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Review Article

PULMONARY FIBROSIS IN SYSTEMIC SCLEROSIS: FROM PATHOGENESIS TO A NEW DIAGNOSTIC ALGHORITHM

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ABSTRACT

Our experience of thoracic ultrasound (TUS) over three decades tells us that the fervent support of TUS in diagnostics put forward in recent publications is misplaced, potentially engendering misinformation and possibly resulting in misleading recommendations or even poor best-practice guidelines. Although it is tempting to champion diagnostic TUS in patients with fibrosis, misplaced confidence in the accuracy of lung US findings (e.g., "ring down" or B-lines) could lead to misdiagnosis, not to mention legal controversy. The use of thoracic US has been proposed in patients with systemic sclerosis, whose pulmonary function tests do not reliably identify early interstitial lung involvement. Actually, in this as in every interstitial lung disease, high-resolution computed tomography (HRCT) is the golden standard for diagnosis. However, cost and radiation exposure rule out its usual utilization as screening test. Such a high specificity allows to exclude interstitial involvement in patients with systemic sclerosis but without lung disease. The latter characteristic suggests that chest US could be a useful complementary technique in the management of patients with systemic sclerosis, allowing to screen for interstitial lung involvement better than pulmonary function tests. Consequently we believe, that, at least in patients without lung and heart co-morbidities, US could be a valuable tool to select those to refer for HRCT thoracic scan in the follow-up of the disease, with sensible saving in terms of cost and radiation exposure.

INTRODUCTION

Systemic sclerosis (SSc) is a multisystemic autoimmune connective tissue disease characterized by endothelial dysfunction resulting in a micro and macro-vascular damage leading respectively to an excessive cutaneous and visceral fibrosis and luminal narrowing and eventually vascular occlusion Gabrielli A (2009). The incidence of SSc is about 20 new cases per million per year and the prevalence is more than 250 patients per million Medsger TA (1994) and Morrow WJM (1999). The visceral involvement is responsible for decreased survival in SSc patients Steen VD (2007). The most common cause of death in SSc is pulmonary fibrosis, from interstitial lung disease (ILD), which generally develops relatively soon after the disease onset and is more common in cases of diffuse cutaneous systemic sclerosis (dSSc) featuring anti-Scl-70 antibodies Nihtyanova SI (2014), Tyndall AJ (2010) and Mayes MD (2003). Fibrosis in SSc can potentially affect any organ or system, and is detected at autopsy in over 90% of pulmonary impairment Domsic RT (2011). Unsurprisingly therefore, it is considered the patho-anatomical *hallmark* of advanced-stage SSc instead of autoimmunity and vasculopathy that precede fibrosis.

Pathogenesis and Histophatology Patterns

It is possible to hypothesize an interaction between environmental and genetic factors other than both an innate and adaptive immune response in the pathogenesis of SSc. The process responsible for fibrosis onset is not yet well known, but it is undoubtedly mediated by uncontrolled and amplified

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fibroblast activity leading to an over-accumulation of both extracellular matrix (ECM) and collagen fibres, in addition to an uncontrolled structural remodelling, in stark contrast to normal conditions where the fibroblast repair system is tightly controlled and self-limiting. The endothelial damage and the consequent development of sclerodermal micro-angiopathy is the prime agent of the fibrotic scleroderma disorder Kalaleh MB (1979). Several hypotheses have been advanced on the origin of endothelial damage. However, none completely convincing. It has been advanced a pathogenetic role of parvovirus B19, of Helicobacter pylori and cytomegalovirus Maul GG (1989), Lunardi C (2006), Lunardi C (2000) and Farina A (2014). Nevertheless, the pathogenetic role of anti-endothelial cell antibodies (AECA), found in 40-80% of cases Mihai C (2010), which are capable to determine endothelial apoptosis in vitro Ahmed SS (2006) and Del Papa N (2010) and to increase the expression of endothelial adhesion molecule appears to be more convincing Corallo C (2015).

