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Evaluation of serum lidocaine/monoethylglycylxylidide concentration to assess shunt closure in dogs with extrahepatic portosystemic shunts

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Abstract

Background: Liver function tests do not always normalize despite successful attenuation of extrahepatic portosystemic shunts (EHPSS).

Objectives: Assess the lidocaine/monoethylglycylxylidide (MEGX) test to determine liver perfusion after EHPSS closure.

Animals: Twenty dogs with EHPSS.

Methods: A prospective cohort study was performed and all dogs were tested at diagnosis, 1, 3, and 6 months postoperatively. After collecting a baseline blood sample (T0), 1 mg/kg body weight of lidocaine was injected intravenously. Fifteen (T15) and 30 minutes (T30) later, blood was collected. Plasma concentrations of lidocaine and its metabolites MEGX and glycylxylidide (GX) were determined, using a high-performance liquid chromatography with electrospray ionization tandem mass spectrometry method. Three months postoperatively, transsplenic portal scintigraphy was performed to determine EHPSS closure.

Results: At T15, median MEGX concentrations were higher in dogs with closed EHPSS compared to diagnosis (33.73 ng/mL [21.11-66.44 ng/mL] vs 13.74 ng/mL [7.25-21.93 ng/mL]; $P < .001$), but were not different (12.28 ng/mL [10.62-23.17 ng/mL] vs 13.74 ng/mL [7.25-21.93 ng/mL]) in dogs with persistent shunting. Sensitivity to determine shunt closure for MEGX at T15 was 96.2% (95% confidence interval [CI]: 78.4-99.8) and specificity 82.8% (95% CI: 63.5-93.5).

Conclusions and Clinical Importance: The lidocaine/MEGX test is a promising, rapid, and noninvasive blood test that seems helpful to differentiate dogs with closed EHPSS and dogs with persistent shunting after gradual attenuation.

KEYWORDS
blood test, canine, liver dysfunction, vascular anomaly

Abbreviations: AUROC, area under the curve of receiver operating characteristics; CI, confidence interval; CTA, computed tomography angiography; EHPSS, extrahepatic portosystemic shunt; GX, glycylxylidide; LOD, limit of detection; LOQ, limit of quantification; MAPSS, multiple acquired portosystemic shunts; MEGX, monoethylglycylxylidide; PSS, portosystemic shunt; ROC, receiver operating characteristics; T0, blood sample at time zero (baseline); T15, blood sample 15 minutes after lidocaine injection; T30, blood sample 30 minutes after lidocaine injection; TSPS, transsplenic portal scintigraphy.

1 | INTRODUCTION

Portosystemic shunts (PSS), in which an aberrant vessel causes blood to bypass the liver, are the most common congenital vascular anomalies affecting the liver in small animals.[1] Surgical attenuation is regarded as the best treatment method, but it is unclear which surgical treatment provides the best long-term outcome.[2,3] Gradual attenuation of extrahepatic portosystemic shunts (EHPSS) is commonly performed to allow the liver to gradually adapt to the increased blood flow, consequently reducing the risk of developing multiple acquired portosystemic shunts (MAPSS).[4,5] Medical imaging techniques such as Doppler ultrasonography, computed tomography angiography (CTA), portovenography, or scintigraphy can be used to determine EHPSS closure.[6-10] All these imaging techniques require specialized equipment, sedation, or anesthesia and well-trained people to obtain and interpret the images.[1] Furthermore, normalization of liver function through increased liver perfusion rather than shunt occlusion is the goal of treatment. Commonly used liver function tests, such as measurement of blood ammonia concentration after feed withholding, ammonia tolerance test, and serum bile acids give false-negative as well as false-positive results after surgery.[11,12] The measurement of protein C activity is a potentially useful test in dogs after surgical attenuation of PSS,[13,14] yet, further research is warranted.

Lidocaine is a commonly used analgesic drug in small animals, which is metabolized in the liver by cytochrome P450 to monoethylglycylxylidide (MEGX) and subsequently to glycylxylidide (GX).[15] In human medicine, the hepatic metabolism of lidocaine is used as a dynamic liver function test.[16-19] As MEGX accurately reflects the severity of hepatic dysfunction, it is not only a valuable test for quantitative evaluation of the liver dysfunction but it is also used as a prognostic predictor of liver diseases in adults.[20] Advantages of the use of lidocaine are its short elimination half-life and its high hepatic extraction ratio, that is blood flow dependent,[17] making it an attractive candidate for assessment of liver function in dogs with EHPSS. The lidocaine/MEGX test has already been evaluated in healthy dogs and lidocaine and its metabolites are well tolerated and measurable in this species.[21] This test seems to be promising to indicate hepatic function in small numbers of dogs undergoing partial hepatectomy and subsequent autotransplantation of liver tissue,[22] dogs with experimentally induced liver fibrosis,[23] and dogs that underwent ligation of a portal branche and portocaval anastomosis.[24] Although dosages of up to 10 mg/kg can be safely used in healthy dogs,[25] the tolerated dose might be far lower in dogs with portosystemic shunting. Experimental studies showed that doses of 1 to 1.5 mg/kg of lidocaine are well tolerated in dogs with hepatic dysfunction.[22-24]

