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Citation

Velde, N. M. van de, Koeks, Z., Signorelli, M., Verwey, N., Overzier, M., Bakker, J. A., ... Niks, E. H. (2022). Longitudinal assessment of creatine kinase, creatine/creatinine ratio and myostatin as monitoring biomarkers in Becker muscular dystrophy. *Neurology*. doi:10.1212/WNL.0000000000201609

Version: Accepted Manuscript

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Downloaded from: <https://hdl.handle.net/1887/3564395>

Note: To cite this publication please use the final published version (if applicable).

OPEN

Neurology[®]

The most widely read and highly cited peer-reviewed neurology journal
The Official Journal of the American Academy of Neurology



Neurology Publish Ahead of Print
DOI: 10.1212/WNL.000000000201609

Longitudinal Assessment of Creatine Kinase, Creatine/Creatinine_{ratio} and Myostatin as Monitoring Biomarkers in Becker Muscular Dystrophy

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Neurology[®] Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.

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Figure Count:

5

Table Count:

1

Search Terms:

[185] Muscle disease, Becker muscular dystrophy, biomarkers, prognostic, serum

Acknowledgment:

The authors are grateful to all the patients who participated in this study.

Study Funding:

The study was funded by Grant 113302001 from The Netherlands Organization for Health Research and Development (Hague The Netherlands) and by Stichting Spieren for Spieren (grant number SvS15). Analysis of the biomarkers was funded by Solid Biosciences Inc.

Disclosures:

N. M. van de Velde reports no relevant disclosures; Z. Koeks reports no relevant disclosures; M. Signorelli reports no relevant disclosures; N. Verwey reports no relevant disclosures; M. Overzier reports no relevant disclosures; J.A. Bakker reports no relevant disclosures; G. Sajeev is an employee of Analysis Group, a consulting company that received funding from Solid Biosciences to support this study; J. Signorovitch is an employee of Analysis Group, a consulting company that received funding from Solid Biosciences to support this study; V. Ricotti is a cofounder of DiNAQOR AG; J.J.G.M.

Verschuuren reports no relevant disclosures; K.J. Brown is an employee and stock holder of Solid Biosciences Inc; P. Spitali has served as scientific advisor of Astellas, Bioclinica BV, Epirium, Esperare, Marathon, PTC, Sarepta, Solid Bio, Summit therapeutics PLC, D-RSC consortium. All reimbursements were received by the LUMC. No personal financial benefits were received; E.H. Niks has been site principal investigator for clinical trials conducted by BioMarin, GSK, Eli Lilly, Santhera Pharmaceuticals, Italfarmaco SpA, Roche Pharma, Reveragen, NS Pharma, Fibrogen, Alexion and Argnx outside the submitted work. He also reports consultancies for BioMarin, Summit, PTC therapeutics, WAVE Life Sciences, Edgewise, Epirium Bio, Janssen and Regenxbio. All reimbursements were received by the LUMC. No personal financial benefits were received.

Preprint DOI:

Received Date:

2022-01-26

Accepted Date:

2022-10-11

Handling Editor Statement:

Submitted and externally peer reviewed. The handling editor was Renee Shellhaas, MD, MS.

Abstract

Background and Objectives: The slow and variable disease progression of Becker muscular dystrophy (BMD) urges the development of biomarkers to facilitate clinical trials. We explored changes in three muscle-enriched biomarkers in serum of BMD patients over 4 years-time and studied associations with disease severity, disease progression and dystrophin levels in BMD.

Methods: We quantitatively measured creatine kinase (CK) using the IFCC reference method, creatine/creatinine_{ratio} (Cr/Crn) using liquid chromatography – tandem mass spectrometry (LC-MS/MS) and myostatin with ELISA in serum, and assessed functional performance using North Star Ambulatory Assessment (NSAA), ten-meter run velocity (TMRv), six-minute walking test (6MWT), and Forced Vital Capacity (FVC) in a 4-year prospective natural history study. Dystrophin levels were quantified in the tibialis anterior muscle using capillary Western immunoassay. The correlation

between biomarkers, age, functional performance, mean annual change, and prediction of concurrent functional performance were analyzed using linear mixed models.

Results: Thirty-four patients with 106 visits were included. Eight patients were non-ambulant at baseline. Cr/Crn and myostatin were highly patient-specific (intraclass correlation coefficient for both=0.960). Cr/Crn was strongly negatively correlated, while myostatin was strongly positively correlated with NSAA, TMRv and 6MWT (Cr/Crn rho=-0.869 to -0.801 and myostatin rho=0.792 to 0.842, all $p<0.001$). CK showed a negative association with age ($p=0.0002$) but was not associated with patients' performance. Cr/Crn and myostatin correlated moderately with average annual change of 6MWT (rho=-0.532 and 0.555, $p=0.02$). Dystrophin levels did not correlate with the selected biomarkers nor with performance. Cr/Crn, myostatin and age could explain up to 75% of the variance of concurrent functional performance of the NSAA, TMRv and 6MWT.

