pulmonary hypertension; Q: quartile; RF: rheumatoid factor; s: soluble; SSc: systemic sclerosis; TACI: transmembrane activator and CAML interactor.

Results are expressed as median (first quartile; third quartile).

Effect sizes were calculated using the Cohen d. Absolute values of 0.20–0.49 represent a small change; values of 0.50–0.79 a medium change; and values of ³ 0.80 a large change. Groups were matched on age (± 5 years) and gender. Given the exploratory nature of this analysis, p-values were not corrected for multiplicity.

Table 6. Associations between serum levels of soluble markers of B cell activation and clinical markers of PH assessed among the 18 SSc-PAH patients from the validation cohort.

	RF (IU/mL)	β2m (mg/L)	IgA (g/L)	IgG (g/L)	IgM (g/L)	BAFF (pg/mL)	APRIL (pg/mL)	sBCMA (pg/mL)	sTACI (pg/mL)	sCD23 (pg/mL)	sCD25 (pg/mL)	sCD27 (pg/mL)	CXCL13 (pg/mL)
Nt-pro-BNP (pg/mL)	p=0.18 ρ=0.34 (-0.18;0.7)	p=0.37 ρ=0.23 (-0.29;0.64)	p=0.35 ρ=-0.24 (-0.64;0.28)	p=0.34 ρ=-0.25 (-0.65;0.27)	p=0.50 ρ=-0.18 (-0.60;0.34)	**p=0.01** ρ=0.62 (0.17;0.84)	p=0.10 ρ=0.41 (-0.10;0.74)	p=0.26 ρ=0.29 (-0.23;0.67)	p=0.56 ρ=0.15 (-0.36;0.59)	p=0.62 ρ=0.13 (-0.38;0.57)	p=0.24 ρ=0.30 (-0.22;0.68)	p=0.82 ρ=0.06 (-0.43;0.52)	p=0.57 ρ=0.15 (-0.36;0.58)
Peak TRV (m/s)	p=0.79 ρ=0.08 (-0.46;0.56)	p=0.34 ρ=0.27 (-0.29;0.68)	p=0.19 ρ=-0.36 (-0.73;0.2)	p=0.24 ρ=-0.32 (-0.71;0.24)	**p=0.02** ρ=-0.59 (-0.84;-0.09)	p=0.09 ρ=0.45 (-0.1;0.77)	p=0.06 ρ=0.50 (-0.04;0.80)	p=0.18 ρ=0.37 (-0.19;0.73)	p=0.06 ρ=0.49 (-0.05;0.79)	p=0.69 ρ=0.11 (-0.43;0.59)	p=0.47 ρ=0.20 (-0.35;0.64)	p=0.77 ρ=-0.08 (-0.57;0.45)	p=0.44 ρ=0.21 (-0.34;0.65)
FVC/DLCO ratio	p=0.65 ρ=-0.12 (-0.56;0.39)	p=0.08 ρ=0.43 (-0.07;0.75)	p=0.68 ρ=0.11 (-0.40;0.56)	p=0.10 ρ=-0.41 (-0.74;0.10)	p=0.53 ρ=-0.16 (-0.59;0.35)	**p=0.01** ρ=0.60 (0.15;0.83)	p=0.73 ρ=0.09 (-0.41;0.54)	p=0.95 ρ=0.02 (-0.47;0.49)	p=0.28 ρ=0.28 (-0.24;0.66)	p=0.83 ρ=0.06 (-0.44;0.52)	p=0.45 ρ=0.20 (-0.32;0.62)	p=0.93 ρ=-0.02 (-0.50;0.46)	p=0.99 ρ=0.004 (-0.48;0.48)

APRIL: a proliferation-inducing ligand; BAFF: B-cell-activating factor; BCMA: B-cell maturation antigen; CD: cluster of differentiation; CXCL13: C-X-C motif chemokine 13; DLCO: diffusing capacity of the lung for carbon monoxide; FVC: forced vital capacity; Ig: immunoglobulin; IU: international unit; Nt-pro-BNP: N-terminal prohormone of brain natriuretic peptide; PH: pulmonary hypertension; PAH: pulmonary arterial hypertension; RF: rheumatoid factor; s: soluble; SSc: systemic sclerosis; TACI: transmembrane activator and CAML interactor; TRV: tricuspid regurgitation velocity; b2m: β2-microglobulin.

All analyses are adjusted for age. Given the exploratory nature of this analysis, p-values were not corrected for multiplicity.

Effect sizes are expressed as the partial Spearman correlation coefficient r (with 95% confidence interval). The usual interpretation is that an absolute value of correlation between 0.00 to 0.19 is very weak; 0.20 to 0.39 weak; 0.40 to 0.59 moderate; 0.60 to 0.79 strong; and greater than 0.80 very strong.

Table 7. Concentrations of angiogenic mediators in B cell culture supernatants from healthy controls, and SSc patients with and without PAH from the validation cohort.

		Healthy controls *vs* SSc patients				SSc-no PAH *vs* SSc-PAH patients			
		HC (N=9)	SSc (N=18)	Effect size	*p*	SSc-no PH (N=9)	SSc-PAH (N=9)	Effect size	*p*
Angiogenin (pg/mL) median (Q1;Q3)	No stim	23.35 (1.28 ; 25.09)	29.01 (24.51 ; 33.30)	1.36 (0.48 ; 2.24)	**0.008**	29.01 (23.40 ; 31.71)	29.01 (25.09 ; 34.86)	0.21 (-0.72 ; 1.13)	0.69
	Stim	25.66 (1.28 ; 29.01)	34.08 (29.55 ; 38.44)	1.49 (0.60 ; 2.38)	**0.004**	31.40 (30.10 ; 36.41)	37.43 (29.55 ; 38.44)	0.29 (-0.64 ; 1.21)	0.57
Angiopoietin 1 (pg/mL) median (Q1;Q3)	No stim	136.3 (39.20 ; 156.9)	167.1 (146.6 ; 197.7)	1.15 (0.29 ; 2.00)	**0.02**	172.3 (167.1 ; 197.7)	156.9 (146.6 ; 187.6)	-0.33 (-1.26 ; 0.60)	0.51
	Stim	105.2 (54.68 ; 136.3)	149.2 (146.6 ; 212.9)	1.00 (0.16 ; 1.84)	**0.03**	146.6 (146.6 ; 212.9)	156.9 (136.3 ; 187.6)	-0.06 (-0.99 ; 0.86)	0.93
Angiopoietin 2 (pg/mL) median (Q1;Q3)	No stim	599.0 (589.4 ; 612.6)	606.5 (592.9 ; 620.4)	0.05 (-0.75 ; 0.85)	0.92	608.2 (592.9 ; 620.4)	604.9 (595.9 ; 614.3)	-0.08 (-1.01 ; 0.84)	0.89
	Stim	589.4 (577.5 ; 604.9)	565.2 (552.9 ; 595.9)	-0.92 (-1.76 ; -0.08)	**0.05**	565.2 (559.1 ; 612.6)	565.2 (552.9 ; 583.6)	-0.37 (-1.30 ; 0.56)	0.45
ANGPTL-6 (pg/mL) median (Q1;Q3)	No stim	106.5 (106.5 ; 136.4)	110.8 (89.03 ; 192.8)	0.03 (-0.77 ; 0.83)	0.96	106.5 (89.03 ; 175.6)	115.2 (106.5 ; 192.8)	0.18 (-0.74 ; 1.11)	0.72
	Stim	227.1 (192.8 ; 388.9)	218.6 (201.4 ; 261.3)	-0.2 (-1.00 ; 0.6)	0.62	227.1 (201.4 ; 227.1)	210.0 (210.0 ; 261.3)	-0.08 (-1.01 ; 0.84)	0.89
VEGFR-1 (pg/mL) median (Q1;Q3)	No stim	6.16 (0.35 ; 6.16)	6.16 (6.16 ; 6.16)	0.46 (-0.35 ; 1.27)	0.26	6.16 (6.16 ; 6.16)	6.16 (6.16 ; 6.16)	0.00 (-0.92 ; 0.92)	1.00
	Stim	24.29 (15.40 ; 66.24)	94.49 (39.36 ; 138.4)	0.90 (0.07 ; 1.74)	**0.04**	93.28 (39.36 ; 138.4)	95.70 (51.02 ; 138.4)	0.08 (-0.84 ; 1.01)	0.89
PDGF-AA (pg/mL) median (Q1;Q3)	No stim	1.21 (0.42 ; 1.99)	2.71 (1.21 ; 5.87)	0.95 (0.11 ; 1.79)	**0.04**	2.71 (1.22 ; 3.78)	1.79 (1.21 ; 5.87)	-0.10 (-1.03 ; 0.82)	0.86
	Stim	56.54 (33.29 ; 62.46)	73.97 (47.33 ; 96.53)	0.77 (-0.06 ; 1.59)	0.09	74.88 (63.92 ; 97.90)	57.15 (47.33 ; 83.17)	-0.31 (-1.24 ; 0.62)	0.54
L-selectin (pg/mL) median (Q1;Q3)	No stim	1988 (1042 ; 1988)	1988 (1988 ; 3329)	0.46 (-0.35 ; 1.27)	0.27	1988 (1988 ; 3329)	1988 (1988 ; 2939)	-0.17 (-1.09 ; 0.76)	0.75
	Stim	2939 (1988 ; 2961)	3539 (2939 ; 3684)	1.34 (0.47 ; 2.22)	**0.007**	3329 (2939 ; 3684)	3684 (3184 ; 3684)	0.19 (-0.74 ; 1.12)	0.71
MMP-8 (pg/mL) median (Q1;Q3)	No stim	260.4 (92.43 ; 274.9)	274.9 (195.7 ; 328.4)	0.68 (-0.14 ; 1.50)	0.12	274.9 (274.9 ; 328.4)	213.1 (195.7 ; 288.9)	-0.27 (-1.19 ; 0.66)	0.59
	Stim	177.2 (92.43 ; 229.6)	252.9 (177.2 ; 274.9)	1.00 (0.16 ; 1.84)	**0.04**	260.4 (245.3 ; 274.9)	229.6 (177.2 ; 274.9)	-0.06 (-0.99 ; 0.86)	0.93
MMP-9 (pg/mL) median (Q1;Q3)	No stim	111.4 (77.59 ; 198.1)	56.22 (39.83 ; 164.7)	-0.30 (-1.11 ; 0.50)	0.49	53.64 (39.83 ; 164.7)	58.80 (46.75 ; 161.7)	0.16 (-0.76 ; 1.09)	0.76
	Stim	260.7 (198.1 ; 293.9)	345.4 (118.2 ; 618.1)	0.30 (-0.51 ; 1.10)	0.50	406.5 (323.4 ; 513.5)	163.4 (118.2 ; 618.1)	-0.14 (-1.07 ; 0.78)	0.79
TIMP-1 (pg/mL) median (Q1;Q3)	No stim	19.28 (19.28 ; 26.57)	62.09 (19.28 ; 135.4)	0.94 (0.10 ; 1.78)	**0.05**	52.86 (26.57 ; 95.98)	81.82 (19.28 ; 135.4)	0.02 (-0.90 ; 0.94)	1.00
	Stim	135.4 (128.2 ; 237.5)	251.1 (194.9 ; 376.7)	0.99 (0.14 ; 1.83)	**0.03**	219.4 (206.0 ; 339.9)	259.6 (145.2 ; 376.7)	0.02 (-0.90 ; 0.94)	1.00

ANGPTL-6: angiopoietin-like protein 6; HC: healthy control; MMP: matrix metallopeptidase; PAH: pulmonary arterial hypertension; PDGF-AA: platelet derived growth factor AA; SSc: systemic sclerosis; stim: stimulation; TIMP-1: tissue inhibitor of metalloproteinases; VEGFR-1: vascular endothelial growth factor receptor 1.

Results are pooled from 2 independent experiments and expressed as median (first quartile; third quartile).

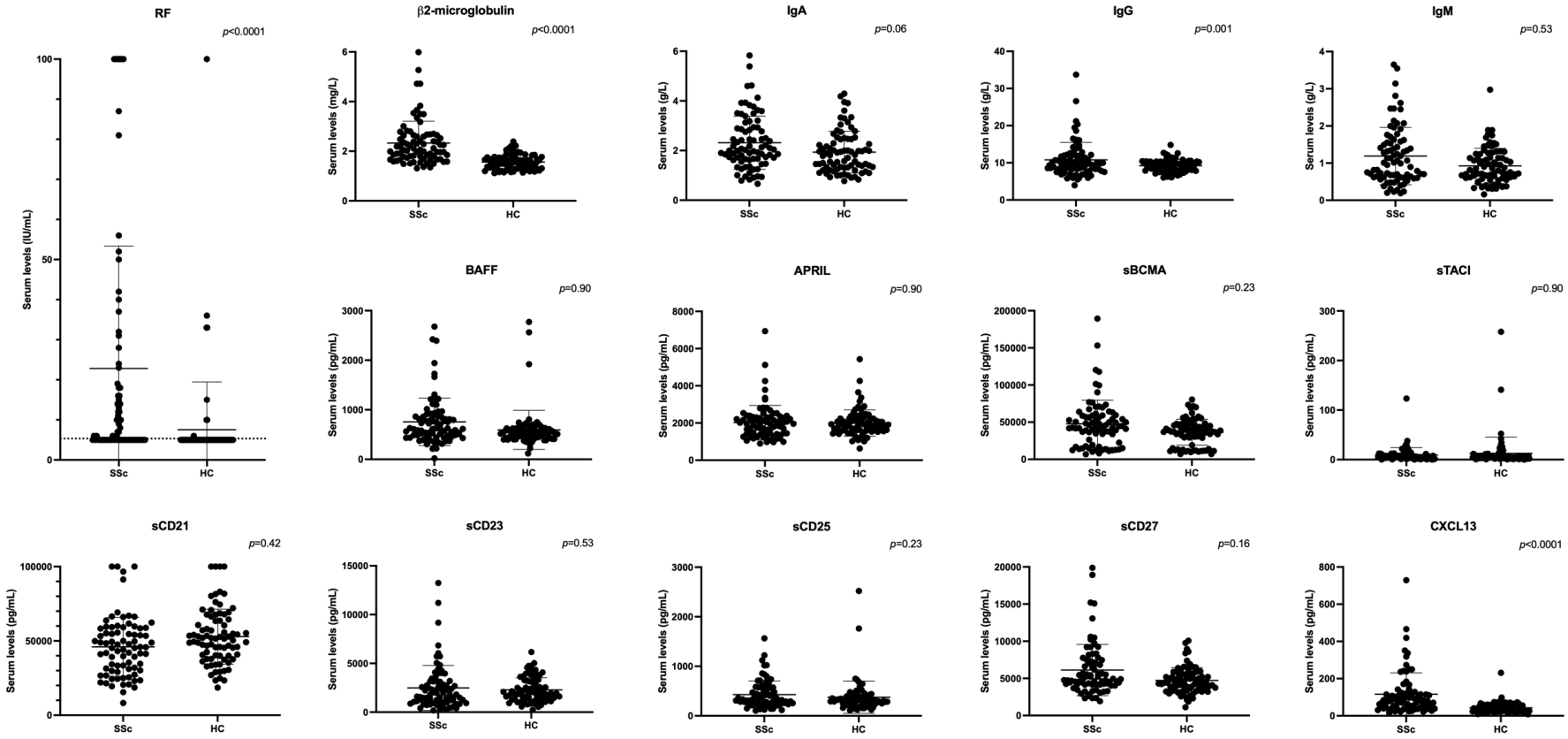
Effect sizes were calculated using the Cohen d. Absolute values of 0.20–0.49 represent a small change; values of 0.50–0.79 a medium change; and values of ³ 0.80 a large change. Given the exploratory nature of this analysis, p-values were not corrected for multiplicity.

Table 8. Synthetic view of the associations identified between soluble markers of B cell activation and clinical markers of PH in the discovery and validation cohorts.

Discovery cohort		Validation cohort	
PH *vs* no PH	**IgG** **RF**	PAH *vs* no PAH	**IgG** **β2-microglobulin** **sBCMA** **sCD23** **sCD27**
Nt-pro-BNP	**β2-microglobulin** sBCMA	Nt-pro-BNP	**BAFF**
Peak TRV	**BAFF** **RF**	Peak TRV	BAFF **IgM**
DLCO	**β2-microglobulin** BAFF sCD23 **RF**	FVC/DLCO	β2-microglobulin **BAFF** IgG

BAFF: B-cell-activating factor; BCMA: B-cell maturation antigen; CD: cluster of differentiation; DLCO: diffusing capacity of the lung for carbon monoxide; Nt-pro-BNP: N-terminal prohormone of brain natriuretic peptide; PAH: pulmonary arterial hypertension; PH: pulmonary hypertension; RF: rheumatoid factor; s: soluble; TRV: tricuspid regurgitation velocity.
Bold characters denote statistical significance (p< 0.05). Regular characters denote trends for statistical significance (p< 0.10).

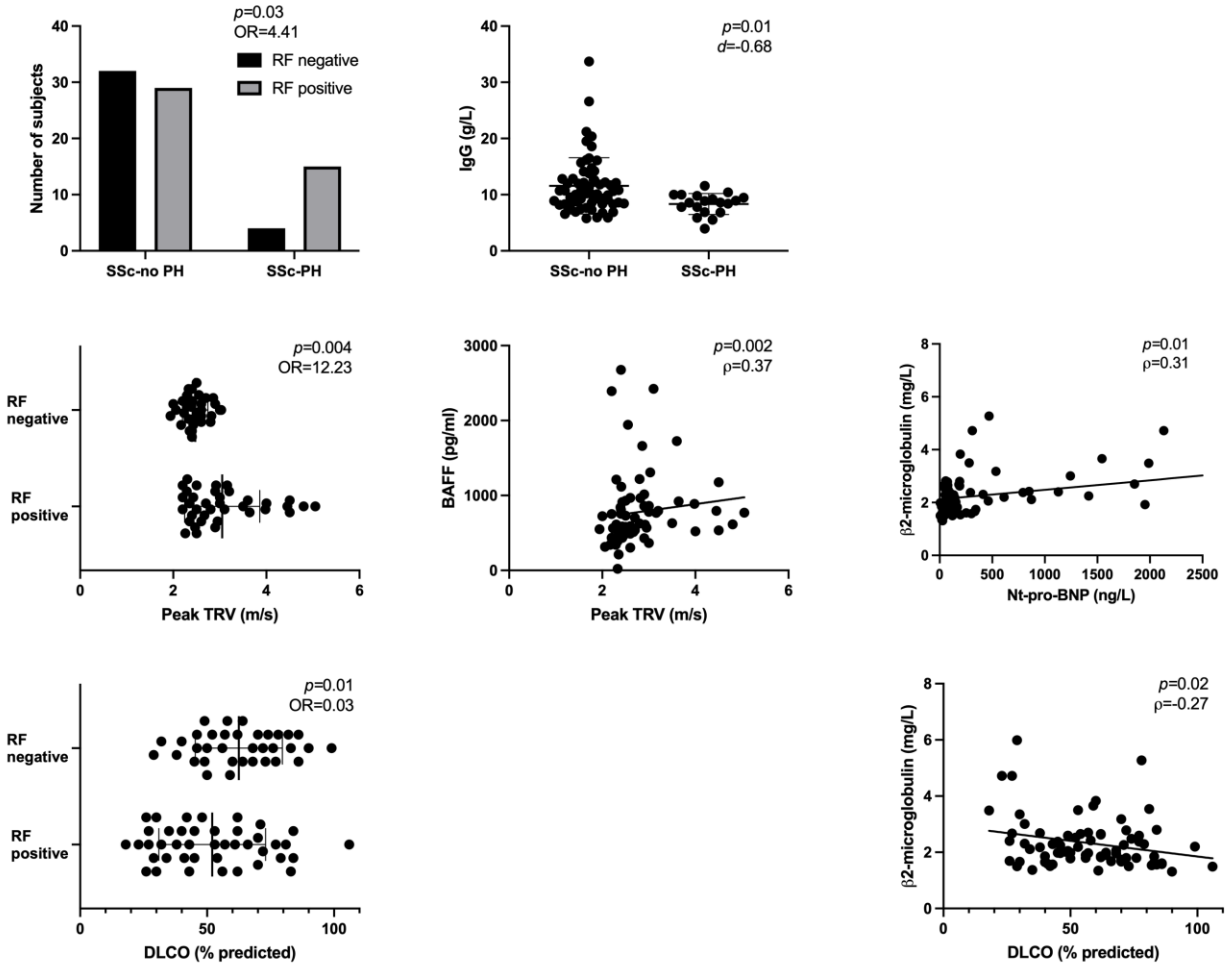
Figure 1. Serum levels of soluble markers of B cell activation in SSc patients and healthy controls (discovery cohort).



APRIL: a proliferation-inducing ligand; BAFF: B-cell-activating factor; BCMA: B-cell maturation antigen; CD: cluster of differentiation; CXCL13: C-X-C motif chemokine 13; HC: healthy controls; Ig: immunoglobulin; RF: rheumatoid factor; s: soluble; SSc: systemic sclerosis; TACI: transmembrane activator and CAML interactor.

The dotted line on the RF panel represents the threshold for RF positivity. Error bars display means and standard deviations. p-values are adjusted for age, gender and multiplicity. The p-value displayed on the RF panel refers to the analysis using RF as a binary variable.

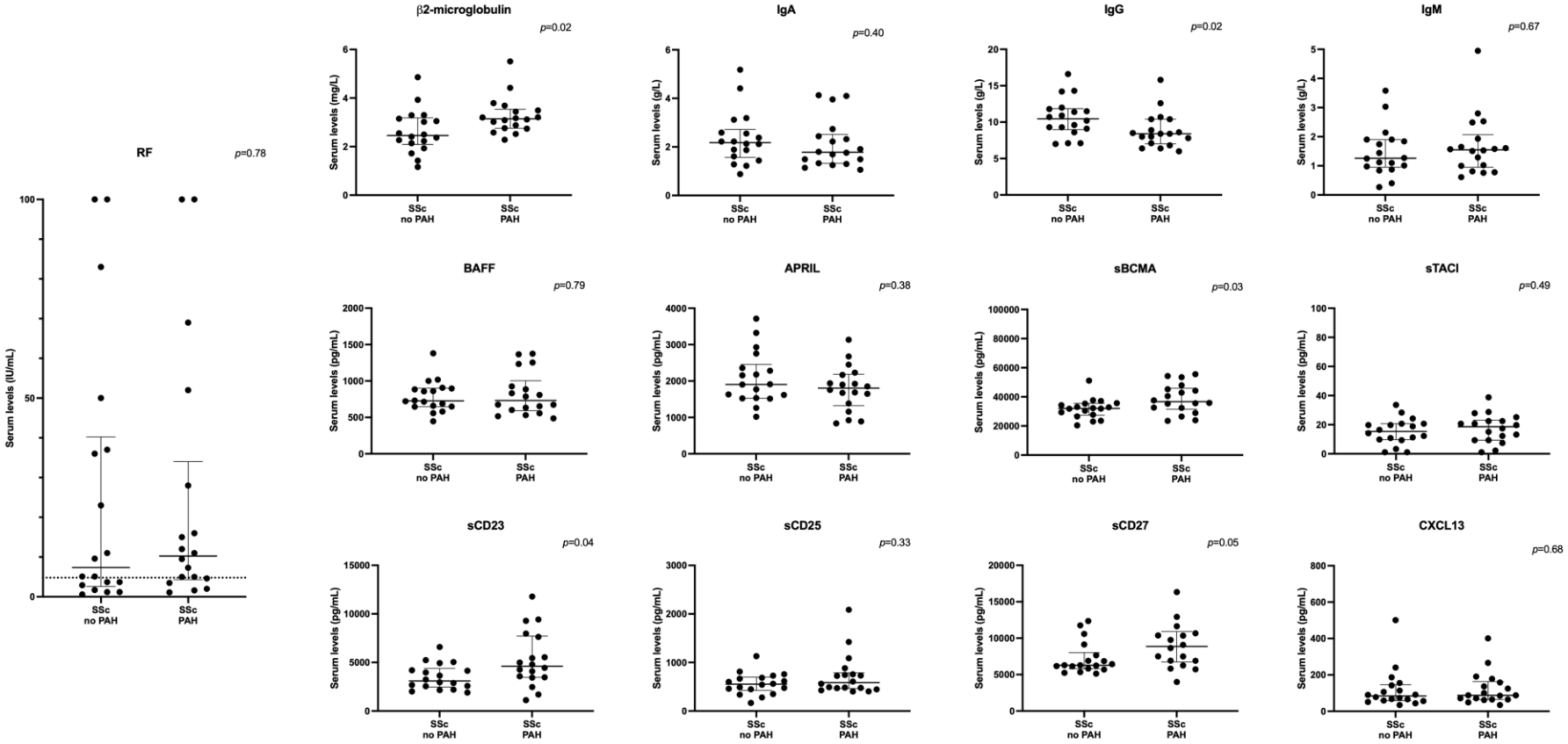
Figure 2. Significant associations between serum levels of soluble markers of B cell activation and SSc characteristics related to PH, assessed among the 80 SSc patients from the discovery cohort.



