Galectin-1: a Potential Biomarker Differentiating Between Early Rheumatoid Arthritis and Spondyloarthritis

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Research article

Keywords: Spondyloarthritis, galectin 1, biomarker, rheumatoid arthritis, early arthritis

Posted Date: July 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-695957/v1>

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Abstract

Background: Galectin 1 (Gal1) is a lectin highly expressed in immune cells that plays a key immunoregulatory role in autoimmunity and resolution of chronic inflammation. Immune-mediated inflammatory diseases (IMIDs) are a broad group of disorders that share pathogenic mechanisms involving components of acquired and innate immunity. Although IMIDs can develop at onset similar features such as peripheral arthritis, they show differences in their evolution and response to treatments. Therefore, additional diagnostic biomarkers are needed in order to get a better classification of patients with early arthritis, especially those not fulfilling specific classification criteria. In this regard, we have recently described that Gal1 serum levels are increased in rheumatoid arthritis (RA) patients compared to healthy donors (HD). Thus, the objective of this work was to evaluate Gal1 levels in serum and synovial fluid from spondyloarthritis (SpA) patients in comparison with RA patients in order to determine their value as a diagnostic biomarker.

Methods: We studied Gal1 levels in serum samples from SpA (n=55) and RA patients (n=52). In addition, 49 HD were studied. We also measured Gal1 synovial fluid levels in RA (n=26), osteoarthritis (OA) (n=26) and peripheral SpA (n=26). In SpA patients, clinical parameters were also collected in order to evaluate their association with Gal1 serum levels.

Results: We found that SpA patients showed significantly lower Gal1 serum levels than RA patients and similar levels to the general population. In SpA patients, Gal1 synovial fluid levels were similar to those of OA patients and significantly lower than in RA patients. In addition, we did not find any correlation between Gal1 serum levels and clinical parameters of severity in SpA patients.

Conclusions: Our results suggest that Gal1 might be a potential diagnostic biomarker of RA that could allow distinguishing between SpA and RA patients.

Background

Immune-mediated inflammatory diseases (IMIDs) are a broad group of diseases, including psoriasis, rheumatoid arthritis (RA), inflammatory bowel disease, ankylosing spondylitis (AS) and connective tissue diseases (CTDs), among others, that can cause peripheral arthritis. IMIDs share pathogenic mechanisms involving components of adaptive and innate immunity.

RA is the most common IMID causing arthritis. It is considered a complex disease in which the interaction between genetic, environmental and stochastic factors leads to breakdown of self-recognition resulting in chronic infiltration of the synovial membrane by a wide variety of immune cells. These infiltrating cells cause chronic production of proinflammatory cytokines (tumour necrosis factor [TNF]- α , interleukin [IL]-1 β and IL-6) and metalloproteinases that, in absence of treatment, leads to joint destruction due to cartilage degradation and bone erosion (1, 2) .

Spondyloarthritis (SpA), with an estimated prevalence between 0.4 and 1.9%, is the second most frequent group of IMIDs causing arthritis (3, 4). Early diagnosis of SpA is challenging due to the absence of pathognomonic features, which may lead to a delay in treatment causing a large symptomatic burden and as a consequence functional disability during the most productive years of working life (5).

There is strong evidence supporting that early detection of an IMID and prescription of immunomodulatory treatment within the first year after the onset of symptoms provide a better chance of good response and a greater possibility of reaching remission. This has been clearly established for RA (6, 7) and there is some evidence indicating that it could also be true for other IMIDs (8, 9). In this regard, during the last 2 decades early arthritis clinics have been established in many clinical settings all around the world. These clinics have improved research in RA and SpA, as well as the implementation of early treatment in these patients (10). Nevertheless, the early recruitment of patients leads to the classification of a higher percentage of cases as undifferentiated arthritis (UA), who usually receive methotrexate as the initial treatment. However, subsequent immunomodulatory treatments may be based on different mechanisms such as cytokine blockade (for TNF, IL-6, IL-17, IL-12/23 and B lymphocyte stimulator [BlyS]), B-cell depletion or costimulation blockade, depending on the type of underlying IMID. Therefore, to improve therapeutic choice, additional diagnostic biomarkers are needed to better classify patients with early arthritis, especially those not fulfilling specific classification criteria.

