Measurement of Cardiac Troponin I in Downer Cows in Dairy Herds around Tehran

Shahram Javadian Kutanaee, Mahdi Sakha, Iraj Sohrabi Haghdoost, Mohammad Gholi Nadalian

INTRODUCTION

Downer cow syndrome is a complication of recumbency associated with milk fever [21]. Downer cow syndrome is characterized by inability of an animal to stand from recumbency voluntarily. The most common cause of downer cow syndrome is hypocalemia [milk fever] [1-4] but it is also caused by injuries, muscle damage, macro mineral deficiencies, toxic mastitis or metritis [7]. Approximately 58% occurred within 1 day of parturition and 37% occurred during the first 100 days of lactation[21].

Acute focal myocarditis may occur in about 10% of cases resulting in tachycardia, arrhythmia, and the unfavorable response to IV calcium salts observed in some downer cows.[21]

Cardiac troponin is part of the cardiac contractile apparatus, the troponin –tropomyosin complex. The complex is comprised of three structurally and functionally different troponins (troponin I, T and C) and mediates the interaction between actin and myosin.[3] Troponins occur in cardiac and skeletal muscle, but not in smooth muscles.[8] Cardiac troponin I (cTnI) is a small (~ 20 kDa) myofibrillar protein associated with the thin filaments of sarcomeres and is the only troponin uniquely expressed in the myocardium.[10] Phosphorylated cTnI inhibits the activity of actin-myosin ATPase preventing myofibrillar contraction during specific phases of the cardiac cycle inducing muscle relaxation.[10] Cardiac troponin T (cTnT) has structural functions in the binding cardiac troponin C (cTnC) and the troponin complex to tropomyosin.[10]

Analysis of cardiac troponin I (cTnI) is considered the“gold standard” for noninvasive diagnosis of myocardial injury in humans,[3,18] It has replaced previously used cardiac biomarkers such as the MB isoenzyme of creatine kinase (CK-MB) because of its unique and high myocardial sensitivity and specificity. The creatine-kinase (CK) and the isoenzyme of CK (CK-MB), have limited value in detecting myocardial injury due to their lack of tissue specificity and sensitivity.[17] They are also found in the myocardium, skeletal muscle tissue and gastrointestinal tract and are less specific to detect myocardial injury in the presence of skeletal...
muscle damage, whereas the measurement of cTnI remains unaffected.[15] Even though the specificity of CK-MB can be enhanced by calculating the CK-MB/CK ratio, the use of this ratio markedly reduces the sensitivity for detection of myocardial injury in patients with concurrent cardiac and skeletal muscle injury.[22] Cardiac troponins have been shown to be a useful cardiac index even in patients with skeletal muscle myopathies.[16]


In the past, the diagnosis of myocardial injury in cattle has been made on the basis of results of physical examination; cardiac auscultation; and, more specifically, by radiography, electrocardiography, and echocardiography. However, diagnostic modalities such as radiography and cardiac ultrasound may not be readily available to the veterinarian. Therefore, easy-to-perform, reliable, and specific tests to detect myocardial injury in cattle are needed. Bovine cTnI has high amino acid sequence homology [496%] with human cTnI[20]. Therefore, it is anticipated that antibodies against human cTnI used in commercially available immunoassays will cross-react with bovine cTnI. In healthy cows serum cTnI concentration of ≤ 0.04 ng/ml have been reported[13]. As of now, the diagnosis of myocardial injury in food animal medicine has traditionally relied on physical examination, cardiac auscultation and further investigations using radiography, electrocardiography [ECG], and echocardiography. Although electrocardiography is an invaluable diagnostic tool, by itself it is neither sensitive nor specific enough to make a definitive diagnosis of myocardial injury[12]. Lack of generally available hospital-standard diagnostic equipment, such as cardiac ultrasound machines or ECG, to the bovine veterinarian in the field suggests that a simple blood test such as the measurement of cTnI could potentially have a significant impact on the ante-mortem assessment of the presence and extent of myocardial injury. Myocardial damage in cattle can have infectious, toxic (such as calcium overdose injection or electrolyte imbalance), nutritional and traumatic etiologies. Examples include but are not limited to vitamin E and selenium deficiency, traumatic-recticulo-pericarditis, and ionophore and glycoside intoxication. The purposes of this study were to evaluate cardiac damage in downer cows by measurement of bovine cTnI, CPK-MB, CPK (creatine phospho kinase), AST (aspartate aminotransferase), LDH (lactate dehydrogenase), Ca(calcium) and P(phosphorus) serum activities to show that cTnI will increase in cattle with myocardial injury in downer cows. We hypothesized that a commercially available immunoassay is highly sensitive and specific for the detection of bovine cTnI and that increased serum concentrations of cTnI are associated with myocardial necrosis in cattle.

MATERIALS AND METHODS

Animal selection:
Thirty Holstein cows (6 of which were first-calf heifers) from 5 industrial dairy farms in Iran were used. Cows that became recumbent were eligible for inclusion in the study and were referred by the local veterinarians who serviced the herds. All cows were sampled 24 hours after recumbency and after first treatments were administered. Downer cows in the present study had failed to rise within 24 h after the 1st treatment.