Moreover, the ischemia-reperfusion phenomena, which are secondary to the Raynaud's phenomenon, are capable of releasing the reactive oxygen species (ROS) that, in the presence of a defect of the endocellular synthesis of antioxidant agents to balance its aggressive effect, may determine endothelial damage by oxidizing the proteins and peroxidizing the lipids. It is also possible a direct release of ROS by mesenchymal cells in SSc Servettaz A (2007) and Sambo P (2001). A defect of angiogenesis was also found in the SSc despite the high concentration of pro-angiogenic factors such as the vascular endothelial growth factor (VEGF) and of its receptors (VEGFR1 and VEGFR2) secondary to the progressive reduction of the micro-vascular shaft Distler O (2004), Davies CA (2004) and Mackiewicz (2002).

Following the onset of endothelial damage, many physiological functions of the endothelium (platelet aggregation, lymphocyte and monocyte adhesion/migration, fibrinolysis/coagulation, vasodilatation/vasoconstriction, vascular smooth muscle cell proliferation) become functionally imbalanced due to the gross functional activation of endothelin-1 (ET-1) Vancheeswaran R (1994) and Morelli S (1995) (vasoconstriction, vascular remodelling promoter, inducer of fibroblast proliferation) whose function is also amplified by an abnormal tissue clearance of its receptors (ETR-A and ETR-B), thromboxane (TXA2) (platelet aggregation promoter), monocyte-colony stimulating factor (MCSF), plasminogen activator inhibitor-1 (PAI-1, a prothrombotic agent), platelet-derived growth factor (PDGF) with an accelerating function to the fibroblast activity Xu S (1998) and Shi-wen X (2007) and growth factors (TGF-beta and others) able to transdifferentiate endothelial cells in mesenchymal cells (*endothelial-mesenchimal transition* - EndoMT) with proliferative and micro-occlusive tendency characterized by myofibroblast phenotype expressing alpha-SMA. Jimenez SA (2016), Trojanowska M (2008), Ghosh AK (2012) and Widyantoro B (2010)

This leads to vasoconstriction, vascular remodelling, inflammation and generation of a pro-thrombotic state. At the same time, activation and extravascular diapedesis of the circulating lymphocyte and monocyte system, modulated by several pro-inflammatory cytokines (TNF-alfa and adhesion molecules (P- and E-selectins first, followed by ICAM1 and VCAM1), result in the later development of tissue fibrosis mainly for a TH2-type extravascular immune network amplification (B-cell hyperactivity).

Therefore, the primary effectors of fibroblast stimulation and pro-fibrotic tissue macrophagia seem to be PDGF, ET1 Corallo C (2015), idrossi-triptamina (5HT), IL6 and, especially, the pleiotropic TGF-beta Fang F (2016).

Local fibroblast hyperactivity, thought to be governed by an inhibition of apoptosis (up-regulated Bcl2 and down-regulated Bax), could then be responsible for an uncontrolled production of fibrous tissue in the interstitial lung tissue Laplante P (2005). The TGF-beta which is considered the primary regulator of the ongoing fibrosis in SSc, Meng XM (2016) is a cytokine secreted by various cells (fibroblasts, myofibroblasts, T cells, monocytes, macrophages and platelets) as an inactive precursor and targeted to specific locations in the extracellular matrix linked to a TGF-beta binding protein. It's activation occurs thanks to the protease and integrin released by various cells, including the fibroblasts. Mauviel A (2005) and Bhattacharyya S (2005)

The cascade signalling subsequently activated by TGF are complex and mainly involve a canonical pathway connected to the cytosolic activation of SMAD 2 and 3 (after the phosphorylation of its receptor TGF-beta Type I) and to their modulators activators (SMAD and the nuclear receptor NR4A1) and inhibitors (SMAD7 that blocks the SMAD signal), and a not-canonical pathway of intra-cellular activation involving ERK2 and 1, p38, PI3K, AKT and TAK. A further intracellular system of signal transduction TGF-beta involves the mediation of EGR1, Massagué J (2006) non-receptor tyrosine kinases ABL1 (c-ABL) and FAK and the inactivation of transcriptional repressors as peroxisome proliferator-activated receptor (PPAR-gamma), friend leukemia integration 1 (FLI1) and the Kruppel-like factor (KLF) family. Wei J (2010) and Asano Y (2004) The PDGF is an important pro-fibrotic factor released primarily by platelets but also from endothelial cells, macrophages and fibroblasts. It has a chemotactic and mitogenic function on fibroblasts. In addition, It also stimulates the synthesis of the extracellular matrix products and the secretion of profibrotic factors such as TGF-beta, IL6 and monocyte chemoattractant protein-1. The profibrotic activity is mediated by the phosphorylation of the PDGF receptors. Antibodies that stimulates the receptor of PDGF have been identified in the serum of patients with scleroderma. These antibodies activates, for longer than the PDGF itself, the Ha-Ras-ERK 1/2 cascade that leads to the release of ROS and to an increase in the synthesis of extracellular matrix and transform the normal fibroblast phenotype in a scleroderma phenotype. The release of ROS can amplify the profibrotic response by both activating the Wnt system and through the determined damage on DNA and the amplification of TGF-beta secretion Trojanowska M (2008), Bhattacharyya S (2012), Olson L (2009) and Svegliati S (2014). The IL6, mainly secreted by B-cells and macrophages, directly stimulates the fibroblasts to release collagen Kitaba S (2012). The direct or indirect inhibition of IL6 is today one of the main targets in the SSc therapy. The H5T or serotonin is contained in large quantities in the platelets. Its biological function is to facilitate wound healing through its profibrotic action that follows the activation of platelets after endothelial damage. The persistent vasculopathic stimulus in course of SSc

