

Serum protein biomarker signature of Duchenne muscular dystrophy

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Abstract

In contrast to invasive skeletal muscle biopsies and the associated complexity of tissue sampling techniques and potential detrimental side effects, the alternative application of liquid biopsy procedures has considerable advantages concerning minimal invasiveness, repeated sampling options, assay robustness and cost effectiveness. This article outlines the current status of serum biomarkers used for diagnosing and characterizing Duchenne muscular dystrophy (DMD), a primary muscle wasting disease of early childhood due to primary abnormalities in the extremely large *DMD* gene. Reviewed are important aspects of the discovery, characterization and diagnostic value of biofluid-based protein markers of dystrophinopathy. This includes an overview of traditional general skeletal muscle damage markers, such as creatine kinase, myoglobin and lactate dehydrogenase, which have been used for many decades in clinical applications to evaluate patients with muscular weakness. In addition, this article outlines the biochemical identification of novel biomarker candidates focusing on the usage of mass spectrometry-based proteomic surveys to establish comprehensive profiles of protein alterations in dystrophinopathy. Pathoproteomic serum markers of myonecrosis with great potential for improved patient screening, differential diagnosis, stage-specific prognosis and therapeutic monitoring include specific isoforms of muscle-derived cytosolic proteins, such as carbonic anhydrase isoform CA3 and fatty acid binding protein FABP3, as well as sarcomeric proteins, including specific isoforms of myosin light chain, myosin binding protein, troponin, and myomesin, in addition to peptide fragments derived from the giant protein titin. Biofluid-associated marker proteins of reactive myofibrosis include the extracellular matrix proteins fibronectin, osteopontin, collagen and matrix-metalloproteinases.

Key Words: dystrophinopathy, liquid biopsy, muscle proteomics, myofibrosis, myonecrosis.

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A large number of inherited or acquired human disorders are associated with abnormalities in contractile myofibre abundance, regulation and/or physiological functioning.¹ One of the most severely progressive genetic skeletal muscle wasting disorders of early childhood is Duchenne muscular dystrophy (DMD),² which is characterized by primary abnormalities in the highly complex and multi-promoter *DMD* gene.³ Mutations trigger the almost complete loss of the full-length *DMD* gene product, the dystrophin protein isoform Dp427-M of the membrane cytoskeleton, and the concomitant reduction of dystrophin-associated glycoproteins at the weakened

sarcolemma membrane.⁴ The neuromuscular abnormalities in DMD are characterized by sarcolemmal leakage and surface membrane instability rendering muscles more susceptible to progressive myonecrosis, which is followed by chronic inflammation, reactive myofibrosis, fat substitution and abnormal myofibre regeneration.^{5,6} DMD patients can also suffer from a variety of additional multi-system complications including cognitive deficiencies, cardio-respiratory failure, kidney damage, liver dysfunction and gastro-intestinal symptoms.⁷ Newborn screening, detection of early signs of neuromuscular weakness, the differential diagnosis of different forms

of dystrophinopathy and the evaluation of genetic carrier status with varying degrees of symptoms can be carried out by numerous tests. This includes physical examinations, strength/walk motor assessments, blood enzyme assays, the histological and immunochemical examination of skeletal muscle biopsies, neurological electro-diagnostics and various imaging technologies.⁸⁻¹²

To decisively reduce the clinical diagnostic/prognostic complexity and considerable financial burden of these diverse tests, there is a need for the development of simpler, streamlined and more cost-effective biofluid-based assay systems that are ideally minimally invasive in nature.¹³ In general, direct or indirect damage of skeletal muscles often leads to the release of distinct muscle-associated or muscle-derived proteins into the circulatory system. The active or passive shedding of muscle proteins can be linked to strenuous physical exercise, traumatic injury, work-related musculoskeletal damage, sepsis, drug abuse, excessive alcohol consumption, certain medications, co-morbidities of the neuromuscular system during systemic disease or primary muscular disorders.¹⁴⁻¹⁸

It is therefore critical to identify DMD disease-specific serum biomarkers that exhibit only a limited overlap with other neuromuscular disorders or activity-dependent changes.

