Serial Lipocalin 2 and Oncostatin M levels reflect inflammation status and treatment response in axial spondyloarthritis

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Abstract

Background: Informative serum biomarkers for monitoring inflammatory activity and treatment responses in axial spondyloarthritis (axSpA) are lacking. We assessed whether Lipocalin 2 (LCN2) and Oncostatin M (OSM), both have roles in inflammation and bone remodeling, may accurately reflect chronic inflammation and treatment response in axSpA. Previous reports in animal models showed involvement of LCN2 and OSM in gut inflammation. We asked whether they also play a role in human axSpA.

Methods. Analysis of a longitudinal observational axSpA cohort (286 patients) with yearly clinical assessments and concurrent measurements of serum LCN2 and OSM (1204 serum samples) for up to 12 years. Biomarkers levels were correlated with MRI scoring and treatment response.

Results. Persistent and transient elevation of LCN2 and OSM were observed in axSpA patients. Persistent elevation of LCN2 or OSM, but not CRP, was correlated with sacroiliac joint MRI SPARCC scores (Pearson's correlation $p = 0.0005$ and 0.005 for LCN2 and OSM respectively). We observed both concordant and discordant patterns of LCN2 and OSM in relationship to back pain, the cardinal clinical symptom in axSpA. 26% (73/286) of the patients remained both clinically and serologically active (CASA). 14% (40/286) of them remained clinically active with back pain, but were serologically quiescent (CASQ). 60% (173/286) of the patients became clinically quiescent, with back pain resolved, but 53% (92/173) of them were serologically active (CQSA). With respect to treatment responses, transient elevation of LCN2 or OSM over time was predictive of better response to all treatments. The data suggest that failure to normalize LCN2 and OSM indicates persistent chronic inflammation, as reflected by positive MRI imaging of the sacroiliac joints. Prevalence of severe ankyloses is comparable in CQSA and CASA patients, indicating that disease progresses even when back pain may be controlled (CQ)

Conclusion. In axSpA, persistent LCN2 and/or OSM elevation reflects chronic SIJ inflammation and suboptimal treatment response. In our cohort, half of the currently deemed clinically quiescent patients with back pain resolved, continued to demonstrate chronic inflammation. LCN2 and OSM profiling outperforms CRP as a predictive measure and provides an objective assessment of chronic local inflammation in axSpA patients.

Background

Ankylosing spondylitis (AS), a subgroup of axial spondyloarthritis (axSpA), is a progressive debilitating disease. Both genetic and environmental effects such as microbial factors contribute to the pathogenesis of AS. To date, there is no available model which adequately characterizes the biological determinants for axSpA development.

Current management of AS is challenging: First, there is a lack of informative serological markers to assess treatment response. Current parameters used to evaluate treatment effectiveness include BASDAI and C-reactive protein (CRP) which is elevated in less than 30% of AS patients with active disease [1]. The

AS disease activity score (ASDAS) incorporates some core elements of the BASDAI with the CRP [2]. Better biomarkers are needed for personalized management of axSpA patients. Secondly, TNFi therapies while not being curative, are highly effective in controlling symptoms such as back pain, and improving quality of life of most patients. At least 30% of AS patients are not responsive to, or are intolerant of TNFi [3]. Biomarkers which accurately reflect local joint inflammation and treatment response could aid clinicians in better disease management.

Research on the gut-joint axis in AS is emerging. Aside from immune cells [4], we explore the role of pleiotropic factors in AS pathogenesis. Lipocalin 2 (LCN2) is an acute phase protein released in response to microbial triggers [5]. LCN2 is produced in multiple cell types in different tissues (including gut, joint and liver) and has both pro- and anti-inflammatory properties which are context-dependent [6–10]. Elevated LCN2 levels have been reported in patients with IBD [10], and psoriasis [11], which are common AS comorbidities. We have recently shown that in patients with concurrent AS and IBD, elevated LCN2 was associated with coexisting ankylosis and gut inflammation [12]. Here, we show that LCN2 is a local inflammatory biomarker which reflects treatment responses in AS patients.

