Article:

EVALUATING SAFETY LABORATORY SIGNALS IN NEUROMUSCULAR CLINICAL TRIALS

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ABSTRACT

Interpretation of clinical safety laboratory data in neuromuscular disease populations presents unique challenges due to disease-related physiological alterations that affect biomarker production, distribution, and clearance. Progressive muscle loss, thus limiting altered protein metabolism, and reduced mobility can significantly distort commonly used indicators of renal and hepatic function, limiting the reliability of standard reference ranges. These complexities are particularly relevant in modern therapeutic development, including antisense oligonucleotide (ASO) and phosphorodiamidate morpholino oligomers (PMO) programs, where accurate differentiation between disease-related effects, pharmacologic activity, and true organ toxicity is critical.

This article provides a practical framework for interpreting renal, electrolyte, and hepatic laboratory parameters in muscle disease clinical trials. Key considerations include impact of reduced muscle mass on creatinine-based estimates of glomerular filtration rate (eGFR), the role and limitations of alternative biomarkers such as cystatin C, and the influence of concomitant treatments (e.g., corticosteroids) on laboratory values. Emphasis is placed on integrating mechanistic understanding with context-specific assessment strategies to mitigate misinterpretation.

By applying these principles, Sponsors and investigators can enhance the accuracy of safety signal detection, reduce unnecessary treatment interruptions, and support more informed clinical decision making in neuromuscular therapeutic development.

INTRODUCTION

Interpretation of clinical safety laboratory data in neuromuscular disease populations presents unique challenges that are not fully addressed by conventional reference frameworks. Progressive muscle loss, altered protein turnover, reduced mobility, and disease-specific pathophysiology can substantially modify the production, distribution, and clearance of commonly used biomarkers. As a result, laboratory values that are typically considered reliable indicators of renal or hepatic function in the general population may be misleading when applied to individuals with muscle disease, including Duchenne Muscular Dystrophy and related disorders.

These complexities are increasingly relevant in the context of modern therapeutic development, particularly for antisense oligonucleotide (ASO) and phosphorodiamidate morpholino oligomers (PMO) programs, where accurate attribution of laboratory abnormalities is essential for distinguishing disease-related physiology, pharmacologic effects, and true organ toxicity. Misinterpretation may lead to unnecessary treatment interruption, inappropriate safety signaling, or failure to recognize clinically meaningful risk.

Emphasis is placed on:

- Mechanistic understanding of biomarker behavior
- Recognition of disease and treatment-related confounders
- The value of integrated and context-specific assessment strategies

By applying these principles, Sponsors and investigators can improve the accuracy of safety signal detection and support more informed clinical development decisions in neuromuscular therapeutics.

UNDERSTANDING RENAL GLOMERULAR FILTRATION RATE (GFR)

In muscle disease populations, interpretation of renal function requires careful adjustment of conventional safety lab approaches. Reduced muscle mass lowers creatinine production, leading to creatinine-based eGFR calculations to systematically overestimate true glomerular filtration.

Serum cystatin C is not influenced by muscle loss and is therefore often the preferred biomarker for estimating GFR in this setting; however, its interpretation is not without nuance, as systemic corticosteroids can increase cystatin C concentrations and may contribute to modest underestimation of renal function in chronically treated patients, including many with Duchenne muscular dystrophy.

Both creatinine and cystatin C-based equations also rely on steady state assumptions and may lag behind true renal function during acute changes. While exogenous tracer methods such as iohexol or radiolabeled DTPA clearance can provide more direct assessment, they are not routinely feasible in clinical trials. This is often due to operational complexity, radiation exposure considerations, and sensitivity to sampling schedules and analytic methodology. Sponsors should account for disease-related and treatment-related factors that may influence renal safety signals, including GFR reductions associated with recurrent rhabdomyolysis, potential glomerular effects of oligonucleotide therapeutics, and concomitant medications such as intravenous bisphosphonates that may independently reduce GFR. Therefore, longitudinal trends and clinical context are more informative than single GFR.

