Safety and disease monitoring biomarkers in Duchenne muscular dystrophy: results from a Phase II trial

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Aim: Evaluate the utility of glutamate dehydrogenase (GLDH) and cardiac troponin I as safety biomarkers, and creatine kinase and muscle injury panel as muscle health biomarkers in Duchenne muscular dystrophy. **Patients & methods:** Data were collected during a Phase II trial of domagrozumab. **Results:** GLDH was a more specific biomarker for liver injury than alanine aminotransferase. Cardiac troponin I elevations were variable and not sustained, limiting its applicability as a biomarker. Muscle injury panel biomarkers were no more informative than creatine kinase as a muscle health biomarker. **Conclusion:** Results support the use of GLDH as a specific biomarker for liver injury in patients with Duchenne muscular dystrophy.
Clinical trial registration: ClinicalTrials.gov, NCT02310763.

Duchenne muscular dystrophy (DMD), an X-linked disorder caused by loss of function mutations in dystrophin, is among the most common and severe muscular dystrophies. Dystrophin is a critical component of a protein complex that is responsible for stabilizing the plasma membrane of muscle fibers [1]. The lack of functional dystrophin over time leads to sarcolemma instability and degeneration of muscle fibers, which are replaced by fibrosis and fat [1]. Availability of sensitive and specific biomarkers is important for developing therapies, in addition to monitoring available therapies. There is currently an unmet need for not only disease monitoring biomarkers to understand progression of the disease, but also for specific safety biomarkers that can accurately diagnose liver health in patients with DMD.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are commonly used in clinical practice for routine safety monitoring and determination of risk of drug-induced liver injury (DILI) [2]. However, these serum enzymes have limitations in monitoring patients with DMD as they are also released from skeletal muscle, and they are markedly elevated in this population of patients. Elevated levels of ALT and AST due to underlying muscle pathology can make diagnosis of DILI challenging. These enzymes are broadly produced in multiple tissues and are elevated from membrane instability and leakage from dystrophic muscle, and during muscle degeneration [3,4].

Identifying liver-specific, noninvasive biomarkers may increase the precision of detecting DILI in the background of muscle disease. The recently completed trial of domagrozumab in DMD [5], provided the opportunity to study the performance of novel soluble safety and muscle health biomarkers in this rare muscle disease. This trial used

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a sequential, short interval of soluble biomarkers sampling and assessed biomarker variability independent of changes in functional status. Serum glutamate dehydrogenase (GDH or GLDH), an emerging liver injury-specific biomarker [6], was evaluated as a safety biomarker in this trial. GLDH is a mitochondrial enzyme that converts glutamate to α-ketoglutarate and is primarily found in the hepatocytes, with only trace amounts in skeletal muscles. The specificity of GLDH for liver injury has been demonstrated in preclinical species [7]. The US FDA has recognized the importance of GLDH as a specific biomarker of liver injury in human subjects with underlying muscle injury or degeneration [8] and recently accepted the qualification plan submitted by the Critical Path Institute's (C-Path) Predictive Safety Testing Consortium. Similarly, the EMA has issued a letter of support to encourage the further study of GLDH for the monitoring of liver injury [9]. These are major milestones toward qualifying GLDH as a safety biomarker in this specific context of use.

Assessment of cardiac safety includes monitoring cardiac troponin I (cTnI), a commonly used and FDA-qualified cardiac biomarker. cTnI is elevated in some patients with DMD [10]. A recent study showed correlation between serum cTnI levels and two cardiac MRI biomarkers (late gadolinium enhancement and native T1) of myocardial disease [11]. However, the clinical utility, context and significance have not been studied serially in an interventional clinical trial.

Creatine kinase (CK) in the serum is the conventional biomarker of skeletal muscle injury, but investigations to identify improved biomarkers continue [12]. CK also varies substantially from day to day in those with DMD and is believed to depend on activity level [13,14]. A skeletal muscle injury panel (MIP) of biomarkers consisting of sTnI, FABP3, creatine kinase muscle-type (CKM) and Myl3), has been proposed as more specific and sensitive of skeletal muscle injury based on recent comprehensive nonclinical studies using mechanistically distinct myotoxicants and controls. This biomarker panel has been extensively characterized and validated [15,16,17,18] and the FDA has provided a letter of support toward their utility as safety biomarkers in nonclinical studies. Encouragingly, the MIP has been recently evaluated in patients with genetic muscle diseases [12,19,20].