BAFF: B-cell-activating factor; DLCO: diffusing capacity of the lung for carbon monoxide; Ig: immunoglobulin; Nt-pro-BNP: N-terminal prohormone of brain natriuretic peptide; OR: odds ratio; PH: pulmonary hypertension; RF: rheumatoid factor; SSc: systemic sclerosis; TRV: tricuspid regurgitation velocity.

Error bars display means and standard deviations. p-values are adjusted for age, gender and immunosuppressive drugs.

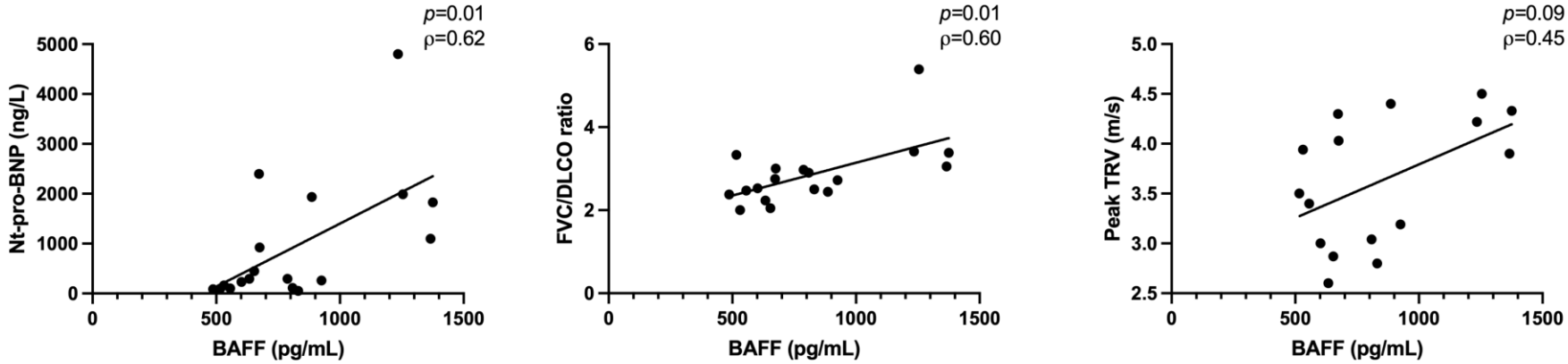
Figure 3. Serum levels of soluble markers of B cell activation in SSc patients with and without PAH (validation cohort).



APRIL: a proliferation-inducing ligand; BAFF: B-cell-activating factor; BCMA: B-cell maturation antigen; CD: cluster of differentiation; CXCL13: C-X-C motif chemokine 13; HC: healthy controls; Ig: immunoglobulin; PAH: pulmonary arterial hypertension; RF: rheumatoid factor; s: soluble; SSc: systemic sclerosis; TACI: transmembrane activator and CAML interactor.

The dotted line on the RF panel represents the threshold for RF positivity. Error bars display means and standard deviations.

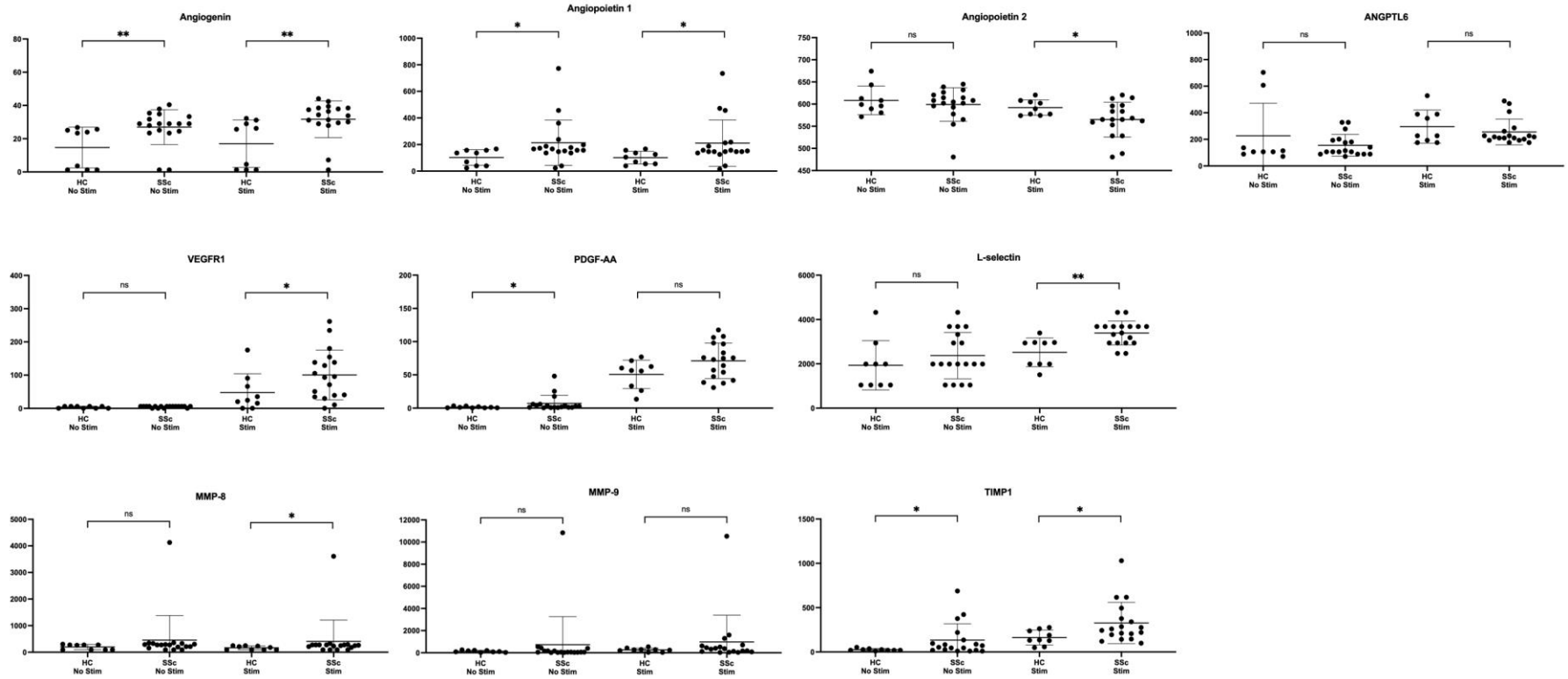
Figure 4. Associations between BAFF and clinical markers of PH, assessed among the 18 SSc-PAH patients from the validation cohort.



BAFF: B-cell-activating factor; DLCO: diffusing capacity of the lung for carbon monoxide; FVC: forced vital capacity; Nt-pro-BNP: N-terminal prohormone of brain natriuretic peptide; PAH: pulmonary arterial hypertension; SSc: systemic sclerosis; TRV: tricuspid regurgitation velocity.

Error bars display means and standard deviations. p-values are adjusted for age.

Figure 5. Concentrations of angiogenic mediators in B cell culture supernatants from SSc patients and healthy controls.



ANGPTL-6: angiopoietin-like protein 6; HC: healthy control; MMP: matrix metallopeptidase; PDGF-AA: platelet derived growth factor AA; SSc: systemic sclerosis; stim: stimulation; TIMP-1: tissue inhibitor of metalloproteinases; VEGFR-1: vascular endothelial growth factor receptor 1.

*Results are pooled from 2 independent experiments. Error bars display means and standard deviations. Levels of significance are pictured as follows: *** for p≤0.001, ** for p≤0.01, * for p≤0.05, ns for p>0.05. Given the exploratory nature of this analysis, p-values were not corrected for multiplicity.*

REFERENCES

1. Pokeerbux MR, Giovannelli J, Dauchet L, Mouthon L, Agard C, Lega JC, Allanore Y, Jego P, Bienvenu B, Berthier S, et al. Survival and prognosis factors in systemic sclerosis: data of a French multicenter cohort, systematic review, and meta-analysis of the literature. *Arthritis Res Ther* (2019) 21:86. doi: 10.1186/s13075-019-1867-1
2. Hachulla E, Launay D. Diagnosis and classification of systemic sclerosis. *Clin Rev Allergy Immunol* (2011) 40:78–83. doi: 10.1007/s12016-010-8198-y
3. Morell-Dubois S, Condette-Wojtasik G, Clerson P, Berezné A, Launay D, Lambert M, Maillard-Lefebvre H, Hatron P-Y, Hachulla E. [Complaints, needs of patients with systemic sclerosis: a better understanding for a better care]. *Rev Médecine Interne* (2011) 32:537–543. doi: 10.1016/j.revmed.2011.02.004
4. Lefèvre G, Dauchet L, Hachulla E, Montani D, Sobanski V, Lambert M, Hatron P-Y, Humbert M, Launay D. Survival and Prognostic Factors in Systemic Sclerosis-Associated Pulmonary Hypertension: A Systematic Review and Meta-Analysis: Survival and Prognosis in SSc-Associated Pulmonary Hypertension. *Arthritis Rheum* (2013) 65:2412–2423. doi: 10.1002/art.38029
5. Launay D, Humbert M, Berezne A, Cottin V, Allanore Y, Couderc L-J, Bletry O, Yaici A, Hatron P-Y, Mouthon L, et al. Clinical characteristics and survival in systemic sclerosis-related pulmonary hypertension associated with interstitial lung disease. *Chest* (2011) 140:1016–1024. doi: 10.1378/chest.10-2473
6. Dumoitier N, Lofek S, Mouthon L. Pathophysiology of systemic sclerosis: State of the art in 2014. *Presse Médicale* (2014) 43:e267–e278. doi: 10.1016/j.lpm.2014.08.001
7. Sanges S, Guerrier T, Launay D, Lefèvre G, Labalette M, Forestier A, Sobanski V, Corli J, Hauspie C, Jendoubi M, et al. Role of B cells in the pathogenesis of systemic sclerosis. *Rev Médecine Interne* (2017) 38:113–124. doi: 10.1016/j.revmed.2016.02.016
8. Kill A, Riemekasten G. Functional Autoantibodies in Systemic Sclerosis Pathogenesis. *Curr Rheumatol Rep* (2015) 17: doi: 10.1007/s11926-015-0505-4
9. Sanges S, Jendoubi M, Kavian N, Hauspie C, Speca S, Crave J-C, Guerrier T, Lefèvre G, Sobanski V, Savina A, et al. B Cell Homeostasis and Functional Properties Are Altered in an Hypochlorous Acid-Induced Murine Model of Systemic Sclerosis. *Front Immunol* (2017) 8: doi: 10.3389/fimmu.2017.00053
10. Forestier A, Guerrier T, Jouvray M, Giovannelli J, Lefèvre G, Sobanski V, Hauspie C, Hachulla E, Hatron P-Y, Zéphir H, et al. Altered B lymphocyte homeostasis and functions in systemic sclerosis. *Autoimmun Rev* (2018) 17:244–255. doi: 10.1016/j.autrev.2017.10.015
11. Du AX, Gniadecki R, Osman M. Biomarkers of B cell activation in autoimmune connective tissue diseases: More than markers of disease activity. *Clin Biochem* (2022) 100:1–12. doi: 10.1016/j.clinbiochem.2021.11.009
12. Salazar-Camarena DC, Ortiz-Lazareno PC, Cruz A, Oregon-Romero E, Machado-Contreras JR, Muñoz-Valle JF, Orozco-López M, Marín-Rosales M, Palafox-Sánchez CA. Association of BAFF, APRIL serum levels, BAFF-R, TACI and BCMA expression on peripheral B-cell subsets with clinical manifestations in systemic lupus erythematosus. *Lupus* (2016) 25:582–592. doi: 10.1177/0961203315608254
13. Laurent SA, Hoffmann FS, Kuhn P-H, Cheng Q, Chu Y, Schmidt-Supprian M, Hauck SM, Schuh E, Krumbholz M, Rübsamen H, et al. γ-Secretase directly sheds the survival receptor BCMA from plasma cells. *Nat Commun* (2015) 6:7333. doi: 10.1038/ncomms8333
14. Hoffmann FS, Kuhn P-H, Laurent SA, Hauck SM, Berer K, Wendlinger SA, Krumbholz M, Khademi M, Olsson T, Dreyling M, et al. The Immunoregulator Soluble TACI Is Released by ADAM10 and Reflects B Cell Activation in Autoimmunity. *J Immunol* (2015) 194:542–552. doi: 10.4049/jimmunol.1402070
15. Hegazy M, Darwish H, Darweesh H, El-Shehaby A, Emad Y. Raised serum level of APRIL in patients with systemic lupus erythematosus: correlations with disease activity indices. *Clin Immunol Orlando Fla* (2010) 135:118–124. doi: 10.1016/j.clim.2009.12.012
16. Morel J, Roubille C, Planelles L, Rocha C, Fernandez L, Lukas C, Hahne M, Combe B. Serum levels of tumour necrosis factor family members a proliferation-inducing ligand (APRIL) and B lymphocyte stimulator (BLyS) are inversely correlated in systemic lupus erythematosus. *Ann Rheum Dis* (2009) 68:997–1002. doi: 10.1136/ard.2008.090928
17. Koyama T, Tsukamoto H, Miyagi Y, Himeji D, Otsuka J, Miyagawa H, Harada M, Horiuchi T. Raised serum APRIL levels in patients with systemic lupus erythematosus. *Ann Rheum Dis* (2005) 64:1065–1067. doi: 10.1136/ard.2004.022491
18. Treamtrakanpon W, Tantivitayakul P, Benjachat T, Somparn P, Kittikowit W, Eiam-ong S, Leelahavanichkul A, Hirankarn N, Avihingsanon Y. APRIL, a proliferation-inducing ligand, as a potential marker of lupus nephritis. *Arthritis Res Ther* (2012) 14:R252. doi: 10.1186/ar4095
19. Breen EC, Hussain SK, Magpantay L, Jacobson LP, Detels R, Rabkin CS, Kaslow RA, Variakojis D, Bream JH, Rinaldo CR, et al. B-Cell Stimulatory Cytokines and Markers of Immune Activation Are Elevated Several Years

Prior to the Diagnosis of Systemic AIDS–Associated Non-Hodgkin B-Cell Lymphoma. *Cancer Epidemiol Biomarkers Prev* (2011) 20:1303–1314. doi: 10.1158/1055-9965.EPI-11-0037
20. De Roos AJ, Mirick DK, Edlefsen KL, LaCroix AZ, Kopecky KJ, Madeleine MM, Magpantay L, Martínez-Maza O. Markers of B-cell activation in relation to risk of non-Hodgkin lymphoma. *Cancer Res* (2012) 72:4733–4743. doi: 10.1158/0008-5472.CAN-12-1639
21. Monach PA, Warner RL, Tomasson G, Specks U, Stone JH, Ding L, Fervenza FC, Fessler BJ, Hoffman GS, Iklé D, et al. Serum proteins reflecting inflammation, injury and repair as biomarkers of disease activity in ANCA-associated vasculitis. *Ann Rheum Dis* (2013) 72:1342–1350. doi: 10.1136/annrheumdis-2012-201981
22. Land J, Abdulahad WH, Sanders J-SF, Stegeman CA, Heeringa P, Rutgers A. Regulatory and effector B cell cytokine production in patients with relapsing granulomatosis with polyangiitis. *Arthritis Res Ther* (2016) 18:84. doi: 10.1186/s13075-016-0978-1
23. Nocturne G, Seror R, Fogel O, Belkhir R, Boudaoud S, Saraux A, Larroche C, Le Guern V, Gottenberg JE, Mariette X. CXCL13 and CCL11 Serum Levels and Lymphoma and Disease Activity in Primary Sjögren's Syndrome. *Arthritis Rheumatol Hoboken NJ* (2015) 67:3226–3233. doi: 10.1002/art.39315
24. Jones JD, Hamilton BJ, Challener GJ, de Brum-Fernandes AJ, Cossette P, Liang P, Masetto A, Ménard HA, Carrier N, Boyle DL, et al. Serum C-X-C motif chemokine 13 is elevated in early and established rheumatoid arthritis and correlates with rheumatoid factor levels. *Arthritis Res Ther* (2014) 16:R103. doi: 10.1186/ar4552
25. Hafez SS, Saad WES, Shedid NH. B-cell-attracting chemokine-1 (BCA-1/CXCL13) in systemic lupus erythematosus, its correlation to disease activity and renal involvement. *Egypt J Immunol* (2014) 21:23–32.
26. Greisen SR, Schelde KK, Rasmussen TK, Kragstrup TW, Stengaard-Pedersen K, Hetland ML, Hørslev-Petersen K, Junker P, Østergaard M, Deleuran B, et al. CXCL13 predicts disease activity in early rheumatoid arthritis and could be an indicator of the therapeutic "window of opportunity." *Arthritis Res Ther* (2014) 16:434. doi: 10.1186/s13075-014-0434-z
27. Lanteri A, Sobanski V, Langlois C, Lefèvre G, Hauspie C, Sanges S, Lambert M, Morell-Dubois S, Hatron P-Y, Hachulla E, et al. Serum free light chains of immunoglobulins as biomarkers for systemic sclerosis characteristics, activity and severity. *Autoimmun Rev* (2014) 13:974–980. doi: 10.1016/j.autrev.2014.07.003
28. Bassyouni IH, Azab NA, El-Dakrony E-HM, Fawzi MMT, Ghanoum R, Bassyouni RH. Elevated serum levels of a proliferation-inducing ligand in patients with systemic sclerosis: Possible association with myositis? *Joint Bone Spine* (2011) 78:56–61. doi: 10.1016/j.jbspin.2010.05.004
29. Matsushita T, Fujimoto M, Hasegawa M, Tanaka C, Kumada S, Ogawa F, Takehara K, Sato S. Elevated serum APRIL levels in patients with systemic sclerosis: distinct profiles of systemic sclerosis categorized by APRIL and BAFF. *J Rheumatol* (2007) 34:2056–2062.
30. Pagano L, Paoletti S, Afa G, Marra R, Garcovich A, Bizzi B. Serum beta 2-microglobulin in systemic sclerosis. *Clin Rheumatol* (1985) 4:286–289. doi: 10.1007/BF02031609
31. Minh VN, Hau KT, Takashi M, Ha VN, Bao LH, Huyen ML, Huu DL, Van TN, Gandolfi M, Satolli F, et al. Efficacy of BAFF in Monitoring Treatment Response in Early Vietnamese Systemic Sclerosis Patients. *Open Access Maced J Med Sci* (2019) 7:264–268. doi: 10.3889/oamjms.2019.070
32. Bosello S, Basile U, De Lorenzis E, Gulli F, Canestrari G, Napodano C, Parisi F, Pocino K, Di Mario C, Tolusso B, et al. Free light chains of immunoglobulins in patients with systemic sclerosis: correlations with lung involvement and inflammatory milieu. *J Clin Pathol* (2018) 71:620–625. doi: 10.1136/jclinpath-2017-204656
33. Wutte N, Kovacs G, Berghold A, Reiter H, Aberer W, Aberer E. CXCL13 and B-cell activating factor as putative biomarkers in systemic sclerosis. *Br J Dermatol* (2013) 169:723–725. doi: 10.1111/bjd.12411
34. Abdo MS, Mohammed RHA, Raslan HM, Gaber SM. Serum B-cell activating factor assessment in a population of Egyptian patients with systemic sclerosis. *Int J Rheum Dis* (2013) 16:148–156. doi: 10.1111/1756-185x.12019
35. Fawzy SM, Gheita TA, El-Nabarawy E, El-Demellawy HH, Shaker OG. Serum BAFF level and its correlations with various disease parameters in patients with systemic sclerosis and systemic lupus erythematosus. *Egypt Rheumatol* (2011) 33:45–51. doi: 10.1016/j.ejr.2010.12.001
36. Matsushita T, Hasegawa M, Yanaba K, Kodera M, Takehara K, Sato S. Elevated serum BAFF levels in patients with systemic sclerosis: Enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum* (2006) 54:192–201. doi: 10.1002/art.21526
37. Taniguchi T, Miyagawa T, Toyama S, Yamashita T, Nakamura K, Saigusa R, Ichimura Y, Takahashi T, Toyama T, Yoshizaki A, et al. CXCL13 produced by macrophages due to Fli1 deficiency may contribute to the development of tissue fibrosis, vasculopathy and immune activation in systemic sclerosis. *Exp Dermatol* (2018) 27:1030–1037. doi: 10.1111/exd.13724
38. Zhang H, Li P, Wu D, Xu D, Hou Y, Wang Q, Li M, Li Y, Zeng X, Zhang F, et al. Serum IgG Subclasses in Autoimmune Diseases. *Medicine (Baltimore)* (2015) 94:e387. doi: 10.1097/MD.0000000000000387

39. Wielosz E, Majdan M, Dryglewska M, Zwolak R. Anti-CCP antibodies and rheumatoid factor in systemic sclerosis:Prevalence and relationships with joint manifestations. *Adv Clin Exp Med* (2018) 27:1253–1257. doi: 10.17219/acem/69921
40. Horimoto AMC, Costa IP da. Sobreposição de esclerose sistêmica e artrite reumatoide: uma entidade clínica distinta? *Rev Bras Reumatol* (2016) 56:287–298. doi: 10.1016/j.rbr.2014.12.011
41. Avouac J, Clements PJ, Khanna D, Furst DE, Allanore Y. Articular involvement in systemic sclerosis. *Rheumatology* (2012) 51:1347–1356. doi: 10.1093/rheumatology/kes041
42. Arslan Tas D, Erken E, Sakalli H, Yucel AE. Evaluating hand in systemic sclerosis. *Rheumatol Int* (2012) 32:3581–3586. doi: 10.1007/s00296-011-2205-3
43. Ueda-Hayakawa I, Hasegawa M, Kumada S, Tanaka C, Komura K, Hamaguchi Y, Takehara K, Fujimoto M. Usefulness of anti-cyclic citrullinated peptide antibody and rheumatoid factor to detect rheumatoid arthritis in patients with systemic sclerosis. *Rheumatology* (2010) 49:2135–2139. doi: 10.1093/rheumatology/keq205
44. Ingegnoli F, Galbiati V, Zeni S, Meani L, Zahalkova L, Lubatti C, Soldi A, Paresce E, Murgo A, Crapanzano C, et al. Use of antibodies recognizing cyclic citrullinated peptide in the differential diagnosis of joint involvement in systemic sclerosis. *Clin Rheumatol* (2007) 26:510–514. doi: 10.1007/s10067-006-0313-3
45. Allali F, Tahiri L, Senjari A, Abouqal R, Hajjaj-Hassouni N. Erosive Arthropathy in systemic sclerosis. *BMC Public Health* (2007) 7:260. doi: 10.1186/1471-2458-7-260
46. Tomita M, Kadono T, Yazawa N, Kawashima T, Tamaki Z, Ashida R, Ohmatsu H, Asano Y, Sugaya M, Kubo M, et al. Serum levels of soluble CD21 in patients with systemic sclerosis. *Rheumatol Int* (2012) 32:317–321. doi: 10.1007/s00296-010-1610-3
47. Yoshikawa T, Nanba T, Kato H, Hori K, Inamoto T, Kumagai S, Yodoi J. Soluble Fc epsilon RII/CD23 in patients with autoimmune diseases and Epstein-Barr virus-related disorders: analysis by ELISA for soluble Fc epsilon RII/CD23. *ImmunoMethods* (1994) 4:65–71.
48. Bocchino M, Bruzzese D, D'Alto M, Argiento P, Borgia A, Capaccio A, Romeo E, Russo B, Sanduzzi A, Valente T, et al. Performance of a new quantitative computed tomography index for interstitial lung disease assessment in systemic sclerosis. *Sci Rep* (2019) 9:9468. doi: 10.1038/s41598-019-45990-7
49. Valentini G, Vettori S, Cuomo G, Iudici M, D'Abrosca V, Capocotta D, Del Genio G, Santoriello C, Cozzolino D. Early systemic sclerosis: short-term disease evolution and factors predicting the development of new manifestations of organ involvement. *Arthritis Res Ther* (2012) 14:R188. doi: 10.1186/ar4019
50. Bečvář R, Štork J, Pešáková V, Stáňová A, Hulejová H, Rysová L, Zatloukalová A, Zatloukal P, Jáchymová M, Pourová L. Clinical Correlations of Potential Activity Markers in Systemic Sclerosis. *Ann N Y Acad Sci* (2005) 1051:404–412. doi: 10.1196/annals.1361.082
51. Dziankowska-Bartkowiak B, Zalewska A, Sysa-Jedrzejowska A. Duration of Raynaud's phenomenon is negatively correlated with serum levels of interleukin 10 (IL-10), soluble receptor of interleukin 2 (sIL2R), and sFas in systemic sclerosis patients. *Med Sci Monit Int Med J Exp Clin Res* (2004) 10:CR202-208.
52. Lis AD, Brzezińska-Wcisło LA. [Soluble receptors of cytokines in sera of patients with systemic sclerosis--clinical correlation]. *Wiadomosci Lek Wars Pol 1960* (2003) 56:532–536.
53. Lee YJ, Shin KC, Kang SW, Lee EB, Kim HA, Song YW. Type III procollagen N-terminal propeptide, soluble interleukin-2 receptor, and von Willebrand factor in systemic sclerosis. *Clin Exp Rheumatol* (2001) 19:69–74.
54. Gardinali M, Pozzi MR, Bernareggi M, Montani N, Allevi E, Catena L, Cugno M, Bottasso B, Stabilini R. Treatment of Raynaud's phenomenon with intravenous prostaglandin E1alpha-cyclodextrin improves endothelial cell injury in systemic sclerosis. *J Rheumatol* (2001) 28:786–794.
55. Søndergaard K, Stengaard-Pedersen K, Zachariae H, Heickendorff L, Deleuran M, Deleuran B. Soluble intercellular adhesion molecule-1 (sICAM-1) and soluble interleukin-2 receptors (sIL-2R) in scleroderma skin. *Br J Rheumatol* (1998) 37:304–310.
56. Steen VD, Engel EE, Charley MR, Medsger TA. Soluble serum interleukin 2 receptors in patients with systemic sclerosis. *J Rheumatol* (1996) 23:646–649.
57. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, Matucci-Cerinic M, Naden RP, Medsger TA, Carreira PE, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis* (2013) 72:1747–1755. doi: 10.1136/annrheumdis-2013-204424
58. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA, Rowell N, Wollheim F. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* (1988) 15:202–205.
59. Goh NSL, Desai SR, Veeraraghavan S, Hansell DM, Copley SJ, Maher TM, Corte TJ, Sander CR, Ratoff J, Devaraj A, et al. Interstitial lung disease in systemic sclerosis: a simple staging system. *Am J Respir Crit Care Med* (2008) 177:1248–1254. doi: 10.1164/rccm.200706-877OC

60. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, Williams PG, Souza R. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* (2019) 53:1801913. doi: 10.1183/13993003.01913-2018
61. Valentini G, Rossa AD, Bombardieri S, Bencivelli W, Silman AJ, D'Angelo S, Cerinic MM, Belch JF, Black CM, Bruhlmann P, et al. European multicentre study to define disease activity criteria for systemic sclerosis. II. Identification of disease activity variables and development of preliminary activity indexes. *Ann Rheum Dis* (2001) 60:592–598. doi: 10.1136/ard.60.6.592
62. Medsger TA, Bombardieri S, Czirjak L, Scorza R, Rossa AD, Bencivelli W. Assessment of disease severity and prognosis in systemic sclerosis. *Clin Exp Rheumatol* (2003) 21:S42–S46.
63. Georges C, Chassany O, Mouthon L, Tiev K, Toledano C, Meyer O, Marjanovic Z, Heneggar C, Papo T, Crickx B, et al. Validation of French version of the Scleroderma Health Assessment Questionnaire (SSc HAQ). *Clin Rheumatol* (2005) 24:3–10. doi: 10.1007/s10067-004-0942-3
64. Cohen J. Eta-Squared and Partial Eta-Squared in Fixed Factor Anova Designs. *Educ Psychol Meas* (1973) 33:107–112. doi: 10.1177/001316447303300111
65. Scherlinger M, Lutz J, Galli G, Richez C, Gottenberg J-E, Sibilia J, Arnaud L, Blanco P, Schaeverbeke T, Chatelus E, et al. Systemic sclerosis overlap and non-overlap syndromes share clinical characteristics but differ in prognosis and treatments. *Semin Arthritis Rheum* (2021) 51:36–42. doi: 10.1016/j.semarthrit.2020.10.009
66. Wielosz E, Majdan M, Dryglewska M, Targońska-Stępniak B. Overlap syndromes in systemic sclerosis. *Adv Dermatol Allergol* (2018) 35:246–250. doi: 10.5114/ada.2018.72662
67. Moinzadeh P, Aberer E, Ahmadi-Simab K, Blank N, Distler JHW, Fierlbeck G, Genth E, Guenther C, Hein R, Henes J, et al. Disease progression in systemic sclerosis-overlap syndrome is significantly different from limited and diffuse cutaneous systemic sclerosis. *Ann Rheum Dis* (2015) 74:730–737. doi: 10.1136/annrheumdis-2013-204487
68. Balbir-Gurman A, Braun-Moscovici Y. Scleroderma Overlap Syndrome. (2011) 13:7.
69. Matsushita T, Hasegawa M, Yanaba K, Kodera M, Takehara K, Sato S. Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum* (2006) 54:192–201. doi: 10.1002/art.21526
70. Hebbar M, Lassalle P, Delneste Y, Hatron PY, Devulder B, Tonnel AB, Janin A. Assessment of anti-endothelial cell antibodies in systemic sclerosis and Sjögren's syndrome. *Ann Rheum Dis* (1997) 56:230–234.
71. Negi VS, Tripathy NK, Misra R, Nityanand S. Antiendothelial cell antibodies in scleroderma correlate with severe digital ischemia and pulmonary arterial hypertension. *J Rheumatol* (1998) 25:462–466.
72. Pignone A, Scaletti C, Matucci-Cerinic M, Vázquez-Abad D, Meroni PL, Del Papa N, Falcini F, Generini S, Rothfield N, Cagnoni M. Anti-endothelial cell antibodies in systemic sclerosis: significant association with vascular involvement and alveolo-capillary impairment. *Clin Exp Rheumatol* (1998) 16:527–532.
73. Ihn H, Sato S, Fujimoto M, Igarashi A, Yazawa N, Kubo M, Kikuchi K, Takehara K, Tamaki K. Characterization of autoantibodies to endothelial cells in systemic sclerosis (SSc): association with pulmonary fibrosis. *Clin Exp Immunol* (2000) 119:203–209. doi: 10.1046/j.1365-2249.2000.01115.x
74. Wusirika R, Ferri C, Marin M, Knight DA, Waldman WJ, Ross P, Magro CM. The Assessment of Anti–Endothelial Cell Antibodies in Scleroderma-Associated Pulmonary Fibrosis. *Am J Clin Pathol* (2003) 120:596–606.
75. Tamby MC. Anti-endothelial cell antibodies in idiopathic and systemic sclerosis associated pulmonary arterial hypertension. *Thorax* (2005) 60:765–772. doi: 10.1136/thx.2004.029082
76. Riccieri V, Germano V, Alessandri C, Vasile M, Ceccarelli F, Sciarra I, Di Franco M, Spadaro A, Valesini G. More severe nailfold capillaroscopy findings and anti-endothelial cell antibodies. Are they useful tools for prognostic use in systemic sclerosis? *Clin Exp Rheumatol* (2008) 26:992–997.
77. Riemekasten G, Philippe A, Näther M, Slowinski T, Müller DN, Heidecke H, Matucci-Cerinic M, Czirják L, Lukitsch I, Becker M, et al. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. *Ann Rheum Dis* (2011) 70:530–536. doi: 10.1136/ard.2010.135772
78. Becker MO, Kill A, Kutsche M, Guenther J, Rose A, Tabeling C, Witzenrath M, Kühl AA, Heidecke H, Ghofrani HA, et al. Vascular receptor autoantibodies in pulmonary arterial hypertension associated with systemic sclerosis. *Am J Respir Crit Care Med* (2014) 190:808–817. doi: 10.1164/rccm.201403-0442OC
79. Günther J, Kill A, Becker MO, Heidecke H, Rademacher J, Siegert E, Radi¿ M, Burmester G-R, Dragun D, Riemekasten G. Angiotensin receptor type 1 and endothelin receptor type A on immune cells mediate migration and the expression of IL-8 and CCL18 when stimulated by autoantibodies from systemic sclerosis patients. *Arthritis Res Ther* (2014) 16:R65. doi: 10.1186/ar4503
80. Kill A, Tabeling C, Undeutsch R, Kühl AA, Günther J, Radic M, Becker MO, Heidecke H, Worm M, Witzenrath M, et al. Autoantibodies to angiotensin and endothelin receptors in systemic sclerosis induce cellular and systemic events associated with disease pathogenesis. *Arthritis Res Ther* (2014) 16:R29. doi: 10.1186/ar4457

81. Avouac J, Riemekasten G, Meune C, Ruiz B, Kahan A, Allanore Y. Autoantibodies against Endothelin 1 Type A Receptor Are Strong Predictors of Digital Ulcers in Systemic Sclerosis. *J Rheumatol* (2015) 42:1801–1807. doi: 10.3899/jrheum.150061
82. Jin R, Kaneko H, Suzuki H, Arai T, Teramoto T, Fukao T, Kondo N. Age-related changes in BAFF and APRIL profiles and upregulation of BAFF and APRIL expression in patients with primary antibody deficiency. *Int J Mol Med* (2008) doi: 10.3892/ijmm.21.2.233
83. Zollars E, Bienkowska J, Czerkowicz J, Allaire N, Ranger AM, Magder L, Petri M. BAFF (B cell activating factor) transcript level in peripheral blood of patients with SLE is associated with same-day disease activity as well as global activity over the next year. *Lupus Sci Med* (2015) 2:e000063–e000063. doi: 10.1136/lupus-2014-000063
84. Taniguchi T, Miyagawa T, Toyama S, Yamashita T, Nakamura K, Saigusa R, Ichimura Y, Takahashi T, Toyama T, Yoshizaki A, et al. CXCL13 produced by macrophages due to Fli1 deficiency may contribute to the development of tissue fibrosis, vasculopathy and immune activation in systemic sclerosis. *Exp Dermatol* (2018) 27:1030–1037. doi: 10.1111/exd.13724
85. Becvár R, Stork J, Pesáková V, Stánová A, Hulejová H, Rysová L, Zatloukalová A, Zatloukal P, Jáchymová M, Pourová L. Clinical correlations of potential activity markers in systemic sclerosis. *Ann N Y Acad Sci* (2005) 1051:404–412. doi: 10.1196/annals.1361.082
86. Koudstaal T, van Uden D, van Hulst J a. C, Heukels P, Bergen IM, Geenen LW, Baggen VJM, van den Bosch AE, van den Toorn LM, Chandoesing PP, et al. Plasma markers in pulmonary hypertension subgroups correlate with patient survival. *Respir Res* (2021) 22:137. doi: 10.1186/s12931-021-01716-w
87. Olsson KM, Olle S, Fuge J, Welte T, Hoeper MM, Lerch C, Maegel L, Haller H, Jonigk D, Schiffer L. CXCL13 in idiopathic pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. *Respir Res* (2016) 17:21. doi: 10.1186/s12931-016-0336-5
88. Launay D, Hachulla E, Hatron P-Y, Jais X, Simonneau G, Humbert M. Pulmonary arterial hypertension: a rare complication of primary Sjögren syndrome: report of 9 new cases and review of the literature. *Medicine (Baltimore)* (2007) 86:299–315.
89. Yan S, Li M, Wang H, Yang X, Zhao J, Wang Q, Liu Y, Lai J, Tian Z, Song H, et al. Characteristics and risk factors of pulmonary arterial hypertension in patients with primary Sjögren's syndrome. *Int J Rheum Dis* (2018) 21:1068–1075. doi: 10.1111/1756-185X.13290
90. Shinya Y, Hiraide T, Momoi M, Goto S, Suzuki H, Katsumata Y, Kurebayashi Y, Endo J, Sano M, Fukuda K, et al. TNFRSF13B c.226G>A (p.Gly76Ser) as a Novel Causative Mutation for Pulmonary Arterial Hypertension. *J Am Heart Assoc Cardiovasc Cerebrovasc Dis* (2021) 10:e019245. doi: 10.1161/JAHA.120.019245
91. Bourcy CFA de, Dekker CL, Davis MM, Nicolls MR, Quake SR. Dynamics of the human antibody repertoire after B cell depletion in systemic sclerosis. *Sci Immunol* (2017) 2:eaan8289. doi: 10.1126/sciimmunol.aan8289
92. López-Cacho JM, Gallardo S, Posada M, Aguerri M, Calzada D, Mayayo T, González-Rodríguez ML, Rabasco AM, Lahoz C, Cárdaba B. Association of immunological cell profiles with specific clinical phenotypes of scleroderma disease. *BioMed Res Int* (2014) 2014:148293. doi: 10.1155/2014/148293
93. Zhang T, Huang C, Luo H, Li J, Huang H, Liu X, Zhan S. Identification of key genes and immune profile in limited cutaneous systemic sclerosis-associated pulmonary arterial hypertension by bioinformatics analysis. *Life Sci* (2021) 271:119151. doi: 10.1016/j.lfs.2021.119151
94. Marrapodi R, Pellicano C, Radicchio G, Leodori G, Colantuono S, Iacolare A, Gigante A, Visentini M, Rosato E. CD21low B cells in systemic sclerosis: A possible marker of vascular complications. *Clin Immunol Orlando Fla* (2020) 213:108364. doi: 10.1016/j.clim.2020.108364
95. Zamanian RT, Badesch D, Chung L, Domsic RT, Medsger T, Pinckney A, Keyes-Elstein L, D'Aveta C, Spychala M, White RJ, et al. Safety and Efficacy of B-Cell Depletion with Rituximab for the Treatment of Systemic Sclerosis Associated Pulmonary Arterial Hypertension: A Multi-center, Double-blind, Randomized, Placebo-controlled Trial. *Am J Respir Crit Care Med* (2021) doi: 10.1164/rccm.202009-3481OC
96. Guedez L, Mansoor A, Birkedal-Hansen B, Lim MS, Fukushima P, Venzon D, Stetler-Stevenson WG, Stetler-Stevenson M. Tissue inhibitor of metalloproteinases 1 regulation of interleukin-10 in B-cell differentiation and lymphomagenesis. *Blood* (2001) 97:1796–1802. doi: 10.1182/blood.V97.6.1796
97. Mo X, Du S, Chen X, Wang Y, Liu X, Zhang C, Zhu C, Ding L, Li Y, Tong Y, et al. Lactate Induces Production of the tRNAHis Half to Promote B-lymphoblastic Cell Proliferation. *Mol Ther* (2020) 28:2442–2457. doi: 10.1016/j.ymthe.2020.09.010
98. Lin S-J, Wu S-W, Chou Y-C, Lin J-H, Huang Y-C, Chen M-R, Ma N, Tsai C-H. Novel expression and regulation of TIMP-1 in Epstein Barr virus-infected cells and its impact on cell survival. *Virology* (2015) 481:24–33. doi: 10.1016/j.virol.2015.02.015

99. Zhou Y, Liu X, Xu L, Tseng H, Cao Y, Jiang J, Ciccarelli BT, Yang G, Patterson CJ, Hunter ZR, et al. Matrix metalloproteinase-8 is overexpressed in Waldenström's macroglobulinemia cells, and specific inhibition of this metalloproteinase blocks release of soluble CD27. *Clin Lymphoma Myeloma Leuk* (2011) 11:172–175. doi: 10.3816/CLML.2011.n.041
100. Maffei R, Martinelli S, Castelli I, Santachiara R, Zucchini P, Fontana M, Fiorcari S, Bonacorsi G, Ilariucci F, Torelli G, et al. Increased angiogenesis induced by chronic lymphocytic leukemia B cells is mediated by leukemia-derived Ang2 and VEGF. *Leuk Res* (2010) 34:312–321. doi: 10.1016/j.leukres.2009.06.023
101. Sadagopan S, Sharma-Walia N, Veettil MV, Bottero V, Levine R, Vart RJ, Chandran B. Kaposi's sarcoma-associated herpesvirus upregulates angiogenin during infection of human dermal microvascular endothelial cells, which induces 45S rRNA synthesis, antiapoptosis, cell proliferation, migration, and angiogenesis. *J Virol* (2009) 83:3342–3364. doi: 10.1128/JVI.02052-08
102. Hüttmann A, Klein-Hitpass L, Thomale J, Deenen R, Carpinteiro A, Nückel H, Ebeling P, Führer A, Edelmann J, Sellmann L, et al. Gene expression signatures separate B-cell chronic lymphocytic leukaemia prognostic subgroups defined by ZAP-70 and CD38 expression status. *Leukemia* (2006) 20:1774–1782. doi: 10.1038/sj.leu.2404363
103. Guedez L, Martinez A, Zhao S, Vivero A, Pittaluga S, Stetler-Stevenson M, Raffeld M, Stetler-Stevenson WG. Tissue inhibitor of metalloproteinase 1 (TIMP-1) promotes plasmablastic differentiation of a Burkitt lymphoma cell line: implications in the pathogenesis of plasmacytic/plasmablastic tumors. *Blood* (2005) 105:1660–1668.
104. Kay N, Bone N, Tschumper R, Howell K, Geyer S, Dewald G, Hanson C, Jelinek D. B-CLL cells are capable of synthesis and secretion of both pro- and anti-angiogenic molecules. *Leukemia* (2002) 16:911–919.
105. Wahlgren J, Maisi P, Sorsa T, Sutinen M, Tervahartiala T, Pirilä E, Teronen O, Hietanen J, Tjäderhane L, Salo T. Expression and induction of collagenases (MMP-8 and -13) in plasma cells associated with bone-destructive lesions. *J Pathol* (2001) 194:217–224. doi: 10.1002/path.854
106. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature* (2011) 473:298–307. doi: 10.1038/nature
107. Rabquer BJ, Koch AE. Angiogenesis and Vasculopathy in Systemic Sclerosis: Evolving Concepts. *Curr Rheumatol Rep* (2012) 14:56–63. doi: 10.1007/s11926-011-0219-1
108. Cantatore FP, Maruotti N, Corrado A, Ribatti D. Angiogenesis Dysregulation in the Pathogenesis of Systemic Sclerosis. *BioMed Res Int* (2017) 2017:1–6. doi: 10.1155/2017/5345673
109. Liakouli V, Cipriani P, Marrelli A, Alvaro S, Ruscitti P, Giacomelli R. Angiogenic cytokines and growth factors in systemic sclerosis. *Autoimmun Rev* (2011) 10:590–594. doi: 10.1016/j.autrev.2011.04.019
110. Dziankowska-Bartkowiak B, Gerlicz-Kowalczuk Z, Waszczykowska E. Angiogenin and SDF-1α serum concentration in patients with systemic sclerosis in relation to clinical status. *Arch Med Sci AMS* (2011) 7:92–96. doi: 10.5114/aoms.2011.20610
111. Michalska-Jakubus M, Kowal-Bielecka O, Chodorowska G, Bielecki M, Krasowska D. Angiopoietins-1 and -2 are differentially expressed in the sera of patients with systemic sclerosis: high angiopoietin-2 levels are associated with greater severity and higher activity of the disease. *Rheumatol Oxf Engl* (2011) 50:746–755. doi: 10.1093/rheumatology/keq392
112. Flower VA, Barratt SL, Ward S, Pauling JD. The Role of Vascular Endothelial Growth Factor in Systemic Sclerosis. *Curr Rheumatol Rev* 15:99–109.
113. Trojanowska M. Role of PDGF in fibrotic diseases and systemic sclerosis. *Rheumatology* (2008) 47:v2–v4. doi: 10.1093/rheumatology/ken265
114. Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, Fraticelli P, Sambo P, Funaro A, Kazlauskas A, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med* (2006) 354:2667–2676. doi: 10.1056/NEJMoa052955
115. Young-Min SA, Beeton C, Laughton R, Plumpton T, Bartram S, Murphy G, Black C, Cawston TE. Serum TIMP-1, TIMP-2, and MMP-1 in patients with systemic sclerosis, primary Raynaud's phenomenon, and in normal controls. *Ann Rheum Dis* (2001) 60:846–851.
116. Leong E, Bezuhly M, Marshall JS. Distinct Metalloproteinase Expression and Functions in Systemic Sclerosis and Fibrosis: What We Know and the Potential for Intervention. *Front Physiol* (2021) 12:fphys.2021.72745

Notre travail portant sur les biomarqueurs LB a donc permis de mettre en évidence une association entre activation lymphocytaire B et HTAP chez les patients SSc. Ce résultat original nous a conduit à réaliser une revue exhaustive de la littérature, afin de déterminer si d'autres arguments cliniques ou expérimentaux soutenaient l'implication du LB dans l'apparition et la pérennisation de la vasculopathie pulmonaire. Nous avons élargi le champ de cette revue pour inclure l'ensemble des HTP du groupe 1, et notamment l'HTAP idiopathique pour lesquelles de nombreuses données suggèrent un rôle crucial de l'immunité.

Ce travail a été réalisé en collaboration avec l'équipe du Pr Mark Nicolls à *Stanford University* et est soumis pour publication dans *European Respiratory Journal*.

RESUME DETAILLE EN FRANÇAIS

Il existe un besoin non satisfait de nouvelles stratégies thérapeutiques ciblant des voies alternatives pour améliorer le pronostic des patients atteints d'hypertension artérielle pulmonaire (HTAP). Dans la mesure où l'immunité semble jouer un rôle majeur dans le développement et la progression des lésions vasculaires pulmonaires associée à l'HTAP, nous avons examiné la contribution potentielle des lymphocytes B (LB) dans sa physiopathologie et évalué la pertinence des thérapies ciblées sur LB.

L'homéostasie des LB circulants est altérée chez les patients atteints d'HTAP, avec une lymphopénie B, une distribution anormale des sous-populations B (expansion des formes naïves, des plasmablastes et des plasmocytes ; contraction du compartiment B mémoire) et des stigmates d'activation chronique. En effet, les LB sont intensément recrutés de la périphérie vers les poumons du fait de la sécrétion locale de chimiokines, et sont activés par plusieurs mécanismes : interaction des auto-antigènes vasculaires pulmonaires avec leur BCR spécifique ; signaux de costimulation fournis par les lymphocytes Tfh (production d'IL-21), les lymphocytes Th2 et les mastocytes (production d'IL-4, IL-6 et IL-13) ; signaux de survie accrus provenant du système BAFF. Ce processus aboutit à la formation de centres germinatifs au sein d'organes lymphoïdes tertiaires périvasculaires et à la production locale d'auto-anticorps pathogènes ciblant les vaisseaux pulmonaires (cellules endothéliales, cellules musculaires lisses, fibroblastes adventitiels, récepteurs de l'angiotensine-II/endothéline-1 et récepteurs BMPR/ALK). Les LB médient également leurs effets par une production accrue de cytokines pro-inflammatoires, des propriétés anti-inflammatoires réduites par les LB régulateurs, l'activation du complément induite par les IgG et l'activation de mastocytes induite par les IgE.

Les approches de médecine de précision ciblant l'immunité des LB constituent une perspective prometteuse pour certaines formes d'HTAP, comme le suggèrent l'efficacité du traitement anti-CD20 dans différents modèles expérimentaux et un essai clinique récent sur le rituximab dans l'HTAP associée à la SSc.

B cells in pulmonary arterial hypertension: friend, foe or bystander?

Sébastien Sanges[1,2,3,4,5,6,7*], MD, MS; Wen Tian[6,7*], PhD; Sylvain Dubucquoi[1,2,8], MD, PhD; David Launay[1,2,3,4,5,6,7**], MD, PhD; Mark Nicolls[6,7**], MD.

(1) Univ. Lille, U1286 – INFINITE – Institute for Translational Research in Inflammation, F-59000 Lille, France
(2) INSERM, F-59000 Lille, France
(3) CHU Lille, Département de Médecine Interne et Immunologie Clinique, F-59000 Lille, France
(4) Centre National de Référence Maladies Auto-immunes Systémiques Rares du Nord et Nord-Ouest de France (CeRAINO), F-59000 Lille, France
(5) Health Care Provider of the European Reference Network on Rare Connective Tissue and Musculoskeletal Diseases Network (ReCONNET), F-59000 Lille, France
(6) Veteran Affairs Palo Alto Health Care System, Palo Alto, California, USA.
(7) Division of Pulmonary, Allergy, and Critical Care Medicine, Stanford University, School of Medicine, Stanford, California, USA.
(8) CHU Lille, Institut d'Immunologie, Pôle de Biologie Pathologie Génétique, F-59000 Lille, France

*Both authors contributed equally and share co-first authorship.
**Both authors contributed equally and share co-last authorship.