would affect the stimulation of fibroblasts with the release of the extracellular matrix. The over-expression, in patients with scleroderma, of 5HT2B fibroblast receptors capable of causing the release of TGF-beta, would explain its pathogenetic role in that pathology. Dees C (2011)

In SSc, pulmonary fibrosis generally commences in the peripheral and inferior/posterior sites of the interstitial lung tissue, spreading upwards and outwards as the disease progresses. The fact that these sites are the first affected is presumably due to mechanical and circulatory conditions in this area (greater haemodynamic flux, more susceptible to modifications in endothoracic pressure) favouring the onset of sclerodermal microangiopathy. In SSc patients, this fibrotic reaction in the interstitial tissue generally follows, within a variable timeframe, a fairly acute alveolitis. In the initial phases of ILD, the alveolar wall features exudative oedema and the infiltration of plasma cells, macrophages and eosinophils, and at a later stage the interstitial tissue expands due to the local accumulation of collagen and other connective tissue proteins, thereby bringing about the fibrosis Bouros D (2002), Vivero M (2015) and de Lauretis A (2011).

From a histopathological perspective, many different types of ILD-related lesion can be present in SSc. A typical histolological picture is a non-specific interstitial pneumonia (NSIP) characterized by a moderate inflammation and an uniform interstitial fibrosis. The high resolution CT image (HRCT) shows symmetric sub-pleural anomalies, ground glass opacity and linear or reticular irregular opacity and scattered micronodules mainly located in the lower lobes. Fujita J (2001) and Solomon JJ (2013)

Less common is an usual interstitial pneumonia (UIP), typical of idiopathic fibrosis, with scattered fibroblast centres and uneven fibrosis. The CT picture is characterized by sub-pleural reticular opacity, macrocystic honeycombing and traction bronchiectasis. Desai SR (2004) and MacDonald SL (2001) The sub-pleural reticular opacities increasingly distribute from apex to base. Mink SN (2012) More rarely still, bronchiolitis obliterans organizing pneumonia (BOOP) and lymphocyte interstitial pneumonia-like (LIP) (overlapping with Sjögren's syndrome) cases are also seen. Taylor JG (2003) and Bridges AJ (1992)

Cigarette-smoking SSc patients, as well as having an increased risk and more severely deteriorating evolution of ILD, are particularly susceptible to desquamative interstitial pneumonia (DIP), whose histological signs are therefore evident upon histological testing. More seldom, in less histologically expressive forms, the signs of cryptogenic interstitial pneumonia (COP), can be detected, while acute interstitial pneumonia (AIP), comparable to a lesion from acute respiratory distress, is more common in the hyperacute forms.