The objective of these analyses in a dense sampling paradigm using the longitudinal setting of a 96-week clinical trial [5] was twofold: to evaluate the utility of cTnI and GLDH as acute safety biomarkers for drug-induced cardiac and liver injury, respectively, in patients with DMD; and to evaluate the correlation between CK, sTnI, FABP3, CKM and Myl3 and muscle health [19,20].

Materials & methods

Patients & study design

As described previously [5], this was a Phase II, randomized, double-blind, two-period (48 weeks each) study of domagrozumab (intravenous every 4 weeks) versus placebo in patients with DMD (ClinicalTrials.gov: NCT02310763). Patients were randomized into one of three treatment sequences (SEQ). In SEQ1, patients (n = 41) were treated with escalated doses of domagrozumab (5, 20 and 40 mg/kg) in the first period and with domagrozumab (40 mg/kg) in the second period. In SEQ2, patients (n = 39) were treated with escalated doses of domagrozumab (5, 20 and 40 mg/kg) in the first period and with placebo in the second period. In SEQ3, patients (n = 40) were treated with placebo in the first period and dose-escalated domagrozumab (5, 20 and 40 mg/kg) in the second period.

Except for the cTnI data, which were collected over the two periods of the study (96 weeks) and reported per treatment sequence, all data described here were collected during the first period (first 48 weeks). In this period, 80 patients were treated with domagrozumab within-patient dose escalation (5, 20 and 40 mg/kg), and 40 patients were treated with placebo. The study included ambulatory boys, 6 to <16 years of age, who were able to perform the four-stair climb in 2.5 to ≤12 seconds at screening, and were treated with glucocorticosteroids for ≥6 months with stable regimen for ≥3 months prior to screening.

Sample collection

Blood samples for GLDH, CK, ALT, AST and MIP biomarkers were collected at baseline and Weeks 5, 9, 13, 17, 21, 25, 29, 33, 37, 41 and 45. Blood samples for cTnI were collected at baseline, Weeks 17, 33, 49, 65, 81, 97, and at early withdrawal from study. Blood collections were performed per the study site's local standard operating procedures. Whole blood samples were processed to serum or plasma by centrifugation. The serum or plasma was removed and stored frozen at -80°C until testing. Samples for CK, ALT, AST and cTnI were transported on dry ice to Covance Central Laboratory Services (IN, USA). The MIP biomarkers in serum samples were assayed at Pfizer Bioanalytics Laboratory (CT, USA). The serum aliquot designated for GLDH testing was forwarded to Pfizer Clinical Pathology Laboratory.

Safety biomarkers

Measurement of serum ALT, AST and CK enzymatic activity were performed using validated assay methods on Roche cobas® instrumentation and Roche Diagnostics reagent systems (Roche Diagnostics, IN, USA). Serum GLDH activity was quantified using Randox GLDH reagent kit (catalog #GL441; Randox Laboratories Ltd, Crumlin, UK) on an ADVIA 1800 (Siemens Healthineers, NY, USA). The ADVIA Centaur XP TnI-Ultra method was used to evaluated the levels of cTnI in serum. Other cardiac testing included 12-lead ECG and cardiac MRI with gadolinium or echocardiography (ECHO; if cardiac MRI was not available at the site) to evaluate left-ventricular ejection fraction. ECG was assessed concurrently with cTnI, and MRI/ECHO was assessed annually at Weeks 49, 97, and at early withdrawal.

