Review

One year in review 2016: systemic sclerosis

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ABSTRACT

Systemic sclerosis is a rare acquired systemic disease characterised by a complex pathogenesis and multi organ involvement. Every year, novel insights into the pathogenesis, diagnosis and treatment of this severe disease are published. Herewith, we provide an overview of the most significant literature contributions published over the last year.

Introduction

Systemic sclerosis (SSc) is a chronic disabling disease characterised by three cardinal pathogenetic features, microvascular involvement, activation of the immune system and increase of extracellular matrix deposition in the skin and internal organs (1).

In this manuscript we will provide our annual update of the recent advances in the pathogenesis, diagnosis and treatment of systemic sclerosis. 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 July 2015 and June 2016.

Recent insights into the pathogenesis of SSc

Currently, the mechanisms involved in SSc pathogenesis remain unknown, but there is growing evidence suggesting a close connection between environmental factors and SSc pathogenesis. In particular, the majority of the data available suggests that environmental factors may play a pivotal role in the pathogenesis of SSc. Therefore, it is possible to hypothesise a complex pathogenetic mechanism in which environmental and genetic factors interact with each other, with a regulatory epigenetic mechanism (2). These mechanisms do not involve alterations in DNA sequence, but include changes in the expression of DNA (including DNA methylation, histones modifications), and changes in the expression of several microRNA (miRNA) (3).

These epigenetic alterations are potentially reversible and could be counteracted by treatment with epigenetic modifier molecules (2, 4, 5).

DNA methylation is one of the most widely studied epigenetic mechanism in SSc (4). Performing a genome-wide DNA methylation study in dermal fibroblasts of twelve SSc Patients, Altorok *et al*. (6) identified thousands of differentially methylated cytosines in SSc. However, only 203 differentially methylated cytosine sites were present in both cutaneous diffuse and limited SSc (dSSc/lSSc). Common hypomethylation was detected in the gene encoding for integrin-α9, a membrane glycoprotein that mediates cell-cell and cell-matrix adhesion. The consequence of hypomethylation of the gene can produce an overexpression of integrins that contributes to myofibroblast differentiation (7) and activation of transforming growth factor β (TGF-β) (8). Hypomethylation was detected also in genes encoding for collagen, that was overexpressed in SSc fibroblasts, and in some transcription factors (6). Moreover, a recent paper (9) reported the demethylation of lysine 27 on histone H3 in $CD4^+$ T cells of patients with SSc: this demethylation may lead to over-activation of several proteins such as CD40L, CD70 and CD11a, related to the pathogenesis of SSc.

In the pathogenesis of SSc, some regulatory proteins, implicated in several cellular processes such as transcription, apoptosis and cellular metabolism, can be involved. Sirtuin-1 (Sirt1) is a regulatory protein involved in deacetylation of histones and SMAD proteins and is involved in the expression of antioxidants. In a recent study, Zerr *et al*. (10) demonstrated that Sirt1 is a crucial regulator of TGF-β signalling in the

skin of SSc patients and in bleomycin-induced murine model; knockdown of Sirt1 inhibited the release of collagen in fibroblasts by reducing TGF-β signalling. The authors suggest that Sirt1 plays an important role in SSc pathogenesis and, although Sirt1 is down-regulated in SSc, this decrease seems to be insufficient to counterbalance the extensive and persistent activation of TGF-β signalling fibroblasts.

Micro-RNAs (miRNAs) are a large group of non-coding RNA molecules involved in the post-transcriptional regulation of multiple target gene expression. Recent reports suggest that miRNAs are key elements in the pathogenesis of SSc. Circulating miRNAs have been found to be different between SSc patients and healthy subjects, but also between lSSc and dSSc patients, and between patients with different autoantibodies (11). Iwamoto reports that miRNA-193b is one of the most significantly downregulated-miRNAs in SSc fibroblasts (12). The decrease of miRNA-193b is related to an increase of urokinase-type plasminogen actrivator (uPA). uPA was strongly expressed in vascular smooth muscle cells in SSc and inhibited apoptosis of human pulmonary artery smooth muscle cells and can contribute to the proliferative vasculopathy characteristic of SSc. Another recent study reported that miRNA-135b is involved in collagen induction in fibroblasts and, in SSc patients, miRNA-135b is repressed by methylation (13). miRNAs can also represent a novel approach for the treatment of SSc and promising studies have recently been made on miRNA-29a (14) and miRNA-155 (15) that seem to be able to reduce dermal fibrosis in experimental models.

Traditionally, in the pathogenesis of SSc, a combination of autoimmunity, vascular damage and fibrosis of skin and internal organs are considered to be involved (16), although it has not been established which of these processes is primarily responsible for the disease.