Discussion: Both Cr/Crn and myostatin could potentially serve as monitoring biomarkers in BMD as higher Cr/Crn and lower myostatin were associated with lower motor performance and predictive of concurrent functional performance when combined with age. Future studies are needed to more precisely determine the context of use of these biomarkers.

Introduction

Becker muscular dystrophy (BMD) is characterized by progressive muscle weakness caused by reduced levels of dystrophin with abnormal molecular weight¹. The conduction of clinical trials has been challenging in BMD, as the low incidence (around 1:18:000 male live births, which is one third of Duchenne muscular dystrophy (DMD)) severely hampers patient recruitment². Also, high functional variability and slow disease progression lead to difficulties capturing potential drug efficacy during a trial³. Therefore, objective biomarkers are needed that could facilitate trial design and conduction⁴. For example, prognostic biomarkers could enrich trials by selecting patients with

greater likelihood of having a clinical event, thereby allowing to reduce the sample size as recently outlined⁵.

Circulating biomarkers are of interest due to easy accessibility, limited patient burden, and relatively low costs. They may also provide a representation of the overall condition of patients. To identify possible blood-derived biomarkers in Duchenne muscular dystrophy (DMD) and BMD, analyses have been performed via several methods including large-scale exploratory “-omics” approaches and more targeted approaches, e.g. investigation of a limited number of molecules based on hypotheses or previous research⁶⁻¹¹. Markers related to muscle structure or integrity are of specific interest due to the replacement of muscle tissue by fat a prominent sign of pathology in a muscle wasting condition like BMD. Creatine kinase (CK), commonly used as a diagnostic biomarker in muscular dystrophies, may not be optimal to monitor the disease due to the fact that its serum levels change due to both the membrane pathology and the amount of remaining muscle mass, next to other factors like seasonal variation and dependence on physical activity¹². Alternatively, creatine, derived from diet or synthesized in liver, is nonenzymatically converted to creatinine in muscle at a constant rate. Therefore, the conversion of creatine to creatinine is more closely related to the amount of preserved muscle tissue where this conversion takes place. Similarly, myostatin relates to muscle mass as it is produced and released by skeletal muscle tissue. It also acts as an inhibitor of excessive muscle growth¹³. Indeed, creatine/creatinine_{ratio} levels were increased in DMD patients, while serum myostatin was lower in BMD and DMD compared to healthy controls¹⁴⁻¹⁸. Although these cross-sectional studies provide evidence that creatine/creatinine_{ratio} and myostatin may be suitable as biomarkers, their relation to functional performance over a longer period of time still has to be largely demonstrated. Such data are important to more closely define the potential context of use of circulating biomarkers.

We quantified CK, creatine/creatinine_{ratio}, and myostatin as biomarkers in a longitudinal natural history study of adult BMD patients, and assessed correlations with disease severity, disease progression, and the ability to predict concurrent performance.

Materials and methods

Patient characteristics and study protocol

Participants were recruited from the Dutch Dystrophinopathy Database in a 4-year prospective BMD natural history study conducted at the Leiden University Medical Center between 2014 and 2019¹⁹. Male subjects of age ≥ 18 diagnosed with BMD based on the following criteria were included in the study: genetic confirmation (in-frame mutation), or / another mutation in the *DMD* gene with a mild clinical phenotype (ambulant >16 years without steroid treatment). The study protocol consisted of four yearly one-day visits with venous blood sampling and measurement of several functional tests including the North Star Ambulatory Assessment (NSAA), 10-meter walk/run test velocity (TMRv), the 6-minute walk test (6MWT), and pulmonary function (forced vital capacity percentage predicted, FVC%). Functional tests were performed by two trained observers as previously described^{20, 21}. The NSAA was also performed in patients that lost ambulation during the study to capture decline of function in this scale. These patients could only score points on neck flexion (0-2 points) or sit from supine (0-2 points). Patients could also consent for a biopsy from the tibialis anterior muscle (TA), taken at baseline or year one of the study.

Standard Protocol Approvals, Registrations, and Patient Consents

The clinical data and human tissue have been obtained, stored and handled in strict accordance with relevant guidelines and regulations. The study was approved by the medical ethical committee (METC) of the LUMC. Written informed consent was obtained from all participants.

Blood sampling and analysis

Blood samples were allowed to clot for 0.5-2 hours at room temperature in serum collection tubes (SST tubes, 3.5 ml). Samples were further centrifuged for 10 minutes at 20°C at 2350 RCF. Aliquots of 1.5ml of serum were then frozen at -80°C pending use. Analysis of creatine and creatinine was performed using the method described by Bodamer et al²². In brief serum samples were mixed with the internal standard solutions (d3-creatine and d3-creatinine). Samples were deproteinized with

acetonitrile. Supernatants were dried under nitrogen and derivatized with a mixture of butanol/acetylchloride. Creatine was converted to its butylester, while creatinine remains underivatized, both were measured in $\mu\text{mol/l}$. Samples were dried again under nitrogen and reconstituted in the mobile phase. The compounds were separated using a Symmetry C18 column and detected in MRM mode using tandem mass spectrometry.