Aim of this article on serum biomarkers

This article reviews the current status of established serum markers of DMD versus novel protein indicators of this devastating skeletal muscle disease. In the future, serum biomarkers will be especially crucial for the therapeutic monitoring of novel treatment approaches that utilize genome editing, stop codon read-through, antisense oligonucleotide-based exon skipping, gene transfer via adeno-associated viruses that express micro-dystrophins, and/or new types of pharmacotherapy.¹⁹⁻²³

The discovery of novel biomarker candidates is outlined in this article, which has been carried out mostly by peptide Mass Spectrometry (MS),²⁴ or aptamer-based proteomic SOMAscan methodology.²⁵ Detailed descriptions of the various preparative and analytical methodologies that are routinely used in MS-based skeletal muscle proteomics have recently been published,²⁴ including their application for studying the molecular and cellular pathogenesis of DMD and associated pathophysiological crosstalk and multi-system dysfunctions.²⁶

Biofluid protein biomarkers

Characteristic changes in the abundance and/or biochemical activity of marker proteins in bodily fluids can be used to develop superior liquid biopsy assays to improve diagnostics and prognostics, as well as the continuous monitoring of therapeutic success and the potential occurrence of adverse side effects.²⁷ A large variety of biofluids are currently investigated for their suitability to contain appropriate biomarker signatures, including serum, plasma, saliva, urine, cerebrospinal fluid, tears, lymph, broncho-

alveolar lavage fluid, gastric juice, bile, pancreatic juice, aqueous humour, sweat, menstrual flow, breast milk, nipple aspirate, amniotic fluid, follicular fluid, vaginal secretions, sperm, ear cerumen, gland exudes, synovial fluid, nasal mucus, alveolar fluid and breath condensate.²⁸ The application of body fluid proteomics has greatly enhanced this exciting field of pathobiochemical research and established an unprecedented capability for the unbiased large-scale and high-throughput discovery of novel disease biomarker candidates.²⁹⁻³¹

Biofluid-associated protein biomarkers are ideally specific proteoforms of distinct protein species, or their representative peptide fragments, which are: (i) characterized by discrete biochemical, physiological or cellular activities/features that can be easily and cost-effectively measured, (ii) cell or tissue specific in their expression pattern, (iii) enriched in a restricted subcellular location, (iv) actively or passively released following particular types of cellular damage, (v) shed into easily assessable biofluids for minimally invasive monitoring, (vi) being suitable for repeated sampling procedures, (vii) are of robust and sensitive nature for optimum assaying, (viii) specific for a particular pathophysiological phenotype, (ix) suitable for disease screening, differential diagnostics, prognosis, evaluation of unwanted side effects and therapeutic monitoring, and (x) being an integral part of a meaningful and comprehensive biomarker signature.¹³

Established and clinically approved indicators of disease processes can be used in a variety of biomedical applications as population screening markers, differential diagnostic markers, tissue injury markers for the evaluation of disease severity, prognostic markers that also take into account potential co-morbidity/multi-system effects, therapeutic monitoring markers that encompass the determination of adverse side effects, and clinical outcome markers (Figure 1).^{15-18,32}

A variety of protein species, which are highly enriched in skeletal muscles, exhibit many of the above outlined characteristics of muscle damage markers, and can be used alone or in combination for the development of more reliable biomarker tests of muscular disorders such as DMD.

Skeletal muscle damage markers

General markers of muscle injury

In clinical medicine, occupational health and sports physiology, serum markers of skeletal muscle damage are routinely used to assess the degree of myofibre injury.¹³⁻¹⁸ One of the most extreme forms of muscle disintegration is rhabdomyolysis,³³ a condition that is characterized by the massive release of muscle proteins and electrolytes often causing harmful downstream effects on kidney, lung and heart function.¹⁸ Frequently used skeletal muscle damage markers include creatine kinase (CK), myoglobin (MB), aspartate transaminase (AST) and glycolysis-associated enzymes such as lactate dehydrogenase (LDH).¹⁶

Figure 2 gives an overview of general skeletal muscle protein biomarkers released into the circulatory system following myofibre damage¹⁶⁻¹⁸, as observed in DMD,¹⁰ and

associated complications, such as myofibrosis.³⁴ The majority of the listed marker proteins are currently only used for research purposes. A minority of the displayed protein species are clinically approved for evaluating muscle injury, such as CK, MB, AST and LDH.^{16,18}

Creatine kinase

One of the most frequently employed clinical indicators of general damage to the muscular system is measuring the blood-based activity levels of the enzyme CK.¹⁶ CK catalyses the reversible conversion of ATP and creatine to ADP and phosphocreatine. Five CK isoforms exist in the body in the cytosol and mitochondria. In the cytosol, combinations of muscle (M) and brain (B) type subunits result in 3 isoenzymes, i.e. CK-MM, CK-MB and CK-BB.³⁵ The cytosolic CK enzymes provide an energy reservoir for ATP regeneration. The mitochondrial CK isoenzymes consist of the sarcomeric (mtCK_s) and the ubiquitous form (mtCK_u), and are involved in the direct formation of phosphocreatine from ATP in the mitochondrial intermembrane space.¹⁸