Immunological activity, especially of T cells, is considered to play an important role in the development of the vascular abnormalities and fibrosis observed in SSc(17). Activated oligoclonal $CD8^+$ T cells may be detected in the blood and lungs of SSc patients and effector memory $CD8^+$ T cells may be involved in the pathogenesis of organ involvement in SSc. In a recent study, Tsuakamoto *et al*. reported an elevation of the expression CD226, a protein related to immune response and cell adhesion, in $CD8^+$ T cells of scleroderma patients. $CD226^+CD8^+$ T cells produce high amounts of cytokines and a high expression of CD226 on T cells correlated with the severity of skin sclerosis and interstitial lung disease in SSc patients (18).

Anti-topoisomerase-I (Scl70) antibodies have high specificity for SSc and are present in up to 45% of patients. Recent data reported that in all the enrolled Scl70+ SSc patients topoisomerase-I reactive $CD4^+$ cells are present and these cells presented a distinct pro-inflammatory Th17 polarised phenotype. Topoisomerase-I reactive cells are significantly increased in subjects with interstitial lung disease (ILD) and were associated with the decline of pulmonary volumes (19). The authors concluded that the quantification of these autoreactive T cells may be used to predict the presence and progression of ILD in SSc.

Abnormal homeostasis and function of B cells are probably involved in the onset of SSc and there is evidence of the participation of B cells in the pathogenesis of the disease (20). Matsushita *et al*. in a recent paper, reported that regulatory B cells (Breg), producing interleukin-10, are significantly lower in SSc patients compared to healthy controls and lower Breg cells levels correlate with the presence of ILD; moreover, Breg cells were found to be increased in patients with dSSc after treatment, and were inversely correlated with disease activity of SSc (21). A recently published paper confirmed the phenotypical and functional impairment of Breg cells and reported impaired activation of some transcription factors, suggesting that B cell autoaggression could act as an immunopathogenic factor in SSc (22). Also, the memory B cells, especially non-switched memory B cells, are reduced in SSc patients, particularly in those with dSSc, and may serve as a biomarker for this subgroup of the disease (23).

Several data support the idea that TGF-β plays a crucial role in the pathogenesis of SSc, particularly through the activation of collagen production that leads to fibrosis. The overexpression of TGF-β in SSc patients, as previously reported, may be strictly connected with epigenetic modifications of some regulatory proteins, like Sirt1 (10). Moreover, TGF-β pathways are altered in SSc and it is possible to observe an increase of IL-13 synthesis in patients' T cells, involved in the collagen production, while in healthy subjects IL-13 synthesis is decreased by TGF-β (24). Recently, an interesting novel relationship between TGF-β and other molecules has been reported, opening up a large number of potential therapeutic targets for SSc. In an experimental model, soluble guanylatecyclase (sGC) reduced the TGF-β dependent fibroblast activation and collagen release (25). Moreover the activating transcription factor 3 (ATF3) (26) and a calcium binding protein (S100A4) (27) have been reported to be closely related to the fibrosis induced by TGF-β in SSc patients and murine animal models.

TGF-β and ET-1 can play a major role in the production of collagen by myofibrobast in SSc. Macitentan, an endothelin (ET) receptor antagonist that blocks two endothelin receptor subtypes, ET-A and ET-B, interfered with the activation of fibroblasts induced both by ET-1 and TGF-β (28). Moreover, on *in vitro* analysis, macitentan was reported to be able to inhibit the transition of endothelial cells to mesenchymal cells, a process probably involved in SSc pathogenesis (29). In a recent work, Milara *et al*. reported the effect of the inhibition of endothelial-mesenchymal transition promoted by sildenafil (30). This capacity to inhibit the accumulation of fibroblasts and therefore the deposition of collagen promoted by macitentan and sildenafil, may help to explain the beneficial effects of these drugs in pulmonary involvement in SSc-patients.

Recent insights into clinical manifestations of SSc

Autoantibodies

SSc encompasses a wide range of clinical subtypes that are linked by com-

mon clinical features. A common issue in the management of this disease is that of defining the disease course precociously, in order to predict outcome and to establish the best therapeutic strategy and the timing of follow-up as early as possible (31).