Both LCN2 and oncostatin M (OSM) have known functions in inflammation and bone remodeling [13, 14], the hallmark of AS. In view of these common features, we propose that both LCN2- and OSM- associated pathways are involved in AS pathogenesis. There is a mechanistic basis to support this perspective: 1. *ank/ank* mice showed higher serum LCN2 in mutant mice with gut involvement [12]; 2. Intestinal inflammation in mice was driven by OSM [15]. To address whether these inflammation mechanisms play a similar role in human axSpA pathogenesis, we conducted an observational study.

Traditionally, single point measurement of all relevant clinical parameters from large cohorts of axSpA patients were used to decipher patterns with sophisticated statistical methods, and multiple clinical parameters have been used to reflect disease activity. The major limitations of this approach relate to the lack of understanding the biological connectivity among the multiple factors identified and the inability to stratify patients into more homogeneous subgroups. An alternate approach has recently been used to implicate a possible role of endothelin-1 in systemic sclerosis using repeated measurements of serological markers [16]. We chose to use the latter approach and performed a longitudinal association study of 286 axSpA patients (200AS and 86 nr-axSpA). In our study, we used the term axSpA to encompass AS (i.e. radiographic axSpA) and nr-axSpA patients as one entity. We evaluated the association of LCN2 and OSM levels with a single key clinical symptom (back pain) in treatment response of these patients.

Methods

Patients

286 axSpA patients (200 AS and 86 nr-axSpA) followed yearly (as per protocol for up to 12 years) with serum banking and concurrent clinical parameters in the Toronto Western Hospital Spondylitis Clinic, were assessed. Suppl. Table 1 summarizes demographic features of this cohort.

Study Approval

The study was approved by University Health Network (UHN) research ethics committee. All participating patients provided written informed consent. A written informed consent was received from participants prior to inclusion in the study. Participants were identified by number.

MRI scoring

37 axSpA patients with radiographic sacroiliitis scores of ≤ 3 and involvement of either LCN2 or OSM (but not both), had MRI assessments. Spondyloarthritis Research Consortium of Canada (SPARCC) scoring and Berlin spinal joint scoring [17-19] were evaluated. Scoring were done independently by two readers (IS and SL). The mean scores were used for correlation analysis with LCN2 or OSM levels (Pearson's correlation and Spearman's Rho [nonparametric] correlation).

Quantification of serum LCN2 and OSM levels by ELISA

The sequential samples (stored at -70°C) from each patient were thawed and analyzed at the same time to minimize assessment variabilities. Both LCN2 and OSM levels were measured by ELISA according to manufacturer's protocol (LCN2 ELISA kit: R & D Systems, DLCN20; OSM ELISA kit: Thermo Scientific, EHOSM).

Statistics

One-way analysis of variance (ANOVA), student's t-test were carried using GraphPad Prism program. A p -value of less than 0.05 was considered significant. Data are presented as mean $\pm$ standard error. Calculators from socsistatistics.com were used for Chi-square tests, Pearson's correlation coefficient, and Spearman's Rho correlation.

Results

Presence of two types of LCN2 (L) and OSM (O) elevation patterns in axSpA patients

Annual serial measurements of LCN2 and OSM levels (over a course of at least 4 years) from 286 patients, revealed two patterns of elevation: persistent or transient. In this study persistent elevation (p) is defined as elevation of LCN2 (Lp) or OSM (Op) levels which are sustained over a period of at least 2 years. Transient elevation (t) is defined as a single elevation over a period ≥ 2 years (Lt and Ot). 43% (123/286) of axSpA patients have involvement of the LCN2 pathway alone (Lp & Lt). 9% (27/286) have involvement of the OSM pathway alone (Op & Ot). 26% (74/286) have involvement of both pathways. The remaining 22% (62/286) have normal LCN2 and OSM levels (LnOn; Suppl. Table 2).

Patients with involvement of LCN2 pathway alone had no detectable OSM levels (On). Significantly higher LCN2 levels (mean $\pm$ SE) are found in those with persistent LCN2 (Lp) elevation (LpOn vs LtOn:

259 ng/ml $\pm$ 9 vs 193 ng/ml $\pm$ 7; $p < 0.0001$). LnOn patients have normal LCN2 levels (114 ng/ml $\pm$ 3; 150 ng/ml being the cutoff) and undetectable OSM levels (Fig. 1A).

