Usefulness of serum cardiac troponin I concentration as a marker of survival of harbor seal (Phoca vitulina) pups during rehabilitation

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OBJECTIVE
To measure serum cardiac troponin I (cTnI) concentrations in orphaned harbor seal (Phoca vitulina) pups at various points during rehabilitation in a seal rescue center and determine whether cTnI concentration was associated with survival during rehabilitation and duration of rehabilitation.

DESIGN
Serial cross-sectional study.

ANIMALS
Fifty-five 2- to 9-day-old harbor seal pups.

PROCEDURES
Blood samples for serum cTnI concentration measurement, CBC, and serum biochemical analysis were obtained from seal pups at admission into a seal rescue center, after 2 weeks of rehabilitation at the center, and prior to release. Serum cTnI concentrations were compared between seals that did or did not survive rehabilitation.

RESULTS
Median serum cTnI concentration was highest at admission (0.03 ng/mL). After 2 weeks, the median value was 0.01 ng/mL; prior to release, it was 0.01 ng/mL. Seal pups that were found to have died during or after rehabilitation (n = 7) had a significantly higher median serum cTnI concentration at admission (0.06 ng/mL) than did seal pups that survived rehabilitation (and for which the postrelease fate was unknown; 48; 0.03 ng/mL). No correlation was identified between serum cTnI concentration and duration of rehabilitation.

CONCLUSIONS AND CLINICAL RELEVANCE
The results of this study suggested some degree of myocardial injury was present in most of the orphaned seal pups admitted for rehabilitation. Measurement of serum cTnI concentration in seal pups at admission might provide prognostic information about their likelihood of survival during or after rehabilitation. (J Am Vet Med Assoc 2016;249:1428–1435)

Harbor seals (Phoca vitulina) are the most widely distributed pinnipeds throughout the coastal waters of the Northern Hemisphere. Increasing commercial and leisure use of coastal areas1–3 is likely to influence the seals' habitat,4 and stagnation and decline in the size of most large harbor seal colonies have been observed in UK waters over recent decades.3,5 During the pupping season, severe weather conditions and, potentially, human interferences result in orphaned seal pups.2,3,6–9 Live orphaned pups are admitted to seal rescue centers, where 50% to 80% of pups are successfully rehabilitated.10–12 Common causes of death identified through postmortem evaluation are pneumonia, sepsis, and malnutrition.10–15

Detection of disease in seals before death is challenging. Seals rarely have clinical signs, and results of routine blood testing are frequently unremarkable.11–14 Furthermore, age-related changes in CBC and serum biochemical values during rehabilitation need to be considered,11,13,15–17 and reference ranges reported for one seal population might not be applicable for another population living in a different environment.11,12,15,16 More advanced diagnostic methods, such as blood gas analysis14 and leukocyte function tests,12 are also unable to identify seals with a greater risk of death than others.

Cardiac troponin I is a sensitive and specific marker for myocardial cell damage.18,19 This troponin isoform is part of the troponin complex, which consists of troponins I, C, and T and is important for excitation-contraction coupling. Most troponins are myofibril bound, but approximately 5% to 8% of troponins I and T are free within the cytosol. After cell membrane damage, troponins I and T are released into the systemic circulation, followed by myofibril-bound cTnI, indicating irreversible myo-

ABBREVIATIONS
cTnI Cardiac troponin I
IQR Interquartile range
cardiac cell damage. The circulating cTnI concentration therefore reflects the severity and persistence of that damage.20

In human medicine, serum or plasma cTnI concentration is used to detect myocardial injury and predict the likelihood of death.21-23 Similarly in veterinary medicine, serum or plasma cTnI concentration increases with cardiac diseases in dogs, cats, and horses.24-28 On the other hand, serum cTnI concentrations in California sea lions with degenerative cardiomyopathy caused by domoic acid intoxication are no different from those of sea lions that die of other causes.29