Autoantibodies in SSc are highly heterogeneous and reflect the multiplicity of the disease, therefore an autoantibody profile is a useful aid in refining and sub-typing the disease, as recently strengthened by the inclusion of SSc-specific autoantibodies in the American College of Rheumatology (ACR) classification criteria (32). In the last decades, a number of new associations with distinct clinical features have been unveiled. For example anti U11/U12 RNP, Scl-70, TH/T0 and TRIM 20/ Ro52, have been related to the risk of ILD (33, 34) and/or poor survival (34) conversely RNA pol III have been associated with diffuse disease and a marked increased risk of SRC but a low rate of ILD (33). Disease classification based solely on skin involvement may be difficult in clinical settings, either because skin disease might not be clinically apparent in the earliest stages (31), or because the skin may show an unpredictable pattern of involvement and pace in a continuum spectrum that is not always included in the classical dichotomous classification of diffuse and limited disease. Therefore, classification of the disease by identifying the dominant scleroderma specific autoantibody expressed, in addition to skin and organ involvement, have been proposed. Patterson and co-authors analysed a cohort of 505 Australian SSc patients with a commercial line immunoassay. The patients were grouped by principal component analysis into 5 clusters: CENP, RNAPIII strong and weak, Topo1 and other. CENP, RNAP III and topo 1 were mutually exclusive, but nearly half of the patients in this cohort expressed multiple autoantibodies. Given the suspicion that antibodies in SSc may either be pathogenic themselves or are a unique biomarker for the underlying autoimmune disease process, this approach is intuitively more likely to account for some of the clinical heterogeneity seen among patients with a specific circulating autoantibody. While the identified clusters were defined largely by the dominant scleroderma-specific antibody expressed (namely, anti-topoisomerase 1, anti-centromere A or B, and anti-RNA polymerase III), the analysis also unveiled two distinct subgroups among patients with RNA-polymerase III antibodies, based on the concentration of the antibody. The authors go on to demonstrate phenotypic separation between these subgroups across most clinical outcomes examined and argue that this autoantibody-driven categorisation may be more meaningful than the traditional limited or diffuse clinical division (33, 35).

Another clue to the usefulness of subtyping the disease based on a more refined autoantibody profile, is the observation that ACA positive Sjögren patients show a unique reactivity to centromeric C protein and heterochromatin protein 1, not exhibited by SSc patients without Sjögren or SSc patients with siccasymptoms but not full blown Sjögren (36). This preliminary observation seem to strengthen our previous report of a subset of "ACA positive/limited scleroderma SS overlap syndrome", characterised by a distinct clinical profile, with a milder course of the disease counterweighted by higher risk of lymphoma (37).

Organ involvement

Skin

The Modified Rodnan Skin Score (MRSS) is a good indicator of improvement or progression in SSc and is a suitable measure for use in clinica trials. Skin involvement is a frequent feature in SSc subjects. MRSS remains the most widespread method for the objective assessment of skin involvement bothin clinical practice and in research settings (1). However, the assessment of skin involvement by physical examination has a number of limitations, such as the difficulty in distinguishing at times between thick, hard and hidebound skin that carries a high intra- and inter-rater variability. The intra-observer variability and within patient SD values can be significantly reduced by repeated teaching (38). Another way to reduce variability is to assess skin involvement by instruments capable of sampling objective measures, such as skin texture and thickening by high frequency ultrasound (39) or stiffness, by virtual touch imaging and quantification (VTIQ) (40). Both measures were highly correlated to the Rodnan skin score in cross sectional assessment, while high frequency ultrasound was a reliable measure of skin thickness also after a follow-up of more than one year. The correlation between US and Rodnan skin score was better in the earliest stages of the disease, a possible explanation is that skin is thick at the beginning of the disease, while in the later stages, the abnormalities of skin are mostly related to hardening and hidebound (39).

Another frequent feature of skin involvement of SSc is calcinosis, or the deposition of calcium in the subcutaneous tissue. This manifestation may be asymptomatic, but sometimes it is associated with increased risk of complications, such as the appearance of skin ulcers, limited range of motion if the location is in strict contact with the periarticular area or other manifestations such as neck pain, abdominal pain, radicular dysfunction, weakness of the limbs in the rarer spine calcinosis (41).

A tentative classification of calcinosis according to clinical and radiological features, may helpindiscovering a number of clinical associations, such as the correlation of mousse calcinosis with pulmonary hypertension, stone calcinosis with lung involvement and net calcinosis with smouldering and slow to heal skin ulcers (42).

Vascular involvement

Vasculopathy is a central key feature in the pathogenesis of SSc, including digital ulcers (DUs) and many organ-based complications (43).

A growing number of data indicate that the DU burden not only has an impact on quality of life and function, but is strictly correlated with the severity of the disease and outcome (44).

Up to one half of patients with SSc will develop DUs at some time during the disease (45) and among these, almost three quarters will have developed their first ulcer within 5 years of being diag-