Investigation Tools

Examination of the gross anatomy of the lung in SSc involves *thoracic x-ray*, which often appears normal in the initial phases of the ILD but at later stages generally shows reticular opaque areas at the bases and posterior subpleural sites. Only in the more advanced phases is the characteristic 'honeycomb' appearance evident using this technique. Nonetheless, and despite its fairly low success rate in identifying pulmonary fibrosis (roughly 70%), thoracic x-ray is given an important role in the SSc classification criteria. Strollo D (2010)

That being said, early diagnosis of ILD is now possible, even in cases where conventional x-ray detects nothing amiss, by means of a more advanced radiography technique, *high-resolution computed tomography* (HRCT), a fact which has understandably prompted its use becoming routine Wells AU (2014) and Pignone A (1992). Indeed, under HRCT, 90% of SSc patients show signs of ILD at various stages, results similar to those determined upon autopsy. Schurawitzki H (1990)

An additional advantage to the routine use of HRCT is that it also radically reduces the need for bronchoalveolar lavage (BAL) and lung biopsy, highly invasive techniques that are now reserved for few, select cases Behr J (2012). Under HRCT, definite indicators of fibrosis are linear instances of sub-pleural opacity, parenchymal reticulation and a 'honeycomb' appearance. Desai SR (2004) Nevertheless, NSID tends to feature scarce evidence of a honeycomb pattern, in contrast to, for example, UIP. NSID also differs from UIP in the uniformity of the radiological appearance of the lung alveoli interstitial tissue and its homogeneous progression. In contrast, UIP shows a more evident honeycomb pattern and a lack of homogeneity in the radiological, anatomical and pathological appearance Goldin JG (2008) and Patiwetwitoon S (2012).

In cases of ILD where a 'ground glass' pattern is detected, this is generally indicative of active phlogosis with cellular infiltration and interstitial and alveolar oedema, although the possibility of the gravitational role with consequent vascular and interstitial 'filling' needs to be considered. Hence CT slices should be taken with the patient in a prone or supine position to clarify the situation. The progression of ILD can be evaluated by the combined use of HRCT and the *Warrick score*. In this way both the pulmonary alterations (scored 1–5) and the number of lung lobes affected can be evaluated (1–3). Warrick JH (1992) Furthermore, the HRCT patterns seem to correlate well with the histological lesions.[62]

Functional evaluation of the SSc lung involves routine use of spirometry to determine its diffusing capacity (DLCO). Although this is not an absolute predictor, it can indicate interstitial lung tissue involvement, even in early-stage of ILD (except in cases of reduced inspiration volume, in which DLCO is naturally reduced) Suliman YA (2015).

The *6-minute-walk distance test* (6 MWT), wherein the distance covered in this time is evaluated in metres and monitored every 6–12 months, is a reliable indicator of the disease progression in terms of both anatomy and function Vandecasteele E (2016) and Rizzi M (2015). Despite its invasiveness, BAL can be used to exclude an infectious alveolar process in cases of pulmonary disease and fever. 17-24% of BAL in asymptomatic SS patients can give positive cultures. Identification of clinically unexpected infection is of particular importance when an immunosuppressive treatment is being considered. Scleroderma alveolitis can be diagnosed in this manner as the presence of a 3–6 fold increase in cell number with respect to the normal value of $1x10^6$ cells with T CD8+ prevalence in BAL fluid Hesselstrand R (2013), Vasakova M (2009) and Meyer Kc (2011). An increase in

neutrophils of 4% or greater with respect to the norm and a 2 % increase in eosinophils is also a common finding in sclerodermal alveolitis. A marked prevalence of neutrophils is more common in the more extensive disease rather than quantifies the severity of interstitial inflammation and is associated with worse outcome. Goh NS (2007)

Rarer, and more benign, is a relative increase in lymphocytes of more than 10-15% with respect to the norm. Much more information may lie the TH1/TH2/TH17/TReg balancement in BAL to better define interstitial disease and its clinical grade in the near future. Meyer KC (2012)

Another invasive technique, *lung biopsy* is given a secondary role in the diagnosis of sclerodermal ILD. Indeed, in theory, it should only be resorted to in cases in which pulmonary histology is essential to consent prognosis evaluation (the prognosis in NSID is better than in UIP). Bouros D (2002) and Vivero M (2015)

