RATIO OF URINARY $\alpha$-AMYLASE ACTIVITY TO CREATININE CONCENTRATION: A NEW DIAGNOSTIC MARKER FOR PANCREATITIS IN DOGS?

BARBARA FOJUT-PALKA, ANNA WINNICKA$^1$, AND JÓZEF SZAREK$^2$

Animal Clinic, 03-358 Warsaw, Poland
$^1$Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, 02-776 Warsaw, Poland
$^2$Department of Forensic and Administration of Veterinary Medicine, Faculty of Veterinary Medicine, University of Warmia and Mazury, 10-719 Olsztyn, Poland

anna_winnicka@sggw.pl

Received: February 16, 2011    Accepted: May 6, 2011

Abstract

The hypothesis that acute pancreatitis in dogs could be diagnosed based on the ratio of urinary $\alpha$-amylase activity to creatinine concentration (U-A/C) was tested. The study was performed on 292 dogs. Based on clinical, laboratory, and imaging findings, the dogs were divided into the following groups: 34 healthy patients serving as the control, 48 sick dogs with U-A/C>2.9, and 210 sick dogs with U-A/C<2.9. The sick dogs were subsequently divided into subgroups according to their diseases. The results of blood and urine analyses of sick dogs were compared to those of healthy dogs. The subgroups of dogs with U-A/C>2.9 differed from the control group in the same way: their serum and urine $\alpha$-amylase activity and total urinary protein levels were significantly higher (P<0.001), and their urine specific gravity and urinary creatinine concentration were significantly lower (P<0.001). Acute or chronic pancreatitis was confirmed in many of the sick dogs with U-A/C>2.9 that were tested by ultrasonography (48%, 10/21) and histopathology (100%, 7/7). The findings might suggest that U-A/C ratio higher than 2.9 could serve as a cut-off value for diagnosing pancreatitis in dogs (excluding advanced chronic pancreatitis), regardless of concurrent diseases.

Key words: canine, pancreatitis, $\alpha$-amylase, creatinine, urine.

In veterinary medicine it is generally accepted that healthy dogs exhibit very little to no $\alpha$-amylase activity in the urine (U-AMY). Furthermore, even in experimentally induced acute pancreatitis (AP), the value of U-AMY activity does not tend to show a significant increase. Thus, it is believed that in dogs U-AMY values are not clinically useful (1, 3, 13, 14, 21). Akuzawa et al. (1) found that the amylase-creatinine clearance ratio did not increase significantly following pancreatitis induction in dogs. Whereas, Turgut et al. (24) reported that the ratio of urinary $\alpha$-amylase activity to creatinine concentration (U-A/C) was more diagnostically relevant than serum $\alpha$-amylase activity (S-AMY) for experimentally induced AP in dogs, when a cut-off value of U-A/C >5 was used.

In the presented study, the hypothesis that a diagnosis of AP in dogs could be made when the U-A/C ratio exceeds 2.9 was tested. The U-A/C ratio cut-off value of 2.9 was based on our previous clinical observations. In our clinical practice, we have observed that the U-A/C ratio was always below 2.9 in sick dogs with non-pancreatic diseases, whereas the U-A/C ratio was always above 2.9 in sick dogs with clinically recognised AP, confirmed by clinical and laboratory examinations, and ultrasonography, when the tests were conducted within 2d from the disease onset.

Material and Methods

Two hundred and ninety-two dogs of both sexes and various breeds, aged 11 months to 17 years, with body weights ranging from 4.6 to 60.0 kg, which were submitted to the Small Animal Veterinary Clinic in Warsaw, Poland, between January 2004 and January 2008 with various spontaneous diseases, were used in the study. All dogs were examined, and their data (e.g., age, breed, sex, body weight and condition, clinical signs, diagnosis, treatment) were recorded. Blood and urine samples were collected at the first presentation to the clinic (within 24-48 h from the onset of clinical signs of any disease). In each dog, U-AMY and urinary creatinine concentrations (U-Crn) were determined, and the U-A/C ratio was calculated. All patients were classified into the following categories: healthy, sick with U-A/C < 2.9, sick with U-A/C > 2.9 and exclusions.

The results of blood and urine analyses from sick dogs with U-A/C > 2.9 and U-A/C < 2.9 were
compared to those of healthy dogs and searched for common and distinct characteristics that could permit the identification of sick dogs with U-A/C > 2.9 from those with U-A/C < 2.9. When possible, autopsies were performed on dogs with U-A/C > 2.9 that were euthanised due to poor prognosis. In many cases, radiography, abdominal ultrasonography, and other examinations were carried out. In this study, AP in dogs were clinically recognised based on history, results of physical examinations, the presence of severe clinical signs, results of CBC and biochemistry profiles, and ultrasonography.

