AB0158

IMPACT OF JAK INHIBITORS ON MACROPHAGE POLARISATION: PERSPECTIVES FOR SYSTEMIC SCLEROSIS

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Background: Macrophage can adopt various phenotypes and activation states according to their surrounding microenvironment. M1 or inflammatory macrophages are generated under IFN γ /LPS signaling and express the membrane marker CD86. Different subtypes of M2 macrophages are also described: M2a macrophages (generated under IL4/IL13 signaling) and characterized by a high expression of CD206 and pro-fibrotic properties and, M2c macrophages (generated under IL10 and/or glucocorticoid signaling), considered as anti-inflammatory resolving macrophages. There is growing interest in the role of macrophages in the pathogenesis of Systemic Sclerosis (SSc). Recent studies highlight that macrophages from fibrotic tissues such as lung or skin from SSc patients have a M2 phenotype whereas, in blood-monocytes derived macrophages (MDM), SSc MDM have a mixed signature associating M1 and M2 characteristics. Jak inhibitors are treatments used in rheumatoid arthritis and that can variously target signals that could be involved both in M1 and in M2 polarisation.

Objectives: This study evaluates the impact of three Jak inhibitors on the polarisation state of human MDM in vitro.

Methods: Blood monocytes from healthy donors (HD) were differentiated with M-CSF (for 7 days) in MDM and pre-treated by ruxolitinib (Jak2-Jak1 inhibitor), tofacitinib (Jak3 inhibitor) or itacitinib (Jak1 inhibitor) (1 μ M for all). They were then polarised into M1i (IFN γ , 20 μ g/mL), M1Li (IFN γ +LPS, 20 μ g/mL), M2a (IL4+IL13; 20 μ g/mL), M2c (IL10, 20 μ g/mL) and M2c(dex) (IL10+dexamethasone, 10nM). The impact of each Jak inhibitor on phenotype (flow cytometry), gene expression (qPCR) and cytokine secretion (ELISA) was evaluated in each polarisation state.

Results: Concerning phenotypes, all Jak inhibitors reduced the expression of the M1i and M1Li marker CD86, but ruxolitinib had a higher effect. Only ruxolitinib reduced the expression of the M1i marker MHCII. All Jak inhibitors reduced the expression of CD206 in M2a. They had no impact on the expression of CD163, CD204 in any M2 conditions. Key M1 genes were repressed by all Jak inhibitors, such as CXCL10, IL6 or TNF α with a more significant effect of ruxolitinib. All Jak inhibitors reduced the gene expression of CXCL13 and SOCS3 in M2c. Secretion levels of IL6 and CCL18 were also repressed, with a more significant effect of ruxolitinib.

Conclusion: Jak inhibitors can limit M1 and M2 polarisation state in vitro, with a more significant effect of the Jak2-Jak1 inhibitor ruxolitinib. The relevance of these results in MDM from SSc patients and in vivo models of SSc is still to be determined.

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AB0159

INTERLEUKIN-16 PLAYS A ROLE IN THE PATHOGENESIS OF SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune disorder with chronic and persistent inflammation. Interleukin-16 was originally described as a factor that could attract activated T cells in humans [1]. Elevated amounts of IL-16 have been demonstrated in SSc [2].

Objectives: This study was undertaken to find out if IL-16 is associated with clinical characteristics of SSc.

Methods: IL-16 was measured by Elisa in serum of patients with SSc (n=119) and healthy controls (n=50). Further, the presence of active IL-16 in mononuclear cells from peripheral blood of SSc patients (n=10) was examined by Western blot. Statistical analyses were done employing Graph Pad prism software (v 8). Patients with SSc were characterized based upon epidemiological and clinical parameters.

Results: The serum concentration of IL-16 was higher in patients with SSc than in healthy controls (272.7 $\pm$ 165.4 vs 172.8 $\pm$ 64.84 pg/ml, p<0.0001). Further, the difference in the IL-16 serum concentration was more prominent in females (265.6 $\pm$ 174.2 vs 160.1 $\pm$ 53.37 pg/ml, p=0.0002) than in males (287.1 $\pm$ 144.1 vs 187.6 $\pm$ 74.64 pg/ml, p=0.0034). In addition, the concentration of IL-16 was elevated in patients with diffuse SSc compared to limited SSc (p=0.0206). The concentration of IL-16 in serum of SSc patients positively correlated with CRP (n=115, r=0.49, p<0.0001). There was a weak positive correlation between IL-16 in serum of SSc patients and the mRSS (n=112, r=0.22, p=0.0175). Noteworthy, the concentration of IL-16 was heightened in SSc patients with lung fibrosis compared to SSc patients without lung fibrosis (p=0.009). The ROC value of SSc patients with lung fibrosis was 0.64 (95%CI: 0.58-0.83). Moreover, active IL-16 derived from peripheral blood mononuclear cells (PBMC) of SSc patients with lung fibrosis was present in higher amounts compared to PBMC of SSc patients without lung fibrosis (5 vs 5, p=0.0557).