RENAL TUBULAR INJURY: MECHANISMS, MARKERS, AND MISCLASSIFICATION RISKS

Renal tubular injury in muscle disease trials requires nuanced interpretation of urinary protein findings. Urinary protein can arise from glomerular leakage or from impaired proximal tubular reabsorption, with albuminuria, more typically associated with glomerular injury, whereas increased non-albumin or total protein often reflects tubular dysfunction.

Spot urine measurements introduce additional complexity because albumin and total protein concentrations are influenced by urine dilution. Normalization to urine creatinine is common practice, yet in populations with low muscle mass, reduced urine creatinine can bias UACR and UPCR values upward or render ratios uninterpretable. Orthostatic proteinuria, particularly in adolescent males, may further confound interpretation, while 24-hour urine collections are frequently limited by incomplete collection, especially in pediatric or functionally impaired populations.

Investigational product-related factors also warrants consideration. Intravenously administered ASOs are often excreted at high levels in early post-dose urine, where they may interfere with standard protein assays, compete with physiologic protein reuptake in proximal tubules, and in some cases be associated with tubular toxicity. Together, these factors underscore the importance of context-specific biomarker strategies and cautious attribution of renal safety signals.

COLLECTING SYSTEM FINDINGS

Renal collecting system findings in muscle disease populations reflect both disease-related physiology and treatment context and should be interpreted with attention to mechanisms that may not represent intrinsic nephrotoxicity. Crystalluria (oxalate or uric acid) is frequently observed, and the risk of nephrolithiasis is increased by dehydration, which often results from reduced mobility and delayed access to toileting.

Preclinical observations have additionally identified ureteral transitional cell neoplasia in association with certain exon-skipping antisense oligonucleotides, a finding potentially linked to urinary stasis and crystalluria rather than direct urothelial toxicity. In parallel, emerging kidney safety biomarker panels provide complementary mechanistic insight into renal injury pathways beyond conventional laboratory measures. Composite panels of qualified urinary biomarkers offer a structured framework for detecting and characterizing renal effects, supporting more informed attribution of safety signals in muscle disease clinical development.

RENAL ADDITIONAL MARKERS

Regulatory-qualified kidney safety biomarker panels provide mechanistic insight into glomerular, tubular, and collecting system injury, complementing standard safety labs, and supporting more accurate interpretation and attribution of renal signals *(see table below)*.

Acronym	Unique ID	Description
CLU	Urinary Clusterin (P10909)	A heterodimeric highly conserved secreted glycoprotein expressed in the proximal and distal tubules, glomerulus and collecting duct.
CysC	Cystatin-C (P01034)	A small serum protein produced by all nucleated cells and found in most tissues and body fluids. CysC is freely filtered by the glomerulus and almost completely reabsorbed and catabolized in healthy renal tubular epithelium.
KIM-1	Kidney Injury Molecule –1 (Q96D42)	A type I transmembrane glycoprotein containing an ectodomain consisting of an immunoglobulin-like domain and a mucin domain that is strongly induced by ischemic and toxic insults to the kidney.
NAG	N-acetyl-beta-D-glucosaminidase (O60502)	A large lysosomal enzyme with two isoforms and is mainly expressed in proximal tubules.
NGAL	Neutrophil gelatinase-associated lipocalin (P80188)	Expressed in various tissues at low levels with upregulated transcription in tubuloepithelial cells following ischemic and nephrotoxic kidney injuries.
OPN	Osteopontin (P10451)	A highly acidic glycoprotein expressed by many tissues that acts as a macrophage adhesion and chemotactic molecule.