Gal1 belongs to the lectin family of proteins that bind β -galactoside carbohydrates (11, 12). It might be involved in RA pathogenesis (13) since it is highly expressed in immune cells (14) acting as a novel regulatory checkpoint that links innate and adaptive responses (15). Recently, we have confirmed that patients with RA present increased Gal1 serum level (16, 17). However, to date there is no solid information on the role that Gal1 may play in SpA. Therefore, the objective of this work was to determine potential differences between Gal1 serum levels in SpA patients, RA patients and healthy donors (HD), in order to determine whether this molecule could be useful as a diagnostic biomarker of either RA or SpA.

Patients And Methods

The current study included 55 spondyloarthritis patients classified in different subpopulations:

Early spondyloarthritis population

This subpopulation comprised 19 early SpA patients from The Princesa Register of Spondyloarthritis with Early Onset (PERSEO). PERSEO protocol includes seven prospective visits (baseline, 1, 2, 4, 6, 8 and 10 years). Clinical data and samples obtained from 76 visits corresponding to the first four years of follow-up of these patients were available for this study.

PERSEO inclusion criteria are inflammatory back pain (IBP) for more than 3 months and less than 2 years, and symptom onset before the age of 45. Socio-demographic, clinical, therapeutic and laboratory data are recorded and included in an electronic database. Biological samples are collected at each visit

and stored at -80°C at the Instituto de Investigación Sanitaria La Princesa (IIS-IP) Biobank. A more detailed description of the register has been previously published (18). At each visit during the follow-up, patients of PERSEO are checked for fulfilment of the Assessment of SpondyloArthritis International Society (ASAS) criteria for SpA (19). All patients included in the study fulfilled ASAS criteria for SpA, patients with other diagnoses were excluded from the study except those who finally were classified as mechanical low back pain patients, which were included in the control population group.

Long term Ankylosing spondylitis population

The second SpA subpopulation comprised 21 patients with long term ankylosing spondyloarthritis (AS) assessed in a single cross-sectional visit. All patients fulfilled the New York classification criteria for ankylosing spondylitis (20). In order to homogenize variables, the information collected in this visit was the same as that collected in early SpA. Biological samples were also collected and stored as described for PERSEO.

Psoriatic arthritis

Finally, the third SpA subpopulation comprised 15 psoriatic arthritis (PsA) patients from the Princesa Early Arthritis Register Longitudinal (PEARL) study. More detailed description of PEARL protocol is provided below.

Early rheumatoid arthritis population

Fifty-two patients from the Princesa Early Arthritis Register Longitudinal (PEARL) study who fulfilled 1987 ACR classification criteria (21) after two years of follow-up were used as RA group to be compared with SpA.

The PEARL study comprises patients with one or more swollen joints and symptoms with ≤ 1 year of evolution. The register protocol includes 4 visits during a 2-year follow-up (0, 6, 12 and 24 months). Socio-demographic, clinical, therapeutic and laboratory data are recorded and included in an electronic database. Biological samples are collected at each visit and stored at - 80°C at the IIS-IP Biobank for translational research. More detailed description of PEARL protocol has been previously published (22).

Healthy donors

Serum samples from 49 healthy donors were included in this study, of whom 24 were mechanical low back pain patients from the PERSEO population, 20 were patients from PEARL population in which it was excluded the presence of arthritis or any autoimmune disorder, and the remaining controls were obtained from IIS-IP Biobank.

Synovial fluid samples

Synovial fluid (SF) samples were obtained from therapeutic or diagnostic arthrocentesis. Patients gave their oral informed consent for the use of these samples for research purposes, which was registered in the clinical chart. Samples contaminated with blood were discarded. Samples were centrifuged at 2,000

rpm during 20 minutes at room temperature and the cell-free supernatants were collected and stored at -80 °C until analysis. The samples were anonymised and only information about diagnosis was collected. For this study, samples from 26 RA patients, 26 OA patients and 26 peripheral SpA patients were analysed.

Measurement of serum and synovial fluid Gal1

Gal1 serum and SF levels were measured using Quantikine Human Gal1 Immunoassay (R&D Systems, Minneapolis, USA). The procedure was performed according to the manufacturer's instructions. Absorbance was measured in a spectrophotometer (Innogenetics Diagnostica y terapeutica S.A.U, Barcelona, Spain) at 450 nm with correction at 620 nm. Measurements for all samples were performed in duplicate. In order to minimize the influence of inter-assay variability we included samples from all study groups in every plate.