Data collected when the cows were examined included age, parity, date of calving, recent health and production problems, time (h) from the onset of recumbency, and vital signs. A thorough physical examination was performed on each cow including rectal temperature, pulse rate, inspection of mucous membranes and examination for mastitis, metritis and bone fractures. Then blood samples was collected. Cows without signs of disease other than hypocalcemia were then treated with 500 ml of calcium- magnesium- phosphorous solution (CMP) IV, 250 mL of 40% calcium borogluconate SC, 20 mL of phosphorous IV, 500 mL of 50% dextrose IV, and 250 mL propylene glycol PO.

The 30 cows that were finally included in the study (from a total of 150 initially referred) fit the definition of “downer cows.” These cows were between 1 and 90 days after calving. None of them had a history of musculoskeletal injury, nor showed evidence of other disease (such as, fever, vaginal discharge, mastitis).

Sample collection:
Blood samples were collected from the jugular vein of each cow using an 18-gauge needle into glass tubes without anticoagulant. After clotting for 30-45 min, serum was separated at the farm by centrifugation at 1600 x g for 15 min, transferred to plastic vials, and transported at 4°C to the laboratory, where it was stored frozen at −20°C. Frozen serum was analyzed for aspartate aminotransferase (AST), creatine phosphokinase (CPK), CPK-MB, Troponin I (cTnI ) and lactate dehydrogenase (LDH), calcium(Ca) and phosphorous(P) concentrations.

Serum biochemical analysis:
Spectrophotometric kinetic methods were used to determine serum activities of aspartate aminotransferase (AST) (25), creatine phosphokinase (CPK) (11) and CPK-MB. Troponin I was determined using the
immunoassay methods. All measurements were obtained at a temperature of 30°C. All blood samples were analyzed for serum total Calcium (Ca) concentrations by flame atomic absorption spectrophotometry. Total serum concentration of phosphorus (P) was determined using the heteropoly acid-blue method [2].

Statistical analysis:
Analysis was performed using a commercial software program (SPSS, version 16.0; SPSS, Chicago, Illinois, USA). The Pearson rank bivariate correlation was used to investigate the relationship between variables. Finally, a Paired Samples T test was used to compare medians of biochemical parameter measurements in the 2 different clinical outcome groups (normal—abnormal). For all tests, values of P < 0.05 were considered significant.

Results:
Clinical outcome:
The serum biochemical results of these 30 downer cows are presented in Table 1. Classification of cows according to the clinical and biochemical outcome and the severity of cardiac damage is shown in Table 1.

Table 1: Blood parameters test results from normal downer cows (normal cardiac parameters) and downer cows with cardiac damage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Mean</th>
<th>CPK-MB Mean</th>
<th>cTnI (ng/ml) Mean</th>
<th>Ca (mg/dl) Mean</th>
<th>P (mg/dl) Mean</th>
<th>LDH (U/L) Mean</th>
<th>CPK (U/L) Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=6</td>
<td>171.6</td>
<td>80.16</td>
<td>0.015</td>
<td>7.6</td>
<td>4.75</td>
<td>1673</td>
<td>932</td>
</tr>
<tr>
<td>Mild and Moderate cardiac damage n=15</td>
<td>260.8</td>
<td>190.93</td>
<td>0.15</td>
<td>7.86</td>
<td>5.64</td>
<td>2948.6</td>
<td>1137.73</td>
</tr>
<tr>
<td>Severe cardiac damage n=9</td>
<td>152.3</td>
<td>127.77</td>
<td>1.9</td>
<td>7.94</td>
<td>5.04</td>
<td>3235.44</td>
<td>1668.66</td>
</tr>
</tbody>
</table>

AST — aspartate aminotransferase, cTnI — Serum cardiac Troponin I, Ca — calcium, P — phosphorus, LDH — lactate dehydrogenase, CPK — creatine phosphokinase.

The time frame for their recovery was between 1 and 6 days after 1st treatment. The 21 cows (70%) could not rise and culled by 7 days after 1st treatment that the 18 cows had high concentration of cTnI.

For 7 parameters (AST, cTnI, Ca, P, and LDH, CPK) there were significantly different parameter distribution medians in animals that were normal versus abnormal (Table 2).

Table 2: Median values of the parameters with statistically significantly different means (P < 0.05) between the two outcome groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Med</th>
<th>CPK-MB Med</th>
<th>cTnI (ng/ml) Med</th>
<th>Ca (mg/dl) Med</th>
<th>P (mg/dl) Med</th>
<th>LDH (U/L) Med</th>
<th>CPK (U/L) Med</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=6</td>
<td>69</td>
<td>39</td>
<td>0.015</td>
<td>10.50</td>
<td>6.60</td>
<td>1257</td>
<td>87</td>
</tr>
<tr>
<td>Abnormal</td>
<td>281.50</td>
<td>141</td>
<td>0.2</td>
<td>7.05</td>
<td>4.8</td>
<td>3151</td>
<td>1568.50</td>
</tr>
</tbody>
</table>

AST — aspartate aminotransferase, cTnI — Serum cardiac Troponin I, Ca — calcium, P — phosphorus, LDH — lactate dehydrogenase, CPK — creatine phosphokinase.

