Rheumatoid arthritis – etiology, pathogenesis and animal models

AB0108 RELATIONSHIP BETWEEN PROGRANULIN AND MUSCULOSKELETAL ULTRASOUND IN RHEUMATOID ARTHRITIS
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Background: Progranulin (PGRN) is a pluripotent, secretory growth factor, mainly present in cells of the epithelium, central nervous system and immune system. PGRN known to directly regulate regulatory T cells and recently found to be upregulated in Rheumatoid Arthritis (RA) and related to its activity. No attempts was made to explore the relation between PGRN and RA activity measured by clinical and Ultrasound score (US).

Objectives: To explore PGRN levels in the serum of patients with RA, and its correlation with disease activity assessed clinically combined by ultrasonography (US).

Methods: RA patients: 52 RA patients were included in this study. They were consecutively recruited from the outpatient clinic of Rheumatology Department for regular follow-up at Fayoum University Hospitals. Patients were classified according to the 2010 ACR/EULAR criteria. Control group: 20 age and sex matched healthy volunteers were recruited as controls for level of serum PGRN.

Informed consent was obtained from all patients and controls for inclusion in the study. All patients were subjected to full history taking, and clinical examination. Assessment of disease activity by modified DAS-28 score. Progranulin is measured by an ELISA Kit (Elabscience), this kit uses sandwich-ELISA as the method. The ultrasound examination was performed with GE LOGIQ 7 PRO equipment using a linear transducer with 18 MHz frequency. The patients were examined according to the German US7 score in 7 joint areas.

Results: Demographic and clinical data of the RA group: Females represents 90.4% (47 of 52), Age ranged from 21–72 years, mean age was 42.8±10.4 years. Disease duration ranged from 1 to 30 years, median 4 years. Their mean ESR levels was 58±14 (Range: 5–70). The median of Ultrasound score assessment was 8 while the mean was 8.7±3.6 (Range: 0–33).

Correlation analysis: The correlations between PRGN levels and other continuous variables were evaluated using Spearman’s rank correlation. There was high statistically significant correlation between PRGN level and USK score (r=0.57, p<0.001). Also, there was high statistically significant correlation between PRGN level and ESR (r=0.67, p<0.001) and between PRGN level and DAS 28 (r=0.74, p<0.001). There was high statistically significant correlation between USS score and ESR level (r=0.54, p<0.001) and between USS score and DAS 28 (r=0.68, p<0.001).

Conclusions: The serum Progranulin levels were higher in the serum of RA patients than age and sex matched controls. It is positively correlated with diseases activity measured by DAS 28, ESR and Ultrasound activity measured by German US7 score. Serum PGRN levels may be a useful biomarker in RA disease. Ultrasound positively correlated with ESR and DAS 28 in our cohort of RA patients.

REFERENCE:

Disclosure of Interest: None declared

AB0109 CHARACTERISATION AND COMPARISON OF SERUM BIOMARKERS IN PATIENTS WITH RHEUMATOID ARTHRITIS VERSUS OSTEOARTHRITIS USING MASS SPECTROMETRY
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Background: There is increasing interest in biomarkers in medicine, especially for their use as a diagnostic tool and in disease outcome prognosis. Rheumatoid arthritis (RA) is a chronic autoimmune disease with important debilitating outcome pathologies as well as being able to determine their potential interactors in their cellular pathway. Objectives: To characterise biomarkers using mass spectrometry, from sera of patients with RA and make a comparison between RA, osteoarthritis (OA) and healthy controls.

Methods: Blood was collected from 26 subjects, 12 patients with RA, 12 patients with OA, and 2 HC, centrifuged and sera was collected in dedicated tubes. Sera were separated by SDS-PAGE electrophoresis and stained with Coomassie staining. The samples were subjected to in gel digestion followed by LC/MS analysis. The obtained spectra were searched with Proteome Discoverer v1.4 using SEQUEST algorithm.

Results: A total of 985 proteins were identified, from which 323 were specific to the RA study group and 159 were characteristic to the OA group. Prevalent peptides found in the RA group were EGF-containing fibulin-like extracellular matrix protein 2, isofrom DeltaLf of lactotransferrin, BPI fold-containing family B member 1 and isofrom 2 of BPI fold-containing family A member 1. In the OA study group there was a prevalence in homeobox protein TGF1, major facilitator superfamily domain-containing protein 9, methyltransferase-like protein 23, a fragment of the salivary acidic proline-rich phosphoprotein, serine/threonine-protein phosphatase 4 regulatory subunit 1, zinc finger protein 229.