Increased CK levels are routinely used for the preliminary diagnosis of neuromuscular disorders, including DMD.^{35,36} Bottom-up proteomics can detect cytosolic M-type CK (*CKM* gene), cytosolic B-type CK (*CKB* gene) and mitochondrial CK (*CKMT2* gene) in serum samples.³⁷⁻³⁹ Advantages of CK assays are their low cost and suitability for high-throughput testing. The amount of serum CK cor-

relates well with the degree of skeletal muscle damage.^{15,35} Of note, large-scale newborn screening of elevated CK levels has been successfully applied for the early diagnosis of DMD cases, combined with confirmatory genetic analysis.⁴⁰⁻⁴² Disadvantages of CK tests are bioanalytical issues with serum CK measurements that are associated with inter-individual and ethnic variabilities, an uneven tissue distribution of CK in different skeletal muscles, and a relatively low detection sensitivity in the case of minor muscle injury.⁴³⁻⁴⁵

Myoglobin

As an essential oxygen storage and transportation element, the cytoplasmic haemoprotein MB reversibly binds oxygen in heart and skeletal muscles.⁴⁶ MB is of central physiological importance for intracellular oxygen buffering, supporting oxygen diffusion and facilitating the mitochondrial process of oxidative phosphorylation.⁴⁷ However, MB is also implicated to interact with glycolytic metabolites, being involved in the sequestration and trafficking of lipids, and serving as a modulating sensor of nitric oxide-responsive signalling pathways,⁴⁸ besides its canonical oxygen binding function.⁴⁶ Importantly, the drastic increase of MB levels in blood and urine is a reliable indicator of rhabdomyolysis.^{18,33}

An analytical issue with serum MB is the overlap between its release from both heart and skeletal muscles following tissue injury. However, this problem can be addressed by

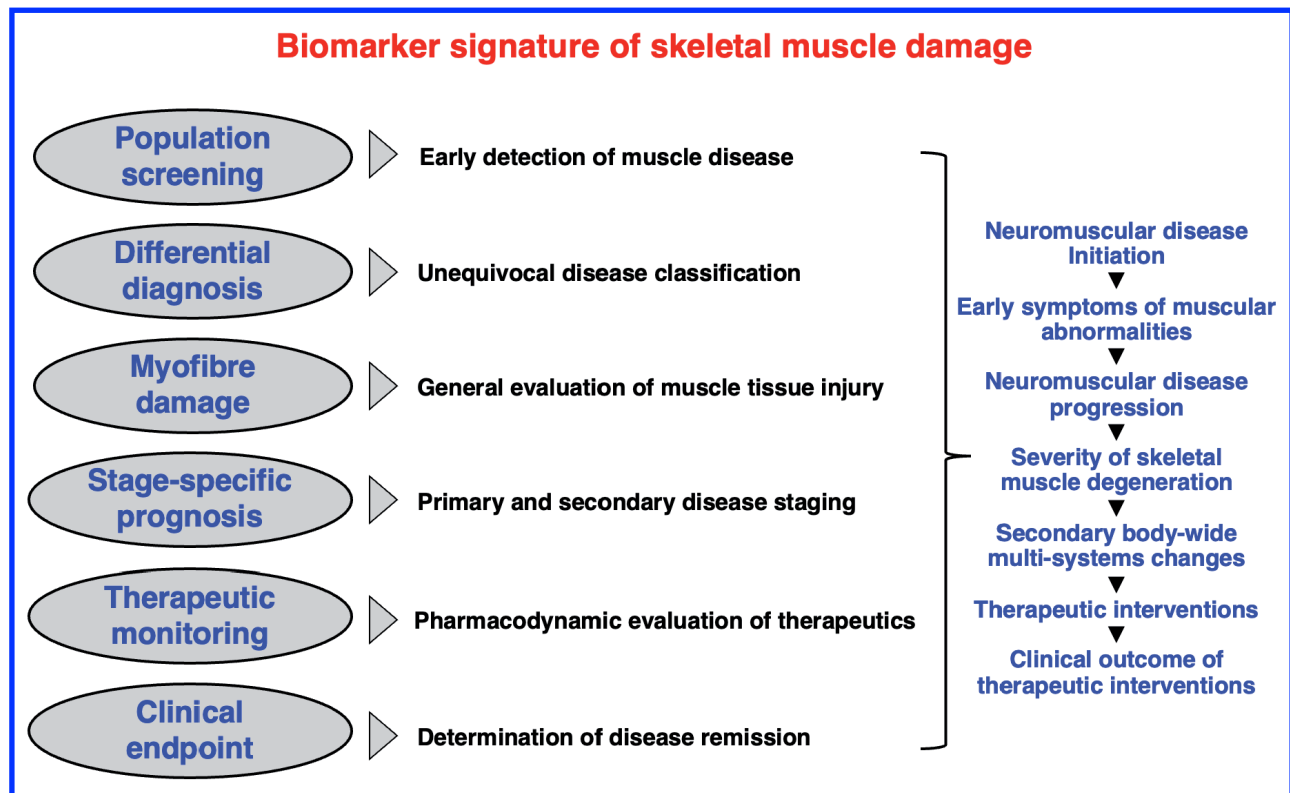


Figure 1. Summary of biomarker categories used to evaluate skeletal muscle damage.