Statistical analysis

Statistical analyses were performed using Stata 14.0 for Windows (Stata Corp LP, College Station, TX, USA). Most quantitative variables followed a non-normal distribution, so they were represented as median and interquartile range (IQR), and the Mann Whitney or Kruskal-Wallis tests was used to analyse significant differences. Qualitative variables were described as proportions, and the χ^2 or Fisher's exact test was used to compare categorical variables. The Pearson correlation test was applied to analyse correlation between quantitative variables.

Multivariate analyses

In order to identify those variables associated with variation in Gal1 serum levels we fitted a multivariate linear regression analysis. The first model was performed by adding all variables with a *p* value < 0.15 in the bivariate analysis. The final models were constructed removing those variables with *p* value > 0.15, based on the Wald test analysis of sequential models. Once the best model was obtained, several variables related with assessment of SpA were forced in the model in order to determine whether there was an association with Gal1 or not.

Considering that Gal1 serum levels significantly increased with age, to achieve a more accurate graphical representation, we obtained age-adjusted values of serum Gal1 using the command "margins" of Stata once the multivariable analysis was fitted.

Finally, to assess the ability of Gal1 serum levels to discriminate between SpA patients and RA patients we performed Receiver Operating Characteristic (ROC) curve analysis through the command *roctab*. For development of ROC curves the option *graph* was used. Each cut-off point was selected on the basis of the best trade-off values between sensitivity, specificity, cases correctly classified and positive (LR+) and negative (LR-) likelihood ratios obtained using the command *roctab* with the option *detail*.

Results

Study population

In order to compare Gal1 serum levels between SpA, RA patients and healthy donors (HD), we measured Gal1 levels in serum from 55 SpA patients (early spondyloarthritis n = 19; psoriatic arthritis n = 15; ankylosing spondylitis n = 21), 52 RA patients and 49 HD. The characteristics of the different populations are shown in Table 1 and Supplementary (Suppl.). Table 1. SpA patients were significantly younger, and had longer disease duration at the beginning of the follow-up and a higher frequency of males compared to RA. Due to these differences, we analysed whether variables such as gender or age influenced Gal1 serum levels. Gal1 serum levels significantly increased with age, whereas no significant differences were observed by gender (Suppl. Figures 1A and 1B). Therefore, age was taken into consideration in subsequent analyses of Gal1 levels.

Table 1

Baseline characteristics of the study populations. n: number; p50: median or percentile 50; p25-p75: range between percentiles 25 and 75 or interquartile range; SpA: spondyloarthritis; RA: rheumatoid arthritis; ACPA: anti-citrullinated protein antibodies; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; ASDAS: Ankylosing spondylitis disease activity index; DAS28: disease activity score estimated with 28 joint count; HAQ: baseline health assessment questionnaire.

	Healthy donors	SpA patients	RA patients	p-value
	(n = 49)	(n = 55)	(n = 52)	
Female; n (%)	30 (61.22)	25 (45.45)	44 (84.62)	< 0.001
Age; p50 [p25-p75]	35.36 [31.63–55.36]	49.79 [38–57.55]	54.06 [43.95–69.75]	< 0.001
Disease duration (months); p50 [p25-p75]		22 [6– 57.55]	4.96 [2.53–8.83]	< 0.001
HLA-B27 positive; n (%)		38 (69.09)		
RF positive; n(%)			37 (71.15)	
ACPA positive; n(%)			31 (59.62)	
BASDAI; p50 [p25-p75]		3.5 [1.95–4.6]		
BASFI ; p50 [p25-p75]		1.85 [1.2–3.1]		
ASDAS ; p50 [p25-p75]		2.17 [1.5–3.05]		
DAS28; p50 [p25-p75]			4.85 [3.45–5.78]	
HAQ; p50 [p25-p75]			1.12 [0.625–1.5]	

Gal1 serum levels are similar in HD and in SpA patients and lower in early RA patients.