Recently, cTnI has gained attention as a marker for myocardial cell damage in noncardiac diseases.20,30 Myocardial injury, which is common in critically ill humans, can be caused by systemic inflammation, hemodynamic changes, and intermittent myocardial ischemia.30,31 Similar findings have been observed in several animal species with noncardiac diseases, in which high serum cTnI concentration has been associated with short- and long-term mortality rates.19,20,32-38

The usefulness of cTnI as a potential marker for systemic disease and its association with outcome has not yet been investigated in seals, and such information could be valuable for predicting whether injured or diseased pups might be successfully rehabilitated. The objective of the study reported here was to investigate changes in serum cTnI concentration in harbor seal pups at different points during rehabilitation and to test whether serum cTnI concentrations were associated with survival during rehabilitation or duration of rehabilitation.

Materials and Methods

Animals

The study protocol was approved by the University of Bristol Committee on Research Ethics. Seal pups that had been abandoned by their mothers and collected from the German Wadden Sea coastline of Schleswig-Holstein from June 9 through July 1, 2013, were included in the study. Licensed hunters and specifically trained personnel assess these pups initially at their location and collect the ones that are considered likely to survive and fulfill specific criteria that are published in an official register from the provincial government of Schleswig-Holstein.39

On admission to the rescue center, seal pups were physically evaluated, and basic data such as body weight, sex, rectal temperature, and respiratory rate were recorded as reported elsewhere.14 Blood samples were collected from the epidural venous sinus for performance of a CBC and serum biochemical analysis as previously reported.14 Age was estimated from body weight and states of pelage, umbilical regression, and tooth development.12-40 The seal pups were estimated to be between 2 and 9 days old (mean, 5.7 days; median, 7 days).

After admission, all seal pups received antimicrobial treatment (amoxicillin-clavulanic acid) for the first 1 to 2 weeks to treat and prevent potential infections. Individual medical management plans were devised on the basis of CBC, serum biochemical, and physical examination findings obtained at admission and after 2 weeks of rehabilitation. Plans included administration of various fluids, antimicrobials, NSAIDs, corticosteroid drugs, or topical agents or a combination of these treatments. Once the seal pups were considered stable, they were kept in small groups.

The seal pups were initially tube-fed a milk replacer and mashed herring before being weaned to whole herring. When the pups had reached a target body weight of approximately 15 kg (33 lb) and were able to eat independently, they were brought together into 1 group for the remainder of the rehabilitation period to promote social interactions. Seal pups weighing >25 kg (55 lb) and that were considered healthy on the basis of physical examination, CBC, and serum biochemical findings were released back into the wild.

After 2 weeks of rehabilitation and again prior to release of seal pups back into the wild, a veterinarian performed a physical examination of each that included body weight measurement, cardiac auscultation, physical inspection, respiratory rate measurement, and blood sample collection for CBC and serum biochemical analysis. Following rehabilitation, seal pups that were alive were released back into the wild. Duration of rehabilitation, body weight at release from the center, and weight gain per day was recorded for each seal that was released back into the wild. For seal pups that died during rehabilitation, this information was obtained until their death.

CBC and serum biochemical analysis

Blood samples for CBC were collected into EDTA tubes and immediately analyzed; samples for serum cTnI measurement and other serum biochemical analyses were collected into serum separation tubes. Serum separation tubes were kept at room temperature (approx 23°C) for 30 minutes to 1 hour before centrifugation at 1,500 X g for 15 minutes. The samples were divided into aliquots, and 1- to 2-mL portions were stored at -20°C until analysis at a commercial laboratory.5

Complete blood cell counts were performed in house by use of a hematologic analyzer and consisted of differential counts of leukocytes (WBCs, granulocytes, monocytes, and lymphocytes), RBCs, and platelets and measurement of hemoglobin concentration and Hct. Serum biochemical analysis was performed by use of a modular analyzer and consisted of triglycerides, cholesterol, total protein, and albumin concentrations; albumin-to-globulin ratio; and lipase, α-amylase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, and glutamate dehydrogenase activities. Measurements were also made of serum to-
Serum cTnI concentration

