Measurement of urinary 11-dehydro-thromboxane B₂ excretion in dogs with gastric dilatation-volvulus

Wendy I. Baltzer, DVM, PhD; Maureen A. McMichael, DVM; Craig G. Ruaux, BVSc, PhD; Laura Noaker, DVM; Jörg M. Steiner, Dr med vet, PhD; David A. Williams, VetMB, PhD

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dehydrogenase enzyme, urinary concentrations of 11-dTXB₂ are considered a more accurate indicator of in vivo TXA₂ production than either TXB₂ in plasma (which can be generated by platelets during blood collection) or TXB₂ in urine (which can be generated by the kidneys and then excreted without metabolism). 11-Dehydro-thromboxane B₂ production peaks within 5 minutes and decreases by half by 60 minutes following IV infusion of TXB₂. 11-Dehydro-thromboxane B₂ is thus considered a useful indicator of TXA₂ production in vivo in dogs. 

The purposes of the study reported here were to compare the concentrations of 11-dTXB₂ (a stable metabolite of TXA₂) in the urine of healthy nonsurgical control dogs, surgical control dogs undergoing OHE, and dogs undergoing surgical correction of naturally occurring GDV and to validate a commercial immunoassay kit for measuring 11-dTXB₂ in canine urine samples. We hypothesized that dogs with naturally occurring GDV would have elevated urinary concentrations of 11-dTXB₂. We further hypothesized that increasing urinary 11-dTXB₂ excretion in dogs following corrective surgery for GDV would indicate an increased probability of postoperative complications. In addition, validation of the commercial immunoassay kit for measuring 11-dTXB₂ in canine urine samples will aid in further investigation of thromboxane metabolism in disease states in dogs.

Materials and Methods

Animals and urine sample collection—This study was conducted in a manner consistent with the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. Urine samples were obtained by free catch, manual expression of the urinary bladder under anesthesia, or urinary bladder catheterization as appropriate from 15 healthy nonsurgical control dogs, 12 healthy dogs undergoing OHE (ie, surgical control dogs), and 32 dogs with GDV (ie, GDV dogs) that subsequently underwent surgical derotation of the stomach and exploratory laparotomy with gastropexy.

Urine samples from nonsurgical control dogs were obtained by free catch. Nonsurgical control dogs were healthy dogs that were volunteered by students and faculty of the Veterinary Teaching Hospital at the College of Veterinary Medicine and Biomedical Sciences, Texas A&M University. Urine samples from dogs undergoing OHE and those with GDV were obtained prior to commencement of surgical procedures and approximately 1 hour after surgery.

Urine samples from GDV dogs were obtained at the time of hospital admission, prior to initiation of any gastric decompression or fluid therapy. These patients had not received previous gastric decompression or fluid therapy before hospital admission. Urine samples were obtained 1 hour after the stomach was manually derotated by the surgeon, although time between preoperative sample collection and postoperative sample collection varied among affected dogs depending on how much time elapsed before the stomach was derotated.

Preoperative urine samples from dogs undergoing OHE were obtained by manual expression of the bladder following induction of anesthesia, whereas postoperative urine samples were obtained by free catch when the dogs were walked following recovery from anesthesia. In this study, urine samples from dogs that underwent OHE were unused portions obtained by free catch or expression of the bladder that had been collected in accordance with the standard of care for the practices. The time between collection of preoperative and postoperative urine samples for dogs that underwent OHE varied depending on how long it took the dog to recover from anesthesia (on average between 1 to 2 hours).

Preoperative urine samples from GDV dogs were obtained by either cystocentesis or direct catheterization of the urinary bladder as part of the routine workup. Postoperative urine samples were obtained from indwelling urinary collection systems placed as part of the routine postoperative management. Samples contaminated with blood were excluded from the study to avoid contamination with 11-dTXB₂ produced by inflamed urinary tract tissue. 

Urine samples collected by use of indwelling catheters have been previously used for determination of 11-dTXB₂ in experimental dogs, with no apparent interference from TXB₂ originating in the urinary tract. For GDV dogs, the collection of urine samples was part of the standard of care for each practice. Urine samples from GDV dogs were obtained from patients admitted to the teaching hospital at the College of Veterinary Medicine and Biomedical Sciences at Texas A&M University and also at a local private emergency clinic.

