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● *Original Contribution*

ULTRASOUND TISSUE CHARACTERIZATION DOES NOT DIFFERENTIATE GENOTYPE, BUT INDEXES EJECTION FRACTION DETERIORATION IN BECKER MUSCULAR DYSTROPHY

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Abstract—The aims of the study were, first, to assess whether myocardial ultrasound tissue characterization (UTC) in Becker muscular dystrophy (BMD) can be used to differentiate between patients with deletions and those without deletions; and second, to determine whether UTC is helpful in diagnosing the evolution of left ventricular dysfunction, a precursor of dilated cardiomyopathy. Both cyclic variation of integrated backscatter and calibrated integrated backscatter (cIBS) were assessed in 87 patients with BMD and 70 controls. The average follow-up in BMD patients was 48 ± 12 mo. UTC analysis was repeated only in a subgroup of 40 BMD patients randomly selected from the larger overall group (15 with and 25 without left ventricular dysfunction). Discrimination between BMD patients with and without dystrophin gene deletion was not possible on the basis of UTC data: average cvIBS was 5.2 ± 1.2 and 5.5 ± 1.4 dB, and average cIBS was 29.9 ± 4.7 and 29.6 ± 5.8, respectively, significantly different ($p < 0.001$) only from controls (8.6 ± 0.5 and 24.6 ± 1.2 dB). In patients developing left ventricular dysfunction during follow-up, cIBS increased to 31.3 ± 5.4 dB, but not significantly ($p = 0.08$). The highest cIBS values (34.6 ± 5.3 dB, $p < 0.09$ vs. baseline, $p < 0.01$ vs BMD patients without left ventricular dysfunction) were seen in the presence of severe left ventricular dysfunction. Multivariate statistics indicated that an absolute change of 6 dB in cIBS is associated with a high probability of left ventricular dysfunction. UTC analysis does not differentiate BMD patients with or without dystrophin gene deletion, but may be useful in indexing left ventricular dysfunction during follow-up. (E-mail: giglio.echo@libero.it)

Key Words: **Becker muscular dystrophy, Myocardial tissue characterization, Cardiomyopathy, Genetics.**

INTRODUCTION

The early prediction of cardiomyopathy in patients affected by X-linked Becker muscular dystrophy (BMD) is a crucial goal, because the prognosis of clinically overt cardiomyopathy is poor. BMD is due to in-frame, mainly intragenic deletions, duplications or missense mutations in the dystrophin gene. Cardiac involvement may be associated, from absence of symptoms to overt dilated cardiomyopathy (DCM). When severe DCM is observed in young BMD patients with normal skeletal muscle function, it becomes the first cause of death, and heart transplantation represents the ultimate therapeutic choice (Komanapalli et al. 2006).

In BMD patients without cardiac involvement, controversy exists over when to start cardioprotective therapy aimed at preventing or postponing DCM. This calls for non-invasive, relatively inexpensive techniques to help decide whether and when cardioprotective therapy needs to be added (McNally 2007). Among risk factors for DCM onset and evolution in BMD patients, genotyping has been considered, but there are no conclusive reports linking cardiac phenotype and genetics in BMD patients without deletions (Melacini et al. 1993; Nigro et al. 1995).

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Myocardial ultrasound tissue characterization (UTC) is a 2-D echocardiographic (2-D Echo) technique whereby non-invasive cardiac tissue analysis is achieved (Naito et al. 1995). The aims of the study described here were, first, to assess whether UTC can differentiate between BMD patients with deletions and those without deletions; and second, to determine whether UTC is helpful in diagnosing the evolution of DCM.