Serum cTnI concentration was measured by use of a high-sensitivity 3-site immunoassay. The assay was validated by use of pooled serum samples from adult harbor seals, and the intra- and interassay coefficients of variation were 7.9% and 9.1%, respectively. Two control samples were prepared from pooled serum samples, one providing a low cTnI concentration and the other a high concentration, and these samples were run with each analysis. The minimum detection limit of the assay reported by the manufacturer was 0.006 ng of cTnI/mL, and the upper limit was 50 ng/mL.

Statistical analysis

Statistical analysis was performed by use of statistical software. Basic descriptive statistics were computed for all measured variables (body weight, rectal temperature, respiratory rate, duration of rehabilitation, and CBC, serum biochemical, and cTnI values). Body weight, rectal temperature, and duration of rehabilitation were normally distributed and are reported as mean, SD, and range. Respiratory rate and CBC, serum biochemical, and cTnI values were not normally distributed and are reported as median, IQR, and range.

To test for differences among the 3 points at which blood samples were collected from the seal pups (at admission, after 2 weeks of rehabilitation, and prior to release), 1-way ANOVA with repeated measures was performed for the normally distributed data and the Friedman test was performed for nonnormally distributed data. Seal pups were then grouped as those that died during or after rehabilitation, or those that were successfully rehabilitated and were not found dead after release, and the Student t test and Mann-Whitney U test were used to test for differences between these 2 groups. Relationships were also examined between serum cTnI concentrations at all 3 measurement points and body weight at admission, weight gain, duration of rehabilitation, and CBC and serum biochemical values by construction of scatterplots and calculation of the Spearman ρ value. Values of P < 0.05 were considered significant for all analyses.

Results

Animals

Fifty-five seal pups (21 males and 34 females) were included in the study. Four pups (2 males and 2 females) died during rehabilitation at 4, 24, 31, or 53 days after admission. Three seal pups were known to have died after release back into the wild. One of these pups was found weak, dehydrated, and hypothermic (rectal temperature, 34°C [93.2°F]; range, 34.0° to 35.5°C [92.8° to 94.9°F]), and median respiratory rate was 22 breaths/min (IQR, 18 to 26 breaths/min; range, 12 to 42 breaths/min). Mean body weight of the 7 seal pups that were known to have died was 9.2 kg (20.2 lb; SD, 0.4 kg [0.9 lb]; range, 8.2 to 11.3 kg [18.0 to 24.9 lb]). Mean rectal temperature was 37.4°C (99.3°F; SD, 0.6°C [1.0°F]; range, 35.6° to 38.9°C [96° to 102°F]), and median respiratory rate was 22 breaths/min (IQR, 18 to 26 breaths/min; range, 12 to 42 breaths/min). Mean body weight of the 7 seal pups that were known to have died was 9.2 kg (20.2 lb; SD, 0.4 kg [0.9 lb]; range, 8.2 to 11.3 kg [18.0 to 24.9 lb]). Mean rectal temperature was 37.1°C (99.8°F; SD, 0.4°C [0.7°F]; range, 36.6° to 37.5°C [97.9° to 99.5°F]), and median respiratory rate was 18 breaths/min (IQR, 18 to 24 breaths/min; range, 16 to 28 breaths/min), and these values did not differ significantly from those of the seal pups that were presumed to have survived.

