B3: "Is there an immune proteome in PJI?"

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Response/Recommendation: Yes, there is an immune proteome in PJI, and our understanding of this proteome continues to grow and evolve.

Level of Evidence: Expert Opinion
Delegate Vote: Agree: [% vote], Disagree: [%], Abstain: [%]

Rationale: Periprosthetic joint infection (PJI) remains a major cause of arthroplasty failure after total knee (TKA) or hip arthroplasty (THA). While the understanding of the underlying mechanisms of PJI development and persistence continues to evolve, so does the technology available to study all aspects of the PJI process. The role of the immune system in PJI is also becoming more evident, introducing the idea of an immune proteome in PJI. Conceptually, the immune proteome is the collection of proteins produced by the human immune system, including antibodies, cytokines, chemokines, and other immune cell signaling molecules, in response to an antigen challenge. Recently, proteomic profiling of host compartments in PJI has garnered interest in advancing knowledge and improving diagnostic capabilities to help address the difficult-to-distinguish PJI cases[2; 3]. Given the complexity of the host, it is important to understand the different compartments/tissues sampled, including—locally—synovial tissue, bone, implant sonicate fluid, and synovial fluid and—systemically—peripheral blood. Each of these sampling locations will have unique proteomic profiles compared to other compartments. While all have been studied in various capacities with respect to the proteome, serum and synovial fluid have been the most predominantly studied to date, likely due to their accessibility and current role in PJI diagnosis.

Recent high-throughput approaches such as mass-spectrometry (MS), protein pathway array, multiplexed assays (Luminex, Meso-scale Discovery [MSD], Simoa, etc) have advanced the capacity to study the proteome in an more complete and unbiased fashion, overcoming some limitations to techniques such as ELISA, immunohistochemistry staining (IHC), western blot, and others also employed to study the proteome. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based profiling paired with bioinformatic interpretation has been utilized to differentiate PJI from aseptic failure [2; 4-7]. From these studies, three of which involved synovial fluid and one implant sonicate fluid, several proteins were identified as increased in PJI compared to aseptic revision in each case, including proteinase3 (PRTN3), myeloid cell nuclear differentiation antigen (MNDA), and lactoferrin (LTF). However, there was some discrepancy between studies potentially based on sample location (synovial fluid vs. tissue [bone] vs. sonicate fluid) with MNDA and PRTN3 in particular [2; 4-7]. Further overlap between studies involved increased abundance of certain proteins in PJI compared to aseptic revision—to varying degrees— including C-reactive protein (CRP), S100A8/A9, CORO1A, HISTH2HBL, SERPINB1, PGD, APCS, ACTN1, APOD, CAP1, LRG1, ANXA2, PRG4, CRTAC1, LCN2, MPO, CTDG, MMP9, and PYGL [2; 4-7]. To summarize these findings, based on pathway enrichment analyses, the pathways that were enriched in PJI cases generally involved haemostasis, innate immune system, extracellular matrix organization, and protein metabolism [2; 4-7]. Many of these proteins have recently gained attention and interest in multiple fields of medicine and various conditions.

When focusing on alternative, non-MS methods of investigating the proteome, synovial fluid is a popular target for study of multiplex assays and ELISA assessment of

signaling molecules in particular. For example, interleukin-6 (IL-6), has been the subject of many studies over the years, and is consistently found to be elevated in PJI compared to aseptic revision [3; 8-15]. Furthermore, IL-6 has been shown to be elevated in PJI compared to aseptic revision for total shoulder arthroplasty, thus demonstrating analogous findings in a distinct joint when compared to the literature concerning the hip and knee [16]. One study did find no change in IL-6 for TKA/THA PJI vs aseptic revision, highlighting the differences in measurement techniques and the impact of assay design/manufacturer [17]. Additional proteins commonly—but not always— found to be increased in PJI vs aseptic revision include IL-8, IL-1β, IL-10, CRP, IFN-γ, TNF-α, and many other cytokines/chemokines involved in the immune response/signaling [8-11; 14; 17; 18]. Additionally, within the synovial fluid, multiple individual proteins have been identified as increased in PJI, including Lipocalin-2 [19], complement proteins (C1q, C3b/C3i, C4b, C5, C5a, MBL, properdin) [20], antimicrobial peptides (LL-37, HBD-3) [10], and those currently included in at least one PJI defining criteria such as leukocyte esterase [21] and alpha-defensin [22]. The data clearly supports proteomic variation between PJI and aseptic revision in the synovial fluid, and our understanding of these variations continues to evolve with technological and study design improvements.