Corresponding author: Prof. Mark Robert Nicolls, MD, Division of Pulmonary, Allergy and Critical Care Medicine, Stanford University, 1701 Page Mill Road, Palo Alto, CA 94304
E-mail: mnicolls@stanford.edu

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a severe disease characterized by progressive thickening and obliteration of pulmonary vessels, resulting in increased vascular resistance, elevated pulmonary artery (PA) pressures, and right heart failure [1]. Current treatments mostly rely on vasodilating sometimes occluded vessels and have limited effectiveness in reversing vascular remodeling or preventing new lesion formation. Therefore, there is an urgent need for innovative therapeutic strategies that can modify the natural course of the disease.

Extensive research has recently emphasized the crucial role of immunity in the development and progression of pulmonary vascular alterations in PAH. Notably, B cells have attracted particular attention due to their potential as therapeutic targets, as they can be specifically reduced by the same treatments used for hematological malignancies and autoimmune disorders [2]. As such, it appears relevant to examine the extent to which the cellular and humoral arms of B cell immunity contribute to the pathogenic events occurring during PAH.

This review summarizes the emerging evidence regarding the involvement of B cells in the pathogenesis of PAH, highlighting activating mechanisms, effector functions, and therapeutic potential.

1. ABNORMAL B CELL HOMEOSTASIS IN PAH

Several lines of evidence suggest abnormal B cell homeostasis in PAH. Although not consistently observed [3–5], most studies have reported lower circulating B cell counts in idiopathic (i)PAH and connective tissue diseases (CTD)-PAH compared to healthy controls (HC) [6–9]. B cell subset distribution is also altered: typical modifications include an expansion of antigen (Ag)-unexperienced populations (mostly mature naïve B cells), plasmablasts, and plasma cells (especially immunoglobulin (Ig)A^+); findings that contrast with a contraction of the memory B cell compartment (particularly non-switched forms) [4, 5, 10, 11]. These anomalies correlate with survival in patients with heritable (h) and iPAH [12]. The lower levels of circulating memory B cells may be explained by an enhanced pulmonary recruitment, as they are found in increased proportions within the lungs of hypoxic rats [13].

A recently identified B cell population, associated with autoimmune processes and characterized by a specific membrane phenotype (CD27/IgD double negativity, low CD21 expression), is expanded in iPAH patients [11]. Their levels are similar in systemic sclerosis (SSc) patients with and without PAH [14], but correlate with clinical markers of pulmonary hypertension (PH) [15].

Moreover, several observations suggest that circulating B cells are activated in PAH. B cells from SSc-PAH patients display an increased expression of CD25 and are prone to apoptosis [7]. Genes involved in B cell-specific processes are the most differentially-expressed genes in peripheral blood mononuclear cells (PBMCs) from SSc-PAH compared to SSc-no PAH and HC [8, 9]. In iPAH, B cells display a distinct mRNA expression profile, characterized by upregulation of genes involved in inflammation and immune response, compared to HC [16].

2. ACTIVATING MECHANISMS OF B CELLS IN PAH

B cell activation classically requires the engagement of a specific Ag to its cognate membrane B cell receptor (BCR) [17]. Costimulatory signals are mandatory and usually provided by Ag-specific $CD4^+$ T cells through direct cell-cell interactions and cytokine production [17]. In the field of autoimmune diseases, interactions between ligands and receptors from the B-cell activating factor (BAFF) system are also of specific importance to drive survival and proliferation of autoreactive B cells [18].

2.1. Abnormal BCR signaling

The BCR platform is a complex signaling system consisting of a membrane Ig, a transmembrane signal transducer (the CD79A-CD79B heterodimer), a constellation of kinases that mediate downstream intracellular signaling (such as Bruton tyrosine kinase (BTK)), and several membrane co-receptors that act as positive (CD19, CD21) or negative (CD22, CD35) signal regulators [19]. Ag engagement on its cognate BCR triggers this signaling cascade leading to B cell activation through various intracellular pathways (notably the BTK-nuclear factor-kappa B (NFκB) axis) [19].

Gene expression studies performed on PBMCs from SSc-PAH patients and lungs from various PAH patients reveal a significant upregulation of genes involved in BCR signaling and NFκB pathway in the PAH groups [8, 20]. In lungs from rats with monocrotaline (MCT)-induced PAH, genes related to B cell activation, and especially BCR signaling mediators, display the strongest correlations with hemodynamic and histologic markers of PAH severity [21]. These anomalies become more pronounced with the course of the experimental disease. Pathway analysis reveals significant dysregulation of several key components of the BCR complex (such as upregulation of CD79A/B, BTK, CD19 and CD21) [21].

Among the various components involved in the abnormal BCR signaling observed during human and experimental PAH, BTK was the focus of recent attention. In iPAH and CTD-PAH patients, intracellular BTK expression is increased in total B cells, as well as in the naïve and memory subsets, and correlates with the presence of circulating auto-antibodies (Abs) [5]. Moreover, B-cell-specific BTK overexpression in transgenic mice exposed to bleomycin induces a PH phenotype, leads to accumulation of memory B cells and ASCs in mediastinal lymph nodes and stimulates production of serum Abs targeting endothelial Ags [5]. In MCT rats, treatment by a BTK inhibitor attenuates right ventricle (RV) systolic pressure (SP)

elevation, pulmonary vascular remodeling, RV hypertrophy and endothelium-mesenchymal transition, although these effects could be mediated by inhibition of macrophage BTK [22].

2.2. Abnormal costimulatory signals

In addition to Ag-BCR interaction, second costimulatory signals are mandatory to induce B cell proliferation and differentiation. These are usually provided by direct cell-cell interactions with cognate T helper (Th) cells, as well as by specific cytokine environments produced by various sources [17, 23].

2.2.1. Th2 cells, IL-4, IL-6, and IL-13

Th2 cells and their related cytokines (such as IL-4, IL-6 and IL-13) seem to play a major role in B cell activation in PAH. Preferential class-switching towards IgE, a hallmark of Th2 stimulation, is frequently noted in PAH. Increased IgE serum levels and IgE^+ B cells are observed in the blood of PAH patients and the lungs of several experimental models [24, 25].

Single-cell RNA sequencing performed on the lungs of hypoxia-induced PH (HPH) mice reveals a significant infiltration of Th2 cells with upregulated genes involved in the IL-4 pathway, an increased expression of costimulatory receptors in both T cells (CD28) and B cells (CD86 and CD40), as well as differential expression of genes involved in B cell activation and IgE class-switching (GLε) [25]. In a similar model induced by combined hypoxia and ovalbumin exposure, Th2 inhibition due to CD294 deficiency or IL-4/IL-13 neutralization decreases lung infiltration by IgE^+ B cells and attenuated PH [24]. Overall, these data suggest that Th2 cells contribute to B cell activation and IgE class-switching through direct interactions and cytokine production.

Increased IgE production leads to mast cell priming and activation through binding of FcεRI receptors [26]. Interestingly, lung infiltration by mast cells is a prominent feature of the disease in human and experimental PAH; and IL-4, IL-6 and IL-13 were identified as the main mediators produced by mast cells after IgE stimulation in animal models [25, 27–31]. Mast cell inhibition in MCT rats results in lower B cell lung infiltration, lower circulating auto-Abs and normalized serum IgG levels [29]. Most of these effects are also observed in animals treated by anti-IL6 Abs [29]. Overall, these data indicate that Th2 cytokines produced by mast cells also participate in B cell activation in PAH [27, 29].

2.2.2. Tfh cells and IL-21

Although T follicular helper (Tfh) cells are mainly located next to germinal centers (GC) in lymphoid organs, a circulating equivalent (cTfh) to this population has recently been identified in human peripheral blood [32], that facilitated their study in PAH.

In SSc patients, cTfh cell counts are higher in the case of PAH and correlate with circulating plasma cell counts [33]. SSc cTfh cells also produce more IL-21 and CXCL13 (a B cell chemokine) than HC. In coculture experiments using cTfh and B cells from SSc and HC, SSc cTfh cells induce more plasmablast differentiation and IgG/M production than SSc non-cTfh T cells and HC cTfh cells. This effect is neutralized when cocultures are performed with an IL-21 antagonist.

In iPAH patients, cTfh levels have been described as increased [11], or decreased but with higher cTfh17 subset [5]. BTK intracellular levels in B cells positively correlate with the proportion of several cTfh subsets [5]. Considered collectively, these data suggest that Tfh cells promote B cell recruitment and activation, plasmablast differentiation and Ig production, through an IL21-mediated mechanism.

2.3. Abnormal production of B cell survival signals: the BAFF system

The BAFF system consists of several protein members of the tumor-necrosis factor (TNF) superfamily: 2 ligands, BAFF and a proliferation-induced ligand (APRIL); and 3 receptors, transmembrane activator and CAML interactor (TACI), B-cell maturation antigen (BCMA) and BAFF-receptor (BAFF-R). BAFF-R is expressed on most B cells beyond the pre-B cell stage with the exception of antibody-secreting cells (ASCs), but can only bind BAFF. TACI and BCMA expression is restricted to activated B cells, memory B cells and ASCs, but they bind both BAFF and APRIL. Fixation of the ligands on their receptors promote the survival, proliferation, and differentiation of peripheral B cells through activation of the NFκB pathway. Compared with normal B cells, the generation of autoreactive B cells is more dependent on the BAFF system [18].

The role of the BAFF system in PAH B cell activation has yet to be thoroughly investigated. Serum BAFF concentrations have been assessed in several SSc cohorts: while BAFF levels are similar in patients with and without PH, they strongly correlate with clinical markers of PH severity in SSc-PAH patients [34–37]. A pathogenic variant in the gene coding for TACI has recently been identified as a new causative mutation for PAH, possibly by promoting

dysregulated vascular inflammation [38]. BAFF-R is also one of the most upregulated gene in the lungs of MCT rats [21].

Interestingly, the BAFF system has also been extensively implicated in the pathogenesis of atherosclerosis, another cardiovascular disease that involves inflammation and shares common mechanisms with PAH [39]. In humans, mutations in BAFF-R are the second most prominent candidate gene in determining cardiovascular risk [40]. In experimental models, animals that are BAFF-R-deficient or treated by anti-BAFF-R Abs display reduced atherosclerosis [41–43]. Paradoxically, mice treated by anti-BAFF Abs or TACI-deficient have increased atherosclerosis, which was explained by unexpected extra-B cell effects: BAFF ligates to macrophage TACI and represses its production of pro-atherogenic CXCL10; and APRIL binds endothelial heparan-sulfate proteoglycans and limits the constitution of atherosclerotic plaques [44, 45]. Finally, endothelial progenitor cells (EPCs) isolated from SLE patients PBMCs express BAFF-R, TACI and BCMA. Incubation with BAFF induces their apoptosis, which is rescued by belimumab, an anti-BAFF Ab [46]. Whether these processes are similarly at play in the pulmonary vascular bed of PAH has yet to be explored. These data collectively suggest that there are pleiotropic effects of the BAFF system in vascular diseases; effects that go beyond B cells themselves and could also involve activated signaling pathways in endothelial cells that may recapitulate certain B cell signaling pathways.

3. EFFECTOR MECHANISMS OF B CELLS IN PAH

B cell immunity encompasses both the direct cellular actions of cells and Abs; both of these arms may be involved in PAH pathogenesis. B cells give rise to plasmablasts which subsequently differentiate into non-dividing plasma cells. Plasma cells synthesize Igs whose effects are either Ag-specific (direct consequences of Ag stimulation or inhibition due to the Ab binding) or Ag-nonspecific (opsonization, *i.e.,* tagging Ag for phagocytes; antibody-dependent cellular cytotoxicity, *i.e.,* tagging Ag for cytotoxic cells; and complement activation). Additionally, another effector mechanism, specific to IgEs, consists in mast cell priming and activation through FcεRI receptor fixation.

Recently, other important B cell properties were acknowledged, namely cytokine/growth factor production and direct cell-cell interactions. Through these newly described functions, B cells were recognized as major regulators of immune responses, but also can also potentially act on non-immune processes [47]. We speculate below on how Abs and B cells may contribute to pulmonary vascular pathology in PAH.

3.1. Production of auto-antibodies

The importance of auto-Abs in the constitution and perpetuation of vascular lesions in PAH was highlighted by several experimental observations. Passive transfer of serum IgG from various PH models to healthy animals is sufficient to induce pulmonary vascular remodeling and hemodynamic alterations [48–50]. Conversely, Ig-deficient mice are resistant to hypoxia-induced PH [51].

3.1.1. Global antibody repertoire: serum immunoglobulins

In the UK national iPAH cohort, serum levels of IgG, IgM, IgA, IgG1, IgG2, and IgG4 are identical to those of HC [52]. Only IgG3 titers are significantly increased, possibly due to preferential class-switching induced by elevated IL-21 concentrations [52]. When unsupervised clustering was applied on the whole blood transcriptome from this cohort, 3 patient subgroups are delineated that display incremental risks of mortality: interestingly, the genes, whose expression allows the best discrimination between clusters, are Ig genes; here, higher expression was associated with better survival [12]. Although not measured in this cohort, previous studies reported elevated IgE levels in iPAH patients, due to skewed isotype switching induced by Th2 cytokines [24, 25]. Few data are available regarding patients with

CTD-PAH, with conflicting reports of elevated IgA [53] and decreased IgG [37] levels in SSc-PAH (compared to SSc without PH).

3.1.2. Global antibody repertoire: BCR diversity

Anomalies of the BCR repertoire in PAH have been highlighted by 2 recent works, one focusing on total B cells from SSc-PAH patients [10] and the other on plasmablasts from iPAH patients [4], that yielded similar results. First, in an analysis of the complementarity-determining regions 3 (CDR3; the Ig segment responsible for Ag specificity), no common sequence among patients was revealed; this finding argues against a shared antigenic motif driving the humoral response. Alternatively, this result could also indicate immunological idiosyncrasy, *i.e.,* that different Ag (or different epitopes of the same Ag) are driving the immune response in each patient. Second, analysis of the VDJ segments, the Ig region involved in Ag binding and supporting the diversity of the Ab repertoire, indicates over- and under-used recombinations, suggesting small clonal expansions driven by chronic exposure to specific Ags. Third, quantification of mutation loads in the VDJ segments reveals a higher number of fixed mutations, suggestive of increased selective sweeps and sustained affinity maturation. Finally, monoclonal Abs generated from representative iPAH plasmablast clones bind multiple proteins on an auto-Ag microarray, indicating that the PAH Ab repertoire is autoreactive (Abs target self-Ags) and polyreactive (a single Ab targets multiple Ags, possibly by recognizing shared post-translational modifications). These results are consistent with a persistent humoral response polarized against specific auto-Ags that are probably different from one patient to another.

3.1.3. Ag-specific antibodies and properties: conventional auto-antibodies

The frequent occurrence of conventional auto-Abs in PAH has long been documented, with older studies already observing antinuclear Ab positivity in about 40% of iPAH patients [54, 55]. In patients with CTDs, certain auto-Ab specificities are associated with an increased risk of PAH occurrence (anti-centromere and anti-U1-ribonucleoprotein (RNP) Abs in SSc [56]; anti-phospholipid, anti-sicca syndrome (SS)A/SSB and anti-U1RNP Abs in SLE [57, 58]) and with PAH prognosis (improved survival in anti-U1RNP-positive CTD-PAH patients [59]). In the UK national iPAH cohort, several conventional Ab specificities (notably targeting cardiolipin, histones, SSB, RNP complex or thyroid Ags) occur more frequently than in HC [52]. Their serum

levels allow to delineate 3 distinct clusters of patients ("high", "intermediate" and "low" auto-Ab positivity), with the "high Ab" cluster having a more severe PAH phenotype but better survival [52].

Whether these conventional auto-Abs, that are specific to intracellular Ags, can exert a direct pathogenic role remains a controversial issue [60], although some research suggests their ability to act on endothelial cells (ECs) in CTD patients. Stimulation of human umbilical vein (HUV)ECs by immune complexes (ICs) containing SSc Abs induces endothelial activation with different characteristics according to each Ab specificity, and through distinct intracellular pathways [61]. Incubation of human PA (HPA)ECs with serum IgG from anti-U1-RNP-positive CTD patients specifically induces a pro-adhesive phenotype [62].

3.1.4. Ag-specific antibodies and properties: functional auto-antibodies

Aside from conventional routinely-tested auto-Abs, several Ab specificities have been subsequently identified (*Table 1*), with more evidence supporting a pathogenic role in PAH [63]. These Abs are usually designated based on the location of their putative target Ag, either on a vascular cell (anti-EC, anti-smooth muscle cell (SMC) and anti-fibroblast (Fb) Abs) or a vascular membrane receptor (members of the G-protein-coupled receptor (GPCR) or the transforming growth factor (TGF)β receptor superfamilies).

- <u>Anti-endothelial cell antibodies</u>

Prevalence. There are considerable variations in the reported prevalence of anti-EC Abs in PAH, due different detection methods, EC substrates and patient ethnic background. In a European population, the frequency of anti-EC Abs is estimated at 62% (IgG) and 45% (IgM) in iPAH, 78% (IgG) and 61% (IgM) in CTD-PAH, and 33% (IgG) and 20% (IgM) in HC using a HUVEC-based ELISA [64]. In Chinese cohorts, their prevalence in CTD-PAH, CTD-no PH and HC is 63%, 41% and 5% respectively using a HPAEC-based ELISA [65]; and 82%, 73% and 20% respectively using Western blot [66]. Most studies have usually observed higher Ab titers in PAH patients compared to HC, and in similar range between PAH groups; however, comparisons of CTD patients with and without PAH have yielded conflicting results [64–70].

Targets. Serum IgG from iPAH and SSc-PAH patients express distinct reactivity profiles with macrovascular and microvascular EC Ags [71], indicating different targets depending on EC types and underlying disease. If HUVECs are used, anti-EC Abs from both patient groups

recognize cytoskeletal (lamin A/C, tubulin-β chain, vimentin) and ribosomal proteins [72]. In an animal model of PH, serum IgA from experimental mice specifically stains PA intima, indicating a reactivity against endothelial Ags at the pulmonary level [73].

Effects. Stimulation of various endothelial cell-lines with serum IgG from seropositive PAH patients consistently activates ECs into a pro-inflammatory (increased IL-6, IL-8, CCL2 and CCL5 expression) and pro-adhesive (increased E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 production) phenotype [4, 65, 69, 70, 74]. No effect is observed in endothelin-1 expression [4]. Conversely to previous observations in other CTDs, serum IgG from seropositive PAH patients does not induce EC apoptosis.[75, 76].

- Anti-smooth muscle cell antibodies

Prevalence. Anti-SMC Abs are detected by immunofluorescence on permeabilized human aortic SMCs in the serum of iPAH, SSc-PAH and SSc-no PH patients, but not HC. The prevalence of anti- stress-induced phosphoprotein 1 (STIP1) Abs, one of the major anti-SMC idiotypes (see below), is estimated at 84% in SSc-no PH, 76% in SSc-PAH and 24% in iPAH (compared with 3% in HC) [77].

Targets. When immunoblotted against mammary arterial SMC Ags, serum IgG from SSc-PAH and iPAH patients more frequently recognizes STIP1 and α-enolase than those of HC. Other targets also include cytoskeletal (such as tropomyosin α-1 chain) and mRNA regulation (such as KH-Type Splicing Regulatory Protein (KSRP)) proteins [77].

Effects. Serum IgG from SSc-PAH and iPAH patients induces SMC contraction within a collagen matrix, conversely to those of HC [77]. Serum IgG from an anti-α-enolase Ab-positive SLE-PAH patient promoted lamellipodia formation and migration of PA-SMCs, which was reversed by removal of the auto-Abs from the IgG fraction and by treatment with an α-enolase inhibitor [78]. As STIP1 is a co-chaperone of heat shock protein (HSP)70, a major regulator of SMC homeostasis, anti-STIP1 Abs could also modulate SMC migration by modifying the interactions between these 2 proteins [77].

- Anti-fibroblast antibodies

Prevalence. Anti-Fb Abs are detected in 40% of iPAH patients, 30% of SSc-PAH patients, 15% of SSc-no PH patients and 3% of HC, using a human dermal Fb-based ELISA assay [79].

Immunofluorescence performed on human PA-Fbs confirms significant staining with plasma from PH patients, which is not observed with HC [48].

Targets. By immunoblotting serum IgG from PAH patients against human dermal Fb proteome, several Ags were identified as putative targets of anti-Fb Abs [80]. These proteins are involved in various biological processes: cytoskeletal organization (vimentin, calumenin, phosphoinositide 3-kinase (PI3K)), cell contraction (tropomyosin 1), oxidative stress (HSP27 and 70) and other key cellular pathways [80]. Additionally, plasma from MCT rats labels PA adventitia, suggesting reactivity against Fb Ags at the pulmonary level; and vimentin, PI3K and HSP27 are also confirmed as targets to anti-Fb Abs in this model [48].

Effects. Human PA-Fbs stimulated by anti-Fb Ab-positive plasma from PAH patients acquire a pro-inflammatory (increased IL-1β and IL-6 secretion) and pro-adhesive (enhanced ICAM-1 expression) properties [48]. In SSc patients, anti-Fb Abs induce a myofibroblast phenotype with production of reactive oxygen species and extracellular matrix (ECM) remodeling [81, 82]. Additionally, Abs directed against fibrillin-1, a major component of ECM that regulates TGF-β signaling by sequestering TGF-β1 molecules, have been detected in iPAH sera [83]: these Abs can indirectly activate Fbs by liberating the TGF-β1 proteins trapped within the ECM microfibrils [84].

- Anti-GPCR antibodies: anti-AT1R and ETAR antibodies

Abs against GPCR have been described in various conditions and in health, and are considered natural components of the immune system [85, 86]. They form a network of closely intercorrelated Abs that targets structurally and functionally related molecules, such as vascular, neuronal or chemokine receptors. Gender, age and diseases (such as SSc) modify Ab titers and correlations, in a way that suggests alterations to homeostasis (disease-specific modification of the overall network) rather than production driven by exposure to an auto-Ag. [85]. In PAH, Abs targeting angiotensin receptor 1 (AT1R), endothelin receptor A (ETAR) and B (ETBR), α1-adrenergic receptor, sphingosine-1-phosphate receptor 2 (S1PR2) and S1PR3 were reported [50, 87–92]; but only anti-angiotensin and endothelin receptors Abs were thoroughly investigated [50, 87, 88].

Prevalence. Anti-AT1R and ETAR Abs occur more frequently in patients with SSc-PAH (69% and 65%, respectively) and CTD-PAH (63% and 55%, respectively) than in iPAH (21% and 11%, respectively) [50, 87]. Anti-ETBR Abs are also found at higher concentrations in SSc-PAH (but

not iPAH patients) compared to HC [88]. Anti-AT1R and ETAR Abs show weak-to-inexistent correlation with hemodynamics but predict the occurrence of PAH in SSc patients and mortality in SSc-PAH patients [50, 87, 93].

Targets. The epitopes targeted by anti-AT1R, anti-ETAR and anti-ETBR Abs have not been investigated so far.

Effects. Anti-AT1R/ETAR Ab display agonistic properties on their target receptors. Indeed, stimulation of rat PA segments with seropositive SSc-PAH serum increases endothelial cytosolic Ca^{2+} concentration (a downstream signal of AT1R/ETAR activation) and amplifies vasoconstriction induced by angiotensin and endothelin exposure [50]. These effects are blocked by pretreatment with valsartan and sitaxsentan [50]. Anti-ETAR Abs also increase HUVEC permeability and expression of vascular endothelial growth factor (VEGF)-A and platelet-derived growth factor (PDGF)-B, as well as human PA-SMC proliferation and expression of PDGF-Rβ; both of which are annulled by exposure to bosentan [87]. Transfer of anti-AT1R Abs induce pulmonary vasculopathy in healthy mice [94]; and transfer of anti-ETAR Abs worsen hemodynamics and vascular remodeling in MCT rats [87]. In SSc patients, anti-AT1R/ETAR Ab-positive serum induces pro-inflammatory (increased expression of IL-8 and CXCL8, increased neutrophil migration and activation) and pro-adhesive (increased expression of VCAM-1) properties in human microvascular ECs [95].

The effects of anti-AT1R and anti-ETAR Abs may extend beyond the vasculature. Indeed, AT1R and ETAR are expressed by normal human PBMCs. In SSc patients, serum IgG induce T cell chemotaxis and PBMC secretion of IL-8 and CCL-18, both of which are reduced by exposure to AT1R and ETAR antagonists [96]. The functional properties of anti-ETBR Abs have not been reported. As ETBR-deficient mice develop PAH, an inhibitory activity on its target receptor could be hypothesized [88].