Mean duration of rehabilitation for the 51 seal pups presumed to have survived was 70 days (SD, 22 days; range, 31 to 119 days). Prior to release, the mean body weight of the seal pups was 29.3 kg (64.5 lb; SD, 2.4 kg [5.3 lb]; range, 25.2 to 35.8 kg [55.4 to 78.8 lb]), and mean weight gain per day was 0.27 kg (0.59 lb; SD, 0.06 kg [0.13 lb]; range, 0.22 to 0.43 kg [0.48 to 0.95 lb]).
Serum cTnI concentration

Serum cTnI concentration was highest at center admission (median, 0.03 ng/mL; IQR, 0.02 to 0.06 ng/mL; range, 0.01 to 0.30 ng/mL) and lowest after 2 weeks of rehabilitation (median, 0.01 ng/mL; IQR, 0.0 to 0.02 ng/mL; range, 0.0 to 0.06 ng/mL). The difference between median values at the first and subsequent measurement points was significant (P < 0.001; Figure 1). Serum cTnI concentrations in 6 seal pups were in the upper quartile (cTnI ≥ 0.06 ng/mL) at admission and remained elevated prior to release, with a median value of 0.03 ng/mL (range, 0.02 to 0.04 ng/mL).

Seal pups that were known to have died during or after rehabilitation (n = 7) had significantly (P = 0.02) higher serum cTnI concentrations (median, 0.06 ng/mL; IQR, 0.03 to 0.13 ng/mL; range, 0.03 to 0.22 ng/mL) at admission than did the remaining seal pups (n = 48; median, 0.03 ng/mL; IQR, 0.02 to 0.05 ng/mL; range, 0.01 to 0.30 ng/mL; Figure 2). The seal pup that was euthanized after release back into the wild had a cTnI value of 0.06 ng/mL prior to release; the 2 seal pups that were found dead after release had cTnI values of 0.02 and 0.00 ng/mL. Serum cTnI concentrations measured at admission, after 2 weeks of rehabilitation, and prior to release were not correlated with duration of rehabilitation, body weight at admission, or weight gain.

CBC and serum biochemical values

Significant differences were identified among measurement points for most CBC and serum biochemical values (Tables 1 and 2). The CBC values for RBC count, hemoglobin concentration, and Hct were highest at admission; values for WBC, granulocyte, and monocyte counts were highest after 2 weeks of rehabilitation; and values for platelet and lymphocyte counts were highest prior to release back into the wild. Serum biochemical values for albumin-to-globulin ratio, activities of alkaline phosphatase and alanine aminotransferase, and concentrations of total bilirubin, creatinine, albumin, phosphorus, magnesium, iron, and fructosamine were highest at admission; values for activities of γ-glutamyltransferase and lipase and concentrations of BUN, sodium, and total calcium were highest after 2 weeks of rehabilitation; and values for activities of aspartate aminotransferase, creatine kinase as well as for concentrations of triglycerides, cholesterol, total protein, and globulin were highest prior to release. Only serum potassium concentration remained relatively unchanged with time (P = 0.08).

Table 1—Values of CBC analytes of harbor seal (Phoca vitulina) pups measured at admission to a seal rescue center, after 2 weeks of rehabilitation, and prior to release back into the wild.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Admission (n = 55)</th>
<th>2 weeks (n = 54)</th>
<th>Release (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Range</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>RBCs (X 10^12 cells/L)</td>
<td>6.0 (5.7–6.5)</td>
<td>4.85–6.9</td>
<td>5.7 (5.3–6.0)</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>215 (200–230)</td>
<td>166–246</td>
<td>204.5 (190–220)</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>61.9 (57.8–66.7)</td>
<td>48.8–69.5</td>
<td>57.2 (53.3–61.9)</td>
</tr>
<tr>
<td>WBC (X 10^9 cells/L)</td>
<td>7.1 (6.2–8.5)</td>
<td>4.9–20.1</td>
<td>14.3 (11.4–18.5)</td>
</tr>
<tr>
<td>Lymphocytes (X 10^9 cells/L)</td>
<td>2.2 (1.8–2.8)</td>
<td>1.1–7.2</td>
<td>2.5 (2.1–2.9)</td>
</tr>
<tr>
<td>Monocytes (X 10^9 cells/L)</td>
<td>0.3 (0.2–0.4)</td>
<td>0–0.9</td>
<td>0.4 (0.3–0.5)</td>
</tr>
<tr>
<td>Granulocytes (X 10^9 cells/L)</td>
<td>4.3 (3.9–5.4)</td>
<td>0.5–14.9</td>
<td>11.8 (8.6–16.0)</td>
</tr>
</tbody>
</table>

