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Assessment of Myocardial Fibrosis Using Advanced Echocardiography in Patients With Systemic Lupus Erythematosus: a Pilot Study

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Assessment of Myocardial Fibrosis Using Advanced Echocardiography in Patients With Systemic Lupus Erythematosus: a Pilot Study
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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease, which is characterized by a multi-organ involvement and increased mortality, mainly due to cardiovascular complications. Myocardial fibrosis (MF) is common in SLE, affecting up to ~30% of these patients. Cardiac magnetic resonance imaging allows an accurate assessment of myocardial tissue in SLE patients, but it is costly, time consuming, and unfit for patients with coexisting chronic kidney disease. Recent advanced echocardiographic techniques allow an accurate assessment of MF. In particular, speckle tracking echocardiography (STE) is a reproducible technique that provides information about MF by detecting abnormalities in myocardial active deformation. Scar imaging echocardiography with ultrasound multi-pulse scheme (eSCAR) is another novel technique that has been validated for detecting ischemic myocardial scars in patients with prior acute myocardial infarction.

Aim: To examine whether STE and eSCAR may detect the presence of subclinical myocardial involvement in patients with SLE.

Methods: We consecutively recruited 29 patients (M/F=3/26; age 45±11 years) with established SLE, who had a disease duration of 15±10 years. Their median SLE Disease Activity Index (SLEDAI) score was 2 (0-6). Patients with current cardiac symptoms or prior history of any heart disease were excluded from the study. We also recruited a sample of 32 control individuals, who were comparable for age, sex and traditional cardiovascular risk factors to the cases. All participants underwent a complete echocardiography examination, using both STE and eSCAR.

Results: Global longitudinal strain (GLS) was significantly impaired in most myocardial segments in SLE patients than in control subjects, except for the myocardial apical region that was comparable between the two groups. Higher SLEDAI was associated with an impaired GLS-4 chamber (r=0.470, p=0.01) and GLS infero-septal wall (r=0.464, p=0.01). A higher daily dosage of prednisone was also associated with an impaired GLS in the infero-septal myocardial

segment (r=0.414, p=0.02). Myocardial scar by eSCAR was observed in 5 (17%) out of 29 SLE patients, mainly in the infero-septal myocardial segment. A significant association was found between the infero-septal GLS and the presence of scar by eSCAR technique (r=0.569, p<0.001).

Conclusions: Advanced echocardiography techniques detected the presence of subclinical myocardial dysfunction in SLE patients with no history of cardiac disease compared to controls. An 'apical sparing' GLS pattern was also observed in SLE patients, with possible important diagnostic implications. In about one fifth of SLE patients a myocardial scar by eSCAR technique was identified, mainly in the infero-septal segments. Larger prospective studies are certainly needed to confirm these findings and to better elucidate the diagnostic and prognostic significance of advanced echocardiography techniques (including GLS and eSCAR) in patients with SLE.

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1 INTRODUCTION

1.1 Systemic Lupus Erythematosus

1.1.1 Epidemiology

Systemic lupus erythematosus (SLE) is an autoimmune chronic disease with a multi-organ involvement and a broad range of clinical features (1). The reported prevalence of SLE in the United States is 20-150 cases per 100,000. Both geographical and racial issues may affect the prevalence of SLE and the severity of its clinical manifestations. SLE is more common among African-Americans and other non-white populations (2). Black, East Asian, South Asian and Hispanic subjects with SLE usually have a more severe disease, with a greater number of clinical manifestations and worse clinical outcomes. This can be explained by an unfavourable genetic risk burden associated with increased autoantibody reactivity compared to white individuals (3). Moreover, the prevalence of SLE is higher in women than in men: the so-called "gender bias". In a young population of reproductive age, the female-to-male ratio ranges from 7:1 to 15:1. However, the female predominance isn't as significant in children (female-to-male ratio 3:1) and in older individuals, especially in post-menopausal women (4). The background for this "gender bias" is not fully understood yet, but it seems to be the result of a complex interaction between sex hormones, (epi-)genetics, and possibly even the gut microbiota composition (5). In 65% of patients with SLE, the disease onset occurs between the ages of 16 and 55 years, whereas 20% of cases occur before age 16 and approximately 15% of patients will be diagnosed with late-onset SLE (first diagnosed 50 years of age or older) (6). Older adults often have milder manifestations of the disease that may result in a delayed diagnosis (7). That said, it is important to note that improvements in both diagnostic techniques and treatment strategies have markedly increased the overall survival rates of this patient population over the last decade (from a 4-year survival rate of 50% in the 1950s to a 20-year survival rate of approximately 80% of SLE patients in the 2000s) (8).

1.1.2 Etiology

The etiology of SLE remains largely unclear, but it is multifactorial (Figure 1). In SLE, the innate and adaptive immune systems induce an inappropriate response to nucleic acid-containing cellular particles, with appearance of autoantibody and immune complexes (IC). Apparently, SLE patients do not clear early apoptotic cells effectively: these cells release auto-antigens that help drive the faulty immune response (9). However, since the presence of antinuclear antibodies (ANA) is quite common in the healthy population, even in the absence of overt disease, the clinical manifestations of SLE are probably the result of a complex interaction between genetic, immunologic, hormonal and environmental factors. Genetic susceptibility to SLE is inherited as a complex trait and some studies also suggested that several genes play a role, most of them with a small effect on risk (10). When enough genetic polymorphisms aggregate in a subject, they may achieve a threshold for SLE susceptibility. Genetic factors include deficiencies of complement components, polymorphisms in some DNA repair genes and at the major histocompatibility (MHC) locus. Other predisposing genetic variants are associated with innate immunity, most of which are involved with interferon (IFN)-alpha pathways. Also epigenetic changes may be important in SLE pathogenesis, such as hypomethylation of DNA.

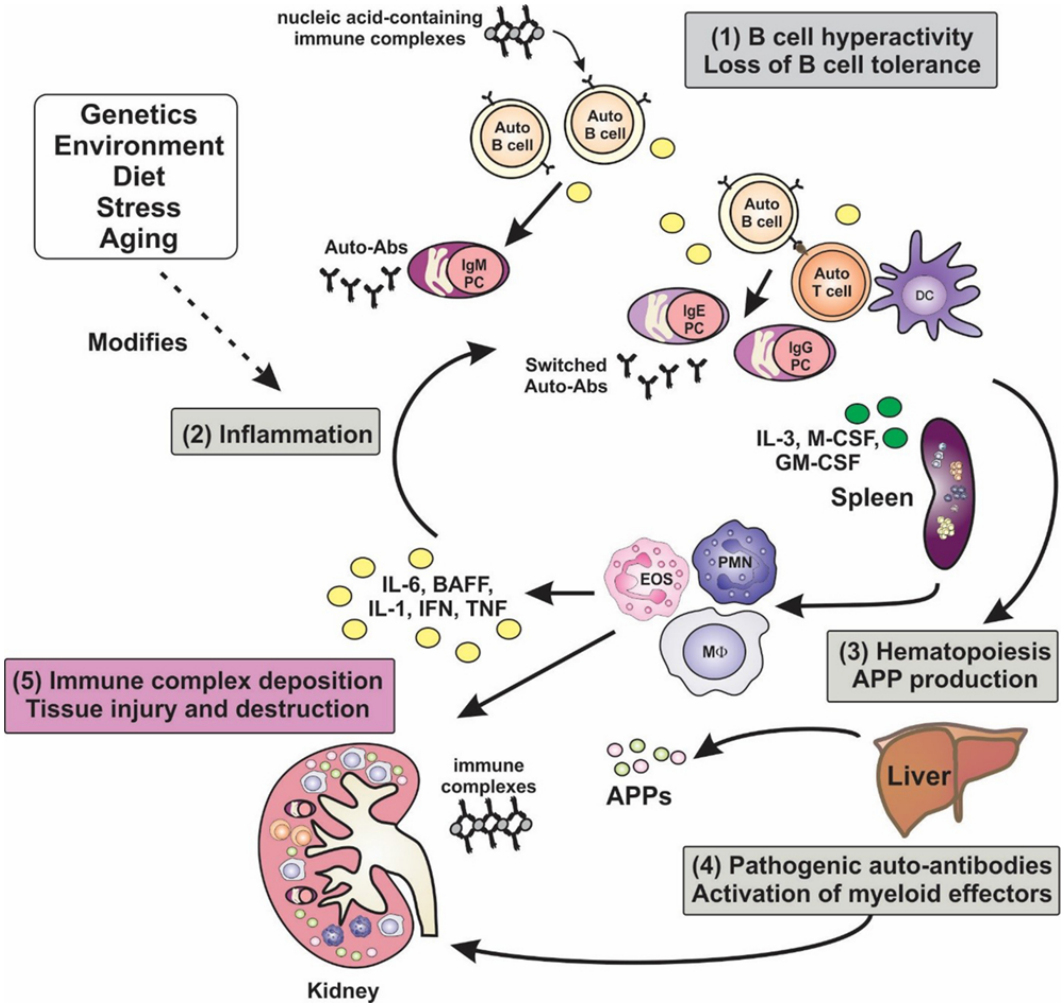

Figure 1. Overview of SLE pathogenesis. The disease is characterized by hyperactive B-cells and loss of B-cell tolerance. Immune complexes containing nucleic acid autoantigens promote inflammation. Proinflammatory cytokines drive T-cell activation and dendritic cell maturation. Moreover, they stimulate extramedullary hematopoiesis with a growth of innate immune cells and induce the production of acute-phase proteins (APPs). Autoantibodies are deposited in many organ tissues, leading to tissue injury and destruction. Many factors, including environment, diet, genetic factors and stress, can modify disease course and severity (11).