**Urine and blood samples collection.** For laboratory testing, the first morning urine was sampled. Blood samples were always taken at the same morning. Samples were obtained from all dogs after fasting for 10-12 h and were tested within 2 h after sampling at the Veterinary Diagnostic Laboratory LAB-WET in Warsaw.

**Laboratory tests.** Urinalysis included physical, chemical, and sediment examination. U-AMY was measured in the supernatant using a POINTE 180 biochemical auto-analyser (Pointe Sc., Inc., USA). Haematological analyses included complete blood cell counts and blood smear analysis. Biochemical serum tests included measurements of α-amylase and lipase activity, and standard biochemical parameters. In some cases, when diagnosis needed to be confirmed, additional biochemical parameters were determined.

**Ultrasonography.** Acute and chronic pancreatitis (CP) were diagnosed based on the criteria described by Hess et al. (9) and Hori et al. (10).

**Histopathology.** The pancreas was obtained immediately after death to avoid post-mortem autolysis. Acute and chronic pancreatitis were diagnosed based on the criteria established by Kyogoku (15).

**Statistical analysis.** Data are presented as mean values and standard deviations (mean ± SD). Comparisons of the means were made using Student’s t-test or Mann-Whitney’s U test, as data sets had failed the Kolmogorov-Smirnov normality test. Correlations were calculated using Pearson’s or Spearman’s correlation coefficients. For all analyses, P-values < 0.05 were considered significant.

**Results**

Thirty-four dogs were classified as clinically healthy and served as a control group (group C). Group B consisted of 48 sick dogs with U-A/C >2.9, which were subsequently divided into three subgroups according to renal efficiency: B1 (normal renal function, n=28), B2 (acute renal failure, n=8), and B3 (chronic renal failure, n=12). Four dogs with clinically recognised AP were identified in group B. The remaining 210 sick dogs with U-A/C <2.9 (group A) were divided into 14 subgroups according to their diseases: A1-A12 (different diseases with normal renal function, n=164), A13 (acute renal failure, n=20), and A14 (chronic renal failure, n=26). Subgroups A13 and A14 served as additional controls for subgroups B2 and B3, respectively.

**Serum α-amylase activity.** Among subgroups A1-A12, only subgroups A1 and A8 had serum α-amylase levels that were different from control group C, but none of the dogs in these subgroups had α-amylase levels in the upper normal range (reference <1,850 U/L). Three percent (5/157) of the dogs in subgroups A1-A12, 30% (13/43) of the dogs in subgroups A13 and A14, 26% (6/23) of the dogs in subgroup B1, 57% (4/7) of the dogs in subgroup B2, and 92% (11/12) of the dogs in subgroup B3 had hyperamylasaemia.

**Urine α-amylase activity.** In subgroups A1-A12, the maximum U-AMY value was 16-fold higher than the maximum value for group C, whereas the highest value of U-AMY in group B was 220-fold above the maximum value for group C. The U-AMY values were similar among the dogs in group A with U-A/C < 2.9, and more than 45% of the dogs in this group had U-AMY values within the reference range; in contrast, the minimum U-AMY value in group B was three-fold higher than the upper reference range (Table 1).

There was a statistically significant difference in U-AMY values between female and male dogs only in subgroup A7 [4.48 ±2.37 vs. 34.05 ±21.14, t(10) = - 4.11, P<0.01]. A significant correlation between U-AMY and body weight, age, and S-AMY was identified in dogs from subgroup B3 (r=0.66, P<0.05), subgroup A12 (r=0.73, P<0.05), and subgroup A3 (r=0.42, P<0.01), respectively, whereas such correlations were not observed in the other subgroups.

**Urine total protein concentration (U-TP).** Twenty-five percent (41/164) of the dogs in subgroups A1-A12, 45% (19/42) of the dogs in subgroups A13 and A14, and 79% (38/48) of the dogs in group B had increased U-TP levels relative to dogs in group C. The high U-TP values correlated with the high U-AMY values, but there was no correlation between these two parameters in group B dogs with U-A/C >2.9: subgroup B1 (r=0.11), subgroup B2 (r=0.06), and subgroup B3 (r=0.40), in contrast to the control groups of dogs with U-A/C <2.9, including group C (r=0.39, P<0.05), subgroup A13 (r=0.56, P<0.05) and subgroup A14 (r=0.44, P<0.05).