Significant (Friedman test, P < 0.001) differences were identified among measurement points for all CBC analytes. No reference ranges are available for seals in the circumstances described in this report.
Table 2—Values of serum biochemical analytes of harbor seal pups measured at admission to a seal rescue center, after 2 weeks of rehabilitation, and prior to release back into the wild.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Admission (n = 55)</th>
<th>2 weeks (n = 54)</th>
<th>Release (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Range</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.87 (0.77–0.98)</td>
<td>0.59–1.73</td>
<td>1.70 (1.00–2.52)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>7.5 (5.9–9)</td>
<td>4.1–11.5</td>
<td>6.5 (5.6–7.2)</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>60.3 (58–61.8)</td>
<td>53.7–67.9</td>
<td>57.1 (52.5–59.5)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>48.1 (45.9–50.2)</td>
<td>39.1–52.9</td>
<td>39.5 (38.0–41.0)</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>12.5 (10.6–14.0)</td>
<td>8.3–17.5</td>
<td>17.6 (16.2–18.9)</td>
</tr>
<tr>
<td>Albumin-to-globulin ratio</td>
<td>4.0 (3.4–4.8)</td>
<td>2.2–6.1</td>
<td>2.26 (2.13–2.39)</td>
</tr>
<tr>
<td>Lipase (U/L)</td>
<td>25.1 (19.1–32.5)</td>
<td>13.9–60.9</td>
<td>121.7 (99.0–170.3)</td>
</tr>
<tr>
<td>α-Amylase (U/L)</td>
<td>304 (263–321)</td>
<td>198–399</td>
<td>284 (255–313)</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>122 (94–146)</td>
<td>64–266</td>
<td>68 (57–76)</td>
</tr>
<tr>
<td>Alumina aminotransferase (U/L)</td>
<td>48.3 (32.3–71.3)</td>
<td>15.5–191.3</td>
<td>23.7 (20.1–28.4)</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>38.3 (27.5–53.5)</td>
<td>17.6–98.3</td>
<td>28.3 (23.4–33.6)</td>
</tr>
<tr>
<td>γ-Glutamyltransferase (U/L)</td>
<td>13.8 (10.8–19.0)</td>
<td>0.01–77.6</td>
<td>14.8 (11.9–18.8)</td>
</tr>
<tr>
<td>Glutamate dehydrogenase (U/L)</td>
<td>11.4 (8.7–15.9)</td>
<td>5–29.6</td>
<td>20.0 (15.9–24.7)</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>182.0 (16.6–329.0)</td>
<td>6.5–102</td>
<td>1.05 (0.01–2.62)</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>16.9 (14.6–19.1)</td>
<td>9.8–35.6</td>
<td>21.6 (19.3–23.3)</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>43.0 (36.0–48.0)</td>
<td>26.0–67.0</td>
<td>22.0 (18.9–24.7)</td>
</tr>
<tr>
<td>Fructosamine (µmol/L)</td>
<td>220 (207–233)</td>
<td>186–206</td>
<td>172 (163–179)</td>
</tr>
<tr>
<td>Creatine kinase (µ/L)</td>
<td>124.4 (87.0–171.8)</td>
<td>57.9–679.9</td>
<td>75.2 (45.7–171.75)</td>
</tr>
<tr>
<td>Total calcium (mmol/L)</td>
<td>2.4 (2.3–2.4)</td>
<td>2.0–2.7</td>
<td>2.6 (2.5–2.5)</td>
</tr>
<tr>
<td>Iron (µmol/L)</td>
<td>52.3 (35.7–62)</td>
<td>12.8–82.8</td>
<td>42.5 (29.5–58.3)</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.3 (4.1–4.7)</td>
<td>3.4–5.4</td>
<td>4.5 (4.2–4.7)</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>1.2 (1.1–1.3)</td>
<td>1.0–1.5</td>
<td>1.0 (0.9–1.0)</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>148 (146–150)</td>
<td>144–154</td>
<td>152 (151–154)</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>2.6 (2.4–2.8)</td>
<td>1.9–3.3</td>
<td>2.2 (2.0–2.3)</td>
</tr>
</tbody>
</table>

