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Original Article

Composite Biomarkers for Assessing Duchenne Muscular Dystrophy: An Initial Assessment

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ABSTRACT

BACKGROUND: Compared with individual parameters, composite biomarkers may provide a more effective means for monitoring disease progression and the effects of therapy in clinical trials than single measures. In this study, we built composite biomarkers for use in Duchenne muscular dystrophy by combining values from two objective measures of disease severity: electrical impedance myography and quantitative ultrasound and evaluating how well they correlated to standard functional measures. **METHODS:** Using data from an ongoing study of electrical impedance myography and quantitative ultrasound in 31 Duchenne muscular dystrophy and 26 healthy boys aged 2-14 years, we combined data sets by first creating *z* scores based on the normal subject data and then using simple mathematical operations (addition and multiplication) to create composite measures. These composite scores were then correlated to age and standard measures of function including the 6-minute walk test, the North Star Ambulatory Assessment, and handheld dynamometry. **RESULTS:** Combining data sets resulted in stronger correlations with all four outcomes than for either electrical impedance myography or quantitative ultrasound alone in six of eight instances. These improvements reached statistical significance ($P < 0.05$) in several cases. For example, the correlation coefficient for the composite measure with the North Star Ambulatory Assessment was 0.79 but was only 0.66 and 0.67 (respectively) for gray scale level and electrical impedance myography separately. **CONCLUSIONS:** Arithmetically derived composite scores can provide stronger correlations to functional measures than isolated biomarkers. Longitudinal study of such composite markers in Duchenne muscular dystrophy clinical trials is warranted.

Keywords: electrical impedance myography, quantitative ultrasound, Duchenne muscular dystrophy, biomarker, outcome measure, composite

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Introduction

A variety of potential therapeutic approaches are currently being studied in Duchenne muscular dystrophy (DMD), including exon-skipping strategies,[1] gene therapy,[2] myostatin inhibitors,[3] and antifibrotic agents.[4] Some of these, especially the exon-skipping approaches, are already demonstrating impressive potential value and may ultimately help in converting progressive DMD into a disease similar to Becker muscular dystrophy.[1] To date, potential therapies are being assessed with clinical outcome measures such as the 6-minute walk test (6MWT) and the North Star Ambulatory Assessment (NSAA).[5,6] Although these measures are useful, they are limited in a number of respects. First, they have inherent variability, are limited by effort and mood, and can only be completed in ambulatory boys. Moreover, such methods typically only indicate decline in children about $\geq$7 years of age and thus cannot provide data in younger children who may be most responsive to treatment; this reduces the inclusivity of most

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clinical trials. Moreover, these measures may not have been sensitive enough to detect therapy effects in two recent trials.[7]

Rapid, safe, and objective surrogate measures that correlate strongly to disease status could potentially find wide use in Phase II and III clinical trials in DMD. Imaging, such as magnetic resonance imaging, has also been proposed as a potential outcome measure[8]; however, it is limited by cost and lengthy image acquisition time, which may be difficult for children. Quantitative ultrasound (QUS)[9] and electrical impedance myography (EIM)[10] are two attractive, objective candidates for evaluating neuromuscular pathology. Ultrasound can be quantified by measuring the gray scale level (GSL), which reflects the degree of brightness in the muscle. In DMD, fibrosis and fatty infiltration result in brighter images and higher GSL values.[11] EIM is a painless, noninvasive tool that relies on the application of a small current and measurement of surface voltages. EIM detects properties of healthy muscle, including age-related increases in muscle fiber size resulting in increasing muscle capacitance, which are lost in DMD.[12] We recently studied cross-sectional data in DMD and identified that both modalities provided excellent discrimination between DMD and control subjects and correlated with the NSAA in children with DMD.[13,14] However, the two measures only correlated moderately with one another ($R_{Spearman} = -0.40$, $P = 0.054$), and thus, the two methods provide complementary data on disease status. This is perhaps not unexpected because QUS relies on backscattered acoustic energy, whereas EIM relies on transmitted electrical energy. Accordingly, here we study the concept of creating a composite measure of disease status by combining data from these two modalities, an approach that has been used with success in magnetic resonance imaging studies in multiple sclerosis.[15,16] This strategy has the potential to result in new, sensitive outcome measures that could be used in future clinical trials to facilitate drug development in DMD.

