

Unraveling the heterogeneity of chronic inflammatory diseases: Lessons learned from axial spondyloarthritis (axSpA)

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Commentary

A key feature of chronic inflammatory disease is heterogeneity. For clinicians, this poses problems not only in disease diagnosis and assessment, but also in providing personalized disease management for the patients. There are at least two explanations for disease heterogeneity: First, different patient subgroups have different etiologies and subsequent pathways involvement, leading to a similar clinical outcome. Secondly, patients have similar etiologies but with different pathway modulations in patient subgroups, resulting in different degree of clinical outcome. Even with these two scenarios, they are not mutually exclusive.

The lack of understanding of the different pathogenic pathways leading to similar clinical outcome for individual inflammatory disease is the major hurdle to unravel disease heterogeneity. We have recently published two articles on axial spondyloarthritis (axSpA) [1,2] which serve as examples of a successful approach to address this issue.

It has been known for decades that two key factors (HLA-B27 positivity and maleness) are prominent in axSpA patients with more severe disease [3-5]. The end-stage of radiographic axSpA (r-axSpA) patients is complete spinal fusion and most of these patients are HLA-B27 positive male patients. Despite huge efforts in both genetic and immunological research on delineating the role of B27 positivity and male sex in r-axSpA pathogenesis, it remains unclear how these two factors contribute to spinal fusion.

In Search of Appropriate Serological Biomarkers Which Reflect SIJ Inflammation and Spinal Progression

For selection of more homogeneous patient subgroups, informative biomarkers are required. Clinically, CRP serves as the most commonly used inflammatory biomarker [6]. CRP has been shown to be a predictor of r-axSpA radiographic progression especially in men [7,8]. However, CRP has long been viewed as a biomarker of systemic inflammation. More appropriate inflammatory biomarkers for axSpA would be those relevant to local inflammation in the joints, as most axSpA patients do not have systemic inflammation. We recently identified LCN2 and OSM as informative biomarkers for local inflammation at target sites [1]. Serum LCN2 and OSM levels correlate with MRI SPARCC SIJ scores and thus reflect SIJ inflammatory activity. In our cohort, there is no correlation of CRP with MRI SPARCC SIJ scores, supporting the concept that CRP does not accurately reflect local joint inflammation.

Chronic inflammation in axSpA patients is associated with persistent elevation of LCN2 and OSM levels in the sera [1]. In axSpA, the predominant pathogenic pathway appears to involve LCN2. Fewer patients have involvement of the OSM pathway. In about 200 axSpA patients with persistently elevated LCN2/OSM levels, 55% have involvement only in the LCN2 related pathway; 12% have involvement only in the OSM pathway and 33% have involvement in both pathways.

Our finding that three acute phase proteins (LCN2, OSM and CRP) were elevated persistently or transiently in our cohort of axSpA patients, together with the observation that worse treatment

response were detected in patients with persistent LCN2/OSM elevations, support our hypothesis that persistent elevation of these biomarkers is pro-inflammatory [1]. Delay in normalization of acute phase proteins in axSpA likely perpetuates chronic inflammation. In this regard, LCN2 and OSM pathway involvement represents early events in axSpA pathogenesis. Persistently elevated LCN2/OSM impacted the treatment response outcome (back pain) in our cohort of axSpA patients, but persistently elevated CRP appeared to have less association with the treatment response outcome [1]. Previous report in the literature showed poor correlation of CRP with clinical response of biologic treatments [9]. In our studies [1], the profiling outcomes of treatment response were not affected by cofactors such as HLA-B27 status, gender and comorbidities. This observation likely suggests that axSpA patients have common early pathogenic events. In subsequent clinical course of the disease, heterogeneity is influenced by HLA-B27 status and gender [2].