Figure 1: *Six Urinary Biomarkers*[1]

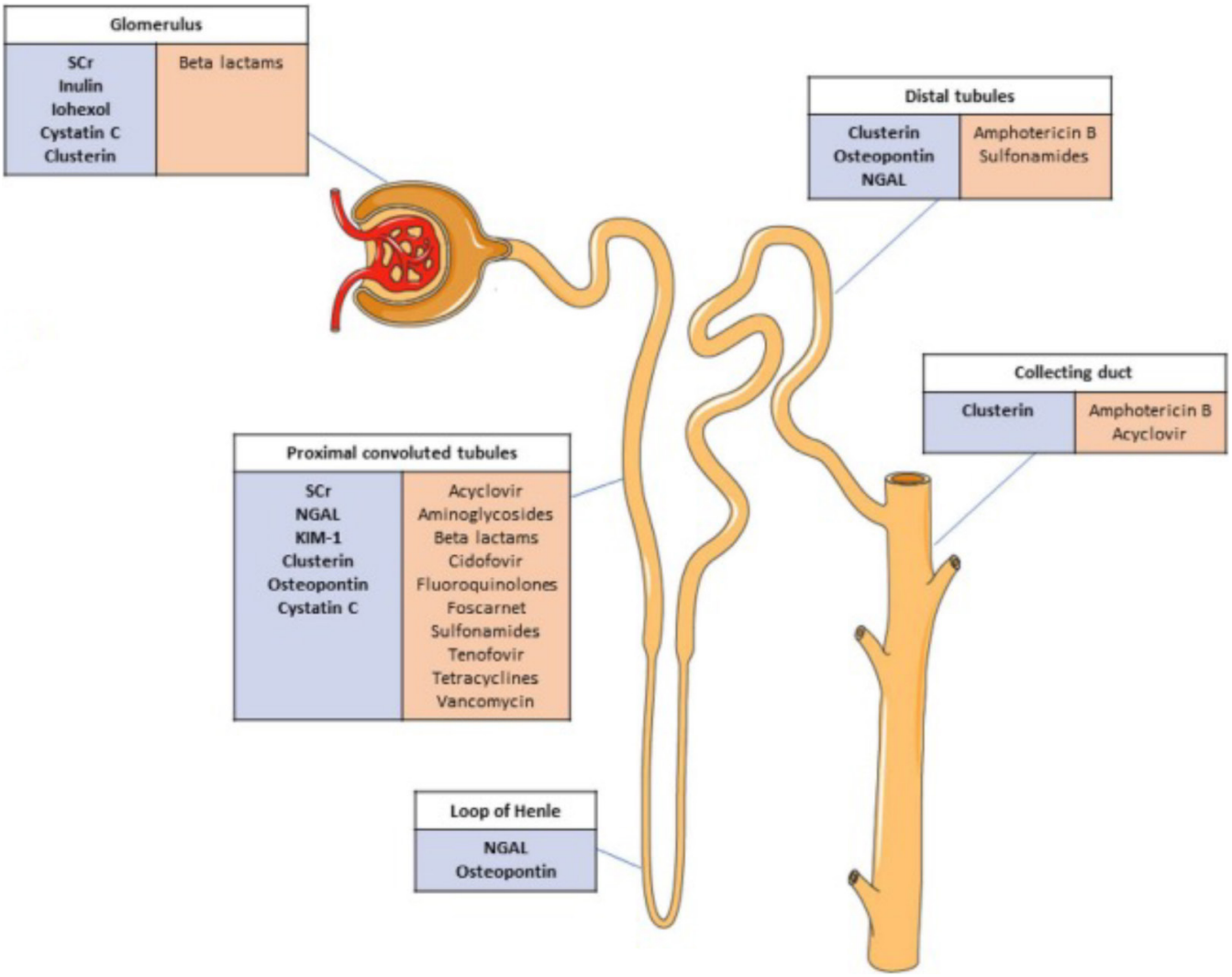

Figure 2: *Past, Present, and Future Biomarkers of Kidney Function and Injury: The Relationship with Antibiotics. Int J Antimicrob Agents. 2024 Nov;64(5): 1073322*[3]

ELECTROLYTE DISTURBANCES IN ASO AND PMO THERAPY

For Sponsors developing ASO therapies in neuromuscular disease, particularly PMOs, emerging renal electrolyte considerations are becoming an important aspect of clinical strategy. While PMOs are engineered for improved stability and tissue distribution, cellular uptake remains limited, promoting the development of peptide-conjugated PMOs (PPMOs) to enhance delivery. Clinical experience with PPMOs has demonstrated a signal of renal magnesium wasting, with potential effects on calcium and potassium homeostasis, underscoring the need for proactive monitoring strategies. Assessment should include serum magnesium trends, electrocardiographic changes, and evaluation of fractional excretion of magnesium.