Conclusions: Our results show a prevalence of peptides linked to cell migration, proliferation and activation in the RA sera as compared to peptides related to cell turnover and tissue senescence observed in OA sera. We believe further mass spectrometry studies of biomarkers in larger cohorts to be a promising tool for diagnosis and outcome prognosis in RA.

Disclosure of Interest: None declared
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AB0110 SERUM AND SALIVARY IGA1 AND IGA2 ACMA SUBCLASSES IN ESTABLISHED RHEUMATOID ARTHRITIS AND ASSOCIATIONS TO SMOKING
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Background: A prevailing hypothesis regarding the pathogenesis of rheumatoid arthritis (RA) involves an association between mucosal immunity and the development of RA. A pathogenic role for antibodies against citrullinated peptides (ACPAs) is assumed, and there are indications of mucosal immunisation in the lungs, the oral mucosa and the intestinal mucosa preceding overt RA.1 Iga antibodies are produced both locally, at mucosal membranes, and systemically. Among the two Iga subtypes, IgA1 dominates in serum IgA2 in mucosal secretions. We have previously reported the presence of ACPA of IgA isotype in both serum and saliva of RA patients, and that Iga ACPA is associated with cigarette smoking.2

Objectives: To investigate the presence and levels of mucosal and circulating IgA1 and IgA2 isotypes of ACPA in patients with RA, and to investigate their association to cigarette smoking habits.

Methods: Patients with established RA, mean disease duration of 12.2 years (n=196), and healthy controls (n=101), included in the Secretory Antibodies in Rheumatoid Arthritis (SARA) study were analysed by enzyme immunoassays regarding total IgA ACPA and the subclasses IgA1 and IgA2 ACPA in serum and saliva. The results are presented as delta-values of optical density (OD) between each IgA ACPA subclass and the corresponding arginine peptide.

Results: Serum IgA1 ACPA was detected in 44% of the RA patients and serum IgA2 in 39%. 10% of the RA patients had detectable salivary IgA1 and/or IgA2 ACPA. Both serum and salivary IgA2 levels were higher among smokers than never-smokers, while this association was not seen for IgA1 class antibodies.

Abstract AB0110 – Table 1. Levels of serum and salivary IgA anti-CCP subclasses among RA patients, comparing ever smokers and never smokers.

<table>
<thead>
<tr>
<th>Antibody level</th>
<th>Smokers (n=85)</th>
<th>Never smokers (n=93)</th>
<th>T-test (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgA aCCP, AU/mL (SD)</td>
<td>100 (211)</td>
<td>39 (59)</td>
<td>0.013</td>
</tr>
<tr>
<td>Serum IgA1 aCCP, AU/mL (SD)</td>
<td>101 (328)</td>
<td>33 (43)</td>
<td>0.061</td>
</tr>
<tr>
<td>Serum IgA2 aCCP, AU/mL (SD)</td>
<td>333 (745)</td>
<td>140 (156)</td>
<td>0.022</td>
</tr>
<tr>
<td>Salivary IgA aCCP*</td>
<td>0.31 (0.62)</td>
<td>0.21 (0.65)</td>
<td>0.271</td>
</tr>
<tr>
<td>Salivary IgA1 aCCP**</td>
<td>0.29 (0.55)</td>
<td>0.21 (0.46)</td>
<td>0.283</td>
</tr>
<tr>
<td>Salivary IgA2 aCCP*</td>
<td>0.24 (0.38)</td>
<td>0.14 (0.17)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

aCCP=Antibodies to Cyclic Citrullinated Peptides, SD=Standard Deviation

*Delta value between Optical Density (OD) value for CCP (Cyclic Citrullinated Peptide) and OD for CAP (Cyclic Arginine Peptide), the corresponding arginine peptide.

Conclusions: In this study of patients with established RA, IgA2 ACPA but not IgA1 ACPA was associated to cigarette smoking. As IgA2 predominates over IgA1 in mucosal secretions, this finding strengthens the hypothesis that smoking via mucosal ACPA production is one pathway to develop RA.

REFERENCES:


Disclosure of Interest: None declared


AB0111 MEMBRANE TNF EXPRESSION ON MONOCYTES AND DIFFERENTIATION OF MONOCYTES INTO M2-M1 MACROPHAGES: 2 NEW BIOMARKERS OF RHEUMATOID ARTHRITIS

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Background: Three monocyte subsets have been described based on their CD14 and CD16 expression profiles, the subpopulation CD14+CD16- being expanded in rheumatoid arthritis (RA) patients. Macrophages contribute in situ to the RA pathogenesis. They can display various states of activation or polarization. Two distinct states of polarisation for macrophages have been recognised: the "classically activated macrophage phenotype" (M1) and the "alternatively activated macrophage phenotype" (M2). To sum-up, M1 are considered to be pro-inflammatory and M2 to be regulatory and anti-inflammatory.