The Diagnostic Role of Ultrasound

Transthoracic Ultrasound (TUS) is a relatively non-invasive imaging technique that is being used increasingly for the study of diseases affecting the pleura and the peripheral regions of the lungs, also due to its reliability, reproducibility and relatively low cost. Nonetheless, its diagnostic sensitivity is operator-dependent, and transthoracic US is greatly limited by the presence of air in the lungs, as well as the osseous structures of the ribcage. That being said, it is an extremely useful tool for examining pathologies confined to the diaphragm, the thoracic walls (abscesses, fistulae and tumours), the parietal pleura (atelectasis, masses or lesions in the lung and surrounding tissue), the upper anterior mediastinum (tumours, lymphomas, cysts), and the visceral pleura, particularly in the assessment of pleural effusions. Moreover, this technique is an indispensable aid and guide for several surgical (pleural and lung biopsy and pleural drainage) and therapeutic procedures. Dietrich CF (2003) and Sperandeo M (2014) In SSc patients, pulmonary fibrosis initially involves the lower posterior subpleural regions of the lung area, which are largely accessible to ultrasound, despite some limitations. For instance, in cases of pleuritis, blebs, emphysema, obesity and other cardio-pulmonar co-morbidity, the capacity of this technique to successfully diagnose pulmonary fibrosis at an early stage is much reduced. Song GG (2016)

The recent meta-analysis study by Song G. *et al*. concluded the fascinating hypothesis that thoracic ultrasonography (TUS) has a high diagnostic accuracy and correlates well with HRCT findings displayed in interstitial lung diseases related to connective tissue diseases. This study raises some relevant concerns: due to the anatomical constraints of the thoracic cage, TUS (in optimal conditions) assesses only 70% of lung surface, and even in areas subject to TUS examination, only alterations closely related to the pleural surface may be visualized Reissing A (2009). This condition partly explains the overall rather low sensitivity of TUS in the study of interstitial lung diseases. Furthermore, the authors did not evaluated relevant technical aspects which could significantly affect their results.

In fact, in their meta-analysis, the study was limited by the different ultrasound devices employed, the probes used and the different measuring points of the B-lines. Moreover the authors did not evaluated the different operators (inter-observer variability), the time gain compensation setting, the presence or not of tissue harmonic and electronic focus of machine, which in turn may all affect the ultrasonographic pattern (number and/or type-score of B lines) Trovato GM (2013). Noteworthy, B-lines are artifacts or error in images and are commonly generated behind the pleural line due to the difference of acoustic impedance between soft tissue and gas or between fluid and gas, in conclusion these create almost a complete reflection of the incident beam Mathis G (1997).

Interfaces of this type are likely to be more evident in a number of pleuro-pulmonary diseases, including cardiogenic pulmonary edema, pneumonia, COPD exacerbation, ARDS, asthma, neoplastic lymphangitis, interstitial lung diseases, because they are associated with an increase of fluids and impairment of ventilation in the lungs or also an increase of connective / fibrotic tissue in chronic fibrotic diseases. Therefore the number of B-lines were not useful in distinguishing between these conditions for low specificity Sperandeo M (2012) and Sperandeo M (2014). In our experience the HRCT discrepancy compared to ultrasound is observed in most cases of peribronchiolar fibrosis accompanied by mild/absent subpleural fibrosis (Figure 1). In these cases, TUS shows no significant artifacts at the point where there is fibrosis on HRCT with a poorly specific irregular thickening of pleural line in complete absence of ring down artifacts.