Patients with involvement of OSM pathway alone had normal LCN2 levels (LnOp & LnOt: 97 ng/ml $\pm$ 6 & 78 ng/ml $\pm$ 11). Higher OSM levels are found in those with persistent OSM (Op) elevation (LnOp vs LnOt: 463 pg/ml $\pm$ 110 vs 61 pg/ml $\pm$ 14), though it is not significantly different. This may be due to the spread of levels in LnOp patients and too few LnOt patients for the comparison (Fig. 1B).

There are two groups of patients with involvement of both LCN2 and OSM pathways: Lp and Lt; each of which has 2 subgroups: Op and Ot. Comparison of patients LpOp vs LpOt reveals no significant difference in LCN2 and OSM levels (LCN2 levels in LpOp vs LpOt: 229 ng/ml $\pm$ 13 vs 227 ng/ml $\pm$ 18; OSM levels in LpOp vs LpOt: 575 pg/ml $\pm$ 125 vs 178 pg/ml $\pm$ 90; Fig. 1C). Comparison of patients LtOp vs LtOt shows no difference in LCN2 levels (LtOp vs LtOt: 186 ng/ml $\pm$ 8 vs 181 ng/ml $\pm$ 7); but there is a significant difference in OSM levels (LtOp vs LtOt: 978 pg/ml $\pm$ 20 vs 103 pg/ml $\pm$ 34, $p=0.0006$; Fig. 1D). It appears that only when LCN2 is transiently elevated, persistently elevated OSM levels (Op) are much higher than transiently elevated OSM levels (Ot). This result also implicates interactions between LCN2 and OSM in patients with involvement of both pathways. For this reason, the remaining results will focus just on analyses of patients who have involvement in a single pathway, either LCN2 or OSM. This approach has generated more informative insights with less complexities due to cross talks between pathways. Suppl. Table 3 summarized the different categories of patients.

The above results on the two patterns of elevation were based on annual measurements of LCN2 or OSM. We asked whether the elevation patterns could be established in less than a year. About 12% (34/286) of the axSpA patients in our cohort had more frequent assessment mainly due to acute increased back pain. Suppl. Fig. 1 showed that the pattern of LCN2 or OSM elevation or normalization could be established with repeat measurements 3 months apart.

MRI evidence of sacroiliac joint (SIJ) involvement is correlated with elevated LCN2, and OSM in axSpA patients

We previously showed that there is a relationship between circulating LCN2 and ankyloses [12]. In this study, we asked whether LCN2 plays a role in SIJ and/or axial inflammation. For this purpose, we selected axSpA patients with SIJ scores 3 or lower, as ankylosis was absent in these patients (mSASS=0)

For comparison of LCN2/OSM levels with MRI scores (SPARCC SIJ or Berlin spine score) [17-19], we used 3 patient subgroups based on their pathway involvement signature: LpOn (patients with persistent LCN2; n=12); LnOp (patients with persistent OSM; n=11); and LnOn (patients with normal LCN2 and OSM n=14). MRI taken within 12 months of the time of biomarker measurements were used for this analysis. In all axSpA patients, significant correlations are present between LCN2 levels and SPARCC SIJ scores (Fig. 2A; Pearson's correlation coefficient = 0.7, $p = 0.0005$; Spearman's Rho = 0.8, $p=0.0001$). Similarly, for LnOp and LnOn patients, there is a significant correlation between OSM levels and SPARCC SIJ scores (Pearson's correlation coefficient = 0.55, $p = 0.005$; Spearman's Rho: 0.57, $p=0.003$; Fig. 2B). Using

Pearson's correlation analysis, there is no correlation between LCN2 or OSM levels and Berlin Spine scores in the respective patient groups. However, for LpOn and LnOn patients, there is significant correlation between LCN2 levels and Berlin Spine scores using Spearman's Rho correlation analysis (Spearman's Rho: 0.5, $p=0.009$). These results suggest that persistent elevation of LCN2 or OSM is associated mainly with SIJ inflammation.