Gal1 serum levels were not significantly different in HD and SpA patients ($p = 0.94$, Fig. 1A); however, the Gal1 serum levels in both populations were significantly lower than those of RA patients ($p < 0.001$, Fig. 1A). Even after adjustment by gender and age, the differences by diagnosis remained significant (Table 2). In addition, no significant differences were found in Gal1 serum levels between the different subgroups of SpA (early SpA, AS, PsA) and HD but they were significantly lower than those of RA patients (Suppl. Figure 2 and Suppl. Table 2).

Table 2
Relationship between Gal1 (ng/ml) serum levels and diagnosis. Coeff. Coefficient; CI. Confidence interval

	β Coeff. (95% CI)	p-value
Female gender	-1.09 (-3.53 to 1.35)	0.37
Age (years)		
< 45	Reference	
45–65	1.16 (-1.40 to 3.73)	0.372
> 65	2.92 (-0.24 to 6.08)	0.07
Diagnosis		
Spondyloarthritis	Reference	
Healthy donors	-0.43 (-3.23 to 2.36)	0.75
Rheumatoid arthritis	6.54 (3.61 to 9.47)	< 0.001

In view of these results, the next step was to determine the capability of Gal1 serum level to discriminate between RA and SpA patients. The ROC analysis (AUC = 0.743) showed that a Gal1 concentration above 17.95 ng/ml had 72% sensitivity and 72.73% specificity to differentiate those diagnosis (Fig. 1B; LR + 2.64 and LR- 0.38).

Furthermore, we decided to analyse whether Gal1 serum levels enabled us to distinguish between patients with early SpA and patients with mechanical low back pain throughout the follow-up (clinical characteristics in Suppl. Table 3). Our data did not show significant differences in baseline Gal1 expression levels between both groups, and no significant differences were observed in Gal1 serum levels during the follow-up in early SpA patients (p-trend = 0.53, Fig. 2A).

Gal1 serum levels are not associated with disease activity or disability in Early SpA

Despite the absence of differences along the follow-up, we aimed to determine if Gal1 serum levels could be a biomarker of disease severity. Thus, we studied if Gal1 serum levels correlated with either disease activity assessed by ASDAS (Ankylosing spondylitis disease activity score) or BASDAI (Bath ankylosing spondylitis disease activity index) and disability evaluated by BASFI (Bath ankylosing spondylitis functional index). Disease activity decreased along the follow-up in response to treatment (Fig. 2B and 2D). However, Gal1 levels were similar in HD and early SpA patients and did not fluctuate throughout follow-up (Fig. 2A). Moreover, no significant correlation was observed between ASDAS or BASDAI and Gal1 serum levels in early SpA patients (Fig. 2E and 2G). A similar finding was observed with disability, BASFI score improved during the follow-up (Fig. 2C), whereas no significant correlation was observed between BASFI and Gal1 serum levels (Fig. 2F).

Gal1 synovial fluid levels are different in distinct arthritis types.

Finally, in order to confirm the previous results locally at the joint compartment, we compared Gal1 levels in SF of 26 patients with RA, 26 with OA and 26 with peripheral SpA. We observed that SpA patients had similar Gal1 SF levels than OA patients ($p = 0.66$, Fig. 3). However, RA patients had significantly higher Gal1 levels than OA or SpA patients ($p < 0.001$, Fig. 3).

Discussion

One of the main concerns in the management of IMIDs is early detection and diagnosis in order to treat them as soon as possible since this ensures a higher probability to achieve complete remission. In this scenario, the main result of our work shows that Gal1 levels in both serum and SF are significantly lower in patients with SpA compared to RA patients. This result raises the possibility that measurement of Gal1 in biological fluids could be a useful diagnostic tool. In this regard, several studies have reported and validated that serum Gal1 levels are increased in RA patients compared to healthy donors (16, 17). However, as far as we know, Gal1 has not been previously studied in SpA. This approach is needed to verify whether increased levels of Gal1 are specific of RA or a common finding to different IMIDs.