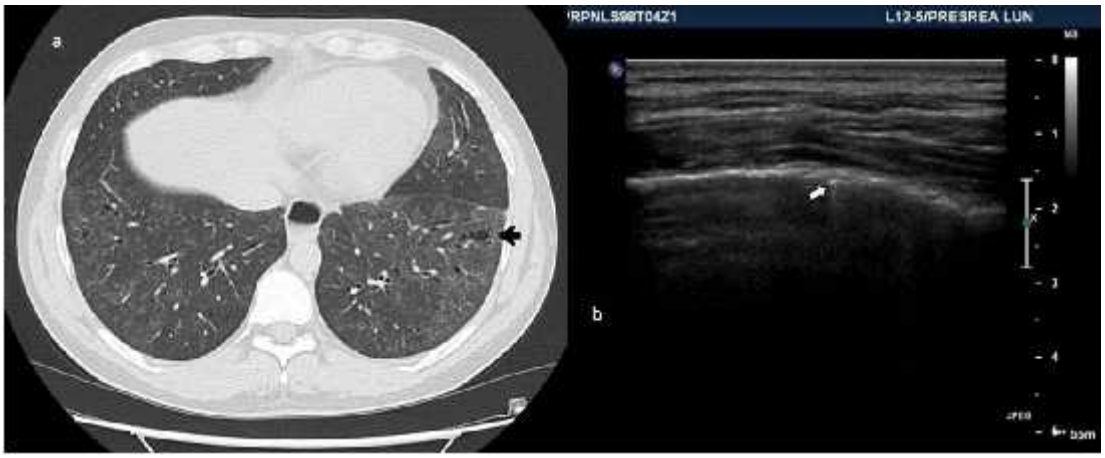

Figure 1 a HRCT: peribronchiolar fibrosis accompanied by mild/absent subpleural fibrosis. b: ultrasound postero-lateral transthoracic scan: absence of B line artefacts and irregular thickening of hyperechoic pleural line.

For these reasons, in our experience included in meta-analysis study, the evaluation of B-lines (ring-down artifacts) and comet tail is a speculative and unproductive practice because these artifact are not specific signs of lung injury and are common in many conditions and their 'count' shows a high variability with an excessively great standard deviation. Indeed, B lines are present sometimes excessively in identical cases of several conditions, such as pulmonary oedema, chronic obstructive pulmonary disease exacerbation (COPD), acute exacerbations of asthma, pleural effusion in congestive heart failure, carcinomatous lymphangitis, pneumonias, acute/subacute or chronic interstitial lung diseases Zanforlin A (2014) and Rea G (2015).

fibrosis. Previous studies investigated the employment of US in interstitial lung diseases, Sperandeo M (2009) showing that these give rise to typical, although not specific, signs such as diffuse and/or irregular thickening of the pleural line, subpleural nodules, and the reduction of the 'gliding' or 'sliding sign' (that is the physiological shift of the pleural line with respiratory movements). In particular, the use of thoracic US has been proposed in patients with systemic sclerosis, Trovato GM (2014) whose pulmonary function tests do not reliably identify early interstitial lung involvement Suliman YA (2015). Actually, in this as in every interstitial lung disease, high-resolution computed tomography (HRCT) is the golden standard for diagnosis.

