Results: Among differentially expressed renal microRNAs in LN, miR-127-3p was reduced in renal tissues of patients with LN. The miR-127-3p suppressed the fluorescein gene expression of ISRE induced by ISRE, and the phosphorylation of STAT1 and STAT2. By microarray analysis, we found that most ISG was inhibited by miR127-3p in IFN-stimulated Hela cells. The functional deletion of miR-127-3p enhanced the IFN response in human primary mesangial cells, which was manifested by enhanced ISRE mediated reporter gene expression, enhanced STAT2 phosphorylation and increased ISG expression. In addition, we found that JAK1, the upstream tyrosine kinase of STAT1 and STAT2, was a new target molecule for miR-127-3p.

Conclusion: Our study shows that miR-127-3p can inhibit IFN signal transduction by targeting JAK1. The decreased expression of miR-127-3p in the kidney is associated with an overactive IFN response in the renal tissue of patients with LN. Subsequent mouse model studies indicate the therapeutic potential of miR-127-3p in treating lupus associated organ damage.

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AB0198B BENEFIT OF CRITHIDIA TESTS IN DIAGNOSING CONNECTIVE TISSUE DISEASES

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Background: The diagnosis of systemic lupus erythematosus (SLE) relies on autoantibody testing, including double stranded DNA (dsDNA), the testing of which has evolved over time from the FARR assay through to ELISA/ELIA. dsDNA assays can pick up non-specific single stranded DNA as false positives and can give occasional false negative results. To evaluate the implications of this we analysed our hospital's use of crithidia testing as a confirmatory assay of dsDNA results.

Objectives: Investigate the relation between dsDNA Abs measured by Crithidia test and ELIA. Define the clinical role for Crithidia testing.

Methods: All crithidia tests for an 8 months from January 2017 to August 2017 were reviewed and results of ANA, ENA, dsDNA and complement collected. Data were collected regarding referral to rheumatology and where possible rheumatology clinic letters were reviewed regarding final diagnosis.

Results: One hundred and four crithidia tests were undertaken of which 91 were negative and 13 positive. Sixteen of the 104 patients were ANA negative, 18 ANA 1:100, 70 ANA >=1:400, and 14 patients had a positive ENA. Positive crithidias had a dsDNA range from 2 to 333 and negative crithidia from <1 to 131 however positive crithidias were more likely to have higher dsDNA and ANA titres. Of the 65 patients seen by rheumatology 4 did not have available notes for analysis. Ten of the 13 crithidia positive patients were seen by rheumatology, 6 diagnosed as SLE, 1 as TNF induced lupus, 1 MCTD, 1 RA and 1 had no clinical evidence of autoimmune disease. 88 patients were dsDNA positive (cut off of >=9) of whom 11 were also crithidia positive. Of the 13 positive crithidia results, 2 had a negative dsDNA level, one of whom was ANA 1:400 Ro positive and diagnosed with RA, the other was diagnosed as TNF induced lupus. Of the crithidia negative patients the median dsDNA level was 20, a range of diagnoses were made including 5 patients with SLE who had a positive Ro or La, 3 patients with SLE who were ENA negative, 4 UCTD, 2 MCTD, 2 with GCA/PMR, 3 with RA and 5 with inflammatory arthropathy. Twenty patients were documented as having no evidence of connective tissue disorders (CTDs), others remain under assessment.

Conclusion: We have shown a significant number of patients using our ELIA assay are dsDNA positive and crithidia negative. In patients with crithidia positivity autoimmune disease is more likely to be diagnosed. Crithidia testing appears to influence whether or not a patient will be referred to rheumatology, with negative crithidias less likely to be referred. This highlights some of the limitations of dsDNA ELISA testing however

there is a clear role for this assay as depicted above in preventing over diagnosis of CTDs and unnecessary commitment to immunosuppression.

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Systemic sclerosis, myositis and related syndromes – etiology, pathogenesis and animal models

AB0199 ASSESSING DISABILITY AND QUALITY OF LIFE IN ALGERIAN SYSTEMIC SCLEROSIS PATIENTS: CONSTRUCT VALIDITIES OF THE ARAB HAND FUNCTION INDEX AND HEALTH ASSESSMENT QUESTIONNAIRE (HAQ)

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Objectives: To assess the construct validity of the Arab Hand Function Index (AHFI), an Arabic and adapted version of the Cochin hand functional scale (CHFS) and an Arabic version of the Health Assessment Questionnaire (HAQ) in SSc.