- Anti-TGF-βR antibodies: anti-BMPR/ALK antibodies

Studies performed in Asian populations have identified specific patterns of reactivity against bone morphogenetic protein (BMP) receptor (BMPR) and activin receptor-like kinase (ALK) [97, 98]: presence of anti-ALK1 Abs in SLE-PAH patients (conversely to SLE-no PAH and HC), but not in MCTD-PAH and iPAH patients; presence of anti-BMPR1A Abs in SLE-PAH patients (conversely to SLE-no PH and HC); absence of anti-BMPR2 Abs in SLE-PAH, MCTD-PAH and iPAH. In the UK national iPAH cohort, patient sera were screened for reactivity against

various members of the BMPR/ALK signaling pathway [52]. Anti-BMPR2 Abs are detected in a small fraction of iPAH patients, and none of the HC. Anti-BMPR2 Abs recognize the extracellular domain of the protein and attenuate BMP4 signaling in PA-SMCs.

3.1.5. Functions of Abs not specific to known Ags

Among the various Ag-nonspecific effector functions of Abs, two mechanisms appear of particular importance in PAH: IgG-mediated complement activation and IgE-mediated mast cell activation. A recent study highlighted the critical role of the complement cascade, and especially its alternative pathway, in driving perivascular inflammation in the lungs of iPAH patients and various PH models [51]. Interestingly, IgM and IgG deposits co-localize with C4 and C3 staining in lungs of HPH mice; and Ig-deficient animals are protected against the complement activation, vascular changes and RVSP elevation caused by hypoxia. These effects are restored by immune reconstitution with polyvalent IgG from HC, suggesting a possible contribution of physiologically occurring so-called “natural Abs”.

Another recent work demonstrated the importance of IgE-induced mast cell activation in the pathogenesis of PAH [25]. In HPH mice and MCT rats, treatment by anti-IgE Abs or mast cell-specific FcεRI deletion normalizes hemodynamics, pulmonary vascular thickening and muscularization, as well as IL-6 and IL-13 expression in lung tissues.

3.2. Production of cytokines/growth factors and direct cell-cell interactions

3.2.1. Pro-inflammatory properties: effector B cells

B cells have been increasingly recognized as a major source of pro-inflammatory cytokines (notably IL-6 and interferon (IFN)-γ) in autoimmune diseases in recent years [99]. For instance, circulating B cells from SSc patients produce more IL-6 than those from HC [100, 101].

In PAH, however, their pro-inflammatory properties have not been a focus of attention. In a novel model of SSc-PAH induced by P-selectin glycoprotein ligand-1 (PSGL-1) deficiency in female mice, IFN-γ-producing B cells infiltrate the lungs in higher proportion than in control animals [102]. In a rat PAH model induced by a double-hit of anti-VEGF Sugen-5416 injection and ovalbumin immunization, B cell depletion is associated with lower IL-6 expression in lungs [103].

3.2.2. Anti-inflammatory properties: regulatory B cells

The term “Bregs” (regulatory B cells) refers to a heterogeneous group of various B cell subsets, all involved in suppressing immune responses and maintaining immune tolerance through distinct mechanisms (secretion of IL-10, IL-35 and TGF-β, direct cellular interactions mediated by CD1d and programmed death-ligand 1 (PD-L1)) [104]. As such, they play a crucial role in the emergence of the autoreactivity observed in various autoimmune diseases.

In SSc-PAH patients, circulating levels of $CD24^{hi}$ $CD27^{+}$ B regs are decreased compared to SSc-no PAH patients [14]. HPH mice have decreased Breg levels in the spleen and peripheral blood, and decreased blimp-1 (a major Breg transcription factor) expression in the lungs [105]. Adoptive transfer of Bregs improves hemodynamics and pulmonary vascular remodeling in HPH mice. In co-culture experiments, Bregs favor $CD4^{+}$ differentiation into T follicular regulator (Tfr) cells at the expanse of Tfh cells; and inhibit hypoxia-induced PA-SMC proliferation.

3.2.3. Angio-active properties: angiogenic B cells?

A novel angiogenic B cell population have been reported in patients with melanoma and eosinophilic esophagitis, as well as in HC [106]. This subset, characterized by a specific membrane phenotype ($IgG4^{+}$ $CD49b^{+}$ $CD73^{+}$ B cells), can produce proangiogenic cytokines (such as VEGF and PDGFA) and promote EC tube formation.

The presence and putative role of this subset has yet to be investigated in PAH. However, indirect evidence may suggest a direct action of B cells on the vasculature in these patients. In SSc, B cells produce angiogenic mediators (such as angiogenin and angiopoietin 1) in greater proportion than HC, but without difference in patients with and without PAH [37]. Various plasma cell proliferative disorders are characterized by elevated VEGF levels and vascular manifestations, including PAH [107].

4. LOCAL INVOLVEMENT OF B CELLS IN PAH LUNGS: FUNCTIONAL TERTIARY LYMPHOID ORGANS AS A MODEL RECAPITULATING B CELL PARTICIPATION IN PAH

Although most available data stem from experiments using peripheral blood samples, several studies have evaluated B cells and Abs directly within PAH lungs.

4.1. B cell infiltration and Ig deposits are observed in PAH lungs

B cell infiltration has been reported in the lungs of PAH patients and several PH models [6, 30, 31, 48, 108–114]. They typically form lymphoid aggregates located in perivascular areas (within the adventitial layer; distributed throughout the whole arterial tree and around plexiform lesions) but also less frequently in peri-bronchial areas. Solitary infiltrating B cells are rarely seen and, when present, usually localize within PA intima, or between ECs in plexiform lesions. B cell lung infiltration in PAH is probably an early event, as it preceded pulmonary vascular remodeling and RVSP elevation in a model of PAH induced by Sugen-5416 administration in athymic rats [110].

Similarly, local Ab deposition in the form of ICs has also been observed in human and experimental PAH and mostly localizes in PA intima and adventitia [6, 51]. The main target Ag of ICs isolated from the lungs of iPAH patients was recently identified as SAM domain and HD domain-containing protein 1 (SAMHD1), a cellular enzyme expressed by ECs in reaction to the increased replication of an endogenous retrovirus called HERV-K [115]. Interestingly, other candidates also include cytoskeletal and ribosomal proteins, reminiscent of the targets recognized by anti-EC and anti-Fb Abs from the peripheral blood of PAH patients (see above). Similarly, serum from two distinct PAH models stains PA intima and adventitia when applied on lung samples from diseased animals [48, 113]. Overall, these data suggest that the circulating auto-Ab in PAH may actually target lung self-Ags.

4.2. Lung B cells organize as functional tertiary lymphoid organs and are the main source of local and circulating auto-antibodies targeting pulmonary vascular self-antigens in PAH

B cell aggregates in PAH lungs adopt a classic conformation of tertiary lymphoid organs (TLOs), characterized by a central B-cell follicle that contains follicular dendritic cells (FDCs), and a well-segregated $CD4^+$ T cell peripheral zone embedded in a network of fibroblastic reticular cells (FRCs) and supplied by high-endothelial venules [6, 48, 113, 114]. Lung TLOs in

PAH appears to provide an adequate model that recapitulates the involvement of B cells in the pathogenesis of PAH (***Figure 1***).

TLO generation is usually initiated by the interaction of lymphoid tissue inducer cells (hypothesized to have a mast cell phenotype in PAH) and lymphoid tissue organizer cells (typically FDCs and FRCs) through the lymphotoxin (LT)α/β-LTβR couple [6]. This process results in the local production of lymphorganogenic chemokines (especially CXCL13, CCL19, CCL20 and CCL21 in PAH) by FRCs and FDCs, that recruit circulating $CXCR5^+$ and/or $CCR7^+$ T cells and B cells (which accounts for the decreased B cell levels and altered subset distribution observed in these patients) [6]. A local expansion in lymphatic vasculature is also observed [6], that allows pulmonary self-Ags from damaged vessels to circulate and activate B cells through interactions with their cognate BCR. Strong co-stimulatory signals are locally provided by Th2 cells located in T-cell zones, mast cells surrounding lymphatics and IL-21-producing Tfh within B cell follicles [6]. Although not specifically studied in PAH, the BAFF system is also involved in B cell activation during tertiary lymphoid neogenesis in autoimmune diseases [116]. As such, it appears that the main factors incriminated in B cell activation in PAH can be locally available within TLOs.

B cell activation in TLOs leads to a GC reaction, as suggested by the presence of b-cell lymphoma (Bcl)2^- $Bcl6^+$ $Ki67^+$ B cells (characteristic of GC B cells) within follicles, the strong over-expression of activation-induced cytidine deaminase (AID) (supporting the occurrence of somatic hypermutations and class-switch recombinations), and the presence of $CD138^+$ plasma cells and IgG^+/IgA^+ cells (indicating generation of ASCs and memory B cells, both terminal products of the GC reaction) [6, 48, 113]. These data suggest that TLOs are functional and act as a local producer of auto-Abs, which mainly targets auto-Ag expressed by PA ECs and Fbs. Interestingly, experimental interventions preventing lung TLO generation in MCT rats diminish serum auto-Ab titers, identifying them as the source of the circulating auto-Abs observed in PAH [48].

5. B CELL-TARGETED THERAPIES IN PAH

As evidence supporting their pathogenic role have accumulated over the last decades, several works have tried to assess the potential of B cells as a therapeutic target in PAH. Various approaches were investigated in both human patients and experimental models of the disease, the most studied so far remaining B cell depletion induced by rituximab (RTX), an anti-CD20 monoclonal Ab.

5.1. Rituximab

Preliminary evidence suggesting an efficacy of RTX in PAH originated from animal studies. Indeed, anti-CD20 therapy improves hemodynamics, RV hypertrophy and pulmonary vascular remodeling in several models of PH [103, 105, 114]. Treatment also limits IC deposition and IL-6, VEGF and hypoxia-induced factor (HIF)-1α expression in the lungs of Sugen-5416/ovalbumin rats [103]; and annulled the increase in survival and proliferation observed in PAECs incubated with MCT plasma [114]. Concurrently, several case reports also described a clinical improvement in CTD-PAH and iPAH patients treated by RTX for indications other than the pulmonary vascular disease [117–121].

More recently, an NIH multi-center randomized placebo-controlled trial tried to assess the safety and efficacy of RTX as an add-on therapy in SSc-PAH patients [122]. In the primary analysis based on a model using longitudinal data through week 24, the adjusted mean change in the 6-minute walking distance (6MWD) from baseline to week 24, the primary outcome measure of this study, does not differ significantly between arms (p=0.12). However, in a secondary analysis using a model with 6MWD data through week 48, the RTX arm is superior to placebo (p=0.03). Treatment appears to be safe and well tolerated. Machine learning identified a subgroup of patients, characterized by low levels of rheumatoid factor, IL-12, and IL-17, that gains the greatest benefit from RTX.

Interpreting these results is challenging because of several unexpected methodological issues that hindered the conduct of this trial. First, the study was underpowered because of insufficient recruitment, due to stringent inclusion criteria and competing industry-sponsored protocols. Second, the primary outcome measure was modified from hemodynamic improvement to 6MWD variation during the course of the study, because baseline PVR were lower than expected in SSc-PAH patients, rendering the original primary outcome measure much less useful. Interestingly however, an independent re-analysis of the trial data focused

on the subgroup that displayed the biomarker signature predictive of RTX efficacy and revealed noteworthy findings. Specifically, patients receiving RTX exhibited a 6MWD variation of +80m (compared to -21m with placebo) and a PVR variation of -1.06 Wood Units (WU) (compared to +1.5 WU with placebo) [123].

Finally, these equivocal clinical findings may appear contradictory to experimental observations of RTX efficacy. However, it should be noted that, in studies using animal models of PH, anti-CD20 therapy was started at the time of disease induction, making it more of a prophylaxis than a curative treatment [103, 105, 114]. As such, RTX could be an interesting option to prevent PAH occurrence in high-risk individuals such CTD patients, or, alternatively, as an adjunctive therapy in select patients with the biomarkers suggesting RTX-responsiveness. At the very least, RTX treatment appears to be safe for patients who are being appropriately monitored for hypersensitivity reactions. Further studies are warranted to further determine the relevance and place of B cell depletion within PAH therapeutic arsenal.

5.2. Other B cell-oriented strategies

5.2.1. Proteasome inhibitors

Proteasome inhibitors (such as bortezomib) were initially developed to target plasma cells in multiple myeloma, but their use have since then been extended to autoimmune diseases [124]. In several animal models of PH, proteasome inhibition improves hemodynamics, RV hypertrophy, pulmonary vascular remodeling and survival [125–131]. Although a direct effect on PA ECs and SMCs can explain these outcomes, a possible mediation through plasma cell inhibition was not investigated.

5.2.2. BTK inhibitors

BTK inhibition improves hemodynamics, RV hypertrophy, PA remodeling and fibrosis and endothelial-to-mesenchymal transition in MCT rats [22]. Although BTK expression mostly co-localizes with macrophages in this model, it remains possible that these results are also mediated by an effect through B-cell BTK. In SSc patients, ibrutinib reduces the production of pro-inflammatory cytokines and auto-Abs by peripheral B cells without modifying their IL-10 secretion [132].

5.2.3. Anti-CD22 antibody

In HPH mice, treatment by anti-CD22 Ab worsens hemodynamics, RV hypertrophy and pulmonary vascular remodeling, by inducing a specific depletion of Bregs [105].

5.2.4. Vaccination against endothelin receptors

Vaccine immunization against the second extracellular loop of ETAR leads to the generation of inhibitory Abs in MCT rats (conversely to naturally-occurring anti-ETAR Abs, that are agonistic) [133]. Anti-ETAR vaccination is associated with improved clinical (hemodynamics, RV hypertrophy) and pathophysiological (PA thickening, proliferation, inflammation and fibrosis) outcomes with no obvious side effects [133, 134].

5.2.5. Immunoadsorption

The rationale for an effect of immunoadsorption in PAH relies on the removal of pathogenic Igs, notably anti-ETAR and anti-α1-adrenergic receptor Abs [90]. Its efficacy is suggested by two small studies, that report modest improvements in hemodynamics, RV function, exercise capacity and patient-reported outcomes [90, 91].

CONCLUSION

In conclusion, B cells are emerging as potential key immunological players in the pathogenesis of PAH. Their aberrant activation contributes to the inflammatory milieu and vascular remodeling characteristic of this devastating disease through various mechanisms, including auto-Ab production, cytokine release, and direct cell interactions. Targeting B cells and their associated pathways may offer promising therapeutic avenues for the treatment of PAH. Ultimately, a deeper understanding of the role of B cells in PAH may pave the way for personalized and targeted approaches that improve patient outcomes and quality of life.

	Frequency	Targets	Effects
Vascular cells			
Anti-endothelial cell [64–66, 69, 71, 72, 74, 76]	iPAH (62%), CTD-PAH (63-82%), HC (5-33%)	Lamin A/C Tubulin-β chain Vimentin	Induction of pro-inflammatory (increased IL-6, IL-8, CCL2 and CCL5 expression) and pro-adhesive (increased E-selectin, ICAM-1 and VCAM-1 production) properties in ECs
Anti-smooth muscle cell [77, 78]	More frequent in SSc-PAH, SSc-no PAH and iPAH than in HC	STIP1 α-enolase	Induction of contraction and proliferation of SMCs
Anti-fibroblast [79, 80]	iPAH (40%), SSc-PAH (30%), SSc-no PAH (15%), HC (3%)	Vimentin, calumenin, PI3K Tropomyosin 1 HSP-27, HSP-70, G6PD	Induction of pro-inflammatory (increased IL-1β and IL-6 secretion), pro-adhesive (enhanced ICAM-1 expression) and pro-fibrotic (production of ROS and ECM remodeling) properties in Fbs
Vascular receptors: GPCRs			
Anti-AT1R/ETAR [50, 87, 93, 95, 96]	*Anti-AT1R:* SSc-PAH (69%), CTD-PAH (63%), iPAH (21%) *Anti-ETAR:* SSc-PAH (65%), CTD-PAH (55%), iPAH (11%) Positivity predicts occurrence of PAH in SSc and mortality in SSc-PAH	AT1R ETAR	Agonistic properties on AT1R/ETAR: • *ECs:* vasoconstriction, permeability, expression of VEGF-A and PDGF-B, pro-inflammatory (IL-8 and CXCL8 production, neutrophil recruitment) and pro-adhesive (VCAM-1 expression) properties • *SMCs:* proliferation and expression of PDGF-Rβ
Anti-ETBR [88]	Higher titers in SSc-PAH than HC (but not iPAH)	ETBR	Not investigated (hypothesized to be antagonistic, as ETBR-deficient mice develop a PAH phenotype)
Anti-α1AR [90, 91]	Pre-capillary PH (95%), with PAH (100%)	α1-adrenergic receptor	Activation of α1AR on rat cardiomyocytes, inducing long-lasting stimulatory effects without receptor desensitization (contrary to its natural agonist)
Anti-S1PR [92]	*Anti-S1PR1:* SSc-PAH (16%), SSc-no PAH (18%), HC (3%) *Anti-S1PR2:* SSc-PAH (26%), SSc-no PAH (15%), HC (4%) *Anti-S1PR3:* SSc-PAH (28%), SSc-no PAH (18%), HC (8%)	S1P receptors	Not investigated (anti-S1PR2/3 hypothesized to be agonistic, promoting PA-SMC proliferation and medial thickening)
Vascular receptors: TGF-βRs			
Anti-BMPR2 [52, 97, 98]	i/hPAH (0-1.4%), CTD (0%), HC (0%)	Extracellular domain of BMPR2	Diminution of BMP4 signaling in PA-SMCs
Anti-BMPR1A [98]	Higher titers in SLE-PAH than in SLE-no PAH and HC	BMPR1A (=ALK3)	Not investigated (hypothesized to interfere with ligand binding)
Anti-ALK1 [97, 98]	No detection in MCTD-PAH and iPAH Higher titers in SLE-PAH than in SLE-no PAH and HC	ALK1	Not investigated (hypothesized to interfere with ligand binding)
Other			

Anti-fibrillin 1 [83, 84]	iPAH (93%), anorexigen PAH (67%), HC (2%)	N-terminal fragment of fibrillin 1	Release of sequestered TGF-β1 from fibrillin-1-containing microfibrils in the ECM, leading to fibroblast activation into a profibrotic phenotype
Anti-ACE 2 [135]	More frequent in CTD patients with vasculopathy (including PAH) than those without	ACE 2	Inhibition of ACE-2 activity (which transforms AngII into Ang(1-7), an Ang isoform with vasodilating and antiproliferative properties)

Table 1. Functional auto-antibodies in PAH

α1AR: α1-adrenergic receptor; ACE: angiotensin converting enzyme; ALK: activin receptor-like kinase; Ang: angiotensin; AT1R: angiotensin receptor 1; BMP: bone morphogenetic protein; BMPR: bone morphogenetic protein receptor; CCL: C-C motif chemokine ligand; CTD: connective tissue disease; CXCL: chemokine (C-X-C motif) ligand; EC: endothelial cell; ECM: extracellular matrix; ETAR: endothelin receptor A; ETBR: endothelin receptor B; Fb: fibroblast; G6PD: glucose-6-phosphate dehydrogenase; GPCR: G-protein coupled receptor; h: heritable; HC: healthy control; HSP: heat shock protein; i: idiopathic; ICAM: intercellular adhesion molecule; IL: interleukin; MCTD: mixed connective tissue disease; PA: pulmonary artery; PAH: pulmonary arterial hypertension; PDGF-R: platelet-derived growth factor receptor; PDGF: platelet-derived growth factor; PH: pulmonary hypertension; PI3K: phosphoinositide 3-kinase; ROS: reactive oxygen species; S1PR: sphingosine-1-phosphate receptor; SLE: systemic lupus erythematosus; SMC: smooth muscle cell; SSc: systemic sclerosis; STIP1: stress-induced phosphoprotein 1; TGF-βR: transforming growth factor β receptor; TGF-β: transforming growth factor β; VCAM: vascular cell adhesion molecule; VEGF: vascular endothelial growth factor.

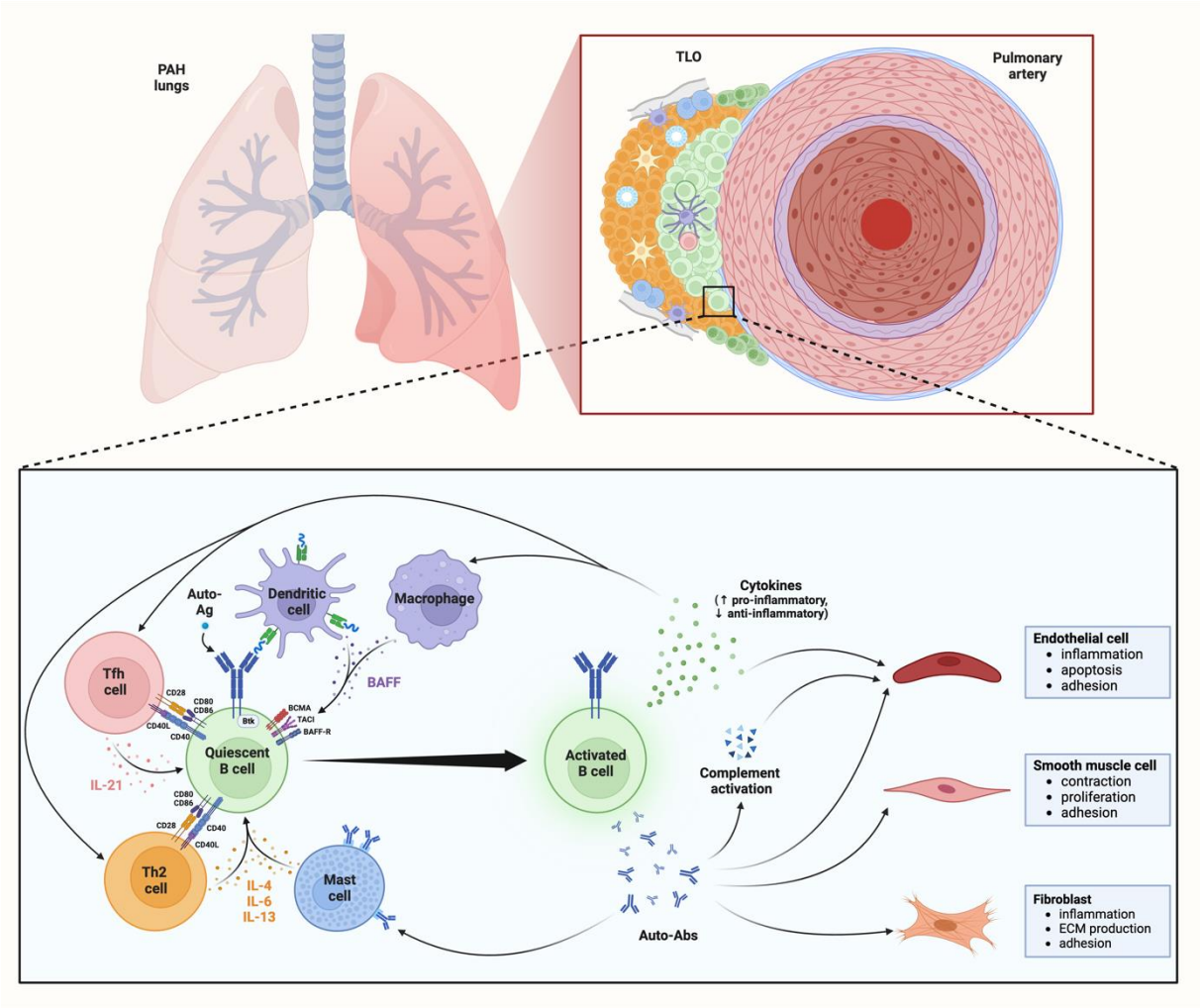


Figure 3. B cells in PAH: a proposed model.

In PAH patients, B cells are recruited to the lungs and activated by several mechanisms: interaction of lung vascular auto-antigens with their cognate BCR; co-stimulatory signals provided by Tfh cells (IL-21), Th2 cells and mast cells (IL-4, IL-6 and IL-13); and increased survival signals provided by the BAFF system. This process results in the formation of germinal centres within perivascular tertiary lymphoid organs (TLOs) and in the local production of pathogenic auto-antibodies targeting the pulmonary vasculature (endothelial cells, smooth muscle cells, adventitial fibroblasts). B cells also mediate their effects through enhanced production of pro-inflammatory cytokines, reduced anti-inflammatory properties by regulatory B cells, IgG-induced complement activation, and IgE-induced mast cell activation.