Conclusion: Our results confirm and extend previous data by showing not only an increased concentration of IL-16 in the circulation of SSc patients, but new findings pointing towards a role of IL-16 for contributing to lung fibrosis in SSc.

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AB0160

CARDIAC AUTONOMIC NEUROPATHY PREVALENCE IN A COHORT OF SYSTEMIC SCLEROSIS (SSC) PATIENTS

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Background: Systemic sclerosis is a rare disease determining a damage to the connective tissue and, consequently, an involvement of several organs. Besides the damage of the connective tissue, preminent is also the small vessels injury, detectable by videocapillaroscopy. Some authors report that the vascular damage may be also responsible of a cardiovascular impairment as cardiac autonomic disease (CAN) and heart rate variability [1].

Objectives: Our study aims to assess the presence and entity of CAN in patients with systemic sclerosis (SSc).

Methods: This is a pilot prospective cohort study. We enrolled 28 patients in a period of six months, from May 2019 to November 2019, afferent to the outpatient clinic of internal medicine and immunology of the Primo Policlinico of Naples, with definite SSc diagnosis in absence of other comorbidities. All patients underwent diagnostic tests for autonomic cardiac neuropathy (NAC) and videocapillaroscopy. In particular, four test were performed to search for the presence of NAC: orthostatic hypotension, deep breathing, lying to standing and Valsalva maneuver. Each test was corrected for age and diagnosis was made in the case at least two tests resulted positive. Primary endpoint of the study was the assessment of the prevalence of autonomic cardiac neuropathy in the study population.

Results: Our cohort was mainly characterized by females (92.9%), with a median age of 58.5 years [IQR: 49-64.8 yrs.] and a median duration of the disease of 4 years [IQR 2-13 yrs.]. The observed prevalence of NAC was equal to the 46.4% (13 cases). In addition, we evaluated the potential association of NAC with age, duration of disease, gastrointestinal dysmotility, sicca syndrome, cutaneous involvement and type of videocapillaroscopy pattern, from which no statistically significant result emerged. Hence, a further analysis, by using a time-dependent Cox regression model (with the duration of disease as time covariate), was performed on the same variables. From this model a

significant association emerged in particular between the presence of NAC and the active videocapillaroscopy pattern (OR 6.23; 95% CI: 1.058-36.71, $p=0.043$).

Conclusion: Though current data in the literature on this topic are poor, cardiac autonomic neuropathy is among the clinical manifestations of SSc. In our study population, though the limited sample size, we observed a high percentage of patients with autonomic cardiac neuropathy, which seems much more frequent with the increase in the duration of disease and based on the type of videocapillaroscopy pattern.

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AB0161 CLONAL HEMATOPOIESIS IS INCREASED AND NOT RELATED TO AGING IN SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis, microangiopathy and immune dysfunctions including dysregulation of proinflammatory cytokines. Clonal hematopoiesis of indeterminate potential (CHIP) is defined by the acquisition of somatic mutations in hematopoietic stem cells leading to detectable clones in the blood. Recent data have shown a higher risk of cardiovascular disease in patients with CHIP resulting from increased production of proinflammatory cytokines and accelerated atherosclerosis. Eventual links between CHIP and autoimmune diseases are undetermined.

Objectives: The aim of our study was to evaluate the prevalence of CHIP in SSc patients and its association with clinical phenotype.

Methods: Forty-one genes frequently mutated in myeloid malignancies were sequenced in peripheral blood mononuclear cells from 90 SSc patients and from 44 healthy donors.

Results: A total of 15 somatic variants was detected in 13/90 SSc patients (14%) and 4 somatic variants in 4/44 (9%) HD ($p=0.58$). The prevalence of CHIP was significantly higher in younger SSc patients than in HD: 25% (6/24) vs 4% (1/26) ($p=0.045$) under 50 years and 17% (7/42) vs 3% (1/38) ($p=0.065$) under 60 years. The prevalence of CHIP in patients over 70 years was similar in SSc patients and healthy donors

For SSc patients the most common mutations occurred in *DNMT3A* (7 variants). Other variants involved *ATM*, *SF3B1*, *SETBP1*, *TET2*, *TP53*, *NF1* or *CBL*. The distribution of gene mutations was overall comparable in SSc patients and in previously described CHIP series (3)

In most SSc patients, we identified a single CHIP mutation. Several mutations were detected in two SSc patients: *SETBP1* and *NF1* in one and, *TET2* and *ATM* in the other. Clonal mutations included missense ($n=10$), nonsense ($n=3$), frameshift ($n=1$) and a single splice site mutation. In all HD we detected a single CHIP mutation which occurred in *DNMT3A*, *TP53* and *CSF3R*