Analysis of CK (U/L) was performed by colorimetric (IFCC) method on the Roche Cobas 8000. Analysis of myostatin (pg/mL) was performed by ELISA using kit (cat. n. DGDF80) including the activation kit (cat. n. DY010) and quality control set 794 (cat. n. QC98). All products were obtained from R&D Systems, Bio-Techne, Minneapolis, Minnesota, United States, and procedures were performed according to manufacturer's instructions. Calculation of the myostatin values was performed using a Four Parameter Logistic (4PL) Regression.

All analysis were done in duplo. The average of the samples was used in the analysis. Samples were excluded when the coefficient of variation (CV) exceeded 30%.

Dystrophin quantification

Dystrophin percentage was quantified from available muscle biopsies of the TA muscle using capillary Western immunoassay as previously described²³. In 13 patients a biopsy was available at baseline. In seven other patients, muscle biopsies had been performed four to five years prior to baseline as part of a previous natural history study, yielding a total of 20 patients with dystrophin quantification²⁴.

Statistical analysis

The longitudinal trajectories of creatine/creatinine_{ratio}, CK and myostatin were modelled using a linear mixed model²⁵ for the log-transformed value of the biomarker, where we included a patient-specific random intercept and age as fixed effect covariate. Patient-specific random slopes were not added as there were too few patients and data points available. The models were estimated using restricted

maximum likelihood. The level of heterogeneity of each biomarker across patients was studied using the intraclass correlation coefficient (ICC). In a linear mixed model with random intercept, the ICC is an estimate of the correlation between repeated measurements from the same patient, and it provides an indication of how well the trajectories from different patients are separated. The association of the biomarkers with age was tested using an F test with degrees of freedom determined using Satterwhite's correction²⁶. Multiple testing corrections were implemented using the Benjamini-Hockberg method for all the performed analysis²⁷.

For patients with at least two measurements of a given functional test, the average yearly change of the functional test was estimated by fitting a linear regression model with functional test as response and time (in years) as covariate to all data points available for that patient.

Correlations of biomarkers with disease severity, disease progression and dystrophin percentage across patients were assessed by measuring the Pearson correlation between the patient-specific random intercepts of the biomarkers and those of the functional tests. Random intercepts are then interpreted as the deviation of each patients from the mean of the studied cohort.

The prediction of concurrent performance was calculated using linear models with different combinations of predictors (age, creatine/creatinine_{ratio}, CK, myostatin and dystrophin). Predictive performance was measured with an optimism-corrected R² based on the bootstrap²⁸.

Data availability

Anonymized data can be made available to qualified investigators on request.

Results

Thirty-six patients were included in the natural history study. One patient did not consent for blood sampling and another patient was excluded due to withdrawal of consent after the baseline visit. The remaining 34 patients had a total of 123 visits. Serum samples were missing from 17 visits of 13 patients. CK levels were not analyzed at the fourth-year follow-up visit for 19 patients as no

association with functional tests was found in the preliminary analysis of the first three study years. In seven more visits, myostatin levels could not be determined (in four visits not enough sample was left, in three visits the CV% was too high). Thus, creatine/creatinine_{ratio}, myostatin and CK levels were available at 106, 99 and 87 visits respectively.

Baseline characteristics of patients are shown in Table 1. Eight patients were non-ambulant (defined as inability to perform the TMR without support) at baseline. Three patients lost ambulation during the study. The functional ability of the patients varied widely. For example, among the ambulant patients, the NSAA score at baseline ranged from 5 to 34 points. FVC was above 80% in 26 of the 34 patients. Two patients were on nocturnal ventilatory support.

Association of biomarkers with age and cross-correlation across biomarkers

The ICC of creatine/creatinine_{ratio} and myostatin were both high 0.960 (Figure 1, A and B), indicating that the values of these two markers are strongly patient-specific, and highly heterogeneous across patients. Both biomarkers were not associated with age (adjusted p-values=0.626 and 0.147 respectively). CK was less patient specific (ICC=0.550, Figure 1C) and showed a negative association with age (adjusted p=0.0002). The estimates from the mixed model for all the biomarkers are outlined in eTable 1. The values of creatine/creatinine_{ratio} and myostatin correlated highly with each other ($\rho=-0.85$, adjusted p<0.001, Figure 2A and showed low correlations to CK (adjusted p=0.362 and p=0.034 respectively, Figure 2, B and C).