Ab: antibody; Ag: antigen; BAFF: B cell activating factor; BAFF-R: BAFF-receptor; BCMA: B-cell maturation antigen; BCR: B cell receptor; CD: cluster of differentiation; IL: interleukin; PAH: pulmonary arterial hypertension; TACI: transmembrane activator and CAML interactor; Tfh: T follicular helper cell; Th: T helper cell; TLO: tertiary lymphoid organ.

REFERENCES

1. Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, Olschewski AJ, Pullamsetti SS, Schermuly RT, Stenmark KR, Rabinovitch M. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur. Respir. J.* 2019; 53: 1801887.
2. Sanges S, Guerrier T, Launay D, Lefèvre G, Labalette M, Forestier A, Sobanski V, Corli J, Hauspie C, Jendoubi M, Yakoub-Agha I, Hatron P-Y, Hachulla E, Dubucquoi S. Role of B cells in the pathogenesis of systemic sclerosis. *Rev. Médecine Interne* 2017; 38: 113–124.
3. Edwards AL, Gunningham SP, Clare GC, Hayman MW, Smith M, Frampton CM, Robinson BA, Troughton RW, Beckert LE. Professional killer cell deficiencies and decreased survival in pulmonary arterial hypertension. *Respirology* 2013; 18: 1271–1277.
4. Blum LK, Cao RRL, Sweatt AJ, Bill M, Lahey LJ, Hsi AC, Lee CS, Kongpachith S, Ju C-H, Mao R, Wong HH, Nicolls MR, Zamanian RT, Robinson WH. Circulating plasmablasts are elevated and produce pathogenic anti-endothelial cell autoantibodies in idiopathic pulmonary arterial hypertension. *Eur. J. Immunol.* 2018; 48: 874–884.
5. Heukels P, Corneth OBJ, Uden D van, Hulst JAC van, Toorn LM van den, Bosch AE van den, Wijsenbeek MS, Boomars KA, Kool M, Hendriks RW. Loss of immune homeostasis in patients with idiopathic pulmonary arterial hypertension. *Thorax* BMJ Publishing Group Ltd; 2021; 76: 1209–1218.
6. Perros F, Dorfmüller P, Montani D, Hammad H, Waelput W, Girerd B, Raymond N, Mercier O, Mussot S, Cohen-Kaminsky S, Humbert M, Lambrecht BN. Pulmonary Lymphoid Neogenesis in Idiopathic Pulmonary Arterial Hypertension. *Am. J. Respir. Crit. Care Med.* 2012; 185: 311–321.
7. López-Cacho JM, Gallardo S, Posada M, Aguerri M, Calzada D, Mayayo T, González-Rodríguez ML, Rabasco AM, Lahoz C, Cárdaba B. Association of immunological cell profiles with specific clinical phenotypes of scleroderma disease. *BioMed Res. Int.* 2014; 2014: 148293.
8. Zhang T, Huang C, Luo H, Li J, Huang H, Liu X, Zhan S. Identification of key genes and immune profile in limited cutaneous systemic sclerosis-associated pulmonary arterial hypertension by bioinformatics analysis. *Life Sci.* 2021; 271: 119151.
9. Pendergrass SA, Hayes E, Farina G, Lemaire R, Farber HW, Whitfield ML, Lafyatis R. Limited Systemic Sclerosis Patients with Pulmonary Arterial Hypertension Show Biomarkers of Inflammation and Vascular Injury. *PLOS ONE* Public Library of Science; 2010; 5: e12106.
10. de Bourcy CFA, Dekker CL, Davis MM, Nicolls MR, Quake SR. Dynamics of the human antibody repertoire after B cell depletion in systemic sclerosis. *Sci. Immunol.* 2017; 2.
11. Jones RJ, De Bie EMDD, Groves E, Zalewska KI, Swietlik EM, Treacy CM, Martin JM, Polwarth G, Li W, Guo J, Baxendale HE, Coleman S, Savinykh N, Coghlan JG, Corris PA, Howard LS, Johnson MK, Church C, Kiely DG, Lawrie A, Lordan JL, Mackenzie Ross RV, Pepke Zaba J, Wilkins MR, Wort SJ, Fiorillo E, Orrù V, Cucca F, Rhodes CJ, Gräf S, et al. Autoimmunity Is a Significant Feature of Idiopathic Pulmonary Arterial Hypertension. *Am. J. Respir. Crit. Care Med.* 2022; 206: 81–93.
12. Kariotis S, Jammeh E, Swietlik EM, Pickworth JA, Rhodes CJ, Otero P, Wharton J, Iremonger J, Dunning MJ, Pandya D, Mascarenhas TS, Errington N, Thompson AAR, Romanoski CE, Rischard F, Garcia JGN, Yuan JX-J, An T-HS, Desai AA, Coghlan G, Lordan J, Corris PA, Howard LS, Condliffe R, Kiely DG, Church C, Pepke-Zaba J, Toshner M, Wort S, Gräf S, et al. Biological heterogeneity in idiopathic pulmonary arterial hypertension identified through unsupervised transcriptomic profiling of whole blood. *Nat. Commun.* Nature Publishing Group; 2021; 12: 7104.
13. Li C, Xia J, Yiminniyaze R, Dong L, Li S. Hub Genes and Immune Cell Infiltration in Hypoxia-Induced Pulmonary Hypertension: Bioinformatics Analysis and In Vivo Validation. *Comb. Chem. High Throughput Screen.* 26: 2085–2097.
14. Ricard L, Malard F, Riviere S, Laurent C, Fain O, Mohty M, Gaugler B, Mekinian A. Regulatory B cell imbalance correlates with Tfh expansion in systemic sclerosis. *Clin. Exp. Rheumatol.* 2021; 39 Suppl 131: 20–24.
15. Marrapodi R, Pellicano C, Radicchio G, Leodori G, Colantuono S, Iacolare A, Gigante A, Visentini M, Rosato E. CD21low B cells in systemic sclerosis: A possible marker of vascular complications. *Clin. Immunol. Orlando Fla* 2020; 213: 108364.
16. Ulrich S, Taraseviciene-Stewart L, Huber LC, Speich R, Voelkel N. Peripheral blood B lymphocytes derived from patients with idiopathic pulmonary arterial hypertension express a different RNA pattern compared with healthy controls: a cross sectional study. *Respir. Res.* 2008; 9: 20.
17. Cyster JG, Allen CDC. B Cell Responses: Cell Interaction Dynamics and Decisions. *Cell* 2019; 177: 524–540.
18. Zhang Y, Tian J, Xiao F, Zheng L, Zhu X, Wu L, Zhao C, Wang S, Rui K, Zou H, Lu L. B cell-activating factor

and its targeted therapy in autoimmune diseases. *Cytokine Growth Factor Rev.* 2022; 64: 57–70.
19. Young RM, Staudt LM. Targeting pathological B cell receptor signalling in lymphoid malignancies. *Nat. Rev. Drug Discov.* Nature Publishing Group; 2013; 12: 229–243.
20. Duo M, Liu Z, Zhang Y, Li P, Weng S, Xu H, Wang Y, Jiang T, Wu R, Cheng Z. Construction of a diagnostic signature and immune landscape of pulmonary arterial hypertension. *Front. Cardiovasc. Med.* [Internet] 2022 [cited 2023 May 16]; 9Available from: https://www.frontiersin.org/articles/10.3389/fcvm.2022.940894.
21. Chen Y, Wu C, Wang X, Zhou X, Kang K, Cao Z, Yang Y, Zhong Y, Xiao G. Weighted gene co-expression network analysis identifies dysregulated B-cell receptor signaling pathway and novel genes in pulmonary arterial hypertension. *Front. Cardiovasc. Med.* 2022; 9: 909399.
22. Yu M, Wu X, Peng L, Yang M, Zhou H, Xu J, Wang J, Wang H, Xie W, Kong H. Inhibition of Bruton's Tyrosine Kinase Alleviates Monocrotaline-Induced Pulmonary Arterial Hypertension by Modulating Macrophage Polarization. *Oxid. Med. Cell. Longev.* 2022; 2022: 6526036.
23. Vazquez MI, Catalan-Dibene J, Zlotnik A. B cells responses and cytokine production are regulated by their immune microenvironment. *Cytokine* 2015; 74: 318–326.
24. Chen G, Zuo S, Tang J, Zuo C, Jia D, Liu Q, Liu G, Zhu Q, Wang Y, Zhang J, Shen Y, Chen D, Yuan P, Qin Z, Ruan C, Ye J, Wang X-J, Zhou Y, Gao P, Zhang P, Liu J, Jing Z-C, Lu A, Yu Y. Inhibition of CRTH2-mediated Th2 activation attenuates pulmonary hypertension in mice. *J. Exp. Med.* 2018; 215: 2175–2195.
25. Shu T, Liu Y, Zhou Y, Zhou Z, Li B, Xing Y, Yang P, Pang J, Li J, Song X, Ning X, Qi X, Xiong C, Yang H, Chen Q, Chen J, Yu Y, Wang J, Wang C. Inhibition of immunoglobulin E attenuates pulmonary hypertension. *Nat. Cardiovasc. Res.* Nature Publishing Group; 2022; 1: 665–678.
26. Krystel-Whittemore M, Dileepan KN, Wood JG. Mast Cell: A Multi-Functional Master Cell. *Front. Immunol.* [Internet] 2016 [cited 2023 May 9]; 6Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2015.00620.
27. Taraseviciene-Stewart L, Nicolls MR, Kraskauskas D, Scerbavicius R, Burns N, Cool C, Wood K, Parr JE, Boackle SA, Voelkel NF. Absence of T cells confers increased pulmonary arterial hypertension and vascular remodeling. *Am. J. Respir. Crit. Care Med.* 2007; 175: 1280–1289.
28. Hoffmann J, Yin J, Kukucka M, Yin N, Saarikko I, Sterner-Kock A, Fujii H, Leong-Poi H, Kuppe H, Schermuly RT, Kuebler WM. Mast cells promote lung vascular remodelling in pulmonary hypertension. *Eur. Respir. J.* 2011; 37: 1400–1410.
29. Breitling S, Hui Z, Zabini D, Hu Y, Hoffmann J, Goldenberg NM, Tabuchi A, Buelow R, Dos Santos C, Kuebler WM. The mast cell-B cell axis in lung vascular remodeling and pulmonary hypertension. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2017; 312: L710–L721.
30. Savai R, Pullamsetti SS, Kolbe J, Bieniek E, Voswinckel R, Fink L, Scheed A, Ritter C, Dahal BK, Vater A, Klussmann S, Ghofrani HA, Weissmann N, Klepetko W, Banat GA, Seeger W, Grimminger F, Schermuly RT. Immune and Inflammatory Cell Involvement in the Pathology of Idiopathic Pulmonary Arterial Hypertension. *Am. J. Respir. Crit. Care Med.* 2012; 186: 897–908.
31. Mansueto G, Di Napoli M, Campobasso CP, Slevin M. Pulmonary arterial hypertension (PAH) from autopsy study: T-cells, B-cells and mastocytes detection as morphological evidence of immunologically mediated pathogenesis. *Pathol. - Res. Pract.* 2021; 225: 153552.
32. Morita R, Schmitt N, Bentebibel S-E, Ranganathan R, Bourdery L, Zurawski G, Foucat E, Dullaers M, Oh S, Sabzghabaei N, Lavecchio EM, Punaro M, Pascual V, Banchereau J, Ueno H. Human Blood CXCR5+CD4+ T Cells Are Counterparts of T Follicular Cells and Contain Specific Subsets that Differentially Support Antibody Secretion. *Immunity* Elsevier; 2011; 34: 108–121.
33. Ricard L, Jachiet V, Malard F, Ye Y, Stocker N, Rivière S, Senet P, Monfort J-B, Fain O, Mohty M, Gaugler B, Mekinian A. Circulating follicular helper T cells are increased in systemic sclerosis and promote plasmablast differentiation through the IL-21 pathway which can be inhibited by ruxolitinib. *Ann. Rheum. Dis.* 2019; 78: 539–550.
34. Abdo MS, Mohammed RHA, Raslan HM, Gaber SM. Serum B-cell activating factor assessment in a population of Egyptian patients with systemic sclerosis. *Int. J. Rheum. Dis.* 2013; 16: 148–156.
35. Wutte N, Kovacs G, Berghold A, Reiter H, Aberer W, Aberer E. CXCL13 and B-cell activating factor as putative biomarkers in systemic sclerosis. *Br. J. Dermatol.* 2013; 169: 723–725.
36. Minh VN, Hau KT, Takashi M, Ha VN, Bao LH, Huyen ML, Huu DL, Van TN, Gandolfi M, Satolli F, Feliciani C, Tirant M, Vojvodic A, Lotti T. Efficacy of BAFF in Monitoring Treatment Response in Early Vietnamese Systemic Sclerosis Patients. *Open Access Maced. J. Med. Sci.* 2019; 7: 264–268.
37. Sanges S, Guerrier T, Duhamel A, Guilbert L, Hauspie C, Largy A, Balden M, Podevin C, Lefèvre G, Jendoubi M, Speca S, Hachulla É, Sobanski V, Dubucquoi S, Launay D. Soluble markers of B cell activation suggest a role of B cells in the pathogenesis of systemic sclerosis-associated pulmonary arterial hypertension. *Front.*

Immunol. 2022; 13: 954007.
38. Shinya Y, Hiraide T, Momoi M, Goto S, Suzuki H, Katsumata Y, Kurebayashi Y, Endo J, Sano M, Fukuda K, Kosaki K, Kataoka M. TNFRSF13B c.226G>A (p.Gly76Ser) as a Novel Causative Mutation for Pulmonary Arterial Hypertension. *J. Am. Heart Assoc. Cardiovasc. Cerebrovasc. Dis.* 2021; 10: e019245.
39. Fu M, Song J. Single-Cell Transcriptomics Reveals the Cellular Heterogeneity of Cardiovascular Diseases. *Front. Cardiovasc. Med.* 2021; 8: 643519.
40. Huan T, Zhang B, Wang Z, Joehanes R, Zhu J, Johnson AD, Ying S, Munson PJ, Raghavachari N, Wang R, Liu P, Courchesne P, Hwang S-J, Assimes TL, McPherson R, Samani NJ, Schunkert H, Coronary ARteryDIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) Consortium, International Consortium for Blood Pressure GWAS (ICBP), Meng Q, Suver C, O'Donnell CJ, Derry J, Yang X, Levy D. A systems biology framework identifies molecular underpinnings of coronary heart disease. *Arterioscler. Thromb. Vasc. Biol.* 2013; 33: 1427–1434.
41. Kyaw T, Tay C, Hosseini H, Kanellakis P, Gadowski T, MacKay F, Tipping P, Bobik A, Toh B-H. Depletion of B2 but not B1a B cells in BAFF receptor-deficient ApoE mice attenuates atherosclerosis by potently ameliorating arterial inflammation. *PloS One* 2012; 7: e29371.
42. Sage AP, Tsiantoulas D, Baker L, Harrison J, Masters L, Murphy D, Loinard C, Binder CJ, Mallat Z. BAFF receptor deficiency reduces the development of atherosclerosis in mice--brief report. *Arterioscler. Thromb. Vasc. Biol.* 2012; 32: 1573–1576.
43. Kyaw T, Cui P, Tay C, Kanellakis P, Hosseini H, Liu E, Rolink AG, Tipping P, Bobik A, Toh B-H. BAFF Receptor mAb Treatment Ameliorates Development and Progression of Atherosclerosis in Hyperlipidemic ApoE−/− Mice. *PLoS ONE* 2013; 8: e60430.
44. Tsiantoulas D, Sage AP, Göderle L, Ozsvar-Kozma M, Murphy D, Porsch F, Pasterkamp G, Menche J, Schneider P, Mallat Z, Binder CJ. B Cell–Activating Factor Neutralization Aggravates Atherosclerosis. *Circulation* American Heart Association; 2018; 138: 2263–2273.
45. Tsiantoulas D, Eslami M, Obermayer G, Clement M, Smeets D, Mayer FJ, Kiss MG, Enders L, Weißer J, Göderle L, Lambert J, Frommlet F, Mueller A, Hendrikx T, Ozsvar-Kozma M, Porsch F, Willen L, Afonyushkin T, Murphy JE, Fogelstrand P, Donzé O, Pasterkamp G, Hoke M, Kubicek S, Jørgensen HF, Danchin N, Simon T, Scharnagl H, März W, Borén J, et al. APRIL limits atherosclerosis by binding to heparan sulfate proteoglycans. *Nature* 2021; 597: 92–96.
46. Spinelli FR, Barbati C, Cecarelli F, Morello F, Colasanti T, Vomero M, Massaro L, Orefice V, Alessandri C, Valesini G, Conti F. B lymphocyte stimulator modulates number and function of endothelial progenitor cells in systemic lupus erythematosus. *Arthritis Res. Ther.* 2019; 21: 245.
47. DiLillo DJ, Horikawa M, Tedder TF. B-lymphocyte effector functions in health and disease. *Immunol. Res.* 2011; 49: 281–292.
48. Colvin KL, Cripe PJ, Ivy DD, Stenmark KR, Yeager ME. Bronchus-associated Lymphoid Tissue in Pulmonary Hypertension Produces Pathologic Autoantibodies. *Am. J. Respir. Crit. Care Med.* 2013; 188: 1126–1136.
49. Park S-H, Chen W-C, Durmus N, Bleck B, Reibman J, Riemekasten G, Grunig G. The Effects of Antigen-Specific IgG1 Antibody for the Pulmonary-Hypertension-Phenotype and B Cells for Inflammation in Mice Exposed to Antigen and Fine Particles from Air Pollution. *PLOS ONE* 2015; 10: e0129910.
50. Becker MO, Kill A, Kutsche M, Guenther J, Rose A, Tabeling C, Witzenrath M, Kühl AA, Heidecke H, Ghofrani HA, Tiede H, Schermuly RT, Nickel N, Hoeper MM, Lukitsch I, Gollasch M, Kuebler WM, Bock S, Burmester GR, Dragun D, Riemekasten G. Vascular receptor autoantibodies in pulmonary arterial hypertension associated with systemic sclerosis. *Am. J. Respir. Crit. Care Med.* 2014; 190: 808–817.
51. Frid MG, McKeon BA, Thurman JM, Maron BA, Li M, Zhang H, Kumar S, Sullivan T, Laskowsky J, Fini MA, Hu S, Tuder RM, Gandjeva A, Wilkins MR, Rhodes CJ, Ghataorhe P, Leopold JA, Wang R-S, Holers VM, Stenmark KR. Immunoglobulin-driven Complement Activation Regulates Proinflammatory Remodeling in Pulmonary Hypertension. *Am. J. Respir. Crit. Care Med.* 2020; 201: 224–239.
52. Jones RJ, De Bie EMDD, Groves E, Zalewska KI, Swietlik EM, Treacy CM, Martin JM, Polwarth G, Li W, Guo J, Baxendale HE, Coleman S, Savinykh N, Coghlan JG, Corris PA, Howard LS, Johnson MK, Church C, Kiely DG, Lawrie A, Lordan JL, Mackenzie Ross RV, Pepke Zaba J, Wilkins MR, Wort SJ, Fiorillo E, Orrù V, Cucca F, Rhodes CJ, Gräf S, et al. Autoimmunity Is a Significant Feature of Idiopathic Pulmonary Arterial Hypertension. *Am. J. Respir. Crit. Care Med.* American Thoracic Society - AJRCCM; 2022; 206: 81–93.
53. Huang J, Li M, Tian Z, Hsieh E, Wang Q, Liu Y, Xu D, Hou Y, Zhao J, Guo X, Lai J, Hu C, Song N, Sun Q, Sun Q, Zhang F, Zhao Y, Zeng X. Clinical and laboratory characteristics of systemic sclerosis patients with pulmonary arterial hypertension in China. *Clin. Exp. Rheumatol.* 2014; 32: S-115-121.
54. Rich S, Kieras K, Hart K, Groves BM, Stobo JD, Brundage BH. Antinuclear antibodies in primary pulmonary hypertension. *J. Am. Coll. Cardiol.* 1986; 8: 1307–1311.

55. Yanai-Landau H, Amital H, Bar-Dayan Y, Levy Y, Gur H, Lin HC, Alosachie IJ, Peter JB, Shoenfeld Y. Autoimmune Aspects of Primary Pulmonary Hypertension. *Pathobiology* 1995; 63: 71–75.
56. Jiang Y, Turk MA, Pope JE. Factors associated with pulmonary arterial hypertension (PAH) in systemic sclerosis (SSc). *Autoimmun. Rev.* 2020; 19: 102602.
57. Hachulla E, Jais X, Cinquetti G, Clerson P, Rottat L, Launay D, Cottin V, Habib G, Prevot G, Chabanne C, Foïs E, Amoura Z, Mouthon L, Le Guern V, Montani D, Simonneau G, Humbert M, Sobanski V, Sitbon O, French Collaborators Recruiting Members(∗). Pulmonary Arterial Hypertension Associated With Systemic Lupus Erythematosus: Results From the French Pulmonary Hypertension Registry. *Chest* 2018; 153: 143–151.
58. Ruiz-Irastorza G, Garmendia M, Villar I, Egurbide M-V, Aguirre C. Pulmonary hypertension in systemic lupus erythematosus: prevalence, predictors and diagnostic strategy. *Autoimmun. Rev.* 2013; 12: 410–415.
59. Sobanski V, Giovannelli J, Lynch BM, Schreiber BE, Nihtyanova SI, Harvey J, Handler CE, Denton CP, Coghlan JG. Characteristics and Survival of Anti-U1 RNP Antibody-Positive Patients With Connective Tissue Disease-Associated Pulmonary Arterial Hypertension. *Arthritis Rheumatol. Hoboken NJ* 2016; 68: 484–493.
60. Chepy A, Bourel L, Koether V, Launay D, Dubucquoi S, Sobanski V. Can Antinuclear Antibodies Have a Pathogenic Role in Systemic Sclerosis? *Front. Immunol.* [Internet] 2022 [cited 2023 May 9]; 13Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2022.930970.
61. Raschi E, Privitera D, Bodio C, Lonati PA, Borghi MO, Ingegnoli F, Meroni PL, Chighizola CB. Scleroderma-specific autoantibodies embedded in immune complexes mediate endothelial damage: an early event in the pathogenesis of systemic sclerosis. *Arthritis Res. Ther.* 2020; 22: 265.
62. Okawa-Takatsuji M, Aotsuka S, Fujinami M, Uwatoko S, Kinoshita M, Sumiya M. Up-regulation of intercellular adhesion molecule-1 (ICAM-1), endothelial leucocyte adhesion molecule-1 (ELAM-1) and class II MHC molecules on pulmonary artery endothelial cells by antibodies against U1-ribonucleoprotein. *Clin. Exp. Immunol.* 1999; 116: 174–180.
63. Shu T, Xing Y, Wang J. Autoimmunity in Pulmonary Arterial Hypertension: Evidence for Local Immunoglobulin Production. *Front. Cardiovasc. Med.* 2021; 8: 680109.
64. Arends SJ, Damoiseaux J, Duijvestijn A, Debrus-Palmans L, Boomars K, Broers B, Tervaert JWC, Paassen P van. Prevalence of anti-endothelial cell antibodies in idiopathic pulmonary arterial hypertension. *Eur. Respir. J.* 2010; 35: 923–925.
65. Liu X-D, Guo S-Y, Yang L-L, Zhang X-L, Fu W-Y, Wang X-F. Anti-endothelial cell antibodies in connective tissue diseases associated with pulmonary arterial hypertension. *J. Thorac. Dis.* 2014; 6: 497–502.
66. Li M, Ai J, Tian Z, Fang Q, Zheng W, Zeng X, Zeng X. Prevalence of Anti-endothelial Cell Antibodies in Patients with Pulmonary Arterial Hypertension Associated with Connective Tissue Diseases. *Chin. Med. Sci. J.* 2010; 25: 27–31.
67. Yoshio T, Masuyama J, Sumiya M, Minota S, Kano S. Antiendothelial cell antibodies and their relation to pulmonary hypertension in systemic lupus erythematosus. *J. Rheumatol.* 1994; 21: 2058–2063.
68. Negi VS, Tripathy NK, Misra R, Nityanand S. Antiendothelial cell antibodies in scleroderma correlate with severe digital ischemia and pulmonary arterial hypertension. *J. Rheumatol.* 1998; 25: 462–466.
69. Bodolay E, Csipo I, Gál I, Sipka S, Gyimesi E, Szekanecz Z, Szegedi G. Anti-endothelial cell antibodies in mixed connective tissue disease: frequency and association with clinical symptoms. *Clin. Exp. Rheumatol.* 2004; 22: 409–415.
70. Vegh J, Szodoray P, Kappelmayer J, Csipo I, Udvardy M, Lakos G, Aleksza M, Soltesz P, Szilágyi A, Zeher M, Szegedi G, Bodolay E. Clinical and immunoserological characteristics of mixed connective tissue disease associated with pulmonary arterial hypertension. *Scand. J. Immunol.* 2006; 64: 69–76.
71. Tamby MC. Anti-endothelial cell antibodies in idiopathic and systemic sclerosis associated pulmonary arterial hypertension. *Thorax* 2005; 60: 765–772.
72. Dib H, Tamby MC, Bussone G, Regent A, Berezné A, Lafine C, Broussard C, Simonneau G, Guillevin L, Witko-Sarsat V, Humbert M, Mouthon L. Targets of anti-endothelial cell antibodies in pulmonary hypertension and scleroderma. *Eur. Respir. J.* 2012; 39: 1405–1414.
73. Koudstaal T, van Hulst JAC, Das T, Neys SFH, Merkus D, Bergen IM, de Raaf MA, Bogaard HJ, Boon L, van Loo G, Aerts JGJV, Boomars KA, Kool M, Hendriks RW. DNGR1-Cre-mediated Deletion of Tnfaip3/A20 in Conventional Dendritic Cells Induces Pulmonary Hypertension in Mice. *Am. J. Respir. Cell Mol. Biol.* 2020; 63: 665–680.
74. Arends SJ, Damoiseaux JGMC, Duijvestijn AM, Debrus-Palmans L, Boomars KA, Rocca H-PB-L, Tervaert JWC, Paassen P van. Functional implications of IgG anti-endothelial cell antibodies in pulmonary arterial hypertension. *Autoimmunity* 2013; 46: 463–470.
75. Mihai C, Tervaert JWC. Anti-endothelial cell antibodies in systemic sclerosis. *Ann. Rheum. Dis.* 2010; 69: 319–324.