measuring the ratio between serum MB and the CA3 isoform of cytosolic carbonic anhydrase. Since the CA3 iso-enzyme is only expressed in skeletal muscle, but not cardiac myocytes, the determination of CA3, MB and the CA3/MB ratio in serum specimens can differentiate between the involvement of the heart versus skeletal muscles in particular disease processes.⁴⁹

MS-based proteomics can conveniently measure alterations in blood MB levels.³⁷⁻³⁹ The proteomic screening of serum from dystrophic patients and animal models of DMD has clearly confirmed an elevated MB concentration due to skeletal muscle degeneration.^{37,50-52}

Glycolytic enzymes

Glycolytic enzymes represent ideal skeletal muscle markers, since they are highly abundant in the sarcosolic fraction as determined by proteomics,⁵³⁻⁵⁵ and they easily shed from damaged myofibres resulting in high levels of these types of enzymes in the circulatory system.^{36,37} Biochemical and MS-based proteomic analyses of serum samples can be employed to routinely detect glycolysis-associated enzymes, such as LDH (encoded by *LDHA* and *LDHB* genes).^{37-39,56} LDH mediates the conversion of pyruvate to lactate under anaerobic conditions. In dystrophic patients and animal models of DMD, increased levels of

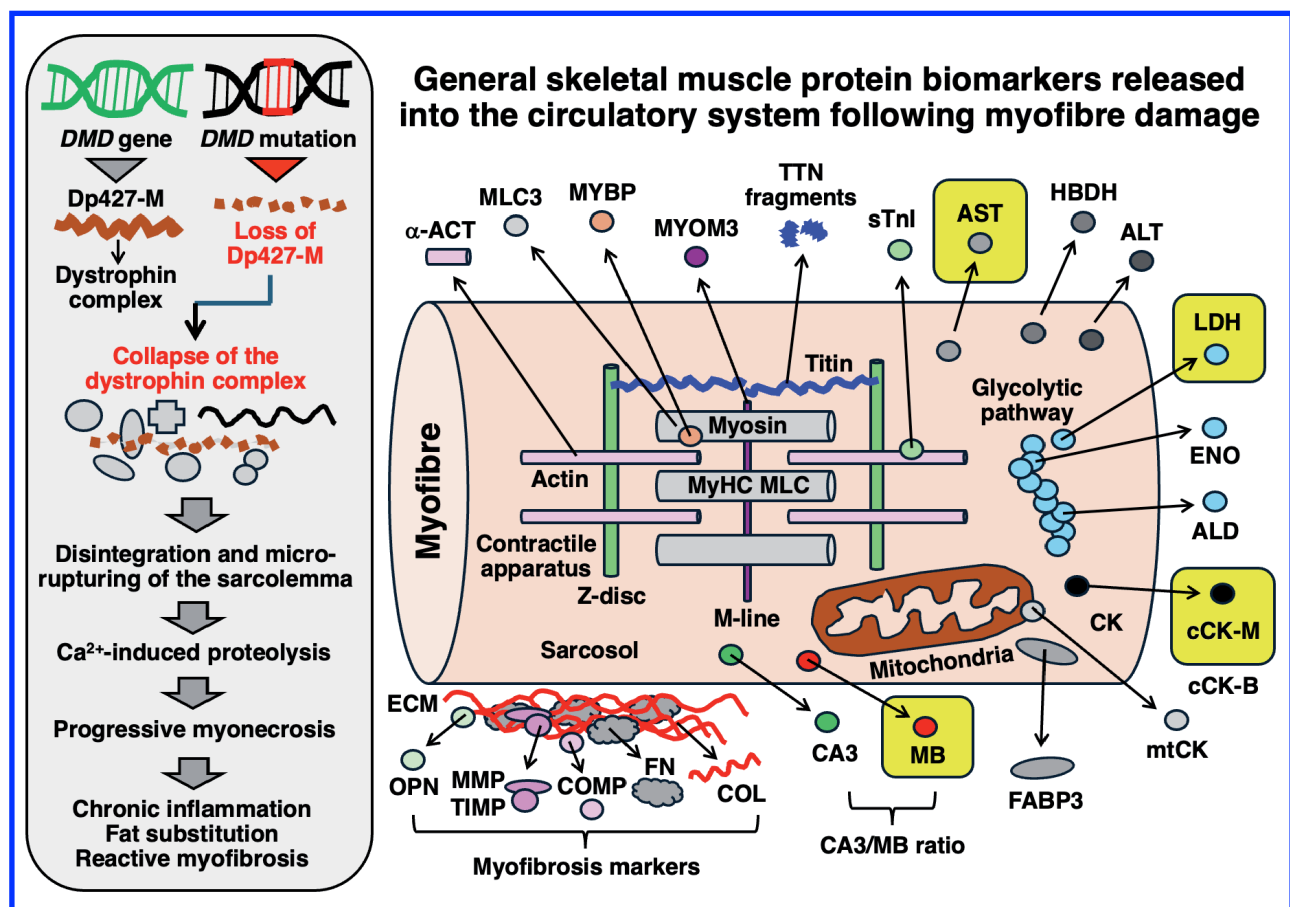


Figure 2. Overview of major proteins that are actively or passively released from dystrophic skeletal muscle tissues. On the left is shown a flowchart of major steps that are involved in the molecular and cellular pathogenesis of dystrophinopathy ranging from the primary abnormality in the DMD gene to complex alterations in dystrophic skeletal muscles, including myonecrosis, chronic inflammation, fat substitution and reactive myofibrosis. On the right are listed major muscle-derived serum protein species that exhibit elevated levels in X-linked muscular dystrophy and have been routinely identified by biochemical and proteomic assays. The yellow boxes in the diagram highlight clinically approved protein markers that are frequently employed to evaluate skeletal muscle damage.