Association of treatment outcome with pathway involvement

Since persistent elevation of LCN2 or OSM reflects SIJ inflammation, we asked whether there is any association of LCN2 or OSM levels with the central clinical symptom back pain in the evaluation of treatment response of the patients. Using sequential LCN2 and OSM measurements and sequential back pain scores (question 2 of BASDAI survey) [20], concordant vs discordant association were observed in responses to all treatments (both with and without TNFi). Patients with the concordant pattern include those who were clinically quiescent and serologically quiescent (**CQSQ**) vs clinically active and serologically active (**CASA**). **CQSQ** patients had normalized LCN2 and/or OSM levels as well as reduced back pain scores (<4) after treatments. **CASA** patients remained having persistently elevated LCN2 and/or OSM, and back pain scores (>4) after treatments. Patients with the discordant pattern include those who were **CQSA** vs **CASQ**. **CQSA** patients had back pain resolved but remained serologically active. **CASQ** patients had persistent back pain even though they were serologically quiescent. Persistent back pain in **CASQ** patients is likely not due to involvement of LCN2 and OSM pathways. Alternatively it could reflect a possibly non-inflammatory nature of back pain in **CASQ** patients.

We questioned how the pathways involving LCN2 or OSM might affect the outcome of treatments, both with and without TNFi. We first compared treatment response in patients having involvement of the LCN2 pathway alone. For patients with persistent LCN2 elevation (LpOn), profiling indicated that both concordant and discordant patterns were observed. Patients with concordant response were mostly **CASA** (35% [29/82]). Only 12% [10/82] are **CQSQ**. Most of the patients with discordant response are **CQSA** (pain resolved but LCN2 remained elevated; 50% [41/82]). Only two patients with discordant response are **CASQ** (pain persisted but with normal LCN2; 2% [2/82]; Table 1A Left Panel). Similarly, both concordant and discordant treatment responses are observed in patients with transient LCN2 elevation (LtOn). However, for patients with concordant treatment responses, significantly more of them are responders [**CQSQ**] (LpOn vs LtOn; **CQSQ**: 11% vs 71%; **CASA**: 35% vs 7%; $\chi^2 = 27.9$, $p<0.00001$; Table 1A Right Panel).

Out of 62 LnOn patients, 58% (36/62) are deemed responders [**CQSQ**] as defined by normal LCN2, undetectable OSM and low back pain scores. The remaining 42% (26/62) showed discordant treatment response, having the **CASQ** pattern as both LCN2 and OSM were persistently normal, but back pain persisted (Table 1A Left Panel).

Profiling treatment responses in patients with involvement of OSM (O) pathway alone (LnOp and LnOt) revealed differences compared with those found in LpOn patients with involvement of LCN2 (L) alone. All LnOp patients with concordant treatment responses are **CASA** (63% [15/24]; with persisting elevated OSM levels and back pain >4). Those with discordant treatment response are all **CQSA** (pain resolved but

elevated OSM levels persisted; 38% [9/24]; Table 1B). Unlike LnOp patients, all LnOt patients in our cohort (n=3) are **CQSQ** with both pain resolved and OSM levels normalized. Thus, among patients with involvement of only one pathway (either LCN2 or OSM alone; comprising 43% and 9% of our cohort respectively; Table 1), transient elevation of LCN2 or OSM during the disease course appears to be an indicator of better response to all treatments (Table 1A & 1B). In addition, current treatments are less effective in LnOp patients. None of the 24 LnOP patients in our cohorts were **CQSQ**, although about half of them are **CQSA** with back pain resolved but OSM elevation persisted (Table 1B).

Table 1 Association of treatment outcome with LCN2 and OSM in axSpA with a single pathway involvement. A. Left Panel: Comparison of treatments outcome in patients with normal OSM but persistent LCN2 elevation (LpOn) vs. transient LCN2 elevation (LtOn) vs. normal LCN2 (LnOn). Right Panel: Comparison of treatments outcome in patients with normal OSM but persistent LCN2 elevation (LpOn) vs. transient LCN2 elevation (LtOn). B. Comparison of treatments outcome in patients with normal LCN2 but persistent OSM elevation (LnOp) vs. transient OSM elevation (LnOt). CQSQ: clinically quiescent, serologically quiescent; CASA: clinically active, serologically active; CQSA: clinically quiescent, serologically active; CASQ: clinically active, serologically quiescent. Pearson's Chi-square test was used.