Skeletal muscle injury panel

The MIP biomarkers included the analytes sTnI, FABP3 and Myl3 measured using the Meso Scale Discovery (MSD) Muscle Injury Panel 1 reagent kit (catalog #K15181C; MSD, MD, USA) and CKM using the MSD Muscle Injury Panel 2 reagent kit (catalog #K15180C). Both reagent kits are immunoassay platforms that utilize an electrochemiluminescent detection method. The assays were performed as per the manufacturer's instructions and were fit-for-purpose validated. Serum samples were diluted to be within the dynamic range of the assays. Samples for sTnI, FABP3 and Myl3 quantification were diluted 1:8 prior to analysis in kit diluent. Samples for CKM measurement were initially diluted 1:600 in kit diluent with some samples requiring up to a 1:1800 sample dilution to be within the dynamic range of the assay. Samples had undergone one or two freeze–thaw cycles prior to quantitation.

Statistical analysis

Samples from placebo- and domagrozumab-treated patients were combined for these analyses, except for the cTnI data, which were analyzed per treatment sequence. Correlation analyses to evaluate relationships between the biomarkers were performed using Spearman's correlation. These analyses included individual patient data at all time points through Week 49. cTnI values are shown over the 96 weeks by SEQ.

The upper limit of normal (ULN) varied depending on age: ALT was 34 U/l for 6–10 years old and 43 U/l for 10–16 years old; AST was 59 U/l for 6–7 years old and 40 U/l for 7–16 years old; CK was 158 U/l for 6–7 years old, 354 for 7–10 years old and 363 U/l for 10–13 years old. The ULN for GLDH was 10 U/l. For cTnI, the lower limit of quantification was 0.03 ng/ml, which was reported as the ULN. No age-related differences in ULN were reported for cTnI nor GLDH. No standardized ULN are available for the other parameters. Biomarker parameters were analyzed over time using simple linear regression with 99% prediction intervals.
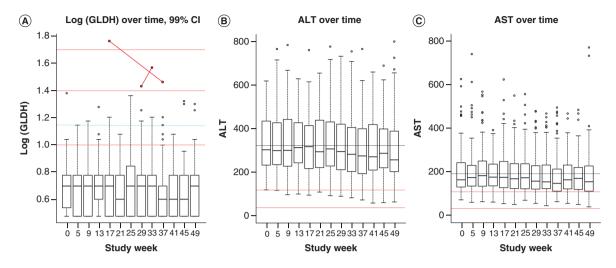
Results

GLDH versus ALT & AST

The mean values of AST and ALT remained relatively stable over time through Week 49 (Figure 1). No patient had increased total bilirubin >2× ULN. No patient had abnormal serum concentration (>2× ULN) of γ-glutamyl transferase (GGT), a biomarker of injury of the hepatobiliary system. Two patients had GGT values above ULN (1.04–1.71) during the study at various visits, but these were not reported as clinically significant.

GLDH levels showed some variability over time, with two patients having levels above 2.5× ULN during period 1 (Figure 1). One patient (SEQ1) had one report of GLDH >5× ULN while on 5 mg/kg, and one report of >2.5× ULN while on 40 mg/kg domagrozumab). This patient had elevated total bilirubin (1.25–1.5 mg/dl) which started in the first period and was subsequently diagnosed with Gilbert's syndrome. The second patient (SEQ3) had a transient elevation of GLDH >2.5× ULN at screening (data not shown), and two elevations of >2.5× ULN in the first period while on placebo. Both of these patients, and one additional patient (SEQ1), each had one report of GLDH >2.5× ULN during the second period while on domagrozumab (data not shown).

GLDH levels were generally within the normal range while both ALT and AST were generally markedly elevated. (Figure 2). CK moderately correlated with ALT (r = 0.63, p < 0.01), but weakly correlated with GLDH (r = 0.34, p < 0.01; Supplementary Figure 1). No drug-induced liver adverse events (AEs) were reported in this study.