Urine samples obtained at the local private emergency clinic were stored and shipped overnight at 4°C to Texas A&M University. At Texas A&M University, all urine samples were immediately frozen and stored at –80°C until analyzed. Thromboxane B₂ is stable at –20° to –80°C, and overnight shipping on ice does not cause significant changes in concentrations. Other authors have reported that storage of urine at room temperature (approx 25°C) to 4°C for 12 to 24 hours, before being frozen at either –20° or –80°C, leads to no significant changes in 11-dTXB₂ concentrations.

Anesthetic protocol—All dogs that underwent OHE were anesthetized with tiletamine-zolazepam (10 mg/kg, IM) with atropine (0.04 mg/kg, IM), maintained on isoflurane inhalant, and received butorphanol (0.2 mg/kg, IM) for postoperative pain management. The induction method and postoperative pain management used for GDV dogs varied at the discretion of the clinician; however, all dogs were maintained on isoflurane inhalant anesthesia.

Outcome data and complications—Outcome data were recorded for all GDV dogs. Outcome data included perioperative mortality (defined as death within 24 hours of surgery); tissue resection (partial gastrectomy, splenectomy, or both); and the occurrence of postoperative complications including gastric necrosis, cardiac arrhythmias, and death. Dogs were defined as having complications related to GDV if any tissue resection was required or if one of the following conditions was recorded: gastric necrosis, cardiac arrhythmias, or death. On the basis of these categories, GDV dogs were assigned to 2 groups: those with no complications (n = 23) and those with complications (n = 9).

Analytic methods—Urinary concentrations of 11-dTXB₂ were determined by use of a commercial competitive enzyme immunoassay kit and reported as picograms per milliliter of urine. Samples were analyzed in duplicate and batched to reduce interassay variation. This assay has undergone extensive analytic validation by the manufacturers and has no substantial cross-reactivity with TXA₂, TXB₂, or related metabolites, with only 0.09% cross-reactivity with prostaglandin D₂, 0.05% cross-reactivity with TXB₂, and < 0.01% cross-reactivity with 2,3-dinor-TXB₂.

Because 11-dTXB₂ is not a species-specific compound, our in-house validation of this assay was aimed at confirming its usefulness and providing optimization for use of the assay with canine urine samples. In-house validation of the assay included assessment of dilutional parallelism of canine urine samples (ie, 5 samples were used at 3 dilutions for determin-
nation of the correct sample dilution to be within the working range of the assay), assessment of the correlation between direct analysis of canine urine samples and analysis following solid-phase extraction of 11-dTxB₂ from canine urine (ie, 9 samples were used to assess the presence of cross-reaction substances in canine urine), and assessment of interassay variation (ie, 3 samples were used on 3 separate runs).

Urinary Cr concentrations were determined for all samples at an external laboratory⁷ by use of an automated dry chemistry system⁸ and are reported as milligrams per deciliter of urine. To account for individual variations in glomerular filtration rate and urinary concentrating capacity, urinary 11-dTxB₂ concentrations were normalized to the urinary Cr concentration by calculation of the 11-dTxB₂-to-Cr ratio, as previously described.⁹

Statistical analysis—Analyses were performed with a commercial software package.⁴ Data sets were tested for consistency with a Gaussian distribution by use of the D'Agostino-Pearson omnibus normality test. Several data sets were not normally distributed; thus, nonparametric analytic methods were used for all analyses of 11-dTxB₂-to-Cr ratios. Differences in ratios among urine samples from nonsurgical control dogs, surgical control dogs, and GDV dogs were analyzed before and after surgery by use of the Kruskal-Wallis test, followed by the Dunn multiple comparison post hoc test. Wilcoxon matched pairs tests were used to compare preoperative and postoperative 11-dTxB₂-to-Cr ratios between GDV dogs with and without complications. Ages of dogs within all groups were compared by use of a 1-way ANOVA, whereas ages of GDV dogs with and without complications were compared by use of a 2-tailed Student t test. The use of preoperative and postoperative urine samples from GDV dogs to predict the need for tissue resection or occurrence of postoperative complications was analyzed by use of ROC analysis.

For all analyses, the null hypothesis was that no significant differences existed among groups. The null hypothesis was rejected and significance assigned with values of P < 0.05.