During the last decades, it has been shown that IMIDs share immunopathogenic mechanisms, resulting in inflammation frequently located at joints. Thus, different IMIDs can present, at the beginning of the disease, with similar symptoms at articular level. Although some disease-modifying antirheumatic drugs (DMARDs) such as methotrexate, sulfasalazine or leflunomide can be useful for the treatment of peripheral arthritis, there are subtle differences in its response to these drugs, and specially to biological treatments (19, 23). Therefore, additional diagnostic biomarkers are needed in order to better classify patients with peripheral involvement (e.g., peripheral arthritis, enthesopathy or dactylitis) not fulfilling specific classification criteria (24). Psoriatic arthritis can be a paradigmatic example for this situation, due to its characteristic absence of autoantibodies and rare HLA-B27 positivity. Our data show that patients with PsA show lower Gal1 levels in both serum and SF compared to RA. Furthermore, considering SpA as a whole group, a Gal1 serum level higher than 17.95 ng/ml showed a good performance to discriminate SpA patients from those with RA. This cut-off is very similar to that proposed in our previous study in which Gal1 serum level higher than 19 ng/ml discriminated properly RA patients and HD (17). The differences between both studies could be due to inter-assay coefficient of variation (8.5% approximately according to the ELISA kit manufacturer).

Interestingly, a subpopulation of the current study is included in a longitudinal cohort of patients with early SpA (PERSEO). As it happened with the longitudinal study of early arthritis (PEARL) (17), no relevant variation in Gal1 serum levels was observed across the follow-up in PERSEO, suggesting that Gal1 serum levels are not altered by treatment or disease duration. Therefore, measurement of Gal1 serum levels could be useful as a diagnostic biomarker at any time during the disease course.

On the other hand, using Gal1 as a diagnostic biomarker has some limitations. One of them, described in this work, is the increase of Gal1 serum levels with age. Other authors have already reported an increase

in this protein with age and even proposed a role for endogenous Gal1 as a key immune regulator that promotes immune tolerance and prevents age-dependent spontaneous autoimmunity (25). In our study, differences in Gal1 levels between RA and SpA patients remained significant after adjustment by age. However, the increase with age raises the question of whether different cut-offs would be necessary depending on age. Another limitation is the sample size and heterogeneity of the study population, especially because it was composed of three different SpA subpopulations. However, all three subpopulations behaved very similarly in terms of Gal1 serum levels. In addition, validation studies in other SpA populations, as well as characterization of Gal1 serum levels in CTD such as systemic lupus erythematosus, systemic sclerosis or Sjögren syndrome are needed.

Conclusions

The principal aim of this study was to evaluate the usefulness of Gal1 as a potential biomarker in SpA, and compare it with that obtained in RA. The main findings of this work are: (i) Gal1 serum levels could be a possible diagnostic biomarker to differentiate between SpA and RA. (ii) Gal1 serum levels do not correlate with clinical severity parameters in SpA patients.

Abbreviations

Galectin 1: Gal1; Immune-mediated inflammatory diseases: IMIDs; Rheumatoid arthritis: RA; Healthy donors: HD; Spondyloarthritis: SpA; Ankylosing spondylitis: AS; Instituto de Investigación Sanitaria La Princesa :IIS-IP; Princesa Early Arthritis Register Longitudinal: PEARL; Princesa Register of Spondyloarthritis with Early Onset: PERSEO; Undifferentiated arthritis: UA; Synovial fluid: SF; osteoarthritis: OA; Interquartile range: IQR; Receiver Operating Characteristic: ROC; Area Under curve: AUC; Likelihood ratios: LR; ASDAS: Ankylosing spondylitis disease activity score; BASDAI: Bath ankylosing spondylitis disease activity index; BASFI: Bath ankylosing spondylitis functional index; Inflammatory back pain: IBP; CTD: connective tissue disease; TNF: tumour necrosis factor; IL: interleukin; BLYS: B lymphocyte stimulator; ASAS: Assessment of spondyloarthritis international society; PsA: Psoriatic arthritis; Suppl.: supplementary; DMARDs: disease modifying antirheumatic drugs; CI: confidence interval

Declarations

Ethics approval and consent to participate

The present study was conducted according to the principles expressed in the Helsinki Declaration of 1983 and it was approved by the Research Ethics Committee of Hospital Universitario de La Princesa (PI-467;September 9th, 2010, PI-518; March 28th, 2011). All patients included were over 18 years old and signed a written informed consent at study entry; samples and data from patients included in this study were provided by the IIS-IP Biobank (ISCIII B.0000763). All samples were processed following standard operating procedures with the appropriate approval of the Ethics and Scientific Committees.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author (isidoro.ga@ser.es) on reasonable request.