Cardiac troponin I

cTnI concentration was elevated (>0.03 ng/ml) in approximately 20% of patients at baseline (range, 0.04–1.43 ng/ml). Elevations (range, 0.04–3.53 ng/ml) were not sustained over time (Figure 3). Most elevated cTnI

Figure 1.   Glutamate dehydrogenase, alanine aminotransferase and aspartate aminotransferase over time. All time points included through Week 49. **(A)** GLDH figure on the log_{10} scale. Three red reference lines indicate 1×, 2.5× and 5× ULN. Two patients with values >2.5× ULN are connected with red lines. The blue dashed line indicates the upper limit of the 99% prediction interval. In the ALT **(B)** and AST **(C)** panels, the two red reference lines indicate 1× and 3× ULN, and the black line indicates the overall mean.
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GLDH: Glutamate dehydrogenase; ULN: Upper limit of normal.
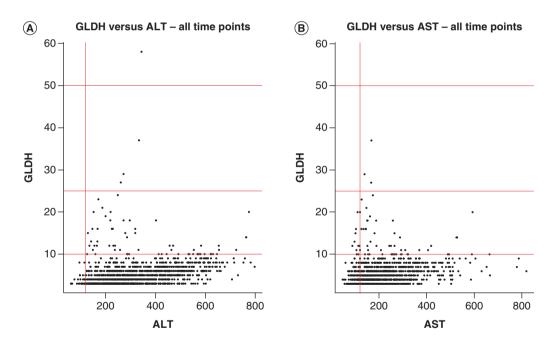

Figure 2.   Glutamate dehydrogenase versus alanine aminotransferase and aspartate aminotransferase. Figure shows individual patient data at all time points through Week 49. Vertical reference line indicates 3× ULN for both **(A)** ALT and **(B)** AST: ~120 U/l. Three horizontal reference lines indicate ULN (10 U/l), 2.5× ULN (25 U/l) and 5× ULN (50 U/l) for GLDH.
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GLDH: Glutamate dehydrogenase; ULN: Upper limit of normal.

values ranged between 0.04 and 0.94 ng/ml, with 11 measurements between 1.21 and 3.53 ng/ml. In most patients with normal cTnI values at baseline, the levels remained normal throughout the study. None of the patients with elevated cTnI had clinically meaningful changes in symptomatology or ECG (assessed concurrently with cTnI) or on cardiac MRI/ECHO (assessed annually) performed by the treating physicians and reviewed by the unblinded
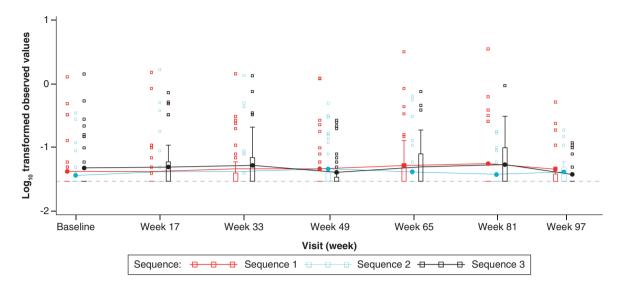
Figure 3.   Cardiac troponin I elevations by treatment sequence. Solid circles denote arithmetic mean. Squares denote an outlier. Dashed line refers to upper limit of the test. Box plot provides median and 25/75% quartiles with whiskers to the last point within 1.5× interquartile range.

external data monitoring committee. The AE of troponin increase was reported in two patients in the first period (placebo, n = 2; domagrozumab, n = 2), and in four patients in the second period (placebo, n = 1; domagrozumab, n = 3). All of the AEs of troponin increase were mild in severity.

CK, CKM & MIP

All biomarkers (CK, CKM, FABP3, Myl3 and sTnI) remained relatively stable through Week 49 (Supplementary Figure 2). There was a nonsignificant progressive decline in mean Myl3 with domagrozumab versus placebo across the three sequence groups over the 96 weeks of the study (data not shown). As expected, there was a strong correlation between CK and CKM (r = 0.91, p < 0.01; Supplementary Figure 3). CK had moderate correlation with FABP3 (r = 0.40) and Myl3 (r = 0.46), and strong correlation with sTnI (r = 0.76); all correlations were significant (p < 0.01; Supplementary Figure 4).

