Review

One year in review 2018: systemic sclerosis

M. Orlandi[1], S. Barsotti[2,3], G. Lepri[1], V. Codullo[4],
M. Di Battista[3], S. Guiducci[1], A. Della Rossa[3]

[1]Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Florence, Italy;
[2]Department of Medical Biotechnologies, University of Siena, Italy;
[3]Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Pisa, Italy;
[4]Rheumatology A Department, Hopital Cochin, Paris, France.

Martina Orlandi, MD
Simone Barsotti, MD
Gemma Lepri, MD
Veronica Codullo, MD
Marco di Battista, MD
Serena Guiducci, MD, PhD, PH
Alessandra Della Rossa, MD

Please address correspondence to:
Dr Alessandra Della Rossa,
Rheumatology Unit,
University of Pisa,
Via Roma 67,
56126 Pisa, Italy.
E-mail: a.dellarossa@ao-pisa.toscana.it

Received on September 14, 2018; accepted on September 17, 2018.

Clin Exp Rheumatol 2018; 36 (Suppl. 113): S3-S23.

Key words: systemic sclerosis, pathogenesis, diagnosis, treatment

Competing interests: none declared.

ABSTRACT

Systemic sclerosis is a rare acquired systemic disease characterised by a complex pathogenesis and multi organ involvement. Every year the scientific world contributes to enrich the knowledge on the pathogenesis, clinical manifestations, diagnosis and treatment of this complex and severe disease. Herewith, we provide an overview of the most significant literature contributions published over the last year.

Introduction

Systemic sclerosis (SSc) is a chronic, multisystem disease with distinctive pathogenetic features, comprising vascular derangement, immune system activation, tissue fibrosis and heterogeneous clinical profile.

In this manuscript we will provide our annual update of the recent advances in the pathogenesis, diagnosis and treatment of SSc, analysing both the limited cutaneous form (lcSSc) and the diffuse cutaneous form (dcSSc). A systematic MedLine search has been performed using the term "systemic sclerosis" (MeSH terms and semantic search), focusing on the most relevant contributions to the medical literature published between August 2017 and June 2018.

Recent insights into the pathogenesis of SSc

Genetics and epigenetics

As mentioned in our previous review, SSc has been found to be associated with alleles of human leukocyte antigen (HLA) genes especially class II. Oka A *et al*. (1) recently studied the DNA from 318 unrelated SSc patients, all with anti-topoisomerase I positivity, and from 561 unrelated controls. Results confirmed that the alleles DPB1*09:01 and *13:01 had a significant association with SSc. In addition, the authors genetically identified rs17847931 in retinoid X receptor beta (RXRB) as an SSc susceptibility variant and suggested RXRB as a gene involved in antifibrotic activity in the skin. The HLA DRB1 allele group DRB1*11 that has been described in SSc Caucasian patients. It encodes for the β1 chain of the HLA-DR molecule whose its third hypervariable region (3rd HVR) seems to have an important role in T-cell recognition. Gentil *et al*. (2) recently investigated the DRβ1 3rd HVR, charge, and parental transmission in SSc patients compared to healthy controls enrolling a total of 306 unrelated families (121 SSc and 185 controls). Data from this study suggested a skewed parental inheritance of HLA-DRB1 alleles showing a reduction in paternal transmission of DRB1 alleles encoding a +2 charge 3rd HVR in SSc population compared to the control group. Contrarily, an increase of paternal transmission when charge was 0 was observed. According with these results, an epigenetic modulation of HLA may be assumed in SSc, but further investigations are needed. The major aetiopathogenesis hypothesis of SSc is that environmental factors may be a trigger in genetically susceptible patients leading to the beginning of the inflammatory cascade. The DNA methylation, an epigenetic mechanism, is influenced by inherited DNA and environmental factors therefore it may represent a link between the environment and the genetical predisposition. For these reasons, Hudson *et al*. (3) evaluated the DNA methylation signatures in SSc patients (n 19) and other rheumatic disease (13 patients with rheumatoid arthritis and 12 with systemic lupus erythematous) compared to 8 controls using $CD4^+$ T cells isolated from blood samples. Authors identified 33 differentially methylated and ex-

pressed genes in these autoimmune diseases when compared to controls, the gene mostly expressed in patients was CD1C. Regarding the functional analysis, they found that the lipid metabolism, molecular transport, small molecule biochemistry was the top network identified. Altogether, results from this recent study may indicate the presence of novel cellular targets of interest. Rezaei R *et al*. (4) suggested that hypomethylation of the interferon regulatory factor 7 (IRF7) promoter might play a role in SSc pathogenesis, probably through promoting the IRF7 expression in the peripheral blood mononuclear cells (PBMCs) of patients with SSc. In PBMCs of 50 limited cutaneous patients (lcSSc), the CpG2 hypomethylation was significantly associated with increased SSc risk and the mRNA expression of IRF7 was higher than controls. Moreover, overall promoter methylation and mRNA level of IRF7 were significantly correlated with each other. Bergmann *et al*. (5) reported that the expression of Jumonji domain-containing protein 3 J (MJD3) was increased in fibroblasts in SSc skin and in experimental fibrosis in a transforming growth factor beta (TGFβ)-dependent manner. JMJD3 is capable to reverse the trimethylation of histone H3 on lysine 27 modification which is implicated in fibroblast activation. Moreover, they demonstrated that pharmacological targeted inhibition of JMJD3 ameliorated bleomycin-induced and topoI-induced fibrosis in well-tolerated doses. Regarding gene profile in SSc, last year microarray experiments revealed that the matrix metalloproteinase 10 (MMP-10) gene is up-regulated in SSc-associated pulmonary hypertension (PH) endothelial progenitor cell (EPC)-derived endothelial cells (ECs); the treatment of Fra-2-Tg mice with neutralising anti-MMP10 antibodies reverse established PH and markedly reduced pulmonary vascular remodelling (6). In a small sample of limited SSc patients was demonstrated a decrease in transcriptional activity of some genes of the nuclear factor ϰB (NF-ϰB) family suggesting that a disregulation of intracellular signal transduction by NF-κB may have a central role both in the beginning and in the late stage of SSc (7). Integrative genomics has yielded powerful tissue-specific functional networks modelling the interaction of genes in each microenvironment. Taroni *et al*. (8) recently investigated if each SSc organ manifestation has distinct disease signatures at the molecular level. Results from this study indicated a common immune–fibrotic axis—indicative of profibrotic macrophages in peripheral blood, skin, lung and esophagus but with different activated macrophages in each SSc tissue and different functional consequences. Therefore, the diversity of organ microenvironment seemed to give different stimuli to the infiltrated macrophages determining their profibrotic phenotype, as suspected for skin macrophages when compared to the lung ones. P-glycoprotein (P-gp) is a product of ABCB1 gene that includes 29 exones and the activity of P-gp seems to be influenced by ABCB1 polymorphism. Recently, attention has been focused on the possible role of P-gp in immunologic processes, as the expression of ABCB1 is increased by proinflammatory cytokines. With these premises, a recent study aimed to investigate a possible relation between ABCB1 polymorphism and SSc morbidity in 61 SSc Polish patients compared with 100 healthy controls. ABCB1 genotypes and alleles for polymorphisms C1236T, G2677T/A and C3435T did not show a significant different frequency in the two populations. Authors detected a significant higher occurrence of the haplotype 1236 C-2677 G-3435 in SSc than in controls suggesting a possible increased risk pf SSc in patients with this haplotype (9). Killer immunoglobulin-like receptors (KIRs), cell surface proteins on NK cells specific for allelic forms of HLA class I molecules, have been investigated in SSc pathogenesis. A recent study investigated the possible association between KIR and HLA genes and SSc in Irian population enrolling 451 controls and 279 SSc subjects. The interaction between KIR genes and HLA seemed to modify the susceptibility to the disease and authors showed a correlation between this interaction and incidence rate of SSc. The combination of KIR3DL receptor and HLA-BW4 ligand having treonine as an 80th amino acid appeared to have a confounding effect compared to KIR3DL-HLABW4-A1 (10). In 455 Iranian SSc patients, single nucleotide polymorphisms (SNPs) of genes encoding CD247 and CD226 (whose play important roles in signalling of lymphocytes) have not been associated with the risk SSc (11). Several gene polymorphisms involved in regulatory T cell function have been identified in many autoimmune diseases, including SSc. Moreover, dysregulation of co-stimulatory and/or co-inhibitory signals, including ICOS signalling, can lead to autoimmunity. In 166 cohort of SSc patients the FOXP3 rs2294020, ICOS rs6726035 and ICOSL rs378299 SNPs were studied and no effect on SSc susceptibility was found. The occurrence of FOXP3 rs2294020 in female patients was associated with decreased time to progression from early to definite SSc so that this SNP may be considered a disease-modifying gene-variant rather than a disease-susceptibility SNP in SSc (12). Mutations of the keratin 1 gene (KRT1) were reported to associate with skin diseases. Recently in a Chinese population of 164 systemic lupus erythematous (SLE) and 99 SSc patients the genotype of KRT1 was determined and the results showed that the mutant with G at SNP rs14024 was associated with the high risk to SLE and SSc while the deletion allele at rs267607656 was associated with the low risk to SSc. The haplogenotype, Del-/MU+ was associated with high susceptibility to SLE and SSc but the haplogenotype Del+/MU- was associated with resistance to SLE and SSc (13). The crucial role of the signal transducer and activator transcription 4 (STAT4) in the pathogenesis of SSc was recently confirmed in 102 Russian SSc patients. In that pilot study STAT4 rs7574865 polymorphism is associated with SSs, the dc-SSc, the presence of interstitial lung disease (ILD), cardiac injury, and anti-topoisomerase I antibodies positivity (14). Transcriptomics of SSc skin biopsies has revealed sets of pro-fibrotic genes strongly enriched in diseased as compared to normal biopsies suggesting the role of

the in vivo microenvironment in maintaining the pathological myofibroblasts phenotypes in SSc. An American group, through a novel method for quantitative image analysis of dermal collagen ultrastructure with genome-wide transcriptomic analysis, demonstrated that collagen bundle alignment is a feature of dcSSc skin and is associated with a cell migration gene signature. Moreover, these cells show increased directed migration on aligned extracellular matrix (ECM) fibers that is dependent on expression of Arhgdib (Rho GDP-dissociation inhibitor 2) (15). Studies have demonstrated the significant genetic component of SSc and genome-wide association studies (GWAS) may probably identify unknown susceptibility genes in SSc, as already happened for rheumatoid arthritis. GWAS for SSc using 716 Japanese cases,2 and 1797 controls were recently performed by Terao *et al*. (16) in whose study meta-analysis of GWAS using the previous GWAS from the French population was performed. Results showed two novel susceptibility loci, moreover associated with other autoimmune diseases: GSDMA and PRDM1. Gorlova OY *et al*. (17) published data from ImmunoChip and GWAS genotyping studies in White and African American people (1833 white sample and 3466 controls and 291 African American sample, and 260 controls). The gene-level analysis identified four novel candidate genes (STAT1, FCGR2C, NIPSNAP3B, and SCT) significantly associated with SSc in Whites and 4 genes (SERBP1, PINX1, TMEM175 and EXOC2) suggestively associated with SSc. Some of these genes (FCGR2C, SERBP1, EXOC2) have been shown to be involved in immune response. Otherwise the comparison of the results on Whites with those from African Americans demonstrated that only TNFAIP3, FCGR2C and PINX1 were nominally significant in African Americans. Recently attention has focused on exosomes, microvecicles that play a crucial role in the intercellular communication determining the target cells phenotype. They contain different macromolecules as microRNAs (miRNAs) a non-coding RNA able to regulate gene expression and for this reason, recent studies evaluated the role of miRNAs in the pathogenesis of fibrosis in SSc. The study of Wermuth *et al*. (18) analysed the miRNAs in exosomes of 6 SSc patients (3lcSSc and 3 dcSSc) and evaluated their ability to alter the phenotype of normal human dermal fibroblast *in vitro*. The results showed a significant increase of six profibrotic miRNAs level in SSc patients compared to the controls and of three others in dcSSc. On the other hand, twelve antifibrotic miRNAs were lower in SSc population, and two others in dcSSc, compared to healthy controls. Even regarding the antifibrotic miRNAs, eight were lower in lcSSc compared to dcSSc. The same study compared fibroblasts treated with exosomes from SSc patients and from controls, confirming that the first ones induce the expression of genes associated to a profibrotic phenotype and encoding interstitial collagens. miRNA-202-3p whose target is matrix metallopeptidase (MMP) is another miRNA that has been found to be increased in involved skin in SSc patients. Zhou *et al*. (19) suggested miRNA-202-3p as a profibrotic miRNA in SSc as it was found to be upregulated in SSc skin tissue and primary fibroblast with a consequent increase in collagen deposition. In addition, authors showed that miRNA-202-3p levels were inversely related to MMP1 expression.Another recent study investigated the role of miRNAs in the SSc pathogenesis identifying six miRNAs and four mRNAs crucial in SSc pathogenesis. As indicated by authors, the identified miRNAs seem to be involved in the regulation of TGF-β, Toll-like receptor (TLR) and Want signalling pathways and these data suggested their role in the SSc pathogenesis. Five miRNAs (miR-146b, miR-130b, miR-21, miR-31 and miR34a) were higher, on the contrary miR-145 was lower, in SSc skin and fibroblasts and also in in the normal ones and in endothelial cells stimulated with SSc serum (20). miRNA-155 plays a role in pulmonary fibrosis and its expression can be induced by interleukin (IL)-1β. SSc fibroblasts have activated inflammasomes that are integrally involved in mediating the myofibroblast phenotype. Artlett *et al*. (21) demonstrated that microRNAs -155 (miR-155) is upregulated in SSc, especially in SSc lung fibroblasts and that its expression was dependent on inflammasome activation. Moreover, in the absence of miR-155, inflammasome-mediated collagen production is blocked confirming the key role of this miRNA in the pathogenesis of fibrosis. Plasmacytoid dendritic cells (PDCs) are a critical source of type I interferons (IFNs) and their disregolation can contribute to the persistent type I IFN signature that we can see in SSc. Rossato *et al*. (22) showed that miR-618 was over-espressed in PDCs from SSc patients, including those with early disease without skin fibrosis. *In vitro* the up-regulation of miR-618 reduced the development of PDCs from CD34+ cells and enhanced their ability to secrete IFNα and it can explain the type I IFN signature observed in SSc patients. Regarding the link between SSc and malignancies, of growing interest in recent years, to cite a recent study of Dolcino *et al*. (23) even regarding the possible role of miRNAs. In the PBMCs derived from patients with SSc, this study demonstrated the presence of modulated genes linked to the pathogenesis of SSc and to carcinogenesis process and the presence of miRNAs (miR-21-5p, miR-92a-3p, and on miR-155-5p, miR 126-3p and miR-16-5p) that can play a predisposing role in the development of malignancies in SSc.