Correlation of biomarkers with disease severity, disease progression and dystrophin percentage

In ambulant patients, the NSAA, TMRv and 6MWT showed strong negative correlations to creatine/creatinine_{ratio} ($\rho<-0.80$, adjusted p<0.001, Figure 3, A and C, and eFigure 1) and strong positive correlations to myostatin ($\rho>0.79$, adjusted p<0.001, Figure 3, B and D, and eFigure 1). As the NSAA was also performed in patients that lost ambulation during the study, we also correlated the biomarkers to the NSAA scores of only ambulant patients. Creatine/creatinine_{ratio}, and myostatin were

still highly correlated to NSAA ($\rho=-0.83$ and $\rho=0.85$, respectively, adjusted $p<0.001$). The functional tests did not correlate to CK ($\rho\leq 0.25$, adjusted $p>0.33$, eFigure 1). The FVC correlated moderately to creatine/creatinine_{ratio} ($\rho=-0.45$, adjusted $p=0.02$), myostatin ($\rho=0.58$, adjusted $p=0.001$) and CK ($\rho=0.55$, adjusted $p=0.003$).

Creatine/creatinine_{ratio} and myostatin correlated moderately with the average yearly change of the 6MWT ($\rho=-0.53$, adjusted $p=0.02$ and $\rho=0.56$, adjusted $p=0.016$ respectively, Figure 4, A–C). All other correlations between the three biomarkers and average yearly change of functional tests and pulmonary function were weak ($-0.27<\rho<0.10$, adjusted $p>0.39$) eFigure 2, A–D). None of the three biomarkers correlated with dystrophin levels in muscle, quantified in the tibialis anterior muscle ($\rho<0.17$, eFigure 3, A–C).

Prediction of concurrent functional performance using age, biomarkers and dystrophin percentage

The explained variance of concurrent performance, as assessed by NSAA, TMRv and 6MWT, using each predictor separately was highest using myostatin (bootstrap-corrected $R^2\approx 50\%$ and creatinine/creatinine_{ratio} (bootstrap-corrected $R^2 = 55-60\%$)(Figure 5). A combination of age and these two biomarkers increased the explained variance to about 75%. Dystrophin percentage did not seem to improve the prediction, although only 20 patients had dystrophin data available. Concurrent pulmonary function (FVC%) could be predicted around 35% by creatinine/creatinine_{ratio}, 10% by myostatin and 30% by the biomarkers combined with age (data not shown).

Discussion

In this prospective four year study we aimed to investigate whether creatine/creatinine_{ratio}, myostatin and CK could be biomarkers of disease severity and progression in BMD. Creatine/creatinine_{ratio} and myostatin, but not CK, were highly patient-specific and correlated to disease severity but not with disease progression over four years. The explained variation of concurrent functional performance by

these biomarkers together with age was up to 75%. Our study demonstrates that creatine/creatinine_{ratio} and myostatin may be candidate monitoring biomarkers in BMD.

The lack of outcome measures capable of showing changes within the duration of a trial in rare and slowly progressive diseases has hindered the development and evaluation of potential drugs in clinical trials. Monitoring biomarkers have been defined by the Food and Drug Administration as a biomarker that is measured over time²⁹. Monitoring biomarkers in general may serve multiple purposes, including to detect therapeutic effect or disease progression, or to determine how a drug is metabolized. Therefore, this term covers several types of biomarkers, including prognostic, predictive, response (pharmacodynamic) and safety biomarkers. Creatine used in the creatine/creatinine ratio is derived from diet, but can also be synthesized in the liver using guanidinoacetic acid and S-adenosyl-methionine. It functions as an energy buffer in high energy demanding tissues such as skeletal muscle tissue. In periods of low muscle activity, creatine is phosphorylated to create phosphocreatine by the transfer of a phosphate group from adenosine triphosphate (ATP) to creatine. This reversible transfer is catalysed by CK and yields adenosine diphosphate (ADP) and a phosphate group. In periods of high energy demand in muscle cells, the phosphor group of phosphocreatine is transferred from ADP to create ATP and can occur within seconds of intense muscular effort³⁰. Creatine is nonenzymatically converted to creatinine in human muscle tissue in a constate rate of about 1.7% per day and is then released into the blood and cleared by the kidneys³¹. With loss of muscle mass that is typical for muscular dystrophies, less creatine is converted into creatinine leading to an increased ratio of creatine to creatinine. Serum creatinine has been shown to be reduced in several neuromuscular diseases including DMD, BMD and spinal muscular atrophy^{14, 15, 32-34}. Two studies in a large cohort of mainly pediatric dystrophinopathy patients demonstrated that creatinine levels distinguished DMD from BMD, and that it was inversely correlated to function; creatinine was also associated with disease progression in a smaller longitudinal subset with deteriorating DMD (n=32) and BMD (n=4) patients with two time points spanning multiple years^{32, 33}. Myostatin (or growth differentiation factor 8, GDF8) is a member of the transforming growth factor β (TGF- β) superfamily and acts as a muscle growth inhibitor³⁵. It is produced and released by myocytes and fibroblast residing in skeletal muscle