Methods

Subjects and recruitment

The recruitment process has been described previously.[13] The Boston Children's Hospital Institutional Review Board approved the protocol. Patients provided written consent, and children provided verbal assent. Boys with DMD and healthy boys aged 2-14 years were recruited.

EIM and ultrasound measurements

The methods for GSL and EIM acquisition have also been described previously.[13] Briefly, six muscles, including deltoids, biceps, wrist flexors, quadriceps, tibialis anterior, and medial gastrocnemius, were measured transversely relative to the long axis of each muscle. Each subject underwent a maximum of three measurements at baseline, 6, and 12 months. EIM measurements were obtained with the Imp SFB7 (ImpediMed, Inc, Sydney, Australia) using a custom handheld array,[17] with three different probe sizes being used depending on the child's size. The array dimensions were as follows: small, 4 × 1.5 cm; medium, 5 × 2 cm; and large, 7 × 2.5 cm. Ultrasound images were obtained using the Terason t3000 system (Teracorp, Inc, Burlington, MA) with a 10 MHz probe. All images were converted to JPEG files and analyzed using Matlab (MathWorks, Inc, Natick, MA) to obtain the brightness of the region of interest, measured as median GSL.[18] The region of interest was defined as a region of fixed dimensions (130 × 64 pixels) and placed in the area of muscle directly below the subcutaneous fat layer. For both EIM and US, measurements were performed on the same muscles and locations. For this analysis, and for simplicity, data from all six muscles were averaged and the six-muscle average values for EIM and QUS used in all analyses.

Standard functional measures

The 6MWT, NSAA, and handheld dynamometry (HHD) were all performed by an experienced pediatric physical therapist (A.P.). For HHD, shoulder abduction, elbow flexion, forearm flexion, knee extension, foot dorsiflexion, and foot plantar flexion were measured each three times. The highest value obtained for each muscle was then averaged across all the muscles to provide a single average HHD score for each subject.

Data analysis, including creation of z and composite scores

EIM phase is measured in degrees, and GSL is dimensionless. Thus, to create a composite score from these independent variables, we developed z scores based on the healthy subject data, in which individual measurement values are replaced by values that are measured in standard deviations relative to the group mean. Thus a z score of +0.5 for a boy with DMD would indicate that the value was 0.5 standard deviation (SD) more than the group mean for healthy boys; a z score of −0.5 would be 0.5 SD less than the healthy boy mean. To do so, we first confirmed a relatively normal distribution for the EIM and GSL healthy subject data. The six-muscle average values for all healthy subjects and the associated SDs for both EIM and GSL were then obtained separately. The difference between raw individual DMD patient data points and the mean healthy subject value was calculated and then divided by the SD for the normal subject data, providing a z score for each DMD patient's averaged six-muscle data point for both EIM and GSL separately.

However, worsening disease in the United States is accompanied by elevations in GSL and thus positive z scores, whereas worsening of disease with EIM results in lower phase values and thus negative z scores. Accordingly, EIM scores were multiplied by −1 to make the direction of change for both z scores consistent. The final composite score was created via simple arithmetic combinations: either adding or multiplying the EIM and GSL z scores. The output was then correlated to age, NSAA, 6MWT, and HHD (via Spearman analysis), and the results compared with correlations for the individual EIM and US data. Steiger Z test was used to compare rho values to determine if the differences between values were significant at the $P < 0.05$ level.[19]

Results

Subject demographics

We obtained EIM and QUS measurements on 31 subjects with DMD and 26 healthy controls in which both sets of data were acquired. DMD subjects had a median age of 7.81 +/− 3.42 years, and healthy boys had a median age of 7.40 +/− 2.6 years (t test, $P = 0.89$). Data were included from a total of 65 visits with the DMD patients and 64 visits with the normal subjects. There were 30 data points for NSAA measurements, 17 for 6MWT, and 14 for HHD testing (because children could only undergo age-appropriate testing).

Correlations with surrogate measures of disease

The results of a comparison between correlation analyses of GSL and EIM and the composite scores are summarized in Table and Figure. In short, in six of eight combinations of data sets, both approaches for creating composite scores (adding and multiplying) resulted in stronger correlation