Discussion

Serum ALT activity is commonly used in clinical practice to monitor for signs of DILI. However, in patients with underlying muscle disease, the increased serum activity of ALT from DILI is confounded by ALT released from the damaged muscles. The lack of liver specificity of ALT highlights the need for a specific biomarker in patients with DMD. GLDH is emerging as a specific biomarker of liver injury in individuals with underlying muscle damage. Therefore, we evaluated the potential of GLDH to monitor for signs of liver injury in patients with DMD who participated in the domagrozumab Phase II trial. Our results suggest that, compared with ALT and AST, GLDH may be a more specific biomarker to monitor for signs of liver injury in patients with DMD. The poor correlation between GLDH and ALT, AST or CK, a widely accepted biomarker of skeletal muscle injury, suggests that serum GLDH is not affected by muscle injury, whereas the moderate correlation between ALT and CK, further demonstrates the lack of ALT specificity to liver injury in this population. These results are consistent with those of Schomaker *et al.* who reported that GLDH levels in patients with DMD were equivalent to those in healthy subjects, whereas ALT levels were significantly higher in patients with DMD compared with healthy subjects [21]. Because no liver injury occurred during the current study based on Hy's law criteria, the elevated levels of ALT and AST, and the lack of elevation in GLDH, suggests that GLDH may be more specific to rule out possible DILI.

GGT is another biomarker that is often used to detect liver injury. GGT activity is elevated in response to drug-induced injury of the liver biliary system, which accounts for 23% of DILI. However, GGT elevation alone is not sufficient to diagnose hepatocellular injury, but may confirm hepatic origin when elevations in alkaline phosphatase are detected [22]. Given that no abnormal serum GGT levels were reported in this study, future studies

may be needed to confirm whether GLDH is a more sensitive biomarker of DILI compared with GGT in patients with DMD.

In this study, we evaluated the value of serum cTnI as a safety biomarker in patients with DMD. Our results showed that in most patients, normal cTnI values at baseline remained normal throughout the study. Elevated cTnI values at baseline were not predictive of subsequent values in the trial. Over the course of the study, sustained cTnI elevations were infrequent. Because cardiac MRI/ECHO data were not collected at all timepoints of cTnI data collection, this study is not powered to evaluate whether cTnI elevations are able to predict a decline in cardiac function by left-ventricular ejection fraction.

Sensitive and specific noninvasive biomarkers are needed to detect DMD disease progression and response to treatment. We evaluated the utility of the recently suggested panel of biomarkers for skeletal muscle injury, which consists of sTnI, FABP3, CKM and Myl3, and was found to be more specific than total serum CK and correlated with efficacy end points in DMD [19]. Our results showed that the mean levels of these biomarkers or total serum CK did not change significantly throughout the study. CK correlated significantly with the MIP biomarkers; however, a comparison of the sensitivity and specificity of the MIP panel and CK is not possible in the context of DMD since there is always ongoing muscle injury. Given the wide range of the eligibility criteria in this study, it is possible that MIP may be a better predictor of disease progression in subgroups of these patients or in a different phase of the disease.

The study has several limitations. There were no liver injuries reported during this study and, hence, the benefit of detecting liver injury with GLDH in patients with DMD has to be further confirmed in prospective clinical trials and potentially over a longer period of time. Secondly, these analyses were conducted in patients with DMD with no comparison to healthy subjects, which could have further highlighted the specificity of GLDH over ALT in patients with DMD. Lastly, the correlation analyses included a large number of data points, which may have contributed to the statistical significance reached.

Conclusion

We investigated the utility of several biomarkers in detecting safety and DMD muscle health. Our results support the use of GLDH as a specific biomarker for liver injury in patients with DMD. cTnI values in this study fluctuated in both directions and were not sufficient to predict cardiomyopathy progression without other supportive clinical evidence. Furthermore, in this study population, MIP was not shown to be more specific than CK in detecting skeletal muscle injury in patients with DMD. Additional analyses may be required to determine the value of MIP in specific subgroups of patients with DMD or mechanistically different forms of muscle diseases.

