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

Nathalie Berghen^{1,2}, Ellen De Langhe^{1,2}, Rik Lories^{1,2}, ¹KU Leuven, Department of Development and Regeneration, Leuven, Belgium; ²UZ Leuven, Division of Rheumatology, Leuven, Belgium

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.

Acknowledgement: RNA sequencing and analysis was performed by VIB Nucleomics Core (www.nucleomics.be).

Disclosure of Interests: Nathalie Berghen: None declared, Ellen De Langhe: None declared, Rik Lories Consultant for: Abbvie, Celgene, Eli-Lilly, Janssen, Merck, Novartis, Pfizer, UCB

DOI: 10.1136/annrheumdis-2019-eular.1023

AB0201

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

Cecilia Chighizola¹, Daniela Privitera^{1,2}, Maria Orietta Borghi^{1,2}, Pier Luigi Meroni¹, Elena Raschi¹, ¹Istituto Auxologico Italiano, Milan, Italy; ²University of Milan, Department of Clinical Sciences and Community Health, Milan, Italy

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

REFERENCE:

[1] Raschi E, Chighizola CB, Cesana L, et al. Arthritis Res Ther 2018;20:187

Disclosure of Interests: Cecilia Chighizola Speakers bureau: Inova Diagnostics, Daniela Privitera: None declared, Maria Orietta Borghi: None declared, Pier Luigi Meroni Consultant for: Inova, Thermofisher, Teofarma, Elena Raschi: None declared

DOI: 10.1136/annrheumdis-2019-eular.5575

AB0202 DYSPHAGIA: POOR PROGNOSIS IN IDIOPATHIC INFLAMMATORY MYOPATHIES

Ana Carolina Costi, Claudia Pena, Adriana Testi, Pierina Sansinanea, Mercedes García. Hospital Interzonal General de Agudos General José de San Martín, Buenos Aires, La Plata, Argentina

Background: Dysphagia has been reported to occur in 10% to 73% of these patients and can be present at any time during the disease process (1).

Objectives: The primary objective of the study was to evaluate the prevalence of dysphagia in a cohort of patients with idiopathic inflammatory myopathy (IM) and to evaluate factors associated with the presence of dysphagia. The secondary objective was to evaluate the factors associated with severe dysphagia.

Methods: Retrospective, observational study, which included patients with a diagnosis of IM according to modified classification criteria of Bohan and Peter (1992-2018). Demographic data, clinical characteristics, laboratory data, autoantibodies, imaging studies, videodeglutition, muscle biopsy and EMG were recorded.

Severe dysphagia was considered: one in which oral feeding was contraindicated and/or which required nasogastric tube feeding (SNG) either by clinical evaluation or by videodeglutition study. The rest of the patients with dysphagia who did not present a contraindication to oral intake during the course of the disease were considered mild/moderate dysphagia.

Results: 94/110 patients were included, 76% female, mean age at diagnosis: 48 years (SD $\pm$ 14). Idiopathic dermatomyositis was the most frequent subtype of myopathy (64%). Dysphagia occurred in 53/94 patients (56.4%) and it was presented at the beginning of the disease in 31/94 (32%). Severe dysphagia was found in (22/94) 23%.

When analyzing the clinical features of patients with myopathy and dysphagia, it was found that Idiopathic dermatomyositis was the most frequent IM in these patients (71%). Patients with dysphagia presented: proximal muscle weakness 90%, neck muscles weakness 47%, and respiratory muscle weakness 27%.

Treatment received: 90/94 (97%) oral glucocorticoids, mean dose 48 mg of prednisone (Range 4 -100 mg.), pulses of Intravenous methylprednisolone was indicated in 25 patients (27.5%). The main steroid sparing agents used were: 72% methotrexate, followed by 33% azathioprine.

Significant association was found between dysphagia and weakness of neck muscles, respiratory muscles, of glucocorticoid pulses, gamma globulin and mortality (data not shown). In the Logistic Regression analysis, no variable was independently associated with the presence of dysphagia.

When analyzing the relationship of severe dysphagia and factors associated, a significant association was found with the requirement of mechanical ventilation, hospitalization in an intensive care unit, serious infections, neoplasia and mortality (Table 1). In the multivariate analysis: no associated factors were found independently.

Table 1.

