ORS Consensus Article: Aging and OA: Question #7 - What are the minimum outcome measures required to make conclusions about OA from aging in pre-clinical models (e.g. histology, pain, activity, microbiome, ultrastructure of the joint tissues, subchondral bone changes, et al.).

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RECOMMENDATION: Studies of aging-related OA should employ a minimum outcome set consisting of at least one mechanistically relevant change beyond normal aging from two or more of the following domains: (1) structural tissue measures, (2) behavioral/functional measures, or (3) molecular/systemic measures. These domains were chosen because aging-related OA changes are variable, and measures from multiple modalities are likely needed to distinguish them from natural aging changes. Furthermore, reliance on structural criteria biases assessments toward moderate and late-stage disease while missing biologically meaningful, incipient OA changes that occur in important joint tissues (e.g. cartilage, synovium/joint capsule).

LEVEL OF EVIDENCE: Expert Opinion

RATIONALE: Diagnosing OA in clinical populations within the context of aging is complicated by OA's inherent pathologic heterogeneity[1–3] and the confounding effects of natural aging[4,5], which produces similar tissue changes. OA is distinguished from healthy aging by severity and multi-tissue involvement, often apparent in late stages but challenging at onset or early OA. Clinical OA diagnosis typically requires structural joint changes (e.g., osteophytes, joint space narrowing) and symptoms (pain, functional limitation), yet radiographic OA can be present asymptomatically across joints in 20–50% of adults over 60.[1,6–10] These criteria bias current clinical definitions toward moderate/late OA, with limited tools for early detection, and is mirrored in veterinary patient populations (cats, dogs, horses).[11–13]Thus, when studying age-related OA in which the transition from age-related tissue changes to pathology is likely gradual, subtle, and not well-defined, early tissue and symptom changes across myriad biological systems should be considered, though they may not be the gold standard outcome metrics used to evaluate other models of moderate/late OA.

Preclinical Modeling Landscape

Common preclinical OA models include biomechanics manipulation (post-traumatic, immobilization), metabolic/obesity-induced, transgenic, chemically induced, and spontaneous aging. [14–21] Each has strengths and limitations in modelling different aspects of OA pathology which should be considered when designing age-related OA studies. Given the diversity of pathogeneses, heterogeneity of outcomes is substantial.[21] Defining which pathological changes are unique to age-related OA, versus those driven by other model mechanisms in aged animals, is essential to disentangle aging effects from model-specific biology.

Defining Aging-Related OA and the Need for a Minimum Outcome Measures

Aging-related OA encompasses: (**1**) spontaneous OA in aging animals, and (**2**) age effects superimposed on other OA models (e.g., post-traumatic OA in aged vs. young). Natural aging features include progressive cartilage matrix thinning and proteoglycan loss; subchondral bone sclerosis and osteophytes; joint capsule fibrosis; low-grade synovial inflammation; altered sensory nerve density with heightened nociceptor sensitization; and systemic immune/microbiome shifts toward chronic low-grade inflammation.[4,22–25] These changes transition to

OA pathology when tissue dysfunction exceeds compensatory capacity. Preclinical models are well positioned to identify this transition to early OA and to define OA onset, where clinical studies struggle. Because aging and OA changes overlap, a minimum outcome set that distinguishes healthy aging from OA is needed.

Core Structural Outcome Measures

Studies should account for independent aging effects on joint tissues when assessing OA-related degeneration. At minimum, studies should include quantitative cartilage, subchondral bone, and joint capsule/synovial measures. Histology remains the gold standard for cartilage and synovium;[3,13,26–30] micro-computed tomography (μCT) is the gold standard for bone.[31–35] Researchers should choose the most rigorous method feasible without compromising other assessments and standardize sampling planes, regions of interest, and timing. Where available, researchers should use advanced image analysis (e.g., validated ML pipelines for cartilage segmentation and quantitative bone histomorphometry) to improve speed, sensitivity, and reproducibility.[36–39]