Hormonal factors play a crucial role in SLE. Female predominance of SLE is well known and is apparently related to the immunoregulatory function of sex hormones (4). Estrogen stimulates immune system activation through various pathways, and serum prolactin levels are higher in patients with SLE than in controls. Moreover, lupus flares have been associated with hyperprolactinemia (12).

There are many immune defects in patients with SLE, involving both innate and adaptive immune system activation. As already mentioned, in SLE a defective autoimmune response takes place and the main mediators are autoantibodies and

ICs. Autoantigens are released by necrotic and apoptotic cells. Phagocytosis and clearing of apoptotic and necrotic cell-derived materials are defective in SLE, allowing persistence of antigens and ICs. Therefore, B-cells are more persistently activated into producing auto-antibodies, and driven into maturation both by B-cell activating factor (BAFF) and by activated helper T-cells producing cytokines, like interleukin (IL)-6 and IL-10 (13).

Also the environment may play a role in the etiology of SLE possibly via its adverse effects on the immune system and epigenetic changes (14). Identified factors include some viruses that may stimulate antigen-specific cells in the immune network (for example, the Epstein-Barr virus); ultraviolet (UV) light inducing DNA breaks that might alter gene expression, generate nucleic acid fragments or lead to apoptotic or necrotic cell death; occupational exposure to silica dust, for its capacity to function as an adjuvant for heightening immune response; cigarette smoking; and use of certain medications (i.e. drug-induced SLE).

1.1.3 Clinical Manifestations

SLE presents a wide range of clinical and serologic manifestations that may affect virtually any organ. The disease course often follows a characteristic pattern of relapses and remissions and typically develops over a long period of time.

Almost all patients with SLE exhibit constitutional symptoms, such as malaise, fatigue, fever, and weight loss during the course of the disease. Arthritis and arthralgias occur in over 90% of cases and are often one of the first clinical manifestations. They tend to be migratory, symmetrical and polyarticular. Moreover, they usually are mildly painful and rarely cause articular bone erosions and deformations (15). Many patients with SLE also have skin and mucous membrane lesions, including discoid and malar lesions, alopecia, periungual erythema, nailfold infarcts, photosensitivity, and splinter hemorrhages. Renal involvement clinically occurs in about 50% of SLE patients and is an important cause of morbidity and mortality. Several forms of glomerulonephritis can occur (16), and renal biopsy is useful to stage the severity of kidney damage. SLE patients are also affected by several vascular abnormalities, including Raynaud phenomenon, vasculitis, and thromboembolic disease. Cardiac disease is a

common condition in SLE and involves the pericardium, myocardium, valves, conduction system, and coronary arteries (17). Pericarditis, with or without pericardial effusion, is the most common cardiac manifestation of SLE, occurring in about 25% of SLE patients. Clinically apparent myocarditis is rare but it may be severe. Libman-Sacks endocarditis is usually clinically silent, but it can produce valve regurgitation and can serve as a source of emboli. Patients with SLE also have an increased risk of coronary artery disease (CAD), which is the most common cause of death amongst patients with late-onset or long-standing SLE. Cardiac manifestations in SLE will be reviewed in more details in other sections of this PhD thesis. SLE-related gastrointestinal abnormalities can involve almost any organ along the gastrointestinal tract. However, the majority of symptoms are related to adverse medication reactions or infection. Pulmonary manifestations of SLE include pleuritis (with or without effusion), pneumonitis, interstitial lung disease, pulmonary hypertension, shrinking lung syndrome, and alveolar hemorrhage. Neuropsychiatric involvement of SLE consists of a broad range of neurologic and psychiatric manifestations, including cognitive dysfunction, organic brain syndromes, delirium, psychosis, seizures, headache, and/or peripheral neuropathies. Hematologic abnormalities can affect all three blood cell lines and include anemia, leukopenia, and thrombocytopenia. Lymphadenopathy and splenomegaly can also be observed.

1.1.4 Diagnosis

The diagnosis of SLE is based on the recognition of characteristic symptoms and signs as well as supportive serologic tests and after excluding alternative diagnoses. This is often challenging due to the wide variability in the clinical expression of SLE. Several classification criteria have been developed, mainly for research purposes in order to categorizing patients for inclusion in research studies (18-21). The most recent classification criteria are the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) criteria. They have been developed to improve the identification of SLE patients with early- or new-onset disease. This classification requires the presence of ANA positivity as an entry criterion, added to seven clinical and three immunological criteria, each of which with a grading from 2 to 10 points. Patients are considered

affected by SLE if they have a score of 10 or more points. Previous classification criteria are the 2012 Systemic Lupus International Collaborating Clinics (SLICC) and the 1997 American College of Rheumatology (ACR) criteria. The 2012 SLICC classification requires at least 4 out of 17 criteria (including at least one among the 11 clinical and one among the 6 immunological features). A diagnosis of SLE can be made also in the case lupus nephritis is confirmed by kidney biopsy in the presence of ANA or anti-dsDNA antibodies. The 2012 SLICC criteria had an improved sensitivity but lower specificity than the 1997 ACR classification criteria; moreover the SLE diagnosis resulted delayed in a significant number of patients (22). The 1997 ACR criteria are still largely used in clinical practice. A patient is considered to have SLE if 4 or more manifestations are present, either consecutively or simultaneously. The 1997 ACR version replaced the 1982 ACR criteria, mainly adding the antiphospholipid antibodies to the first ground-breaking effort that shaped SLE science. The classification criteria for SLE are summarized in Table I.

Table I. Classification criteria for systemic lupus erythematosus.

ACR 1982	ACR 1997	SLICC 2012	EULAR/ACR 2019	
			Mucocutaneous	
1. Malar rash		1. Acute cutaneous LE*	Acute cutaneous LE	6
		or SCLE	SCLE	4
2. Discoid rash		2. Chronic cutaneous LE*	Discoid LE	4
3. Photosensitivity				
4. Oral ulcers		3. Oral ulcers	Oral ulcers	2
		or nasal ulcers		
		4. Non-scarring alopecia	Non-scarring alopecia	2
5. Arthritis		5. Synovitis	Joint involvement	6
6. Serositis		6. Serositis	Serosal	
a) Pleuritis		Pleuritis	Effusion	5
b) Pericarditis		or pericarditis	Acute pericarditis	6
7. Renal disorder		7. Renal	Renal	
a) Persistent proteinuria		Proteinuria	Proteinuria	4
b) Cellular casts		or red cell casts		
		Histology compatible with lupus nephritis	ISN/RPS II/V	8
			ISN/RPS III/IV	10
8.Neurologic disorder		8. Neurologic	Neuropsychiatric	
a) Seizures		Seizures	Seizure	5
b) Psychosis		Psychosis	Psychosis	3
		Mononeuritis multiplex		
		Myelitis		
		Peripheral or cranial neuropathy		
		Acute confusional state	Delirium	2
9. Hematologic disorder			Hematologic	
a) Hemolytic anemia		9. Hemolytic anemia	Coombs+ hemolytic anemia	4
b) Leukopenia		10. Leukopenia	Leukopenia	3
c) Lymphopenia		or lymphopenia		
d) Thrombocytopenia		11. Thrombocytopenia	Thrombocytopenia	4
10. Immunologic disorder				
a) LE cell preparation				
			SLE-specific antibodies	
b) Anti-DNA	a) Anti-DNA	12. Anti-dsDNA	Anti-dsDNA	6
c) Anti-Sm	b) Anti-Sm	13. Anti-Sm	Anti-Sm	6
d) False-positive syphilis serology	c) Anti-phospholipid	14. Anti-phospholipid	Anti-phospholipid	2
		15. Low complements	Low complement	
			C3 or C4 low	3
			C3 and C4 low	4
		16. Coombs test without hemolytic anemia		
11. ANA	11. ANA	17. ANA	Entry criterion ANA	

ACR: American College of Rheumatology, ANA: antinuclear antibody, C: complement component, EULAR: European League against Rheumatism, ISN/RPS: International Society of Nephrology / Renal Pathology Society classification, LE: Lupus Erythematosus, SCLE: subacute cutaneous lupus erythematosus, SLE: Systemic Lupus Erythematosus, SLICC: Systemic Lupus International Collaborating Clinics. (23)

1.1.5 Treatment

The aims of treatment for SLE are to prevent organ damage and complications while minimizing drug toxicity, therefore ensuring long-term survival and good quality of life (24). Management is individualized and guided by the predominant disease manifestations. An accurate assessment of both disease activity and severity is essential, as well as monitoring the patient's response to previous and ongoing therapeutic interventions. A close follow-up protocol favours higher compliance to therapy and a better control of drug-induced side effects. The treatment strategy includes both pharmacological and non-pharmacological components (Figure 2). Non-pharmacological therapy refers to a series of measures that should be implemented in order to prevent or limit the disease manifestations and its comorbidities. Important among these measures are the avoidance of sun exposure and adoption of strong sunscreen protection, as well as weight control, regular exercise and smoking cessation. Smoking not only is associated with more active disease (25), but also seems to reduce the efficacy of drug therapy (26).