tissue³⁶. Myostatin circulates in latent form in the blood as a complex of two C-terminal dimers and two N-terminal inhibitory prodomains. When the prodomains are cleaved, the myostatin dimer becomes active and can bind to its receptor complex activin type IIB (ActRIIB) and activin receptor-like kinase (Alk) 4 or Alk5 type 1 receptor in muscle tissue. Upon binding, myostatin inhibits muscle growth in three different pathways; myogenesis is downregulated via mitogen-activated protein kinase activation (MAPK) and via Smad 2/3 phosphorylation and subsequent nuclear translocation, and protein synthesis is reduced by inhibition of mammalian target of rapamycin (mTOR) signaling³⁷. Inhibition of the myostatin pathway has been shown to lead to muscle hypertrophy in mice models for various muscular dystrophies³⁸⁻⁴¹. Circulating myostatin levels seemed to be lower in patients with clinically more severe disease like DMD and SMA compared to for example BMD and FSHD^{16, 17}. Moreover, its levels seemed to decrease with age in patients with DMD. Thus, downregulation of myostatin may be due to an intrinsic disease process in patients with clinically more severe disease, or it may be a direct consequence of the amount of muscle mass (e.g. the amount of muscle tissue that is able to produce myostatin), or a combination of both.

The results of our study are in line with a previous finding in DMD that higher creatine/creatinine_{ratio} is related to lower motor performance measured by functional performance¹⁵. Lower myostatin levels correlated weakly to decreased function in DMD and limb girdle muscular dystrophy type 2B patients¹⁶. These results could be replicated in our BMD cohort when only taking baseline measurements into account for myostatin ($\rho=-0.410$, $p=0.052$, data not shown) but not for creatine/creatinine_{ratio} ($\rho=0.279$, $p=0.177$, data not shown). In addition, myostatin was significantly lower in non-ambulant DMD and BMD patients compared to patients that were still able to walk¹⁶. However, given the progressive nature of diseases such as DMD and BMD, it is difficult to demonstrate a relationship between a biomarker and functional performance in cross-sectional studies, where age alone is often associated with functional decline. In our study, a lower creatine/creatinine_{ratio} and higher myostatin levels were related to better functional performance after correction for age, therefore supporting the conclusion of these markers being associated with concurrent performance on top of the effect of age.

Despite the strong correlation of myostatin and creatine/creatinine_{ratio} with performance, we observed a moderate and significant association between the biomarkers and the average yearly decline only for the 6MWT, while the correlation with the NSAA and TMRv were weak and not significant. The 6MWT was originally developed as a tool to measure the global and integrated response of all systems involved during exercise such as the pulmonary and cardiovascular systems, and muscle metabolism⁴². It functions as a measure of endurance, while the TMRv measures transient peak activity and the NSAA is an important measure of daily life activities. While biomarkers such as creatine/creatinine_{ratio} and myostatin reflect change in endurance over the years rather than change in transient peak activity or daily life activities, it is possible that the lack of clear functional decline for part of the patients in any functional measurement in our study (manuscript in preparation) over the four studied years does not allow to identify these changes in performance tests. This is especially true for functional scales, such as the NSAA, where floor/ceiling effects were observed, therefore masking the possibility to detect yearly functional changes and therefore the correlation with the biomarkers.

The prediction of concurrent functional performance was more accurate using myostatin and creatine/creatinine_{ratio} together with age compared to when a single predictor was used. These results for prediction of concurrent function are in line with previous studies in which several prognostic prediction models for future changes in function were developed in effort to optimize trial design in DMD. These models included up to 1,137 patients with 23,305 observations and reported more accurate prediction of future change in functional performance using multiple predictors as opposed to a single predictor. The explained variance of future performance was up to 50-60% using combined clinical factors such as multiple measures of ambulatory function and steroid use⁴³⁻⁴⁵. Despite the smaller number of individuals and observation, our models resulted in a high prediction accuracy indicating that the addition of creatine, creatinine and myostatin leads to an improvement in accuracy of performance prediction. Furthermore, the absence of additive accuracy of dystrophin to the prediction is in line with previous studies from our group and others demonstrating that dystrophin percentage has a limited correlation with functional performance, although dystrophin percentage was

only available in about half of the BMD patients in our study^{24, 46}. In one other study with 52 patients, dystrophin showed moderate positive correlations with several functional measures⁴⁷.

Several limitations should be acknowledged. Withdrawal of blood was variable in and between patients during the study day, taking place either before or after the clinical assessments. We included only ambulant assessments in the analysis. Dystrophin was only available cross sectionally in a subset of the patients. This may influence the accuracy of the prediction of concurrent performance. We could not control this study for dietary/supplemental intake and body lean mass. This may effect total biomarker levels and should be investigated in future studies. While our study includes longitudinal serum samples and clinical data required to identify a potential monitoring biomarkers, the presented data show small changes over time for both creatine/creatinine_{ratio} and myostatin and functional scales. This currently limits the possibility to more closely define the context of use for these substances.