Significant (Friedman test, P < 0.001) differences were identified among measurement points for all serum biochemical analytes except potassium (P = 0.08). No reference ranges are available for seals in the circumstances described in this report.

No significant differences in CBC and serum biochemical values at admission were identified between seal pups that were known to have died and those that were presumed to have survived. No significant correlations were detected between serum cTnI concentration and any other serum biochemical or CBC values at any measurement point.

**Discussion**

To the authors’ knowledge, the study reported here represents the first prospective study in which serum cTnI concentration was measured in seal pups at various points during rehabilitation. Serum cTnI concentrations varied significantly during rehabilitation, with values highest at admission and lowest after 2 weeks of rehabilitation and prior to release back into the wild. Pups that were found to have died during rehabilitation or after release had significantly higher serum cTnI concentrations than did pups that were successfully rehabilitated and were not found dead after release.

The generally high cTnI concentrations identified in seal pups at admission into the Seal Center Friedrichskoog in Germany suggested a degree of myocardial injury most likely secondary to systemic diseases, as has been reported for other species. High serum cTnI concentrations have been detected in dogs, cows, and horses with noncardiac diseases.52,54,56,57 Similarly, serum cTnI concentrations in California sea lions with degenerative cardiomyopathy are no different from those of sea lions that died from other causes (renal failure or trauma), suggesting an influence of systemic diseases on the heart in that species as well.59

Indirect effects on the heart caused by ischemia, systemic activation of the inflammatory system, metabolic derangement, fluid shift, tachycardia, and an increase in sympathetic tone have been suspected to cause cTnI release.20,31,41 Limited ischemia followed by reoxygenation results in membranous bleb formation and nonrecurring release of cytoplasmic contents.20 When ischemia persists, these blebs grow and eventually rupture and cell necrosis occurs. However, reoxygenation before bleb rupture causes reabsorption or shedding of blebs into the circulation. Shedding of blebs results in release of cytoplasmic bleb contents and an intermittently high circulating cTnI concentration.20 Most seal pups are weak, malnourished, and dehydrated at admission into seal rescue centers.10,12 This compromised state and the unfamiliar environment result in an increase in sympathetic tone, which is reflected by sinus tachycardia.42 Myocardial ischemia and cTnI release are therefore possible.
Seal pups that were known to have died during or after rehabilitation had significantly higher serum cTnI concentrations at admission than did pups that survived rehabilitation and were not found dead after release back into the wild. No other CBC or serum biochemical values differed significantly between seal pups that were found to have died and those that were presumed to have survived. This suggests that measurement of serum cTnI concentration in seal pups at admission into a seal rescue center might provide prognostic information on the likelihood of long-term survival. In particular, 3 seal pups died after having been considered healthy and being released back into the wild. However, not all pups with a high serum cTnI concentration died during rehabilitation and there was no correlation of cTnI concentration with duration of rehabilitation. This finding was consistent with previous reports\(^{52,54}\) that not all animals with a high serum cTnI concentration will die.

Intermittent ischemia and less severe disease (as causes of increases in cTnI values) as well as a more mature immune system could have been responsible for a faster recovery and unremarkable duration of rehabilitation for the seal pups of the present study.\(^{36,43}\) High serum cTnI concentration at admission did not automatically indicate that a seal pup would die or fare worse than other seal pups during rehabilitation in the present study; however, a high concentration should raise suspicion that a seal pup might be at higher risk of death than others.