Abbreviations used: ACT, actin; ALD, aldolase; ALT, alanine aminotransferase; AST, aspartate transaminase; c, cytosolic; CA3, carbonic anhydrase 3; CK, creatine kinase; COL, collagen; COMP, cartilage oligomeric matrix protein; ECM, extracellular matrix; ENO, enolase; FABP3, fatty acid binding protein 3; FN, fibronectin; HBDH, hydroxybutyrate dehydrogenase; LDH, lactate dehydrogenase; MB, myoglobin; MLC3, myosin light chain 3; MMP, matrix metalloproteinase; mt, mitochondrial; MYBP, myosin binding protein; MYOM3, myomesin 3; MyHC, myosin heavy chain; OPN, osteopontin; TIMP, tissue inhibitor of metalloproteinases; TnI, troponin subunit I; TTN, titin.

serum LDH, in addition to aldolase, enolase and pyruvate kinase, were identified by both enzyme assays, often in combination with elevated CK,⁵⁷⁻⁵⁹ and proteomic surveys.^{37,38,50-52}

Proteomic muscle damage markers

Mass spectrometric analysis of biofluids

The large-scale and MS-based proteomic analysis of bodily fluids has led to the comprehensive establishment of liquid biopsy markers in health and disease.²⁷⁻³¹ A large array of several thousand identified serum proteins spans a wide dynamic range of concentrations.³⁹ This proteomic atlas of the plasma/serum proteome can now serve as an advanced search tool for biofluid marker candidates.⁵⁶

Proteomic biomarkers of dystrophinopathy

MS-based profiling studies of dystrophic skeletal muscles have revealed considerable changes in proteins involved in myofibre contraction, the regulation of the physiological coupling of excitation, contraction and relaxation, ion homeostasis, bioenergetic pathways, the organization of the cytoskeletal network and the cellular stress response.⁶⁰⁻⁶⁷ Thus, the collapse of the dystrophin-glycoprotein complex,⁶⁸ which acts as a signalling and stabilization node at the plasmalemma and costameres in healthy skeletal muscles,⁶⁹ causes surface membrane micro-rupturing, sarcolemmal Ca^{2+} -influx, Ca^{2+} -dependent proteolysis, weakened lateral force transmission and impaired excitation-contraction coupling.⁷⁰ These massive alterations in the proteomic profile of dystrophic skeletal muscles are partially reflected by alterations in the circulatory system showing distinct changes in the abundance of certain skeletal muscle-derived proteoforms in plasma/serum, saliva and urine.^{37,38,49-52,71-87}

