Conclusion: This trial is the first to report that MRI tenosynovitis independently predicts both X-ray and MRI damage progression in RA patients in clinical remission. Further studies are needed to confirm MRI-determined tenosynovitis as predictor of progressive joint destruction in RA clinical remission.

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FRIDAY, 14 JUNE 2019

Complement and autoimmunity – emerging therapeutic opportunities

OP0332

COMPLEMENT FACTOR EXPRESSION AND ACTIVATION IN OSTEOARTHRITIS JOINT COMPARTMENTS

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Background: Several studies have identified a central role for the activation of the complement system in the pathogenesis of OA, finding an abnormally high expression and activation of complement in human osteoarthritic joints and the specific contribution of complement (C) components 5 and 6 and the membrane attack complex (MAC) (1). The complement system consists of several proteins which contribute to an enzymatic cascade ending in lysis. Two main, different pathways converge into C3 factor activation. In the classical pathway C4 is involved, while in alternative pathways Factor-b (FB) is involved.

Objectives: The aim of this study was to investigate the expression of complement factors (C3, C4 and FB) and activation fragments (C3a, C5a, FBa, MAC) in OA joint tissues.

Methods: Osteochondral biopsies from 33 patients (16 OA, 12 Rheumatoid Arthritis-RA and 15 Posttraumatic cases-PT) who underwent knee, hip or shoulder arthroplasty were collected. Immunohistochemistry and quantitative Imaging analysis were performed to analyze C3, C4 and CFB expression. Cartilage pathology was evaluated utilizing Mankin score. Chondrocytes, synoviocytes, cartilage and synovial biopsies were obtained from 11 OA patients undergoing joint replacement surgery. Cultures were set up with or without pro-inflammatory (IL-1 β) stimulus. Culture supernatants were analyzed for C3a, C5a, CFBa and C5b-9 production using enzyme-linked immuno-sorbent assay (ELISA). Statistical analysis was performed utilizing Wilcoxon matched pairs test and Mann Whitney's test, using Graph Pad Prism software.

Results: In osteochondral biopsies, C factor expression was located in bone marrow and in a few subchondral bone cells. C3 was the most expressed factor in all

patient subtypes while factor C4 had a very low expression, except for some sporadic cases. (OA: C3 vs FactorB $p=0.0280$, C3 vs C4 $p=0.0005$; RA: C3 vs FactorB $p=0.0269$, C3 vs C4 $p=0.0005$; PT: C3 vs FactorB $p=ns$, C3 vs C4 $p=0.0001$) CFB was significantly augmented in PT and was not significantly different between OA and RA. C factors expression did not correlate with Mankin score or with the joint type (knee, hip or shoulder). Cell positivity was mainly present in the subchondral bone (bone marrow) whereas cartilage was almost negative. As to activation fragments, C3a was the most produced factor (vs C5a and CFBa $p<0.0005$) but no differences were found between cultured chondrocytes and synoviocytes (vs synovial tissue $p<0.0005$) and its production was not stimulated by proinflammatory stimulus. C5a production in cartilage was significantly higher than in chondrocytes and synovial tissue (both $p<0.0005$) but no differences were found between cartilage and synoviocytes. CFBa was induced by IL-1 β in synovial tissue. C5b-9 production was undetectable in chondrocytes and synoviocytes cell culture supernatants. In cartilage C5b-9 production was decreased by IL-1 β stimulus while in synovium it was increased, although not significantly.

Conclusion: Complement factors were expressed mainly in bone marrow and in a few subchondral bone cells, but not in cartilage. Complement activating fragments are produced by chondrocytes and synoviocytes. The dissimilar expression of activating fragments by different cell types could suggest various C activating mechanisms in joint tissues.

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SATURDAY, 15 JUNE 2019

Novel autoantibodies in RMD: a neverending quest?