Summary points

- There is currently an unmet need for biomarkers to understand progression of the Duchenne muscular dystrophy (DMD) and for specific safety biomarkers that can accurately diagnose liver health in these patients.
- The objectives of this study were to evaluate the utility of glutamate dehydrogenase and cardiac troponin I as acute safety biomarkers, and creatine kinase (CK), sTnI, FABP3, creatine kinase muscle-type and Myl3 as biomarkers of muscle health in DMD.
- A sequential, short interval of soluble biomarkers sampling were collected during a 96-week Phase II trial of domagrozumab in patient with DMD.
- The study included ambulatory boys aged 6 to <16 years.
- Our results suggest that glutamate dehydrogenase may be a more specific biomarker to monitor for signs of liver injury in patients with DMD compared with alanine aminotransferase, which is a commonly used biomarker for liver injury.
- The elevations observed in cardiac troponin I did not predict a greater decline in cardiac function by left-ventricular ejection fraction.
- CK correlated significantly with sTnI, FABP3, creatine kinase muscle-type and Myl3, suggesting that muscle injury panels may not be more sensitive than CK in detecting skeletal muscle injury in patients with DMD.
- Additional analyses may be required to determine the value of muscle injury panels in specific subgroups of patients with muscular dystrophies or within DMD.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/bmm-2021-0222

Author contributions

All authors contributed to study concepts and design, data analysis and interpretation; KR Wagner, M Guglieri, F Muntoni contributed to data acquisition; J Palmer contributed to statistical analysis; all authors contributed to manuscript preparation, editing and approval of final draft.

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Financial & competing interests disclosure

Ethical conduct of research

The protocol and informed consent were approved by the institutional review board or ethics committee at each study center. The study was conducted in accordance with the Declaration of Helsinki, International Ethical Guidelines for Biomedical Research Involving Human Subjects, and guidelines for Good Clinical Practices. Parent or legal guardians provided written, informed consent prior to study initiation.

Data sharing statement

Upon request, and subject to certain criteria, conditions and exceptions (see https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information), Pfizer will provide access to individual de-identified participant data from Pfizer-sponsored global interventional clinical studies conducted for medicines, vaccines and medical devices (1) for indications that have been approved in the USA and/or EU or (2) in programs that have been terminated (i.e., development for all indications has been discontinued). Pfizer will also consider requests for the protocol, data dictionary and statistical analysis plan. Data may be requested from Pfizer trials 24 months after study completion. The de-identified participant data will be made available to researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. To gain access, data requestors must enter into a data access agreement with Pfizer.

Open access

References

Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

1. Blake DJ, Weir A, Newey SE, Davies KE. Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol. Rev.* 82(2), 291–329 (2002).

2. Aubrecht J, Schomaker SJ, Amacher DE. Emerging hepatotoxicity biomarkers and their potential to improve understanding and management of drug-induced liver injury. *Genome Med.* 5(9), 85 (2013).

3. Tay SK, Ong HT, Low PS. Transaminitis in Duchenne's muscular dystrophy. *Ann. Acad. Med. Singapore.* 29(6), 719–722 (2000).

4.   McMillan HJ, Gregas M, Darras BT, Kang PB. Serum transaminase levels in boys with Duchenne and Becker muscular dystrophy. *Pediatrics* 127(1), e132–e136 (2011).

5.   Wagner KR, Abdel-Hamid HZ, Mah JK *et al.* Randomized Phase II trial and open-label extension of domagrozumab in Duchenne muscular dystrophy. *Neuromuscul. Disord.* 30(6), 492–502 (2020).

6.   Schomaker S, Warner R, Bock J *et al.* Assessment of emerging biomarkers of liver injury in human subjects. *Toxicol. Sci.* 132(2), 276–283 (2013).

•   **Evaluates the utility of glutamate dehydrogenase (GLDH) as indicators of liver injury in healthy subjects, those with liver impairments, and in cases of acetaminophen poisoning.**

7.   O'Brien PJ, Slaughter MR, Polley SR, Kramer K. Advantages of glutamate dehydrogenase as a blood biomarker of acute hepatic injury in rats. *Lab. Anim.* 36(3), 313–321 (2002).

