SUPPLEMENTARY DATA

IRRITABLE BOWEL SYNDROME SYMPTOMS IN AXIAL SPONDYLOARTHRITIS MORE COMMON THAN AMONG HEALTHY CONTROLS – IS IT AN OVERLOOKED COMORBIDITY?

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THE SPARTAKUS STUDY

All patients with ≥1 outpatient visit to the Department of Rheumatology, Skåne University Hospital, 2011-2014, with an ICD-10 diagnosis compatible with axial spondyloarthritis (axSpA) (M45.9, Ankylosing spondylitis; M46.0, Spinal enthesopathy; M46.1, Sacroiliitis; M46.8, Other specified inflammatory spondylopathies; and M46.9, Unspecified inflammatory spondylopathies), and residing within a defined area of southern Sweden, were invited to enroll in a cross-sectional study, SPARTAKUS (SPondylARtrit TvÄrsnittsKohort Universitetssjukhuset i Skåne). Since the SPARTAKUS study focuses on axSpA, patients with undifferentiated spondyloarthritis (SpA) diagnoses (M46.8 and M46.9) had to report back pain ≥3 months with onset before age 45 to be eligible. Enrolment started November 2015 and is ongoing as of September 2019.

Here, the first 182 consecutive patients classified as non-radiographic axSpA (nr-axSpA; n=63) or ankylosing spondylitis (AS; n=119) are analysed, after excluding cases with known concomitant inflammatory bowel disease (IBD; n=22). A control group (n=50) without rheumatologic diseases or IBD and frequency-matched for sex and age was included, recruited among colleagues/friends/relatives of the authors.

Study protocol

All patients included in the SPARTAKUS study underwent a structured four-hour study visit according to a predefined protocol, comprising patient questionnaires (including information for classification, gastrointestinal symptoms [using the ROME III questionnaire [1]], disease activity, physical function, and health-related quality-of-life), clinical examinations by a rheumatologist, physiotherapist, and occupational therapist, and sampling of blood, faeces, and urine. Patients were assessed regarding fulfilment of 1990 criteria for fibromyalgia (report of widespread pain ≥3 months in combination with ≥11/18 tender points at evaluation).[2]

Classification algorithm

To assess fulfilment of the Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axSpA,[3] and enable subclassification into nr-axSpA or AS (according to ASAS or modified New York criteria, respectively [3, 4]), the following decision and work-up algorithm was used:

- Any previously performed plain X-rays and/or magnetic resonance imaging (MRI) scans of the sacroiliac (SI) joints were reexamined and scored. If previous SI-joint X-rays

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were either absent or negative for fulfilment of modified New York criteria ≥24 months prior to the study visit, a new SI-joint X-ray was performed and scored.

- For patients in whom AS was thereby ruled out, and for whom nr-axSpA according to ASAS criteria could not be confirmed based on HLA-B27 positivity and/or previous MRI findings and clinical characteristics, a new SI-joint MRI was also performed and scored (unless such an examination was already available and ASAS negative within the last 24 months).
- All X-ray and MRI scorings were done by one experienced musculoskeletal radiologist (MG).

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Supplementary Table S1. Characteristics of the axSpA patients and controls