Variable	severe dysphagia (present) n=22 (%)	severe dysphagia (absent) n=72 (%)	p	OR	IC
Weak neck muscles	15 (68%)	18 (25%)	<0.001	8.86	2.75-8.86
Weakness of respiratory muscles	10 (45%)	6 (8%)	<0.001	9.5	2.8-32
pulses of corticosteroids	17 (77%)	8 (11%)	<0.001	25	7.5-89.5
intravenous gammaglobulin	10 (45%)	7 (9%)	<0.001	7.73	2.46-24
intensive care unit	8 (36%)	8 (11%)	<0.001	4.57	1.46-14.26
mechanical ventilation	6 (27%)	5 (7%)	<0.001	5.02	1.36-18.55
death	16 (72%)	10 (14%)	<0.001	16.53	5.89-90

neoplasms	6 (27%)	9 (12%)	<0.001	2.62	0.65-9.2
serious infections	11 (50%)	10 (14%)	<0.002	4.95	1.71-14

Conclusion: in patients with IM 56% course with dysphagia at some point in the evolution. Of which 23% of cases were characterized as severe dysphagia. Both dysphagia in general and severe dysphagia were associated with parameters of severity, high cost and poor prognosis. However, in the analysis of multiple variables, this relationship could not be demonstrated.

REFERENCE:

[1] Mayo Clin Proc. • April 2007;82(4):441-447

Disclosure of Interests: ANA CAROLINA COSTI: None declared, Claudia Pena: None declared, Adriana Testi: None declared, Pierina Sansinanea: None declared, Mercedes García Grant/research support from: GSK, Speakers bureau: GSK

DOI: 10.1136/annrheumdis-2019-eular.6093

AB0203 INTRAPATELET ANGIOGENIC AND GROWTH FACTORS IN SYSTEMIC SCLEROSIS

Bryan-Josué Flores Robles¹, María-Alejandra Sánchez², José Luis Bueno Cabrera², Lucía Nuñez Martín-Buitrago², Cristina Gómez Casado³, Juan Mulero Mendoza², Hilda Godoy², Valvanera Pinillos¹, Ezitzen Labrador-Sánchez¹, Juan Antonio López-Martín¹, Angel Elena-Ibáñez¹, Leticia Merino-Meléndez¹, José Luis Andréu Sánchez². ¹Hospital San Pedro, Logroño, La Rioja, Spain; ²Puerta de Hierro Hospital, Madrid, Spain; ³Institute of Applied Molecular Medicine (IMMA) San Pablo-CEU University Madrid, Molecular Medicine, Madrid, Spain

Background: Systemic sclerosis (SE) is a serious disease of the connective tissue characterized by dysfunction of the microcirculation. Angiogenesis is a complex process regulated by both angiogenic and angiostatic factors. Normally the functions of these factors are under an adequate balance, however, under certain conditions these factors can be induced initiating disorganized angiogenic phenomena.

Objectives: For the first time, the levels of intraplatelet growth and angiogenic factors (normalized by platelet number and volumen) are described in patients with systemic sclerosis.

Methods: We included 23 patients with systemic sclerosis (ACR/EULAR) and 16 controls. Patients underwent a platelet-apheresis to obtain platelet-rich plasma (PRP) and platelet-poor plasma (PPP). The samples were subjected to a freeze-thaw process to break the cell membranes and release the platelet content. Subsequently the various factors were measured (VEGF- α , PDGF $\beta\beta$, HGF, FGF2, G-CSF, MCP-1, IL-1 α , IL-1 β , IL-8, IL-6, IL-13). Finally, the results were normalized according to the value of the plaquetocrit (number and volumen of platelets). We compared the levels in platelet-rich plasma and in platelet-poor plasma. A cut-off value p <0.05.

Results: The mean age of the patients was 52 years $\pm$ 9.71, with a duration of the disease of 8 years, 74% were patients with limited SE, 70% of the patients had pulmonary involvement. Both patients and controls, the TFG- β was 12 times more concentrated in PRP than in PPP (p <0.0001), IL-1 β 13 times more (p <0.0001), IL-6 10 times more (p <0.0001), G-CSF 11 times more (p <0.0001), VEGF- α more concentrated (11.6 times) p <0.005. When the PRP of the patients with scleroderma was compared with the PRP of the controls, only differences were found in the VEGF concentrations, being decreased in the patients with scleroderma respect to controls (p <0.0001). Table 1 and 2.

Conclusion: The levels of VEGF- α (intraplatelet) are lower in patients with SE vs controls (p <0.0001). The intraplatelet values of the growth and angiogenic factors are higher than the plasma (patients and controls), finally, the plasma levels of these factors are similar in patients compared with controls. In almost all studies until today the measured levels have been carried out in serum, which are not precise since, in serum, several cells and lysate residues can alter the values, therefore, quantification in plasma is important. The present work is the result of the doctoral thesis of the main author.

Table 1. Growth factors values (23 patients with SE and 16 controls)

Factor	PPP (pg/mL)	PRP (pg/mL)	p
TFG- β 1	21149,7	237185,2	<0.0001
IL-1 β	7,7	117	<0.0001