The systematic immunochemical, biochemical and proteomic screening of serum from DMD patients and animal models of dystrophinopathy has confirmed elevated levels of previously characterized muscle damage markers such as M-type CK, LDH and MB, and identified novel marker candidates, including the MDH2 isoform of malate dehydrogenase, CA3, fibronectin (FN), fatty acid binding protein FABP3, fast troponin TnI (TNNI2), myosin light chain MYL3, fragments of the giant muscle protein titin (TTN), the molecular chaperone Hsp70 (HSPA1A), mitogen-activated protein kinase MAPK12, and Ca^{2+} /calmodulin-dependent protein kinase CAMK2A.^{49-52,71-87} The measurement of serum inflammatory cytokines in dystrophic dog models suggests that elevated levels of C-C motif chemokine ligand 2 (CCL2) could be useful disease biomarkers of dystrophinopathy.^{88,89} In addition, a high concentration of the acute phase protein haptoglobin, which is mostly produced in the liver, was discovered in the serum from an animal model of dystrophinopathy.³⁷ Interestingly, amino-terminal fragments of the extracellular dystrophin-association glycoprotein alpha-dystroglycan were detected in serum by ELISA tests, making this crucial laminin/merosin-binding protein of the dystrophin-glycoprotein complex a potential biofluid marker of

DMD.⁹⁰ A recent study indicates that changes in proteins encoded by the *RGMA*, *EHMT2*, *ART3*, *ANTXR2* and *DLK1* genes are associated with an increased risk of limb clinical milestones in DMD.⁹¹

Carbonic anhydrase CA3

Elevated levels of CA3, a key muscle enzyme with anti-oxidative function that is intrinsically involved in the physiological maintenance of the intracellular pH-value, were established to occur in the serum of DMD patients.⁴⁹ This agrees with previous non-proteomic screening studies.⁹²⁻⁹⁴ A variety of CA isoforms, which catalyse the reversible hydration of carbon dioxide, exist in subtypes of contractile tissues.⁴⁹ The establishment of fibre type specific markers is important for the cell biological classification of skeletal muscles in health and disease. In the human musculature, slow-twitching fibers that exhibit oxidative metabolism are of type I, predominantly fast-twitching fibres with an oxidative-glycolytic bioenergetic profile are of type IIa, and fast-twitching fibres with high levels of glycolysis are of type IIx.⁹⁵ Fibre types can be differentiated by their expression of the myosin heavy chain (MyHC) isoforms MyHC-1, MyHC-2a and MyHC-2x in type I, IIa and IIx fibers, respectively.⁹⁶ In addition to the differential MyHC expression, cytosolic CA3 can also be utilized to characterize fibre type specification. CA3 is significantly enriched in slow-twitching type I myofibres, making it a reliable marker enzyme of fibre type distribution and skeletal muscle adaptations due to changed functional demands or pathophysiological changes that are associated with fibre type shifting.^{97,98} Of note, in DMD, CA3 levels were shown to be decreased in muscle tissues and elevated in serum.^{37,49,51,52,75}

Fatty acid binding protein FABP3

The muscle/heart isoform of the fatty acid transporter, FABP3 (H-FABP), belongs to the family of lipid chaperones that mediate extra- and intracellular movements of hydrophobic metabolites.⁸⁴ Voluntary muscles express high levels of FABP3 in addition to FABP1, FABP2, FABP4 and FABP5, which are involved in bioenergetic metabolism and cellular signalling processes. Systematic proteomic surveys of FABP isoforms in skeletal muscle, in combination with heart, kidney, liver and serum, revealed that dystrophinopathies are associated with considerable changes in metabolite transportation and fatty acid metabolism in DMD.⁸⁴

Dystrophin-deficient cardiac and skeletal muscle tissues contain decreased concentration of FABP3, but serum from DMD patients and dystrophic animal models shows elevated FABP3 levels.^{37,49,51,52,75} Interestingly, animal models of DMD exhibit increased FABP5 in the liver and FABP1 in the kidney in association with ectopic fat deposition.⁹⁹⁻¹⁰¹ This indicates that impaired liver function and renal failure that may occur at advanced stages of DMD,^{2,7} in combination with abnormalities in the gastrointestinal tract and chronic inflammation,¹⁰²⁻¹⁰⁴ could be associated with abnormal fat metabolism. This is most likely due to impaired cardiovascular functioning and abnormal circulation which

starves organs of the proper supply with nutrients, metabolites, oxygen, hormones and signalling factors.⁸⁴

Sarcomeric proteins

The complex arrangement of actin/nebulin-containing thin filaments with their regulatory troponin and tropomyosin complexes, myosin-containing thick filaments, auxiliary TTN filaments, Z-disc complexes and M-line proteins has been extensively characterized by proteomics.^{105,106} Degenerative muscle diseases are characterized by disintegration of the acto-myosin complex and associated sarcomeric proteins. It is therefore not surprising that the release of contractile proteins from damaged myofibres in DMD has been confirmed by MS-based studies.^{37,50-52,75} Of special interest are fragments of TTN, an abundant high-molecular-mass protein that belongs to the class of giant muscle proteins,¹⁰⁷ TTN of apparent 3,900 kDa is a half-sarcomere spanning protein that functions as a mechanical stabilizer of the contractile apparatus and is involved in sarcomere assembly and maintenance, as well as the provision of muscle elasticity.¹⁰⁸ Degradation of TTN and the release of peptide fragments are clearly associated with DMD.^{86,87} TTN-derived peptides have been

