Q16. Are there systemic biomarkers of pain that could be used (in parallel with or as surrogates for pain behaviors) in pre-clinical models to define pain phenotypes/chronicity and/or therapeutic subtype?

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Response: No. In OA preclinical models, understanding the systemic biomarkers related to pain is crucial for developing translational strategies that connect laboratory findings to clinical applications. These biomarkers can be obtained from various sources, including blood, cerebrospinal fluid (CSF), urine, saliva, or peripheral tissues. They may provide insights into neuroimmune status, neural activity indicators, metabolic changes, and stress axis signaling. Biomarkers may facilitate the discovery of novel pain mediators and mechanisms leading to new therapeutic targets. Biomarkers may also be used to stratify cohorts into high- or low-biomarker status prior to interventions. However, most biomarkers currently require collection methods that add additional stress during handling, which may confound the data. Additionally, systemic or local biomarkers may change in response to changes in joint structure, not necessarily pain. Overcoming challenges such as species differences, assay standardization, and data integration will be crucial for the successful application of these biomarkers in preclinical OA research.

Level of Evidence: Moderate

Delegate Vote:

Rationale: Pain, especially inflammatory and neuropathic pain, is associated with peripheral and central immune activation and glial signaling. Despite the promising potential of systemic biomarkers, several challenges remain in adopting these approaches in pre-clinical OA models: **(1) Species Differences and Assay Sensitivity:** Although many biomarker targets are conserved between humans and mice, interspecies variations in expression levels, kinetics, and assay sensitivity require careful calibration. Assays must be rigorously validated in the animal model to ensure comparable sensitivity and specificity. **(2) Heterogeneity of Pain Measurement:** Pre-clinical pain assessment relies on behavioral tests that may not fully capture the multidimensional nature of pain. Variability in such tests can confound the interpretation of biomarker correlations. **(3) Temporal Dynamics:** The dynamic nature of biomarker expression necessitates longitudinal sampling. However, repeated sampling in small-animal models can be technically challenging and may affect animal behavior or welfare. **(4) Integrative Data Analysis:** Combining molecular data from serum with behavioral pain metrics requires robust statistical models and machine learning algorithms to define pain phenotypes accurately. Integrative studies must be designed with sufficient power to detect subtle correlations and causal relationships.

Implementation Strategy for Pre-Clinical Studies: To use biomarkers in parallel with behavior, several strategies exist. **(1) Longitudinal Sampling**: Unlike terminal tissue collection, serial collection of blood/plasma/urine/saliva in the same animal. This strategy allows correlating the trajectory of a biomarker with the trajectory of behavior (allodynia developing) or with improvements following interventions. **(2) The "Silent" Pain Check**: cortisol/corticosterone (stress markers) can confirm if the animal is in distress versus just sedated. **(3) Therapeutic Stratification**: Before randomizing animals to treatment, collect biomarker(s) and segregate them by biomarker levels (e.g., High-BDNF group vs. Low-BDNF group). This mimics human clinical trials and can explain why a drug works in only 50% of a cohort (responders vs. non-responders).

Current and Emerging Biomarkers:

1. Genetic and Epigenetic Markers (microRNAs): This is currently the most promising area for defining chronicity and neuropathic phenotypes. Unlike proteins, which degrade quickly, circulating microRNAs (miRNAs) are stable in serum/plasma and reflect gene expression changes driving central sensitization.[1] The utility of these biomarkers includes phenotyping to differentiate neuropathic from inflammatory pain

(distinct miRNA signatures exist for each) and determining chronicity by tracking the transition from acute injury to chronic maintenance, as miRNA profiles shift over weeks in rodent models.

2. Neurotrophic Factors & Neuropeptides: These markers are best used to define therapeutic subtypes, specifically, whether the pain is driven by central sensitization or peripheral drive. Systemic levels of Brain-Derived Neurotrophic Factor (BDNF) in plasma have been correlated with the severity of mono-iodoacetate (MIA) -induced OA pain behaviors. [2] Calcitonin Gene-Related Peptide (CGRP) is a validated biomarker for migraine models and is increasingly used in OA models. [3] Substance P and bradykinin neuropeptides also correlate with OA pain in a rat model. [1] The neurotrophic factor artemin has been shown to be associated with naturally occurring OA-pain states in dogs and cats, and synovial fluid concentrations have been shown to correlate inversely with joint pain.[4]

3. Inflammatory Mediators (Cytokines/Chemokines): These are the most used, but the least specific for pain itself. They are best used as surrogates for the driver of the pain, rather than the pain experience. Candidates include IL-6, TNF, IL-1β, IL-17A, IFN-γ, IL-10; CCL2 (MCP-1), CXCL1/2, CXCL10, CRP, C3/C5 fragments, among others. The utility lies in therapeutic subtyping, such as defining an "Inflammatory Profile." The downside to using these biomarkers is that stress alone during specimen handling and collection can spike these markers in mice, confounding the data.

4. Metabolomics and Lipids: This is an emerging field for defining therapeutic subtypes of OA related to the endocannabinoid system.[5] Serum Endocannabinoid (AEA, 2-AG) levels can be inversely correlated with pain thresholds. Lysophosphatidic Acid (LPA) is a lipid mediator that is strongly linked to neuropathic pain and joint damage.[6]

5. Neuroendocrine/autonomic stress axis (plasma/saliva/urine): Biomarkers here include serum or saliva corticosterone/cortisol, catecholamine metabolites in urine, and heart rate variability as a readout of the balance of the sympathetic and parasympathetic nervous system and pain. Lower HRV is associated with higher cortisol and chronic pain.[7]

6. Multi-omics Approaches: Integrating proteomics, genomics, and metabolomics offers the promise of a comprehensive biomarker panel that can capture the complexity of OA pain phenotypes. Such multi-omics approaches might uncover novel biomarkers that better predict therapeutic responses and chronic pain.

Summary: Integrating systemic biomarkers into pre-clinical OA pain evaluation offers a transformative opportunity. Combining serum markers with behavioral assessments enables more nuanced, predictive insights into OA pain phenotypes and chronicity. This enhances model precision and supports personalized therapies for specific pain subtypes. Challenges include sample variability and multiomic data complexity, highlighting the need for standardization and innovative analysis. Future steps involve translational studies, non-invasive sampling, and creating validated composite biosignatures for preclinical and clinical use. Standardized protocols for biomarker measurement, including sample collection, assay conditions, and behavioral tests, are crucial for reproducibility. Longitudinal studies linking behavioral and biomarker changes will clarify biological mechanisms of pain. Machine learning and multivariate methods can reveal complex relationships between biomarkers and behavior. Integrating diverse biological data into biosignatures furthers the goal of precision medicine in OA pain management. Overall, systemic biomarkers could revolutionize pain phenotyping in preclinical models and improve clinical outcomes.

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