OP0333

RISING ACPA IGG VARIABLE DOMAIN GLYCOSYLATION PRE-DISEASE ASSOCIATES WITH AN INCREASE IN AUTOANTIBODY LEVELS AND THE DEVELOPMENT OF RHEUMATOID ARTHRITIS

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Background: Anti-citrullinated protein antibodies (ACPA) are present in the majority of rheumatoid arthritis (RA) patients (60-70%) and play a pivotal role in disease development¹. ACPA IgGs are unique in a way that they are abundantly N-glycosylated within their variable regions². These variable domain (V-domain) glycans are found on over 90% of ACPAs present in sera from RA-patients³. To undergo N-linked glycosylation, a consensus sequence in the protein backbone is required (N-X-S/T, where X $\neq$ P)⁴. Recently, it was shown that the N-linked glycosylation sites in ACPA-IgG V-domains are introduced during somatic hypermutation and that the introduction of such sites likely conveys a selective advantage to ACPA expressing B-cells⁵. However, it is currently unknown, whether ACPA-expressing B-cells already introduced glycosylation-sites into their V-domains before disease onset or when ACPA-V-domain glycosylation occurs.

Objectives: Investigate the appearance of ACPA V-domain glycosylation in pre-symptomatic individuals and RA patients.

Methods: In a case-control study, individuals (n=201) from the Medical Biobank, who were sampled before onset of symptoms (mean $\pm$ SEM predating time; 5.8 $\pm$ 0.3 years) (140 aCCP+ and 61 aCCP-), and after diagnosis of RA (n=99, 94 aCCP+ and 5 aCCP-) and randomly selected control samples (n=43, 3 aCCP+ and 40 aCCP-) were analyzed for their ACPA IgG V-domain glycosylation levels. ACPA IgGs were affinity purified, N-linked glycans released, and 2-AA labeled for further analysis using UHPLC⁶. Data calibration and integration was performed and a cut-off defined. Samples below the cut-off were determined as non-detectable and excluded from the analysis. The percentage V-domain glycosylation was calculated as described previously³. Statistical calculations were performed using SPSS.

Results: Our data indicated that ACPA IgG V-domain glycans are already present years before symptom onset, in pre-symptomatic individuals who subsequently will develop RA. Analysis of matched pairs showed a significant increase of ACPA V-domain glycosylation in RA patients compared to individuals pre-disease ($p<0.001$). The results showed that ACPA N-glycosylation was correlated with anti-CCP concentrations pre-disease ($r_s=0.504$, $p<0.001$), while no such association can be found after RA onset. Further, V-domain glycosylation levels increase closer to symptom onset.

Table 1. Impact of serologic features on markers of active SLE

	Subset	N	Mean/Median C3	p value	Mean/Median C4	p value	% with Low C3/C4	p value	% DNA +	p value
Baseline N=1019	ALL	1019	1056		186		39.2%		35.8%	
	ENA+	670	990	**	162	**	42.1%	#0.001	43.6%	#0.001
	ENA-	349	1130	0.001	210	0.001	23.8%		30.7%	
	DNA+	399	890	**	130	**	63.9%	#0.001	-	na
	DNA-	620	1130	0.001	210	0.001	22.9%		-	
	ENA+ DNA-	378	1117	**	198	**	25.9%	**	-	na
	ENA- DNA-	242	1211	0.001	237	0.001	18.2%	0.001	-	
Screen N=783	ALL	783		***		***	32.1%		24.9%	
	ANA neg	190	1230	<0.001	230	<0.001	15.3%	#0.073	7.4%	#0.026
	ANA=1:80	74	1130		228		25.7%	comp	17.6%	comp
	ANA=1:160	104	1110		210		25.0%	ns	19.2%	ns
	ANA=1:320	97	1110		190		29.9%	ns	22.7%	ns
	ANA=1:640	104	960		152		47.1%	#0.006	34.6%	#0.019
	ANA=1:1280	88	924		160		45.4%	#0.015	35.2%	#0.019
	ANA=1:2560	52	920		160		48.1%	#0.016	44.2%	#0.002
	ANA≥1:5120	74	959		135		45.9%	#0.016	48.6%	#0.001
	ANA - ENA +	61	1122	*	203	**	21.3%	ns	13.1%	ns
	ANA - ENA -	129	1254	0.007	258	0.001	12.4%		4.7%	
	ANA≥1:80 or DNA+	607	1020	**	170	**	37.7%	#0.012	-	na
	DNA+ or ENA+ or ANA≥1:640	318	940	0.001	152	0.001	46.5%		-

Figure 1 %Variable. domain glycosylation of matched paired samples before and after disease onset.