In conclusion, creatine/creatinine_{ratio} and myostatin may be used as monitoring biomarkers in BMD as higher creatine/creatinine_{ratio} and lower myostatin were associated with patients performance after correction for age, and they improved prediction of concurrent functional performance when combined with age. A longer follow-up, increasing the sample size and/or focusing on patients with a more similar rate of functional decline is needed to get more insight in the longitudinal relationship of creatine/creatinine_{ratio} and myostatin with function and disease milestones in BMD to get more insight in their potential to serve as monitoring biomarkers. In addition, the correlation between the serum biomarkers and quantitative MRI measures should be investigated as it has been shown that muscle MRI may also be used as biomarker for disease progression in DMD and BMD^{48, 49}.

<http://links.lww.com/WNL/C505>

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Table 1. Baseline characteristics	
Variable	BMD patients (n=34)
Age (years)	42.2 (13.0)
BMI (kg/m ²)	25.3 (4.3)
Ambulant, n	26 (76.5%)
Mutation, n	
Del 03-04	2 (5.9%)
Del 03-07	1 (2.9%)
Del 05-09	1 (2.9%)
Del 11-18	1 (2.9%)
Del 30-44	1 (2.9%)
Del 45-47	10 (29.4%)
Del 45-48	2 (5.9%)
Del 45-49	1 (2.9%)
Del 45-53	1 (2.9%)
Del 45-55	4 (11.8%)
Del 48-49	2 (5.9%)
Dup 03	2 (5.9%)
Dup 02-07	1 (2.9%)
Dup 14-42	1 (2.9%)
Dup 16-41	1 (2.9%)
Exon 6: c.419T>A p.(Leu140His)	1 (2.9%)
Exon 26: c.3515G>A p.(Trp1172*)	1 (2.9%)
Exon 29: c.3940C>T p.(Arg1314*)	1 (2.9%)
NSAA (points)**	28.0 (5.0 – 34.0)
10 meter run/walk (m/s) ***	1.0 (0.0 – 4.2)
6MWT (meters)*	329.5 (0 – 650.0)
FVC* (%)	91.0 (19.0 – 118.0)
Dystrophin percentage (n=20)	38.9 (18.6-86.4)
Data are shown as mean (SD) or median (range). BMI; body mass index, FVC; forced vital capacity percent predicted, NSAA; North Star Ambulatory Assessment, 6MWT; 6-minute walk test. *n=32 **n=24 ***n=33	

Figure 1. Change over time biomarkers in BMD patients. Creatine/creatinine_{ratio} (A), myostatin (B) and creatine kinase (C). Creatine/creatinine_{ratio} and myostatin were highly patient specific. Only creatine kinase declined significantly over time (adjusted p=0.002). BMD = Becker muscular dystrophy Cr/Crn = Creatine/creatinine_{ratio}, CK = Creatine kinase

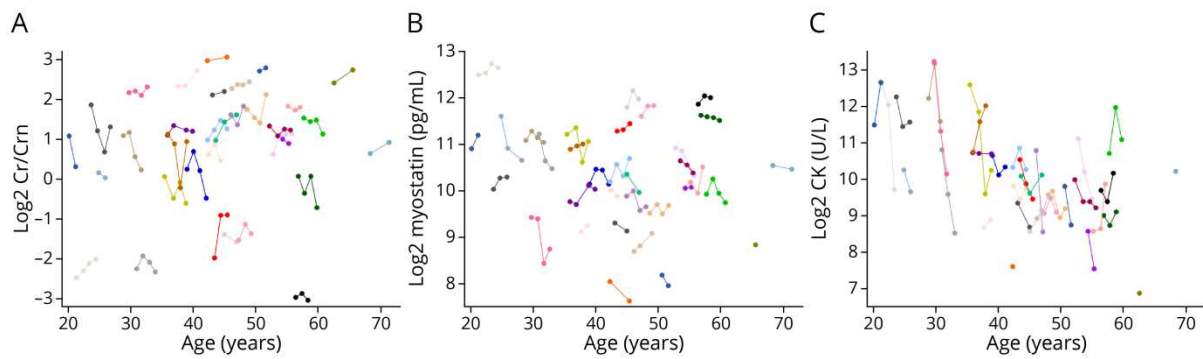


Figure 2. Cross-correlation of biomarkers in BMD patients. creatine/creatinine_{ratio} (A), myostatin (B) and creatine kinase (C). There was high cross correlation between creatine/creatinine_{ratio} and myostatin (A) but not with CK (B,C). BMD = Becker muscular dystrophy Cr/Crn = Creatine/creatinine_{ratio}, CK = Creatine kinase