**Urine specific gravity (U-SG) and creatinine concentration.** The U-SG and U-Cr values in 29% (48/164) and 35% (58/164) of sick dogs from subgroups A1-A12, respectively, were lower than the lowest levels in group C (1.018 1/L and 90.0 mg/dL, respectively). In the remaining five subgroups (A13, A14, B1, B2, and B3), the mean U-SG and U-Cr values were also lower than those in group C dogs and more patients had U-SG and U-Cr values lower than minimum values in group C: subgroup A13 had 75% (12/16) and 77% (10/13), subgroup A14 had 65% (17/26) and 68% (17/25), subgroup B1 had 36% (10/28) and 61% (17/28), subgroup B2 had 88% (7/8) and 100% (8/8), and subgroup B3 had 92% (11/12) and 83% (10/12), respectively.
Table 1

U-AMY and U-A/C levels in all groups and subgroups of dogs: C (healthy), A1-A12 (sick with normal renal function), A13 (acute renal failure), A14 (chronic renal failure), B1 (sick with normal renal function), B2 (acute renal failure), and B3 (chronic renal failure)

<table>
<thead>
<tr>
<th>Group or subgroup</th>
<th>Number of tests</th>
<th>U-AMY (U/L)</th>
<th>Values of U-A/C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>max.</td>
<td>mean ±SD</td>
</tr>
<tr>
<td>C</td>
<td>34</td>
<td>0.4</td>
<td>21.0</td>
</tr>
<tr>
<td>A1</td>
<td>16</td>
<td>0.8</td>
<td>113.9</td>
</tr>
<tr>
<td>A2</td>
<td>18</td>
<td>1.2</td>
<td>112.0</td>
</tr>
<tr>
<td>A3</td>
<td>37</td>
<td>0.4</td>
<td>276.0</td>
</tr>
<tr>
<td>A4</td>
<td>11</td>
<td>0.4</td>
<td>146.5</td>
</tr>
<tr>
<td>A5</td>
<td>16</td>
<td>1.2</td>
<td>33.5</td>
</tr>
<tr>
<td>A6</td>
<td>12</td>
<td>0.0</td>
<td>42.0</td>
</tr>
<tr>
<td>A7</td>
<td>12</td>
<td>1.6</td>
<td>56.0</td>
</tr>
<tr>
<td>A8</td>
<td>9</td>
<td>0.8</td>
<td>139.0</td>
</tr>
<tr>
<td>A9</td>
<td>8</td>
<td>1.0</td>
<td>295.0</td>
</tr>
<tr>
<td>A10</td>
<td>7</td>
<td>2.8</td>
<td>346.0</td>
</tr>
<tr>
<td>A11</td>
<td>10</td>
<td>1.2</td>
<td>28.2</td>
</tr>
<tr>
<td>A12</td>
<td>8</td>
<td>1.2</td>
<td>113.5</td>
</tr>
<tr>
<td>A13</td>
<td>16</td>
<td>0.4</td>
<td>370.9</td>
</tr>
<tr>
<td>A14</td>
<td>26</td>
<td>3.0</td>
<td>242.8</td>
</tr>
<tr>
<td>B1</td>
<td>28</td>
<td>75.0</td>
<td>4,623.0</td>
</tr>
<tr>
<td>B2</td>
<td>8</td>
<td>267.5</td>
<td>3,855.0</td>
</tr>
<tr>
<td>B3</td>
<td>12</td>
<td>190.0</td>
<td>878.1</td>
</tr>
</tbody>
</table>

U-AMY – urinary α-amylase activity, U-A/C – urinary α-amylase to creatinine ratio.<sup>a</sup> P<0.05, <sup>b</sup> P<0.01, and<sup>c</sup> P<0.001 as compared with group C using Student’s t-test. <sup>d</sup> U Mann-Whitney test. <sup>e</sup>n=13, <sup>f</sup>n=25.

**Urinary α-amylase activity to creatinine concentration ratio.** Only two dogs in group C had U-A/C ratios above 0.05. Forty-five percent (91/202) of the dogs in group A had U-A/C values above 0.15, and the highest value was 17-fold higher than the maximum U-A/C value in group C. In contrast, among the dogs in group B, the minimum U-A/C value was 19-fold above the maximum value in group C (Table 1).

**Histopathology.** Of the 48 dogs with U/A-C >2.9 (group B), twelve were euthanised and pancreatic histology was available from seven of them. Pancreatitis was confirmed in all the cases: AP in one dog with clinically recognised disease (U-A/C was 9.16) and CP in six remaining dogs suffering from different diseases, including splenic tumour (one case), liver diseases (two cases), acute renal failure (two cases), and chronic renal failure (two cases).