Vasculopathy and endothelium

The endothelial injury and the consequent disruption of its homeostasis is one of the most important step in the SSc pathogenesis. For this reason, studies focused on the role of endothelial progenitor cells (EPCs) recruited to repair the endothelium. A population of 45 SSc patients and of 41 controls was enrolled in the study of Benyamine *et al*. (24) that, using flow cytometry, found CD34+CD45-EPC mobilisation as a marker of vascular activation and disease severity. The authors also quantified circulating endothelial microparticles (EMPs) that had higher levels in plasma of SSc patients comparted to controls however without a correlation with the disease severity. Between the

endothelial biomarkers, s-Fractalkine was found to be elevated in SSc compared to controls (together with vascular endothelial growth factor (VEGF) and endothelin-1 (ET-1). In addition, s-Fractalkine showed a correlation with $CD34^{+}CD45$-EPCs levels suggesting new markers for the endothelial injury in SSc pathogenesis (24). In the last years, attention has been focused on endothelial-to-mesenchymal transition (EndoMT) a process for which endothelial cells (ECs) change their phenotype acquiring ECM-producing myofibroblast features. This process has been studied assuming its role in fibrotic diseases pathogenesis. For this reason, a recent study of Manetti *et al*. (25) suggested a potential role of EndoMT in SSc skin fibrosis pathogenesis as it seems to occur in the dermal endothelium of SSc leading to the development of fibrosis. In the study, skin section of SSc patients and of controls were immunostained for endothelial CD31 or vascular endothelial (VE)-cadherin and α-smooth muscle actin (α-SMA) myofibroblast marker. Results from this first part of their work showed that in SSc derma there were EndoMT cells that expressed both EC and myofibroblast marker, in controls this datum was detected at minimum levels. Authors also investigated EndoMT in two mouse models: mice with bleomycin-induced dermal fibrosis and uPAR-deficient mice. In these animal models EndoMT cells were assessed with both marker combination (CD31 o VE-cadherin and α-SMA) and lower levels of these cells were found in control mice compared to the higher levels detected in the bleomycin treatment group and similar results were found in the uPAR one. The angiopoietin (Ang)/Tie2 system is a key regulator of vascular biology. The expression of membrane bound (mb) Tie2 and Ang-1 ensures vessel stability, whereas Ang-2, inducible by VEGF, hypoxia, and inflammation, acts as an antagonist. Tie2 signalling is also attenuated by soluble Tie2 (sTie2), the extracellular domain of the receptor, which is shed upon stimulation with VEGF. Recently the Swiss group confirmed that peripheral microvasculopathy in SSc results from a complex dysregulation of angiogenic signalling networks including the VEGF and the angiopoietin (Ang)/Tie2. They demonstrated that dermal microvessels abundantly expressed Ang-2 and the levels of sTie2 were increased already in early disease while the percentage of the membrane bound (mb) Tie2 (mbTie2)+ microvessels was profoundly decreased. Moreover, both in skin and sera of SSc patients, the Ang1/2 ratio was reduced, especially in patients with digital ulcers (DUs) indicating vessel destabilising conditions (26). Although TGF-β plays a central role in the pathogenesis of SSc, various other growth factors, cytokines, and chemokines play a part in initiating and developing infammation, vasculopathy, and fbrosis in SSc. IL-6 is another critical cytokine deeply associated with the development of SSc together with these cytokines and serum IL-6 levels are significantly higher in SSc patients than in healthy controls and correlate with the severity of dermal and pulmonary fibrosis. New treatment targeting the anti-IL-6 receptor antibody are showing benefit on skin sclerosis confirming the role of IL-6 in SSc fibrosis process as also evidenced by a recent review (27). In addition, Taniguchi *et al*. (28) showed an activation of IL-6/signal transducer in SSc cells, in particular in the endothelial ones. Leukaemia inhibitory factor (LIF) is a member of IL-6 family, which plays pleiotropic roles in vascular remodelling and angiogenesis. Taniguchi *et al*. (29) reported that serum LIF levels were significantly decreased in patients with SSc, especially in those with early disease and with (DUs), suggesting that decreased serum LIF levels may be associated with vasculopathy in SSc. Moreover, they hypotise that Friend leukaemia virus integration 1 (Fli1) deficiency may contribute to the inhibition of LIF-dependent biological effects on SSc endothelial cells by suppressing the expression of LIF, LIF receptor, and gp130. Urotensin II, a potent vasoconstrictor and it is supposed to be linked to Raynaud phenomenon (RP) as it was demonstrated in SLE patients. In a small group of RP secondary to SSc, plasma UII level is decreased and maybe it is correlated to the presence of SSc and not to RP (30). Klotho is a trans-membrane protein that is involved in the control of vessel tone, reparative and fibrotic processes, for this reason it was studied in 69 SSc patients. In these patients, a significant deficit of klotho was found but no significant correlation with clinical, laboratory or instrumental features of the disease, maybe due to the small number of studied patients (31).

Fibrosis

SSc patients have increased collagen and extracellular matrix synthesis driven by fibroblast activation, which is characterised by transformation of fibroblasts to myofibroblasts. This event is phenotypically detected by increased a-SMA expression and synthesis of collagen type 1 (COL1A1). Several factors have been implicated in myofibroblast differentiation, including TGF-β, vascular βlial growth factor, IL-13 activation of STAT6 and TLR ligands. Fibroblasts are a key driver of the fibrotic process through deposition of extracellular matrix. The mechanisms by which fibroblasts are induced to become pro-fibrotic remain unclear. A recent study demonstrated that lcSSc- and dcSSc-derived keratinocytes have pro-fibrotic effects on normal primary fibroblasts as manifested by ASMA and COL1A1 mRNA and protein expression; in a TGF- β – independent manner. Moreover, NF-kB and PPAR-g are dysregulated nodes in both lcSSc and dcSSc, indicating that inhibition of NF-kB or activation of PPAR-g may have therapeutic benefits in SSc (32). TheTGF-β signalling pathway has a central role in inducing pro-fibrogenic cellular programs, as demonstrated by previous studies. The recent study of Lu *et al*. again focused on TGF-β that is known to be involved in the pathogenesis of skin fibrosis in SSc. The small latent TGF-β complex (SLC) forms the large latent complex (LLC) together with the latent TGF-β-binding proteins (LTBPs) that are components of themECM (33). Considering that LTBP-4 seems to be associated with fibrosis-related condition Lu *et al*. (34) tried to elucidate its role in SSc

analysing the LTBP-4 expression in skin and plasma of SSc subjects. 46 SSc and 43 localised scleroderma (LSc) patients were enrolled together with a population of 46 healthy controls. The expression of LTBP-4 was higher in SSc skin patients compared to control. In addition, the ELISA assay showed that also the plasma levels of LTBP-4 was significantly increased in SSc compared to controls (no significant difference was found between LSc and controls). Similar data were detected regarding the plasma levels of TGF-β that were higher in SSc patients compared to control ones, its levels seem to positively correlate with LTBP-4 levels. The study concluded that LTBP-4 may have a crucial role in SSc fibrotic process with an increase collagen production. In fact, the authors also investigated the influence of LTBP-4 on the regulation of collagen gene and protein expression using LTBP-4 knockdown fibroblasts and showed a decrease in type I and III collagen expression. All together these results may suggest LTBP-4 as a new actor in SSc. As known, inflammation, type 2 immunity, and fibrogenic processes are involved in disease development and may be affected by sphingolipids. The sphingolipid sphingosine-1-phosphate (S1P) is elevated in the sera of SSc patients, and its receptor S1P5 is expressed in skin tissue. Schmidt *et al*. in a pilot study proposed that S1P5 deficiency impacts on early-stage dermal Th2 inflammatory responses by affecting longchain ceramide profiles in the skin as well as the composition of cellular infiltrates in low-dose bleomycin (BLM)- induced fibrogenesis in murine skin (35). Sirtuin1 (SIRT1) is a deacetylase which has an anti-inflammatory and antifibrotic activity in various organs and it has already been demonstrated to play an important role in the inflammation induced by TNF-α as SIRT activation inhibit its production. In addition, the mammalian target of rapamycin (mTOR) whose activation has been demonstrated to have a role in inflammation is down-regulated by Sirt1. For this reason, SIRT1 may be an important target in SSc therapies as already suggested by resveratrol (Res) a Sirt1 activator. Given these premises, Zhu *et al*. (36) recently investigated Sirt1 role in SSc using C3H/He mice treated with bleomycin (BLM). Authors showed an up-regulation of Sirt1 in dcSSc fibroblasts, compared to controls, and in BLM-treated mice and an amelioration of cutaneous fibrosis in BLM-treated mice after administration of Res. In addition, mTOR was increased in SSc fibroblasts and in the involved skin of BLM-treated mice, in which treatment with an inhibitor of mTOR was found to inhibit the inflammation and fibrosis. In addition, authors suggested that the activation of SIRT1 by Res caused an inhibition of mTOR expression. All together, these results, confirmed a possible role of Sirt1 in SSc pathogenesis and suggested SIRT a potential therapeutic target. Recently Chu *et al*. (37) supposed that the loss of SIRT1 may participate in the pathogenesis of SSc-related pulmonary fibrosis, and that SIRT1 activation is an effective treatment for both the early (inflammatory) and late (fibrotic) stages of pulmonary fibrosis. They reported that the expression of SIRT1 in peripheral blood mononuclear cells of patients with pulmonary fibrosis secondary to SSc is lower than that in patients with SSc without pulmonary fibrosis. They also showed that in mice models of lung fibrosis, SIRT1 activation reduced collagen production and in human fetal lung fibroblasts, SIRT1 activation inhibited TNF-α-induced inflammatory responses. Sacchetti *et al*. (38) assessed the expression of all the tyrosine phosphatase (PTPs14) in dermal fibroblasts of patients with dcSSc finding that PTP4A1 (a sub-class of three prenylated PTP) mRNA and protein are significantly overexpressed in SSc dermal fibroblasts lines, especially in those derived from patients in the early stage of the disease. PTP4A1 promotes TGFβ signalling in human dermal fibroblasts enhancing the canonical pro-fibrotic TGFβ signalling in these cells and exacerbates experimental fibrosis in mice. They also propose a model of molecular mechanism in which PTP4A1 binds directly to SRC and protects it from excessive degradation and functional inhibition. These results suggest that interfering with the interaction between PTP4A1 and SRC might represent a therapeutic strategy in fibrotic diseases where TGFβ play a pathogenic role. Metalloproteinase (MMP)-1, is the principal enzyme involved in collagen degradation and the synthesis of collagen in connective tissue. Platelet-derived growth factors (PDGFs) are important in fibrosis pathogenesis and their functions is mediated by two receptors: the PDGFRα and PDGFRβ. Studies demonstrated the role of PDGFRα in the skin and organ fibrosis development. Recently, Makino *et al*. (39) confirmed the role of PDGF in SSc pathogenesis evaluating the effect of crenolanib, an inhibitor of PDGFRα, PDGFRβ and demonstrating that accumulation of PDGFR-positive collagen producing fibroblasts was inhibited by crenolanib. The results from this study confirmed the role of PDGF in SSc pathogenesis showing a correlation between its receptor-α mRNA and CCN2 (connective tissue growth factor) and other profibrotic markers and a proliferation of SSc fibroblasts in response to PDGFAA, datum not detected in healthy control fibroblasts. ECs, pericytes (PC) and epithelial cells are supported by the networks formed by collagen type IV produced from ECs and PC and with a blood vassel stabilisation function. Some previous studies reported a correlation between the levels of serum collagen type IV and the disease activity of liver fibrosis in chronic hepatitis or other liver disease. For this reason, a recent study investigated the serum levels of serum collagen IV in a Japanese SSc cohort (n 127, lcSSc 72, dcSSc 55) compared these results with those obtained in a control group (n 30). Data showed higher levels of serum collagen in SSc patients compared to controls and its levels were positively correlated with the extension of skin involvement evaluate by mRSS. Serum collagen was elevated in particular in dcSSc patients with a disease duration <3 years. In addition, the same study investigated the expression of perivascular collagen type IV with immunohistochemical staining revealing a lower

expression in SSc patients (n. 8) compared to 7 controls suggesting its potential role in the vasculopathy and/or skin fibrosis pathogenesis in SSc (40). Metalloproteinase (MMP)-1, is the principal enzyme involved in collagen degradation and the synthesis of collagen in connective tissue. IL-13 which is a T helper type 2 (Th2) cytokine is a major inducer of fibrosis modulating the collagen homeostasis in fibroblasts, and stimulating the production and activation of TGF-β. Recently Brown Lobbins *et al*. (41) suggested that IL-13 regulates MMP-1 expression in response to TNF-α through an serine/threonine kinase B/protein kinase B (Akt)- mediated pathway. They showed that IL-13 suppresses MMP-1 in TNF-α-stimulated dermal fibroblasts and that Akt inhibitor VIII is able to block the suppressive effect of IL-13 on MMP-1 expression in fibroblasts and that Akt inhibitor increases MMP-1 protein synthesis. Previous data suggested a decrease of Fli, a member of the Ets transcription factor family, in SSc fibroblast and endothelial cells not only in the affected skin but also in the non-involved one (42). Interestingly, Takahashi *et al*. recently confirmed the role of Fli1 in the SSc pathogenesis reporting a decrease of its expression in SSc keratinocytes and showing that the deficiency of Fli1 caused a SSc-like molecular phenotype. Data from the same study suggested a role of Fli1 deficiency in the disease autoimmunity and in the development of organ fibrosis. In fact, keratin 14-expressing epithelial cell-specific Fli1 knockout mice, the animal model used in this study, developed SSc fibrotic skin and esophagus involvement principally due to an epithelial activation. In the same animal model, ILD was reported, likely due to thymic abnormality with a suppression of autoimmune regulator (Aire) and the presence of lung antigens autoantibodies (43) Even regarding Fli1 deficiency, another study of Taniguchi *et al*. investigated the effect of Fli1 deficiency on the expression of granulocyte chemotactic protein 2 (CXCL6) known to have increased circulating levels in SSc patients and to be involved in disease vasculopathy. For this study, dermal fibroblasts were isolated from 5 dcSSc subjects and 5 controls and authors showed an increase of CXCL6 in dermal fibroblasts treated with Fli1 siRNA compared to the not treated ones. In addition, levels of CXCL6 positively correlated with mRSS and heart involvement and negatively with lung involvement (% of forced vital capacity (FVC) and % of lung diffusing capacity for carbon monoxide [DLCO]) in SSc patients. Serum CXCL6 levels were found to correlate with the presence of digital ulcers/pitting scars by multiple regression analysis. In conclusion, this study seems to confirm that Fli1 deficiency may induce a SSc-phenotype and suggest a role of CXCL6 in the process leading to fibrosis and also in the vasculopathy in SSc (44). The recent study of Saigusa *et al*. (45) investigated the impact of Fli1 deficiency on galectin-9 expression. Galectin-9 is a pleiotropic immune modulator interacting with a unique glycoprotein ligand TIM-3 that is highly expressed on Th1 cells and minimally on Th17 cells. The inhibition of TIM-3 signalling induces the IFN-γ, IL-17, IL-2 and IL-6. Results from this study showed that galectin-9 was overexpressed in dermal fibroblasts compared to control ones and its levels correlated with the presence of ILD and positively with mRSS. In addition, galectin-9 expression was found to be induced by Fly1 deficiency. Authors also suggested that the overproduction of galectin-9 may suppress the expression of IFN-γ that increased when Fli1+/- dermal fibroblasts were transfected with Lgals9 siRNA.