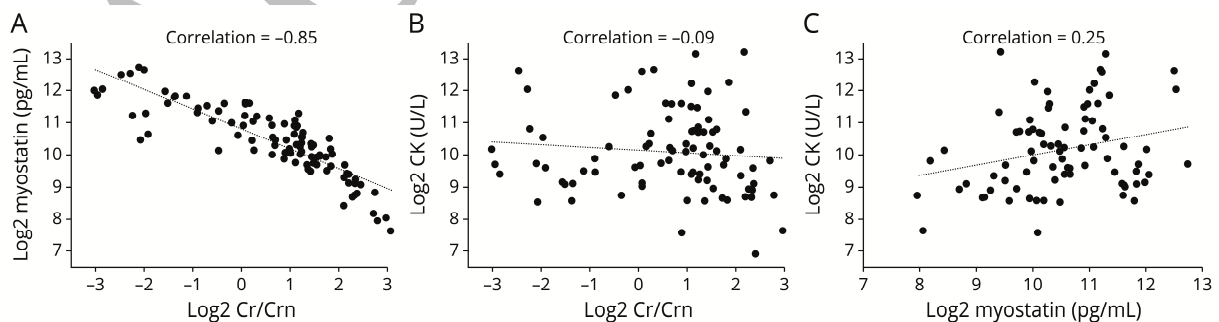


Figure 3. Correlation of biomarkers with functional tests across patients. The random intercepts of the North Star Ambulatory Test (A,B) and Ten meter run velocity (C,D) correlated highly to the random intercepts of creatine/creatinine_{ratio} and myostatin. Cr/Crn = Creatine/creatinine_{ratio}, NSAA = North Star Ambulatory Assessment, TMRv = ten meter run velocity

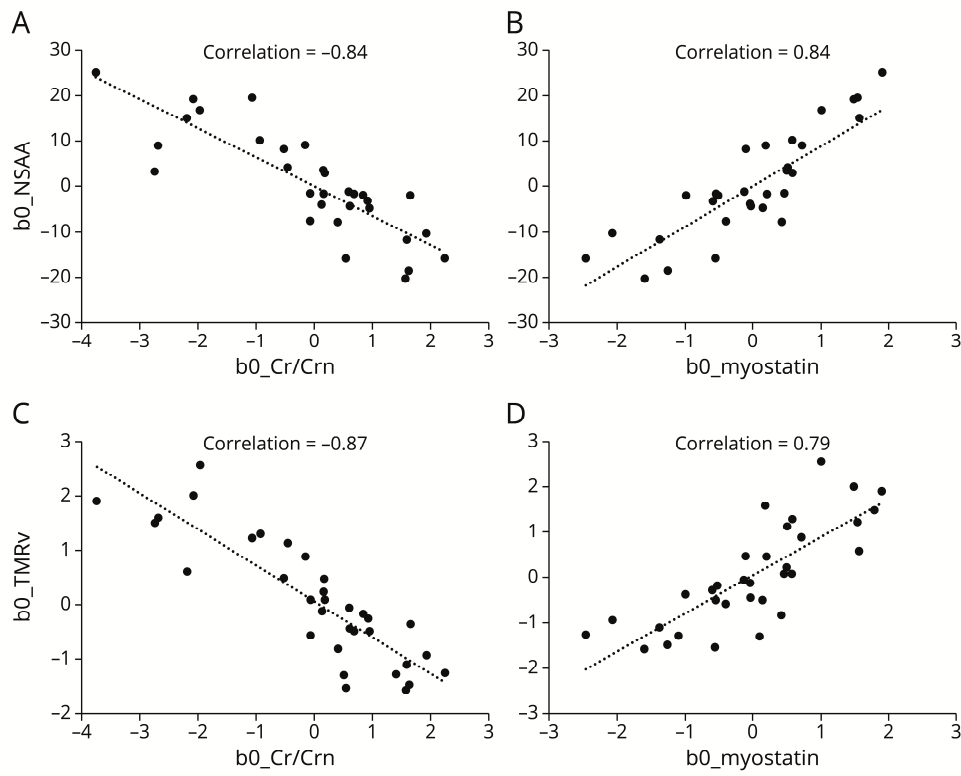


Figure 4. Correlation of biomarkers with average yearly change of the 6MWT in BMD patients.