Results

Study group characteristics—Thirty-two GDV dogs were studied. Dogs with GDV included 5 sexually intact females, 12 spayed females, 6 sexually intact males, and 9 neutered males. Breeds recorded for GDV dogs included German Shepherd Dog (n = 4), Great Dane (3), Springer Spaniel (3), mixed breed (3), Standard Poodle (3), Boxer (2), Chow Chow (2), Dalmatian (2), Golden Retriever (2), and Irish Setter (2). Also included in GDV dogs were 1 representative each of Akita, Chesapeake Bay Retriever, Doberman Pinscher, Gordon Setter, Labrador Retriever, and Saint Bernard. The breeds of dogs that underwent OHE were as follows: Labrador Retriever (n = 3), Dachshund (2), mixed breed (2), Boxer (1), Australian Shepherd (1), and 3 dogs of unknown breed.

The mean ± SD age of the healthy nonsurgical control dogs was 6.3 ± 3.5 years. The mean age of dogs that underwent OHE was 2.5 ± 3.8 years, whereas the mean age of GDV dogs was 7.1 ± 3.1 years. Dogs that underwent OHE were significantly younger than the healthy nonsurgical control dogs and GDV dogs.

No dogs that underwent OHE had intra- or postoperative complications, whereas 9 of the 32 (28.1%) GDV dogs required either splenic or gastric resection or had postoperative complications including perioperative mortality. Two dogs died within 36 hours of surgery; one of these dogs had undergone partial gastrectomy for gastric necrosis at the time of surgery, and the other was not necropsied. The mean age of GDV dogs that had postoperative complications or tissue resection during surgery was 9.2 ± 2.9 years, which was significantly (P = 0.018) older than those that did not have complications or tissue resection (6.6 ± 2.9 years).

Analytic validation—Values reported from the assay of 9 canine urine samples that were assayed in parallel following either solid-phase extraction of 11-dTxB₂, as described in the literature accompanying the kit, or direct dilution with the ELISA buffer had a strong correlation (Pearson correlation coefficient, 0.9937; P < 0.001). Analytic validation of this assay and comparison of this assay to the gold standard gas chromatography–mass spectroscopy method of analysis are outlined in Lellouche et al.¹⁰ Results of the study of 5 samples, diluted at 1:4, 1:8, and 1:16 in the ELISA buffer after analysis, revealed good dilutional parallelism with a mean observed-to-expected ratio of 94.01% (range, 133.9% to 65.9%; SD, 24.21%). An observed-to-expected ratio of 65.9% was obtained from a sample at 1:16 dilution; this sample was at the lower limits of the standard curve of the assay, and this represents a dilution greater than that eventually used in the urine samples from clinically affected dogs.

Interassay variation of 5 samples run on 5 separate occasions was good, with a mean interassay coefficient of variation of 14.38%. For analysis of 11-dTxB₂, interassay coefficients of variation of 12.1% to 20% have been previously reported.¹¹⁻¹³

All subsequent analyses were performed by use of urine samples diluted 1:4 in ELISA buffer. Samples with values outside the linear region of the standard curve were reanalyzed after adjustment of the urine dilution to yield accurate results.

Urinary 11-dTxB₂-to-Cr ratios—The urinary 11-dTxB₂-to-Cr ratio was significantly higher in GDV dogs before and after surgery than in healthy nonsurgical control dogs (median [range], 22.95 [7.0 to 715.5] before surgery and 33.0 [10.3 to 1,682.6] after surgery vs 9.83 [1.7 to 20.7], respectively). The urinary 11-dTxB₂-to-Cr ratios of dogs that underwent OHE were not significantly different from healthy nonsurgical control dogs at either time point, and no apparent increase in urinary 11-dTxB₂-to-Cr ratio was found following OHE (median [range], 15.05 [1.2 to 63.4] before surgery and 15.55 [3.4 to 80.30] after surgery vs 9.83 [1.7 to 20.7], respectively). No significant difference in urinary 11-dTxB₂-to-Cr ratios was found between preoperative urine samples from surgical control dogs and preoperative urine samples from GDV dogs (median [range], 15.05 [1.2 to 63.4] vs 22.95 [7.0 to 715.5], respectively); however, median urinary 11-dTxB₂-to-Cr ratio was significantly higher in postoperative urine samples from GDV dogs than in postoperative urine samples from surgical control dogs (median [range], 33.0 [10.3 to 1,682.6] vs 15.55 [3.4 to 80.3], respectively).