Behavioral and Functional Outcome Measures

Behavior and function are essential analogues to quality-of-life symptoms as well as factors that may exacerbate pathogenesis. Because aging itself[40–45] alters behavior, mobility, and stress reactivity, researchers should include healthy aging controls tested in parallel and document frailty limitations that may preclude some assays. When possible, use operant assays aligned to the modeled joint (e.g., reach/grasp for hand/shoulder; gait for knee/hip). When non-operant assays are necessary, prioritize quantitative tests and minimize stress. Researchers should also consider multidimensional assessment to capture wholistic features of age-related OA that may differ from other models of OA. These can include measures of cognitive/affective-motivational aspects of pain or evoked/somatic pain readouts. Preclinical behavioral and functional assessment is highly sensitive to environmental factors,[46,47] thus researchers should maintain and publish detailed methods and environmental metadata (light/dark cycles, housing, enrichment, handling, time-of-day) pertinent to behavior.

Systemic and Molecular Profiling

We recommend aging-related OA changes should be contextualized within the systemic aging environment, perhaps more so than other models of OA. This aging milieu includes inflammaging cytokines/chemokines, immune cell phenotypes, candidate genetic/epigenetic signatures, microbiome composition and metabolites, circadian health/sleep metrics, and endocrine/metabolic markers. the broader systemic changes associated with aging. While systemic changes associated with OA have been explored for mechanistic insight into disease pathology of other OA models, these changes may be more important in age-related OA because relatively little is known in this area.[5,22,48–54] Researchers should select assays that mechanistically align with the hypothesized pathway and, if possible, include a minimal integrative panel to anchor interpretation across aging processes. When integrating systemic and joint measures, apply appropriate multivariate/statistical models to extract OA-relevant signal from high-variance (“noisy”) data.

Model Selection Across Species

Chosen outcome measures should be ethologically appropriate to the model animal’s natural aging and OA model uniqueness. For example, cats may not participate in certain behavior tests that work well in other animal models, or important molecular assays may not yet exist in for a species.

Rodents, especially mice and rats, dominate preclinical OA literature, but they may not provide the best outcome measures for certain OA hypotheses. Other animals, such as dogs, horses, guinea pigs, cats, and pigs, offer different advantages and drawbacks that should be considered when selecting an animal model, outcome measures, and interpreting findings in age-related OA studies. It is worth noting that veterinary dog, cat, and horse patient populations offer well-documented aging health, genetically diverse populations, and considerable spontaneous and post-traumatic OA prevalence that can be leveraged specifically for age-related OA studies.[12,22,40,41,43,45,51,55–59] Researchers should select species and model fit-for-purpose, considering species

beyond rodents: mechanistic depth vs. translational fidelity, throughput vs. longitudinal imaging/biopsy, and the feasibility of age-related OA detection.

Experimental Design and Statistics

Keeping the feasibility of outcome measures in mind, researchers should always include age-matched controls, both animal sexes, and report key covariates: absolute age and rationale for defining “aged” by species/strain; sex; body weight/condition; behavioral changes outside assays; cage environment/enrichment; and comorbidities (e.g., strain-specific tumor incidence). Some induction methods (e.g., constitutive transgenics) may be incompatible with aging studies due to developmental effects; others may be too slow or surgically demanding for frail animals.

Proposed Minimum Essential Outcome Measures for Aging-Related OA

Operationally, aging-related OA (spontaneous or as an age cofactor in other models) should be defined by converging evidence from two or more domains beyond healthy aging expectations:

1. **Structural tissue pathology** (quantitative histology for cartilage/synovium; µCT for bone; standardized ROIs/timepoints),
2. **Pain/functional impairment** (operant and/or quantitative non-operant), and
3. **Molecular/systemic profiling** (e.g., joint catabolism markers, immune recruitment, microbiome/cytokine panels, circadian/sleep metrics).

This approach intentionally departs from structural-centric clinical criteria to reduce bias against important early age-related changes in OA pathology and to improve translational detection of OA onset.

Conclusion

These recommendations are made within the context of our current knowledge and knowledge gaps of healthy aging in preclinical models.

Studies should prioritize fit-for-purpose models and standardized and quantitative cross-modal endpoints, with transparent reporting (PREPARE/ARRIVE). Structural, functional, and molecular measures carefully distinguished from healthy aging trajectories will enable earlier and more accurate identification of aging-related OA.

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