	AxSpA n=182	Nr-axSpA n=63	AS n=119	Controls [a] n=50
Male sex, n (%)	100 (55%)	21 (33%)	79 (66%)	26 (52%)
Age, yrs	51 (13)	46 (12)	54 (13)	50 (13)
Symptom duration, yrs	25 (14)	20 (11)	28 (14)	
Family history of SpA, n (%)	74 (41%)	24 (38%)	50 (42%)	
HLA-B27 positive, n (%)	152 (85%)	55 (87%)	97 (84%)	
Back pain ≥3 months:				
With onset <45 years, n (%)	175 (96%)	63 (100%)	112 (94%)	
Improved by exercise and not relieved by rest, n (%)	146 (80%)	51 (81%)	95 (81%)	
Inflammatory back pain (ASAS definition), n (%)	158 (87%)	55 (87%)	103 (87%)	
Sagittal lumbar flexion (Modified Schober´s test), cm	4.2 (1.6)	4.4 (1.2)	4.1 (1.7)	
Lateral lumbar flexion, cm [b]	14 (5.3)	15 (4.8)	13 (5.4)	
Chest expansion, cm	4.8 (1.8)	5.1 (1.9)	4.6 (1.8)	
Sacroiliitis on plain X-ray, n (%)	119 (65%)	0 (0%)	119 (100%)	
SI-joint MRI available, n (%)	93 (51%)	40 (63%)	53 (45%)	
SI-joint bone marrow edema on MRI, n (%) [c]	46 (50%)	18 (45%)	28 (53%)	
Peripheral arthritis, n (%)	94 (52%)	36 (57%)	58 (49%)	
Dactylitis, n (%)	20 (11%)	13 (21%)	7 (5.9%)	
Heel enthesitis, n (%)	79 (43%)	31 (49%)	48 (40%)	
History of uveitis, n (%)	69 (38%)	18 (29%)	51 (43%)	
Skin and/or nail psoriasis, n (%)	13 (7.1%)	5 (7.9%)	8 (6.7%)	
Inflammatory bowel disease, n (%) [d]	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Fibromyalgia, n (%) [e]	18 (10%)	8 (13%)	10 (8.8%)	
Good response of back pain to NSAID, n (%)	141 (77%)	47 (75%)	94 (79%)	
VAS global, mm	34 (25)	38 (25)	32 (26)	
VAS pain, mm	34 (26)	37 (25)	32 (27)	
VAS fatigue, mm	37 (28)	41 (29)	35 (27)	
EQ-5D utility [f]	0.71 (0.25)	0.68 (0.25)	0.73 (0.25)	
BASDAI	3.1 (2.3)	3.3 (2.1)	3.0 (2.3)	
BASFI	2.1 (2.2)	2.1 (2.1)	2.2 (2.3)	
BASMI	3.0 (1.5)	2.4 (1.1)	3.2 (1.6)	
Evaluator's VAS global, mm	17 (14)	19 (15)	16 (14)	
ASDAS-CRP	1.9 (0.9)	1.9 (0.9)	1.8 (1.0)	
CRP, mg/L	3.7 (4.9)	2.5 (2.5)	4.2 (5.7)	
F-calprotectin, mg/kg	72 (128)	46 (55)	86 (152)	17 (23)
NSAID-use during last 3 months, n (%)	113 (62%)	37 (59%)	76 (64%)	23 (46%)
Ongoing csDMARDs, n (%)	32 (18%) [g]	13 (21%)	19 (16%) [g]	
Azathioprine, n (%)	1 (0.5%)	1 (1.6%)	0 (0%)	
Hydroxycholoquine, n (%)	1 (0.5%)	1 (1.6%)	0 (0%)	
Methotrexate, n (%)	21 (12%)	8 (13%)	13 (11%)	
Sulfasalazine, n (%)	10 (5.5%)	3 (4.8%)	7 (5.9%)	
Ongoing bDMARDs	81 (45%)	28 (44%)	53 (45%)	
Adalimumab, n (%)	15 (8.2%)	4 (6.3%)	11 (9.2%)	
Certolizumab pegol, n (%)	15 (8.2%)	9 (14%)	6 (5.0%)	
Etanercept, n (%)	28 (15%)	7 (11%)	21 (18%)	
Golimumab, n (%)	6 (3.3%)	2 (3.2%)	4 (3.4%)	
Infliximab, n (%)	12 (6.6%)	4 (6.3%)	8 (6.7%)	
Secukinumab, n (%)	5 (2.7%)	2 (3.2%)	3 (2.5%)	

Mean (SD) if not otherwise stated. [a] Frequency-matched against the axSpA patients for sex and age. [b] Mean of right and left lateral lumbar flexion. [c] Previous or current SI-joint bone marrow edema according to the ASAS definition. [d] Patients with diagnosed IBD were excluded from this study. [e] Assessed by the 1990 fibromyalgia classification criteria.[2] [f] United Kingdom time trade-off based preference set. [g] One AS patient was treated with concomitant methotrexate and sulfasalazine. Missing data among the axSpA patients, n (%): HLA-B27 4 (2%), symptom duration 1 (0.5%), chest expansion 1 (0.5%), fibromyalgia 8 (4%), VAS global/pain/fatigue 5 (3%), EQ-5D 12 (7%), BASDAI 12 (7%), BASFI 16 (9%), BASMI 4 (2%), Evaluator's VAS global 11 (6%), ASDAS-CRP 24 (13%), CRP 19 (10%), F-calprotectin 25 (14%). No missing data among the controls.

