BEYOND CPK: THE NEED FOR A MULTI-BIOMARKER APPROACH IN LIMB-GIRDLE MUSCULAR DYSTROPHY R9

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Abstract
Limb-Girdle Muscular Dystrophy R9 is traditionally monitored using creatine kinase (CK) and aldolase, classic markers of muscle injury. However, these parameters predominantly reflect enzymatic leakage and exhibit considerable biological variability, limiting their utility for monitoring disease progression and therapeutic response [1,2]. Recent evidence indicates that muscular dystrophies involve systemic alterations, including chronic low-grade inflammation, oxidative stress, mitochondrial dysfunction, and muscle protein remodeling—processes that are not adequately captured by isolated enzymatic markers [3–5]. Proteomic studies in muscular dystrophies have identified specific circulating profiles, including structural muscle proteins, inflammatory mediators, and components of energy metabolism, suggesting that multi-biomarker panels may provide greater clinical sensitivity and specificity than CK alone [2,4]. Therefore, the adoption of an integrated multi-biomarker approach—including inflammatory, redox, metabolic, and muscle turnover markers—represents a necessary advancement for more accurate characterization of disease activity and clinical stratification in LGMDR9.

Keywords:
Limb-Girdle Muscular Dystrophy R9; FKTN; Biomarkers; Creatine Kinase; Oxidative Stress; Inflammation; Energy Metabolism.

Introduction

Limb-Girdle Muscular Dystrophy R9 (LGMDR9), formerly classified as LGMD2I, belongs to the heterogeneous group of limb-girdle muscular dystrophies and is characterized by progressive proximal muscle weakness and marked clinical variability. Associated with pathogenic variants in the FKTN gene, the disease involves structural impairment of muscle fibers, altered protein glycosylation, and sarcolemmal membrane instability, ultimately leading to chronic muscle degeneration [1].

Despite advances in molecular characterization, laboratory monitoring remains predominantly based on serum creatine kinase (CK) and aldolase measurements. CK, a cytosolic enzyme released following muscle membrane damage, is widely used as a marker of myocellular injury. However, serum CK levels exhibit substantial interindividual variability and are influenced by factors such as physical activity, disease stage, residual muscle mass, and degree of fibrosis, thereby limiting their correlation with clinical progression [1,5]. Moreover, both CK and aldolase primarily reflect enzymatic leakage and do not provide insight into the underlying pathophysiological mechanisms driving muscle degeneration.

Accumulating evidence indicates that muscular dystrophies involve complex systemic alterations that extend beyond structural muscle damage. Processes such as chronic low-grade inflammation, persistent oxidative stress, mitochondrial dysfunction, and dysregulation of energy metabolism play significant roles in disease progression [6,7]. Activation of inflammatory pathways and increased production of reactive oxygen species contribute to amplification of muscle damage, while alterations in oxidative and glycolytic

metabolism reflect bioenergetic impairment of muscle fibers. These phenomena are not adequately captured by isolated enzymatic markers.

In this context, recent serum proteomic studies and analyses of circulating biomarkers in muscular dystrophies have demonstrated distinct molecular signatures, including structural muscle proteins, inflammatory mediators, and components related to energy metabolism [5,8]. These findings support the hypothesis that multimarker biochemical panels may provide greater sensitivity and specificity for monitoring disease activity, subclinical progression, and therapeutic response.

Thus, maintaining an approach centered exclusively on CK and aldolase appears conceptually limited given the pathophysiological complexity of LGMDR9. The adoption of an integrated strategy based on multiple biochemical biomarkers—encompassing inflammatory, redox, metabolic, and muscle remodeling axes—represents a necessary advancement to improve clinical stratification and to support more individualized therapeutic approaches.

Methods

This study consists of a narrative review of the scientific literature focusing on biochemical biomarkers applicable to the monitoring of Limb-Girdle Muscular Dystrophy R9.

The literature search was conducted in PubMed/MEDLINE, Scopus, and Web of Science databases using controlled descriptors and free-text terms combined with Boolean operators, including “Limb-Girdle Muscular Dystrophy,” “LGMDR9,” “LGMD2I,” “Biomarkers,” “Creatine Kinase,” “Oxidative Stress,” “Inflammation,” and “Energy Metabolism.”

Original studies and review articles published between 2010 and 2025, in English or Portuguese, addressing serum or plasma biomarkers related to limb-girdle muscular dystrophies—with emphasis on LGMDR9—were included. Studies exclusively focused on genetic analyses or based solely on imaging methods were excluded.

Selected studies were analyzed qualitatively. Biomarkers were organized according to pathophysiological axes (muscle damage, inflammation, oxidative stress, energy metabolism, and protein turnover), with particular emphasis on the limitations of creatine kinase and aldolase as isolated markers.

Discussion

Clinical practice in the follow-up of limb-girdle muscular dystrophies remains largely based on the measurement of serum creatine kinase (CK) and, to a lesser extent, aldolase. Although these markers are useful for indicating active muscle damage, their application as isolated indicators of disease progression or therapeutic response presents important limitations. Interindividual variability in CK levels, as well as the influence of physical exercise, disease stage, and residual muscle mass, reduces its specificity as a longitudinal marker of disease activity [1].

The pathophysiology of Limb-Girdle Muscular Dystrophy R9 extends beyond enzymatic leakage. Structural alterations associated with pathogenic variants in the FKTN gene trigger secondary cascades, including persistent inflammatory activation, increased production of reactive oxygen species, and impaired mitochondrial bioenergetics [1,2]. Chronic low-grade inflammation has been described as an amplifier of muscle damage in muscular dystrophies, mediated by cytokines and infiltrating immune cells [3]. Concurrently, oxidative stress contributes to mitochondrial dysfunction and progression of muscle degeneration [2].