nosed with SSc (46).About two-thirds of newly-diagnosed patients will go on to develop new DUs within one year (47). Furthermore, large randomised studies which included over 300 patients suggested that about two-thirds of patients with new DUswill have a recurrence within 16-24 weeks (48, 49). The severity of digital vasculopathy reflects the severity of the disease, as it was shown that patients with chronic and recurrent DUs were at higher risk of being younger, having a lower disease duration at first DU, having a major impact on organ-based complication, and having more complicated ulcers (50). Given the clinical and financial burden, as well as the availability of therapies that can prevent DUs in patients with SSc, there is a growing need to identify reliable and easy to collect risk factors predictive for the development of new DUs. Nailfold videocapillaroscopy (NVC) assessment has been shown to be a reliable method for the prediction of the development of new DUs and the severity of organ involvement (51, 52). However, although highly reproducible in trained personnel, capillaroscopy needs the availability of adequate equipment and a skilled operator. An easy to detect clinical manifestation of SSc, telangiectasia, may prove useful in dichotomising patients to be monitored for vascular complications, since it has been associated with the severity of vascular involvement, DUs and pulmonary arterial hypertension (PAH) (53). However, the entity of this clinical manifestation does not seem to be correlated with NVC (54). The CAP study was the first large, prospective, multinational study to evaluate capillaroscopy and other clinical characteristics to determine the risk factors for the development of new digital ulcers in a period of 6 months in patients with SSc. After prospectively collecting clinical and capillaroscopic data of 623 patients with SSc from 59 centres (14 countries), univariable and multivariable logistic regression analyses eventually identified the strongest independent risk factors for new DUs, capillary density and the presence of critical digital ischaemia at enrolment. The main limitations of this study are linked on the one hand to the definition of critical digital ischaemia, which is not always clear-cut, and on the other hand to the type of patients recruited. Indeed, the prevalence of digital ulcers during the study period in the group of subjects with a history of DU was only 22%, as compared to the expected 50% (48) and the prevalence of "incident" DUs in the cohort with no DU history was only 4.1%. However, this did not give the statistical power to predict the occurrence of new DUs in this subset of patients (55). At present, therefore, the prediction rule of the CAP study is only applicable to SSc patients with a history of DUs. The authors attributed these disappointing results to the improvement of the diagnosis and management of SSc patients and to the recruitment of patients in the no DU history group with a disease duration of up to two years, which may have accounted for a lower incidence of DUs than reported in patients with earlier disease (55, 56).

Another issue to be taken into consideration is that some subjects with SSc can have a very slow progression of capillaroscopy or achieve some kind of stability rather than encompassing the classical three stages described by Cutolo *et al*. (55); these subjects have NVC features that resemble the "slow" pattern first described by Maricq (57). This is in line with the observation of Avouac *et al*. of a "progressive" pattern only in 21% of a prospective cohort of 141 SSc patients (58). Furthermore, a limited number of SSc subjects may have a non-specific pattern at NVC and this seems to mirror a milder disease course and organ involvement (59). To further complicate this puzzle, the therapy may alter the predictive value of NVC by improving the capillaroscopic pattern (60).

Besides NVC, which has earned a pivotal place in the assessment and follow-up of SSc microangiopathy (55), newer techniques to evaluate blood perfusion in a dynamic way would allow a better quantification and assessment of response to treatment. Laser Speckle Contrast Analysis (LASCA) has good reliability of peripheral blood measurements in SSc patients (61). Unfortunately, LASCA, as well as infrared thermography, does not reliably differentiate between primary and secondary Raynaud's phenomenon (62). However, by using this technique, a different pattern of vascular reactivity and flow seems to be useful in the sub-typing SSc subjects (63, 64). Future possible applications are linked to the possibility of dynamically mapping skin areas, to test the response to treatment and to follow-up skin ulcers (64, 65)

Pulmonary involvement

Lung complications are one of the leading causes of death in SSc patients (1). Pulmonary disease is a two-faceted disease encompassing a spectrum that may show features of pure interstitial lung disease (ILD), isolated PAHor a various degree of combination of the two entities. In severe, end-stage ILD, secondary pulmonary hypertension may superimpose (ILD-PH).

While the majority of the patients have a certain degree of ILD on high resolution CT scan (HRCT), this type of involvement is not severe or progressive in all cases. The most challenging effort in managing this disorder is therefore to find a valid instrument for dichotomising the patients to be aggressively treated as early as possible or, alternatively, to be followed through a watch and wait strategy.

Pulmonary function tests (PFTs) are not sensitive in detecting SSc-related ILD, especially in early and asymptomatic cases. The negative likelihood ratio of PFTs remains high, even if more comprehensive definitions of ILD are considered, combining delta-FVC, TLC and DLCO, meaning that a negative result in PFT cannot convincingly rule out the presence of SSc-related ILD. It is thus clear that using PFTs as initial screening tool for ILD would lead clinicians to miss a significant number of patients with ILD and a better instrument is needed for the early detection of this complication. At present, HRCT is the gold standard for the detection of ILD, however, timely screening has to be weighed against unnecessary radiation exposure, particularly in asymptomatic subjects. Lung ultrasound has been increasingly used as a highly sensitive screening instrument for the detection of ILD in SSc subjects. Moreo-

ver, the recent introduction of pleural irregularity (PI) seems to add accuracy in quantifying to some degree disease extent as compared to B-lines (66, 67). The extent of ILD on a baseline HRCT significantly predicts disease course and response to treatment of SSc-associated ILD (68). Patients with moderate to extensive ILD, whether assessed by computer-aided (CAD) scores or visual ones, have been shown to have a larger decline in FVC. Fibrosis in HRCT predicts response to treatment (69). In addition, given the absence of PH, DLCO provides the best overall estimate of HRCT-measured lung disease in pooled patients from scleroderma lung study I and II (68).