Biomarkers from early pathogenic events can facilitate not only earlier diagnosis of the disease, but also tracking of disease progression. In a previous publication [10] 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. Delay in normalization of acute phase proteins in axSpA patients likely reflects a global defect with involvement of multiple acute phase proteins. There may be a minority of patients in whom LCN2 and OSM are less informative. However, we prefer the interpretation that contrary to haptoglobin for example, both LCN2 and OSM have known functions in joint inflammation and bone remodeling and thus, they are the predominant pathological factors in axSpA. Not all elevated serological biomarkers would have pathological effects, a scenario similar to elevated autoantibodies in autoimmune patients in general.

LCN2 is a pleiotropic factor found in multiple tissues including joint, gut and liver [11]. Elevated serum LCN2 levels have been reported in multiple inflammatory diseases [12,13]. How then is disease specificity conferred in individuals with elevated LCN2 levels? In our studies [1,2], we have been measuring LCN2 homodimer/oligomers in the sera. LCN2 forms a hetero-complex with MMP9 (LM). MMP9 is expressed in inflammatory cells and osteoclasts and thus could modulate skeletal cell differentiation during bone repair [14]. *In vitro*, MMP9 has increased enzymatic activity when complexed with LCN2, partly due to being protected from degradation [15].

As with LCN2, axSpA patients have elevated LM levels as well [2]. Based on L/LM elevation profiles, there are 3 subgroups of axSpA patients: (a) Both LCN2 and LM levels are elevated (L+LM+; 52%) (b) only LCN2 levels are elevated (L+; 22%) and (c) only LM levels are elevated (LM+; 6%). In patients with elevated LCN2 and LM levels (L+LM+), Both L and LM levels correlate significantly with MRI SPARCC SIJ scores, implying that both L and LM reflects SIJ inflammation, the cardinal feature of axSpA. However, using Pearson's correlation, LM but not L is significantly correlated with MRI Berlin Spine scores, implying that LM might be a better biomarker for spinal inflammation than LCN2. Indirectly, this observation suggests that LM elevation might occur subsequent to L elevation since in axSpA, SIJ inflammation precedes spinal inflammation. Taken together, elevated L levels reflect mainly SIJ inflammation while elevated LM

levels reflect both SIJ and spinal inflammation [2]. Thus, LM is an informative biomarker for spondylitis progression.

Gender Differences

In the literature, gender differences in the availability of MMP9 in target tissues have been reported [16,17]. To avoid complication of data interpretation, first, our analysis was focused on axSpA patients with no detectable OSM [2]. Secondly, to assess the role of LM in joint inflammation, we analyzed r-axSpA patients with minimal ankylosis in the event that LM has roles in spinal ankylosis [2]. While all r-axSpA patients have similarly elevated L levels, irrespective of gender; female patients have significantly lower LM levels compared to male patients. There are distinctive L+LM+ or L+ patterns which can distinguish clinically aggressive vs milder course of disease respectively. Among patients with minimal spinal ankylosis, 65% (35/54) and 17% (9/54) male r-axSpA patients are L+LM+ and L+ respectively; while 32% (7/22) and 64% (14/22) female patients are L+LM+ and L+ respectively, supporting the well-known clinical observations that most male patients had more severe joint inflammation and disease progression. Male L+LM+ patients had higher L and LM levels compared to those with L+ or LM+ patterns, again implying L+LM+ patients have more severe joint inflammation.

HLA-B27 Status Differences in Male r-axSpA Patients

To assess the role of L and LM in spinal ankylosis, we asked whether L/LM levels might reflect spinal radiographic progression in OSM negative r-axSpA male patients [2]. Our cohort has less than 30 female r-axSpA patient to warrant a reliable assessment of this issue; and thus, analyses were focused on male patients.

In male r-axSpA patients, there are significant differences in the elevated L vs LM profiles between B27+ve vs B27-ve patients [2]. Compared to B27-ve patients, B27+ve patients had higher L and LM levels [2]. Profiling was carried out separately in two subgroups based on the status of spinal ankylosis (mSASSS<10 vs mSASSS>11) in both B27+ve and B27-ve patients.