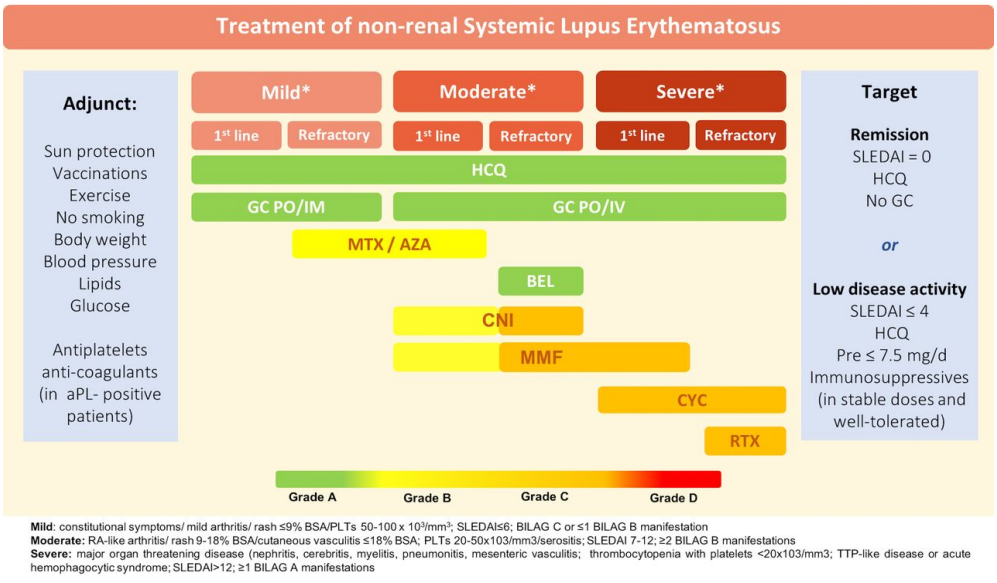

Figure 2. Treatment of non-renal SLE recommended drugs with respective grading of recommendation. aPL, antiphospholipid antibodies; AZA, azathioprine; BEL, belimumab; BILAG: British Isles Lupus Assessment Group disease activity index; CNIs, calcineurin inhibitors; CYC, cyclophosphamide; GC, glucocorticoids; HCQ, hydroxychloroquine; IM, intramuscular; MMF, mycophenolate mofetil; MTX, methotrexate; Pre, prednisone; PO, per os; RTX, rituximab; PLTs: Platelets; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index. (27)

As for the pharmacological treatment, scientific guidelines recommend indefinite therapy with an antimalarial (hydroxychloroquine or chloroquine) in all SLE patients, unless contraindicated (28). Hydroxychloroquine is considered the "backbone" of SLE treatment thanks to its ability to improve long-term survival of SLE patients. Its prognostic action is mediated by the reduction of disease activity, number of flares, damage accrual and cumulative dose of glucocorticoids, as well as the reduction in the risk of thrombosis and vascular disease and protection against infections and neoplastic diseases (29) (30) (31) (32). Additional therapy is based upon the severity of disease and the combination of manifestations. Glucocorticoids are the most useful drugs to induce a rapid remission in the setting of active disease. However, glucocorticoids are also a cause of toxicity in SLE with a dose-related mechanism, causing serious complications such as osteonecrosis, osteoporosis, CAD, infections and an overall increase in mortality (33). Non-steroidal anti-inflammatory drugs (nSAIDs) are commonly used to relieve mild symptoms, such as arthralgia, inflammation, serositis and fever. Lupus nephritis is a risk factor for nSAID-induced acute renal failure; therefore, nSAIDs should be used for the shortest effective period of time, especially in SLE patients with renal disease (34). Other immunosuppressive treatment options allow to reduce daily steroid doses. Methotrexate, an antifolate, can be used in the management of resistant arthritis and cutaneous SLE (35). Cyclophosphamide, an alkylating agent, is indicated in lupus nephritis and in patients with severe neuropsychiatric involvement (36). It is usually used in combination with corticosteroids. Mycophenolate inhibits B- and T-cell proliferation. This drug is effective in the treatment of lupus nephritis in combination with corticosteroids and has less risk of leukopenia compared to cyclophosphamide (37). Azathioprine inhibits DNA synthesis and prevents lymphocyte proliferation in the immune system. It is used as a steroid-sparing agent in moderate to severe lupus (38). In lupus nephritis, after the initial remission of the acute phase, a maintenance therapy with either azathioprine or mycophenolate is usually needed to prevent relapse of the disease.

Other agents have been used in patients with SLE who show an inadequate response to standard-of-care. Belimumab is a human monoclonal antibody that

inhibits a B-cell survival factor (known as BAFF), therefore preventing the formation and survival of memory B cells and plasmablasts making autoantibodies. Belimumab should be considered in patients with active musculoskeletal or cutaneous disease that is unresponsive to standard therapy with glucocorticoids or other immunosuppressive agents (39).

1.2 Cardiovascular involvement in SLE

SLE is associated with several cardiovascular manifestations, virtually involving all anatomic structures of the heart. Cardiac involvement is a leading cause of morbidity and premature death in SLE (40). Despite all-cause mortality in SLE patients has been declining over the past decade, cardiovascular mortality remains essentially unaffected (41). In contrast to systemic involvement, cardiovascular disease in SLE progresses silently (and sub-clinically) for the major part of its evolution. In some SLE patients, however, cardiovascular involvement evolves into manifest cardiac disease. Manifestations include disease of the pericardium, myocardium, valves, conduction system, or coronary arteries (17).

1.2.1 Pericardial disease

The most common cardiac manifestation is pericarditis with or without pericardial effusion, occurring with symptoms in about 25% of SLE patients at some point during the disease course. Asymptomatic pericarditis is even more common and is detected by echocardiography as an incidental finding in up to 40% of SLE patients. Reported prevalence of pericardial disease in autopsy studies reaches approximately 60% of patients with SLE (42). Pericarditis usually occurs at disease onset or when the disease is active in other organs as well (43). Signs and symptoms include the typical positional chest pain, decreased heart sounds, fever, and tachycardia. A pericardial rub can also be heard. An electrocardiogram (ECG) may show PR segment depression and ST segment elevation, as well as inversion of T waves. Chest x-ray may reveal an enlargement of the cardiac silhouette. Echocardiography is the diagnostic test of choice in showing pericardial effusion or thickening, as well as signs of cardiac tamponade. Patients with pericardial effusion should be closely watched for development of cardiac tamponade. The need for pericardiocentesis is usually rare and indicated to drain the fluid if there

are signs of tamponade. Constrictive pericarditis is a rare complication. Cardiac magnetic resonance (CMR) plays a role in the diagnostic process of doubtful cases, thanks to its ability to assess pericardial inflammation and possible concomitant myocarditis (44).

1.2.2 Myocarditis

Myocarditis is a dangerous but often asymptomatic manifestation of SLE, with a prevalence estimated between 8% to 25% (45). As with pericarditis, autopsy studies have reported subclinical myocarditis in a higher percentage of patients (17). Simultaneous involvement of other cardiac structures, particularly the pericardium, may occur in half of SLE cases. Pathological findings include mononuclear cell infiltration and deposition of ICs in the myocardium. Perivascular inflammation appears as the primary lesion (46). Lupus myocarditis may present with fever, dyspnoea, chest pain, resting tachycardia, and signs of heart failure. Non-specific ECG abnormalities (such as diffuse ST and T wave abnormalities) may be also detected. Wall motion abnormalities or global myocardial hypokinesis can be shown on echocardiogram. CMR is crucial in the diagnostic work-up and helps differentiate acute lupus myocarditis from dilated cardiomyopathy, myocardial infarction, vasculitis, and valvular heart disease. Tissue characterization CMR sequences allow to detect myocardial oedema (increased T2-weighted signal), as well as myocardial necrosis/fibrosis observed as late gadolinium enhancement in areas of myocardial inflammation (47). Endomyocardial biopsy is rarely necessary but it might help ruling out other causes before proceeding to immunosuppressive therapy(48).

1.2.3 Valvular heart disease

Valvular heart disease in SLE has a wide variability of presentations and ranges from mitral valve prolapse to nonbacterial thrombotic endocarditis (NBTE), also known as Libman-Sacks endocarditis. Valvular lesions in SLE may appear at any time. Usually their presence does not associate with disease activity.

Mitral valve prolapse appears to occur more frequently in patients with SLE (21% of SLE cases vs 5.5 % of controls) (49). NTBE is a form of noninfective endocarditis typically characterized by the deposition of fibrinous lesions

containing inflammatory cells on heart valves (mostly aortic and mitral). The severity of valvular dysfunction is variable, but the surgical valve replacement is rarely needed. Patients with NBTE are typically asymptomatic until embolization occurs. NBTE pathogenesis is driven by IC deposition. In fact, NBTE is more prevalent among SLE patients with elevated levels of antiphospholipid antibodies (aPL) (50). This finding suggests the adoption of screening echocardiographic examinations in SLE patients with aPL, even without a history of valve dysfunction. Patients with SLE and valve lesions, who are immunosuppressed, may represent a high-risk patient group for bacterial endocarditis, therefore antibiotic prophylaxis should be always used in these patients before invasive procedures associated with bacteremia.

1.2.4 Conduction abnormalities

Cardiac arrhythmias have been reported to be highly prevalent among SLE patients. However, the direct relationship to the underlying disease is unclear and often the arrhythmia is a manifestation of ischemic heart disease or lupus-related cardiomyopathy. A study by Laganá et al. suggested that heart rate variability in SLE may be related to coexisting cardiac autonomic dysfunction (51). Conduction abnormalities in SLE are most commonly recognized as a manifestation of neonatal lupus. Congenital heart blocks can occur in children born to mothers with anti-SSA antibodies, with or without lupus, and result from transplacental passage of maternal anti-SSA antibodies (42). QTc interval prolongation is often observed in SLE patients, also related to the chronic use of hydroxychloroquine, a QTc-prolonging medication. Past researches suggested an association between the presence of anti-Ro/SSA antibodies and QTc interval prolongation in adults (52). However, large studies have not confirmed this association (53).