A Involvement of LCN2 (L) pathway alone

		All treatments (with and without TNFi)			
Pathway involved	LCN2 (L) mean±SE (ng/ml)	Concordant (LCN2 vs back pain)		Discordant (LCN2 vs back pain)	
		CQSQ (%)	CASA (%)	CQSA (%)	CASQ (%)
LpOn (n=82)	259±9	10/82 (12)	29/82 (35)	41/82 (50)	2/82 (2)
LtOn (n=41)	193±7	29/41 (71)	4/41 (10)	1/41 (2)	7/41 (17)
LnOn (n=62)	114±3	36/62 (58)			26/62 (42)

LpOn: more likely CASA			
Rx response	CQSQ	CASA	Chi ² (p-value)
LpOn	10	29	
LtOn	29	4	
			27.9 (<0.00001)

B Involvement of OSM (O) pathway alone

			All treatments (with and without TNFi)				
			Concordant (OSM vs back pain)			Discordant (OSM vs back pain)	
Pathway involved	LCN2 (L) mean±SE (ng/ml)	OSM (O) mean±SE (pg/ml)	CQSQ (%)	CASA (%)		CQSA (%)	CASQ (%)
LnOp (n=24)	97±6	463±110		15/24 (63)		9/24 (38)	
LnOt (n=3)	78±11	61±14	3/3 (100)				

Results on treatment response based on patients receiving TNFi vs no TNFi are summarized in Suppl. Tables 4 & 5. In general, treatment response profiles (with vs without TNFi) are similar. However, for TNFi-treated patients with LCN2 involvement only, LpOn patients with concordant treatment response, compared to LtOn patients, significantly more LtOn patients are responders [**CQSQ**] (LpOn vs LtOn; **CQSQ**:

12% vs 74%; **CASA**: 44% vs 6%; $\chi^2 = 28.5$, $p < 0.00001$; Suppl. Table 4A). Though the trend is similar in patients not treated with TNFi, there are no significant differences. This implicates that TNFi as being more effective in patients with LCN2 pathway involvement.

Profiling of response treatments in AS vs nr-axSpA patients showed similar patterns (data not shown). We also addressed whether HLA B27 status, gender, and comorbidities affected the outcome of treatment response by biomarkers profiling and found that none of these cofactors affected the analyses.

A novel perspective related to defects leading to axSpA development

Acute phase proteins (APP) play a prominent role in the defence mechanisms of the host innate immune system. Both LCN2 and OSM are known APP [21-24]. Transient elevation of APP is thought to play a protective role in host defense. However, failure to normalize APP in a timely manner would have pro-inflammatory consequences, resulting in chronic inflammation. An important implication of this hypothesis is that persistently elevated APP such as LCN2 and OSM lead to SIJ inflammation as reflected by the MRI results. Fig. 3 shows a schematic of this perspective.

CRP, an APP, contributes to systemic inflammation [25]. To further evaluate our hypothesis, we asked whether persistent elevation of CRP could be found in AS patients in our cohort. Nr-axSpA patients are excluded in this analysis as only 2 patients had elevated CRP in our cohort.

Similar to LCN2 and OSM, both transient and persistent elevations of serum CRP are found in AS patients as expected. Patients with any CRP elevation (Cp or Ct) are predominantly found in patients with LCN2 pathway involvement (LpOnCp and LpOnCt; 43% [28/65] and 22% [14/65] respectively of LpOn patients in our cohort). LpOnCp patients had higher LCN2 levels (compared to levels from LpOnCt and LpOnCn patients; $293 \text{ ng/ml} \pm 21$ [LpOnCp] vs $235 \text{ ng/ml} \pm 11$ [LpOnCt] vs $243 \text{ ng/ml} \pm 12$ [LpOnCn]; one-way ANOVA $p = 0.04$; Fig. 4A). LpOnCp patients also had higher CRP levels, compared to levels from LpOnCt patients ($49 \text{ mg/L} \pm 6$ [LpOnCp] vs $27 \text{ mg/L} \pm 5$ [LpOnCt]; $p = 0.02$; Fig. 4B).

We asked whether persistent vs transient CRP elevation might have differential effects on treatment response (with and without TNFi) in LpOn patients (LpOn**Cp** vs LOn**Ct**). LpOn**Cp** patients have higher and lower % of **CASA** and **CQSQ** patients respectively (**CASA**: 36% vs 21%; **CQSQ**: 7% vs 29%; LpOnCp vs LpOnCt respectively; Table 2); though $p = 0.06$ for χ^2 test. Taken together, persistent CRP elevations (Cp) likely have a small effect, if any, on treatment response outcome in LpOn patients.