Methods: We evaluated 100 patients with SSc followed between 2015 and 2017. All patients were white and fulfilled the American College of Rheumatology/European League Against Rheumatism criteria and/or the Leroy and Medsger (2) criteria for SSc. Mean±SD age at the time of evaluation was 47.66±12.28 years. The mean±SD disease duration was 6.54±6.23 years. Forty-one (41%) patients had diffuse cutaneous SSc and 59 (59%) had limited cutaneous SSc. Global hand and wrist mobility were evaluated using the hand functional index (HFI) and Kapandji index. Global disability and hand disability were assessed by an Arabic and adapted version of the HAQ and by the Arab hand functional index, an Arabic and adapted version of the CHFS, respectively. Anxiety and depression significant symptoms were assessed with the Arabic version of Hospital Anxiety and Depression Scale (HADS) anxiety and HADS depression. Construct validity was assessed by convergent and divergent validity (Spearman's rank correlation coefficient) and factor analysis.

Results: The AHFI had correct convergent validity with global disability assessed by the HAQ (0.61) and the HFI (0.55), moderate correlation with the Kapandji Index (-0.48: inverse correlation), depression (HADd) (0.45) and anxiety (HADa) (0.42) and no correlation with the disease duration (0.29) and the age (0.19) (Table 1). Factor analysis (Table 2) extracted 2 factors that accounted for 64.45% of the total variance. The HAQ has a good correlation with hand disability (AHF: 0.61), anxiety (0.57) and the HFI (0.51), a moderate correlation with depression (0.47) and Kapandji index (-0.46) and a little correlation with the age (0.28) and the disease duration (0.10) (Table 1). The factor analysis of the HAQ, extracted 1 factor that accounted for 50.64% of the total variance (Table 2).

Conclusion: In patients with SSc, the AHFI and the Arabic HAQ have good construct validity. The total score of the AHFI explained 61% of the variance of the HAQ.

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Table 1. Convergent and divergent validities of the AHFI and the HAQ for patients with SSc (correlation with other variables)*

Scales	Spearman's correlation Coefficient	P Value
AHFI		
Convergent validity		
HAQ	0.61	<0.001
HFI	0.55	<0.001
Kapandji	- 0.48	<0.001
Divergent validity		
Depression (HADd)	0.45	<0.001
Anxiety (HADa)	0.42	<0.001
Disease duration	0.29	0.003
Age	0.19	0.058

HAQ

Convergent validity

AHFI	0.61	<0.001
HFI	0.51	<0.001
Kapandji	-0.46	<0.001

Divergent validity

Anxiety (HADa)	0.57	<0.001
Depression (HADd)	0.47	<0.001
Disease duration	0.10	0.320
Age	0.28	0.005

Health Assessment Questionnaire; SSc : systemic sclerosis; AHFI: Arab hand functional index; HFI: Hand Functional Index.

Table 2. Factor analysis of the AHFI and HAQ

	Eigenvalue	Variance. %	Cumulative %
AHFI			
Factor 1	5.411	54.114	54.114
Factor 2	1.034	10.338	64.453
HAQ			
Factor 1	4.051	50.638	50.638

AHFI: Arab hand functional index; HAQ: Health Assessment Questionnaire.

AB0200

INHIBITION OF DOT1L ACTIVITY IN HUMAN SKIN FIBROBLASTS: A TRANSCRIPTOME ANALYSIS

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Background: The pathophysiology of fibrosis, a hallmark of diseases like Systemic Sclerosis, is still not well understood. The role of epigenetic factors is increasingly explored. DOT1L, the unique H3K79-methyltransferase, methylates histone 3 at the Lysine residue at position 79, thereby regulating gene expression programs. Inhibition of DOT1L in cartilage and bone has cell-type specific effects on Wnt signaling, a pathway suggested to play an important role in fibrotic disease.

Objectives: To study the changes in the transcriptome of dermal fibroblasts after DOT1L inhibition.

Methods: Primary human dermal fibroblasts isolated from abdominal skin were treated with DOT1L-inhibitor EPZ-5676 or vehicle and stimulated or not with TGF- β or CHIR99021 (activating Wnt signaling). RNAseq was performed using the NextSeq500 as a platform and TruSeq as library prep kit. The preprocessed reads were aligned to the reference genome of Homo-sapiens (Ensembl.GRCh38.88(GRCh38)). Statistical comparative analysis for differential expressed genes (DEG) was done using the edge package in R version 3.20.9. The resulting p-values were corrected for multiple testing with Benjamini-Hochberg to control for false discovery rate (FDR). DEG were selected based on FDR-value less than 0.05 and a fold change of an absolute log₂-ratio larger than 1. Pathway analysis was done using PANTHER14.0. Western Blot for di-methylated H3K79 was performed to confirm successful DOT1L inhibition.

Results: The DOT1L-inhibitor EPZ-5676 effectively reduced H3K79 di-methylation in the treated samples. RNAseq analysis revealed 663 differentially expressed genes in dermal fibroblasts exposed to the DOT1L inhibitor, based on group analysis: 77 genes were downregulated and 556 upregulated. There was no significant interaction between inhibition of DOT1L and stimulation of the cells with TGF- β or CHIR99021. Pathway analysis showed involvement of primarily the integrin signaling, the cadherin pathway and Wnt signaling.