1.2.5 Coronary artery disease

Atherosclerosis with coronary artery disease (CAD) is a significant cause of morbidity and premature death among SLE patients, accounting for up to nearly 30% of all deaths. One of the most striking features of CAD in these patients is the development of premature and accelerated CAD. While older SLE patients have the highest absolute risks of CAD, young women have alarmingly high

relative risks compared to the general population (54) (55). The pathogenesis of accelerated atherosclerosis in SLE is multifactorial but not completely understood. Traditional cardiovascular risk factors, such as diabetes, smoking, hypertension, obesity and hyperlipidemia, are common amongst patients with SLE. However, the increased risk of CAD in SLE cannot be fully accounted for by traditional risk factors; therefore, the disease *itself* and its treatment confer the greatest risk for premature CAD (56). In the literature there is increasing evidence about the role of systemic inflammation in driving premature CAD (57). Autoimmune vascular injury may facilitate atherosclerotic plaque formation through different pathways. Oxidative stress in SLE aggravates chronic inflammation of tissue through the increased production of reactive species and free radicals and leading to dyslipidemia and accelerated atherogenesis (58). Dysfunctional proinflammatory high-density lipoprotein cholesterol may also accelerate low-density lipoprotein oxidation (59). Deposition of ICs also stimulates the accumulation of cholesterol in atherosclerotic plaques (60). Antiphospholipid antibodies may drive atherosclerotic processes as well (61). Treatment-related features of SLE can also contribute to accelerated atherosclerosis. Glucocorticoids are associated with worsening of traditional cardiovascular risk factors, and their chronic use is strongly associated with increased risk of CAD events among patients with SLE (62). Also elevated plasma homocysteine levels, documented in SLE patients and possibly related to diet or treatment, are associated with an increased risk of CAD (63). Finally, renal disease with resulting hypertension also plays a role in the accelerated atherosclerosis related to SLE.

1.3 Diagnosis of subclinical cardiac disease in SLE

Cardiac involvement develops in the majority of patients with SLE during the disease course. These manifestations are the result of a complex interplay between SLE itself, traditional cardiovascular risk factors, and treatment-related effects. The variability of cardiac manifestations in SLE is underscored by the presence of systemic inflammation as the common pathophysiological driver. Cardiovascular complications have become a major cause of death in SLE population, as treatments of other complications have improved. In autopsy studies, histopathological evidence of myocardial disease is found in up to 40% of SLE

patients. However, myocardial involvement is clinically manifest in only 10% of these patients (64), showing how pathways to its recognition and management remain still rudimentary. Clinical management in these patients is primarily guided by systemic symptoms, therefore cardiovascular involvement often remains undetected, as it evolves through years of sustained systemic inflammation. Recent evidence based on tissue characterisation obtained with advanced cardiac imaging, highlights how the main cardiovascular involvement in systemic inflammatory diseases is predominantly non-ischaemic, potentially leading to dilated cardiomyopathy (DCM), heart failure (HF) and arrhythmic presentations (65) (66). Due to the relevant prognostic impact of cardiovascular disease in patients with SLE, there is an increasing interest in the scientific literature into the early detection and screening of cardiovascular involvement during the subclinical and silent phases of SLE. An early identification of subclinical cardiovascular abnormalities remains crucial to improve clinical outcomes in this population of patients.

1.3.1 Echocardiography

Conventional trans-thoracic echocardiography (TTE) is a well-established and widely available imaging technique for the non-invasive detection of cardiovascular complications. It allows an accurate non-invasive assessment of cardiac chamber morphology and volumetry, as well as ventricular systolic and diastolic function and the presence and severity of valvular heart diseases. In addition, advanced echocardiographic features are now available, such as speckle tracking echocardiography (STE), which evaluates and quantifies myocardial active deformation (strain), identifying abnormalities in early and subclinical phases of disease. Due to its wide availability and low cost, TTE represents the cornerstone of screening for cardiac abnormalities in SLE patients. Convincing evidence supports the presence of cardiovascular abnormalities in SLE patients even in the absence of overt cardiac symptoms. A significant reduction of global longitudinal strain (GLS) has been demonstrated in patients with SLE compared to healthy volunteers, denoting early systolic dysfunction before any reduction in left ventricular ejection fraction (LVEF) (67) (68) (69). Also the presence of early-stage, clinically silent LV diastolic dysfunction has been demonstrated in

patients with severe SLE, in terms of increases in LV mass, LV end-diastolic volume, left atrial volume and right heart parameters (70). These subclinical cardiac abnormalities may indicate pathways of myocardial remodelling in the context of systemic inflammation. In fact, GLS appears to be able to derive indirect information about the presence of myocardial fibrosis through the analysis of myocardial dynamics. Indeed, the presence of myocardial fibrosis causes abnormal endocardial thickening by an increase in myocardial stiffness and a consequent reduction of LV strain (71).

1.3.2 Cardiovascular Magnetic Resonance (CMR)

CMR is the non-invasive modality of choice to directly perform myocardial tissue characterisation, as opposed to indirect echocardiographic approaches, such as the analysis of regional or global wall motion and function or STE (72). Late gadolinium enhancement (LGE) is an established technique, which allows to detect myocardial areas with regional extracellular expansion, such as replacement fibrosis and scar. LGE provides important diagnostic and prognostic information in a variety of cardiac conditions, including systemic inflammatory diseases (73) (74). In SLE patients without history of any cardiac disease, various studies confirmed the presence of LGE, predominantly with a mid-wall non-ischemic distribution, consistent with chronic myocardial inflammation, such as an indolent subclinical myocarditis (75) (76). T2-weighted images allow to further characterize the myocardial tissue by identifying the presence of oedema in the context of active myocardial inflammation. Abdel-Aty et al. have shown a higher oedema ratio in SLE patients with active disease, whereas in those in clinical remission this measure was not different from controls (77). There is an emerging role of CMR quantitative parametric mapping (T1 and T2 mapping) in SLE. These novel imaging techniques investigate a diffuse myocardial involvement, including widespread inflammation and diffuse interstitial fibrosis, therefore expanding the ability to detect and decipher the complex pathophysiological pathways of cardiac involvement in SLE (78,79) (80). Although CMR is a very informative and powerful imaging tool, some drawbacks must be acknowledged. The main disadvantages of CMR are its limited availability, high costs, long exam time and the need for repeated patient breath holds. LGE sequences require

gadolinium contrast agent administration, introducing the risk for contrast media reaction. Moreover, gadolinium is contraindicated in patients with severe kidney failure. Some patients may suffer from claustrophobia to a degree that doesn't allow them to tolerate a CMR scan.

For these reasons, echocardiography remains the most common cardiac imaging technique performed in routine clinical practice, due to its wide availability, low cost, and patient acceptance. Recent advanced echocardiographic techniques can also indirectly characterize the myocardial tissue and assess the presence of myocardial fibrosis. STE is a reproducible technique that provides information about myocardial fibrosis by detecting abnormalities in myocardial active deformation. Scar imaging echocardiography with ultrasound multi-pulse scheme (eSCAR) is a novel technique that proved to be effective in detecting ischemic myocardial scars in patients with CAD.

2 AIM OF THE STUDY

The present study aims to investigate whether advanced echocardiographic imaging techniques, including STE and eSCAR, can detect a subclinical cardiac involvement in SLE patients with no history of any cardiac disease.

3 MATERIALS AND METHODS

3.1 Study Design

This is a single-centre cross-sectional study performed at the University Hospital of Verona, Verona, Italy.

The study protocol complies with the Declaration of Helsinki and was approved by the Ethics Commission of Verona and Rovigo (1707CESC) as an ancillary analysis of the study "Cardiovascular ASsessment of IMmunomediated Inflammatory and Rheumatic disOrders: the CASIMIRO study".

The present project is the result of a scientific collaboration between the Cardiology and Rheumatology departments of the University Hospital of Verona. The project has been awarded a grant by the Italian SLE association in the context of the call for funding "Clinical and therapeutic aspects of cardiovascular disease in SLE" edition 2019.

3.2 Study Population

Consecutive patients with an established diagnosis of SLE as per the American College of Rheumatology revised classification criteria, (81) were referred for screening from the local Rheumatology department between August 2019 and March 2020. Patients meeting inclusion and exclusion criteria (Table II) were enrolled to the study (n=29). Prior to the study inclusion, a signed informed consent was obtained from all participants.

Due to the Coronavirus Disease 2019 (COVID-19) pandemic, patient enrolment was suspended before reaching the intended study population of 30 subjects. As soon as the circumstances will allow it, the enrolment will be completed.

A case-control sub-analysis included 32 subjects recruited for the study named "Strain imaging in the evaluation of trastuzumab-related cardiotoxicity in patients with HER-2 positive breast cancer", which served as a control group. These subjects with newly diagnosed breast cancer, but who did not have any prior history of cardiac disease, underwent a baseline echocardiographic examination before any cancer treatment was performed.

Table II. Inclusion and exclusion criteria of the SLE patient group

Inclusion criteria	Exclusion criteria
- Age between 18 and 80 years - Diagnosis of SLE as per the American College of Rheumatology revised classification criteria (81) - Signed informed consent	- Previous or current myocarditis/pericarditis - Systolic or diastolic left ventricular dysfunction on echocardiography - Coronary artery disease, previous myocardial infarction or previous percutaneous or surgical coronary revascularization - Severe heart valve diseases or previous heart valve surgery - Patients with a pacemaker or implantable cardioverter-defibrillator - Life expectancy of less than 2 years for any cause - Congenital heart diseases - End-stage renal failure or kidney transplant - Type 2 or type 1 diabetes mellitus

Case history, SLE disease duration, SLE disease activity, as well as SLE disease-related organ damage, laboratory and treatment data were provided by treating physicians after a routine outpatient visit or during inpatient hospitalisation. SLEDAI score was used (ranging from zero, indicating no activity, to 105 as maximum) for the assessment of SLE disease activity (82).

All participants (both SLE patients and controls) underwent a standard TTE study performed by a single experienced cardiologist, who was blind to clinical and laboratory data of subjects. At the same day of echocardiographic examination, a detailed cardiovascular history was obtained.