**Discussion**

The results of the presented study contradict the generally accepted opinion regarding U-AMY in dogs.
In the studied population, α-amylase activity was detected in all of the urine samples tested. In spite of the fact that information on canine amylasuria is limited, our data have confirmed some previous reports of high α-amylase activity in urine from dogs with pyometra, proteinuria, and renal failure (5, 6, 12). Corazza et al. (5) reported that dogs with renal insufficiency had significantly higher U-AMY levels than those with proteinuria and normal renal function, which correlates with our results (Table 1). Moreover, in our study, the dog with histopathologically confirmed AP had the U-A/C above 9.0. This finding is in agreement with the results of Turgut et al. (24).

Statistical analyses of the results of urine and blood data from the 17 sick dog subgroups showed that only three subgroups of animals with U-A/C >2.9 (B1, B2, and B3) comprising group B differed from the control group C in the same way, based not only on U-A/C values (Table 1), but also on results of serum α-amylase and four urine parameters. Furthermore, group B was the only group that included 10 dogs with pancreatitis confirmed by ultrasonography (including two dogs with clinically recognised AP) and other seven dogs with pancreatitis confirmed by histopathology (including two next dogs with clinically recognised AP). Thus, the distinct characteristic of group B could not be due to a simple chance. Histopathological examination of the pancreas obtained from dogs with U-A/C >2.9 might suggest that animals with a number of different diseases and without overt clinical signs of AP could have coexisting asymptomatic AP or recurrent AP during the course of CP. This is in agreement with previous studies, which demonstrated that pancreatitis is a common and under-diagnosed condition that occurs during the course of many non-pancreatic diseases (4, 9, 17, 18, 23, 25).

Despite the rigorously assumed selection criteria chosen for the purpose of the present study for dogs with normal renal function (subgroups A1-A12 and subgroup B1), we cannot exclude the possibility that in certain cases, an early phase of renal failure might have been occurring, especially in some of the older dogs. It is noteworthy, however, that all of the patients with normal renal function had serum creatinine and urea concentrations within the normal range; only 11 out of 180 sick dogs with normal renal function had hyperamylasemia and a correlation between α-amylase activity in serum and urine was found only in one subgroup of dogs with liver disease (subgroup A3). Thus, amylasuria in many dogs with normal renal function, especially among the dogs of subgroup B1 (Table 1), could not be considered solely as an indication of tubular damage or underlying renal insufficiency associated with a subsequent decrease in the glomerular filtration rate, or as an effect of hyperamylasemia (2, 8, 20). Previous studies have described amylasuria in sick dogs with normal renal function (5, 6, 12, 24), and our findings are in agreement with most of these reports. Finally, our results regarding the U-TP levels in subgroup B1 dogs were similar to those of Hess et al. (9), who found proteinuria in 78% (32/41) of dogs with AP.

In the presented study, U-SG and U-Crn levels in the sick dogs with normal renal function tended to decrease in relation to control group C. However, it is worth noting that all other renal and other-than-renal reasons for decreased urine concentrating ability (e.g., diabetes insipidus, prostatic abscess, severe hepatic diseases) were ruled out. Information about these two parameters (U-SG and U-Crn) in dogs with naturally occurring diseases other than renal failure is limited, but our data are in agreement with the results of relevant previous studies (7-9, 17). Nevertheless, it cannot be excluded that in a few dogs, transitional changes in the water balance/electrolyte equilibrium were taking place during the course of their various diseases, causing a lowered U-SG and, in effect, lower U-Crn.

The results of this study seem to be clinically relevant, because they show a simple, readily available, inexpensive, non-invasive test that might enable a diagnosis of pancreatitis when patients are asymptomatic and have other concurrent diseases. The first morning urine sample is appropriate for analysing protein and creatinine levels (19). Age, sex, and body weight do not affect the levels of creatinine excretion in dogs (1, 7) and in the presented study these three factors do not affect the levels of U-AMY and U-A/C. Akuzawa et al. (1) reported previously that they were unable to detect a significant difference between intact male and female dogs with regard to urinary α-amylase.

Our knowledge of the natural course of pancreatitis in dogs remains limited. Until now, the underlying mechanism associated with α-amylase in serum and urine have remained unknown, since we do not yet understand how renal function influences serum α-amylase activity (11, 13). Much of our current knowledge derives from existing experimental models because of the limitations associated with clinical studies (e.g. limited access to clinical material, such as pancreatic tissue from pancreatitis patients). Recently, commonly used animal models of pancreatitis were evaluated critically, and none of the existing models are completely satisfactory at the present time (16, 22).

Taken together, the results of the presented study can suggest that a U-A/C ratio above 2.9 in the first morning urine sample could be considered a useful tool for the diagnosis of pancreatitis in dogs (excluding late/end stage of chronic pancreatitis), regardless of coexisting diseases and including renal failure.

Acknowledgments: This study was supported by a grant from the State Committee for Scientific Research (No. 2P06 K 029 29).

References


482