In GDV dogs that had intra- or postoperative complications or perioperative mortality, the median urinary 11-dTxB₂-to-Cr ratio was significantly higher than in those GDV dogs that did not have complica-
complications significantly (P < 0.05) better than chance. In this same group of dogs, however, ROC curve analysis indicated that postoperative urinary 11-dTXB2-to-Cr ratios were significantly better at predicting the occurrence of complications than chance, whereas preoperative values were no better than chance at predicting complications. The areas under the ROC curves are 0.67 (95% confidence interval, 0.47 to 0.86) and 0.75 (95% confidence interval, 0.58 to 0.92) for preoperative and postoperative measurements, respectively (Figure 1).

Discussion

Naturally occurring GDV in dogs is an emergency, occurring without obvious premonitory signs and requiring immediate medical and surgical intervention. Our study involved the use of urine samples obtained from dogs with naturally occurring GDV, and as a consequence, several areas exist where variability in the data from our study group may be greater than optimal.

Because GDV was not induced in a controlled manner in the patients used for our study, it is possible that other medications, such as nonsteroidal anti-inflammatory drugs or glucocorticoids, may have been administered to some dogs before they developed GDV. If present, nonsteroidal anti-inflammatory drugs or glucocorticoids at therapeutic concentrations may have reduced thromboxane synthesis and reduced the excretion of 11-dTXB2 in some dogs. As full medical histories were not available for all dogs in the GDV group, histories of exposure to these medications cannot be ruled out.

In an attempt to control for the effect of surgery on changes in thromboxane metabolism, dogs undergoing OHE were also used. A significant age difference existed between dogs that underwent OHE and GDV dogs in our study. The influence, if any, of increasing age on thromboxane metabolism in dogs is unknown.

The use of dogs with experimentally induced GDV typically requires invasive procedures, the number of dogs is often limited by expense, and ethical treatment of experimental dogs typically requires euthanasia as an endpoint of these studies, which makes detection of changes that are associated with increased risk of peri- and postoperative mortality less likely. For these reasons, our study was performed with dogs that had naturally occurring GDV in an attempt to ascertain whether any apparent relationship exists between thromboxane metabolism and clinical outcome. More stringently controlled experimental studies will be necessary to truly isolate the effect of thromboxane metabolism from other confounding variables. Increased variability resulting from these mentioned factors is likely to have reduced the power of our study in the detection of significant differences among groups; thus, conservative interpretation of our findings is appropriate.

Taken together, the results from dilutional parallelism and interassay variation studies demonstrate that the commercial immunoassay kit used for our study is stable and appropriate for the measurement of 11-dTXB2 in canine urine samples. The strong correlation between results obtained after solid-phase purification of 11-dTXB2 from canine urine and the values obtained by direct dilution of the urine samples in ELISA buffer indicates that no apparent substances are present in canine urine samples that cross-react with the anti–11-dTXB2 antibodies used in this kit, and thus, solid-phase purification of 11-dTXB2 from canine urine is not necessary before analysis with this kit.

In an ROC curve, the sensitivity and 1 – specificity of a diagnostic test are plotted at various cutoff values, ranging from extremely specific and insensitive cutoff values at the origin of the curve to entirely inclusive nonspecific cutoff values at the end of the curve. A diagnostic test that is completely unable to detect or predict the diagnosis of interest will have a straight-line curve at an angle of 45° to the x-axis, and the area under the curve of this test will equal 0.5 (the possible range of values for area under the curve being 0.5 to 1.0, with 1.0 representing a perfect diagnostic test).32 From the confidence interval for the calculated area under the curve, the null hypothesis that a test is no better than chance at making a diagnosis or predicting an outcome can be tested.

In our study, results of ROC analysis indicated that postoperative urinary thromboxane excretion was significantly better at predicting postoperative complications than chance, whereas preoperative values were no better than chance at predicting complications. The observation that measurement of postoperative 11-dTXB2 excretion was apparently able to predict postoperative complications, whereas measurement of preoper-
ative urine samples was unable to predict complications, further suggests that the occurrence of complications following surgery for GDV is related to postoperative reperfusion injury rather than the extent of preoperative compromise of the gastrointestinal tract.

Age, sex, and breeds of GDV dogs in our study were similar to those in previous reports. The incidence of death associated with GDV has previously been reported to be as high as 40% to 60%. In a recent retrospective study, a mortality rate of 18% was reported from multiple institutions. In our study, 2 of 32 (6.25%) GDV dogs died or were euthanized within 36 hours of surgical derotation of the stomach. Although these studies are not directly comparable, the overall occurrence of complications in dogs of our study (9/32; 28%) is similar to that observed in the retrospective study reported by Brourman et al.