3.3 Echocardiographic analysis

A standard two-dimensional (2D) echocardiographic study was performed using a standard echocardiography machine (Philips Epiq7; Philips Healthcare, Inc., Andover, MA, USA) equipped with a X5 transducer, with the subject placed in the left lateral decubitus position. Each echocardiographic examination comprised

at least two digital recordings of 2D apical 4-chamber, 2-chamber, and 3-chamber views (each recording containing two cardiac cycles). All echocardiographic images were obtained with a frame rate > 60 frames per second.

The left ventricle contrast opacification (LVO) setting (power-modulation/pulse inversion harmonic imaging [transmit 1.6 MHz/receive 3.2 MHz]) was used for scar detection (eSCAR technique), without any contrast administration (83). With this setting the ‘‘linear’’ signals from normal myocardium are cancelled, while signals from abnormal myocardial tissue (fibrotic/disarrayed myocardium or calcified tis- sues) are enhanced as they have a ‘‘nonlinear’’ response (similar to the nonlinear acoustic behavior of microbubbles). Starting from the 2D standard setting, the “iscan” button, which automatically optimizes gain and time-gain compensation, was used once (set at 0 dB), after which the LVO setting was activated. The LVO setting was finely tuned to an intermediate mechanical index, between 0.40 and 0.47, and general gain set between 70% and 77%, depending on the individual subject echogenicity. This eSCAR setting exponentially enhances the contrast between scar and normal myocardium, allowing detection of myocardial fibrosis.

Echocardiographic exams were saved to a hard disk (as DICOM files) for off-line blinded reading. Recorded echocardiographic variables included LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), LV ejection fraction (LVEF), and left atrial volume (using the biplane Simpson’s method), E/A ratio for diastolic function and medial mitral annulus early diastolic velocity (e’), tricuspid annular systolic velocity and estimation of pulmonary pressures, as well as the presence or absence of valvular heart diseases. Volumetric measures were indexed to body surface area.

Visual analysis of eSCAR images was used for the assessment of the presence/absence and segmental distribution of myocardial scar by a blinded echocardiographer. A 17-segment model was used for assessing segmental distribution of eSCAR signal. Speckle tracking echocardiography (STE) was performed using dedicated commercially available Qlab 9 (cardiac motion quantification (CMQ); Phillips Medical Systems) software package. Longitudinal strain for individual myocardial segments was measured from the apical 4-

chamber, 2-chamber and 3-chamber views (17 segment AHA/ASE model) (84). In the end-diastole, automated border tracking was enabled, before manual adjustment using a point and click approach to ensure that endocardial and epicardial borders were included in the region of interest. In the case of poor tracking, fine-tuning was performed manually after cine-loop playback and tracing was repeated and adjusted until tracking was considered optimal by visual analysis. Individual myocardial segments that returned positive strain values, and those with persistently poor tracking despite manual optimisation were excluded from analysis. Peak strain for the segment was defined as the peak negative value on the time strain curve for the entire cardiac cycle. Peak regional longitudinal strain was measured in 17 myocardial regions and a weighted mean was used to derive global longitudinal strain (GLS).

3.4 Statistical Analysis

Categorical data are expressed as counts (percentages), and continuous variables as means±standard deviation (SD) or medians (range), as appropriate. Continuous variables were analyzed with the Shapiro-Wilk test for testing their normal distribution. The unpaired Student's *t*-test was used to assess normally distributed continuous variables and the Mann-Whitney U test to assess abnormally distributed continuous variables. The chi-squared test or the Fisher's exact test, as appropriate, was used to examine relationships between binary variables. Associations were assessed using both linear regressions and non-parametric correlations. P-values <0.05 were considered to be statistically significant. All calculations were done with the use of the SPSS statistical package version 26.0 (SPSS Inc., Chicago, IL, USA).

4 RESULTS

Demographic and clinical characteristics of patients with established SLE and control subjects are shown in Table III. The two groups of individuals did not significantly differ for age, sex, body mass index and traditional cardiovascular risk factors (although SLE patients tended to have a more atherogenic risk profile). Most of our SLE patients were affected by long-standing lupus, with an age of onset of 30±13 years and an average time since diagnosis of 15±10 years. The clinical manifestations of SLE were quite heterogeneous and the disease activity was relatively low, with 8/29 (27%) patients in disease remission (SLEDAI 0). The most common symptoms were arthritis (69%) and mucocutaneous manifestations (55%). In 38% of our SLE patients leukopenia and/or thrombocytopenia were observed at disease onset. Forty-one percent of patients also had lupus nephritis. Fourteen percent of SLE patients had a history of thromboembolism and two patients had a previous obstetric antiphospholipid syndrome (APS). By study design, none of these patients had clinically manifest cardiac diseases at the time of recruitment. As for medical treatment, hydroxychloroquine was the most used drug (in 83% of SLE patients). Prednisone was also widely used, with an average administered dosage of 3.8±6.2 mg/day. Azathioprine had been used in the past by 52% of patients and in 3% was a current medication; mycophenolate mofetil had been used by 10% of patients and it was a current medication in 34% of our SLE patients.

Table IV summarizes the main echocardiographic characteristics of SLE patients and controls. Compared with controls, LVEDV and LVESV were significantly higher in SLE patients, while LVEF was lower, although the means of these echocardiographic parameters remained within the normal range ($p<0.05$ for all). Notably, there were no significant differences between the two groups in terms of LV mass and left atrial volume, as well as E-wave velocity, A-wave velocity, E/A ratio, E/e' ratio and tricuspid regurgitation peak gradient, but there was a significant difference regarding s' tricuspid wave velocity resulting significantly lower in the SLE patient group ($p=0.01$).

Table III. Demographic and clinical characteristics of SLE patients and controls.

Variable	SLE (n=29)	Controls (n=32)	Significant P Value
Demographic and clinical data			
Age, y	45±11	46±7	0.67
Male sex, n (%)	3 (10)	0 (0)	0.06
Body mass index, Kg/m^2	23±3	23±4	0.9
Smoker, n (%)	5 (17)	8 (25)	0.46
Hypertension, n (%)	8 (28)	3 (9)	0.06
Diabetes mellitus (type 2), n (%)	3 (10)	0 (0)	0.06
Hypercholesterolemia, n (%)	5 (17)	6 (19)	0.87
Time since SLE diagnosis, y	15±10	-	-
Age at disease onset, y	30±13	-	-
SLEDAI	2 (0-6)	-	-
Systemic symptoms			
Flare in the last year, n (%)	13 (45)	-	-
Arthritis, n (%)	20 (69)	-	-
CNS involvement, n (%)	3 (10)	-	-
History of lupus nephritis, n (%)	12 (41)	-	-
Cutaneous manifestations n (%)	16 (55)	-	-
Antiphospholipid syndrome, n (%)	5 (17)	-	-
Pleuritis, n (%)	2 (7)	-	-
Blood markers			
Hemoglobin, mg/L	12.8±1.2	-	-
eGFR, mL/min per m^2	108.7±31.7	-	-
ESR, mm/h	18.4±16.9	-	-
CRP, mg/L	4.1±10.5	-	-
Anti dsDNA (IF), n (%)	14 (48)	-	-
Anti dsDNA (CLIA), (UI/mL)	78.2±121.6	-	-
Anti-ENA, n (%)	21 (72)	-	-
Anti Sm/RNP, n (%)	12 (41)	-	-
Anti Ro-SSA /La-SSB, n (%)	9 (31)	-	-
Complement C3, g/L	78.3±26.3	-	-
Complement C4, g/L	13.8±8.6	-	-
Lupus anticoagulant, n (%)	9 (31)	-	-
aCL IgG/IgM, n (%)	10 (34)	-	-
B2GPI IgG/IgM, n (%)	8 (27)	-	-
Treatment			
Prednisone current dosage, mg/die	3.8±6.2	-	-
Prednisone cumulative dosage, g	26420±28843	-	-
Hydroxychloroquin, n (%)	24 (83)	-	-
Mycophenolate mofetil, n (%)	10 (34)	-	-
Methotrexate, n (%)	4 (14)	-	-
Azathioprine, n (%)	3 (10)	-	-

aCL, anticardiolipin; ANA, antinuclear antibodies; B2GPI, anti-beta2-glycoprotein; CNS, central nervous system; CRP, C reactive protein; dsDNA, double-stranded DNA; eGFR, estimated glomerular filtration rate; ENA, extractable nuclear antigens; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin; SLE, systemic lupus erythematosus; and SLEDAI, SLE Disease Activity Index.

As also shown both in Table IV and in Figure 3, GLS resulted significantly lower in all myocardial segments in SLE patients than in controls, except for the myocardial apical region (−25.1±3 in the SLE group vs. −25.5±3.3 in controls).

Table IV. Echocardiographic characteristics of SLE patients and controls.