Table 2 Association of treatment outcome with CRP in AS patients with LCN2 pathway alone. In patients with the involvement of LCN2 pathway alone, (A) Comparison of treatments outcome in patients with normal CRP (LpOnCn) vs. transient CRP elevation (LpOnCt) vs. persistent CRP elevation (LpOnCp). (B) Comparison of treatments outcome in patients with transient CRP elevation (LpOnCt) vs. persistent CRP elevation (LpOnCp). CQSQ: clinically quiescent, serologically quiescent; CASA: clinically active, serologically active; CQSA: clinically quiescent, serologically active; CASQ: clinically active, serologically quiescent. Pearson's chi-square test was used.

A

All treatments (with and without TNFi)				Concordant (LCN2 vs back pain)		Discordant (LCN2 vs back pain)	
pathways(s) involved	AS (n)	LCN2 (L) mean $\pm$ SE (ng/ml)	CRP (C) mean $\pm$ SE (mg/L)	CQSQ (%)	CASA (%)	CQSA (%)	CASQ (%)
LpOn Cn	23	243 $\pm$ 12	<11 (normal)	3/23 (13)	8/23 (35)	11/23 (48)	1/23 (4)
LpOn Ct	14	235 $\pm$ 11	27 $\pm$ 5	4/14 (29)	3/14 (21)	7/14 (50)	
LpOn Cp	28	293 $\pm$ 21	49 $\pm$ 6	2/28 (7)	10/28 (36)	16/28 (57)	

B

Rx response	CQSQ	CASA	Chi ² (p-value)
LpOnCt	4	3	
LtOnCp	2	10	
			3.4 (0.06)

Discussion

Important findings in study include: (1) the identification of involvement of two APPs, LCN2 and OSM, acting singly or in combination, in axSpA. Elevated baseline LCN2 [12] and OSM [26] levels were reported in axSpA patients. Here, we showed two patterns of LCN2 or/and OSM levels. Persistent elevation of LCN2 alone (LpOn; 43%:123/286) is more prevalent than persistent elevation of OSM alone (LnOp; 9%: 27/286). (2) the first demonstration that LCN2 and OSM levels correlated with MRI SPARCC SIJ scores and thus reflect SIJ inflammation, the cardinal feature of axSpA. Importantly, CRP reflecting systemic inflammation has no correlation with MRI SPARCC SIJ scores (Suppl. Figure 2). (3) the demonstration that profiling of LCN2 and OSM levels, together with concurrent back pain scores during the disease course can effectively predict treatment responses in axSpA patients. Our clinical-serological approach has similarly been used in two diseases: SSc [27] and SLE [28, 29]. CQSA patients were identified in SLE. Our axSpA patients with discordant treatment responses (**CQSA**) had back pain resolved but LCN2 or OSM remained elevated. In our MRI inflammatory scores vs serological levels correlations, 88% (7/8) **CQSA** patients had positive MRI SIJ SPARCC scores, suggesting that persistent LCN2 or OSM levels are associated with SIJ inflammation (data not shown). The single **CQSA** patient who had negative MRI SIJ SPARCC scores, had positive Belin Spine scores. (4) Current axSpA management focuses on symptom (back pain) control. Of the 173 **CQ** patients in this study, 47% (81/173) were **SA** and 9 of them (11%) had mSASS > 50. This is in contrast to only 3% (3/92) **CQSQ** patients had mSASS > 50 ($p = 0.04$; Suppl. Table 6). **CQSA** and **CASA** patients had similar prevalence of patients with mSASS > 50 (11% and 13% respectively), indicating that though back pain may be controlled (**CQ**), the disease may progress if

LCN2/OSM continue to be elevated (**SA**). Thus, LCN2 and OSM profiling provides personalized and more effective treatment.

We used a different approach to assess treatment outcomes. We focused on early event due to LCN2- and OSM-related inflammation and back pain for the symptomatic clinical read-out. Using this strategy, we observed that HLA-B27 positivity, gender and comorbidities involvement did not affect the analyses. Our interpretation is that axSpA patients have common early pathogenic events. Heterogeneity of disease introduced later is influenced by cofactors such as HLA-B27 status, gender and comorbidities. Using this top-down approach, we aim to map the sequences of downstream events which together dictate the final clinical outcomes of axSpA with all the different parameters.

