setting, our results confirm that defSSc might represent an intermediate entity between pre-clinical stages and the most severe subsets of disease, thereby opening new perspectives on SSc pathophysiology and disease interception.

References:
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AB0178 PHENOTYPING OF NATURAL KILLER (NK) RECEPTORS ON NK AND NKT-LIKE CELLS DISCLOSES DEFECTIVE IMMUNE-REGULATORY CAPABILITY IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by dysregulation of the immune system, vasculopathy and fibrosis of the skin and internal organs. Natural Killer ($CD56^+CD3^-$, NK) and NKT-like ($CD56^+CD3^+$) cells display receptors (NKR) whose expression pattern determines their cytotoxic and immune-regulatory activity. The role of NK and NKT-like cells in the dysregulation of the immune system in SSc has not been fully elucidated yet.
Objectives: To improve our knowledge on the contribution of NK, NKT-like and a subset of NKT cells expressing invariant TCR (iNKT) in SSc development, we performed a broad phenotyping of NKR in the circulation of SSc patients, including subjects with pre-clinical SSc.
Methods: NKR were assessed by flow cytometry using two 13-color panels on whole blood of 84 SSc patients and 20 healthy controls (HC). In particular, 15 patients with early SSc (EaSSc) without signs or symptoms of evolutive disease (2001 LeRoy and Medsger criteria[1]), 24 patients with definite SSc without skin or lung fibrosis (defSSc), 26 patients with limited (lcSSc) and 19 patients with diffuse cutaneous SSc (dcSSc) (2013 ACR/EULAR classification criteria for SSc[2]) were included. NK degranulation in response to K562 target cells was assessed in lcSSc and dcSSc patients versus HC.
Results: The number of circulating lymphocytes, NKT-like and iNKT cells - but neither $CD3^+$ T nor NK cells - was reduced in dcSSc versus HC. $NKp46^+$ NK cells co-expressing NKG2D and CD16 were decreased in dcSSc versus HC and EaSSc. Consistently with these observations, dcSSc exhibited lower degranulation capability. $(CD57^+)KIR^+$ and activating NKR-expressing NKT-like cells were diminished in both dcSSc and lcSSc versus HC.
Conclusions: dcSSc patients showed a defective NK cytotoxicity potential, possibly due to the decreased $NKp46^+$ fraction. The regulatory, cytolytic KIR^+ NKT-like fraction was also reduced with a parallel decrease of activating receptors expression in both lcSSc and dcSSc. Overall these results point towards an impairment of NK and NKT-like cells as immune check-points in fibrotic SSc.

References:
[1] LeRoy EC, et al. J Rheumatol 2001.
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Acknowledgements: Supported by a grant from Gruppo Italiano per la Lotta alla Sclerodermia (GILS). MC and TR are partly supported by the VIDI laureate and Dutch Arthritis Foundation (NWO, Netherlands Institute for Science) and ERC starting grant (EU) obtained by TR. The authors would like to thank Koos Gaiser (U-DAIR, LTI, UMC Utrecht) for the technical assistance in developing the 13-color flow cytometry panels; Dr F. Montero, L. Nieto-Gligorovski and E. Gautherot (Beckman Coulter Inc, Marseille) for providing the NKG2A-PB antibody; the NIH Tetramer Facility for providing the CD1d tetramers.
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AB0179 DEGRADATION OF TYPE VII COLLAGEN (C7M) IS ASSOCIATED WITH SYSTEMIC SCLEROSIS – DEVELOPMENT OF A NOVEL NEO-EPITOPE SPECIFIC ASSAY