Immunity and inflammation

Regulatory B cells (Bregs) may prevent the autoimmunity secreting IL-10 which have anti-inflammatory functions suppressing different pro-inflammatory immune cells. Because some results reported a hyperactivation of B cells but a decreased secretion of IL-10 in SSc, a recent study evaluated TIM-1+ B cells in SSc (39 SSc patients and 53 controls). TIM-1 is the T cell Ig and mucin domain protein that some studies demonstrated to be demonstrated to be a marker for IL-10+ Bregs in mice (46). The study of Aravena *et al*. (47) confirmed TIM-1 as a marker to identify Bregs producing IL-10 and showed a great ability of TIM-1+ B cells to suppress the differentiation and the activation of pro-inflammatory cells in the control group (inhibition of IFN-γ, TNF-α and IL-17 production). On the other hand, this capacity was lower in the 39 SSc patients remarking the importance of B cells in the SSc pathogenesis and characterising a subpopulation of B cells that seem to have a crucial role. Another recent study focused on IL-10+ B cells analysing peripheral blood isolated from 12 controls and 26 SSc patients. The authors did not find any correlation between B cells and specific antibodies levels. However, investigating also IL-17+ ant IFNγ T cells an inverse correlation of these cells and B cells was described, for IFN-γ+ $CD3^+$ cells only in dcSSc and not in lcSSc (48). It is thought that the cytokine production by T cells influences the function of fibroblasts and endothelial cells playing a central role in vascular disease and fibrosis development. There is a strong evidence in literature for altered T-cell activation and T helper cells abnormalities in SSc. Several authors have reported higher frequency of Th17 lymphocytes in the peripheral blood of SSc patients and have pointed out the role of these cells as a factor engaged in the pathogenesis of the disease. Th17 cells, firstly described in 2005, produce IL-17A, IL-17F, IL-21, IL-22, and IL-26. Krasimirova *et al*. (49) investigated the T-cell activation in small sample of 24 SSc patients demonstrating that Th 17 cells are up-regulated in patients, especially in the lcSSc and an increased percentage of $CD4^+CD25^-Foxp3^+$ in dcSSc patients. This group also analysed the circulating cytokine profile in SSc describing raised levels of IL-6, TGF-β1 (increased in early stage), IL-10 and IL-17A. Because Treg abnormalities have been found in the SSc pathogenesis, a recent study analysed Treg subpopulations and their cytokine (IL-10 and TGF-β) in the peripheral blood of SSc patients with an early stage of the disease. Results suggested an imbalance of Treg subsets and abnormalities in their cytokines in SSc. The enrolled population was composed

by 26 SSc patients, 7 lcSSc and 19 dcSSc. Authors described a significant elevation of $CD4^+CD25^+Foxp3^+$ Treg cells with a diminished IL-10 production in dcSSc subjects and in SSc patients with anti-topoisomerase I/anti-RNApolII antibodieas and lung fibrosis. CD62L+ Treg cells, that represent a population with an active recirculation into lymph nodes, with less TGF-β production were present in all SSc groups. In addition, authors described a reduced methylation of Treg specific FOXP3 enhancer regions (50). Guggino *et al*. (51) suggested that T helper 9 (Th9) cells and IL-9 are implicated in the pathogenesis of SSc because they found an overexpression of IL-9, IL-9R, IL-4, thymic stromal lymphopoietin (TSLP) and TGF-β in skin tissues of patients (both limited and diffuse SSc). IL-9 is overexpressed in the context of skin infiltrating mononuclear cells and in renal biopsies of patients with SSc and the major source of IL9 was found in Th9 cells in the skin and in PBMCs. The expression of IL9 and Th9 correlated with the modified Rodnan skin score (mRSS) and the stimulation with IL-9 induced the production of anti-systemic scleroderma 70 (Scl70) by B cells. Mekinian *et al*. (52) demonstrated a reduction of the absolute numbers and frequencies of the mucosal-associated invariant T cells (MAIT) and γδ T cells in blood samples from patients with SSc. They failed to prove any correlation of this data with different disease clinical subsets. A Japanese group demonstrated that the serum levels of soluble forms of programmed death 1 (sPD-1) and one of its ligands, soluble PD ligand 2 (sPD-L2) were elevated in SSc T cells, B cells, and macrophages of 91 patients and correlated with the development and the severity of fibrosis. They proposed that PD-L2 acts as a regulator of T cell cytokine production via cognate interactions with T cells and B cells (53).

After an inflammatory reaction has been initiated, rapid innate immunity effector mechanisms have notable potential to cause damage to host tissues. Proteases, enzymes produced mainly by inflammatory phagocytes, provide a perfect example of such action. Recent evidence highlights how innate immune defense can promote autoimmunity and cause damage to host tissues by excessive release of proteases being its effectors. In response to these enzymes, antiproteases belonging to a group of either ‘‘alarm”or ‘‘systemic” inhibitors, are secreted. Alarm antiproteases include secretory leukocyte protease inhibitor (SLPI) and elafin are produced in response to the activation of innate immunity response, mainly in response to IL-1 and TNF and integrate innate and adaptive immunity systems. In 28 patients with SSc Olewicz-Gawlik *et al*. (54) demonstrated SLPI high levels those correlated with lung involvement in SSc (reduction of DLCO and TLC). Xu *et al*. (55) recently suggested that also TNF-like ligand 1A, a member of the TNF superfamily, might have a role in the pathogenesis of SSc. In fact, it has effects on proliferation and activation of immune cells, including helper and regulatory T cells and results from this study showed that SSc patients had higher serum levels of TLA1 compared with controls, in particular patients with an active disease. In addition, TLA1 levels has a positive correlation with cytokines produced by Th17 cells (IL-17 and IL-21). The function of the indoleamine 2,3-dioxygenase (IDO1) enzyme is that to transform tryptophan (TRP) into kinurenins (KYN) a family of molecules whose effects on immune system cells has been reported leading to a suppression of effector T cells and a facilitation of their differentiation to regulatory T cells. B7 costimulatory molecules are expressed on antigen-presenting cells (APCs) and seem to influence the IDO intracellular expression. Legány *et al*. (56) in their recent work studied the expression of B7 molecules and the expression of IDO in patients with SSc (n. 9), Sjögren’s disease (pSS) (n. 15) and in healthy controls (n. 20) supposing that differences in B7 molecules expression might have a role in the diversities of the two diseases pathogenesis. Regarding the CD28 receptors expression, the authors showed that it was lower in SSc as well as in p(SS), similarly in both disease a decreased frequency of PD-1 expressing T cells were found. On the other hand, the frequency of some receptors, like CTLA-4 and ICOS were increase in pSS but not in SSc.

Infectious agents

As know monocytes/macrophages and TLR pathway seem to be involved in the SSc pathogenesis. In the study of Farina *et al*. (57) monocytes from 53 dcSSc and from 34 controls peripheral blood were isolated and infected by Epstein-Barr virus (EBV) in order to investigate if this infection contribute to innate immune activation in SSc. Authors showed an activation of TLR8 by viral lytic genes in monocytes. In addition, EBV seems to be associated with an IFN proinflammatory activation in cells isolated from dcSSc patients suggesting its potential role in the innate immune response. Human cytomegalovirus (HCMV) has been proposed an infectious trigger for SSc, however the definition of its role in the disease pathogenesis is still unknown. Marou *et al*. (58) recently aimed to investigated about immunoreactivity against this virus in SSc enrolling a population composed of 84 SSc, 30 subjects with multiple sclerosis (MS) and 28 controls. In order to conduct their study, authors tested IgG anti-HCMV directed against UL83 epitope founding higher levels in SSc population compared both to MS patients and controls. In addition, anti-UL83 levels were associated to lung fibrosis in SSc patients confirming HCMV possible role in SSc pathogenesis. Different results have been found about UL57, in fact even regarding HCMV, the same group recently investigated the prevalence of antibodies anti-HCMV UL44 and UL57 in SSc patients (n 60), MS subjects (n 40) and controls (n 17). The SSc group was composed by 30 lcSSc and 30 dcSSc patients. Anti-UL 57 was present in all three populations (SSc, MS, control group) without significant differences and its levels did not correlate with disease clinical, immunological and demographic characteristics in SSc population. Authors concluded that these results suggest that the role of HCMV UL57 epitope in SSc pathogenesis has to be investigated and further new studies will be needed (59).

Miscellaneous

Different previous studies tried to identify a correlation between rheumatic diseases and occupational risk factors. Even for SSc environmental factors, as the exposure to heavy metals, have been proposed as risk and trigger factors for the development of the disease. A recent study tried to assess the relationship between SSc and exposure to heavy metals enrolling a population composed by 100 SSc patients and 300 controls. Authors concluded indicating a certain impact of heavy metals in SSc development, as SSc patients had higher levels of some metals in hair samples compared to controls and showing a gender-dependant correlation between SSc and occupational exposure (60).

Clinical features

Serological profile

Since SSc is a chronic, multisystem disabling disease with no definitive cure, the efforts of the scientific community are targeted at the early diagnosis and treatment. It is clear that diagnosis and classification should be based on clinical grounds, but also serological characterisation is a useful adjunct to improve diagnostic power and to better refine prognosis. In this respect, the usefulness of autoantibody profile has been confirmed in a Spanish registry, where it was established the role for autoantibodies anti-RNA polymerase III (RNAP) in increasing the risk of SSc renal crisis, while Scl-70 strongly associate with ILD (61). Autoantibody specificity might be also a biologic filter for cancer risk stratification in SSc patients, as it was demonstrated in Johns Hopkins scleroderma cohort, confirming the role of RNAP for cancer risk definition. Moreover, cancer specific risk may vary depending on scleroderma subtype: patients with RNAP positivity and dcSSc had an increased risk for breast cancer, while those with lcSSc had a higher lung cancer risk (62). Alongside traditional autoantibodies, there is a growing interest in novel biomarkers which could help both in diagnosis and in understanding of the pathogenesis. For example, it was found that serum CD163, a well-accepted marker for activated M2 macrophages, levels are significantly (p<0.001) higher in SSc patients than in controls; suggesting a possible role of M2 macrophages' signalling in SSc pathogenesis (63). It was recently demonstrated that plasma concentrations of resistin, a protein thought to be involved in the inflammatory process, are higher in SSc patients than in controls; moreover, resistin showed a significant association with lung disease, arthralgia, oesophageal involvement and increased C-reactive protein (64). Another study revealed a lower concentration of the protein Klotho in SSc patients' serum as compared with healthy controls, although there is no significant association with clinical, laboratory or instrumental findings (65). It was also found that free light chains (FLC) of immunoglobulins, and specifically isotype K serum levels are elevated in SSc, more in detal is l k-FLC are significantly increased in subjects with restrictive lung disease and correlate with IL-6 levels, namely with a higher degree of inflammation. This finding supports a possible role for B cell activation in the pathogenesis of the disease (66).

Classification of the disease is based on 2013 criteria, which take into account a number of clinical features specific for the disease and include nailfold videocapillaroscopy (NVC) and serological characterisation. However, semistructured interviews randomly assigned to SSc experts have pointed out a series of limitations of the existing view of the disease: the classical division in limited and diffuse subset is almost universally accepted, but this definition may be misleading, since it could lead to misclassification or false prognostic definition, furthermore the limit of above or below the elbows is considered arbitrary. Most of researchers involved in this disease add other classification criteria, such as sine scleroderma SSc (ssSSc), divisions based on autoantibody profile, overlap and juvenile disease. This work has raised the question of some limitations in content validity of the existing criteria and the need of a more modern classification system based not only on clinical and cutaneous subset, but also on novel knowledge on autoantibodies and on the evolution rapidity of the disease (67).

Vascular involvement

One of the hallmarks of SSc is vascular involvement: an intriguing hypothesis is that the disease may be a primitive vascular disorder, with the loss of angiogenic and vasculogenic potential representing a major driver of most of the clinical events during the natural course of the disease. For example, serum endostatin levels, an angiogenesis inhibitor, which raises in severe vascular damage, were found significantly elevated in SSc patients presenting a progressive peripheral microvascular damage, so that endostatin can be considered a reliable marker of skin perfusion and digital arteries damage (68). Vascular complications affect not only capillary bed, but also larger vessels, with obliterative involvement of ulnar and tibial artery. This warrants a thorough assessment involving both micro and macrovascular system in SSc patients (69). In any case, although vascular abnormalities in SSc could represent a plausible risk factor for the development of venous thromboembolism (VTE), a recent study revealed a VTE cumulative incidence comparable between SSc patients and the general population. This suggests that, unlikely RA and SLE, SSc is not an independent risk factor for VTE (70). Microvascular involvement is exploited for the early diagnosis of the disease and NVC represents a milestone in the definition and prognostication of the disease (71). More recently, computed methods to enhance image quality and to standardise quantification of capillary measurements are emerging (72). NVC, in expert hands, is a valid, reliable, reproducible and inexpensive tool which has gained rightfully its place in clinical practice as indispensable diagnostic test for disease definition. In order to provide an idea on how NVC has become a priceless tool, during last year several studies were published regarding its prognostic role in SSc. A Portuguese paper revealed that NVC changes, as capillary loss and avascular areas, have a significant association with the presence of ILD (p=0.008 and p=0.015, respectively). Moreover, avascular areas and capillary loss were associated with a worse pulmonary function, consisting

in a reduction of both FVC and DLCO (73).

A prospective study investigated the progression of organ involvement in SSc patients with a late NVC pattern of microangiopathy at baseline, during a 5-year follow-up. It was found that, even if patients remained with a late NVC pattern, a decrease in absolute capillary number associated with the progressive worsening of pulmonary function and with an increase in renal arterial resistive index and in total DUs (74).

At the same time, a number of reports outline the need of more sophisticated techniques to better assess microvascular involvement in SSc (75).

Laser speckle contrast analysis (LASCA) is gaining growing attention as a reliable instrument useful in mapping skin areas, and also in characterising DUs (76). It was demonstrated a significant inverse relationship between skin blood perfusion measured with LASCA, and dermal thickness, assessed with both skin high frequency ultrasound and mRSS, at the dorsum of the middle phalanx of the third finger of both hands (77). However, these methods are still available only for research purpose.

One of the main complications of vascular involvement is the appearance of DUs. This complication needs a close attention by the clinician, since not only it has a prognostic significance, but also portends poor quality of life and reduced function (78). It was even found that the presence of DUs is significantly predictive of cardiovascular involvement. In fact, SSc patients with DUs tend to present a worse cardiac involvement, with more frequent electrocardiogram alterations, a higher rate of ischaemic events, a greater reduction in left ventricular ejection fraction (EF) and in diastolic function during follow-up (79). The definition of DUs is particularly challenging, since there is no uniform classification system and the heterogeneity of clinical presentation may complicate its approach. A simple operative classification based on prominent pathogenetic mechanism could be useful in DUs classification (80). Regarding lower-limb ulcers in SSc patients, a recent multicentre retrospective case-control study pointed out how approximately half of them is related to venous insufficiency while the other half is due to ischaemic causes with poorer outcomes. Past or concomitant DU and cutaneous sclerosis of the feet were found to be independent risk factors for ischaemic lower-limb ulcers (81). High frequency ultrasound is a feasible and tolerated technique in most SSc patients which may prove useful in better characterising DUs (82). Microvascular alteration is a prominent feature for several organs affected in SSc, in particular kidney, lung and heart. Intrarenal vascular resistance is an early sign of vascular involvement that, no less than NVC, could predict the evolution of the disease and its outcome (83). Scleroderma heart involvement, an ominous as well as insidious manifestation of the disease, stems its origin on myocytes band necrosis due to microvascular damage. A number of efforts addressed at the early recognition of this complication, has outlined the need of more sensitive screening methods than standard echocardiography. In this respect, cardiac magnetic resonance imaging (MRI), although with some limitations due to the method of elaboration, maintains its primacy in detecting early SSc heart involvement (84). In fact, apart from the assessment of ventricular volumes and EF, it is reconfirmed to be the gold standard technique to detect myocardial inflammation, early perfusion defects and myocardial fibrosis (85). A recent study evaluated with cardiac MRI eighty-two SSc patients without any clinical cardiac symptom. Interestingly, according to Lake Louis criteria (positivity if at least 2 on 3 of the examined indices are pathological: T2 ratio, early and late gadolinium enhanced images), nine of them (10.9%) were found positive for myocarditis. Remarkably, no correlation between MRI results and blood inflammatory indices, cardiac troponin T or SSc subgroups was identified. This leads to hypothesise a routinely assessment of SSc patients with cardiac MRI in order to reveal silent heart involvement. An early diagnosis in asymptomatic patients opens new perspectives in the management of a silent and often ominous clinical manifestation, although at present it is not completely clear the correct timing of screening and the prognostic significance of these findings (86). Newer techniques, such as speckle-tracking echocardiography (STE) analysis are promising but still under scrutiny. One of the most studied STE-parameters is the right ventricular longitudinal systolic strain (RVLSS), a deformation index useful in the assessment of regional myocardial contractility. On the basis of an already established RVLSS reduction of both global right ventricular and its lateral wall in SSc patients compared to healthy controls, it was recently detected a RVLSS deterioration in all myocardial layers, with an increasing worsening from the epicardial layer to the mid-myocardial and subendocardial ones. This finding clarifies that all myocardial layers are affected in this connective tissue disease, and not only the subendocardial one (even if it is the most affected), as it would be expected in patients with microvascular involvement (87). STE was also used to demonstrate differences between subgroups of pulmonary arterial hypertension (PAH). In fact, apart from the evidence that global RVLSS is markedly diminished in PAH compared to controls, it was showed that, at similar afterloads, this parameter is significant (p=0.03) worse in SSc-related PAH than in idiopathic PAH. Notably, right ventricular contractile deficit as detected by RVLSS were present despite shorter PAH disease duration in SSc-related PAH patients (88). Another work revealed that global longitudinal strain is impaired in SSc both in right and left ventricles, strengthening the hypothesis that contractility worsening is a direct consequence of primary myocardial involvement rather than a result of PAH. It was also showed how SSc patients with no clinical or traditional echocardiograpic parameters of heart involvement, has global longitudinal strain reduction in over 60% of cases, revealing a subclinical right and left systolic impairment. This data lead to reconsider the prevalence of cardiac involvement in SSc and propose STE as a low-cost, non-invasive and reliable tool in order

to detect early cardiac impairment in SSc patients, who would be otherwise underdiagnosed with traditional echocardiograpic parameters (89). PAH assessment and Recognition of outcomes in Scleroderma (PHAROS) registry is a multicentre prospective cohort of patients with SSc at high risk of developing PAH or with definitive right heart catheter pulmonary hypertension (90). 160 incident group I PAH patients were analysed, and Kaplan-Meier survival curves were generated for the overall cohort and for those who died of PAH. When restricted to PAH-related deaths, 93% of those occurred within 4 years from diagnosis. Male sex (hazard ratio [HR] 3.11), dcSSc (HR 2.12), systolic pulmonary artery pressure (PAP) on echocardiography (HR 1.06), mean PAP on right heart catheterisation (HR 1.03), 6MWD (HR 0.92;), and DLCO (HR 0.65) significantly affected survival on multivariate analysis. Although overall survival in PHAROS was higher than in other SSc-PAH cohorts, PAH accounted for more than one-half of deaths and primarily within the first few years after PAH diagnosis. This warrants a close follow-up of SSc patients to screen for this complication (91).