For B27+ve patients, irrespective of spinal ankylosis status, >85% of them are L+LM+; albeit mSASSS>11 patients had significantly higher L and LM levels compared to mSASSS<10 patients. This observation suggests that more severe spinal ankylosis is associated with higher L and LM levels. Indeed, comparison of L+LM+ patients, mSASSS<10 vs mSASSS 11-55 showed that both L and LM levels (but not CRP) correlate with mSASS scores [2].

In B27+ patients with near fused spines (mSASSS 56-72), L levels remain elevated and correlate with mSASS scores. However, LM levels no longer correlate with mSASS scores, implying that joint inflammation in patients with near fused spine is likely due to L but not LM levels. Indirectly, this observation implies that LM levels are mainly responsible for spinal ankylosis progression. Throughout the disease course, elevation of LCN2 persists, but could fluctuate, irrespective of spinal ankylosis status.

For B27-ve patients, irrespective of spinal ankylosis status, about half of them are L+, but L levels are higher in mSASSS>11 patients [2]. L levels in L+ mSASSS>11 patients also correlate with mSASS scores. Thus, elevated L levels alone (in the absence of LM elevation) is associated with disease progression, albeit likely at a slower rate. In our cohort, out of four B27-ve patients with mSASSS>11, only one had a mSASS score of 20 after having low back pain for 36 years.

Key Messages from Our Novel Findings in AxSpa

1. Chronic joint inflammation in axSpA likely is reflected in the failure to normalize acute-phase proteins, LCN2 (L) and/or OSM in particular, in a timely manner post-acute infection, resulting in pro-inflammatory consequences.
2. Persistent serological elevations of L, LM, and OSM reflect joint inflammation as supported by MRI correlation results.
3. Elevation of L and LM are informative biomarkers (both qualitatively and quantitatively) for SIJ and spinal inflammation, as well as for ankylosing development in r-axSpA patients. These biomarkers outperform CRP which is currently deemed the best biomarker for axSpA.
4. Distinctive L+LM+ or L+ patterns provide explanation for (a) milder disease in female patients and in B27-ve male patients; (b) B27+ve male patients being highly susceptible to severe spinal ankylosis.
5. Significant clinical utilities of measuring serological L, LM and O levels include:
 - (a) Diagnose and reflect different stages of the axSpA disease process.
 - (b) Facilitate prediction of treatment response and disease prognosis.
 - (c) Identify clinically quiescent patients with back pain resolved; but with local inflammation remained ongoing.
 - (d) Enable precise targeted therapy: a need to find appropriate therapy for patients with persistent OSM positivity.

Future Perspectives

Using both repeated and single-point measurements of serologically biomarkers which reflect local inflammation, we have identified critical early events/pathways in axSpA pathogenesis: the LCN2-associated and OSM-associated pathways; and thus, open new avenues for more detailed assessments of the complex axSpA disease. Some examples are illustrated as follows:

1. The use of animal models to find support in our interpretations on the human axSpA studies:
 - (a) Assessing whether transient vs persistent elevations of L, LM and OSM have opposing outcomes relating to joint inflammation (resolution of inflammation vs development of chronic inflammation).
 - (b) Determining how LCN2 and OSM would mediate the link between the local gut and joint inflammation.Research in this aspect would shed light on pathogenesis of not only axSpA, but also IBD.
 - (c) Using B27+ve rodents to evaluate the role of LCN2/OSM in pathogenesis and whether there are gender differences.
2. More detailed human studies: Validation of our observations is needed as they are from retrospective studies. Prospective studies with well-designed parameters are warranted. By grouping patients based on pathway involvement, homogeneous features for patient subgroup stratification could be identified. There are major challenges; the critical one being the need of large patient

cohorts such that minor subgroups would have sufficient cases for meaningful statistical analyses. Collaborative efforts are needed. However, there could be population differences. Peripheral and extramusculoskeletal SpA manifestations have significant geographic regional differences [18].

3. Our approach to unravel early events could be used to investigate other chronic inflammatory diseases such as IBD.

Author Contribution

FT and RD contributed to the article and approved the submitted version.

Conflict of Interest

This work has been included in a PCT application filed by KeyIntel Medical Inc.

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