Variant allele frequencies (VAF) of CHIP mutations ranged from 2 to 18.6% and did not differ between genes (*DNMT3A* or others). Mean age was the same in patients with *DNMT3A* mutations or with other mutations. However, C>T transversions, that have been associated with ageing were more frequent in *DNMT3A* variants than in other genes, suggesting distinct mechanisms for mutation acquisition or clonal selection. No major differences in clinical and laboratory data were observed between SSc patients with or without CHIP. SSc subtypes, disease duration, different organ involvements and the prevalence of ischemic events were not associated with the presence of CHIP, except less frequent pyrosis in patients with CHIP than those without. SSc patients with CHIP had significantly more anti-RNA polymerase III antibodies than those without CHIP ($p=0.045$). At the time of analysis, 45 SSc patients had received a treatment for SSc which consisted in low-dose steroids, hydroxychloroquine, mycophenolate mofetil, cyclophosphamide or methotrexate. SSc patients with CHIP were significantly more exposed to cyclophosphamide (3/13 vs. 3/77) ($p=0.04$) (5, 6.5 and 11 gram respectively between 5 years to 8 years before the NGS sequencing analysis), but among these cyclophosphamide-exposed SSc the age was over 65 in 2/3 of them. When considering all immunosuppressive drugs (cyclophosphamide, methotrexate and mycophenolate mofetil) SSc patients with CHIP were not more exposed than those without

CHIP ($p=0.75$). No patient developed any hematologic malignancy and no cytopenia during the median follow-up of 13 months (0-24 months). One SSc patients with CHIP developed a small lung cancer few months after NGS testing.

Conclusion: Whether CHIP increases the risk to develop SSc or is a consequence of a SSc-derived modified bone marrow micro-environment remains to be explored.

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AB0162 THE SIGNIFICANCE OF M1 AND M2 MONOCYTES IN SYSTEMIC SCLEROSIS

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Background: Recently, the relation between M2 macrophage and fibrosis have been reported in several diseases including systemic sclerosis (SSc). Similar with macrophages, monocytes can be classified into M1 and M2 subset, and the relation of imbalance of these monocytes with disease such as rheumatoid arthritis have been reported^{1,2}.

Objectives: In this study, we attempted to investigate relationship among M1 or M2 monocytes in SSc.

Methods: This study included 23 SSc patients and 20 healthy donors. Using fluorescence-activated cell sorting, we defined CD14, CD68 and CCR2 positive cells as M1 monocytes and CD14, CX3CR1 and CD163 positive cells as M2 monocytes. We examined the ability of cytokines/chemokines secretion of CD14 positive cells from SSc by multiplex bead array assay using MAP human cytokine/chemokine Magnetic Bead Panel which can measure 38 cytokines/chemokines. We next extracted M2 monocytes from CD14-positive cells using FACS, and we used the rest of the CD14 positive cells as M1-dominant monocytes. Then, we evaluated their ability of TGF- β production by multiplex bead array assay.

Results: SSc patients had higher M2/M1 ratio as compared with healthy control (7.00 vs 1.63, $P<0.05$). And, there was tendency that M2/M1 ratio was higher in SSc patients complicated with interstitial pneumonia. Beads array analysis revealed that CCL4 and MCP-1 production from CD14 positive cells which consists M2>M1 (M2/M1 ratio>1) were higher than that from CD14 positive cells which consists M2<M1. Furthermore, the ability of TGF- β secretion of M2 monocytes was higher than that of M1-dominant monocytes.

Conclusion: Our present study suggested that the imbalance of M1/M2 monocytes might contribute to pathogenesis of SSc.

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AB0163 ANTI-KU ANTIBODIES: MUCH MORE THAN SCLEROMYOSITIS

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Background: Initially, anti-Ku antibodies (Ab) were described in patients with overlap syndrome with systemic sclerosis (SSc) and inflammatory myopathy (scleromyositis), although later they have been linked to a wide variety of systemic autoimmune diseases (SAD) questioning its diagnostic value. Recently, the possible existence of 2 different clinical phenotypes associated with these Ab has been described: one with myositis and high risk of interstitial lung disease (ILD) and another with positive anti-dsDNA Ab and glomerulonephritis.

Objectives: To analyze the clinical relevance and the main diagnosis of a serie of patients with positive anti-Ku Ab.

Methods: Descriptive observational study of patients with anti-Ku Ab in two third level hospitals between 2011 and 2019. Their determination was made at the criteria of the requesting physician.

Results: Twenty-three patients (20 women) with a median age of 59 ± 14 years (range, 24-83) and a follow up time (median) of 37 months (1-208) were identified. The main clinical and analytical characteristics, as well as the final clinical