76. Arends SJ, Damoiseaux JGMC, Duijvestijn AM, Debrus-Palmans L, Vroomen M, Boomars KA, Brunner-La Rocca H-P, Reutelingsperger CPM, Cohen Tervaert JW, van Paassen P. Immunoglobulin G anti-endothelial cell antibodies: inducers of endothelial cell apoptosis in pulmonary arterial hypertension? *Clin. Exp. Immunol.* 2013; 174: 433–440.
77. Bussone G, Tamby MC, Calzas C, Kherbeck N, Sahbatou Y, Sanson C, Ghazal K, Dib H, Weksler BB, Broussard C, Verrecchia F, Yaici A, Witko-Sarsat V, Simonneau G, Guillevin L, Humbert M, Mouthon L. IgG from patients with pulmonary arterial hypertension and/or systemic sclerosis binds to vascular smooth muscle cells and induces cell contraction. *Ann. Rheum. Dis.* 2012; 71: 596–605.
78. Kato Y, Kasama T, Soejima M, Kubota T. Anti-enolase 1 antibodies from a patient with systemic lupus erythematosus accompanied by pulmonary arterial hypertension promote migration of pulmonary artery smooth muscle cells. *Immunol. Lett.* 2020; 218: 22–29.
79. Tamby MC, Humbert M, Guilpain P, Servettaz A, Dupin N, Christner JJ, Simonneau G, Fermanian J, Weill B, Guillevin L, Mouthon L. Antibodies to fibroblasts in idiopathic and scleroderma-associated pulmonary hypertension. *Eur. Respir. J.* 2006; 28: 799–807.
80. Terrier B, Tamby MC, Camoin L, Guilpain P, Broussard C, Bussone G, Yaïci A, Hotellier F, Simonneau G, Guillevin L, Humbert M, Mouthon L. Identification of target antigens of antifibroblast antibodies in pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* 2008; 177: 1128–1134.
81. Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, Fraticelli P, Sambo P, Funaro A, Kazlauskas A, Avvedimento EV, Gabrielli A. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N. Engl. J. Med.* 2006; 354: 2667–2676.
82. Chepy A, Vivier S, Bray F, Ternynck C, Meneboo J-P, Figeac M, Filiot A, Guilbert L, Jendoubi M, Rolando C, Launay D, Dubucquoi S, Marot G, Sobanski V. Effects of Immunoglobulins G From Systemic Sclerosis Patients in Normal Dermal Fibroblasts: A Multi-Omics Study. *Front. Immunol.* 2022; 13: 904631.
83. Morse, Antohi, Kasturi, Saito, Fotino, Humbert, Simonneau, Basst, Bona. Fine Specificity of Anti-Fibrillin-1 Autoantibodies in Primary Pulmonary Hypertension Syndrome. *Scand. J. Immunol.* 2000; 51: 607–611.
84. Zhou X, Tan FK, Milewicz DM, Guo X, Bona CA, Arnett FC. Autoantibodies to Fibrillin-1 Activate Normal Human Fibroblasts in Culture through the TGF-β Pathway to Recapitulate the "Scleroderma Phenotype." *J. Immunol.* 2005; 175: 4555–4560.
85. Cabral-Marques O, Marques A, Giil LM, De Vito R, Rademacher J, Günther J, Lange T, Humrich JY, Klapa S, Schinke S, Schimke LF, Marschner G, Pitann S, Adler S, Dechend R, Müller DN, Braicu I, Sehouli J, Schulze-Forster K, Trippel T, Scheibenbogen C, Staff A, Mertens PR, Löbel M, Mastroianni J, Plattfaut C, Gieseler F, Dragun D, Engelhardt BE, Fernandez-Cabezudo MJ, et al. GPCR-specific autoantibody signatures are associated with physiological and pathological immune homeostasis. *Nat. Commun.* Nature Publishing Group; 2018; 9: 5224.
86. Cabral-Marques O, Riemekasten G. Functional autoantibodies targeting G protein-coupled receptors in rheumatic diseases. *Nat. Rev. Rheumatol.* Nature Publishing Group; 2017; 13: 648–656.
87. Guo L, Li M, Chen Y, Wang Q, Tian Z, Pan S, Zeng X, Ye S. Anti–Endothelin Receptor Type A Autoantibodies in Systemic Lupus Erythematosus–Associated Pulmonary Arterial Hypertension. *Arthritis Rheumatol.* 2015; 67: 2394–2402.
88. Tabeling C, González Calera CR, Lienau J, Höppner J, Tschernig T, Kershaw O, Gutbier B, Naujoks J, Herbert J, Opitz B, Gruber AD, Hocher B, Suttorp N, Heidecke H, Burmester G-R, Riemekasten G, Siegert E, Kuebler WM, Witzenrath M. Endothelin B Receptor Immunodynamics in Pulmonary Arterial Hypertension. *Front. Immunol.* 2022; 13: 895501.
89. Okruhlicova L, Morwinski R, Schulze W, Bartel S, Weismann P, Tribulova N, Wallukat G. Autoantibodies against G-protein-coupled receptors modulate heart mast cells. *Cell. Mol. Immunol.* 2007; 4: 127–133.
90. Dandel M, Wallukat G, Englert A, Hetzer R. Immunoadsorption therapy for dilated cardiomyopathy and pulmonary arterial hypertension. *Atheroscler. Suppl.* 2013; 14: 203–211.
91. Nagel C, Ewert R, Egenlauf B, Lehmkuhl HB, Rosenkranz S, Benjamin N, Schwenger V, Herth FJF, Grünig E. Safety and Efficacy of Immunoadsorption as an Add-On to Medical Treatment in Patients with Severe Idiopathic Pulmonary Arterial Hypertension. *Respir. Int. Rev. Thorac. Dis.* 2017; 94: 263–271.
92. Gluschke H, Siegert E, Minich WB, Hackler J, Riemekasten G, Kuebler WM, Simmons S, Schomburg L. Autoimmunity to Sphingosine-1-Phosphate-Receptors in Systemic Sclerosis and Pulmonary Arterial Hypertension. *Front. Immunol.* [Internet] 2022 [cited 2022 Nov 18]; 13Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2022.935787.
93. Riemekasten G, Philippe A, Näther M, Slowinski T, Müller DN, Heidecke H, Matucci-Cerinic M, Czirják L, Lukitsch I, Becker M, Kill A, van Laar JM, Catar R, Luft FC, Burmester GR, Hegner B, Dragun D. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. *Ann. Rheum. Dis.* 2011; 70: 530–536.
94. Yue X, Yin J, Wang X, Heidecke H, Hackel AM, Dong X, Kasper B, Wen L, Zhang L, Schulze-Forster K, Junker

J, Grasshoff H, Müller A, Wallukat G, Schimke I, Zeiner J, Deckstein LM, Mertens N, Kerstein-Staehle A, Hundt JE, Kostenis E, Yu X, Riemekasten G, Petersen F. Induced antibodies directed to the angiotensin receptor type 1 provoke skin and lung inflammation, dermal fibrosis and act species overarching. *Ann. Rheum. Dis.* 2022; 81: 1281–1289.
95. Kill A, Tabeling C, Undeutsch R, Kühl AA, Günther J, Radic M, Becker MO, Heidecke H, Worm M, Witzenrath M, Burmester G-R, Dragun D, Riemekasten G. Autoantibodies to angiotensin and endothelin receptors in systemic sclerosis induce cellular and systemic events associated with disease pathogenesis. *Arthritis Res. Ther.* 2014; 16: R29.
96. Günther J, Kill A, Becker MO, Heidecke H, Rademacher J, Siegert E, Radić M, Burmester G-R, Dragun D, Riemekasten G. Angiotensin receptor type 1 and endothelin receptor type A on immune cells mediate migration and the expression of IL-8 and CCL18 when stimulated by autoantibodies from systemic sclerosis patients. *Arthritis Res. Ther.* 2014; 16: R65.
97. Satoh T, Kimura K, Okano Y, Hirakata M, Kawakami Y, Kuwana M. Lack of circulating autoantibodies to bone morphogenetic protein receptor-II or activin receptor-like kinase 1 in mixed connective tissue disease patients with pulmonary arterial hypertension. *Rheumatology* 2005; 44: 192–196.
98. Xing Y, Zhao J, Zhou M, Jing S, Zhao X, Mao P, Qian J, Huang C, Tian Z, Wang Q, Zeng X, Li M, Yang J. The LPS induced pyroptosis exacerbates BMPR2 signaling deficiency to potentiate SLE-PAH. *FASEB J.* 2021; 35: e22044.
99. Matsushita T. Regulatory and effector B cells: Friends or foes? *J. Dermatol. Sci.* 2019; 93: 2–7.
100. Dumoitier N, Chaigne B, Régent A, Lofek S, Mhibik M, Dorfmüller P, Terrier B, London J, Bérezné A, Tamas N, Varin-Blank N, Mouthon L. Scleroderma Peripheral B Lymphocytes Secrete Interleukin-6 and Transforming Growth Factor β and Activate Fibroblasts. *Arthritis Rheumatol. Hoboken NJ* 2017; 69: 1078–1089.
101. Matsushita T, Hasegawa M, Yanaba K, Kodera M, Takehara K, Sato S. Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum.* 2006; 54: 192–201.
102. González-Tajuelo R, de la Fuente-Fernández M, Morales-Cano D, Muñoz-Callejas A, González-Sánchez E, Silván J, Serrador JM, Cadenas S, Barreira B, Espartero-Santos M, Gamallo C, Vicente-Rabaneda EF, Castañeda S, Pérez-Vizcaíno F, Cogolludo Á, Jiménez-Borreguero LJ, Urzainqui A. Spontaneous Pulmonary Hypertension Associated With Systemic Sclerosis in P-Selectin Glycoprotein Ligand 1-Deficient Mice. *Arthritis Rheumatol. Hoboken NJ* 2020; 72: 477–487.
103. Mizuno S, Farkas L, Al Husseini A, Farkas D, Gomez-Arroyo J, Kraskauskas D, Nicolls MR, Cool CD, Bogaard HJ, Voelkel NF. Severe Pulmonary Arterial Hypertension Induced by SU5416 and Ovalbumin Immunization. *Am. J. Respir. Cell Mol. Biol.* 2012; 47: 679–687.
104. Catalán D, Mansilla MA, Ferrier A, Soto L, Oleinika K, Aguillón JC, Aravena O. Immunosuppressive Mechanisms of Regulatory B Cells. *Front. Immunol.* [Internet] 2021 [cited 2023 May 11]; 12Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.611795.
105. Li C, Liu P, Yao H, Zhu H, Zhang S, Meng F, Li S, Li G, Peng Y, Gu J, Zhu L, Jiang Y, Dai A. Regulatory B cells protect against chronic hypoxia-induced pulmonary hypertension by modulating the Tfh/Tfr immune balance. *Immunology* 2022; .
106. van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Söllner S, Akdis DG, Rückert B, Akdis CA, Akdis M. IgG4 production is confined to human IL-10–producing regulatory B cells that suppress antigen-specific immune responses. *J. Allergy Clin. Immunol.* 2013; 131: 1204–1212.
107. Rajapreyar I, Joly J, Tallaj J, Pamboukian SV, Assad AH, Lenneman C, Litovsky S, Chatterjee A, Hoopes C, Lenneman A. Pulmonary Vascular Disease Due to Plasma Cell Dyscrasia. *Mayo Clin. Proc. Innov. Qual. Outcomes* 2020; 5: 210–218.
108. Caslin AW, Heath D, Madden B, Yacoub M, Gosney JR, Smith P. The histopathology of 36 cases of plexogenic pulmonary arteriopathy. *Histopathology* 1990; 16: 9–19.
109. Tuder RM, Groves B, Badesch DB, Voelkel NF. Exuberant endothelial cell growth and elements of inflammation are present in plexiform lesions of pulmonary hypertension. *Am. J. Pathol.* 1994; 144: 275–285.
110. Taraseviciene-Stewart L, Nicolls MR, Kraskauskas D, Scerbavicius R, Burns N, Cool C, Wood K, Parr JE, Boackle SA, Voelkel NF. Absence of T cells confers increased pulmonary arterial hypertension and vascular remodeling. *Am. J. Respir. Crit. Care Med.* 2007; 175: 1280–1289.
111. Wagner EM, Sánchez J, McClintock JY, Jenkins J, Moldobaeva A. Inflammation and ischemia-induced lung angiogenesis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2008; 294: L351-357.
112. Larsen K-O, Yndestad A, Sjaastad I, Løberg EM, Goverud IL, Halvorsen B, Jia J, Andreassen AK, Husberg C, Jonasson S, Lipp M, Christensen G, Aukrust P, Skjønsberg OH. Lack of CCR7 induces pulmonary hypertension involving perivascular leukocyte infiltration and inflammation. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* 2011; 301:

L50–L59.
113. Koudstaal T, van Hulst JAC, Das T, Neys SFH, Merkus D, Bergen IM, de Raaf MA, Bogaard HJ, Boon L, van Loo G, Aerts JGJV, Boomars KA, Kool M, Hendriks RW. DNGR1-Cre–mediated Deletion of *Tnfaip3* /A20 in Conventional Dendritic Cells Induces Pulmonary Hypertension in Mice. *Am. J. Respir. Cell Mol. Biol.* 2020; 63: 665–680.
114. Breitling S, Hui Z, Zabini D, Hu Y, Hoffmann J, Goldenberg NM, Tabuchi A, Buelow R, Dos Santos C, Kuebler WM. The mast cell–B cell axis in lung vascular remodeling and pulmonary hypertension. *Am. J. Physiol.-Lung Cell. Mol. Physiol.* American Physiological Society; 2017; 312: L710–L721.
115. Saito T, Miyagawa K, Chen S-Y, Tamosiuniene R, Wang L, Sharpe O, Samayoa E, Harada D, Moonen J-RAJ, Cao A, Chen P-I, Hennigs JK, Gu M, Li CG, Leib RD, Li D, Adams CM, Del Rosario PA, Bill M, Haddad F, Montoya JG, Robinson WH, Fantl WJ, Nolan GP, Zamanian RT, Nicolls MR, Chiu CY, Ariza ME, Rabinovitch M. Upregulation of Human Endogenous Retrovirus-K Is Linked to Immunity and Inflammation in Pulmonary Arterial Hypertension. *Circulation* 2017; 136: 1920–1935.
116. Kang S, Fedoriw Y, Brenneman EK, Truong YK, Kikly K, Vilen BJ. BAFF Induces Tertiary Lymphoid Structures and Positions T Cells within the Glomeruli during Lupus Nephritis. *J. Immunol. Baltim. Md 1950* 2017; 198: 2602–2611.
117. Hennigan S, Channick RN, Silverman GJ. Rituximab treatment of pulmonary arterial hypertension associated with systemic lupus erythematosus: a case report. *Lupus* 2008; 17: 754–756.
118. Braun-Moscovici Y, Butbul-Aviel Y, Guralnik L, Toledano K, Markovits D, Rozin A, Nahir MA, Balbir-Gurman A. Rituximab: rescue therapy in life-threatening complications or refractory autoimmune diseases: a single center experience. *Rheumatol. Int.* 2012; 33: 1495–1504.
119. Padilla-Ibarra J, Sanchez-Ortiz A, Sandoval-Castro C, Ramos-Remus C. Rituximab treatment for pulmonary arterial hypertension in adult-onset Still's disease. *Clin. Exp. Rheumatol.* 2013; 31: 657–658.
120. Harbaum L, Hennigs JK, Baumann HJ, Bokemeyer C, Olschewski H, Klose H. Complete resolution of idiopathic pulmonary arterial hypertension following chemotherapy. *Eur. Respir. J.* 2014; 43: 1513–1515.
121. Kusaka K, Nakano K, Iwata S, Kubo S, Nishida T, Tanaka Y. Two patients with mixed connective tissue disease complicated by pulmonary arterial hypertension showing contrasting responses to pulmonary vasodilators. *Mod. Rheumatol. Case Rep.* 2020; 4: 253–261.
122. Zamanian RT, Badesch D, Chung L, Domsic RT, Medsger T, Pinckney A, Keyes-Elstein L, D'Aveta C, Spychala M, White RJ, Hassoun PM, Torres F, Sweatt AJ, Molitor JA, Khanna D, Maecker H, Welch B, Goldmuntz E, Nicolls MR. Safety and Efficacy of B-Cell Depletion with Rituximab for the Treatment of Systemic Sclerosis–associated Pulmonary Arterial Hypertension: A Multicenter, Double-Blind, Randomized, Placebo-controlled Trial. *Am. J. Respir. Crit. Care Med.* 2021; 204: 209–221.
123. Zhang Y, Michelakis ED. A Phase-2 NIH-sponsored Randomized Clinical Trial of Rituximab in Scleroderma-associated Pulmonary Arterial Hypertension Did Not Reach Significance for Its Endpoints: End of Story? Not So Fast! *Am. J. Respir. Crit. Care Med.* American Thoracic Society - AJRCCM; 2021; 204: 123–125.
124. Khalesi N, Korani S, Korani M, Johnston TP, Sahebkar A. Bortezomib: a proteasome inhibitor for the treatment of autoimmune diseases. *Inflammopharmacology* 2021; 29: 1291–1306.
125. Kim S-Y, Lee J-H, Huh JW, Kim HJ, Park MK, Ro JY, Oh Y-M, Lee S-D, Lee Y-S. Bortezomib Alleviates Experimental Pulmonary Arterial Hypertension. *Am. J. Respir. Cell Mol. Biol.* American Thoracic Society - AJRCMB; 2012; 47: 698–708.
126. Wang Y-Y, Luan Y, Zhang X, Lin M, Zhang Z-H, Zhu X-B, Ma Y, Wang Y-B. Proteasome inhibitor PS-341 attenuates flow-induced pulmonary arterial hypertension. *Clin. Exp. Med.* 2014; 14: 321–329.
127. Zhang X, Wang Z-S, Luan Y, Lin M, Zhu X-B, Ma Y, Zhang Z-H, Wang Y-B. The effect of PS-341 on pulmonary vascular remodeling in high blood flow-induced pulmonary hypertension. *Int. J. Mol. Med.* Spandidos Publications; 2014; 33: 105–110.
128. Zhang J, Lu W, Chen Y, Jiang Q, Yang K, Li M, Wang Z, Duan X, Xu L, Tang H, Sun D, Wang J. Bortezomib alleviates experimental pulmonary hypertension by regulating intracellular calcium homeostasis in PASMCs. *Am. J. Physiol. Cell Physiol.* 2016; 311: C482-497.
129. Zhu Y, Wu Y, Shi W, Wang J, Yan X, Wang Q, Liu Y, Yang L, Gao L, Li M. Inhibition of ubiquitin proteasome function prevents monocrotaline-induced pulmonary arterial remodeling. *Life Sci.* 2017; 173: 36–42.
130. Wang C, Li Y, Xu L, Zhang Q, Gegentuya null, Tian G. Bortezomib Inhibits Hypoxia-Induced Proliferation by Suppressing Caveolin-1/SOCE/[Ca2+]i Signaling Axis in Human PASMCs. *BioMed Res. Int.* 2021; 2021: 5551504.
131. Chen I-C, Liu Y-C, Wu Y-H, Lo S-H, Wang S-C, Li C-Y, Dai Z-K, Hsu J-H, Yeh C-Y, Tseng Y-H. Proteasome Inhibitors Decrease the Viability of Pulmonary Arterial Smooth Muscle Cells by Restoring Mitofusin-2 Expression under Hypoxic Conditions. *Biomedicines* 2022; 10: 873.
132. Einhaus J, Pecher A-C, Asteriti E, Schmid H, Secker K-A, Duerr-Stoerzer S, Keppeler H, Klein R,

Schneidawind C, Henes J, Schneidawind D. Inhibition of effector B cells by ibrutinib in systemic sclerosis. *Arthritis Res. Ther.* 2020; 22: 66.
133. Dai Y, Chen X, Song X, Chen X, Ma W, Lin J, Wu H, Hu X, Zhou Y, Zhang H, Liao Y, Qiu Z, Zhou Z. Immunotherapy of Endothelin-1 Receptor Type A for Pulmonary Arterial Hypertension. *J. Am. Coll. Cardiol.* 2019; 73: 2567–2580.
134. Dai Y, Qiu Z, Ma W, Li C, Chen X, Song X, Bai Z, Shi D, Zheng J, Pan G, Liao Y, Liao M, Zhou Z. Long-Term Effect of a Vaccine Targeting Endothelin-1 Receptor Type A in Pulmonary Arterial Hypertension. *Front. Cardiovasc. Med.* 2021; 8: 683436.
135. Takahashi Y, Haga S, Ishizaka Y, Mimori A. Autoantibodies to angiotensin-converting enzyme 2 in patients with connective tissue diseases. *Arthritis Res. Ther.* 2010; 12: R85.

Conclusion

L'HTAP-SSc reste grevée d'une morbi-mortalité majeure (1). Nous avons donc entrepris ce travail de thèse dans l'objectif de mettre en évidence de nouveaux biomarqueurs qui puissent améliorer la caractérisation clinique des patients, identifier des mécanismes physiopathologiques méconnus sous-tendant la maladie et ouvrir de nouvelles pistes thérapeutiques.

En explorant le protéome sérique des patients présentant une HTAP-SSc, nous avons identifié 2 pistes de biomarqueurs associés à la maladie :

- *l'axe chemerin-CMKLR1 : chemerin* paraît être un marqueur de substitution robuste des RVP mesurées lors du cathétérisme cardiaque droit ; et l'axe *chemerin*-CMKLR1 pourrait participer au remodelage vasculaire pulmonaire en stimulant la prolifération des CML-AP ;
- *l'axe BAFF-LB :* BAFF semble associé à différents paramètres d'HTAP (VITmax, CVF/DLCO, Nt-pro-BNP, RVP) ; et les LB pourraient contribuer aux lésions de microangiopathie sclérodermique en produisant des médiateurs pro-angiogéniques, notamment *chemerin* (***Figure 1***).

Afin de nous assurer de la fiabilité de nos biomarqueurs, nous avons reproduit nos résultats dans plusieurs cohortes indépendantes de patients HTAP-SSc. La SSc étant une maladie multi-systémique faisant intervenir d'autres processus physiopathologiques, nous avons également confirmé le lien entre nos biomarqueurs et la maladie vasculaire pulmonaire en les testant dans d'autres phénotypes de patients SSc. Ceci conforte la robustesse de nos résultats et leur potentielle utilité sur un plan diagnostique, pronostique, thérapeutique et physiopathologique.

BIOMARQUEURS DE L'HTAP-SSC : PERSPECTIVES DIAGNOSTIQUES

La prise en charge clinique des patients atteints d'HTAP-SSc reste émaillée de nombreux défis, responsables d'un pronostic sombre (1). La SSc confère un surrisque majeur de survenue d'HTP, et justifie un dépistage régulier des patients (2). Néanmoins, les modalités exactes de ce dépistage, l'estimation précise de la probabilité d'HTP et la stratégie d'adressage au cathétérisme cardiaque droit restent mal définies. On prendra ainsi l'exemple du score DETECT (3) : issu d'une vaste étude internationale ayant inclus 466 patients SSc bénéficiant d'une évaluation hémodynamique systématique, cet algorithme décisionnel se caractérise par une excellente valeur prédictive négative mais une mauvaise valeur prédictive positive, conduisant à la réalisation en excès de nombreux cathétérisme cardiaque droit.