Variable	SLE (n=29)	Controls (n=32)	Significant P Value
Standard echocardiogram			
LV-EDV index, mL/m^2	53.8±11	49.1±6.9	0.04
LV-ESV index, mL/m^2	20.9±5.2	17.9±3.7	0.01
LV ejection fraction, %	61.2±4.2	63.7±2.9	0.009
LV mass index, g/m^2	64±14.7	65±17.6	0.87
LA volume index, mL/m^2	22.8±6.9	24±6.3	0.49
E velocity (cm/s)	74.3±21.7	77.9±17.8	0.47
A velocity (cm/s)	60±18.6	66.6±17.6	0.16
Deceleration time, ms	183.8±74.5	182.5±60.7	0.94
E/A ratio	1.3±0.6	1.2±0.4	0.72
E/E' ratio	6.9±2.5	6.7±2.1	0.85
TRPG, mmHg	17.5±4.1	19.4±4.3	0.29
TAPSE, mm	24.7±7.2	24.3±2.7	0.82
S' tricuspid velocity, cm/s	10.3±5.1	13.2±1.7	0.01
Longitudinal strain			
GLS global (%)	−21±2	−23.9±1.8	<0.0001
GLS 4chamber (%)	−21.5±2.7	−22.8±1.9	0.03
GLS 2chamber (%)	−21.6±2.4	−22.8±2.1	0.04
GLS 3chamber (%)	−20.9±2.6	−22.5±2.4	0.01
GLS base (%)	−19±2.6	−22.8±2.9	<0.0001
GLS mid (%)	−19.5±2	−23.5±3.4	<0.0001
GLS apex (%)	−25.1±3	−25.5±3.3	0.6
GLS anterior (%)	−21.9±2.4	−23.8±4.3	0.03
GLS antero-septal (%)	−22.6±3.2	−25.8±3.6	0.001
GLS infero-septal (%)	−20.9±2.5	−23.5±2.8	<0.0001
GLS inferior (%)	−21.2±2.4	−25±3.5	<0.0001
GLS infero-lateral (%)	−20.3±2.6	−22.6±2.7	0.001
GLS antero-lateral (%)	−21.4±2.7	−23.5±2.7	0.004
eSCAR, n (%)	5 (17)	0 (0)	0.01
eSCAR anterior, n (%)	0 (0)	0 (0)	-
eSCAR antero-septal, n (%)	0 (0)	0 (0)	-
eSCAR infero-septal (%)	5 (17)	0 (0)	0.01
eSCAR inferior (%)	1 (3)	0 (0)	0.29
eSCAR infero-lateral (%)	0 (0)	0 (0)	-
eSCAR antero-lateral (%)	0 (0)	0 (0)	-

EDV, end-diastolic volume; eSCAR, scar imaging echocardiography with ultrasound multi-pulse scheme; ESV, end-systolic volume; LA, left atrium; LV, left ventricular; GLS, global longitudinal strain; TAPSE, tricuspid annular plane excursion; TRPG, tricuspid regurgitation peak gradient

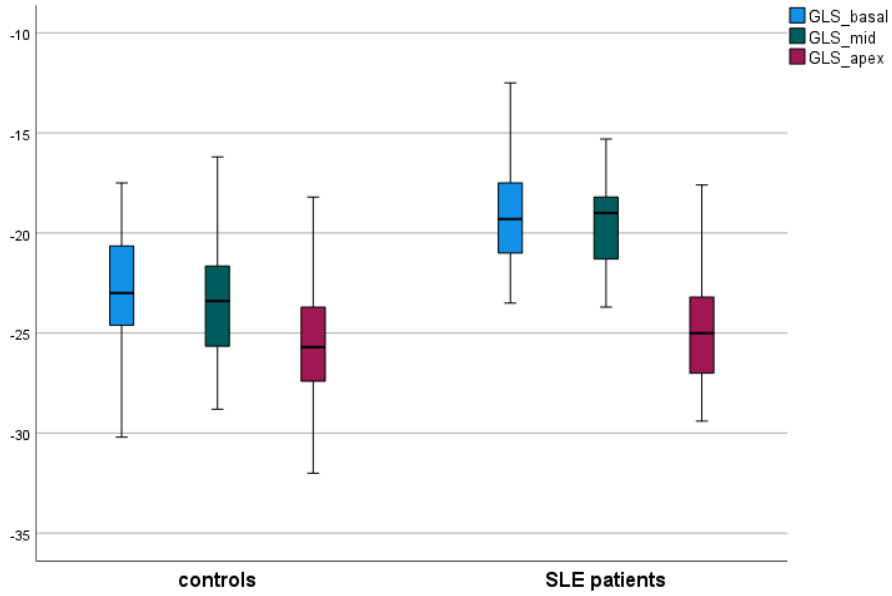

Figure 3. Distribution of GLS in basal, mid and apical myocardial regions both in SLE patients and in control subjects. Data are shown as median and interquartile range in each group.

As shown in Table IV, the presence of myocardial scar by ultrasound eSCAR was detected in 5 out of 29 SLE patients (17% of total). Figure 4 shows the myocardial scar distribution in these 5 patients: myocardial infero-septal segments were involved in all these patients and in one case the inferior myocardial wall was also involved.

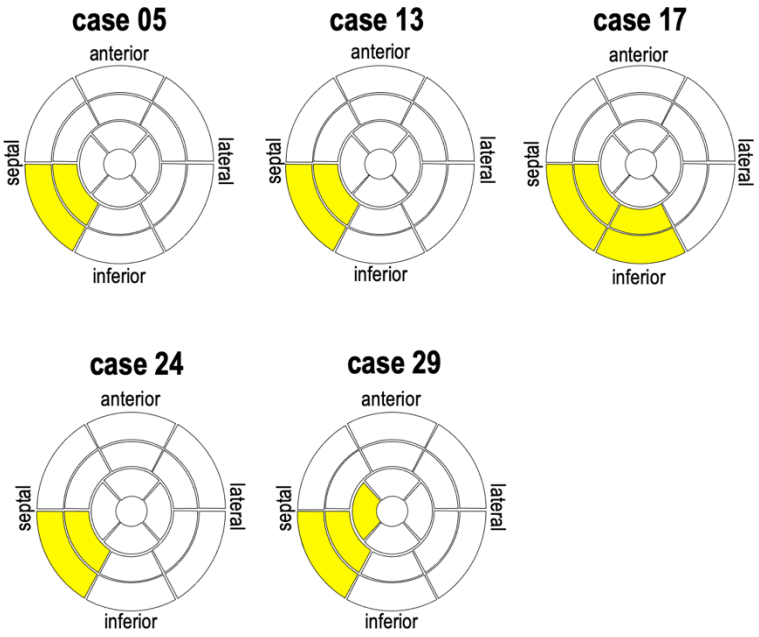

Figure 4. Distribution of myocardial scar by eSCAR technique in 5 SLE patients.

Table V shows the clinical and echocardiographic characteristics of SLE patients, stratified by presence or absence of myocardial scar by eSCAR. Comparing SLE patients with and without myocardial scar by eSCAR, demographic variables were similar in the two groups. There was no significant difference with respect to SLE disease duration, age at onset and SLEDAI; however, in the eSCAR positive group data showed a trend towards an earlier disease onset and a higher disease activity. There were no significant differences in standard echocardiographic parameters. Conversely, most GLS measures were significantly lower in the eSCAR positive group (except for GLS 3-chamber, GLS apex and GLS antero-septal).

Table V. Clinical and echocardiographic characteristics of SLE patients, stratified by presence or absence of myocardial scar by eSCAR technique.

Variable	SLE eSCAR + (n=5)	SLE eSCAR - (n=24)	Significant P Value
Demographic and clinical data			
Age, y	39.1±8.8	46.7±11.1	0.17
Male sex, n (%)	1 (20)	2 (8)	
Body mass index, Kg/m^2	24.1±2.5	23.4±3.2	0.68
Smoker, n (%)	2 (40)	3 (12)	
Hypertension, n (%)	2 (40)	6 (25)	
Diabetes mellitus (type 2), n (%)	0 (0)	3 (12)	
Hypercholesterolemia, n (%)	0 (0)	5 (21)	
SLE-related aspects			
Time since SLE diagnosis, y	12.9±9.2	15.2±10.6	0.66
Age at disease onset, y	26.2±14.4	31.5±13.3	0.43
SLEDAI	5.2±4.6	3.2±3.2	0.27
Flare in the last year, n (%)	3 (60)	10 (42)	
Antiphospholipid syndrome, n (%)	1 (20)	4 (16)	
Prednisone current dosage, mg/die	11.2±12	2.3±2.7	0.17
Prednisone cumulative dosage, g	35210±27379	24589±29361	0.4
Blood markers			
Hemoglobin, mg/L	12.8±0.7	12.8±1.3	0.99
eGFR, mL/min per m2	97.9±50.9	111±27	0.6
ESR, mm/h	18.6±11.2	18.3±18	0.97
CRP, mg/L	1.4±1.2	4.6±11.5	0.54
Complement C3, g/L	72.8±17.2	79.4±28	0.61
Standard echocardiogram			
LV-EDV index, mL/m^2	56.7±18.5	53.3±9.3	0.53
LV-ESV index, mL/m^2	22.4±8	20.7±4.7	0.53
LV ejection fraction, %	60.7±3.2	61.3±4.4	0.76
LV mass index, g/m^2	67.7±20.7	63.3±13.6	0.55
LA volume index, mL/m^2	19.8±7.7	23.5±6.7	0.28
E velocity (cm/s)	76.2±15.1	73.8±23.1	0.82
A velocity (cm/s)	56.5±20.6	60.8±18.6	0.64
Deceleration time , ms	225.2±30.8	175.1±78.4	0.17
E/A ratio	1.5±0.6	1.2±0.5	0.37
E/E' ratio	7.8±3.7	6.7±2.2	0.4

TAPSE, mm	22.2±4	25.2±7.6	0.46
S' velocity, cm/s	12±1.8	9.9±5.5	0.47
Longitudinal strain			
GLS global (%)	−18.4±1.5	−21.6±1.7	0.001
GLS 4chamber (%)	−18.2±2.2	−22.2±2.3	0.002
GLS 2chamber (%)	−18.9±1.9	−22.2±2.1	0.003
GLS 3chamber (%)	−19.8±3.6	−21.1±2.4	0.31
GLS base (%)	−15.7±2.4	−19.7±2.2	0.001
GLS mid (%)	−17.3±1.4	−20±1.9	0.005
GLS apex (%)	−23.1±1.1	−25.5±3.1	0.1
GLS anterior (%)	−18.8±1.9	−22.5±2	0.001
GLS antero-septal (%)	−20.7±2.1	−23.1±3.3	0.13
GLS infero-septal (%)	−17.3±2.1	−21.7±1.9	<0.0001
GLS inferior (%)	−18.5±2.1	−21.7±2.2	0.006
GLS infero-lateral (%)	−18.3±3.3	−20.7±2.4	0.05
GLS antero-lateral (%)	−18.8±2.7	−21.9±2.4	0.01

CRP, C reactive protein; EDV, end-diastolic volume; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; ESV, end-systolic volume; GLS, global longitudinal strain; LA, left atrium; LV, left ventricular; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index; TAPSE, tricuspid annular plane excursion.