In this respect, DETECT algorithm has demonstrated higher utility as a screening tool in SSc subjects in comparison to other screening instruments (92). Evaluating the prevalence of PAH diagnosed by heart catheterisation before and after the institution of DETECT algorithm, a Norwegian study outlined that PAH frequencies were similar (18% in the DETECT cohort and 21% in the previous one). However, in the newer cohort the proportion of patients with borderline hypertension was increased when comparing with patients recruited before DETECT (31% *vs*. 17%), as well as the proportion of patients divided by risk stratification. In fact, in the DETECT group 27% of the patients were classified at low risk (19% in the previous cohort), while 27% at high risk (44% previously). These findings open new scenarios for earlier PAH diagnosis and treatment (93). Simple clinical features at enrolment, such as male sex, prior history of DUs, diffuse subset, FVC/DLCO ratio >1.6 might be useful to predict future vascular complications in unselected SSc patients (94).

It was also found that serum levels of adipsin, which is an adipokine, namely a peptide secreted by adipose tissue with recently identified effects in modulating fibrosis, inflammation and vascular homeostasis, are significantly (p<0.0001) elevated in patients with lcSSc and strongly associate with PAH (p=0.02) (95). In a prospective bicentric cohort of SSc patients included in the DETECT study, Coghlan *et al*. evaluated the incidence and determining factors of PH. Patients without PH at initial catheterisation were systematically followed-up by clinical examination and repeated catheterisation. High pulmonary vascular resistance at baseline, elevated tricuspid regurgitation velocity, low DLCO and enlarged size of inferior vena cava were independent predictors for PH development during follow-up. However, it should be underlined that due to the DETECT inclusion criteria, this cohort is preselected for SSc patients with DLCO <60%, which can limit its generalisability to an unselected SSc population. These patients in fact have a higher prevalence of PH (25%), while PAH seem to be similar (7%) in comparison with other series. Since low DLCO population shows a decline in pulmonary function test, a progression of haemodynamic alterations and a high incidence of PH, it seems reasonable to perform regular clinical assessment including right heart catheterisation in this population until more reliable methods of screening are available (96).

Since PAH in SSc is a heterogeneous condition, as patients may present a spectrum ranging from pure PAH without ILD to PAH due to extensive ILD, Launay et al. evaluated 200 SSc patients with PAH and classified them in homogeneous subgroups according to relevant and simple criteria in order to predict different future survivals. They just split PAH in severe (≥35 mmHg) and in mild-moderate (<35 mmHg), and divided ILD in extensive and in absent-limited on the basis of the staging system proposed by Goh *et al*. (97). A first consideration that can be done is that the presence of a limited ILD has to be considered in the same group of patients without ILD, where the severity of PAH drives the prognosis. They eventually found four simple homogeneous subgroups. The first important distinction concerns patients with extensive ILD, representing a cluster with the worst 3-years survival rate (49.9%). This means that the presence of an extensive ILD, whatever the PAH, is associated with a very severe outcome. All the other patients with absent or limited ILD can be divided according to the presence of severe PAH, individuating a cluster which accounts for a 61.9% 3-years survival, consistent with the poor prognostic features of high pulmonary vascular resistances and low cardiac indexes. Patients with mild-moderate PAH and no extensive ILD represent the most common presentation in SSc and can be further differentiated on the basis of low or normal DLCO, representing two clusters that carry a 3-year survival prognosis of 81.5% and 87.1%, respectively. An unanswered question is whether the last three clusters are distinct subgroups, or a same cluster diagnosed at a different stage of the disease (98).

Lung involvement

Pulmonary involvement is another frequent SSc complication, usually combined the presence of ILD with pulmonary vascular involvement. As previously said, the extremes of the spectrum are represented by pure ILD and pure pulmonary vascular involvement with isolated PAH, but most of the times a combination of the two conditions is present in the same patient (92). Lung function tests are not a sensitive screening tool for lung involvement in SSc, therefore using only this exam in the follow-up, a relevant number of patients with consistent ILD can be missed. Ultrasound B-lines are an excellent non-invasive method for the assessment of ILD in SSc subjects. In a single-centre study of 40 consecutive SSc patients, the US B-lines number and the Warrick score confirmed excellent correlation (Spearman rho: 0.958, p=0.0001). The receiver operating characteristic (ROC) curve analysis revealed that 10 US B-lines are the cut-off point with

the greatest positive likelihood ratio (12:52) for the presence of significant SSc-ILD. Thus, the detection of at least 10 B-lines is highly predictive for the high resolution CT (HRCT) presence of SSc-ILD. In SSc patients, lung US assessment as first imaging tool, may represents an effective model to improve the correct timing of chest HRCT (99). However, in order to assess the severity of lung involvement and the individual risk of progression to end stage disease, it is necessary to perform HRCT scan, which remains the gold standard for the definition of the extension and type of lung involvement. Indeed, in the context of an ILD, the affection of more than one third of lung parenchyma and the presence of reduced FVC at an early stage of the disease, classifies the patient at higher risk of disease progression, thus identifying a subgroup of patients with higher potential advantage from immune suppression (100). In patients with milder disease, however, it is more difficult to establish a cut off point for risk of progression. In this context a recent study assessed the risk of progression in unselected SSc patients with mild lung involvement and found that the presence of arthritis and reduced SpO_2 after 6MWD might represent red flags that independently predict the risk of evolution at follow-up. Outcome measures was derived from a prospective dataset of patients from University centre in Zurich and validated on cohort from 3 different centres across Europe (Oslo, Paris; Berlin). SpO_2 <94% after 6MWD associated with arthritis ever during the follow-up, predicted the progression of ILD at follow-up both in the derivation and in the validation cohort (101). Whilst reduced saturation is intuitively correlated with worsening of lung involvement, it is less clear how the presence of arthritis could represent a link with the risk of future lung impairment in these patients, and possible biases related to concomitant treatments, pattern of ILD or recurrent infections have been claimed (102).

As far as 6MWD is concerned, although it has demonstrated several limitations in SSc subjects and may reflect not only parenchymal but also vascular aspects of lung involvement, it is feasible in the majority of SSc patients and represents a good reference by comparing the single patient values at different time points, rather than rest upon the reference standards at a single observation, since the general population cannot represent a reliable comparison in these subjects (103-104). A study conducted repeating 6-MWT twice, within a minimum 3 months interval among 56 SSc patients, showed strong reproducibility (p<0.0001) of this test and pointed out that factorsi like mRSS, arthralgias and tendon friction rubs, FVC, DLCO, left ventricular EF are independently associated with a lower 6MWD. Considering that 6MWD at first referral was found to be an independent predictor of overall mortality and SSc related mortality, this test is confirmed as a useful tool to assess the overall prognosis in SSc patients (105). To detect progression of ILD, slice reduced sequential HRCT (reduced HRCT) may be an alternative to standard HRCT. In a retrospective study conducted on 60 SSc subjects, reduced HRCT was non-inferior to standard HRCT, with the only exception for the detection of intrapulmonary bronchiectasis, which was significantly lower in reduced HRCT than in standard HRCT (p=0.039). No differences were found comparing visual scores for fibrosis severity and extension from standard and reduced HRCT. Hence, reduced HRCT might be used to detect early disease progression of lung fibrosis in SSc patients (106).

Skin involvement

Skin involvement is a prominent feature of the disease. Numerous data have pointed out a significant prognostic role not only for the extension but also for the pace of skin involvement progression. It is therefore pivotal to predict the pace of progression, in order to timely start treatments and also for the purpose of trial enrichment. Scleroderma Observational Study has set a study to identify patterns and predictors of skin score change in early dcSSc, recording mRSS every 3 months in 326 SSc patients. 'Progressors' were defined as those experiencing a 5-unit and 25% increase in mRSS score over 12 months. Logistic models were fitted to predict progression and, using ROC curves, were compared on the basis of the area under curve (AUC) accuracy and positive predictive value (PPV). 22.5% of the patients progressed, while 77.5% did not. Progressors had shorter disease duration (median 8.1 *vs*. 12.6 months, p=0.001) and lower mRSS (median 19 *vs*. 21 units, p=0.03) than non-progressors. Skin score was highest, and peaked earliest, in the RNAP subgroup. A first predictive model (including mRSS, duration of skin thickening and their interaction) had an accuracy of 60.9%, with AUC of 0.666 and PPV of 33.8%. Adding a variable for RNAP positivity, the model reached an accuracy of 71%, with AUC of 0.711 and PPV of 41% (107). Another common skin feature in SSc patients is the presence of telangiectasias. Telangectasia (TA) is a capillary dilation located over different skin surfaces and several previous reports have correlated the number and extension of TAs to the severity of vascular involvement. Recently, a single-centre study has investigated the distribution and clinical significance of TAs in 106 consecutive SSc patients. The authors pointed out that TAs were predominantly located on the face, hands, and the upper part of the trunk. TAs appeared to be associated with vasculopathy features of SSc, particularly with PAH and soluble endoglin levels (108).

Gastrointestinal involvement and nutrition

GI tract involvement is present in up to 90% of SSc patients, with the esophagus the most frequently affected. It is not correlated to the entity of skin harassment and can be severe and disabling in up to 8–10% of SSc subjects, where it portends a poor outcome. Gastroesophageal reflux is a frequent complaint, however there is no strict association between symptoms and the severity of the organ involvement. Reflux aetiology in SSc is likely multi-factorial with both inheritance and exposures playing a role in the pathogenesis (109-110). Severe GI dysmotility (upper and lower bowel dysfunction till the requirement of enteric or parenteral nutrition) is a life-threatening condition with 85% mortality rate. McMahan *et al*. re-

vealed that SSc patients with severe GI dysmotility are more likely than those with mild symptoms to be male, to have myopathy and sicca symptoms, while baseline features associated with future severe GI dysmotility include male sex and myopathy (111). Malnutrition is a frequent finding in SSc patients: it can be related to GI involvement, to difficulties in correct feeding due to disability, to increased energy expenditures for systemic involvement and eventually it can be related to treatments. While body mass index is not a good indicator of malnutrition, body composition by electric impedance analysis is a more reliable method for its identification. In a single-centre study involving 129 SSc patients, sarcopenia was a common finding, affecting 22.5% of the subjects and was associated with a physical impairment that affects everyday life and participation in work. Interestingly, although age is the main risk factor for sarcopenia in the general population, it did not differ between sarcopenic and non-sarcopenic SSc patients. Instead, the number of immunosuppressive drugs was significantly higher among the sarcopenic subgroup. However, it is no clear to what extent this finding might be related to the severity of the disease or be ascribed to the treatments (112).

Other organ involvements

The urinary tract has been poorly investigated in SSc, but a multicentre study involving 334 SSc subjects revealed that lower urinary tract symptoms (LUTS), ranging from storage to voiding and post-micturition symptoms, are recorded in over 95% of the cases, usually in association with digestive complaints, with strong impact on quality of life (113). Urinary incontinence is another frequent symptom and is significantly associated with limited subset and anticentromere antibodies (ACA) (114). Another frequent but overlooked finding in SSc patients is small fiber involvement of the skin, with neuropathic pain; whose entity seem to be strictly correlated with skin involvement (115). Another aspect of small nerve fiber involvement seem to be inner ear involvement and earing loss. In a small SSc series it was found that hearing loss may be present in over 2/3 of the patient, in clinical practice this aspect is however frequently missed since mild, with prevalent sensory neural hearing loss, abnormal pure tone audiometry and abnormal speech reception threshold as compared to controls (116). Analysing bone mineral density (BMD) of the lumbar spine and deriving spine bone quality using the trabecular bone score (TBS), it was demonstrated that SSc patients show significantly lower BMD and TVS values than healthy controls. This account for a greater risk of osteoporosis and a lower bone quality. TBS values were found to be lower in SSc patients with a late NVC pattern, as compared to active and early ones. Despite there was no statistically significant correlation between TBS values and 25-hydroxyvitamin D levels, it is interesting to notice that these values were progressively lower as NVC pattern progressed (117).

Patient-reported outcome (PRO)

In SSc the failure to find a true DMARD drug and the relative small effect of immunosuppressant or antifibrotic medications on several distinct clinical outcomes in RCT with endpoints on various organ manifestations have always been further hampered by the lack of a patient reported outcome reliably defining the impact on the personal, psychological and social aspects of the disease (92). There are several general patient-reported outcome (PRO) instruments (Health Assessment Questionnaire Disability Index (HAQ-DI), Short Form Health Survey-36 (SF-36)) and SSc-specific PROs, some organ specific [University of California, Los Angeles, Scleroderma Clinical Trials Consortium Gastrointestinal Tract Scale 2.0 (UCLA SCTC GIT 2.0), and the Mouth Handicap Scale in SSc, Cochin Hand Function Scale (CHFS)], and some generalised [the Symptom Burden Index, the UK SSc Functional Score and the scleroderma HAQ (SHAQ)] (118). Most of the PRO used in RCT and/or clinical practice have not always involved the target patient population in the development and therefore are not always adequate measures that can capture involvement in a clinical/symptomatic aspect of the disease nor the patient quality of life (119). This is an aspect that must be taken into account when developing new and targeted PROs for SSc, whose heterogeneity in clinical and symptomatic manifestation is raising the gap between clinicians' and patients' perspectives.

For measures of pain and disability a recent study by Daste *et al*. (120) provided the Patient Acceptable Symptom State (PASS) and the Minimally Clinically important Difference (MCID) for 5 PROs commonly in use in the practice and in RCT: joint pain intensity assessed by a 100-mm visual analog scale (VAS pain), global activity limitation assessed by the HAQ, systemic sclerosis-specific global activity limitation assessed by the scleroderma HAQ (sHAQ), patient-perceived activity limitation assessed by the McMaster-Toronto Arthritis Patient Preference Disability Questionnaire (MACTAR), hand-specific activity limitation assessed by the Cochin Hand Function Scale (0, no limitation, to 90, maximal limitation); and health-related quality of life assessed by the Medical Outcomes Study 36-Item Short Form (SF-36) (See Table I) (120).