On manque ainsi de biomarqueurs permettant de mieux stratifier les patients SSc, à la fois pour évaluer la présence d'une HTP au moment de l'évaluation, mais aussi en termes de risque d'apparition au cours du suivi. Plusieurs études préalables ont tenté de pourvoir ce besoin. Ainsi, Bauer *et al.* ont pu analyser 313 protéines dosés en multiplex sur le sérum des patients inclus dans l'étude DETECT et ont identifié un panel de 8 biomarqueurs (collagen IV, endostatin, IGFBP-2, IGFBP-7, MMP-2, neuropilin-1, Nt-pro-BNP et RAGE) capable de discriminer les patients SSc avec et sans HTAP (4). On notera que, dans cette cohorte, les taux sériques de β2-microglobuline et CXCL13 étaient différentiellement exprimés chez les patients HTAP-SSc ; les taux de BAFF étaient identiques dans les 2 groupes ; et les taux de *chemerin*, bien que dosés, n'étaient pas rapportés par les auteurs. L'étude d'associations avec les paramètres cliniques d'HTAP n'était effectuée qu'avec les 8 protéines sélectionnées et mettait en évidence des corrélations modestes avec les RVP (coefficient *r* de Pearson entre 0,34 et 0,46). D'autres travaux ont identifié plusieurs biomarqueurs candidats, notamment impliqués dans les processus angiogéniques (endoglin, VEGF-A, endothelin-1) et inflammatoires (IFN, IL-6, TNF-α) (5,6). La place exacte de ces nouveaux paramètres biologiques reste cependant à définir pour favoriser leur transfert en routine clinique.

Nous prévoyons ainsi de poursuivre nos travaux en tentant de mieux préciser l'utilité diagnostique potentielle des dosages de *chemerin* et BAFF. Nous doserons les taux sériques de BAFF et *chemerin* de façon longitudinale chez des patients ne présentant pas d'HTP à l'inclusion et développant cette complication au cours du suivi. Nous évaluerons ainsi si une élévation des concentrations de nos biomarqueurs au cours du temps s'associe à l'apparition d'une HTP, et tenteront d'intégrer ces dosages dans les stratégies de dépistage actuelles (associant notamment échographie cardiaque, mesure de la DLCO et du Nt-pro-BNP) afin de déterminer s'ils permettent d'améliorer leur performance diagnostique. En outre, la mise en évidence d'une augmentation progressive des taux de nos biomarqueurs en parallèle de la constitution des lésions vasculaires pulmonaires constituerait un argument fort pour leur implication physiopathologique.

BIOMARQUEURS DE L'HTAP-SSC : PERSPECTIVES PRONOSTIQUES

Les indications d'initiation et d'adaptation thérapeutiques dans l'HTAP-SSc sont guidées par le risque de progression et mortalité à court terme. Actuellement, celui-ci est évalué par un score multimodal, incluant notamment des paramètres cliniques, fonctionnels,

échographiques, hémodynamique et biologiques, et permettant une estimation approximative de la mortalité à 1 an (7) : risque bas (<5%), intermédiaire (5-20%) et élevé (20%). Néanmoins, une importante proportion de patients est classifiée en risque intermédiaire (8) ; et il existe donc un besoin non pourvu de mieux stratifier les patients en termes pronostiques.

Récemment, Boucly *et al.* ont pu tester un panel de 20 biomarqueurs dans 2 cohortes indépendantes de patients atteints d'HTAP idiopathique, héritable ou médicamenteuse (9). De façon assez intéressante, 3 cytokines, β-NGF, CXCL9 et TRAIL, étaient indépendamment associées au pronostic à court terme de l'HTAP, suggérant là encore l'importance du rôle de l'immunité dans la progression de la maladie. Lorsqu'elle était intégrée au modèle actuel d'évaluation du risque, cette signature biologique permettait d'en améliorer la précision.

Nos travaux ont pu démontrer l'existence de corrélation entre nos candidats biomarqueurs et plusieurs paramètres pronostiques de l'HTAP-SSc : *chemerin* et RVP ; BAFF et Nt-pro-BNP. Ainsi, nous nous prévoyons d'étudier la valeur ajoutée de *chemerin* et BAFF dans la stratégie actuelle d'évaluation du risque d'évolution péjorative à court terme. Pour cela, nous étudierons l'association entre les taux initiaux de biomarqueurs et le statut clinique à 1 an, et réaliserons une modélisation du pronostic intégrant les paramètres présents dans le score actuellement utilisé en pratique clinique.

Dans le cas de *chemerin*, la robustesse de son association aux RVP fait poser la question de son utilisation en tant que marqueur de substitution non invasif de l'évaluation hémodynamique au cours du suivi. Ainsi, nous étudierons si la corrélation entre les taux sériques de *chemerin* et les RVP persiste après l'introduction de vasodilatateurs spécifiques, et si la variation de *chemerin* est un reflet adéquat de la variation des RVP sous traitement. Pour cela, nous prolongerons notre collaboration avec l'équipe du centre national de référence HTAP du Kremlin-Bicêtre, afin de collecter des échantillons de sérum prélevés le jour de la réalisation du cathétérisme cardiaque droit des patients HTAP-SSc, et de pouvoir tester nos associations dans des conditions fiables.

BIOMARQUEURS DE L'HTAP-SSC : PERSPECTIVES THERAPEUTIQUES

A l'heure actuelle, les stratégies thérapeutiques dans l'HTAP-SSc reposent essentiellement sur l'utilisation de traitements spécifiques ciblant les voies du NO-GPMc, de l'endothéline ou des prostacyclines (7). L'essentiel de leur effet paraît porté par leurs propriétés

vasodilatatrices ; et leur capacité à agir sur le remodelage vasculaire (prévention de nouvelles lésions, voire réversion des lésions installées) reste hypothétique. L'absence de véritable impact de ces molécules sur l'histoire naturelle de la maladie contribue vraisemblablement à expliquer son pronostic encore sombre. Ainsi, il existe un besoin crucial de développer de nouvelles options thérapeutiques ciblant d'autres processus physiopathologiques.

Plusieurs travaux se sont penchés sur l'utilité de stratégies immunosuppressives dans l'HTAP, notamment associée à la SSc (10). En particulier, un travail récent de l'équipe de Stanford a évalué l'efficacité et la tolérance du rituximab en traitement de l'HTAP-SSc dans un essai randomisé contre placebo (11). La variation du test de marche de 6 minutes entre l'inclusion et la semaine 24, utilisée comme critère de jugement principal, ne différait pas de manière significative entre les 2 bras. Cependant, dans une analyse secondaire utilisant les données jusqu'à la semaine 48, le rituximab était supérieur au placebo. Une approche en *machine learning* identifiait un sous-groupe de patients, caractérisés par de faibles taux de facteur rhumatoïde, d'IL-12 et d'IL-17, comme répondeur au traitement. Sans constituer une preuve formelle de son efficacité, ce travail constitue une preuve de concept invitant à considérer de plus près la place potentielle du rituximab dans l'arsenal thérapeutique de l'HTAP-SSc. De façon intéressante, des données expérimentales ont démontré que le traitement anti-CD20 prévient l'apparition de la maladie dans différents modèles d'HTAP idiopathique, lorsqu'il est utilisé en modalité prophylactique (12,13). Ainsi, il nous a paru pertinent d'étudier la capacité du rituximab à prévenir la survenue d'une HTP chez les patients SSc ne présentant pas de maladie vasculaire pulmonaire connue au moment de l'initiation du traitement anti-CD20. Pour cela, nous avons élaboré l'étude PROPHETS (*Prophylaxis using Rituximab for the Occurrence of Pulmonary HypErTension in Scleroderma*) et eu accès aux données de la base EUSTAR, base européenne colligeant 88 000 visites auprès de 23 000 patients SSc, dont plus de 1 000 ont été exposés au rituximab. Les données sont actuellement en cours d'analyse par notre équipe statistique.

A notre connaissance, les adipokines ne constituent pas pour l'heure une cible thérapeutique à l'étude dans l'HTAP (14). Au vu de nos résultats plaidant pour l'implication de l'axe *chemerin*-CMKLR1 dans le remodelage vasculaire, la question se pose de l'effet d'une inhibition de cette voie physiopathologique sur l'hémodynamique pulmonaire et les lésions de vasculopathie sous-jacentes. Grâce à notre collaboration en cours avec le centre de référence HTAP du Kremlin-Bicêtre, nous prévoyons de tester l'efficacité et la tolérance du

traitement par α-NETA, inhibiteur de CMKLR1, dans un modèle murin d'HTAP idiopathique (rat monocrotaline) dans lequel le rôle de *chemerin* a déjà été souligné. En fonction des résultats obtenus, nous nous poserons la question d'y associer un modèle expérimental plus proche de l'HTAP-SSc, comme la souris $Fra2^{Tg}$.

BIOMARQUEURS DE L'HTAP-SSC : PERSPECTIVES PHYSIOPATHOLOGIQUES

Chemerin : marqueur spécifique du remodelage vasculaire pulmonaire au cours des HTP associés à la SSc ?

Nos travaux ont suggéré le rôle de l'axe *chemerin*-CMKLR1 dans la vasculopathie pulmonaire sous-tendant l'HTAP-SSc, mais également l'absence de l'élévation des taux circulants de *chemerin* dans les autres complications de la maladie, et notamment la fibrose pulmonaire. Ceci fait poser la question de la capacité de *chemerin* à discriminer les HTP de groupe 1 (en lien avec une microangiopathie pulmonaire) et de groupe 3 (liée à une vasoconstriction réactionnelle) chez les patients SSc présentant une fibrose pulmonaire extensive. L'impact clinique en serait majeur, car les modalités thérapeutiques diffèrent entre les groupes (7).

Ainsi, nous souhaitons explorer plus amplement le rôle différentiel de l'axe *chemerin*-CMKLR1 dans les différents phénotypes d'HTP pouvant survenir au cours de la SSc, et tenterons en particulier de déterminer si des anomalies de cet axe peuvent plaider en faveur de l'existence d'une vasculopathie pulmonaire en cas d'association d'HTP et de fibrose pulmonaire étendue chez un même patient. Pour cela, nous nous appuierons sur notre collaboration avec le centre de référence HTAP du Kremlin-Bicêtre, et étudierons l'expression tissulaire de *chemerin* et CMKLR1 sur des explants pulmonaires de patients SSc présentant différents groupes d'HTP.

Implication du LB dans la vasculopathie pulmonaire associée à l'HTAP

Nos travaux, ainsi que de nombreux arguments cliniques et expérimentaux, soutiennent une implication du LB dans la genèse et/ou la pérennisation des lésions vasculaires pulmonaires survenant au cours de l'HTAP (15). Nous nous proposons d'étudier plus précisément la nature de leur contribution sous différents aspects.

- <u>Aspects tissulaires</u>

Dans le modèle actuel, le LB périphériques sont recrutés au sein des poumons HTAP pour former des organes lymphoïdes tertiaires fonctionnels, produisant des anticorps dirigés contre des auto-antigènes de la vascularisation pulmonaire (16). Afin de mieux caractériser les anomalies de leur programme fonctionnel, nous avons initié une collaboration avec le laboratoire du Pr Mark Nicolls à *Stanford University* et avons ainsi pu étudier le transcriptome des LB pulmonaires à l'échelle de la cellule unique (*single-cell RNA sequencing*) dans un modèle murin d'HTAP idiopathique (rat $BMPR2^{+/-}$ exposés au 5LO). Les données sont actuellement en cours d'exploitation auprès de notre équipe bio-informatique.

- Aspects cellulaires

Nos travaux ont suggéré que la participation du LB dans les anomalies vasculaires associées à la SSc pourraient passer par la production de médiateurs angio-actifs. De façon intéressante, une étude récente a mis en évidence l'existence d'une sous-population LB dotée de propriétés angiogéniques et impliquées dans différentes pathologies inflammatoires et cancéreuses (17). Cette population se caractérise par un phénotype membranaire particulier ($CD19^+$ $IgG4^+$ $CD49b^+$ $CD73^+$) et la production de protéines pro-angiogéniques (EGF, CYR61, ADM, FGF2, PDGFA, MDK) capables d'induire la formation de tubes de cellules endothéliales *in vitro*. Nous prévoyons donc d'étudier la présence de cette population dans le sang circulants des patients SSc et de déterminer si elle est particulièrement associée à la survenue de manifestations vasculaires (en particulier l'HTAP). Dans un deuxième temps, cette sous-population LB pourra être isolée, faire l'objet d'une caractérisation fonctionnelle multi-omique, et être mise au contact de cellules endothéliales pulmonaires récupérées lors du cathétérisme cardiaque droit des patients HTAP-SSc.

- Aspects humoraux

L'un des mécanismes principaux par lesquels les LB interagissent avec la circulation pulmonaire passe par la production d'auto-anticorps spécifiques (18). Néanmoins, les conséquences fonctionnelles de ces auto-anticorps restent incertaines. Afin de mieux préciser leur contribution, nous souhaitons mettre au contact de différentes lignées de cellules endothéliales les IgG sériques de patients présentant différents phénotypes (notamment présence d'HTAP) et auto-anticorps de SSc. Nous évaluerons ainsi l'impact différentiel des fractions IgG issues de ces différents profils patient sur le programme fonctionnel de la cellule

endothéliale par une approche combinant transcriptomique et protéomique. Les techniques nécessaires à la réalisation de ce projet sont bien maîtrisées par notre laboratoire et ont fait l'objet d'une publication récente (19).

BAFF et HTAP-SSc : le LB comme seule cible ?

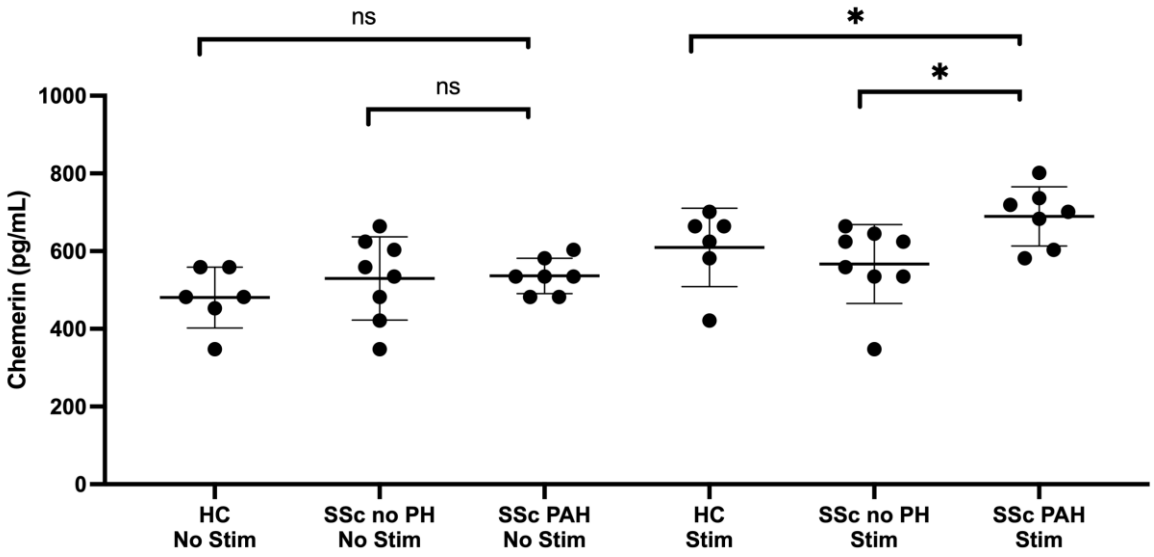
Nos résultats suggèrent donc l'existence d'une association clinique entre BAFF et HTAP au cours de la SSc. De façon intéressante, des travaux précédents ont souligné l'importance de BAFF dans des modèles murins d'athérosclérose, par une action portant non seulement sur les LB, mais également sur les macrophages (20). Un rôle direct sur la cellule endothéliale a également été envisagé au cours du lupus systémique (21).

Afin de mieux préciser le rôle joué par BAFF au cours de l'HTAP-SSc, il pourrait être intéressant d'en étudier les cellules cibles ainsi que les conséquences de son inhibition dans un modèle expérimental de SSc comportant une atteinte vasculaire pulmonaire.

Notre exploration sans *a priori* du protéome sérique des patients HTAP-SSc a donc permis d'identifier 2 pistes prometteuses, à la fois en termes de biomarqueurs cliniques, de mécanismes physiopathologiques et de potentielles cibles thérapeutiques. Ainsi, nous avons souhaité reproduire cette approche en dépistant un panel plus large de biomarqueurs (en spectrométrie de masse) dans différents phénotypes de patients SSc, permettant d'englober l'ensemble des manifestations cliniques de la maladie (projet SCLERO-PROT). Les données sont actuellement en cours d'analyse auprès de notre équipe bio-informatique.

Il est notable que, parmi les 53 biomarqueurs exprimés différentiellement en cas d'HTAP lors de notre exploration protéomique, au moins 10 d'entre eux sont des protéines impliquées dans des processus immunologiques. Ceci renforce l'importance de la participation de l'immunité dans la genèse et/ou la pérennisation des lésions vasculaires pulmonaires, et suggère des interactions jusqu'alors insoupçonnées entre systèmes immunitaire et vasculaire. Mieux comprendre les relations unissant ces 2 composantes de la maladie permettrait d'ouvrir la voie à de nouvelles pistes thérapeutiques, susceptibles de véritablement modifier l'histoire naturelle de l'HTAP-SSc, et à terme d'en changer le pronostic.

Figure 1. Concentration de *chemerin* dans le surnageant de culture de LB circulants issus de témoins sains, de patients SSc avec HTAP et sans HTP, avec et sans stimulation (données non publiées).



Les LB circulants de 6 témoins sains, 8 patients SSc sans HTAP et 7 patients HTAP-SSc étaient isolés par tri magnétique et mis en culture sur une plaque 96-puits (40000 LB/puits) pendant 48h en présence d'une stimulation immunologique (anti-BCR, CpG, CD40L et BAFF) ou en l'absence de stimulation. Les taux de chemerin *dans le surnageant de culture étaient mesurés en Luminex.*

Les axes *chemerin*-CMKLR1 et BAFF-LB constituent des candidats biomarqueurs robustes de l'HTAP-SSc, présentant un potentiel diagnostique et pronostique, ouvrant la voie à de nouvelles possibilités thérapeutiques et suggérant des interactions physiopathologiques insoupçonnées entre immunité et vaisseau.

REFERENCES

1. Pokeerbux MR, Giovannelli J, Dauchet L, Mouthon L, Agard C, Lega JC, et al. Survival and prognosis factors in systemic sclerosis: data of a French multicenter cohort, systematic review, and meta-analysis of the literature. Arthritis Res Ther. 3 avr 2019;21(1):86.
2. Hachulla E, Agard C, Allanore Y, Avouac J, Bader-Meunier B, Belot A, et al. French recommendations for the management of systemic sclerosis. Orphanet J Rare Dis. 26 juill 2021;16(Suppl 2):322.
3. Coghlan JG, Denton CP, Grünig E, Bonderman D, Distler O, Khanna D, et al. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. Ann Rheum Dis. 18 mai 2013;annrheumdis-2013-203301.
4. Bauer Y, Bernard S de, Hickey P, Ballard K, Cruz J, Cornelisse P, et al. Identifying early pulmonary arterial hypertension biomarkers in systemic sclerosis: machine learning on proteomics from the DETECT cohort. European Respiratory Journal [Internet]. 1 juin 2021 [cité 18 sept 2023];57(6). Disponible sur: https://erj.ersjournals.com/content/57/6/2002591
5. Hickey PM, Lawrie A, Condliffe R. Circulating Protein Biomarkers in Systemic Sclerosis Related Pulmonary Arterial Hypertension: A Review of Published Data. Front Med. 6 juin 2018;5:175.
6. Odler B, Foris V, Gungl A, Müller V, Hassoun PM, Kwapiszewska G, et al. Biomarkers for Pulmonary Vascular Remodeling in Systemic Sclerosis: A Pathophysiological Approach. Front Physiol. 19 juin 2018;9:587.
7. Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, et al. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. European Respiratory Journal [Internet]. 1 janv 2022 [cité 2 avr 2023]; Disponible sur: https://erj.ersjournals.com/content/early/2022/08/25/13993003.00879-2022
8. Boucly A, Weatherald J, Savale L, Groote P de, Cottin V, Prévot G, et al. External validation of a refined four-stratum risk assessment score from the French pulmonary hypertension registry. European Respiratory Journal [Internet]. 1 juin 2022 [cité 1 mai 2023];59(6). Disponible sur: https://erj.ersjournals.com/content/59/6/2102419
9. Boucly A, Tu L, Guignabert C, Rhodes C, Groote PD, Prévot G, et al. Cytokines as Prognostic Biomarkers in Pulmonary Arterial Hypertension. European Respiratory Journal [Internet]. 1 janv 2022 [cité 18 sept 2023]; Disponible sur: https://erj.ersjournals.com/content/early/2022/11/17/13993003.01232-2022
10. Ding Y, Qian J, Zhang S, Xu D, Leng X, Zhao J, et al. Immunosuppressive therapy in patients with connective tissue disease-associated pulmonary arterial hypertension: A systematic review. International Journal of Rheumatic Diseases. 2022;25(9):982-90.
11. Zamanian RT, Badesch D, Chung L, Domsic RT, Medsger T, Pinckney A, et al. Safety and Efficacy of B-Cell Depletion with Rituximab for the Treatment of Systemic Sclerosis Associated Pulmonary Arterial Hypertension: A Multi-center, Double-blind, Randomized, Placebo-controlled Trial. Am J Respir Crit Care Med. 2 mars 2021;
12. Breitling S, Hui Z, Zabini D, Hu Y, Hoffmann J, Goldenberg NM, et al. The mast cell-B cell axis in lung vascular remodeling and pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol. 1 mai 2017;312(5):L710-21.
13. Mizuno S, Farkas L, Al Husseini A, Farkas D, Gomez-Arroyo J, Kraskauskas D, et al. Severe Pulmonary Arterial Hypertension Induced by SU5416 and Ovalbumin Immunization. American Journal of Respiratory Cell and Molecular Biology. nov 2012;47(5):679-87.
14. Papathanasiou AE, Spyropoulos F, Michael Z, Joung KE, Briana DD, Malamitsi-Puchner A, et al. Adipokines and Metabolic Regulators in Human and Experimental Pulmonary Arterial Hypertension. Int J Mol Sci. 1 févr 2021;22(3):1435.
15. Tobal R, Potjewijd J, van Empel VPM, Ysermans R, Schurgers LJ, Reutelingsperger CP, et al. Vascular Remodeling in Pulmonary Arterial Hypertension: The Potential Involvement of Innate and Adaptive Immunity. Front Med (Lausanne). 2021;8:806899.
16. Perros F, Dorfmüller P, Montani D, Hammad H, Waelput W, Girerd B, et al. Pulmonary Lymphoid Neogenesis in Idiopathic Pulmonary Arterial Hypertension. Am J Respir Crit Care Med. 1 févr 2012;185(3):311-21.
17. van de Veen W, Globinska A, Jansen K, Straumann A, Kubo T, Verschoor D, et al. A novel proangiogenic B cell subset is increased in cancer and chronic inflammation. Sci Adv. 13 mai 2020;6(20):eaaz3559.
18. Kherbeck N, Tamby MC, Bussone G, Dib H, Perros F, Humbert M, et al. The Role of Inflammation and Autoimmunity in the Pathophysiology of Pulmonary Arterial Hypertension. Clinic Rev Allerg Immunol. 11 mars 2011;44(1):31-8.
19. Chepy A, Vivier S, Bray F, Ternynck C, Meneboo JP, Figeac M, et al. Effects of Immunoglobulins G From Systemic Sclerosis Patients in Normal Dermal Fibroblasts: A Multi-Omics Study. Front Immunol. 29 juin 2022;13:904631.

20. Tsiantoulas D, Sage AP, Göderle L, Ozsvar-Kozma M, Murphy D, Porsch F, et al. B Cell–Activating Factor Neutralization Aggravates Atherosclerosis. Circulation. 13 nov 2018;138(20):2263-73.
21. Spinelli FR, Barbati C, Cecarelli F, Morello F, Colasanti T, Vomero M, et al. B lymphocyte stimulator modulates number and function of endothelial progenitor cells in systemic lupus erythematosus. Arthritis Res Ther. déc 2019;21(1):245.