Patients with transient elevation of either LCN2 or OSM are more likely to have both inflammation and back pain resolved upon treatment (Table 1). There is a need for better therapeutic agents especially for resolving inflammation due to persistent OSM elevation. Half of these patients, irrespective of LCN2 status (LnOp, LtOp, LpOp) were deemed CASA, both pain and LCN2/OSM persisted, and the rest had back pain resolved [**CQSA**; 51% (37/73); but LCN2/OSM persisted] with and without TNFi treatment.

Published literature relating mainly to gut inflammation suggests that the LCN2 pathway is one of the targets for TNFi [30]. It also reflects the inability of TNFi alone to resolve LCN2-mediated inflammation. While TNFi block TNF α , other cytokines may persist to maintain the inflammatory process. IL17 synergizes with IL22 and TNF α to induce LCN2 expression in the colonic epithelium [30] as well as in bone cells [31]. To date, TNFi and IL17i agents seem comparable in clinical efficacy in axSpA [32].

In IBD, the primary cause of TNFi non-responsiveness is due to elevated mucosal OSM levels [15]. Subclinical and clinical gut inflammation are common in AS (about 60% and 10% respectively). It remains unclear what tissues constitute the primary source of high circulating LCN2 and/or OSM in axSpA patients. LCN2 and OSM act as APP in the gut and the kidney respectively [21–24, 33]. We showed that *ank/ank* mice with severe ankylosis [12] had higher serum LCN2 in mutant mice with gut involvement, suggesting that both gut and joints as possible sources of circulating Lcn2. It is unclear whether a similar situation occurs in our axSpA cohort, as our current protocol does not include colonoscopy in these patients without gastrointestinal symptoms.

Our finding that three acute phase proteins (LCN2, OSM and CRP) were found elevated persistently or transiently in our axSpA cohort, together with the observation that better treatment responses were detected from patients with transient elevation, support our hypothesis (Fig. 3) that persistent elevation of these biomarkers is pro-inflammatory. Delay in normalization of acute phase proteins in axSpA patients likely reflects a global defect as multiple acute phase proteins are involved. In our recent publication [34] searching for serum biomarkers associated with MRI and disease activities in golimumab-treated AS patients, there was a correlation between baseline serum levels of acute phase proteins (CRP, haptoglobin and serum amyloid P) with baseline ASDAS, but LCN2 and OSM were not assessed in that study. There may be a minority of patients in whom LCN2 and OSM are less informative, but this is not surprising due

to the heterogeneity of the disease. It remains to be investigated, using animal studies, whether transient vs persistent elevation of LCN2 or OSM have opposing outcomes relating to inflammation.

This report is focussed on the relationship of LCN2 and OSM on chronic inflammation. This is the reason why we chose patients with radiographic sacroiliitis scores of 3 or lower to analyze correlation of LCN2 or OSM levels with MRI inflammation scores. As low numbers of patients with single pathway involvement and MRI assessment were available for this study (n = 23), a larger study is warranted. Though MRI is currently the most sensitive tool for detection of joint inflammation, it is not without limitations such as false positives for patients with low back pain. The use of LCN2 and OSM monitoring serves as a pre-screen to determine whether the costly MRI is needed to confirm findings from LCN2/OSM profiling. We previously showed baseline serum LCN2 levels correlated with mSASS scores in patients with AS alone, and with no concurrent IBD [12]. Due to the low numbers of axSpA patients with OSM involvement in our current cohort, it remains to be investigated whether OSM also plays a role in ankylosis.

There are limitations in retrospective studies, as the data are dependent on available information and our analyses were complicated by some gaps in clinical information. Back pain, a subjective assessment, is used as the key clinical decision making. As gold standard is challenging in axSpA, LCN2 and OSM monitoring can help in this respect. Prospective studies with well-designed parameters are needed to evaluate more rigorously the power and the limitations of LCN2 and OSM as early biomarkers for axSpA patients.

Conclusions

In axSpA, persistent LCN2 and/or OSM elevation reflects chronic SIJ inflammation and suboptimal treatment response. In our cohort, half of the currently deemed clinically quiescent patients with back pain resolved continued to demonstrate chronic inflammation. Importantly LCN2 and OSM profiling outperforms CRP and provides a convenient and objective assessment of chronic local inflammation in axSpA patients. Together with concurrent back pain, LCN2 and OSM profiling provides precision management of axSpA.