These thresholds are essential in evaluating the practical effect of an intervention (pharmacological or non-pharamacological) from the patient perspective, which is unique and complementary to the physician assessment and whose statistical significance is not always a life-changing difference.

PASS itself has been evaluated as an outcome measure alone in a secondary analysis of the Safety and Efficacy of Subcutaneous Tocilizumab in Adults with Systemic Sclerosis (faSScinate) trial (121). Authors have evaluated 3 different ways to evaluate patients' perception of their symptom state: by asking to answer on a 7-point Likert scale with scores from -3 (highly unacceptable) to 3 (highly acceptable) to the question 'Considering all of the ways your scleroderma has affected you over the last week, how acceptable would you rate your level of symptoms?' (PASS 1) or using a dichotomous out-

Table I. Patient acceptable symptom state and minimal clinically important difference of 5 PROs commonly used in clinical practice and randomised controlled trials.

	PASS	MCID		Responsiveness	
		Improvement	Worsening	Effect size	SRM
Joint-pain visual analog scale (0-100)	53.75, (95%CI)34-68	6.74 ± 32.02	-5.08 ± 31.67)	-0.21	-0.19
HAQ (0-3)	1.41, 1.13-1.63	-0.21 ± 0.48	0.04 ± 0.40	-0.31	-0.40
sHAQ (0-3)	1.27, 1.07-1.62	-0.13 ± 0.45	0.14 ± 0.41	-0.29	-0.32
Cochin Hand Function Scale (0-90)	26, 17-37	-3.38 ± 9.87	1.43 ± 13.11	-0.14	-0.21
MACTAR (0-30)	19.4, 17.2-21.9	-5.69 ± 6.79	-2.63 ± 4.84)	-0.66	-0.57

SRM: standardised response means; PASS: patient acceptable symptom state; MCID: minimally clinically important difference. Adapted from reference 3.

come: 'Think about all the ways that your scleroderma has affected you during the last week. If you were to remain for the next few months as you were in the last week, would this be acceptable to you?' and yes/no responses (PASS 2) or with the question 'Has there been a change in how you would describe your level of functional impairment since you started the study?' with responses on a 5-pont Likert scale (from -2 (much worse) to 2 (much better)). At the end of the trial the vast majority of patients had achieved PASS by all the 3 methods (69, 71 and 78% for PASS 1, 2 and 3, respectively), with increasing figures during the trial and with a statistically significant capacity for PASS 1 to discriminate placebo from the active drug. PASS had a moderate correlation to clinical outcomes and PROs of the Fasscinate study such as mRSS, physician global assessment [physician global visual analogue scale (VAS)], patient global assessment (patient global VAS) and HAQ-DI, thus proving its complementarity in patients' assessment and the possibility to be evaluated in future clinical trials of SSc (121). The Scleroderma Patient-centered Intervention Network Cohort Study (SPIN) investigators, an initiative collecting data from English speaking SSc cohorts in Canada, US and UK, reported the application of the National Institutes of Health (NIH) Patient-Reported Outcomes Measurement Information System (PROMIS) v29 in their patients (122). PROMIS29 is part of an initiative of the NIH to collect item banks for measuring patient-reported outcomes across various medical conditions which are standardised to the general US population. PROMIS29 is composed of four items for each of seven domains (physical function, anxiety, depression, fatigue, sleep disturbance, ability to participate in social roles and activities, pain interference), scored on a 5-point scale, plus a single pain intensity item, measured on an 11-point rating scale. Kwakkenbos *et al*. (122), applied PROMIS29 to a total of 696 patients, 87% females and with a long disease duration (9.7±8 years). With respect to the standard (the US population), the mean PROMIS-29v2 domain scores were reported to be 0.7 S.D. lower (worse) for physical function, 0.6 S.D. higher (worse) for pain intensity and fatigue, 0.3 S.D. lower (worse) for social roles, 0.3 S.D. higher (worse) for sleep problems and similar for symptoms of depression (0.1 S.D. higher) and anxiety (0.2 S.D. higher). Construct validity of PROMIS29 was demonstrated by showing significant correlation with legacy score such as the HAQ-DI or the CHFS. It also demonstrated a correlation, significant albeit weak, between older SSc patients and depression or lower scores of anxiety and fatigue. Disease features that were reflected in lower performance score were high mRSS, gastrointestinal (GI) involvement and the presence of joint contractures. Further research should concentrate on finding the minimally important difference (MID) and the sensitivity to change of PROMIS, both key information for the applicability of this PRO in RCT or clinical practice. On the SPIN cohort another patient-centered study was conducted to analyse patient exercise habits by a simple questionnaire (123). Patients responded to a single item related to exercise, "Do you exercise at present?" (yes/no), and if exercising, two additional items were recorded, "On average, how many hours per week do you exercise?" and "What type(s) of exercise do you do?" [walking, jogging, aerobic, swimming, other (specify)]. Approximately half of patients reported exercising with walking being the most common form of exercise. Those who exercised showed significantly better PROMIS-29 scores for function and social roles, lower PROMIS-29 in the psychological effects and pain, HAQ-DI total scores were also significantly lower, indicating less disability. This study especially focusing on the self-reported exercise habits is important to provide indications on specific exercise interventions which are a big part of the non-pharmacological approach to the maintenance of physical and psychological health in SSc and that are often overlooked in the medical approach to the disease (124). For what concerns SSc organ specific manifestations, RP is a key symptom in SSc, objectivated with difficulty in the clinic and relying mainly on PRO for its evaluation. In a recent north-American and British study, SSc patients were involved in focus groups with RP at the centre of discussion to understand the subjective components defining the personal experience of RP (125). In this study a discrepancy has emerged between the currently used PROs for this manifestation (namely the SHAQ RP VAS item and the Raynaud Condition Score (RCS) and the themes emerged from patients participating in the FG. The concept of RP "attacks" was not always representative of the patient experience, and the physical impact was not only confined to "pain" or "painful sores", as mentioned in the

RCS, but encompassed feelings as "tingling", numbness", "hypersensitivity". Neither the other themes emerged in the FG were fully captured by current PROs (*e.g.* impact on daily life, trigger factors, constant vigilance, adaptation, emotional impact) indicating the need of a new measure, which should include patient participation in its development (125). Patients contribution to research in SSc is a key factor for RP and its categorisation for the aim of clinical research. A study by Pauling *et al.* has recently demonstrated that the experience of RP is better described in SSc patients by 4 different patterns of evolution in time. The pattern described by the majority of the patients in the study is that of intermittent longer-lasting attacks (with the circulation in fingers not always returning to normal between attacks). Patterns may evolve in time and correlate well with other PROs as SHAQ, and in particular the VAS GI SHAQ, indicating a relationship between different patients' experience. Further longitudinal studies should clarify whether this is a pathogenetic link or a connection between different pain experiences (126).

Recently, a new skin-related quality of life PRO in patients with SSc has been developed by Man *et al.* the Scleroderma Skin PRO (SSPRO) (127). This includes, in its final version, 18 items obtained by a process consisting on patient focus groups, revisions of expert panels composed by rheumatologists but also dermatologists, psychometrists and biostatisticians and cognitive interviews. SSPRO has been tested in patients with SSc included in a study at the Boston University Medical Center (BUMC). The constructs around which SSPRO is built are physical limitations (PL) related to skin tightness, physical effects (PE), emotional effects (EE) and social effects (SE). Skin severity levels from 1 to 6 were collapsed to three categories: mild, moderate and severe. SSPRO scores were different in limited cutaneous(lc)-SSc and diffuse-cutaneous(dc)-SSc. They also correlated well with the patient-reported skin severity of the SHAQ visual analogue scale (VAS) (construct validity) but interestingly correlation was weak when SSPRO was associated to physician-reported outcomes including physicians' assessment of overall skin disease, global assessment of disease and most importantly, the mRSS. SSPRO showed also internal consistency and moderate-to-high test–retest reliability. The great advantage of SSPRO is the direct SSc patient's participation in its development which helps capturing unique features of the disease with an impact on relevant physical, psychological and social domains (127). It is a new instrument, complementary to other solid and well validated measures, to assess the skin in SSc and that needs further attention (*e.g.* research of the MID) for its promising added value in clinical trials of organ-targeted treatments.

Another sphere of symptoms that are very important to the daily impact of the disease on the patient's quality of life but evaluated with difficulty by objective clinical measures concerns the upper GI tract. In particular, swallowing difficulties (SD) can interfere with one basic life activity and pleasue, eating, but also with oral medication intake and hence patients' adherence to therapy. One recent study by Messerli *et al.* (128) has evaluated the prevalence and pattern of SD in medication intake through a self-reported questionnaire. Authors conducted a systematic literature review (SLR) about SD evaluated in a systematic and structured form as an outcome measure. Items identified by the SLR were included in a questionnaire (named SWAMECO for SWAllowing difficulties with MEdication intake and COping strategies), composed of 30 items covering themes as complaints, intensity of the problem, localisation (mouth, throat, pharynx, oesophagus), coping strategies, adherence. Preliminarly, face and content validity was evaluated in small groups composed of patients and health care professionals, secondly it was proposed to a small cohort of patients (n=46). The final analysis on patients revealed that approximate half of them have SD with medication intake, mostly located at the pharynx (43%) or esophagus (34%) level. As a result, 10% of patients stopped the medication and 40% modified the form of the medication by splitting tablets, crushing pills, opening the capsule etc. This problem was affecting the take of drugs central to SSc therapy such as calcium antagonists or PDE5 inhibitors. The use of PRO is the easiest and more reliable way to quantify this essential symptom and try to adopt medically valid coping methods without altering the drug and potentially increasing toxicity and/or side effects.

Treatment

SSc is associated to a very high social and economic cost, especially in the presence of some clinical manifestations such as lung and GI involvement (128-130). In 2017, a European task force updated the 2009 recommendations for the treatment of SSc; the approaches are divided in 6 major subgroups that include the treatment of RP, DUs, PAH, skin, ILD, renal disease and GI involvement. Despite these new recommendations, the treatment for SSc patients continues to be complex, mainly because the disease is heterogeneous, and it is difficult to target all the three mechanisms that are involved in SSc pathogenesis: the immune system activation, vascular abnormalities and tissue fibrosis (130). Although substantial progresses for the first two mechanisms have been made in last years, there still lack of an effective antifibrotic treatment, even if novel therapies are under investigation.

Immunosuppressive treatment

A better knowledge of the use of existing therapies would be desirable with regard to the immunosuppressive treatment. For example, for corticosteroids too, that are still widely used in SSc mainly for arthritis, myositis and ILD, there are many unknown aspects that should be investigated (131).

Available evidences demonstrated that cyclophosphamide (CTX) remains one of the most prescribed and effective treatments for SSc-ILD (132-133), but it is associated to severe adverse events, including severe immunosuppression, hepatic toxicity and hypogonadism (134), that has been confirmed also in male patients who may have lower lev-

els of testosterone (135). A recent article, published by a Saigusa *et al. (136)*, propose the possibility of identifying the patients responsive to CTX using plasmin-a2-plasmin inhibitor complex (PIC) as a test for quantifying plasmin levels, as in patients responsive to CTX the PIC levels are higher compared to non-responders and decrease significantly after the treatment (136).

Interestingly, recently evidences about the use of mycophenolate mophetyl (MMF) in the treatment of SSc-ILD have been published (137). Reporting results derived from patients enrolled in the scleroderma lung study (SLS) II (138), compared to the placebo arm of the SLS I(139), an improvement in pulmonary function tests (FPTs) and in skin involvement have been reported. Despite the evident biases that can be produced by the use of patients and controls enrolled in different studies, Volkmann and *et al*. reported a significative improvement in dyspnea and PFTs (including FVC and diffusion lung capacity of CO) (140). In addition to the improvement in the PFTs, a recent study reported an improvement also in the percentage of ILD involvement assessed by a novel computerised automated system analysis for high resolution computed tomography (CALIPER)(141). MMF has been reported to be effective also in the treatment of skin involvement in SSc patients, with an improvement of mRSS (140-142), and these results are maintained also during long-term follow-up (142). Rituximab (RTX) is starting to be widely used in the treatment of SSc. In an open-label trial, positive results about RTX compared to conventional immunosuppressants in terms of improvement in the FVC and mRSS have been published (143). These data are confirmed also by a Belgian study that reported also efficacy on the overall disease activity score (144). The treatment has been confirmed to be relatively safe and well tolerated (143-144). A randomised controlled trial for RTX against CTX as first line treatment for ILD-SSc has been proposed (145).

Vasoactive treatment

In the management of SSc, vasoactive treatments are useful in the treatment of different aspects of the disease, mainly for PAH and for RP and DUs (133). PAH in SSc patients represents a major burden for the treatment, as it has a very bad impact on the patients' outcome and prognosis. Several drugs, including endothelin receptors antagonists (ERA), phosphodyestherase-5 (PDE5) inhibitors, prostacyclin analogues and riociguat should be considered to treat SSc-related PAH (133). A combination therapy with ambrisentan and tadalafil could be associated to a better outcome on the left and right ventricular function (146), and the best results for this treatment have been reported in patients treated with the combination as the first therapeutic approach (147-148). The rate of side effects reported with the combination therapy in SSc patients is very high but SAEs are rare and limited (148). The efficacy of tadalafil seems to be associated to changes in gene expression profiles especially for genes regarding the Stat4-dependent, NO-dependent IL-12 pathways and in genes encoding for ECM components; moreover, the gene expression pattern may help to identify patients at higher possibility to respond to the treatment (149). Promising results about for the reduction of morbidity and mortality for selexipag, a selective prostacyclin receptor agonist, in the treatment of patients with PAH have been reported (150). Recently, a subgroup analysis about patients with CTD has been described; despite patients with SSc were more severe compared to other CTD-PAH patients at the baseline evaluation, selexipag allowed to delay the progression of PAH also in this subgroup of patients (151).

Riociguat, a soluble guanylate cyclase stimulator, was reported to improve cardiac and respiratory functionality in SSc patients with efficacy and safety similar to the non-CTD population (152). Additionally, riociguat was effective in the treatment of skin involvement in animal models of SSc and a multinational randomised trial started to check the efficacy of riociguat in skin involvement of SSc patients (153). Despite these promising results, it should be considered that SSc-PAH patients continue to have a worst prognosis compared to patients with idiopathic PAH, as the right ventricle function may not improve even after the institution of a vasodilator therapy (154). Results about vasoactive therapy for the treatment of RP secondary to SSc confirmed that sildenafil is able to increase the finger blood flow assessed by lased doppler imaging and improve RP severity and duration (155). Iloprost remains one of the most effective therapy for long-term treatment of RP and DUs in SSc patients (156), and recently the possibility of a home-based treatment with automated pumps have been tested in a small population with promising results (157). However, as reported in PAH treatment, also in the treatment of RP the association between two drugs, as bosentan and iloprost may be more effective compared to iloprost alone on nailfold capillary architecture (158). The promising results achieved by the treatment with selexipag in SSc-PAH have not been confirmed for the treatment of RP secondary to SSc (159), even if this the results of this study may have been influenced by the relatively short exposure to the drug. In addition to the systemic approach, there may still be the room for topical treatment in SSc-DUs: as reported by Hughes *et al*., the application of topical glycerine trinitrate may improve the blood flow in SSc-DUs (160). A study about local injections of botulin toxin A for secondary RP failed to confirm an improvement of local blood flow in SSc fingers (161), while the results with botulin toxin B are more promising (162).

Antifibrotic drugs

In an open-label trial about dasatinib, a tyrosine kinase inhibitor with potential antifibrotic effect, the drug failed to demonstrate improvement in mRSS or pulmonary function tests, while an improvement of percentage of changes on quantitative HRCT suggested a possible antifibrotic effect, almost in some patients (163).

Despite preclinical evidence suggested that calcium channel blockers (CCBs) may have an anti-fibrotic effect (164), a recent retrospective study did not

confirm these observation in the skin of SSc patients (165).