When comparing SLE patients without myocardial scar by eSCAR with controls, global GLS resulted significantly impaired compared to the SLE group, as well as most GLS measures (except for GLS 2- and 4-chamber, GLS apex and GLS anterior) (Table VI).

Table VI. Echocardiographic characteristics of SLE patients without myocardial scar by eSCAR and controls.

Variable	SLE eSCAR - (n=24)	Controls (n=32)	Significant P Value
Standard echocardiogram			
LV-EDV index, mL/m^2	53.3±9.3	49.1±6.9	0.06
LV-ESV index, mL/m^2	20.7±4.7	17.9±3.7	0.01
LV ejection fraction, %	61.3±4.4	63.7±2.9	0.01
LV mass index, g/m^2	63.3±13.6	65±17.6	0.77
LA volume index, mL/m^2	23.5±6.7	24±6.3	0.76
E velocity (cm/s)	73.8±23.1	77.9±17.8	0.45
A velocity (cm/s)	60.8±18.6	66.6±17.6	0.23
Deceleration time, ms	175.1±78.4	182.5±60.7	0.69
E/A ratio	1.2±0.5	1.2±0.4	0.99
E/E' ratio	6.7±2.2	6.7±2.1	0.89
TAPSE, mm	25.2±7.6	24.3±2.7	0.63
S' tricuspid velocity, cm/s	9.9±5.5	13.2±1.7	0.01
Longitudinal strain			
GLS global (%)	−21.6±1.7	−23.9±1.8	<0.001
GLS 4chamber (%)	−22.2±2.3	−22.8±1.9	0.26
GLS 2chamber (%)	−22.2±2.1	−22.8±2.1	0.28
GLS 3chamber (%)	−21.1±2.4	−22.5±2.4	0.03
GLS base (%)	−19.7±2.2	−22.8±2.9	<0.001
GLS mid (%)	−20±1.9	−23.5±3.4	<0.001
GLS apex (%)	−25.5±3.1	−25.5±3.3	0.98
GLS anterior (%)	−22.5±2	−23.8±4.3	0.14

GLS antero-septal (%)	−23.1±3.3	−25.8±3.6	0.005
GLS infero-septal (%)	−21.7±1.9	−23.5±2.8	0.008
GLS inferior (%)	−21.7±2.2	−25±3.5	<0.001
GLS infero-lateral (%)	−20.7±2.4	−22.6±2.7	0.009
GLS antero-lateral (%)	−21.9±2.4	−23.5±2.7	0.03

EDV, end-diastolic volume; ESV, end-systolic volume; GLS, global longitudinal strain; LA, left atrium; LV, left ventricular; SLE, systemic lupus erythematosus; TAPSE, tricuspid annular plane excursion.

In SLE patients, SLEDAI was significantly associated with GLS 4-chamber (r=0.470, p=0.01) and GLS infero-septal wall (r=0.464, p=0.01). The cumulative prednisone dosage was positively correlated to GLS anterior wall (r=0.371, p=0.04), whereas the daily prednisone dosage was positively associated with GLS 4-chamber (r=0.370, p=0.04), GLS inferior wall (r=0.396, p=0.03), as well as GLS infero-septal wall (r=0.414, p=0.02) (as specifically shown in Figure 5).

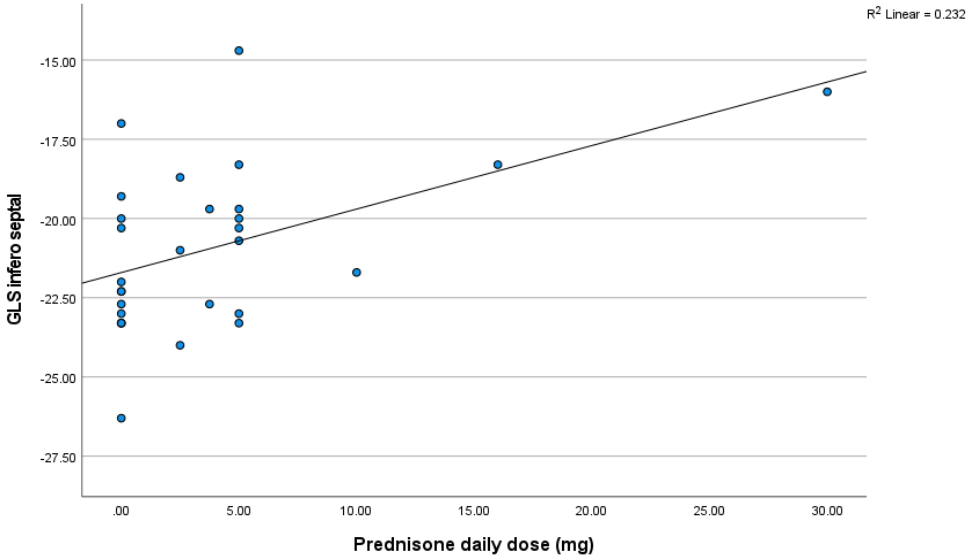

Figure 5. Association between GLS in the infero-septal myocardial wall and the daily dosage of prednisone.

Furthermore, there was also a significant positive association between GLS of the infero-septal wall and the presence of myocardial scar by eSCAR technique (r=0.569, p<0.001) (Figure 6).

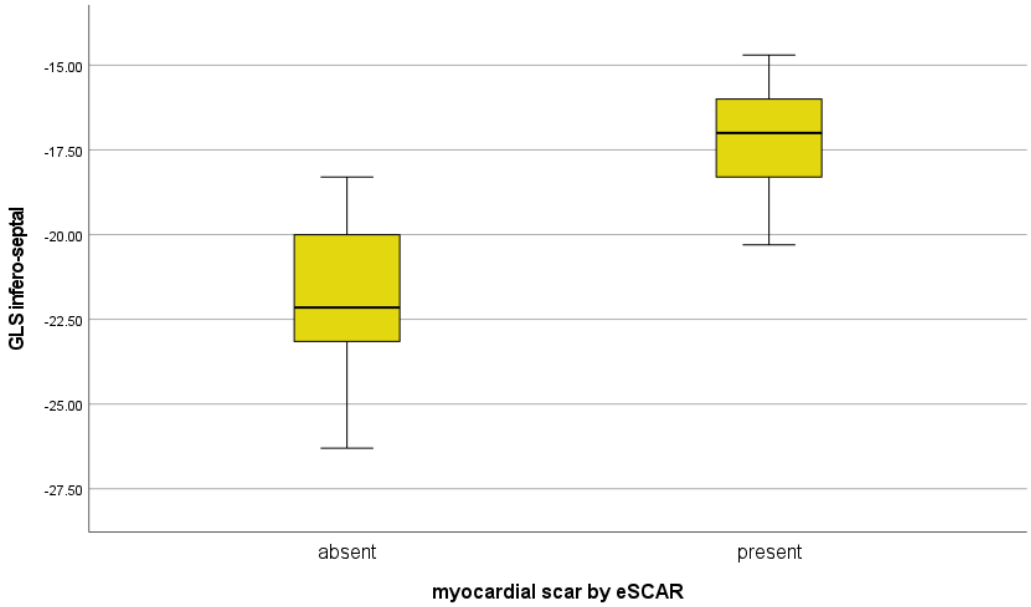

Figure 6. Difference of the GLS in the infero-septal wall in the presence or absence of myocardial scar as detected by eSCAR. Data are shown as median and interquartile range in each group.

5 DISCUSSION

In this pilot cross-sectional study we found that (1) multiple subclinical abnormalities in myocardial structure and function can be identified using advanced echocardiographic imaging techniques (i.e. STE and eSCAR) in SLE patients with no cardiac symptoms and no previous history of any heart disease, despite a preserved global systolic function; (2) SLEDAI and daily prednisone dosage were significantly associated with lower GLS 4-chamber and GLS infero-septal wall; (3) the presence of myocardial scar as assessed by eSCAR technique was observed in nearly 20% of our SLE patients, mainly in the infero-septal myocardial segments; and (4) the presence of myocardial scar by eSCAR was significantly associated with lower GLS of the infero-septal myocardial wall.

Collectively, our findings corroborate and expand previous findings supporting the presence of silent myocardial involvement in SLE patients (67) (68) (69) (70).

Although cardiovascular mortality plays a critical role in SLE, the detection of myocardial involvement in SLE is often difficult in routine clinical practice. Innovative echocardiographic tools may be of help in this research area. STE is a reproducible and well-validated technique used to assess myocardial deformation both at segmental and global levels. Since myocardial pathological changes at the tissue level affect cardiac deformation, information about the underlying myocardium can be indirectly inferred by STE. As discussed previously, the eSCAR technique is a novel advanced echocardiographic tool, which aims to differentiate normal from scarred myocardium through a multi-pulse ultrasound scheme.

Even if cardiac magnetic resonance imaging (CMR) is universally recognized as the “gold standard” for myocardial tissue characterization, due to its ability to identify areas of myocardial replacement fibrosis with the LGE technique, some drawbacks must be acknowledged. CMR is an expensive examination and its availability on the territory is still limited. It has long acquisition times and subjects are required to perform repeated breath holds. Some individuals also suffer from claustrophobia to a degree that doesn’t allow them to tolerate a CMR scan. Furthermore, some metal and electronic devices (such as cardiac pacemakers, implantable defibrillators or cochlear implants) are contraindicated

(85). LGE sequences require gadolinium contrast agent administration, introducing the risk for contrast media reaction. In addition, gadolinium is contraindicated in patients with severe kidney failure due to the risk of nephrogenic systemic fibrosis (86). The percentage of SLE patients with chronic kidney disease due to lupus nephritis varies between 20% and 65%, therefore, the administration of contrast agent could be contraindicated in a relatively high proportion of these patients (87). For these reasons, alternative non-invasive imaging methods, such as STE and eSCAR, may provide interesting insights into cardiovascular pathophysiology in SLE, while overcoming CMR limitations.

