Chapter five

Measurement of troponin T and troponin I levels in carriers of Duchenne and Becker muscular dystrophy with cardiac involvement

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Abstract

Background: At present Troponin T and Troponin I are presumed to be the most cardiac specific markers. Our aim was to establish the value of these markers for diagnosing non-ischemic myocardial damage in Duchenne(DMD) and Becker muscular dystrophy (BMD) carriers.

Methods: Hundred twenty-nine DMD/BMD carriers were included. Cardiological investigations included electrocardiography (ECG) and echocardiography. Laboratory investigations consisted of measurement of serum creatine kinase (CK), and aspartase aminotransferase (ASAT) activity, CK-MB-mass, cardiac Troponin T (cTnT) and cardiac Troponin I (cTnI). Statistic tests were used to analyse the relation between cardiac abnormalities and laboratory measurements.

Results: Seven DMD carriers had dilated cardiomyopathy and 23 DMD/BMD carriers had left ventricular dilation. Twenty-two cases had an elevated CK-MB, but none demonstrated a CK-MB/CKTOTAL ratio above 6%. Total CK was raised in 45%. No elevation was found of cTnI and only one DMD carrier with borderline echocardiographic abnormalities, had slightly increased cTnT.

Conclusion: cTnT and cTnI elevation is very rare (cTnT) or does not occur (cTnI) in DMD/BMD carriers. They can, therefore, not be used as a marker of non-ischemic myocardial damage.

Introduction

Early in the 1960s the World Health Organization recognized the fundamental role of cardiac enzymes for the diagnosis of acute myocardial infarction. Increase in serum creatine kinase (CK) activity, lactate dehydrogenase and its isoenzymes, and myoglobin, however, appeared not to be as sensitive and heart-specific as previously believed. This prompted the search for more sensitive and specific markers. Attention was focussed on the cardiac contractile and regulatory proteins. Most are however co-expressed in slow twitch skeletal muscle fibers. At present, only two candidates for heart-specific markers remain, namely cardiac Troponin I (cTnI) and cardiac Troponin T (cTnT). Recent reports suggest that cTnI may be the most cardiac specific.[130]
Carriers of Duchenne (DMD) and Becker muscular dystrophy (BMD) are at risk for cardiac disease. In a cross-sectional study among definite DMD/BMD carriers (n= 129) 5.4% had dilated cardiomyopathy and 18% had left ventricle dilation.[92] In the present study we analysed both cTnT and cTnI, together with CK, and CK-MB mass to establish the value of these markers in DMD/BMD carriers and their possible association with the presence of non-ischemic myocardial damage.

Material and Methods

Hundred-twenty nine definite carriers of DMD (85) and BMD (44) participated in a cross-sectional study in order to assess the presence of muscle weakness and cardiac involvement.[91,92] Carriers were considered definite when: they were found to be obligate carriers after pedigree analysis (definite X-linked inheritance); when a mutation in the dystrophin gene was found; or when linkage analysis revealed a chance of more than 99% for carriership.
All carriers underwent extensive cardiological examinations, including history taking, physical examination, ECG and transthoracic M-mode and 2-D echocardiography. These studies are described in detail elsewhere.[91,92]
All carriers only participated after informed consent had been given.
After venipuncture 10 cc heparine blood was drawn. Plasma was prepared, aliquotted and frozen at -80E C until analysis for total CK and aspartase aminotranferase (ASAT) activities, CKMB mass, cTnI and cTnT measurements.
Total CK activity (upper reference limit: 193 U/L) was measured at 37°C with a Hitachi 747 analyser (Boehringer Mannheim, Germany) by the method proposed by the International Federation of Clinical Chemistry.[57]

ASAT activity (upper reference limit: 47 U/l) was measured at 37°C with a Hitachi 747 analyser by the method proposed by the International Federation of Clinical Chemistry.
CK-MB mass (upper reference limit: 7 mg/L) was measured on an Immuno I analyser (Bayer, Leverkusen, Germany) using an enzyme labelled sandwich assay. The total imprecision proved to be lower than 2.5%. The analytical sensitivity in human serum proved to be 0.06 mg/l. No interference from CK-MM or CK-BB could be demonstrated.
CK-MB activity was calculated to allow comparison with total CK activity. The CK-MB activity was given as a percentage of the total CK activity. A ratio exceeding 6% was considered suggestive for cardiac involvement.
Cardiac Troponin I (upper reference limit: 0.4 mg/l) was measured with an enzyme-immuno assay on a Stratus II analyser (Dade International, Miami, FA), which uses two monoclonal antibodies specific for cardiac Troponin I.[57] The immunoassay shows no cross-reactivity with human skeletal-muscle Troponin I.[131]
Cardiac Troponin T (upper reference limit: 0.1 mg/l) was measured with an immunoassay on an ES600 analyser (Boehringer Mannheim, Germany). The assay uses a myocardium specific biotinylated antibody as conjugate and a cardiac-specific antibody as the biotinylated component.[132]
Chi-square tests were used to analyse the relation between the different laboratory measurements and age and cardiac abnormalities, respectively. To compare means of serum CK measurements we used t tests or the Mann-Whitney test, when appropriate.