Encouraging preclinical results have been published about antifibrotic treatment as nintedanib and pirfenidone in animal models (166-167), and clinical trials in SSc patients are ongoing (168). Recently, a double-blind, randomised, placebo-controlled clinical trial has been published about the potential use of SAR100842, a potent lysophosphatidic acid 1 receptor antagonist, for the treatment of skin fibrosis in a small group of SSc patients. SAR100842 was relatively well tolerated and allowed a significant reduction of mRSS compared to placebo with a responder rate of 69.2%. These promising results should be confirmed in larger confirmatory trials (169).

Transplant and cellular treatment

Autologous stem cell transplantation (ASCT) may represent a potential therapeutic option for refractory SSc and is now included in the EULAR recommendations (133). The protocols proposed for mobilisation and conditioning regimens are heterogeneous and they could influence transplant related mortality (170) but patients' characteristics, particularly for cardiac dysfunctions, remains the most important prognostic factor for the prognosis and mortality of patients receiving ASCT (170-171). However, despite the mortality for the ASCT procedure is still high, the prognosis in patients with severe disease seems to be favourable (172). Negative prognostic factors for ASCT transplantation response may include the pre-transplant B cell clonal expansion (173) and the presence of a specific epitope for anti-topoisomerase I (489-573) (174). A combined therapeutic approach with CTX, plasmapheresis and mesenchymal stem cells transplantation, could be feasible and beneficial for SSc patients (175). Novel data supports the possibility to candidate SSc patients to lung transplantation for the treatment of ILD or PAH, provided that patients are adequately screened for other organs involvement (176). Renal transplantation in SSc patients with end-stage renal disease may be associated to excellent results for patients and graft survival (177).

Treatment for specific organ involvements

Despite the therapeutic advances in vasoactive treatments, ischaemic DUs continue to represent a major burden for SSc patients and the control of DUs may improve the function of the hands in patients (178). Although the treatment for SSc-DUs cannot disregard the need for a systemic approach, local treatment is crucial for the healing of the wound. Debridement, essential passage for the local approach, can cause severe pain and the application of local lidocaine 4% is associated to a reduction for the pain associated to the procedure and may safely improve the local management of digital wound in SSc (179). A retrospective report confirmed the possible role of hyperbaric oxygen therapy in favourite healing in a small cohort of SSc patients with refractory skin ulcers (180).

GI in SSc if frequent and difficult to treat as the pathogenesis is still not understood (181). For the treatment of GI, prucalopride, a serotonin receptor 4 antagonist, increased the number of intestinal evacuations and improved the GI symptoms in a small randomised trial, although the safety profile is not completely exempt from adverse events (182). Domperidone and algycon may be both equally effective for the treatment of upper GI in SSc patients, and may represents a safe add-on to the standard proton pump inhibitor treatment (183).

References

1. OKA A, ASANO Y, HASEGAWA M *et al*.: RXRB Is an MHC-Encoded Susceptibility Gene Associated with Anti-Topoisomerase I Antibody-Positive Systemic Sclerosis. *J Invest Dermatol* 2017; 137: 1878-86.
2. GENTIL CA, GAMMILL HS, LUU CT, MAYES MD, FURST DE, NELSON JL: Characterization of the HLA-DRβ1 third hypervariable region amino acid sequence according to charge and parental inheritance in systemic sclerosis. *Arthritis Res Ther* 2017; 19: 46.
3. HUDSON M, BERNATSKY S, COLMEGNA I *et al*.: Novel insights into systemic autoimmune rheumatic diseases using shared molecular signatures and an integrative analysis. *Epigenetics* 2017; 12: 433-440.
4. REZAEI R, MAHMOUDI M, GHARIBDOOST F *et al*.: IRF7 gene expression profile and methylation of its promoter region in patients with systemic sclerosis. *Int J Rheum Dis* 2017; 20: 1551-61.
5. BERGMANN C, BRANDT A, MERLEVEDE B *et al*.: The histone demethylase Jumonji domain-containing protein 3 (JMJD3) regulates fibroblast activation in systemic sclerosis. *Ann Rheum Dis* 2018; 77: 150-8.
6. AVOUAC J, GUIGNABERT C, HOFFMANN-VOLD AM *et al*.: Role of Stromelysin 2 (Matrix Metalloproteinase 10) as a Novel Mediator of Vascular Remodeling Underlying Pulmonary Hypertension Associated With Systemic Sclerosis. *Arthritis Rheumatol* 2017; 69: 2209-21.
7. LIS-ŚWIĘTY A, GOLA J, MAZUREK U, BRZEZIŃSKA-WCISŁO L: Transcriptional Activity of Nuclear Factor κB Family Genes in Patients with Systemic Sclerosis. *Ann Clin Lab Sci* 2017; 47: 306-9.
8. TARONI JN, GREENE CS, MARTYANOV V *et al*.: A novel multi-network approach reveals tissue-specific cellular modulators of fibrosis in systemic sclerosis. *Genome Med* 2017; 9: 27.
9. BARAŃSKA M, RYCHLIK-SYCH M, SKRĘTKOWICZ J *et al*.: Genotype and haplotype analysis of ABCB1 at 1236, 2677 and 3435 among systemic sclerosis patients. *Autoimmunity* 2017; 50: 277-82.
10. MAHMOUDI M, FALLAHIAN F, SOBHANI S *et al*.: Analysis of killer cell immunoglobulin-like receptors (KIRs) and their HLA ligand genes polymorphisms in Iranian patients with systemic sclerosis. *Clin Rheumatol* 2017; 36: 853-62.
11. ABBASI F, MANSOURI R, GHARIBDOOST F *et al*.: Association Study of CD226 and CD247 Genes Single Nucleotide Polymorphisms in Iranian Patients with Systemic Sclerosis. *Iran J Allergy Asthma Immunol* 2017; 16: 471-9.
12. D'AMICO F, FIORITO G, SKARMOUTSOU E *et al*.: FOXP3, ICOS and ICOSL gene polymorphisms in systemic sclerosis: FOXP3 rs2294020 is associated with disease progression in a female Italian population. *Immunobiology* 2018; 223: 112-7.
13. LUO W, ZHOU B, LUO Q, FANG H, ZUO X, ZOU Y: Polymorphism of keratin 1 associates with systemic lupus erythematosus and systemic sclerosis in a south Chinese population. *PLoS One* 2017; 12: e0186409.
14. KRYLOV MY, ANANYEVA LP, KONEVA OA *et al*.: The influence of STAT4 rs7574865 (G/T) polymorphism on the risk of clinical and immunological phenotypes of systemic sclerosis in a Russian patient population: Results of a pilot study. *Ter Arkh* 2017; 89: 20-5.
15. CAO L, LAFYATIS R, BURKLY LC: Increased dermal collagen bundle alignment in systemic sclerosis is associated with a cell migration signature and role of Arhgdib in directed fibroblast migration on aligned ECMs. *PLoS One* 2017; 12: e0180751.
16. TARONI JN, GREENE CS, MARTYANOV V *et al*.: A novel multi-network approach reveals tissue-specific cellular modulators of fibrosis in systemic sclerosis. *Genome Med* 2017; 9: 27.
17. GORLOVA OY, LI Y, GORLOV I *et al*.: Gene-level association analysis of systemic sclerosis: A comparison of African-Americans and White populations. *PLoS One*

2018; 13: e0189498.
18. WERMUTH PJ, PIERA-VELAZQUEZ S, JIMENEZ SA: Exosomes isolated from serum of systemic sclerosis patients display alterations in their content of profibrotic and antifibrotic microRNA and induce a profibrotic phenotype in cultulred normal dermal fibroblasts. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S21-30.
19. ZHOU B, ZHU H, LUO H *et al*.: MicroRNA-202-3p regulates scleroderma fibrosis by targeting matrix metalloproteinase 1. *Biomed Pharmacother* 2017; 87: 412-8.
20. ZHOU B, ZUO XX, LI YS *et al*.: Integration of microRNA and mRNA expression profiles in the skin of systemic sclerosis patients. *Sci Rep* 2017; 7: 42899.
21. ARTLETT CM, SASSI-GAHA S, HOPE JL2, FEGHALI-BOSTWICK CA, KATSIKIS PD: Mir-155 is overexpressed in systemic sclerosis fibroblasts and is required for NLRP3 inflammasome-mediated collagen synthesis during fibrosis. *Arthritis Res Ther* 2017; 19: 144.
22. ROSSATO M, AFFANDI AJ, THORDARDOTTIR S *et al*.: Association of MicroRNA-618 Expression With Altered Frequency and Activation of Plasmacytoid Dendritic Cells in Patients With Systemic Sclerosis. *Arthritis Rheumatol* 2017; 69: 1891-902.
23. DOLCINO M, PELOSI A, FIORE PF *et al*.: Gene Profiling in Patients with Systemic Sclerosis Reveals the Presence of Oncogenic Gene Signatures. *Front Immunol* 2018; 9: 449.
24. BENYAMINA A, MAGALON J, COINTE S *et al*.: Increased levels of fractalkine and mobilisation of $CD34^{+}CD45$- endothelial progenitor cells in systemic sclerosis. *Arthritis Res Ther* 2017; 19: 60.
25. MANETTI M, ROMANO E, ROSA I *et al*.: Endothelial-to-mesenchymal transition contributes to endothelial dysfunction and dermal fibrosis in systemic sclerosis. *Ann Rheum Dis* 2017; 76: 924-34.
26. MORITZ F, SCHNIERING J, DISTLER JHW *et al*.: Tie2 as a novel key factor of microangiopathy in systemic sclerosis. *Arthritis Res Ther* 2017; 19: 105.
27. KAWAGUCHI Y: Contribution of Interleukin-6 to the Pathogenesis of Systemic Sclerosis. *J Scleroderma Relat Disord* 2017; 2 (Suppl. 2): S6-S12.
28. TANIGUCHI T, ASANO Y, FUKASAWA T, YOSHIZAKI A, SATO S: Critical contribution of the interleukin-6/signal transducer and activator of transcription 3 axis to vasculopathy associated with systemic sclerosis. *J Dermatol* 2017; 44: 967-71.
29. TANIGUCHI T, MIYAGAWA T, TAMAKI Z *et al*.: A possible implication of reduced levels of LIF, LIFR, and gp130 in vasculopathy related to systemic sclerosis. *Arch Dermatol Res* 2017; 309: 833-42.
30. GÖZEL N, KARATAŞ A, YARDIM M *et al*.: Plasma urotensin II levels in primary Raynaud's phenomenon and systemic sclerosis. *Turk J Med Sci* 2017; 47: 1687-92.
31. TALOTTA R, BONGIOVANNI S, LETIZIA T *et al*.: The role of klotho in systemic sclerosis. *Reumatismo* 2017; 69: 156-63.
32. MCCOY SS, REED TJ, BERTHIER CC *et al*.: Scleroderma keratinocytes promote fibroblast activation independent of transforming growth factor beta. *Rheumatology* (Oxford) 2017; 56: 1970-81.
33. KANTOLA AK, RYYNÄNEN MJ, LHOTA F, KESKI-OJA J, KOLI K: Indipendent regulation of short and long forms of latent TGF-beta binding protein (LTBP)-4 in cultured fibroblasts and huma tissues. *J Cell Physiol* 2010; 223: 727-36.
34. LU J, LIU Q, WANG L *et al*.: Increased expression of latent TGF-β-binding protein 4 affects the fibrotic process in scleroderma by TGF-β/SMAD signalling. *Lab Invest* 2017; 97: 591-601.
35. SCHMIDT KG, HERRERO SAN JUAN M, TRAUTMANN S *et al*.: Sphingosine-1-Phosphate Receptor 5 Modulates Early-Stage Processes during Fibrogenesis in a Mouse Model of Systemic Sclerosis: A Pilot Study. *Front Immunol* 2017; 8: 1242.
36. ZHU X, CHU H, JIANG S *et al*.: Sirt1 ameliorates systemic sclerosis by targeting the mTOR pathway. *J Dermatol Sci* 2017; 87: 149-58.
37. CHU H, JIANG S, LIU Q *et al*.: Sirtuin1 Protects against Systemic Sclerosis-related Pulmonary Fibrosis by Decreasing Proinflammatory and Profibrotic Processes. *Am J Respir Cell Mol Biol* 2018; 58: 28-39.
38. SACCHETTI C, BAI Y, STANFORD SM *et al*.: PTP4A1 promotes TGFβ signaling and fibrosis in systemic sclerosis. *Nat Commun* 2017; 8: 1060.
39. MAKINO K, MAKINO T, STAWSKI L *et al*.: Blockade of PDGF Receptors by Crenolanib Has Therapeutic Effect in Patient Fibroblasts and in Preclinical Models of Systemic Sclerosis. *J Invest Dermatol* 2017; 137: 1671-81.
40. MOTEGI SI, SEKIGUCHI A, FUJIWARA C, TOKI S, ISHIKAWA O: Possible association of elevated serum collagen type IV level with skin sclerosis in systemic sclerosis. *J Dermatol* 2017; 44: 167-72.
41. BROWN LOBBINS ML, SHIVAKUMAR BR, POSTLETHWAITE AE, HASTY KA: Chronic exposure of interleukin-13 suppress the induction of matrix metalloproteinase-1 by tumour necrosis factor α in normal and scleroderma dermal fibroblasts through protein kinase B/Akt. *Clin Exp Immunol* 2018; 191: 84-95.
42. ASANO Y: Epigenetic suppression of Fli1, a potential predisposing factor in the pathogenesis of systemic sclerosis. *Int J Biochem Cell Biol* 2015; 67: 86-91.
43. TAKAHASHI T, ASANO Y, SUGAWARA K *et al*.: Epithelial Fli1 deficiency drives systemic autoimmunity and fibrosis: possible roles in scleroderma. *J Exp Med* 2017; 214: 1129-51.
44. TANIGUCHI T, ASANO Y, NAKAMURA K *et al*.: Fli1 Deficiency Induces CXCL6 Expression in Dermal Fibroblasts and Endothelial Cells, Contributing to the Development of Fibrosis and Vasculopathy in Systemic Sclerosis. *J Rheumatol* 2017; 44: 1198-205.
45. SAIGUSA R, ASANO Y, NAKAMURA K *et al*.: Systemic Sclerosis Dermal Fibroblasts Suppress Th1 Cytokine Production via Galectin-9 Overproduction due to Fli1 Deficiency. *J Invest Dermatol* 2017; 137: 1850-9.
46. DING Q, YEUNG M, CAMIRAD G *et al*.: Regulatory B cells are identified by expression of TIM-1 and can be induced through-TIM-1 ligation to promote tolerance in mice. *J Clin Invest* 2011; 121: 3645-56.
47. ARAVENA O, FERRIER A, MENON M *et al*.: TIM-1 defines a human regulatory B cell population that is altered in frequency and function in systemic sclerosis patients. *Arthritis Res Ther* 2017; 19: 8.
48. MAVROPOULOS A, LIASKOS C, SIMOPOULOU T, BOGDANOS DP, SAKKAS LI: IL-10-producing regulatory B cells (B10 cells), IL-17+ T cells and autoantibodies in systemic sclerosis. *Clin Immunol* 2017; 184: 26-32.
49. KRASIMIROVA E, VELIKOVA T, IVANOVA-TODOROVA E *et al*.: Treg/Th17 cell balance and phytohaemagglutinin activation of T lymphocytes in peripheral blood of systemic sclerosis patients. *World J Exp Med* 2017; 7: 84-96.
50. UGOR E, SIMON D, ALMANZAR G *et al*.: Increased proportions of functionally impaired regulatory T cell subsets in systemic sclerosis. *Clin Immunol* 2017; 184: 54-62.
51. GUGGINO G, LO PIZZO M, DI LIBERTO D *et al*.: Interleukin-9 over-expression and T helper 9 polarization in systemic sclerosis patients. *Clin Exp Immunol* 2017; 190: 208-16.
52. MEKINIAN A, MAHEVAS T, MOHTY M *et al*.: Mucosal-associated Invariant Cells are Deficient in Systemic Sclerosis. *Scand J Immunol* 2017; 86: 216-20.
53. FUKASAWA T, YOSHIZAKI A, EBATA *et al*.: Contribution of Soluble Forms of Programmed Death 1 and Programmed Death Ligand 2 to Disease Severity and Progression in Systemic Sclerosis. *Arthritis Rheumatol* 2017; 69: 1879-90.
54. OLEWICZ-GAWLIK A, TRZYBULSKA D, GRANICZNA K *et al*.: Serum alarm antiproteases in systemic sclerosis patients. *Hum Immunol* 2017; 78: 559-564.
55. XU W, SU L, QING P *et al*.: Elevated levels of TL1A are associated with disease activity in patients with systemic sclerosis. *Clin Rheumatol* 2017; 36: 1317-24.
56. LEGÁNY N, BERTA L, KOVÁCS L, BALOG A, TOLDI G: The role of B7 family costimulatory molecules and indoleamine 2,3-dioxygenase in primary Sjögren's syndrome and systemic sclerosis. *Immunol Res* 2017; 65: 622-9.
57. FARINA A, PERUZZI G, LACCONI V *et al*.: Epstein-Barr virus lytic infection promotes activation of Toll-like receptor 8 innate immune response in systemic sclerosis monocytes. *Arthritis Res Ther* 2017; 19: 39.
58. MAROU E, LIASKOS C, EFTHYMIOU G *et al*.: Increased immunoreactivity against human cytomegalovirus UL83 in systemic sclerosis. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S31-4.
59. MAROU E, LIASKOS C, SIMOPOULOU T *et al*.: Human cytomegalovirus (HCMV) UL44 and UL57 specific antibody responses in anti-HCMV-positive patients with systemic sclerosis. *Clin Rheumatol* 2017; 36: 863-9.