In our study, transthoracic ecochardiography (TTE) examinations, including GLS and eSCAR, resulted feasible in all SLE patients without any side effects. Standard echocardiographic measurements resulted within the normal limits in both SLE patients and controls, however LVEDV and LVESV were significantly increased in SLE patients, whereas LVEF and S' tricuspid wave velocity were decreased compared to controls ($p<0.05$ for all). Our results are in keeping with the published literature. In fact, in a meta-analysis examining the heart involvement in SLE, Chen et al. found a significant increase in LV internal diameter in diastole and a decrease in LVEF among SLE patients compared to controls (88).

In our study, the SLE patient group had impaired global GLS compared to controls, with a highly statistically significant difference ($P<0.001$). Also, other GLS measurements were significantly impaired in the SLE group, except for GLS of the myocardial apical region (−25.1±3 in the SLE group vs −25.5±3.3 in controls). These latter results are in agreement with previous studies showing a subclinical systolic dysfunction in patients with SLE (89) (90) (91). Du Toit et al. (92) reported a significant decrease in GLS in patients with lupus myocarditis compared to healthy controls ($P < 0.001$). Also Nikdoust et al. (69) found an impairment in GLS ($P=0.02$) in SLE patients compared to healthy controls. This finding was further confirmed by Farag et al. (67), who reported a significantly reduced GLS in SLE patients compared to control individuals (−18.95 ± 2.02 vs. −21.4 ± 2.1, $P<0.001$). These investigators also observed that longer disease duration and higher SLEDAI significantly affected GLS. Interestingly, in our

sample of SLE patients, we found that higher SLEDAI was associated with an impaired GLS-4 chamber (r=0.470, p=0.01) and GLS infero-septal wall (r=0.464, p=0.01). Moreover, a higher daily dosage of prednisone was also associated with an impaired GLS 4-chamber (r=0.370, p=0.04) as well as GLS infero-septal wall (r=0.414, p=0.02). This finding denotes how different disease-related aspects might contribute to the subclinical cardiac involvement in SLE, including both systemic inflammation and drug treatments.

We believe that another interesting finding of our study is the relative 'apical sparing' strain pattern of the myocardium, with the presence of myocardial dysfunction in longitudinal deformation, mainly affecting the basal and mid-ventricular areas. In fact, we did not find any significant difference in GLS of the apical region between SLE patients and controls (−25.1±3% vs. −25.5±3.3%). A previous study by Bulut et al (93) reported a similar trend with no significant difference in GLS of the apical myocardial segments. This 'apical sparing' pattern is also a typical finding of cardiac amyloidosis (94), but it has been also identified in other infiltrative myocardial conditions, such as the Danon disease (95) and the Fabry disease (96). A myocardial 'apical sparing' of variable degree has been also reported in other cardiac conditions characterized by myocardial hypertrophy. For example, a prognostic role of an increased apical-to-basal strain has been observed in patients with severe aortic valve stenosis undergoing transcatheter aortic valve replacement (97). The pathophysiological mechanisms for this 'apical sparing' have been investigated extensively, but they remain still unclear. In cardiac amyloidosis, it has been hypothesized, as a possible explanation, the presence of increased total amyloid mass in the basal and mid myocardial segments compared to the apex (98). In other cardiac conditions, possible explanations for the 'apical sparing' might be an imbalanced distribution of myocardial fibrosis and calcium (99), or an enhanced basal remodeling possibly due to flow turbulence in the LV outflow tract; however, to date, these hypotheses are not fully confirmed. That said, we believe that our finding may have important clinical implication in patients with SLE, both in the diagnostic process and as a prognostic marker, but they should be further investigated in future larger studies.

To the best of our knowledge, this is the first study applying the eSCAR technique to a study population other than patients with established coronary heart disease (CAD). A previous report by Gaibazzi et al. showed that eSCAR is an accurate surrogate for the presence of CMR-LGE in patients with a recent acute myocardial infarction (83). In our study, scar was detectable in the septal region, appearing as a myocardial septal stripe, in 5 out of 29 (17%) SLE patients. Moreover, we found that the infero-septal GLS was significantly lower ($P<0.0001$) in eSCAR positive vs. eSCAR negative patients with SLE, and there was also a significant positive correlation between the infero-septal GLS and the presence of scar by eSCAR ($R=0.662$, $p<0.001$). We believe that the presence of eSCAR in our SLE patients might indicate a non-ischemic scar. Some studies by LGE-CMR have recently proven non-ischemic scarring to be more common than ischemic scar in asymptomatic SLE patients with no history of cardiac disease. For example, Puntman et al. reported the presence of myocardial LGE in approximately two thirds of SLE patients, predominantly in the inferolateral-inferior, and infero-septal basal-mid segments, with an intramyocardial or epicardial distribution (76). Winau et al. found LGE in 30% of SLE subjects (of whom 6,7% of ischemic type) (79). Also Burkard et al found LGE-CMR in 30% (9/30) of SLE patients, all with a non-ischemic distribution (100). The LGE-CMR appearance in these studies closely reminds the typical pattern observed in chronic myocarditis or idiopathic DCM, often presenting with a septal intramyocardial stria of infero-lateral epicardial scarring (101). It has been hypothesized that these findings, in asymptomatic SLE patients, may represent the result of an indolent course of peri-myocarditis.

Collectively, our findings agree with these latter observations based on the use of LGE-CMR. In our study, the eSCAR technique has shown the presence of scar, mostly in the infero-septal segment of the myocardium. However, due to the lower spatial resolution compared to LGE-CMR, the myocardial pattern of scar distribution is technically difficult to establish with the eSCAR technique. Therefore, it was not possible to accurately define in our study if the scar was ischemic or non-ischemic in nature. However, it is important to note that if the scar was the result of an ischemic event, we would have expected to find different

coronary territories involved. Instead, considering the scar location and presentation in our subset of SLE patients, a non-ischemic scar appears to be the most probable cause. Moreover, only one eSCAR positive patient was affected by anti-phospholipid syndrome, which is a possible cause of myocardial ischemic events in SLE patients. It could be noted that a previous small report found mostly ischemia-like pattern of myocardial LGE-CMR in patients with SLE (102). However, the population of such study had a history of previous cardiovascular disease and cardiac symptoms, therefore this aspect might largely explain the contrasting finding compared to what was observed in our study.

Another limitation of the eSCAR technique is its possible scar underestimation in apical segments, as already suggested by Gaibazzi et al. (83). A possible explanation for this phenomenon is that superficial myocardial areas, with a shorter distance between the probe and myocardium (such as the LV apex), might show a reduced harmonic signal, since harmonics are generated by ultrasound waves travelling through cardiac tissue. Therefore, the presence of apical scarring could have been missed by eSCAR. However, the fact that GLS was preserved in the apical segments, with no significant differences between SLE patients and controls, makes the hypothesis of a consistent apical scarring underestimation less probable.

The potential clinical implications of our study are that advanced echocardiography techniques may have a potential role in the standard cardiac surveillance and management of patients with SLE. While overcoming the main disadvantages of CMR, such as its limited availability and high costs, these novel imaging techniques can provide important insights into myocardial tissue characterization, the diagnostic and prognostic role of which are to be confirmed in future studies. As for the eSCAR technique, further validation studies are warranted especially in subjects with non-ischemic cardiac involvement. CMR LGE and parametric mapping could help to better define the nature of myocardial damage detected by eSCAR.

STE is an already well-validated technique, largely studied in different clinical settings and it is rapidly moving from the research setting to incorporation into routine clinical practice. The incremental value of STE is maximal among

subjects with standard echocardiographic parameters within the normal range, for example in SLE patients, where the myocardial involvement is often subclinical. Technical improvements in strain imaging, with the advent of new totally automatic software modules, will allow strain analysis in fast and highly reproducible way. Future studies could concentrate on determining if specific strain patterns in SLE could indicate a worse prognosis and if this finding could guide a more aggressive therapeutic management of patients. It would be interesting also to assess the effect of medical therapy used in SLE on STE parameters.

Some important limitations apply to the present study, such as the small sample size and the cross-sectional design of the study. In addition, the control group included only women affected by newly diagnosed breast cancer, although TTE was performed before starting any cancer treatment that could adversely affect cardiac function. Our patients were recruited from a SLE clinic in a tertiary hospital, introducing a possible selection bias on the recruitment of patients with a more severe disease. The eSCAR technique is operator-dependent and the evaluation of myocardial apical segments can be challenging. We could not perform a CMR examination to confirm the presence of LGE in eSCAR positive patients. However, the eSCAR technique has already been validated *versus* LGE-CMR in a population of CAD patients (83).

6 CONCLUSIONS

This pilot cross-sectional study using advanced echocardiographic techniques in SLE patients, has reported the presence of a subclinical myocardial dysfunction in patients with SLE with no cardiac symptoms and no history of myocardial disease. An ‘apical sparing’ GLS pattern was observed in our SLE patients with possible important diagnostic implications. The eSCAR technique identified the presence of scar in nearly 20% of patients with SLE, mainly located in the infero-septal myocardial segments. An impaired infero-septal GLS was also associated to the presence of scar by eSCAR, as well as SLEDAI and daily prednisone dosage. However, further studies in larger cohorts of SLE patients are certainly needed to better elucidate the possible prognostic significance of advanced echocardiography (including GLS and eSCAR) in patients affected by SLE.

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