SAT0050

STANDARDISATION OF SYNOVIAL BIOPSIES ANALYSIS: A EULAR SYNOVITIS STUDY GROUP INITIATIVE USING A DELPHI SURVEY.

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Background: Synovial biopsies are increasingly performed in both clinical setting and translational research. An unmet need in the field is the standardisation of synovial biopsies handling and analysis procedures.

Objectives: The aim of this collaborative work was to create a consensual set of points to consider for handling and analysis of synovial biopsies in clinical practice and translational research through EULAR Synovitis Study Group (ESSG).

Methods: The items were identified and formulated based on a comprehensive literature review. A task force (TF) of EULAR Synovitis Study Group (ESSG) members were constituted and TF members were consulted through a 2-stage eDelphi process. The 2 sequential rounds occurred in 9 months. The first written round occurred in June 2016. Members were sent a written questionnaire containing items divided in 2 parts. The items were identified and formulated based on a comprehensive literature review. The first part of the questionnaire referred to clinical practice containing 5 subsections: biopsy sampling, biopsy handling, histological analysis, staining and immunohistochemistry (IHC), biopsy analysis and pathologist's report. The second part referred to translational research and contained 6 subsections (same 5 plus RNA analysis). Every participant was asked to score each item with a 5 points Likert (0: strongly disagree, 5: strongly agree), comments were allowed for each item. Items with a median score above 3.5 on 5 and a percentage of agreement above 70% were for the next round. Items with lower score were either suppressed or modified according to participants' comments. Anonymized detailed results were circulating through participants between each round. The face to face meeting occurred at ESSG meeting in June 2017.

Results: 27 ESSG members from 19 centres were contacted by email. 20 participants from 17 centres answered (response rate of 74%). Response rates for next rounds were 100%. First questionnaire contained 44 items for Part 1 Clinical practice. 52.3% (23items/44) were selected for the second round based on their score and agreement percentage. 83% (19items/23) were selected for the third round. First questionnaire contained 43 items for the second part about translational research. 44% (19 items/43) were selected for second round based on their score and agreement percentage. 95% (18 items/19) were selected for third round (figure 1). Third oral round allowed to obtain a final set of items unanimously (table 1).

Item	Clinical Practice		Translational Research	
	% Agreement	Median score	% Agreement	Median score
1. Biopsy sampling				
A minimum of 6 synovial biopsies needs to be retrieved in large joints.	95.50	5	77.80	4
A minimum of 6 synovial biopsies needs to be retrieved in small joints.	72.20	5.5	77.80	5
Biopsies shall be retrieved in different areas of the joint, if possible.	83.40	5	83.30	5
If it is clinically relevant, bacteriological, fungal and mycobacteriological assessment should be performed.	94.50	5		
Polymerase chain reaction analysis for ARA 145 should be performed if clinically relevant, especially if empiric antibiotic course has been started.	77.20	4		
If it is clinically relevant, Polymerase chain reaction analysis for Lyme and Whipple diseases should be performed.	77.80	5		
2. Biopsy processing				
The biopsies should be fixed in formalin 4%	88.90	5	72.20	4
At least 2 biopsies should be formalin fixed and paraffin embedded.	72.20	5		
3. Histological criteria				
Synovial biopsy surface should be more than 2.5mm ²	77.80	5	72.20	5
A long tear should be seen.	88.90	5	83.30	5
Morphology of the synovial tissue should be preserved.	94.50	5	94.40	5
4. Staining and immunohistochemistry (IHC)				
H&E staining should always be performed.	100	5	100	5
CD38 staining should be performed.	72.2	4	94.4	5
CD3, CD15 or CD20 staining should be performed.			94.4	5
Additional CD 31 or PNH, CD4, CD8, CD138 staining might be performed depending on the question.	72.2	3.5	83.3	4
In particular clinically relevant cases, additional staining should be performed (CD3, CD20, CD138, CD31 or PNH).	83.4	5		
If performed, IHC results can be given using a semi-quantitative score.	83.4	5		
If infectious arthritis is suspected, CD11 staining should be performed.	39.2	3		
5. Biopsy interpretation and pathologist's report				
A synovitis score should be performed, analysing lining layer hyperplasia, inflammatory infiltrate and resident cell activation (leucocytes, etc.).	88.8	4	83.40	4
Vasculitis should be scored.	66.7	3.5		
The absolute number of leucocytes per mm ² or per high power field should be assessed.			61.10	3.5
Lining layer hyperplasia should be scored.			83.40	4
Synovial pathogenesis should be described.	83.4	5	77.80	5
Presence or absence of lymphoid follicles within the membrane should be described.	83.4	5	83.30	5
Analysis can be semi-quantitative or quantitative depending on the question.			83.30	5
If a semi-quantitative or quantitative analysis is performed for multiple biopsies, an average score should be calculated and given for the analysis of inflammation and vasculature.	72.2	3.5		
The pathologist should mention the presence of granuloma.	88.9	5		
If appropriate and possible, the pathologist should mention the fibrotic diagnosis.	66.7	4		
6. RNA Analysis				
Response of one patient can be pooled for RNA extraction if needed.			100	5