Conclusion: Our analysis revealed that ACPA IgG molecules can harbor V-domain glycans already relatively long time before disease onset. An increase in ACPA V-domain glycosylation, presumably due to the generation of de-novo N-glycosylation sites or the expansion of N-glycosylation site-bearing clones was associated with an increase in ACPA levels. These results suggest that ACPA-expressing B cells gain a selective advantage through the generation V-domain N-glycosylation in parallel with rising ACPA-levels in serum in the phase prior to the development of arthritis.

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OP0334

WHAT SEROLOGIC PROFILING PROVIDES OPTIMAL ENTRY CRITERIA FOR SYSTEMIC LUPUS ERYTHEMATOSUS CLINICAL TRIALS?

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Background: A major concern for SLE clinical trials is the possibility that some patients who technically meet entry criteria may not have active disease. Antinuclear antibody (ANA) is known to lack specificity for SLE, permitting inclusion of

inappropriate patients. Typically, ANA≥1:80 and/or positive anti-double stranded DNA (DNA) are required screening elements, but lately other autoantibody variables have been utilized.

Objectives: To examine serologic features that promote entry of active SLE patients into trials while supporting reasonable recruitment rates.

Methods: Serologic features of 1411 individual subjects who had full laboratory data at screening (N=783) and/or baseline (N=1019) in 4 phase 2 multicenter SLE trials were examined. Complement levels, percent of patients with low C3 or C4, and elevated DNA were used as markers of active disease. Serological profiles consistent with active SLE were tested on screened population for effects on potential screen failure rates.

Results: Of 1019 patients who qualified for the studies (baseline population), 399 (39.2%) were positive for DNA and 670 (65.8%) had anti-Extractable Nuclear Antibodies (ENA). Patients with positive DNA or ENA (even in absence of DNA) had lower complement levels and were more likely to have low C3/C4. (Table 1)

In patients at screening, higher titers of ANA were associated with lower complement levels and higher rates of patients with low C3/C4 and elevated DNA. ENA impacted complement levels even in the absence of ANA. Compared to traditional entry criteria (ANA≥1:80 and/or DNA), patients defined by positive DNA or ENA or ANA≥1:640 had significantly lower complement levels and greater rates of low C3/C4, suggesting a more active patient population. (Table 1)

*t test **ranks ***one way ANOVA on ranks #chi square (comp=comparator) na=not applicable ns=not significant. Data not adjusted for multiple comparisons Compared to a 22.5% screen failure rate with the typical requirement for ANA≥1:80 or DNA, testing DNA or ENA or low C3/C4 as entry criteria resulted in 30.7% exclusion. Adding high titer (≥1:640) ANA as an additional optional criterion would decrease the screen failure to 25.7%. (Table 2)

Table 2. Impact of serological profiles as entry criteria on potential screen failure rate (N = 783)

Entry Criteria	N	Screen Failure Rate
ANA≥1:80 or DNA	607	22.5%
ANA≥1:80 or DNA or anti-Smith	614	21.6%
DNA or ENA	495	36.8%
DNA or ENA or low C3/C4	543	30.7%
DNA or ENA or ANA≥1:640	552	29.5%
DNA or ENA or low C3/C4 or ANA≥1:640	582	25.7%

Conclusion: The following entry criteria may help increase the likelihood that trial subjects have active SLE: ANA≥1:640, or DNA, or ENA, or low C3 or C4. To the extent that a modest loss of recruitment increases the probability of entering true, active SLE patients, these alternative constructs should be considered in future SLE trials.

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