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Supplementary Table S2. Associations between IBS symptoms and disease/treatment characteristics in axSpA patients

	Univariate analyses		Multivariate analysis [a]	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Female sex	3.0 (1.5 to 5.7)	0.001	2.4 (1.2 to 5.0)	0.017
Age ≥50 years [b] (vs. 18-49 years)	1.1 (0.6 to 2.0)	0.872	N.A.	
ASDAS-CRP ≥2.1 (vs. <2.1)	2.3 (1.1 to 4.6)	0.021	2.0 (1.0 to 4.1)	0.063
CRP >3.0 mg/L (vs. ≤3.0 mg/L)	0.6 (0.3 to 1.2)	0.141	N.A.	
F-calprotectin ≥50 mg/kg (vs. <50 mg/kg)	1.5 (0.7 to 3.0)	0.259	N.A.	
NSAID-use [c] (vs. no NSAID-use)	2.2 (1.1 to 4.5)	0.024	1.9 (0.9 to 4.1)	0.120
DMARD-use [d] (vs. no DMARD-use)	1.5 (0.8 to 2.9)	0.187	N.A.	
Comorbid fibromyalgia [e] (vs. no)	4.4 (1.6 to 12.1)	0.004	4.2 (1.3 to 13.3) [g]	0.016
AS (vs. nr-axSpA) [f]	0.8 (0.4 to 1.5)	0.506	N.A.	

[a] Variables displaying univariate associations to IBS symptoms with p-values <0.10 were entered into the multivariate model. [b] Median age in the axSpA group = 50 years. [c] Any during the last 3 months. [d] Conventional and/or biologic DMARD-use at the study visit. [e] Assessed according to the 1990 fibromyalgia classification criteria.[2] [f] Assessed according to the modified New York classification criteria for AS and the ASAS classification criteria for axSpA, respectively. [g] Analysed in a separate model from sex due to multicollinearity (Spearman´s rho >0.3). Missing data, n (%): ASDAS-CRP 24 (13%), CRP 19 (10%), F-calprotectin 25 (14%), fibromyalgia 8 (4%). N.A. not applicable.

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SENSITIVITY ANALYSES

IBS symptoms in axSpA patients versus controls

Regarding the comparison of the proportions of axSpA patients and controls reporting gut symptoms meeting IBS criteria, three different sensitivity analyses were performed:

1) In endoscopy studies, gut inflammation with macro- and/or microscopic lesions reminiscent of Crohn´s disease has been demonstrated in up to ~50-60% of axSpA patients.[5] Furthermore, faecal calprotectin (F-calprotectin) has been shown to be elevated in axSpA patients without known IBD compared to healthy controls.[6] Therefore, to examine whether such low-grade gut inflammation may explain/contribute to the increased risk of IBS symptoms observed in the axSpA group in our main analysis, we performed a sensitivity analysis with additional adjustment (apart from sex and age) for F-calprotectin levels (mg/kg; applied as continuous variable, using logarithmic values due to its skewed distribution). Due to missing F-calprotectin data, 25 patients (but no controls) were excluded from this analysis. Apart from a higher prevalence of previous uveitis (64% versus 34%) and a higher mean score for evaluator´s visual analogue scale for global assessment of disease activity (22 mm versus 16 mm), these excluded patients did not differ significantly from the included ones. Results of this sensitivity analysis showed that 32% of axSpA patients and 16% of controls reported IBS symptoms meeting ROME III criteria (adjusted OR 2.6 [95%CI 1.1-6.2]; p=0.034).

2) Since non-steroidal anti-inflammatory drugs (NSAIDs) – a cornerstone in pharmacological treatment of axSpA – are well-known for their risk to cause gastrointestinal side-effects, a second sensitivity analysis was performed with additional adjustment (apart from sex and age) for NSAID-use during the last 3 months prior to the study visit (dichotomized as no versus any use). No patients/controls had missing information regarding NSAID-use. Results of this sensitivity analysis showed that 30% of axSpA patients and 16% of controls reported IBS symptoms meeting ROME III criteria (adjusted OR 2.2 [95%CI 0.9-5.1]; p=0.067).