Fewer studies have investigated the proteome of the tissue compartment than those studying the synovial fluid. However, the studies investigating tissue (synovium, bone/synovium, and undefined) were also consistent in identifying IL-6 and IL-1β as cytokines increased in PJI cases [23-25]. The other proteomic variations were less consistent between studies within the tissue space, including TNF-α, IL-10, IL-4, among others [23-25]. Additionally, Warren et al. found immune checkpoint protein PD-L1 to be significantly increased in PJI tissues compared to aseptic revisions, but no major difference in PD-1 abundance [26]. Moreover, a recent study demonstrated that checkpoint protein TIM-3 can be leveraged to prognose adverse outcomes in *S. aureus* PJI patients [27]. Tissue analyses are likely to vary widely based on tissue type (bone vs synovium, etc.) and based on variation in processing. Chen et al. performed proteomic analysis of PJI bone tissues and found some similarly abundant proteins in the tissues as were identified in the synovial fluid, namely LTF, and PRTN3, while also finding the complement system proteins to be highly enriched [7]. Thus, even within the tissue space an immune proteome exists in PJI compared to aseptic revisions.

Several studies have investigated systemic variations in proteins in PJI. Among the most widely identified markers increased in peripheral blood/serum of PJI compared to aseptic revision are IL-6 calprotectin, and cell-free DNA (cfDNA) [10; 13; 17; 28; 29]. Other proteins systemically increased in PJI in at least one study include HBD-2, IL-4, IL-17, BAFF, APRIL, and neutrophil elastase [10; 13; 17; 28; 29], while IL-1α, IL-1β, IL-8, G-CSF, and TNF-α [17] as well as LL-37 and HBD-3 showed no change [10]. Interestingly, a recent study highlighted that low serum albumin levels highly correlated with the presence of an *S. aureus* infection in a PJI setting and associated with poor clinical outcomes in these patients [30]. It is unclear how many of these cases had more systemic symptoms that may have contributed to systemic protein variability, however, the current evidence suggests there is also some level of proteomic variability within the peripheral blood/serum in PJI cases compared to aseptic revisions and more work in this area is needed, including potential multi-omics approaches, to further elucidate the extent of the systemic proteome in PJI.

In addition to cytokines, several studies have evaluated the pathogen-specific humoral immune proteome of PJI patients in peripheral blood (serum, plasma, etc.) [31-37]. Recent work has focused on the idea of protective vs. pathogenic immune proteome contributing to clinical outcomes in PJI patients. For instance, patients (from an international biospecimen registry [38]) with high antibody titers against an *S. aureus* heme-scavenging protein IsdB were highly likely to have postsurgical adverse events such as arthrodesis, reinfection, amputation,

and septic death [39; 40]. In sharp contrast, antibodies against the glucosaminidase (Gmd) subunit of *S. aureus* autolysin (Atl) in patients correlated with a marked reduction in adverse outcomes [34]. Additionally, significant reductions in adverse outcome risks in patients were also associated with high IgG titers against other *S. aureus* antigens such as Amd, CHIPS, SCIN, and Hla [39]. Collectively, these studies suggest that the humoral immune proteome can be leveraged as a prognostic tool to determine clinical outcomes in PJI patients.

Conclusion

In total, novel high-throughput approaches paired with more targeted protein investigations indicated there is an immune proteome in PJI compared to aseptic revisions. This proteome involves the synovial fluid, implant sonicate fluid, local tissues, and peripheral blood. As more advanced and unbiased techniques evolve and become more readily available, so too will our understanding of this proteome and how it can be used for diagnosis, treatment, and, ultimately, prevention of PJI in the future.

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