Objectives: Here, we have assessed monocytes subsets and their capacity of differentiation into M2 or M1 macrophages in RA patients and controls.

Methods: PBMCs were isolated and we first determined monocytes subpopulations and looked at expression of membrane TNF (mTNF). After negative selection of monocytes, macrophages were Derived from Monocytes (MDM) by 7 days of culture in the presence of M-CSF (M2 differentiation) or GM-CSF (M1 differentiation). Expression of total macrophages markers (CD11b and CD71) and the M2 macrophage polarisation markers (CD163 and CD206) were evaluated.

Results: We have confirmed that the CD14+CD16- monocytes subset was expanded in RA patients. For the first time, we have demonstrated that mTNF expression was significantly increased only in monocytes in RA patients (CD14+). Moreover, mTNF expression on monocytes correlated with the activity of the disease assessed by DAS28 CRP (Spearman r=0.548 and p=0.0021). We have observed a significant decrease of macrophages induction by M-CSF in RA patients as shown by a decreased expression of CD11b-CD71 (Mean 87.2% HD versus 57.1% RA, p value ****). Among MDM, we have found a specific decreased level of M2 markers (CD206 Mean 78.1% HD versus 27% RA and CD163 56.2% HD versus 37.2% RA) suggesting an impaired maturation to M2 stage in RA patients.

Conclusions: Monocytes from RA patients have an increased expression of mTNF linked to activity of the diseases. RA patients have an impaired maturation of monocytes to M2 macrophages. This might suggest that RA patients have a propensity for preferential maturation towards a pro-inflammatory M1 phenotype thus contributing to synovial inflammation. Deeper characterisation of M1/M2 and effect of the different types of anti-TNF on this differentiation process are on-going and will be presented.

Disclosure of Interest: None declared


AB0112 PATHOGENETIC MECHANISMS IN EARLY RHEUMATOID ARTHRITIS: POSSIBLE CORRELATION BETWEEN TH17 AND T REG CELLS AND GUT MICROBIOTA STRUCTURE: A PILOT STUDY

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Background: In Rheumatoid Arthritis (RA) pathogenesis T helper17 (Th17) and T regulatory cells (Treg) are largely represented.1 Recent studies highlighted the role of intestinal mucosa environment in modulation of T cells function. The composition of gut microbiota influences the Th17/Treg cells balance and the host immune response,2 so the exposure to deranged intestinal microbiota may be crucial in RA.

Objectives: The aim of the study was to compare Th17 and Treg cells and gut microbiota composition in patients with early RA (ERA) and in a control group (CG) at baseline and after treatment.

Methods: Currently, 10 ERA patients and 10 subjects belonging to the CG have been enrolled. All ERA patients were evaluated before (T0) and after 3 months (T1) of treatment with methotrexate (MTX) and glucocorticosteroids (GCS). Blood and faecal samples were collected. After PBMC isolation, staining with conjugated mAbs targeting specific surface and intracellular antigens (CD4 and CD25, IL-17 and FoxP3) were used to distinguish Th17 and Treg cells. The composition of the faecal microbiota has been analysed by Next Generation Sequences on Illumina MiSeq platform, through 16S rDNA V3-V4 targeted sequencing.

Results: At T0, the percentage of Th17 cells was higher in patients than in the CG (p=0.0001) while Treg cells were higher in the CG (p=0.013). At T1, the total number of CD4+ and Th17 cells was decreased (p=0.007, p=0.027) while the frequency of Treg cells increased (p=0.028). A normalisation of Treg cells, with frequencies comparable to CG, was present after treatment. Regarding gut microbiota, at phylum level no difference between patients at T0 and the CG were present but we observed a tendency to decrease in the frequency of Actinobacteria after therapy. Furthermore, the relative abundance of Actinobacteria correlated positively with the circulating levels of Th17 (p=0.012, r=0.59) and with the Th17/Treg at T0 (p=0.010, r=0.6), while Nitrospirae correlated positively with Treg (p=0.028, r=0.68) at T1. A significant increase of the relative abundance in the Lachnospiraceae family in patients at T1 compared with T0 (p=0.042) and CG (p=0.043) was noticed.

Conclusions: Our results highlight the presence of an imbalance between Th17 and Treg cells in patients with ERA. In agreement with literature,3 MTX and GCS