identified in both serum and urine samples from DMD patients and animal models of dystrophinopathy.^{85,109} In addition, myosin binding protein MYBP, troponin subunit TnI, myomesin isoform MYOM3, actin and myosin light chain MLC3 were identified in DMD serum.^{37,50-52,75} An increased concentration of cardiac troponin cTnI is a promising candidate biomarker for late-onset in DMD patients.^{110,111} Besides sarcomeric markers, the elevated levels of interleukin 1 receptor-like 1 protein (ST2) in the serum of cardiomyopathic DMD patients was shown to be a promising for minimally invasive monitoring of cardiac complications in dystrophinopathy.¹¹² The degradation and misfolding of sarcomeric proteins is associated with elevated levels of molecular chaperones in the serum of dystrophic organisms, which agree with a considerable cellular stress response in dystrophic muscles, as confirmed by proteomics.¹¹³⁻¹¹⁵

Myofibrosis markers

The microenvironment of skeletal muscles, which plays a critical role in lateral and longitudinal force transmission, myofiber maintenance and repair mechanisms,¹¹⁶ is characterized by complex layers of the extracellular matrix

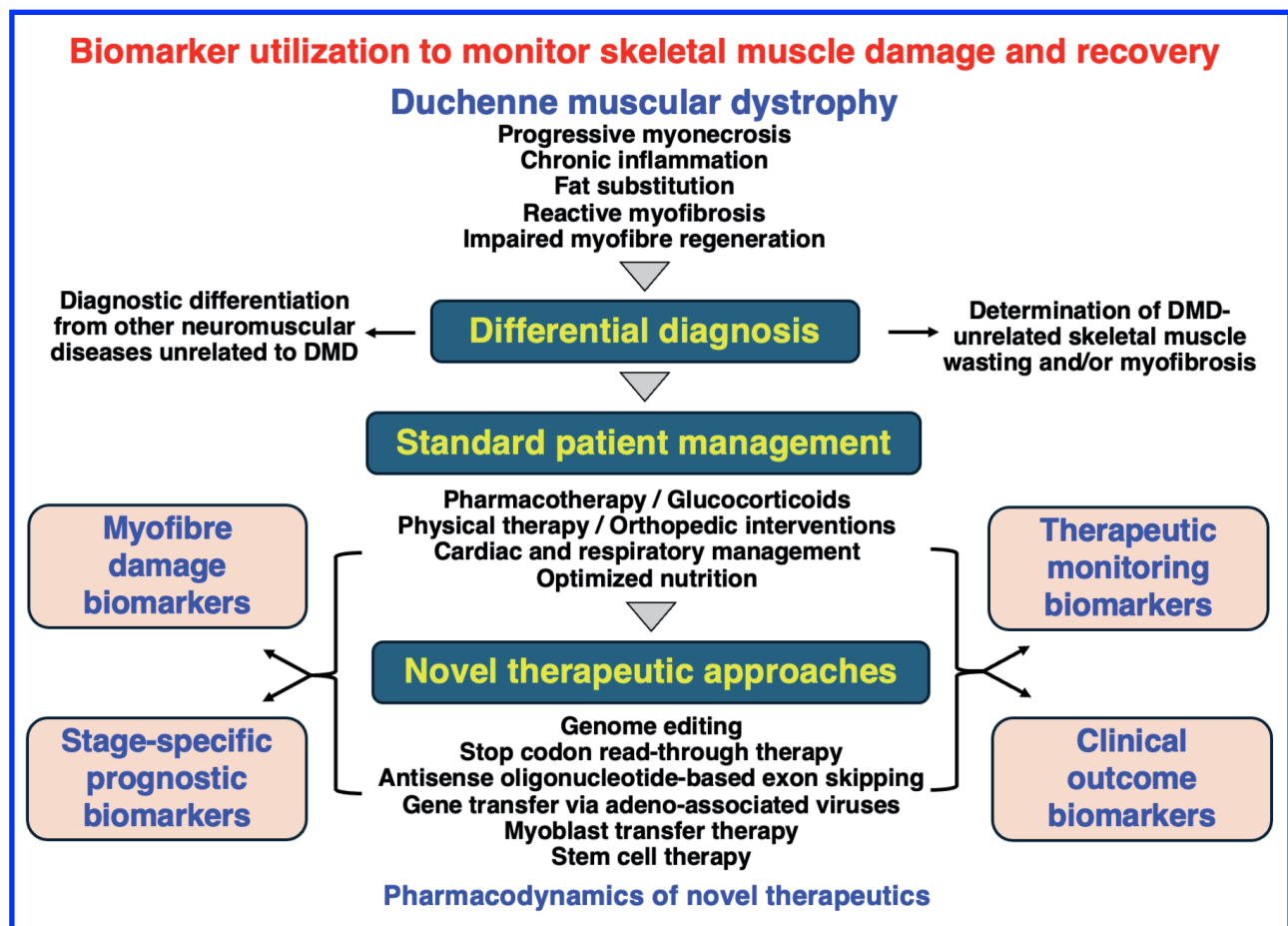


Figure 3. Outline of the usefulness of serum protein biomarkers for the diagnosis, prognosis and therapeutic monitoring of dystrophinopathy.

(ECM).¹¹⁷ The basal lamina and endomysium form the innermost ECM structure which encloses and protects individual myofibres, followed by the perimysium which covers each fasciculus, and the epimysium which surrounds the entire skeletal muscle.¹¹⁸

The MS-based analysis of the extracellular matrix of dystrophic skeletal muscles has identified drastic increases in matricellular proteins, proteoglycans, ECM-associated glycoproteins and various collagens spanning the endomysium, perimysium and epimysium.¹¹⁹⁻¹²¹ This upregulation of ECM components is indicative of reactive myofibrosis,^{122,123} and is reflected by high serum levels of FN, osteopontin, collagen fragments, the cartilage oligomeric matrix protein COMP, and matrix-metalloproteinase MMP-9 plus tissue inhibitors of the TIMP class of proteins.^{37,71,124-126}

Future perspectives

As outlined in Figure 3, novel serum biomarkers of DMD can be utilized in a variety of clinical applications including differential diagnosis, stage-specific prognosis, therapeutic monitoring with repeated sampling options and clinical endpoint measurements. Current patient management and treatment of DMD focuses on pharmacotherapy with glucocorticoids, in combination with physiotherapy, orthopaedic interventions, cardiorespiratory management and nutritional support. The suitability of new therapies, the determination of clinical success and the evaluation of potential adverse effects of novel approaches to treat dystrophinopathy have to be properly assessed. New treatments include improved pharmacotherapy, genome editing, stop codon read-through therapy, antisense oligonucleotide-based exon skipping, gene transfer via adeno-associated viruses, myoblast transfer therapy and stem cell therapy.