The objectives of the current study were to evaluate the potential of the lidocaine/MEGX test to determine liver perfusion, and thus indirectly shunt closure, in dogs with congenital EHPSS after gradual shunt attenuation.

2 | MATERIALS AND METHODS

2.1 | Animals

Twenty client-owned dogs with congenital EHPSS were prospectively enrolled in the study. The study was approved by the ethical (EC2014-179) and deontological committee (2015N03), and all owners signed an informed consent prior to inclusion of their dog.

In all dogs, a routine blood examination, including measurement of blood ammonia and serum bile acid concentrations after feed withholding, was performed at diagnosis. One or more medical imaging techniques (B-mode and Doppler ultrasonography, CTA, or transsplenic portal scintigraphy [TSPS]) were performed to diagnose the EHPSS. Dogs were medically supported for minimally 4 weeks prior to surgery with a combination of a liver diet, lactulose, and metronidazole. Subsequently, all dogs underwent gradual attenuation of the EHPSS by either an ameroid constrictor or thin film banding, depending on the preference of the primary surgeon. The gradual attenuating device was placed as close as possible to the exit of the EHPSS in the systemic circulation; at the level of the omental foramen in dogs with a portocaval shunt, at the level of the peritoneal leaf of the diaphragm close to the exit of the left hepatic vein in dogs with a portophrenic shunt, and intrathoracically in dogs with a portoazygos shunt through a transdiaphragmatic approach as described previously.[26] Medical therapy was continued until the first follow-up visit 1 month postoperatively. Based on the clinical signs, physical examination findings, and results of blood analyses, it was decided whether administration of lactulose, metronidazole, or both lactulose and metronidazole was continued. All dogs received diet designed to support dogs with liver dysfunction until the second follow-up visit, 3 months postoperatively, during which a TSPS was performed to determine EHPSS closure. Shunt fractions of <4.3% were considered normal.[27] Dogs with a closed EHPSS and dogs that developed MAPSS had another follow-up visit 6 months postoperatively. Dogs with persistent shunting indicative of a patent EHPSS were offered a second surgery to completely close the EHPSS. In case TSPS was inconclusive, CTA was offered. Owners of dogs with a closed EHPSS were advised to gradually change to a standard diet. Dogs with persistent shunting that did not undergo a second surgery were advised to keep the dog life-long on a diet designed to support dogs with liver dysfunction alone or in combination with lactulose, metronidazole, or both lactulose and metronidazole depending on the clinical signs and the preference of the attending veterinarian. Dogs that underwent a second surgery had the same follow-up visits as after the first surgery.

2.2 | Lidocaine/MEGX test

The lidocaine/MEGX test was performed in all dogs at diagnosis and at 1, 3, and 6 months postoperatively. Owners were instructed to fast their dog for 12 hours prior to testing. A baseline blood sample (T0) was collected from the jugular vein, and subsequently a peripheral

venous catheter was placed to administer 1 mg/kg body weight of lidocaine (Xylocaine 2%, Aspen Pharma Trading Limited, Dublin, Ireland) followed by 1 mL of sterile saline. Additional venous blood samples were taken from the jugular vein 15 (T15) and 30 minutes (T30) after administration of lidocaine. All blood samples were collected in an EDTA tube and, after centrifugation at 3500*g* during 5 minutes, plasma was stored at −80°C until analysis in batch at the end of the study. Lidocaine, MEGX, and GX concentrations were measured using high-performance liquid chromatography with electrospray ionization tandem mass spectrometry as published earlier.[28] A sample of 100 μL plasma was used for analysis. Limits of quantification (LOQ) of lidocaine, MEGX, and GX were .25, .50, and 2.5 ng/mL, respectively, and limits of detection (LOD) were .08, .18, and .23 ng/mL, respectively.