In a large multicentre study of 264 Australian SSc patients, extensive disease by visual score was correlated to worse outcome, moreover, the decline of PFTs in poor outcome cases was evident in the first 1-4 years of the disease, confirming previous reports (70). A one-year decline in DLCO or KCO of 15% or more was a poor prognostic indicator in ILD patients. Furthermore, dichotomisation of subjects on the basis of HRCT (extensive/limited) and degree of decline of FVC (<10%/>10%) was useful in identifying poor outcome cases (71). These findings confirmed a previous report by the Scleroderma Lung Study indicating extensive fibrosis (>20% in whole lung or >50% in area of maximal involvement) as a sign of poor outcome and progressive disease (72).

The 6-Minute Walking Distance (6-MWD), although not always reliable in SSc patients, due to the frequent coexistence of musculoskeletal involvement, can represent a valuable tool to monitor performance during exercise and to define outcome (73). It has been suggested that, when combined with DLCO and its components, the 6-MWD may potentially indicate patients at risk of developing pulmonary hypertension (74).

Early identification of patients with PH is pivotal in SSc, in the context of a growing number of new therapies, although the outcome remains ominous as compared to other causes of PH (75). Since heart catheterisation is costly, time consuming and difficult for patients to accept, a search for non-invasive screening tools is necessary to select the patients who need to undergo heart catheterisation.

A number of algorithms have been published in the past few years, in general the best performance is accomplished by instruments that combine a comprehensive assessment of PFTs, cardiac peptides and/or echocardiogram (1, 76). Use of asymmetric dimetil arginine (ADMA) in combination with NT-proBNP has been suggested as a sensitive and specific screening tool for non-invasive identification of SSc-PAH (77).

Subclinical RV dysfunction during exercise stress echocardiogram may be a surrogate marker for early pulmonary vascular disease in SSc patients, although the longitudinal value of this tool in identifying patients at risk of full blown PAH remains to be clarified (78, 79).

It needs to be underlined that, according to European Society of Cardiology/European Respiratory Society (ECS/ERS) guidelines (80), right heart catheterisation remains the gold standard to confirm diagnosis and to follow-up CTD-PAH (class 1 PAH), in order to assess haemodynamic and pulmonary artery wedge pressure at the time of diagnosis and after treatment. Vasoreactivity test is no longer recommended in SSc-related PAH. PAH subjects should be referred to tertiary care centres with a multi-professional team, possibly comprising a cardiologist, respiratory care specialist, radiologist, clinical nurse specialist, psychologist and social support, since performing heart catheterisation is technically demanding and may be associated with serious complications and, in general, medical centres with a high volume of patients tend to obtain the best outcomes.

Another issue that needs to be addressed in SSc is the detection of borderline pulmonary arterial pressure in some patients (*i.e.* mean pulmonary artery pressure between 21 and 25 mm Hg with normal wedge pressure). The clinical relevance of this condition is not always clear. Reported data support the evidence that this subgroup may represent an intermediate stage between normal pulmonary arterial pressure and overt pulmonary arterial hypertension, but it is not yet clear either the pattern of evolution of this clinical entity, or whether there are prognostic indicators that can guide the clinician in choosing the best therapeutic and follow-up strategy for this particular subset of patients (81). However, patients presenting with pulmonary arterial pressure (PAP) in this range should be carefully followed, particularly when they are at risk of developing PAH, such as in SSc (80).

Finally, PAH on exercise is not a recommended definition, since there is a poor standardisation of definitions and lack of prospective validation data of outcome (80).

Heart involvement

Cardiac involvement is common in SSc; it has been estimated to occur in almost all scleroderma patients. It is often asymptomatic and insidious, but when it becomes clinically evident it is a well-recognised poor prognostic factor, responsible for up to 30% of SSc-related mortality. Scleroderma heart involvement (SHI) could affect both patients with lcSSc and those with dcSSc, however, recent studies have demonstrated that it might be more prevalent in the diffuse subtype and, in particular, patients with rapid skin thickness progression are at high risk of developing cardiac involvement within three years of the disease onset (45, 82). However many years before the appearance of symptoms, patients with SHI could be totally asymptomatic or could only present systolic or diastolic dysfunction (83). This is in line with autopsy studies, showing evidence of occult myocardial involvement in a large number of SSc patients (84). In routine clinical practice, cardiac assessment is based on clinical evaluation and annual Doppler-echocardiography that is able to detect the development of both PAH and heart complications (85). Unfortunately, neither cardiac symptoms nor ECG or heart echography are sensitive in predicting heart fibrosis (84). Cardiac Magnetic Resonance (cMRI) is increasingly recommended for an accurate assessment of SHI. Indeed it