Proteomic discovery studies have identified circulating structural and metabolic muscle proteins capable of distinguishing individuals with muscular dystrophies from healthy controls, suggesting that multimolecular signatures may offer greater clinical sensitivity than

CK alone [5,8]. These findings support the hypothesis that multimarker panels—including inflammatory, redox, metabolic, and protein turnover biomarkers—may more comprehensively reflect the underlying pathophysiological activity of the disease.
Furthermore, a multi-biomarker approach may enhance clinical stratification and the evaluation of therapeutic interventions in a context of rapidly expanding targeted therapies [1]. However, challenges remain regarding analytical standardization, longitudinal validation specifically in LGMDR9, and the definition of clinical relevance for each individual or combined biomarker.
Thus, the transition from a model centered exclusively on CK and aldolase to an integrated strategy based on multiple biochemical biomarkers represents a paradigm shift in the monitoring of Limb-Girdle Muscular Dystrophy R9, with the potential to improve clinical precision and deepen understanding of disease heterogeneity.

Biochemical Biomarkers and Potential Nutritional Strategies in LGMDR9:

CATEGORY	**BIOMARKER**	**RESULTS ANALYSIS**	**POTENTIAL NUTRITIONAL STRATEGY**	**EVIDENCE**
Muscle Enzimes	Creatine Kinase (CK)	Elevated levels indicate muscle damage and fiber degeneration.	High-Quality Proteins, Leucine, Creatine, Glutamine, and Alanine	Clinical Studies and Reviews
Muscle Enzimes	Aldolase	Elevated levels are associated with muscle degradation.	Protein Supplementation and Essential Amino Acids	Clinical Studies
Muscle Enzimes	Creatine	Reduced levels may reflect energy depletion and catabolism.	Creatine Monohydrate Supplementation	Clinical Studies
Oxidative Stress	Malondialdehyde (MDA)	Elevated levels indicate lipid peroxidation	Antioxidants: Vitamins C and E, Polyphenols	Recent Reviews
Oxidative Stress	Glutathione (GSH)	Reduced levels increase vulnerability to oxidative damage.	Antioxidant Supplements: N-Acetylcysteine (NAC)	Experimental Studies
Oxidative Stress	Superoxide Dismutase (SOD)	Deficiency increases oxidative stress.	Diet Rich in Natural Antioxidants	Experimental Studies
Inflammation	TNF-α (Tumor Necrosis Factor alpha)	Elevated levels indicate chronic systemic inflammation.	Omega-3, Antioxidants, Anti-Inflammatory Diet	Clinical Reviews

Inflammation	IL-6 (Interleukin-6)	Inflammatory and muscle catabolism marker.	Omega-3 and Antioxidant-Rich Diet	Laboratory Studies
Metals and Cofactors	Ferritin	Elevated levels may indicate chronic inflammation, while low ferritin can impair mitochondrial energy metabolism.	Balanced Diet (e.g., Mediterranean Diet)	Laboratory Studies
Energy Metabolism	ATP (Adenosine Triphosphate)	Reduced levels indicate mitochondrial dysfunction.	Coenzyme Q10, Riboflavin, Mitochondrial Support	Experimental Trials
Energy Metabolism	Lactate	Elevated levels indicate predominant anaerobic metabolism.	Protein Adjustments, Low Glycemic Index Carbohydrates	Clinical Studies
Energy Metabolism	Citrate and Malate	Alterations reflect Krebs cycle dysfunction. indicate mitochondrial dysfunction and compromised oxidative metabolism	Coenzyme Q10 and riboflavin supplementation and macronutrient adjustments	Laboratory Studies

It is necessary to further explore how nutritional strategies—including optimized protein intake, specific amino acids, antioxidants, omega-3 fatty acids, and coenzyme Q10 (CoQ10)—may modulate these biochemical pathways. Targeting inflammatory signaling, oxidative stress, mitochondrial dysfunction, and impaired energy metabolism through nutritional interventions may represent an adjuvant approach to attenuate muscle degeneration and support metabolic homeostasis in Limb-Girdle Muscular Dystrophy R9.

Conclusion

Laboratory monitoring of Limb-Girdle Muscular Dystrophy R9 remains largely based on the measurement of creatine kinase (CK) and aldolase. Although these markers are useful for indicating muscle damage, their ability to reflect the overall pathophysiological activity of the disease is limited, particularly given interindividual variability and the influence of factors such as physical exercise and residual muscle mass [1].

LGMDR9 involves complex mechanisms, including persistent inflammatory activation, oxidative stress, and mitochondrial dysfunction, processes that contribute to progressive muscle degeneration and are not adequately captured by isolated enzymatic markers [2,9]. Studies of circulating biomarkers in muscular dystrophies have demonstrated distinct proteomic and metabolic signatures, suggesting that multimarker approaches may offer greater clinical sensitivity and specificity compared with CK alone [2,8].

In this context, the incorporation of integrated biochemical biomarker panels—encompassing inflammatory, redox, and metabolic axes—represents a conceptual advancement in the monitoring of LGMDR9. Such an approach may improve clinical stratification, enable more precise longitudinal assessment, and support the development of targeted therapeutic strategies [1].

Although challenges related to longitudinal validation and analytical standardization persist, the transition from a single-marker model to a multimarker strategy constitutes a paradigm shift in the biochemical follow-up of LGMDR9.

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