Conclusions: We hereby propose a set of consensual points to consider on analysis of synovial biopsies in clinical practice and translational research. This standardisation initiative was conducted through ESSG members using a validated consensus method.

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SAT0051

EXAMINING THE ROLE OF TRPC5 IN OSTEOARTHRITIS PAIN

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Background: Transient receptor potential canonical 5 (TRPC5) is a cation channel that putatively acts as a sensor of one or more chemical factors but the exact biology is not fully known. It was recently found to be expressed in fibroblast-like synoviocytes where TRPC5 activation by endogenous thioredoxin – which has been shown to be elevated in synovial fluid of patients with rheumatoid arthritis – results in the suppression of matrix metalloproteinases secretion.¹ Genetic deletion or pharmacological blockade of TRPC5 receptor in arthritic mice resulted in marked exacerbation of hyperalgesia and critically increased localised inflammation in the synovium, characterised by increased cellular infiltration, secretion of early response cytokines and enhanced synovial vascularisation.² Interestingly, in this study, mRNA expression of TRPC5 was reduced in inflamed human arthritis samples particularly in the synovium from patients with osteoarthritis (OA), highlighting a potential role for TRPC5 in OA.

Objectives: With the aim to investigate putative mechanisms mediated by TRPC5 signalling in OA pain, we have examined the development of monoiodoacetate (MIA)-referred mechanical hypersensitivity and joint pathology in wild type and mice carrying a TRPC5 deletion.

Methods: Wild type and TRPC5 knock-out (TRPC5) mice were intra-articularly injected with 0.75 mg of MIA, and hind-paw mechanical thresholds and weight bearing changes were assessed at regular intervals up to 28 days. Knee joint histochemical analysis of cartilage degradation using toluidine blue staining and synovial inflammation using haematoxylin and eosin staining was also performed.

Results: Both TRPC5 KO and WT mice developed referred mechanical hypersensitivity following MIA injection, however the onset was faster (day 3 vs day 10) in TRPC5 KO mice. By the end of the study, at 28 days after MIA injection, mechanical hypersensitivity had reached similar levels in TRPC5 KO and WT mice. Moreover, at a 0.7 mg MIA dose administered, TRPC5 KO, but not WT mice developed weight bearing asymmetries over the course of the study and most significantly from 14 to 28 days after MIA injection. In paraformaldehyde-fixed joints obtained 28 days post MIA injection from TRPC5 KO and WT mice no significant differences in cartilage degradation or inflammatory content were observed.

Conclusions: This study suggests that deletion of the TRPC5 receptor signalling is associated with a faster onset of referred allodynia and increased guarding/on-going pain like behaviour in a model of OA.

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SAT0052

THE COLL2-1 PEPTIDE OF COLLAGEN TYPE II: A NEW ACTOR OF SYNOVITIS IN OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is characterised by degradation of the extracellular matrix associated with inadequate repair responses including pro-inflammatory pathways of nonspecific natural immune response.

Objectives: We evaluated the inflammatory effect of Coll2–1 peptide in osteoarthritic synoviocytes and rats by comparing peptide-induced inflammatory reaction with the one induced by bovine type II collagen or streptococcal cell wall.

Methods: Human synoviocytes from knee OA patients (n=10) were pre-treated with AS0619 or CLI-095 (500 nM, 1 and 2.5 µM) before a 24 hours treatment with Coll2–1 peptide (¹⁰⁸HRGYPGLDG¹¹⁶; 0.45 or 4.5 nmol). Expression of Interleukin (IL)–8, Vascular Endothelium Growth Factor (VEGF) and phosphorylation of the IκB-α and p65 were evaluated. Either Coll2–1 peptide, bovine type II collagen (CIA), streptococcal cell wall (SCW) or saline solution (100 µl SC or 50 µl IA) were injected into Lewis rats (n=108). The Coll2–1 peptide was subcutaneously injected (SC; 20 and 200 µg/100 µl/animal) or intra-articular (IA; 0.5 and 5 µg/50 µl/animal). The bovine type II collagen was SC injected (200 µg/100 µl/