The seal pup that was euthanized after release in the present study had the highest serum cTnI concentration prior to release. Five other seal pups with initial cTnI concentrations in the upper quartile had lower but still high cTnI concentrations prior to release. High concentrations prior to release are of concern, given that this might indicate either recurrent ischemic events or persistent myocardial cell damage. Repeatedly high serum cTnI concentrations have been associated with poor prognosis in dogs with cardiac disease.\(^{44}\) However, the 2 seal pups that died after release did not have a markedly high serum cTnI concentration prior to release and outcomes for the other pups with high cTnI concentrations during the present study remain unknown, making it difficult to assess the clinical relevance of a high serum cTnI concentration prior to release.

Interestingly, the lowest serum cTnI concentrations in all seal pups were identified at 2 weeks of rehabilitation and thereafter. This suggested that intermittent myocardial ischemia with bleb formation and isolated release of cTnI might be more likely in these seal pups than the ongoing release that is observed with damaged or necrotic myocardial cells.\(^{20}\) Furthermore, sustained release of myofibrill-bound cTnI results in maximum circulating concentration of cTnI that is 5 to 12 times that of cytosolic cTnI.\(^{20}\) However, even mild increases in circulating cTnI concentration might be detrimental in the long term, as has been reported for humans.\(^{44}\) Similarly, for dogs with systemic inflammation, high serum cTnI concentration has been associated with an increase in the likelihood of death within 1 year.\(^{53}\)

Seals live in an environment that is cardiovascularly challenging\(^{39-47}\) because of the need to locate, catch, and consume prey while holding their breath and to withstand hydrostatic pressure as they dive. Therefore, myocardial damage might result in impaired cardiac function insufficient to meet the demand required for diving and foraging or in potentially fatal arrhythmia.\(^{45}\) Serum cTnI concentrations vary in humans and dogs during a diseased state\(^{20,32}\) and could have been the cause for the varying concentrations identified in the seal pups of the present study. The low serum cTnI concentrations after 2 weeks suggested that measurements in seal pups obtained at admission might provide better information than measurements obtained during rehabilitation. A similar pattern has been identified in critically diseased dogs, for which the initial measurement of serum cTnI concentration had stronger prognostic relevance than did subsequent measurements.\(^{32,35}\)

A reference range for serum cTnI concentration in seals has not been established, to the authors’ knowledge. After 2 weeks of rehabilitation in the present study, all seal pups had cTnI values ≤0.01 ng/mL, which suggested that low concentrations might be expected in healthy seals, as has been found in other species, such as dogs, cats, horses, and cows.\(^{25,28,34,37}\) Furthermore, in humans, serum cTnI concentration is reportedly highest at birth and decreases to adult values during the first year of life.\(^{48}\) A reduction in serum cTnI concentration to adult values might occur earlier than this in harbor seal pups; however, such a reduction should typically be stable or a further reduction should occur, rather than a subsequent increase in concentration.

Prior to release, only 53% (27/51) of seal pups had a serum cTnI concentration ≤0.01 ng/mL. Exercise can reportedly result in a mild increase in serum cTnI concentrations in humans and dogs.\(^{49,50}\) and exercise could have also caused slightly higher cTnI values in seal pups prior to release. For the final period of rehabilitation, seal pups were kept in a larger area with a larger pool, and a burst of intense exercise prior to blood sample collection was possible.