Abbreviations

APP: acute phase proteins

ANOVA: one-way analysis of variance

AS: ankylosing spondylitis

ASDAS: AS disease activity score

axSpA: axial spondyloarthritis

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

CASA: clinically active and serologically active

CASQ: clinically active and serologically quiescent

CQSA: clinically quiescent and serologically active

CQSQ: clinically quiescent and serologically quiescent

CRP: C-Reactive Protein

Cp: persistent CRP elevation

Ct: transient CRP elevation

IBD: inflammatory bowel disease

LCN2: Lipocalin 2

Ln: normal LCN2 level

Lp: persistent elevation of LCN2

Lt: transient elevation of LCN2

MRI: magnetic resonance imaging

mSASSS: Modified Stoke AS Scoring System

nr-axSpA: Non-radiographic axial Spondyloarthritis

OSM: Oncostatin M

On: normal OSM level (undetected)

Op: persistent elevation of OSM

Lt: transient elevation of OSM

SIJ: sacroiliac joint

SPARCC: Spondyloarthritis Research Consortium of Canada

TNFi: Tumor Necrosis Factor inhibitor

UHN: University Health Network

Declarations

Ethics approval and consent to participate

All human studies were reviewed and approved by the University Health Network Research Ethics Board. All participating patients provided written informed consent. A written informed consent was received from participants prior to inclusion in the study. Participants were identified by number.

Consent for publication

All participating patients provided written informed consent for publication.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Competing interests

This work has been included as part of a provisional patent application filed by KeyIntel Medical Inc.

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Authors' contributions

FWLT: conceptual planning, designed the study, analyzed data, contributed to the statistical analyses, wrote and edited the manuscript.

RDI: designed the study, researched data, wrote and edited the manuscript.

AL: researched data, contributed to the statistical analyses and edited the manuscript.

IS: contributed to the MRI scoring and data analysis.

HWT and ZZ: designed research studies, assayed the biomarkers, and analyzed data.

FWLT and RDI are the guarantors of this work, and as such, had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis.

All authors read and approved the final manuscript.

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Competing Interests

Findings in this manuscript constitute part of a provisional patent filed by KeyIntel Medical Inc.

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Figures

Figure 1

OSM and/or LCN2 levels in axSpA patients with single or both pathways involvement. A. With LCN2 pathway alone (normal OSM; On): LCN2 levels in patients with persistent (LpOn) vs. transient (LtOn) vs. normal LCN2 (LnOn); B. With OSM pathway alone (normal LCN2; Ln): LCN2 and OSM levels in patients with persistent (LnOp) vs. transient OSM (LnOt); C. With both pathways involved (persistent LCN2; Lp): LCN2 and OSM levels in patients with persistent (LpOp) vs. transient OSM (LpOt); D. With both pathways involved (transient LCN2; Lt): LCN2 and OSM levels in patients with persistent (LtOp) vs. transient OSM (LtOt). One-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison and Student's t test were used. The above results on the two patterns of elevation were based on annual measurements of LCN2 or OSM. We asked whether the elevation patterns could be established in less than a year. About 12% (34/286) of the axSpA patients in our cohort had more frequent assessment mainly due to acute increased back pain. Suppl. Fig. 1 showed that the pattern of LCN2 or OSM elevation or normalization could be established with repeat measurements 3 months apart.

Figure 3

A novel perspective for axSpA development. Upon external triggering agents, acute phase proteins (including LCN2 and OSM) are produced by the innate immune system to combat acute infection. Transient elevation of these proteins is thought to play a protective role in host defense. Nevertheless, failure to normalize these self-defence proteins in a timely manner would cause local chronic inflammation. Eventually, it leads to excessive tissue repair, including ankylosis in spondylitis.

Figure 4

Detection of LCN2 and CRP levels in AS patients with the involvement of LCN2 pathway alone. A. LCN2 levels were compared in patients with normal CRP (LpOnCn) vs. transient CRP elevation (LpOnCt) vs. persistent CRP elevation (LpOnCp). B. LCN2 and CRP levels were compared in patients with transient CRP elevation (LpOnCt) vs. persistent CRP elevation (LpOnCp). One-way analysis of variance followed by Bonferroni's multiple comparison test and Student's t test were used.

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