60. MARIE I, GEHANNO JF, BUBENHEIM M *et al*.: Systemic sclerosis and exposure to heavy metals: A case control study of 100 patients and 300 controls. *Autoimmun Rev* 2017; 16: 223-30.
61. INIESTA ARANDIA N, SIMEÓN-AZNAR CP, GUILLÉN DEL CASTILLO A *et al*.: Influence of antibody profile in clinical features and prognosis in a cohort of Spanish patients with systemic sclerosis. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S98-105.
62. IGUSA T, HUMMERS LK, VISVANATHAN K *et al*.: Autoantibodies and scleroderma phenotype define subgroups at high-risk and low-risk for cancer. *Ann Rheum Dis* 2018; 77: 1179-86.
63. FRANTZ C, PEZET S, AVOUAC J, ALLANORE Y: Soluble CD163 as a Potential Biomarker in Systemic Sclerosis. *Dis Markers* 2018; 2018: 1-5.
64. SAWICKA K, MICHALSKA-JAKUBUS M, KOWAL M, POTEMBSKA 3, KRASOWSKA D: Resistin: a possible biomarker of organ involvement in systemic sclerosis patients? *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S144-50.
65. TALOTTA R, BONGIOVANNI S, LETIZIA T *et al*.: Measurement of Serum Klotho in Systemic Sclerosis. *Dis Markers* 2017; 2017: 1-7.
66. BOSELLO S, BASILE U, DE LORENZIS E *et al*.: Free light chains of immunoglobulins in patients with systemic sclerosis: correlations with lung involvement and inflammatory milieu. *J Clin Pathol* 2018; 71: 620-5.
67. JOHNSON SR, SOOWAMBER ML, FRANSEN J *et al*.: There is a need for new systemic sclerosis subset criteria. A content analytic approach. *Scand J Rheumatol* 2018; 47: 62-70.
68. GIGANTE A, NAVARINI L, MARGIOTTA D, BARBANO B, AFELTRA A, ROSATO E: In systemic sclerosis, microvascular and hands digital arteries damage correlates with serum levels of endostatin. *Microcirculation* 2018; 25: e12449.
69. ALLANORE Y, DISTLER O, MATUCCI-CERINIC M, DENTON CP: Review: Defining a Unified Vascular Phenotype in Systemic Sclerosis. *Arthritis Rheumatol* (Hoboken) 2018; 70: 162-70.
70. JOHNSON SR, HAKAMI N, AHMAD Z, WIJEYSUNDERA DN: Venous Thromboembolism in Systemic Sclerosis: Prevalence, Risk Factors, and Effect on Survival. *J Rheumatol* 2018; 45: 942-6.
71. SOULAIDOPOULOS S, TRIANTAFYLLIDOU E, GARYFALLOS A, KITAS GD, DIMITROULAS T: The role of nailfold capillaroscopy in the assessment of internal organ involvement in systemic sclerosis: A critical review. *Autoimmun Rev* 2017; 16: 787-95.
72. KARBALAIE A, ETEHADTAVAKOL M, ABTAHI F, FATEMI A, EMRANI Z, ERLANDSSON BE: Image enhancement effect on inter and intra-observer reliability of nailfold capillary assessment. *Microvasc Res* 2018; 120: 100-10.
73. CAETANO J, PAULA FS, AMARAL M, OLIVEIRA S, ALVES JD: Nailfold Videocapillaroscopy Changes Are Associated With the Presence and Severity of Systemic Sclerosis-Related Interstitial Lung Disease. *J Clin Rheumatol* 2018.
74. PIZZORNI C, SULLI A, PAOLINO S *et al*.: Progression of Organ Involvement in Systemic Sclerosis Patients with Persistent "Late" Nailfold Capillaroscopic Pattern of Microangiopathy: A Prospective Study. *J Rheumatol* 2017; 44: 1941-2.
75. WILKINSON JD, LEGGETT SA, MARJANOVIC EJ *et al*.: A Multicenter Study of the Validity and Reliability of Responses to Hand Cold Challenge as Measured by Laser Speckle Contrast Imaging and Thermography: Outcome Measures for Systemic Sclerosis-Related Raynaud's Phenomenon. *Arthritis Rheumatol* (Hoboken) 2018; 70: 903-11.
76. CUTOLO M, VANHAECKE A, RUARO B *et al*.: Autoimmunity Reviews Is laser speckle contrast analysis (LASCA) the new kid on the block in systemic sclerosis ? A systematic literature review and pilot study to evaluate reliability of LASCA to measure peripheral blood perfusion in scleroderma patient. *Autoimmun Rev* 2018; 17: 775-80.
77. RUARO B, SULLI A, PIZZORNI C *et al*.: Correlations between blood perfusion and dermal thickness in different skin areas of systemic sclerosis patients. *Microvasc Res* 2018; 115: 28-33.
78. MEUNIER P, DEQUIDT L, BARNETCHE T *et al*.: Increased risk of mortality in systemic sclerosis-associated digital ulcers: a systematic review and meta-analysis. *J Eur Acad Dermatol Venereol* 2018.
79. COPPI F, GIUGGIOLI D, SPINELLA A *et al*.: Cardiac involvement in systemic sclerosis: identification of high-risk patient profiles in different patterns of clinical presentation. *J Cardiovasc Med* (Hagerstown) 2018; 19: 393-5.
80. GIUGGIOLI D, MANFREDI A, LUMETTI F, COLACI M, FERRI C: Scleroderma skin ulcers definition, classification and treatment strategies our experience and review of the literature. *Autoimmun Rev* 2018; 17: 155-64.
81. BOHELAY G, BLAISE S, LEVY P *et al*.: Lower-limb Ulcers in Systemic Sclerosis: A Multicentre Retrospective Case-control Study. *Acta Derm Venereol* 2018; 98: 677-82.
82. HUGHES M, MOORE T, MANNING J, DINSDALE G, HERRICK AL, MURRAY AA: pilot study using high-frequency ultrasound to measure digital ulcers: a possible outcome measure in systemic sclerosis clinical trials? *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S218-9.
83. ROSATO E, GIGANTE A, BARBANO B, GASPERINI ML, CIANCI R, MUSCARITOLI M: Prognostic Factors of Renal Involvement in Systemic Sclerosis. *Kidney Blood Press Res* 2018; 43: 682-9.
84. MAVROGENI S, SCHWITTER J, VAN ROSSUM A *et al*.: Cardiac magnetic resonance imaging in myocardial inflammation in autoimmune rheumatic diseases: An appraisal of the diagnostic strengths and limitations of the Lake Louise criteria. 2018; 252: 216-9.
85. MAVROGENI SI, SCHWITTER J, GARGANI L *et al*.: Cardiovascular magnetic resonance in systemic sclerosis: "Pearls and pitfalls". *Semin Arthritis Rheum* 2017; 47: 79-85.
86. MAVROGENI S, KOUTSOGEORGOPOULOU L, KARABELA G *et al*.: Silent myocarditis in systemic sclerosis detected by cardiovascular magnetic resonance using Lake Louise criteria. *BMC Cardiovasc Disord* 2017; 17: 187.
87. TADIC M, ZLATANOVIC M, CUSPIDI C *et al*.: Systemic sclerosis impacts right heart and cardiac autonomic nervous system. *J Clin Ultrasound* 2018; 46: 188-94.
88. MUKHERJEE M, MERCURIO V, TEDFORD RJ *et al*.: Right ventricular longitudinal strain is diminished in systemic sclerosis compared with idiopathic pulmonary arterial hypertension. *Eur Respir J* 2017; 50: 1701436.
89. GUERRA F, STRONATI G, FISCHIETTI C *et al*.: Global longitudinal strain measured by speckle tracking identifies subclinical heart involvement in patients with systemic sclerosis. *Eur J Prev Cardiol* January 2018.
90. CHUNG L, FAIRCHILD RM, FURST DE *et al*.: Utility of B-type natriuretic peptides in the assessment of patients with systemic sclerosis-associated pulmonary hypertension in the PHAROS registry. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S106-13.
91. KOLSTAD KD, LI S, STEEN V *et al*.: PHAROS Investigators. Long-Term Outcomes in Systemic Sclerosis-Associated Pulmonary Arterial Hypertension From the Pulmonary Hypertension Assessment and Recognition of Outcomes in Scleroderma Registry (PHAROS). *Chest* 2018.
92. BARSOTTI S, BRUNI C, ORLANDI M *et al*.: One year in review 2017: Systemic sclerosis. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S3-20.
93. HOFFMANN-VOLD A-M, FRETHEIM H, MIDTVEDT *et al*.: Frequencies of borderline pulmonary hypertension before and after the DETECT algorithm: results from a prospective systemic sclerosis cohort. *Rheumatology* (Oxford) 2018; 57: 480-7.
94. MECOLI CA, SHAH AA, BOIN F, WIGLEY FM, HUMMERS LK: Vascular complications in systemic sclerosis: a prospective cohort study. *Clin Rheumatol* 2018; 37: 2429-37.
95. KORMAN BD, MARANGONI RG, HINCHCLIFF M *et al*.: Brief Report: Association of Elevated Adipsin Levels With Pulmonary Arterial Hypertension in Systemic Sclerosis. *Arthritis Rheumatol* (Hoboken) 2017; 69: 2062-8.
96. COGHLAN JG, WOLF M, DISTLER O *et al*.: Incidence of pulmonary hypertension and determining factors in patients with systemic sclerosis. *Eur Respir J* 2018.
97. 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.
98. LAUNAY D, MONTANI D, HASSOUN PM *et al*.: Clinical phenotypes and survival of pre-capillary pulmonary hypertension in systemic sclerosis. *PLoS One* 2018; 13: e0197112.
99. TARDELLA M, DI CARLO M, CAROTTI M, FILIPPUCCI E, GRASSI W, SALAFFI F: Ultrasound B-lines in the evaluation of interstitial lung disease in patients with systemic sclerosis: Cut-off point definition for the