is a more sensitive method which can help not only to identify myocardial fibrosis and inflammation, but also to assess myocardial perfusion, coronary reserve and ventricular function (86). However, because it is an expensive and not widely available test, it is performed only in selected patients. Nowadays, it would be essential to identify a sensitive and specific serological marker which could be routinely tested to early diagnose SHI. Several recent studies suggest the role of B-type natriuretic peptides (brain natriuretic peptides (BNP) and N-terminal proBNP) as markers of SHI. In scleroderma patients it has been demonstrated that high serum NT-proBNP is a specific and sensitive marker useful to early diagnose PAH and it is strictly related to the severity of this serious complication (87-89). Furthermore, it has been suggested that increased serum levels of NT-proBNP together with a decrease of lung diffusion carbon monoxide capacity could identify patients at high risk of developing PAH(90). A recent study also showed that high serum levels of NT-proBNP might be detected in scleroderma patients with cardiac involvement, suggesting this peptide as a candidate marker for SHI (85). High sensitivity troponin (HSTn) is a well-recognised indicator of myocardial damage in ischaemic and inflammatory diseases. There are few reports on the utility of this marker in the detection of cardiac involvement in SSc(91), however, HSTn have been claimed as a useful tool for the early diagnosis and stratification of outcome in infiltrative heart disorders, where microvascular damage seems to be one of the main triggers, thus representing a potential instrument for detecting sub-clinical heart involvement also in SSc (92). Speckle-derived strain of the right ventricle (RV) was utilised to detect occult abnormalities in regional and global contractility in SSc patients disclosing a heterogeneous pattern of regional heart strain in SSc, that is not detected by conventional measures of function, suggestive of occult RV myocardial disease (93). Since SHI is common in SSc, but according to a wide analysis of the European Scleroderma Trials and Research group (EUSTAR) database it accounts for only 14% of mortality, the search for outcome indicators for this complication is warranted (94). Arrhythmias are frequent in SSc and portend a poor prognosis, accounting alone for 6% of total deaths. De Luca *et al*. found Ventricular Ectopic Beats (VEBs) in 24% of a series of 100 selected patients with new onset cardiac symptoms. A number of VEBs were correlated with troponin T and left ventricular ejection fraction. The presence of >1190/24 hours VEBs at Holter recording was an independent poor outcome predictor, assessed with a composite index combining sudden cardiac death (SCD) and the need for implantable cardioverter defibrillator (ICD). This finding was highly sensitive and specific in predicting SCD and the need forICD implantation, thus suggesting that a 24-h ECG might be an additional risk stratification technique in subjects with clinically evident cardiac involvement, in order to prevent potential life threatening events (95).

Gastro-intestinal involvement

GI involvement is almost universal in SSc subjects and represents one of the earliest manifestationsof the disease (96). Furthermore, the severity of this involvement leads to a negative outcome in SSc (97).

GI involvement is multifaceted, varying from asymptomatic disease to significant dysmotility causing complications like malabsorption, weight loss and severe malnutrition (98). Oesophageal involvement occurs early in SSc subjects. The lack of correlation between symptoms and severity of gastro oesophageal reflux disease (GORD) and oesophageal dysmotility suggests that formal evaluation with oesophageal manometry and a 24-hour pH study would complement the assessment of upper GI in SSc. Furthermore, the fact that GORD and pulmonary fibrosis often co-exist in patients with SSc and that the patients with pulmonary fibrosis have a higher incidence of reflux disease, suggests that GORD could contribute to the natural history of pulmonary fibrosis. In addition to recurrent micro-aspirations as a contributing factor to the development of pulmonary fibrosis, the occurrence of both severe reflux and pulmonary fibrosis could also simply reflect a more advanced stage of the disease (99). Furthermore, functional anorectal involvement appears to be strictly correlated to oesophageal involvement, suggesting the need for a comprehensive screening of SSc subjects, in order to avoid complications such as fecal incontinence (100).

At present there is no single, validated, specific, objective instrument to assess the global involvement of GI system in SSc. UCLA GIT 2.0, a disease-specific patient-reported questionnaire useful in the initial screening of SSc patients and in follow-up, has been translated and validated in numerous languages, including Italian and Romanian (101).

Small intestinal bacterial overgrowth (SIBO) is a not uncommon, late onset manifestation that should be taken into account in patients with diarrhoea, weight loss, and other less well-defined abdominal complaints. SIBO is one of the main pathogenetic factors of malabsorption, which is associated with 50% mortality over 8.5 years in SSc patients. There are a number of tests used in the diagnosis of SIBO but according to the OMERACT filter, only one test is fully validated in SSc, the hydrogen and methane breath test. Four tests are partially validated, including jejunal cultures, xylose, lactulose tests and 72 hours fecal fat test (102).