Interpretation of renal biomarkers can be complex in Duchenne Muscular Dystrophy due to altered creatinine dynamics and low urine creatinine values, factors that regulatory agencies are increasingly scrutinizing in PMO programs. Through integrated safety monitoring frameworks and deep therapeutic expertise in neuromuscular trials, Medpace partners with Sponsors to anticipate these risks, optimize study design, and generate the high-quality evidence needed to advance innovative therapies with confidence.

HEPATIC SAFETY ASSESSMENT IN THE PRESENCE OF SKELETAL MUSCLE INJURY

Interpretation of liver safety signals requires a differentiated approach because commonly used biomarkers, including ALT, AST, and LDH, are not liver-specific and are frequently elevated due to ongoing skeletal muscle injury, often in the setting of markedly elevated creatine kinase (CK). As a result, safety assessments in ASO and PMO programs benefit from a multimodal monitoring framework. ALT and AST trends can provide useful context when CK remains elevated but stable, given their distinct temporal profiles.

Gamma-glutamyl transferase (GGT), which is not released from muscle, offers additional perspective. Glutamate dehydrogenase (GLDH) is not muscle-derived and may provide a more rapid liver-specific signal, although limited assay availability and lack of standardization pose operational constraints on its routine use in trials. Bilirubin remains an important indicator of clinically meaningful hepatic dysfunction, though increases typically follow earlier biomarker changes. Together, these nuances underscore the importance of tailored safety monitoring strategies that distinguish muscle-driven enzyme elevations from true hepatic risk.

ADDITIONAL CARDIAC MONITORING IN MUSCULAR DYSTROPHIES

Cardiac troponin I (TnI) offers greater cardiac specificity and is generally recommended for monitoring in muscular dystrophies; however, elevations in high-sensitivity TnI have been observed in some patients without clinical or imaging evidence of cardiomyopathy, reinforcing the need for contextual interpretation.

Structural and functional evaluation also presents practical challenges, as echocardiography may be technically limited in older patients due to kyphoscoliosis. Cardiac MRI therefore represents the preferred modality for comprehensive assessment, with late gadolinium enhancement enabling sensitive detection and characterization of myocardial fibrosis.

For Sponsors advancing neuromuscular therapies, these considerations highlight the importance of integrated cardiac monitoring strategies that combine biomarker selection with optimized imaging approaches. Through disease-specific expertise and noninvasive cardiac safety frameworks.

CONCLUSION

Accurate interpretation of safety laboratory data in neuromuscular disease requires an approach that accounts for altered muscle biology, disease progression, and treatment related effects. Conventional biomarkers of renal and hepatic function may not reliably reflect organ injury in this setting and should be evaluated within a broader mechanistic and clinical context. Integration of complementary biomarkers, longitudinal trends, and disease-specific considerations can improve attribution of safety signals and reduce the risk of misclassification.

Applying these principles supports more informed clinical decision making and strengthens the evaluation of emerging therapies in neuromuscular clinical development.

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Dr. Richard D. Scheyer, MD, Sr. Vice President of the Medical Department specializing in Neurology and Pharmacology, is a distinguished board-certified neurologist with more than 30 years of professional medical experience, including over two decades in clinical drug development. A pioneer in translational medicine and early-phase drug development, Dr. Scheyer has a keen interest in the early demonstration of clinical efficacy. He led a team in designing an innovative early development program for next-generation alpha-2 delta ligands and crafted Proof of Concept strategies for novel ion channel blockers. With over 60 published manuscripts and abstracts, his work spans clinical pharmacology and therapeutic activities across various fields. Notably, he launched the first controlled trial of a natural product-derived protein kinase C activator in Alzheimer's disease. Dr. Scheyer's academic credentials include a Bachelor of Science in Physics from Stanford University, a Doctor of Medicine from The State University of New York, Upstate Medical University, a Neurology Residency, and Fellowship Training in Epilepsy at Yale University.

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