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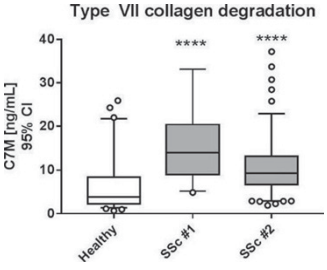
Background: Type VII collagen (col7) is the main component of the anchoring fibrils that connects the basement membrane to the underlying interstitial matrix and has mainly been investigated for its role in blistering skin diseases. It has been investigated for its role in dystrophic epidermolysis bullosa, a severe skin disease. Furthermore, increased levels of type VII collagen in skin has been reported for patients with systemic sclerosis (SSc).
Objectives: The objective was to develop and characterize a blood-based marker assessing col7 degradation in patients with SSc.
Methods: We identified a specific fragment of col7 in serum from COPD patients, which was not found in controls, using mass spectrometry. A monoclonal antibody was raised against the first ten amino acids of the neo-epitope (KLH-CGG-GPPGPPGRLV) and employed in a competitive ELISA (C7M). The C7M assay was validated technically and was subsequently evaluated in 2 cohorts including SSc patients. The first cohort (SSc#1; n=35) consisted of early (<2 years of SSc symptoms; n=16) and late (>10 years of disease with stable skin for at least 6 months, n=19) diffuse SSc patients, while the second cohort (SSc#2; n=119) consisted of limited (n=78) and diffuse (n=41) SSc patients. Serum C7M levels were likewise measured in healthy subjects and compared to the levels of SSc patients using the Kruskal-Wallis test with Dunn's multiple comparisons test comparing healthy individuals with the two SSc cohorts.
Results: A technically robust competitive ELISA (C7M), which was highly specific for a col7 fragment was developed. The assay showed acceptable inter- (13%) and intra-assay (9%) variation, linearity (102% dilution recovery), analyte stability (102% recovery after 4 freeze/thaw cycles), and interference.
The C7M marker was evaluated by comparing serum levels in healthy donors with patients with SSc (Figure). Serum C7M levels were not associated with age, gender, BMI, or disease duration. The geometric mean serum C7M level in healthy donors was 4.6 ng/mL (95% CI 3.7–5.6 ng/mL). The geometric mean serum C7M levels were significantly elevated in both cohorts of patients with SSc (SSc#1, 13.6 ng/mL [95% CI 11.1–16.5], $p<0.0001$; SSc#2, 9.2 ng/mL [95% CI 8.3–10.2], $p<0.0001$). Furthermore, a significant difference were observed between the two cohorts (P=0.05).



Conclusions: The C7M ELISA enabled quantification of type VII collagen degradation in serum. Elevated serum C7M levels indicated that the remodeling of type VII collagen was significantly increased in patients with SSc, suggesting a pathological role.

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AB0180 BIOMARKERS OF EXTRACELLULAR MATRIX REMODELING ARE ASSOCIATED WITH ACUTE EXACERBATIONS OF IDIOPATHIC INTERSTITIAL PNEUMONIA

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Background: Idiopathic interstitial pneumonia (IIP) is characterized by an increased rate of extracellular matrix (ECM) turnover resulting in fibrosis. The pathogenic remodeling includes increased levels of protein synthesis and degradation mediated by proteases such as matrix metalloproteinases (MMPs). Acute exacerbations of IIP (AE-IIP) represent periods of increased disease activity. Pulmonary involvement, especially pulmonary fibrosis, is common in patients suffering from systemic sclerosis (SSc) and ankylosing spondylitis (AS)
Objectives: The objective was to investigate if ECM remodeling was altered during AE-IIP by serological neo-epitope biomarkers.
Methods: Serum samples were collected from patients with IIP at clinically stable disease (S-IIP, n=29) and at AE-IIP (n=68). Of these, 11 and 28 patients, respectively, had idiopathic pulmonary fibrosis (IPF). 28 IIP patients had paired samples. Biomarkers released from MMP-mediated degradation of collagen type I (C1M), III (C3M), IV (C4M), and VI (C6M), elastin (ELM7), versican (VCANM), biglycan (BGM), and C-reactive protein (CRPM) were assessed in serum by competitive ELISAs utilizing neo-epitope specific monoclonal antibodies. Data were analysed using Mann-Whitney test, Wilcoxon test, Spearman's rank correlation, and Kaplan-Meier curves as appropriate.
Results: Mean age of patients was 71 (range 54–86) at AE-IIP and 69 (range 55–83) at S-IIP. Mean forced vital capacity in percentage of predicted value (%FVC) was 55.6% (SD 19.5) at AE-IIP and 79.0% (SD 26.5) at S-IIP. Serum