Nutritional status

Until a few years ago, malnutrition hasdbeen largely overlooked in SSc, since it had been assessed in small cohorts of patients using heterogeneous criteria. Evidence in this field has increased significantly and recent findings on its role as a negative prognostic factor have suggested the need for a systematic screening in every SSc patient with the aim of preventing or correcting it (103). Three main factors may contribute to malnutrition: underfeeding, increased energy expenditure and reduced nutrients availability (*i.e*. malabsorbtion). The inflammatory state, which may be a significant component of the disease, can contribute to both hy-

porexia/anorexia and increased energy requirements. Furthermore, frequent involvement of the GI system can cause reduced intake of food due to oesophageal involvement (reflux, dysphagia, vomiting, oesophagitis or stricture) or functional derangement (reduced oral aperture). In the small intestine, bacterial overgrowth due to luminal content stasis or decreased permeability secondary to intestinal fibrosis, may cause malabsorption. Disability can contribute to reduced intake. Although the Malnutrition Universal Screening Tool (MUST) has not been specifically validated in SSc, it is the only one which has been extensively studied in this disease (103, 104). Incorporating SSc-specific data to the MUST assessment may be of additional value in identifying high risk patients (105). Overall, the prevalence of high nutritional risk in SSc in the current literature is estimated at being around 20% (103). In addition to screening tools, expert recommendations include a set of laboratory parameters to evaluate nutritional statusmore thoroughly, such as haemoglobin, serum carotene, serum folate, serum albumin and prealbumin, zinc, 25-OH vitamin D, methylmalonic acid, vitamin K level or prothrombin time (106). In this respect, serum prealbumin seems to represent an independent predictor of mortality in outpatients with SSc (107).

The first step in the management of subjects with moderate-to-high nutritional risk is nutritional counselling (103). In the case of unsuccessful counselling, or inadequate intake despite counselling, artificial nutritional support becomes necessary. Enteral nutrition represents the first choice, since continuing use of the small intestine is more physiological and prevents infectious complications and organ malfunction. If enteral nutritional support is not feasible or ineffective (*i.e.* severe malnutrition, intestinal pseudo-obstruction), parenteral nutrition may represent an effective alternative intervention (108-110).

However, specific trials addressing the significance of these measures in improving long-term outcome in SSc patients are still lacking and should be planned in the future agenda.

Treatment

Systemic sclerosis is a difficult to handle disease with a variable clinical course and outcome. The multifaceted nature of the disease, with variable extension of skin and internal organ involvement, creates significant challenges for the clinician involved in the management of this disorder. Recently, a comprehensive guideline of the British Society for Rheumatology (BSR) and British Health Professionals in Rheumatology (BHPR) has been published (111). It needs to be outlined that most of the evidences collected were weak, because of the lack of good quality randomised studies on most of the organ-based manifestations of the disease. This stems from the fact that the disease is rare and highly heterogeneous and therefore there is inherent difficulty in setting up clinical studies and obtaining good quality data. In general, it is useful to tailor treatment to the single patient, according to the type of organ involvement, extension of skin involvement and outcome factors. In particular, the authors placed emphasis on the importance to classify SSc patients according to limited and diffuse subsets. Furthermore, it is extremely meaningful to establishdisease onset, in order to identify early disease, particularly in the diffuse subset. Another disease subset to consider is overlap SSc, since a prevalence of around 20% has been claimed (111).

Early diffuse SSc should be offered an immunosuppressive agent: methotrexate (MTX), mycophenolate mofetil (MMF) or cyclophosphamide (CFX), although the strength of the evidence is weak. Autologous stem cell transplantation (ASCT) should be considered in some cases, particularly in subjects at high risk of progression, balancing concerns about treatment toxicity. Other options include CFX and possibly rituximab. Oral steroids can be used in as low a dose as possible to suppress symptoms and with close monitoring of renal function. Azathioprine (AZA) or MMF should be considered as a maintenance therapy after CFX (111).

A number of investigational medications were tested in small open-label or randomised trials in diffuse early SSc.

In a phase 2 randomised trial of subcutaneous tocilizumab in adults with SSc, this drug was not associated with a significant reduction of skin score when compared to placebo after 24 weeks of treatment (112). Moreover, the observation of exacerbation of GI symptoms during this treatment has raised concerns about the use of IL-6 antagonism in SSc, as IL-6 seems to be involved in the maintenance of mucosal integrity in physiological conditions (113).

Tyrosine kinase inhibitors (TKI) are medications of interest in the treatment of SSc because of their ability to inhibit pathways involved in fibrosis. In a small open-label study on ten adult patients with early diffuse SSc, nilotinib was well tolerated by the majority of patients with frequent mild QTc-prolongation. Significant MRSS improvement was observed, but this finding is not conclusive of efficacy of treatment given the open-label study design and small number of patients recruited. Improvers had higher levels of expression of genes associated with TGFBR and PDGFRB signalling at baseline, and a significant decrease in the expression of these genes occurred only in patients with higher MRSS improvement. The findings of this pilot study, although intriguing, warrant more conclusive evaluation (114).