In GDV dogs of our study, urinary excretion of thromboxane metabolites was significantly higher in presurgical samples than in urine samples from healthy nonsurgical control dogs and in postoperative urine samples from dogs undergoing OHE. The potential sources of the urinary 11-dTxB2 detected in preoperative urine samples from GDV dogs are manifold. Increased urinary excretion of thromboxane metabolites in preoperative urine samples from GDV dogs may reflect a state of increased platelet activation, ischemia of the gastrointestinal tract, or both. Ovariohysterectomy was chosen as a surgical control procedure because this is an invasive surgical procedure; yet, it is routinely performed in healthy dogs. No significant difference in urinary measurements were found between surgical control dogs and the healthy nonsurgical control dogs at either pre- or postoperative urine sample collection times, and no apparent difference in urinary excretion of thromboxane metabolites was found as a result of either anesthesia or abdominal surgery in dogs that underwent OHE. In terms of overall invasiveness of OHE, it is less invasive than surgery for GDV, which includes exploratory laparotomy and gastropexy. Sham-operated control dogs undergoing exploratory laparotomy with a larger incision and longer surgical times would provide a more accurate means of evaluating the effects of anesthesia and surgery on thromboxane excretion; however, we did not view this as ethically appropriate for our initial investigation.

Gastric necrosis in GDV has been associated with a high incidence of postoperative complications and mortality and is closely associated with hemostatic abnormalities and disseminated intravascular coagulation. Dogs that had intra- or postoperative complications in our study had a significantly higher excretion of 11-dTxB2 following surgery. Dogs with GDV that had complications were significantly older than dogs with no complications (mean ± SD, 9.9 ± 2.9 years vs 6.6 ± 2.9 years). To our knowledge, no information is currently available regarding the influence of age on thromboxane synthesis and excretion in dogs, and thus, it is possible that the increased excretion of thromboxane metabolites in GDV dogs that had complications following surgery is an epiphenomenon. As experimental ischemia and reperfusion injury of the gastrointestinal tract in dogs has been shown to lead to increases in thromboxane that correlated with the severity of injury and death, we speculate that thromboxane may have been a contributing factor to postoperative complications in our study.

Intestinal production of thromboxane has been documented to increase following reperfusion in experimental dogs with intestinal ischemia, whereas suppression of thromboxane production can protect the intestines from reperfusion injury. Intestinal production of thromboxane increases during and after reperfusion following hemorrhagic shock, whereas no corresponding increase in prostacyclin production is found, which has opposing effects to thromboxane. This imbalance in favor of thromboxane during intestinal reperfusion has been postulated to be a mechanism for further cell damage. The extent of gastric and splenic ischemia that occurs prior to surgical derotation of the stomach may affect the severity of reperfusion injury that follows and thus may explain why removal of necrotic tissue at surgery does not prevent postoperative death or accurately predict postoperative complications.

Dogs with GDV underwent gastric decompression after the preoperative urine sample was obtained, when deemed appropriate by the attending clinician. None of the dogs that underwent OHE were receiving any medications prior to surgery. Although some GDV dogs may have received corticosteroids or nonsteroidal anti-inflammatory drugs prior to presentation for concurrent medical conditions, none of the dogs received any of these medications at the time of hospital admission or at any time during the urine collection period. Because GDV dogs of our study were actual clinical cases, concurrent medical conditions were considered to have a potentially important influence on the amount of 11-dTxB2 excreted and therefore were not controlled. To determine whether thromboxane production plays a role in GDV and whether further investigation is indeed warranted, clinical cases were manipulated by experimental protocols as little as possible. In addition, corticosteroids or nonsteroidal anti-inflammatory drugs would be expected to attenuate any elevations in TXB2 and would therefore act to minimize any significant difference among groups in our study.

Further studies with greater numbers of dogs are needed to determine whether inhibition of the thromboxane synthase enzyme or thromboxane receptor improves postoperative outcome in dogs with GDV. In our study, preoperative urinary 11-dTxB2 concentrations were elevated and postoperative urinary 11-dTxB2 (as early as 1 hour following surgical derotation of the stomach) concentrations had some relationship with the incidence of complications, including tissue necrosis and death. Only 2 dogs died following surgery in our study, and both of these dogs had a large increase in urinary excretion of 11-dTxB2 after surgical derotation of the stomach. Measurement of urinary thromboxane metabolites following surgery may be an additional tool in the future to help predict the development of postoperative complications.
References