3) In the study questionnaire, a few individuals indicated problems with frequent blood in the stool (2 patients), frequent black stool (4 patients) or unexplained weight-loss >4.5 kg (1 control subject and 4 patients, of whom one also reported frequent black stool) during the last 3 months. Four out of these 10 subjects reported gut symptoms meeting ROME III IBS criteria. However, since such “alarm symptoms” may indicate other pathological processes (meaning that the entire ROME III criteria would not be met since this is a reason for non-

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fulfilment), we performed a third sensitivity analysis excluding these 10 subjects. Results of this analysis showed 28% of axSpA patients and 16% of controls to report IBS symptoms meeting ROME III criteria (adjusted OR 2.2 [95%CI 0.9-5.1]; p=0.066).

Associations between IBS symptoms and F-calprotectin in axSpA patients

Although no overall association between the presence of IBS symptoms and F-calprotectin levels among axSpA patients were detected in our main analysis, we conducted a sensitivity analysis to assess whether such associations might be observed with specific IBS symptoms more indicative of bowel inflammation, i.e. more frequent bowel movements or looser stools in conjunction with abdominal discomfort/pain.

As displayed in **Supplementary Table S3**, no difference in the geometric mean of F-calprotectin levels was observed between axSpA patients reporting versus not reporting IBS symptoms. Furthermore, the axSpA group reporting IBS symptoms that included more frequent bowel movements or loose stools at least sometimes, in conjunction with abdominal discomfort/pain, did not differ significantly from axSpA patients without IBS symptoms in regard to F-calprotectin levels. However, when separately assessing the subgroup of patients fulfilling IBS symptom criteria who reported the highest frequencies of loose stools in conjunction with abdominal discomfort/pain (i.e. most of the time or always), F-calprotectin was significantly increased compared to patients without IBS symptoms (p=0.038). This was not the case for patients reporting the most frequent bowel movements. Out of 16 patients in the subgroup with loose stools most of the time or always, 56% (9 patients) displayed F-calprotectin values ≥50 mg/kg (versus 31% in the axSpA group without IBS symptoms).

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Supplementary Table S3. Associations between IBS symptoms and F-calprotectin in axSpA patients

	n	F-calprotectin (mg/kg) Geometric mean (95% CI)	p-value [a]
Patients with no IBS symptoms	107	35 (28 to 43)	N.A.
Patients with IBS symptoms	50	31 (20 to 46)	0.548
Patients with IBS symptoms reporting: [b]			
More frequent bowel movements (any level [c])	45	31 (20 to 48)	0.598
More frequent bowel movements most of the time or always	14	49 (22 to 112)	0.287
Loose stools (any level [c])	47	30 (20 to 47)	0.538
Loose stools most of the time or always	16	66 (33 to 132)	0.038

[a] For comparison of Log_{10} F-calprotectin with patients with no IBS symptoms (by Student´s *t* test). [b] In conjunction with abdominal pain or discomfort. [c] Sometimes, often, most of the time, or always (as opposed to never or rarely). N.A. not applicable. Patients with missing F-calprotectin, n (%): 25 (14%).

Finally, in a review of studies assessing more general populations not restricted to SpA, the pooled sensitivity and specificity of F-calprotectin ELISAs to differentiate between IBD and IBS were estimated to be 93% (range 83%-100%) and 94% (range 60%-100%), respectively, when applying the manufacturers' recommended 50 mg/kg cut-off (as also used in this study).[7] In SpA populations, however, where a large proportion of patients (35-75% in different studies) display elevated F-calprotectin (≥50 mg/kg),[6, 8-11] the situation is likely to be different. Indeed, in a recent study of 64 SpA patients, a F-calprotectin cut-off of 132 mg/kg was found to yield the optimal sensitivity (67%) and specificity (77%) for the detection of small bowel Crohn´s disease, as diagnosed by video capsule endoscopy.[12] Furthermore, in respect to identifying cases with microscopic (histological) bowel inflammation at ileocolonoscopy, another study of 44 SpA patients found a F-calprotectin cut-off of 85 mg/kg to render the optimal sensitivity (64%) and specificity (73%).[13] Similar to the results in our main analysis (Figure 1B; Supplementary Table S2), applying these alternative F-calprotectin cut-offs in our material did not reveal any significant overall associations between IBS symptoms and F-calprotectin levels (unadjusted OR for IBS symptoms in patients with F-calprotectin ≥132 mg/kg: 0.6 [95%CI 0.2-1.9], p=0.356; F-calprotectin ≥85 mg/kg: 1.5 [95%CI 0.7-3.4], p=0.297).

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