In a randomised placebo-controlled study of abatacept in patients with diffuse SSc, mRSS, after adjusting for disease duration, significantly improved in the treatment arm as compared with placebo. Abatacept resulted in decreased CD28 co-stimulatory gene expression in improvers, consistent with its mechanism of action. Improvers mapped to the inflammatory intrinsic subset and showed decreased gene expression in inflammatory pathways, while non-improvers and placebos showed stable or reverse gene expression over 24 weeks (115).

Finally, type I interferons (IFNs) are implicated in the pathophysiology of SSc. Recently, a Phase I open-label trial was conducted with an anti-IFNAR1 receptor antibody (anifrolumab) in adult SSc patients. In this study, the authors aimed to assess the downstream effects of anifrolumab and elucidate the

role of type I IFN in SSc. Serum proteins and extracellular matrix (ECM) markers were measured in relation to IFN pathway activation status and SSc disease activity. The results demonstrated suppressive effects of anifrolumab on T cell activation and collagen accumulation through which tissue fibrosis may be reduced in SSc patients. The relationship between these peripheral markers and the clinical response to anifrolumab should be examined in larger double-blind, placebo-controlled trials (116).

In the management of Raynaud's phenomenon (RP), the first-line treatments are calcium channel blockers, if these are ineffective or not tolerated, alternative option are selective serotonin uptake inhibitors, α-blockers and statins. Phosphodiesterase type 5 inhibitors are being used increasingly for SSc-related RP. In severe or refractory cases iv iloprost and digital sympathectomy should be considered (111).

Digital ulcersrequire a thorough management including local and systemic treatment. Bosentan is not the first recommended choice in the UK (111), however, it should be taken into consideration in SSc patients, since it has been shown to be effective in preventing new DUs (48, 49) and has a potential role in re-modelling the microvasculature, particularly in combination with sildenafil (117). On the other hand, macitentan, a new dual inhibitor of endothelin, has not demonstrated efficacy in reducing the occurrence of new digital ulcers over 16 weeks in the DUAL 1 and DUAL 2 trial, therefore, at present it is not a recommended choice for DUs in SSc (118). Ambrisentan, a single inhibitor of endothelin type A receptor, has been claimed to be a useful treatment for SSc-related digital vasculopathy, although it does not seem to significantly improve digital blood flow assessed by laser Doppler perfusion imaging after 12 weeks (119).

A number of reports state the long-term usefulness of autologous fat grafting in the management of hand and face disability and vascular manifestations in SSc, however, this procedure needs to be confirmed on larger series of patients and is not always feasible, since it needs highly trained personnel with long-term experience and special equipment (120-122).

Because lung involvement is one the leading causes of death in SSc, all SSc patients should be assessed for lung fibrosis. Treatment is determined by the extent and severity and the likelihood of progression to end-stage disease. CPM by iv infusion or oral route or oral MMF are recommended (111). On the basis of scleroderma lung study II, CPM and MMF seem to have comparable effectiveness in SSc ILD, with better tolerability of MMF (123).

As far as the treatment of PAH is concerned, although no studies have specifically addressed the effects of treatments in SSc alone, according to ESC/ERS society guidelines, the first choice in low and intermediate risk subjects is initial monotherapy or initial oral combination therapy (80). If initial monotherapy is chosen, as head to head comparisons among different compounds are not available, no evidence-based first-line monotherapy can be proposed and the choice of the drug might depend on a number of variables, such as the approval status, physician experience and the interaction with background therapy. On the other hand, as head to head comparison between initial combination therapy with ambrisentan plus tadalafil has proven to be superior to initial monotherapy with ambrisentan or tadalafil in delaying clinical failure, a higher grade of recommendation has been given to this initial combination (124).

In the case of inadequate clinical response to initial combination therapy or initial monotherapy, sequential double or triple combination therapy is recommended. However, the combination of riociguat and PDE-5 inhibitors is contraindicated.

Oral anticoagulation is no longer recommended in SSc-PAH, but may be considered on an individual basis and in the presence of thrombophilic predisposition (80).

GI involvement may significantly compromise quality of life and outcome, since it can be associated with significant malnutrition and discomfort. At present there is no definite cure that can revert pathological abnormalities associated with GI involvement and most of the treatments are symptomatic (111).

Recently, significant benefit of GI, muscle and skin involvement by intravenous immunoglobulin infusion has been reported in a small observational cohort of 15 SSc patients (125). By analysing a murine model of bleomycin-induced scleroderma, it was suggested that IVIG treatment may inhibit macrophage recruitment to fibrotic sites by down-regulating MCP-1 and TGF-β products, thus representing a potential anti-fibrotic treatment for SSc (126).

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