METHODS

Study population and clinical evaluation

This study enrolled 87 BMD patients aged 4 to 46 y (mean age: 21 ± 12 y) followed at the Center for Neuromuscular Diseases, Unione Italiana Lotta alla Distrofia Muscolare, Rome, Italy, and 70 age-matched healthy controls (mean age: 20 ± 11 y) (p = NS); the controls had normal electrocardiograms and 2-D echocardiograms. The study was approved by the ethics board of the Catholic University of Rome. Written informed consent was obtained from all patients and controls. Exclusion criteria were hypertension, electrocardiographic changes suggestive of ischemic heart disease, left bundle-branch block, atrial flutter/fibrillation, ventricular arrhythmias, any degree of atrioventricular block, valvular heart disease other than trivial insufficiency and left ventricular (LV) hypertrophy. BMD patients and controls underwent physical examination; surface electrocardiography; assessment, by 2-D echocardiography (2-D Echo), of systolic LV function (average of five consecutive cycles); and segmental wall motion analysis (by two independent observers).

Degree of skeletal muscle involvement was assessed by a neurologist, and all BMD patients were divided into four groups. Forty-one patients (47%) were asymptomatic with increased values of serum creatine kinase and calf hypertrophy; 5 patients (6%) were mildly affected, manifesting some impairment in running and jumping; 30 patients (34%) were moderately affected, with clear strength deficits in specific muscle districts; and 11 patients (13%) were severely affected, that is, not able to walk unaided or wheelchair bound. All clinical assessments and grading were performed by the same observer (E.R.). Both echocardiographic and UTC studies were performed in all patients simultaneously.

Echocardiographic study

All 2-D Echo images were obtained from the parasternal short-axis view (for UTC analysis), by a 2.5 MHz phased-array probe, in the fundamental imaging mode, by the same operator (V.G.) in all patients (SONOS 5500 Philips, Andover, MA, USA). LV systolic function was assessed by calculating, on the apical view, ejection fraction (EF) from LV volumes (derived by the modified Simpson's rule). Rate-corrected velocity of circumferential fiber shortening (V_{cfcm}) was also assessed. LV systolic dysfunction (yes/no) was defined as a LV EF ≤50% to confidently and accurately diagnose abnormal LV function at a clear-cut rounded off point.

Integrated backscatter data acquisition and analysis

For UTC analysis, the mechanical index (0.5), transmit power (+35 dB) and time-gain compensation (140 dB) were set to the same values for all acquisitions in patients and controls. UTC of myocardium was performed by: (i) obtaining from the Sonos 5500 machine, with a built-in and proprietary software, the magnitude of the cyclic variation of integrated backscatter (cvIBS), which is the difference between the average peak (end-diastole) and average nadir (end-systole) values of IBS; and (ii) calculating the calibrated IBS (cIBS), derived from the absolute IBS value also given by the Sonos 5500 machine. To calculate cIBS, the blood pool IBS was used to calibrate myocardial IBS in a semilunar region of interest (ROI) (11 × 11 mm) placed in the LV cavity at the apical level (Naito et al. 1996a). A complete blood cell count was performed both in BMD patients and in control patients. UTC parameters were measured at the basal, mid- (papillary muscles) and apical levels in a semilunar-shaped ROI (9 × 9 mm), manually guided over each individual frame placed in the mid-layer of 16 different LV myocardial segments (Schiller et al. 1989). The semilunar ROI was selected to sample the mid-myocardial layer, where fiber orientation is predominantly circumferential (Greenbaum et al. 1981). Smoothing filters were set to a high level to avoid high-frequency scatter noise, and lateral gain compensation was not used. The time delay value was calculated for each myocardial segment, in both patients and controls, as previously described (Finch-Johnston et al. 1999, 2000). Separate UTC data analyses were performed for the basal, mid- and apical segments. Off-line analysis of cvIBS and cIBS data was performed at baseline and during the follow-up study by two independent operators who were not informed about clinical status. Intra- and inter-observer variability was assessed by selecting 5 BMD patients in whom 16 segments were completely measured. With these 80 data series, cvIBS intra- and inter-observer variability was 4.13% and 4.91%, respectively, whereas cIBS was 2.51% and 1.05%, respectively. Bland–Altman plots provided superposable information.

Dystrophin gene and protein analysis

Immunohistochemical analysis of dystrophin was performed on 8-μm-thick cryostat sections according to an established protocol (Broccolini et al. 2004). BMD diagnosis was performed by DNA analysis of mutations in the dystrophin gene. Mutation detection was carried