presence of significant pulmonary fibrosis. *Medicine* (Baltimore) 2018; 97: e0566.
100. BARSOTTI S, STAGNARO C, D'ASCANIO A, DELLA ROSSA A: One year in review 2016: systemic sclerosis. *Clin Exp Rheumatol* 2016; 34 (Suppl. 100): S3-13.
101. WU W, JORDAN S, BECKER MO *et al*.: Prediction of progression of interstitial lung disease in patients with systemic sclerosis: the SPAR model. *Ann Rheum Dis* 2018; 77: 1326-32.
102. KAVADICHANDA CG, NEGI VS: Can we further SPARkle the SPAR model? *Ann Rheum Dis* 2018.
103. SANGES S, GIOVANNELLI J, SOBANSKI V *et al*.: Factors associated with the 6-minute walk distance in patients with systemic sclerosis. *Arthritis Res Ther* 2017; 19: 279.
104. VANDECASTEELE E, THEVISSEN K, MELSENS K *et al*.: Six-minute walk test in or out in evaluation of systemic sclerosis patients? *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S122-9.
105. PUGNET G, MARJANOVIC Z, DELIGNY C *et al*.: Reproducibility and Utility of the 6-minute Walk Test in Systemic Sclerosis. *J Rheumatol* 2018; 45: 1273-80.
106. NGUYEN-KIM TDL, MAURER B, SULIMAN YA, MORSBACH F, DISTLER O, FRAUENFELDER T: The impact of slice-reduced computed tomography on histogram-based densitometry assessment of lung fibrosis in patients with systemic sclerosis. *J Thorac Dis* 2018; 10: 2142-52.
107. HERRICK AL, PEYTRIGNET S, LUNT M *et al*.: Patterns and predictors of skin score change in early diffuse systemic sclerosis from the European Scleroderma Observational Study. *Ann Rheum Dis* 2018; 77: 563-70.
108. JOUVRAY M, LAUNAY D, DUBUCQUOI S *et al*.: Whole-Body Distribution and Clinical Association of Telangiectases in Systemic Sclerosis. *JAMA Dermatol* 2018; 154: 796-805.
109. KARAMANOLIS GP, DENAXAS K, PANOPOULOS S *et al*.: Severe oesophageal disease and its associations with systemic sclerosis. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S82-5.
110. FRECH TM, FAKOYA L, GAWRON AJ *et al*.: Oesophageal disease in systemic sclerosis: does heritability play a role? *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S86-8.
111. MCMAHAN ZH, PAIK JJ, WIGLEY FM, HUMMERS LK: Determining the Risk Factors and Clinical Features Associated With Severe Gastrointestinal Dysmotility in Systemic Sclerosis. *Arthritis Care Res* (Hoboken) 2018; 70: 1385-92.
112. SIEGERT E, MARCH C, OTTEN L *et al*.: Prevalence of sarcopenia in systemic sclerosis: Assessing body composition and functional disability in patients with systemic sclerosis. *Nutrition* 2018; 55-56: 51-5.
113. JOHN G, AVOUAC J, PIANTONI S *et al*.: Prevalence and Disease-Specific Risk Factors for Lower Urinary Tract Symptoms in Systemic Sclerosis: An International Multicenter Study. *Arthritis Care Res* (Hoboken) 2017; 70: 1218-27.
114. JOHN G, ALLANORE Y, POLITO P *et al*.: The limited cutaneous form of systemic sclerosis is associated with urinary incontinence: an international multicentre study. *Rheumatology* (Oxford) 2017; 56: 1874-83.
115. SOUSA-NEVES J, CERQUEIRA M, SANTOS-FARIA D, AFONSO C, TEIXEIRA F: Neuropathic pain in Systemic Sclerosis patients: A cross-sectional study. *Reumatol Clín* 2018.
116. SHENAVANDEH S, HASHEMI SB, MASOUDI M, NAZARINIA MA, ZARE A: Hearing loss in patients with scleroderma: associations with clinical manifestations and capillaroscopy. *Clin Rheumatol* June 2018; 37: 2439-46.
117. RUARO B, CASABELLA A, PAOLINO S *et al*.: Correlation between bone quality and microvascular damage in systemic sclerosis patients. *Rheumatology* (Oxford) 2018; 57: 1548-54.
118. CASTREJÓN I, GOSSEC L, CARMONA L: The EULAR Outcome Measures Library: an evolutional database of validated patient-reported instruments. *Ann Rheum Dis* 2015; 74: 475-6.
119. PAULING JD, FRECH TM, DOMSIC RT, HUDSON M: Patient participation in patient-reported outcome instrument development in systemic sclerosis. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S184-92.
120. DASTE C, RANNOU F, MOUTHON L *et al*.: Patient acceptable symptom state and minimal clinically important difference for patient-reported outcomes in systemic sclerosis: A secondary analysis of a randomized controlled trial comparing personalized physical therapy to usual care. *Semin Arthritis Rheum* 2018.
121. ARNOLD MB, KHANNA D, DENTON CP *et al*.: Patient acceptable symptom state in scleroderma: results from the tocilizumab compared with placebo trial in active diffuse cutaneous systemic sclerosis. *Rheumatology* (Oxford) 2018; 57: 152-7.
122. KWAKKENBOS L, THOMBS BD, KHANNA D *et al*.: Performance of the Patient-Reported Outcomes Measurement Information System-29 in scleroderma: a Scleroderma Patient-centered Intervention Network Cohort Study. *Rheumatology* (Oxford) 2017; 56: 1302-1311.
123. AZAR M, RICE DB, KWAKKENBOS L *et al*.: Exercise habits and factors associated with exercise in systemic sclerosis: a Scleroderma Patient-centered Intervention Network (SPIN) cohort study. Disabil *Rehabil* 2018; 40: 1997-2003.
124. PAULING JD, DOMSIC RT, SAKETKOO LA *et al*.: Multinational Qualitative Research Study Exploring the Patient Experience of Raynaud's Phenomenon in Systemic Sclerosis. *Arthritis Care Res* (Hoboken) 2018; 70: 1373-84.
125. PAULING JD, REILLY E, SMITH T, FRECH TM: Evolving symptoms of Raynaud's phenomenon in systemic sclerosis are associated with physician and patient-reported assessments of disease severity. *Arthritis Care Res* (Hoboken) 2018.
126. MAN A, CORREA JK, ZIEMEK J, SIMMS RW, FELSON DT, LAFYATIS R: Development and validation of a patient-reported outcome instrument for skin involvement in patients with systemic sclerosis. *Ann Rheum Dis* 2017; 76: 1374-80.
127. MESSERLI M, ASCHWANDEN R, BUSLAU M, HERSBERGER KE, ARNET I: Swallowing difficulties with medication intake assessed with a novel self-report questionnaire in patients with systemic sclerosis - a cross-sectional population study. *Patient Prefer Adherence* 2017; 11: 1687-99.
128. MORRISROE K, STEVENS W, SAHHAR J *et al*.: Quantifying the direct public health care cost of systemic sclerosis: A comprehensive data linkage study. *Medicine* (Baltimore) 2017; 96: e8503
129. FISCHER A, ZIMOVETZ E, LING C, ESSER D, SCHOOF N: Humanistic and cost burden of systemic sclerosis: A review of the literature. *Autoimmun Rev* 2017; 16: 1147-54.
130. AVOUAC J, ALLANORE Y: Targeted immunotherapies in systemic sclerosis. *Clin Exp Rheumatol* 2014; 32 (Suppl. 81):165-72.
131. IUDICI M: What should clinicians know about the use of glucocorticoids in systemic sclerosis? *Mod Rheumatol* 2017; 27: 919-23.
132. BARNES H, HOLLAND AE, WESTALL GP, GOH NS, GLASPOLE IN: Cyclophosphamide for connective tissue disease-associated interstitial lung disease. *Cochrane Database Syst Rev* 2018.
133. KOWAL-BIELECKA O, FRANSEN J, AVOUAC J *et al*.: Update of EULAR recommendations for the treatment of systemic sclerosis. *Ann Rheum Dis* 2017; 76: 1327-39.
134. U.S. FOOD AND DRUG ADMINISTRATION: Cyclophosphamide - product information. 2013.
135. ARNAUD L, NORDIN A, LUNDHOLM H *et al*.: Effect of Corticosteroids and Cyclophosphamide on Sex Hormone Profiles in Male Patients With Systemic Lupus Erythematosus or Systemic Sclerosis. *Arthritis Rheumatol* 2017; 69: 1272-9.
136. SAIGUSA R, ASANO Y, NAKAMURA K *et al*.: Plasma plasmin-alpha2-plasmin inhibitor complex levels may predict the effect of cyclophosphamide for systemic sclerosis-related interstitial lung disease. *Mod Rheumatol* 2017; 27: 618-22.
137. UEDA T, SAKAGAMI T, KIKUCHI T, TAKADA T: Mycophenolate mofetil as a therapeutic agent for interstitial lung diseases in systemic sclerosis. *Respir Investig* 2018; 56: 14-20.
138. TASHKIN DP, ROTH MD, CLEMENTS PJ *et al*.: Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial. *Lancet Respir Med* 2016; 4: 708-19.
139. TASHKIN DP, ELASHOFF R, CLEMENTS PJ *et al*.: Cyclophosphamide versus placebo in scleroderma lung disease. *N Engl J Med* 2006; 354: 2655-66.
140. VOLKMANN ER, TASHKIN DP, LI N *et al*.: Mycophenolate Mofetil Versus Placebo for Systemic Sclerosis-Related Interstitial Lung Disease: An Analysis of Scleroderma Lung Studies I and II. *Arthritis Rheumatol* (Hoboken) 2017; 69: 1451-60.
141. BAQIR M, MAKOL A, OSBORN TG, BARTHOLMAI BJ, RYU JH: Mycophenolate mofetil for scleroderma-related interstitial

lung disease: A real world experience. *PLoS One* 2017; 12: e0177107.
142. BOULOS D, NGIAN G-S, RAJADURAI A *et al*.: Long-term efficacy and tolerability of mycophenolate mofetil therapy in diffuse scleroderma skin disease. *Int J Rheum Dis* 2017; 20: 481-8.
143. DAOUSSIS D, MELISSAROPOULOS K, SAKELLAROPOULOS G *et al*.: A multicenter, open-label, comparative study of B-cell depletion therapy with Rituximab for systemic sclerosis-associated interstitial lung disease. *Semin Arthritis Rheum* 2017; 46: 625-31.
144. MELSENS K, VANDECASTEELE E, DESCHEPPER E *et al*.: Two years follow-up of an open-label pilot study of treatment with rituximab in patients with early diffuse cutaneous systemic sclerosis. *Acta Clin Belg* 2018; 73: 119-125.
145. SAUNDERS P, TSIPOURI V, KEIR GJ *et al*.: Rituximab versus cyclophosphamide for the treatment of connective tissue diseaseassociated interstitial lung disease (RECITAL): Study protocol for a randomised controlled trial. *Trials* 2017; 18: 275.
146. SATO T, AMBALE-VENKATESH B, LIMA JAC *et al*.: The impact of ambrisentan and tadalafil upfront combination therapy on cardiac function in scleroderma associated pulmonary arterial hypertension patients: cardiac magnetic resonance feature tracking study. *Pulm Circ* 2018; 8: 2045893217748307.
147. GALIÈ N, BARBERÀ JA, FROST AE *et al*.: Initial Use of Ambrisentan plus Tadalafil in Pulmonary Arterial Hypertension. *N Engl J Med* 2015; 373: 834-44.
148. COGHLAN JG, GALIÈ N, BARBERÀ JA *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.
149. CHEONG F-Y, GOWER AC, FARBER HW: Changes in gene expression profiles in patients with pulmonary arterial hypertension associated with scleroderma treated with tadalafil. *Semin Arthritis Rheum* 2017; 46: 465-472.
150. SITBON O, CHANNICK R, CHIN KM *et al*.: Selexipag for the Treatment of Pulmonary Arterial Hypertension. *N Engl J Med* 2015; 373: 2522-33.
151. GAINE S, CHIN K, COGHLAN G *et al*.: Selexipag for the treatment of connective tissue disease-associated pulmonary arterial hypertension. *Eur Respir J* 2017; 50.
152. HUMBERT M, COGHLAN JG, GHOFRANI H-A *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.
153. DISTLER O, POPE J, DENTON C *et al*.: RISE-SSc: Riociguat in diffuse cutaneous systemic sclerosis. *Respir Med* 2017; 122 (Suppl.): S14-17.
154. ARGULA RG, KARWA A, LAUER A *et al*.: Differences in Right Ventricular Functional Changes during Treatment between Systemic Sclerosis-associated Pulmonary Arterial Hypertension and Idiopathic Pulmonary Arterial Hypertension. *Ann Am Thorac Soc* 2017; 14: 682-9.
155. ANDRIGUETI FV, EBBING PCC, ARISMENDI MI, KAYSER C: Evaluation of the effect of sildenafil on the microvascular blood flow in patients with systemic sclerosis: a randomised, double-blind, placebo-controlled study. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S151-8.
156. COLACI M, LUMETTI F, GIUGGIOLI D *et al*.: Long-term treatment of scleroderma-related digital ulcers with iloprost: a cohort study. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S179-83.
157. FRATICELLI P, MARTINO GP, MURRI M, MATTIOLI M, GABRIELLI A: A novel iloprost administration method with portable syringe pump for the treatment of acral ulcers and Raynaud's phenomenon in systemic sclerosis patients. A pilot study (ILOPORTA). *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S173-8.
158. CESTELLI V, MANFREDI A, SEBASTIANI M *et al*.: Effect of treatment with iloprost with or without bosentan on nailfold videocapillaroscopic alterations in patients with systemic sclerosis. *Mod Rheumatol* 2017; 27: 110-4.
159. DENTON CP, HACHULLA É, RIEMEKASTEN G *et al*.: Efficacy and Safety of Selexipag in Adults With Raynaud's Phenomenon Secondary to Systemic Sclerosis: A Randomized, Placebo-Controlled, Phase II Study. *Arthritis Rheumatol* (Hoboken) 2017; 69: 2370-9.
160. HUGHES M, MOORE T, MANNING J *et al*.: Reduced perfusion in systemic sclerosis digital ulcers (both fingertip and extensor) can be increased by topical application of glyceryl trinitrate. *Microvasc Res* 2017; 111: 32-6.
161. BELLO RJ, COONEY CM, MELAMED E *et al*.: The Therapeutic Efficacy of Botulinum Toxin in Treating Scleroderma-Associated Raynaud's Phenomenon: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *Arthritis Rheumatol* (Hoboken) 2017; 69: 1661-9.
162. MOTEGI S-I, UEHARA A, YAMADA K *et al*.: Efficacy of Botulinum Toxin B Injection for Raynaud's Phenomenon and Digital Ulcers in Patients with Systemic Sclerosis. *Acta Derm Venereol* 2017; 97: 843-50.
163. MARTYANOV V, KIM G-HJ, HAYES W *et al*.: Novel lung imaging biomarkers and skin gene expression subsetting in dasatinib treatment of systemic sclerosis-associated interstitial lung disease. *PLoS One* 2017; 12: e0187580.
164. MUKHERJEE S, AYAUB EA, MURPHY J *et al*.: Disruption of Calcium Signaling in Fibroblasts and Attenuation of Bleomycin-Induced Fibrosis by Nifedipine. *Am J Respir Cell Mol Biol* 2015; 53: 450-8.
165. LI G, ADACHI JD, CHENG J *et al*.: Relationship between calcium channel blockers and skin fibrosis in patients with systemic sclerosis. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S56-60.
166. HUANG J, MAIER C, ZHANG Y *et al*.: Nintedanib inhibits macrophage activation and ameliorates vascular and fibrotic manifestations in the Fra2 mouse model of systemic sclerosis. *Ann Rheum Dis* 2017; 76: 1941-8.
167. DU J, PAZ K, FLYNN R *et al*.: Pirfenidone ameliorates murine chronic GVHD through inhibition of macrophage infiltration and TGF-beta production. *Blood* 2017; 129: 2570-80.
168. KHANNA D, ALBERA C, FISCHER A *et al*.: An Open-label, Phase II Study of the Safety and Tolerability of Pirfenidone in Patients with Scleroderma-associated Interstitial Lung Disease: the LOTUSS Trial. *J Rheumatol* 2016; 43: 1672-9.
169. ALLANORE Y, DISTLER O, JAGERSCHMIDT A *et al*.: Double-blind, Randomized, 8-week Placebo-controlled followed by a 16-week open label extension study, with the LPA1 receptor antagonist SAR100842 for Patients With Diffuse Cutaneous Systemic Sclerosis. *Arthritis Rheumatol* (Hoboken) 2018.
170. HOST L, NIKPOUR M, CALDERONE A, CANNELL P, RODDY J: Autologous stem cell transplantation in systemic sclerosis: a systematic review. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S198-207.
171. FARGE D, BURT RK, OLIVEIRA M-C *et al*.: Cardiopulmonary assessment of patients with systemic sclerosis for hematopoietic stem cell transplantation: recommendations from the European Society for Blood and Marrow Transplantation Autoimmune Diseases Working Party and collaborating partners. *Bone Marrow Transplant* 2017; 52: 1495-503.
172. DEL PAPA N, ONIDA F, ZACCARA E *et al*.: Autologous hematopoietic stem cell transplantation has better outcomes than conventional therapies in patients with rapidly progressive systemic sclerosis. *Bone Marrow Transplant* 2017; 52: 53-8.
173. FARGE D, ARRUDA LCM, BRIGANT F *et al*.: Long-term immune reconstitution and T cell repertoire analysis after autologous hematopoietic stem cell transplantation in systemic sclerosis patients. *J Hematol Oncol* 2017; 10: 21.
174. HENES J, GLAESER L, KÖTTER I, VOGEL W, KANZ L, KLEIN R: Analysis of anti-topoisomerase I antibodies in patients with systemic sclerosis before and after autologous stem cell transplantation. *Rheumatology* (Oxford) 2017; 56: 451-6.
175. ZHANG H, LIANG J, TANG X *et al*.: Sustained benefit from combined plasmapheresis and allogeneic mesenchymal stem cells transplantation therapy in systemic sclerosis. *Arthritis Res Ther* 2017; 19: 165.
176. SHAH RJ, BOIN F: Lung Transplantation in Patients With Systemic Sclerosis. *Curr Rheumatol Rep* 2017; 19: 23.
177. BERTRAND D, DEHAY J, OTT J *et al*.: Kidney transplantation in patients with systemic sclerosis: a nationwide multicentre study. *Transpl Int* 2017; 30: 256-65.
178. MOUTHON L, CARPENTIER PH, LOK C *et al*.: Controlling the digital ulcerative disease in systemic sclerosis is associated with improved hand function. *Semin Arthritis Rheum* 2017; 46: 759-66.
179. BRASCHI F, BARTOLI F, BRUNI C *et al*.: Lidocaine controls pain and allows safe wound

bed preparation and debridement of digital ulcers in systemic sclerosis: a retrospective study. *Clin Rheumatol* 2017; 36: 209-12.
180. MIRASOGLU B, BAGLI BS, AKTAS S: Hyperbaric oxygen therapy for chronic ulcers in systemic sclerosis - case series. *Int J Dermatol* 2017; 56: 636-40.
181. FURST DE, BRAUN-MOSCOVIC Y, KHANNA D: Points to consider for clinical trials of the gastrointestinal tract in systemic sclerosis. *Rheumatology* (Oxford) 2017; 56 (Suppl. 5): v4-v11.
182. VIGONE B, CARONNI M, SEVERINO A *et al*.: Preliminary safety and efficacy profile of prucalopride in the treatment of systemic sclerosis (SSc)-related intestinal involvement: results from the open label cross-over PROGASS study. *Arthritis Res Ther* 2017; 19: 145.
183. FOOCHAROEN C, CHUNLERTRITH K, MAIRIANG P *et al*.: Effectiveness of add-on therapy with domperidone vs alginic acid in proton pump inhibitor partial response gastro-oesophageal reflux disease in systemic sclerosis: randomized placebo-controlled trial. *Rheumatology* (Oxford) 2017; 56: 214-22.