Duration of rehabilitation, body weight, amount of weight gain, and hemotologic findings of the seal pups in the present study were similar to those in previous reports.\(^{11,12,14}\) No CBC or serum biochemical values differed between seal pups that were found to have died and those presumed to have survived rehabilitation. This confirmed the low sensitivity and specificity of CBC and serum biochemical analysis to detect diseased seals and seals at risk of adverse outcomes, consistent with findings in previous reports.\(^{11,12,15}\) The changes in CBC values during rehabilitation likely reflected the developing immune system, as previously reported for seal pups,\(^{43}\) and could have been influenced by subclinical disease, making it more difficult to identify seals with disease.\(^{11,12,15}\)

Limitations of the study reported here included the low number of seal pups that were found to have died and the unknown outcome of the other seals that were released back into the wild, which hindered assessment of the prognostic value of serum cTnI concentration as marker of survival after rehabilitation. However, seal pups that were known to have died had significantly higher...
serum cTnI concentrations than did the other pups. Post-mortem investigations were not performed to determine cause of death. In addition, physical examination of the seal pups included cardiac auscultation but no additional diagnostic procedures such as echocardiography; therefore, the presence of cardiac disease or early myocardial dysfunction could not be completely excluded. However, considering that these seal pups were from a free-ranging population, they were considered unlikely to have had any cardiac diseases that might be associated with a high serum cTnI concentration.29

The results of the study reported here suggested that most of the orphaned seal pups evaluated had some degree of myocardial injury. Furthermore, a high serum cTnI concentration in seal pups at admission to a rescue center might indicate an increased risk of death during and also after rehabilitation. These preliminary results supported the potential usefulness of serum cTnI concentration as a marker for survival of seal pups after rehabilitation. Additional studies involving larger samples sizes, which might increase the likelihood of disease and death detection, are needed to further explore the usefulness of cTnI values for disease detection and prognostication and to develop reference limits for this analyte in harbor seals.

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Footnotes

b. Laboklin, Bad Kissingen, Germany.
c. Scil Vet ABC, Scil Animal Care GmbH, Viernheim, Germany.
d. Cobas 8000 c701 analyzer, Roche, Mannheim, Germany.
e. ADVIA Centaur Tnl-ultra immunoassay, Siemens, Erlangen, Germany.

References

19. O’Brien PJ. Cardiac troponin is the most effective translational safety biomarker for myocardial injury in cardiototoxicity. Toxicology 2008;245:206–218.


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**From this month’s AJVR**

**Quantification and characterization of pleural fluid in healthy dogs with thoracostomy tubes**

Germaine C. Hung et al

**OBJECTIVE**

To quantify and characterize pleural fluid collected from healthy dogs after placement of a thoracostomy tube (TT).

**ANIMALS**

8 healthy Coonhound-cross dogs (mean ± SD weight, 27.2 ± 1.6 kg).

**PROCEDURES**

Thoracic CT of each dog was performed before placement of a TT and daily thereafter for 7 days. Thoracic fluid volume was calculated from CT images. Effusion was aspirated when detected; volume was recorded, and cytologic analysis and bacterial culture were performed.

**RESULTS**

Mean ± SD volume of pleural effusion detected by CT was 1.43 ± 0.59 mL/kg (range, 0.12 to 3.32 mL/kg). Mean volume collected via aspiration was 0.48 ± 0.84 mL/kg (range, 0 to 2.16 mL/kg). Cytologic analysis yielded results consistent with an exudate, characterized by septic suppurative inflammation in 6 dogs and mixed inflammation in 1 dog; there was insufficient volume for analysis in 1 dog. Sufficient volume was obtained for bacterial culture for 6 dogs, which yielded pure growths of *Staphylococcus pseudintermedius* (n = 3) and *Streptococcus equi* subspecies *zooepidemicus* (2) and mixed growth of both of these species (1). The TT was removed before day 7 in 4 dogs because of pyothorax (n = 3) and irreversible damage to the TT (1).

**CONCLUSIONS AND CLINICAL RELEVANCE**

Presence of a TT induced a minimal volume of pleural effusion in healthy dogs. Pyothorax developed in most dogs between 4 and 6 days after TT placement. On the basis of these findings, a TT should be removed by the fourth day after placement, unless complications are detected sooner. (*Am J Vet Res* 2016;77:1387-1391)