8.   US Food & Drug Administration. Transition summary response letter to the Critical Path Institute's Predictive Safety Testing Consortium Hepatoxicity Working Group (2018). https://www.fda.gov/media/119803/download

9.   European Medicines Agency. Letter of support for glutamate dehydrogenase, a biomarker of hepatocellular liver injury (2017). https://www.ema.europa.eu/en/documents/other/letter-support-glutamate-dehydrogenase-biomarker-hepatocellular-liver-injury_en.pdf

10. Hor KN, Johnston P, Kinnett K *et al.* Progression of Duchenne cardiomyopathy presenting with chest pain and troponin elevation. *J. Neuromuscul. Dis.* 4(4), 307–314 (2017).

11. Voleti S, Olivieri L, Hamann K, Gordish-Dressman H, Spurney C. Troponin I levels correlate with cardiac MR LGE and native T1 values in Duchenne muscular dystrophy cardiomyopathy and identify early disease progression. *Pediatr. Cardiol.* 41(6), 1173–1179 (2020).

•   **Shows significantly increased troponin levels in patients with Duchenne muscular dystrophy with mild late gadolinium enhancement on cardiac MRI compared with those with no late gadolinium enhancement.**

12. Hathout Y, Seol H, Han MH, Zhang A, Brown KJ, Hoffman EP. Clinical utility of serum biomarkers in Duchenne muscular dystrophy. *Clin. Proteomics.* 13, 9 (2016).

•   **Describes advances in development of noninvasive blood circulating biomarkers to assess disease progression and response to therapies in Duchenne muscular dystrophy.**

13. Baird MF, Graham SM, Baker JS, Bickerstaff GF. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. *J. Nutr. Metab.* 2012, 960363 (2012).

14. Jackson MJ, Round JM, Newham DJ, Edwards RH. An examination of some factors influencing creatine kinase in the blood of patients with muscular dystrophy. *Muscle Nerve* 10(1), 15–21 (1987).

15. Burch PM, Greg Hall D, Walker EG *et al.* Evaluation of the relative performance of drug-induced skeletal muscle injury biomarkers in rats. *Toxicol. Sci.* 150(1), 247–256 (2016).

16. Tonomura Y, Matsushima S, Kashiwagi E *et al.* Biomarker panel of cardiac and skeletal muscle troponins, fatty acid binding protein 3 and myosin light chain 3 for the accurate diagnosis of cardiotoxicity and musculoskeletal toxicity in rats. *Toxicology* 302(2–3), 179–189 (2012).

17. Bodie K, Buck WR, Pieh J, Liguori MJ, Popp A. Biomarker evaluation of skeletal muscle toxicity following clofibrate administration in rats. *Exp. Toxicol. Pathol.* 68(5), 289–299 (2016).

18. Maliver P, Festag M, Bennecke M *et al.* Assessment of preclinical liver and skeletal muscle biomarkers following clofibrate administration in Wistar rats. *Toxicol. Pathol.* 45(4), 506–525 (2017).

19. Burch PM, Pogoryelova O, Goldstein R *et al.* Muscle-derived proteins as serum biomarkers for monitoring disease progression in three forms of muscular dystrophy. *J. Neuromuscul. Dis.* 2(3), 241–255 (2015).

••   **Shows that compared with healthy controls, serum concentrations of sTnI, Myl3, FABP3 and creatine kinase muscle-type were all significantly elevated in three forms of muscular dystrophy, including Duchenne muscular dystrophy.**

20. Goldstein RA. Skeletal muscle injury biomarkers: assay qualification efforts and translation to the clinic. *Toxicol. Pathol.* 45(7), 943–951 (2017).

•   **Evaluates the diagnostic performance of a muscle injury biomarker panel and showed that muscle injury panel outperformed the routine biomarkers of creatine kinase and aspartate aminotransferase.**

21. Schomaker S, Potter D, Warner R *et al.* Serum glutamate dehydrogenase activity enables early detection of liver injury in subjects with underlying muscle impairments. *PLoS ONE* 15(5), e0229753 (2020).

••   **Shows that serum GLDH levels were not impacted by muscle injury, and therefore, GLDH has a potential to improve diagnosis of hepatocellular injury in patients with underlying muscle impairments.**

22. European Association for the Study of the Liver, Clinical Practice Guideline Panel. EASL clinical practice guidelines: drug-induced liver injury. *J. Hepatol.* 70(6), 1222–1261 (2019).