Results

Cardiological investigations

Mean age of carriers was 36.9 years (18-58). None had symptoms or signs of ischemic heart disease. Seven carriers (5.4%, all DMD) had echocardiographic evidence of dilated cardiomyopathy (DCM), of whom five had signs or symptoms of congestive heart failure. Furthermore, 23 DMD/BMD carriers (18%) had left ventricle dilatation. In 17 cases (13%) borderline echocardiographic abnormalities, such as wall motion abnormalities or unilateral atrial dilatation, were found. Electrocardiographic abnormalities were seen in 61 carriers (47%). Only 38% had a completely normal investigation of the heart.

Plasma analyses

Total CK-activity was raised in 45% (58 cases): 53% of DMD carriers (45 cases) and

30% of BMD carriers (13 cases). Mean CK was 306 U/l (48-1860). Five of seven carriers with dilated cardiomyopathy had raised CK. Twenty-two (17%) of the DMD/BMD carriers had an elevated CK-MB. None of the carriers demonstrated a CK-MB/CKTOTAL ratio above 6%, including the carriers with dilated cardiomyopathy. None of the DMD/BMD carriers had elevated cTnI, and only one DMD carrier with borderline echocardiographic abnormalities had a slightly increased cTnT (0.16 mg/l).

Statistical analysis

No relation could be demonstrated between cTnI or cTnT and cardiac abnormalities. No significant difference was found in mean CK-activity between carriers with and without muscle weakness. There was a linear correlation between CK-MB and ASAT (r=0.81), and between total CK and (r=0.83). We found no clear correlation between CK and age, but mean CK showed a decreasing linear trend ($p = 0.044$) with increasing age groups. There was no relation between cTnI and CK-MB, cTnI and CK, cTnI and age, cTnT and age, CK-MB and age, or ASAT and age.

Discussion

In this study we measured total-CK and cardiac TnI and TnT in a large group of DMD/BMD carriers. In 1991 and 1993 it was shown that measurements of cTnI and cTnT are superior to conventional measurements of CK-MB mass for the detection of minor myocardial injury of ischemic origin, and it was presumed that these proteins are cardiac-specific. However, in more recent studies cTnT expression was found in a high proportion of skeletal muscle of dialysis patients[130], patients with damaged, regenerating muscle (muscular dystrophy and inflammatory myopathies)[133,134], in skeletal muscle during fetal development, and in normal (nonregenerating) skeletal muscle[135], thus, disclaiming the assumption that cTnT is a cardiac specific marker. cTnI is not expressed in skeletal muscle during fetal development and only sporadically in adult muscle in response to pathological stimuli, such as dialysis, muscular dystrophy, and inflammatory myopathies.[130,136-138] It was therefore presumed to be the most specific of the currently available biochemical markers.

None of our DMD/BMD carriers had an elevated CK-MB/CK-total ratio suggestive of cardiac involvement although 62% showed abnormalities on ECG and/or echocardiography. Only one DMD carrier with borderline echocardiographic abnormality had detectable, but normal cTnT. None of the carriers had detectable cTnI levels.

In only one earlier report[138], cTnI elevation was described in four of 28 patients with chronic muscular diseases, one of them showing left ventricle hypertrophy. Another report describes cTnI elevation in one of 24 dialysis patients without cardiac abnormalities.[130] Whereas cardiac troponin proteins fail to detect non-ischemic cardiac damage in DMD and BMD carriers, these markers could probably be used for carriers suspected of cardiac ischemia. Measurement of CK-MB could have the risk of a false-positive result due to high total-CK.

Some authors have found a negative correlation between CK activity and age in DMD[139] and BMD carriers[140], whereas others did not.[1] We found no such correlation but were able to demonstrate a linear decreasing trend of total CK with increasing age groups of carriers. Surprisingly, only 53% of DMD carriers and 30% of BMD carriers had elevated total CK. Only one earlier study found similar percentages[141], whereas most other studies found raised CK activity in 60-80% in DMD carriers[1,142-146], and in 42-62% of BMD carriers.[140,144,146] These relatively high percentages prompted clinicians in the pre-dystrophin era to calculate the risk of carriership. Perhaps the difference between our study and these carried out before the nineties is due to the fact that in most of these investigations repeat measurements of total CK have been done, whereas we did only one measurement.

In conclusion, elevation of cTnT is extremely rare in carriers of Duchenne and Becker muscular dystrophy and bears no relationship with cardiac abnormalities. cTnI is not elevated in BMD/DMD carriers. Therefore, measurement of these proteins is not helpful to detect non-ischemic myocardial damage in DMD/BMD carriers with cardiac abnormalities. Cardiac TnI and TnT might still be useful in DMD/BMD carriers to detect ischemic cardiac damage when CK-MB fails to discriminate between muscle damage en cardiac ischemia.