^{19-23,32} Thus, to support the clinical evaluation of the functional trajectory of DMD,¹²⁷⁻¹²⁹ it is important to develop reliable liquid biopsy tests.

As recently reviewed by Benemei et al.,¹² crucial aspects of biomarker assays relate to relevance, quantifiability, validity, objectivity, reliability, sensitivity, specificity and precision for measuring diagnostic, prognostic and therapeutic monitoring aspects of DMD. Regarding biofluid markers of skeletal muscle degeneration in dystrophinopathy, ideally these biomarkers exhibit no or only a minimal overlap with other tissue damage markers that are released from the heart, liver, kidney, smooth muscles, the immune system, or the central and peripheral nervous system.

In general, protein biomarker assays should be suitable to evaluate focal abnormalities, as well as the progression of systemic disease. Assay systems should be robust, cost-effective and not prone to disproportionate sampling errors in relation to false negatives or false positives. In biomarker testing, crucial modifying parameters have to be taken into account, such as effects due to the circadian rhythm, potential seasonal variations, patient age, gender, ethnicity, co-morbidities, extensive pharmacotherapy, surgical treatments, nutrition, physical activity, life style and general health status.

List of abbreviations

AST, aspartate transaminase
CA3, carbonic anhydrase isoform 3
CAMK2A, Ca²⁺/calmodulin-dependent protein kinase 2A
CK, creatine kinase
COMP, cartilage oligomeric matrix protein
DMD, Duchenne muscular dystrophy
Dp427-M, muscle dystrophin protein of 427 kDa
ECM, extracellular matrix
ELISA, enzyme-linked immunosorbent assay
FABP, fatty acid binding protein
Hsp, heat shock protein
LDH, lactate dehydrogenase
MAPK12, mitogen-activated protein kinase 12
MB, Myoglobin
MDH2, malate dehydrogenase 2
MMP-9, matrix-metalloproteinase 9
MS, mass spectrometry
mt, mitochondrial
MYL3, myosin light chain 3
MYOM3, myomesin isoform 3
TIMP, tissue inhibitor of metalloproteinases
TnI, troponin subunit TnI

Authors contributions

PD, EN, CT, MZ, DS, and KO were involved in the conceptualization and initiation of this review article. All authors were involved in the writing and final editing of the manuscript.

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Conflict of interest

The authors declare no competing interests.

Ethical publication statement

Not applicable.

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