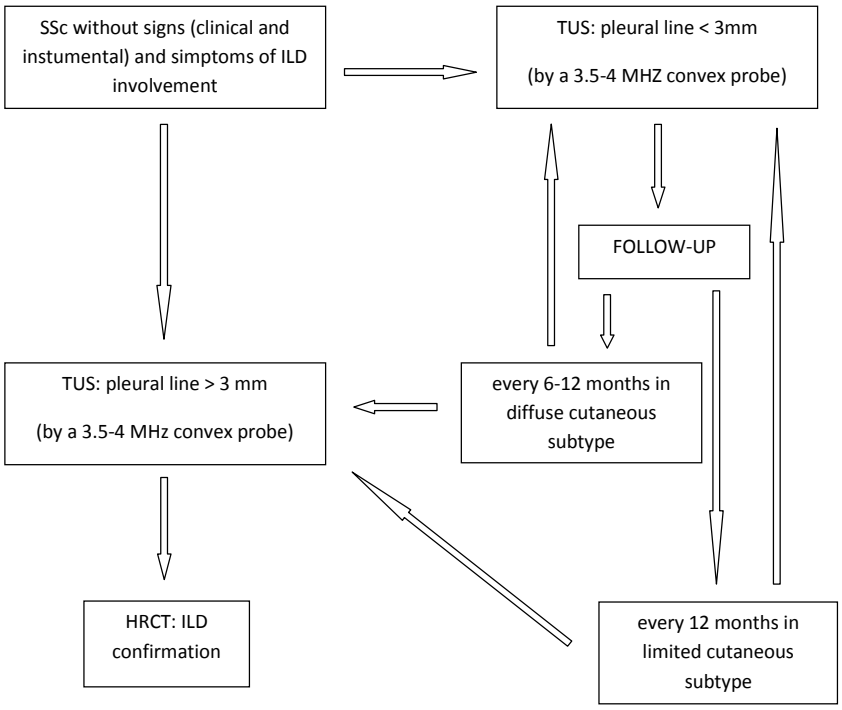

Figure 3 flow chart for TUS screening use in SSc ILD

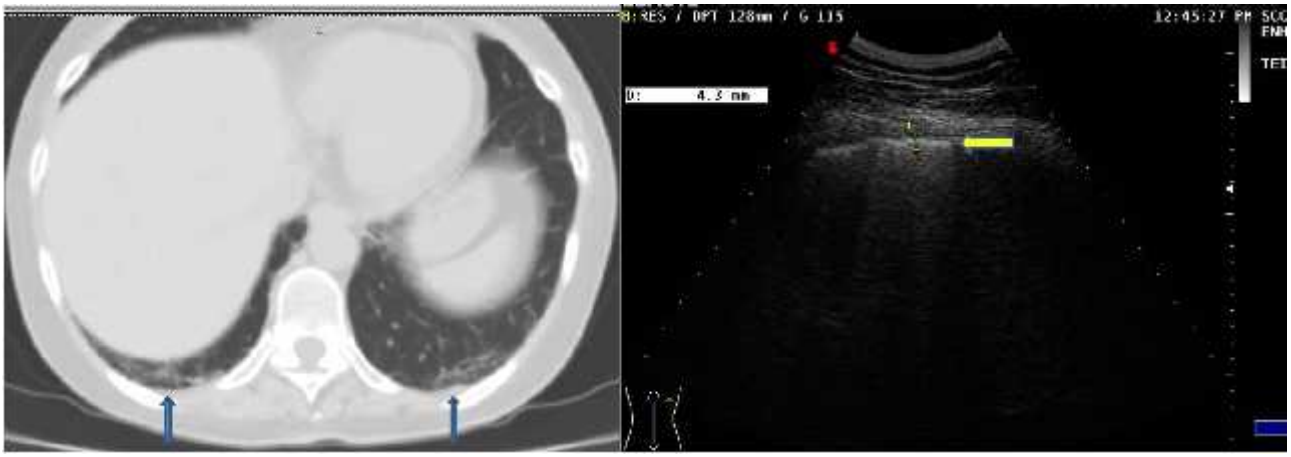

Figure 2 a. reticular-nodular HRCT pattern limited to basal area and transthoracic. b. ultrasound: thickness hyperechoic pleural line (4.3 mm)

Furthermore TUS is not a substitute for HRCT, the latter is able to assess the entire lung parenchyma and interstitial compartment. The HRCT assessment remains the gold standard and the only reliable criterion for diagnosis, treatment and longitudinal evaluation of patients suffering from pulmonary

However, cost and radiation exposure rule out its usual utilization as screening test. On the other hand, US could have relevant clinical utility, because it may easily and adequately explore the subpleural lung zones, which are often involved early in the course of this disease Schneider F (2012) and Barskova T (2013).

In a recent study, Sperandeo M (2015) we compared the findings of chest US to those of HRCT scan in 175 consecutive patients with systemic sclerosis, diagnosed according to ACR/EULAR criteria. In all patients without HRCT signs of interstitial involvement, pleural line thickness was lower than 3.0 mm. Moreover, among the 95 asymptomatic patients with normal pulmonary function tests and single–breath diffusing capacity for carbon monoxide (DLCO), 26 patients had normal HRCT features and pleural line thickness ≤ 3 mm, while the 69 patients with pleural line thickening had reticular or reticular-nodular HRCT pattern limited to basal area (Figure 2).

The sensitivity of pleural line thickness to identify HRCT-detected interstitial lesions ranged from 74.3% for reticular-nodular if the width was >3.5 mm, to 80.0% for reticular pattern with a width of >3.0 to ≤5 mm, and to 90.1% for honeycombing if width was higher than 5.0 mm. Moreover, important is the finding of the specificity was 99% in all cases. Such a very high specificity allows to exclude interstitial involvement in patients with systemic sclerosis but without lung disease. The latter characteristic suggests that chest US could be a useful complementary technique in the management of patients with systemic sclerosis, allowing to screen for interstitial lung involvement better than pulmonary function tests. Patiwetwitoon S (2008) Consequently we believe that, at least in patients without lung and heart co-morbidities, US could be a valuable tool to select those to refer for HRCT thoracic scan, both in the early phases and in the follow-up of the disease, with sensible saving in terms of cost and radiation exposure.

TUS could become only a useful complementary tool in well-trained hands and as part of established validated protocols also designed to lower the overall radiation dose by limiting the number of CT slices used for lung imaging in chest HRCT Sperandeo M (2016).

For all these evidences we propose a flow chart for TUS screening use in SSc ILD (Figure 3).

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