Competing interests

IG-A reports grants from Instituto de Salud Carlos III, during the course of the study; personal fees from Lilly and Sanofi; personal fees and non-financial support from BMS; personal fees and non-financial support from Abbvie; research support, personal fees and non-financial support from Roche Laboratories; non-financial support from MSD, Pfizer and Novartis, not related to the submitted work. SC has received personal fees from Amgen, Lilly, MSD, Pfizer, Roche, Sanofi and SOBI. SC is also assistant professor of the cátedra UAM-Roche, EPID-Future, Universidad Autónoma de Madrid (UAM), Madrid. The remaining authors report no competing interests with the submitted work.

Funding

This work was supported by grants RD16/0011/0012 and PI18/0371 to IG-A, and PI12/01578 to SC from the Ministerio de Economía y Competitividad (Instituto de Salud Carlos III) and co-funded by European regional development fund (ERDF) “A way to make Europe”. NM is currently granted by a RD16/0011/0012. The work of ER-V is currently funded by a grant Rio-Hortega CM19/00149 from the Ministerio de Economía y Competitividad (Instituto de Salud Carlos III) and co-funded by European regional development fund (ERDF) “A way to make Europe”.

Author's contribution

A.T-M, H.dF, A.L and I.G-A designed the study. A.T-M and E.R-V performed laboratory determinations. AM.O, S.C, E.T and I.G-A provided patients and clinical and laboratory data. A.T-M, N.M, A.L and I.G-A performed analysis of the results, interpreted the results and wrote the first draft of the manuscript. H.dF, E.R-V, E.T and S.C revised the manuscript. All authors approved the final version of the manuscript.

Acknowledgments

We thank the patients and the controls for their participation in the study. In addition, we are grateful to collaborating clinicians for their participation in this study. We also acknowledge the excellent editing assistance of Dr. Manuel Gómez Gutiérrez.

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Figures

Figure 1

Spondyloarthritis patients and healthy donors show similar Gal1 serum levels and lower Gal1 serum levels than those in early rheumatoid arthritis patients. (A) Determination of Gal1 serum levels by ELISA in healthy donors, spondyloarthritis (SpA) patients and early rheumatoid arthritis (RA). Gal1 serum levels were adjusted by age as describe in methods. Data are shown as interquartile range (p75 upper edge of box, p25 lower edge, p50 midline) as well as the p95 (line above box) and p5 (line below). Dots represent outliers. Statistical significance was determined with the Kruskal-Wallis test. Significance threshold was set at $p < 0.05$. (B) ROC curve analysis to assess Gal1 capacity to discriminate between SpA patients and RA patients.

Figure 2

Gal1 serum levels do not correlate with clinical severity parameters in early spondyloarthritis. Determination of Gal1 serum levels by ELISA in mechanical low back pain patients and early spondyloarthritis (SpA) patients during the follow-up (A). Gal1 serum levels were adjusted by age as describe in methods. Statistical significance for the trend of Gal1 along the follow-up visits in early SpA patients was determined with the Cuzick's non-parametric test. Significance threshold was set at $p\text{-trend}$

<0.05. Evolution along the follow-up of disease activity assessed by BASDAI (B) or ASDAS-CRP (D), and disability assessed by BASFI (C) in early spondyloarthritis patients. Absence of correlation of Gal1 serum levels with BASDAI (E), BASFI (F) and ASDAS-CRP (G) in early spondyloarthritis patients. In panels A to D, data are shown as interquartile range (p75 upper edge of box, p25 lower edge, p50 midline) as well as the p95 (line above box) and p5 (line below). Dots represent outliers. In panels E to G, data are shown as dot plots and their fitted linear prediction with 95% confidence interval (grey shadow) using the twoway command of Stata with the lfitci option. Pearson correlation test was used to determine the level of significance.

Figure 3

Gal1 synovial fluid levels are different in distinct arthritis populations. Determination of Gal1 synovial fluid levels by ELISA in osteoarthritis patients, spondyloarthritis patients and RA patients. Data are shown

as interquartile range (p75 upper edge of box, p25 lower edge, p50 midline) as well as the p95 (line above box) and p5 (line below). Dots represent outliers. Statistical significance was determined with ANOVA. Significance threshold was set at $p\text{-value} < 0.05$.

Supplementary Files

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