Conclusion: More than 600 differentially expressed genes were discovered by RNA sequencing in human dermal fibroblasts exposed to DOT1L inhibition (independent of TGF- β or CHIR 99021 stimulation). These gene lists and their networks indicate that DOT1L activity affects integrin signaling, the cadherin and Wnt pathway, suggesting a potential impact on the fibrotic process that is leading to pathology in SSc.

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AB0201

THE PATHOGENIC EFFECTS OF IMMUNE COMPLEXES CONTAINING SCLERODERMA-SPECIFIC AUTOANTIBODIES IN ENDOTHELIAL CELLS

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Background: In systemic sclerosis (SSc), autoantibodies provide the most reliable tool to predict disease subset and the pattern of organ involvement.

Objectives: Consistently with the diagnostic and prognostic role of these autoantibodies, our group has previously demonstrated that immune complexes (ICs) containing scleroderma specific autoantibodies elicit a pro-inflammatory and pro-fibrotic signalling cascade in healthy skin fibroblasts, thus contributing to SSc aetiopathogenesis. In addition, we showed that SSc-ICs upregulate Toll-like Receptors (TLRs) and that pre-treatment with DNase and RNase prevent SSc-IC-mediated effects in fibroblasts, thus suggesting that SSc-ICs might engage TLRs via their nucleic acid components. The objective of the present study is to investigate the pathogenicity of SSc-ICs in endothelial cells (ECs).

Methods: Human umbilical vein ECs (HUVECs) have been isolated from umbilical vein and cultured in adequate conditions. ICs have been purified from sera of SSc patients bearing different autoantibody specificities (anti-bodies against centromeric proteins, DNA topoisomerase I, RNA polymerase and Th/To), patients with systemic lupus erythematosus (SLE), primary anti-phospholipid syndrome (PAPS) or healthy controls using poly-ethylen glycol precipitation. Cell cultures have been incubated with pathologic and control ICs and TLR3 [Poly(I:C)] and TLR4 (LPS) agonists. Several parameters have been assessed: mRNA levels of endothelin-1 (ET-1) and type I interferons (IFN- α and IFN- β) have been investigated by real-Time PCR; ICAM-1 expression has been evaluated by cell-ELISA and the secretion of IL-6, IL-8, tumour growth factor (TGF)- β 1 and Pro-Collagen α 1 in culture supernatants has been measured by commercial ELISA kits. The involvement of intracellular signalling pathways culminating with the activation of p38MAPK has been assessed. Furthermore, skin fibroblasts from healthy controls were stimulated with supernatants from HUVEC incubated with scleroderma and control ICs. The mRNA levels of collagen (col) α 1 and matrix metalloproteinases (MMP)-1 and the secretion of TGF- β 1 were evaluated in fibroblasts. Statistical analysis was performed comparing ICs from SSc and disease controls to NHS-ICs.

Results: Stimulation of HUVEC with scleroderma and control ICs resulted in the modulation of study mediators, as detailed in Table 1. When skin fibroblasts from healthy controls were stimulated with supernatants from HUVEC incubated with scleroderma and control ICs, we observed a significant modulation of mediators (Table 2).

Conclusion: These data provide the first demonstration of the pathogenicity of ICs isolated from scleroderma patients with different autoantibody specificities in ECs. According to our preliminary data, the activation of ECs induced by SSc-ICs might ultimately lead to a pro-fibrotic phenotype in healthy skin fibroblasts. SSc-ICs might thus be at the crossroad of scleroderma pathogenesis, contributing to the recruitment of the many cells involved in disease onset.

Table 1. Modulation of study mediators in HUVEC.

	ICAM-1	IL-6	IL-8	TGF- β 1	Coll α 1	ET-1	IFN- α	IFN- β	P38MAPK
Poly(I:C)	***	***	***	NA		NA			***
LPS	***	***	***	NA		NA			***
ATA-IC		*	***	*		**			***
ACA-IC	**	*	***	*					***
ARA-IC		**	***	*					***
anti-Th/To-IC	***	**		*		**			
PAPS-IC	**			NA		***			
SLE-IC			**	NA					**
NHS-IC									

*= p<0.05; **= p<0.001, ***=p<0.0001

Table 2. Modulation of study mediators in fibroblasts.

	TGF- β 1	Coll α 1	MMP-1
TGF- β	NA	***	
ATA-IC	*	**	
ACA-IC	***	**	*
ARA-IC			*
anti-Th/To-IC	***	***	*
NHS-IC			

*= p<0.05; **= p<0.001; ***=p<0.0001