Distribution of downer cows for purposes of different parameters have been shown in Figure 1, Figure 2, Figure 3, Figure 4 and Figure 5.

Fig.1: CPK histogram with normal curve.
Fig. 2: CPK-MB histogram with normal curve.

Fig. 3: Troponin I histogram with normal curve.

Fig. 4: Calcium histogram with normal curve.

Fig. 5: Phosphorus histogram with normal curve.
**Serum biochemical analyses:**

Mean serum cTnI increased significantly with the severity of cardiac damage (Table 1).

Mean serum AST activity was significantly higher in all of downer cows (Table 1) and was significantly correlated with parameters such as CPK-MB and parity (Table3).

Serum CPK activity was very high in 80% of the downer cows, and mean CPK activity increased significantly from the mild and moderate to severe cardiac damage groups (Table 1) and was significantly correlated with parameters such as CPK-MB and Ca (Table 3).

Mean serum LDH activity increased significantly with the severity of cardiac damage (Table 1) and was significantly correlated with parity (Table3).

<table>
<thead>
<tr>
<th>AST</th>
<th>cTnI</th>
<th>CPK</th>
<th>LDH</th>
<th>CPK-MB</th>
<th>Ca</th>
<th>P</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>0.501</td>
<td>-0.527</td>
<td>-0.491</td>
<td>0.545</td>
<td>-0.428</td>
<td></td>
<td>-0.569</td>
</tr>
<tr>
<td>0.6</td>
<td>0.501</td>
<td>0.491</td>
<td>0.545</td>
<td>-0.428</td>
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<td></td>
<td>-0.394</td>
</tr>
<tr>
<td>Ca</td>
<td>-0.527</td>
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<td>0.545</td>
<td>-0.428</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P</td>
<td>-0.569</td>
<td>-0.394</td>
<td>-0.428</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Median serum CPK, CPK-MB, cTnI and LDH concentration was significantly elevated in downer cows with myocarditis. Mean serum cTnI concentration was significantly higher in cows with cardiac damage compared with normal downer cows (Table 1). The cTnI averaged 0.014 in the reference cows, 0.15 in the mild and moderate cardiac damage group and rose to 1.9 for the severe cardiac damage group. (Table 1).

CPK-MB was significantly correlated with AST, CPK, Ca, P and parity (Table 3).

Dowder cows had significantly lower mean serum Calcium and Phosphorus concentrations, but these means were not significantly different between the cardiac damage groups (Table 1).

**Discussion:**

One of the aims of the study was to investigate the occurrence and severity of cardiac damage in downer cows. In this group of 30 downer cows, 70% had calved within first week and 30% calved within first 100 days of sampling. About 80% of dairy cows had biochemical evidence from some degree of cardiac damage that 50%(15 cases) with mild or moderate(0.0 ≤ cTnI ≤ 0.3 ng/ml) and 30%(9 cases) with severe(cTnI≤1ng/ml) cardiac damage (myocarditis) with tachycardia and arrhythmia.

Downer cow syndrome is related to post-parturient disorders like hypocalcemia, fatty liver, ketosis, metritis, which occur more often in older cows. It has been reported to be more common in cows than in heifers (4). In this study 33.33% of downer cows with severe cardiac damage had 1 gestation and 33.33% had 2 gestations and 33.33% had 5-6 gestations.

The lack of postmortem examinations on the downer cows in this study is a limitation of the study, as the cause of death could not be verified.

Serum AST activity is easily elevated in muscle damage [14]. In downer cows with increased AST activity, concurrent analysis of serum CPK activity helps to identify the origin of AST (muscle or liver). In the present study, increases in AST were likely due to muscle damage, because the correlation between serum CPK and AST activity was high.

In 24 cases (80%) of downer cows exist significantly high median CPK-MB activity. The 23 cases (76.66%) of downer cows had increased cTnI and CPK-MB activity together.

The downer cows had significantly lower median serum Ca concentration compared with the reference and healthy fresh cows. This was expected, because hypocalcemia is the most frequent cause of recumbency in fresh cows [1]. In this study 40% of downer cows were hypocalcemic(Ca concentrations below 7.2 mg/dl) despite had been treated for hypocalcemia. Some of the downer cows suffered severe hypocalcemia (Ca concentrations below 6.5 mg/dl). There was no difference in serum Ca between the cardiac damage groups. We speculate that the coexistence of cardiac damage with hypocalcemia may have negatively affected the downer cows and worsened their prognosis.

The Ca/P ratio were abnormal in 21 downer cows (about 70% of cases) and 75% of downer cows with cardiac damage had abnormal Ca/P ratio.

The prognosis of downer cows with cardiac damage was poor.

**REFERENCES**