Average yearly change of the 6MWT correlated moderately with creatine/creatinine_{ratio} (A), and myostatin (B) and the correlation with creatine kinase (C) was weak. 6MWT = 6-minute walking test, BMD = Becker muscular dystrophy, Cr/Crn = Creatine/creatinine_{ratio}, CK = Creatine kinase

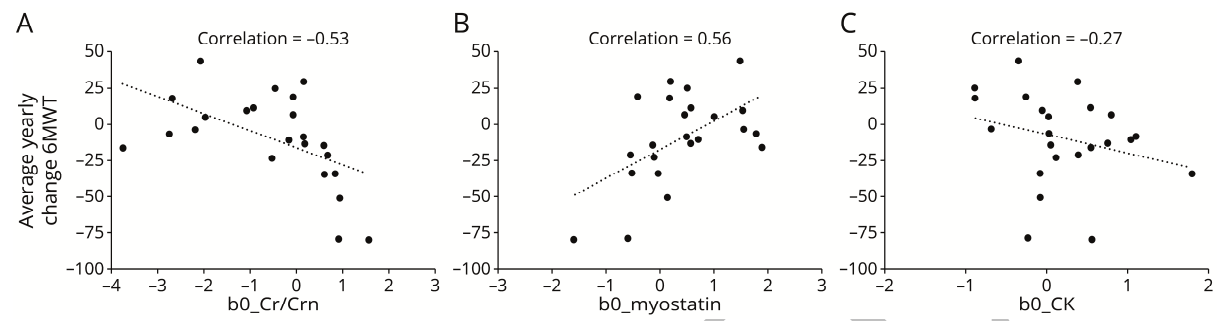
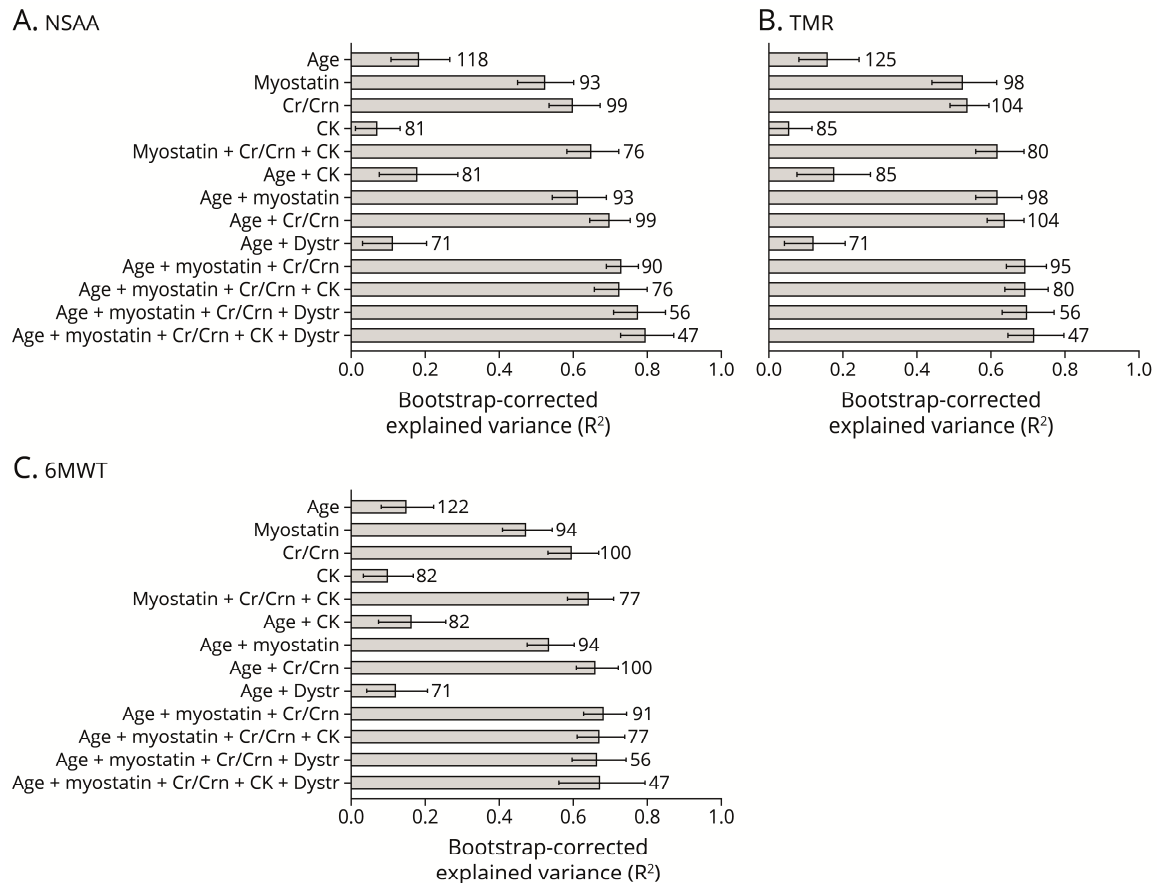


Figure 5. Prediction of concurrent functional performance using age, dystrophin, and biomarkers. Numbers behind bars indicate the amount of available data points for each prediction.

Cr/Crn = creatine/creatinine_{ratio}, CK = creatine kinase, dystyr = dystrophin, NSAA = North Star Ambulatory Assessment, TMR = ten meter run test, 6MWT = 6-minute walking test.



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Neurology published online December 5, 2022

DOI 10.1212/WNL.0000000000201609

This information is current as of December 5, 2022

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