2.3 | Statistical analyses

Statistical analyses were performed using SPSS Statistics 26 (IBM, Armonk, New York). Mann-Whitney *U* tests were used to evaluate differences between dogs with closed and persistent shunting between different metabolites (lidocaine, MEGX, and GX) at different sampling points (T15 and T30) and between different time points (diagnosis, 1, 3, and 6 months postoperatively). Kruskal-Wallis tests were performed separately in dogs with closed EHPSS and dogs with persistent shunting to evaluate the median concentrations of different metabolites at different sampling points over time. Multiple comparison tests with Bonferroni correction were performed in case statistical differences were present. Friedman 2-way analyses with Bonferroni correction were performed to assess the concentrations of different metabolites over time in dogs with closed EHPSS and dogs with persistent shunting. For dogs with closed EHPSS after the first surgery, all different time points were assessed, whereas for dogs that had persistent shunting after the first surgery, only data at diagnosis, 1, and 3 months were assessed.

A receiver operating characteristics (ROC) curve was plotted using values of EHPSS at diagnosis, 3, and 6 months postoperatively. The area under the curve of ROC (AUROC) was calculated with 90% confidence interval (CI) and optimal cutoff values were determined. Sensitivity and specificity to determine shunt closure postoperatively were calculated using 2 × 2 contingency tables and 95% CI were calculated.

3 | RESULTS

3.1 | Study samples

Twenty dogs with EHPSS were enrolled. Several breeds were represented (see Table S1). At diagnosis, median age was 7.5 months (range, 2-74 months) and median body weight was 3.8 kg (range, 1.5-8.4 kg). All information on individual age, body weight, breed, and sex are mentioned in Table S1. Surgical attenuation was performed using an ameroid constrictor in 15 dogs and thin film banding in 5 dogs. After the first surgery, 70% of dogs (n = 14) had a closed EHPSS, whereas 15% (n = 3) developed MAPSS. In all dogs in which MAPSS developed, surgical attenuation was performed using thin film banding. Two dogs had a persistent EHPSS: 1 after ameroid constrictor placement and the other 1 after thin film banding. Both dogs underwent a second surgery during which total ligation was performed, resulting in complete shunt closure and leading to a total closure ratio of 84% (16/19). In 1 dog in which an ameroid constrictor was placed, TSPS was inconclusive because of a poor-quality scan due to unclear reason. The owner of this dog refused further imaging as the dog was clinically doing very well; only data at diagnosis of this dog were used in statistical analysis. Postoperatively, median shunt fraction of dogs with closed EHPSS was 2.5% (1.0%-4.2%) and the median shunt fraction of dogs with persistent shunting was 86.5% (80.6%-96.0%). Not all dogs were represented for all follow-up visits. One dog with MAPSS was not represented for the 6-month follow-up visit and 1 dog with a closed EHPPS was not represented 3 months postoperatively and exceptionally had TSPS at the 6-month follow-up visit.

3.2 | Lidocaine/MEGX test

At T0, lidocaine and its metabolites were not detectable or <LOD. Based on median concentrations of different metabolites at different sampling points, the lidocaine/MEGX test could not differentiate at diagnosis nor at 1 month postoperatively between dogs that were going to have persistent shunting after surgery and dogs in which the EHPSS was going to be closed (Figure 1). At 3 and 6 months postoperatively, to the contrary, significant differences were present between median concentrations of different metabolites at different sampling points (T15 and T30) between dogs with closed EHPSS and dogs with persistent shunting (Figure 1). Friedman tests with post hoc analysis in dogs with closed EHPSS showed differences between diagnosis on the 1 hand and 1, 3, and 6 months postoperatively on the other hand, respectively, for concentrations of lidocaine at T30 (n = 11; P = .001, P = .001, and P = .001, respectively), MEGX at T15 (n = 10; P = .006, P < .001, and P = .006, respectively) GX at T15 (n = 10; P = .001, P < .001, and P = .001, respectively) and GX at T30 (n = 11; P = .005, P < .001, and P < .001, respectively). For MEGX at T30 significant differences were present between diagnosis and 3 and 6 months postoperatively (n = 11; P = .005 and p.048, respectively). In dogs with persistent EHPSS, no significant differences over time were present (n = 5 at T15 and n = 4 at T30).

For each metabolite at each sampling point, a ROC curve was plotted and 2 cutoff values were retained, 1 with optimal combination of sensitivity and specificity, and a second 1 with 100% sensitivity and the highest specificity possible (Table 1). For MEGX at T15, 100% sensitivity could not be reached.

3.3 | Adverse effects and missing data

No observable adverse effects occurred after administration of lidocaine. In 1 dog with an EHPSS that was closed after surgery, no

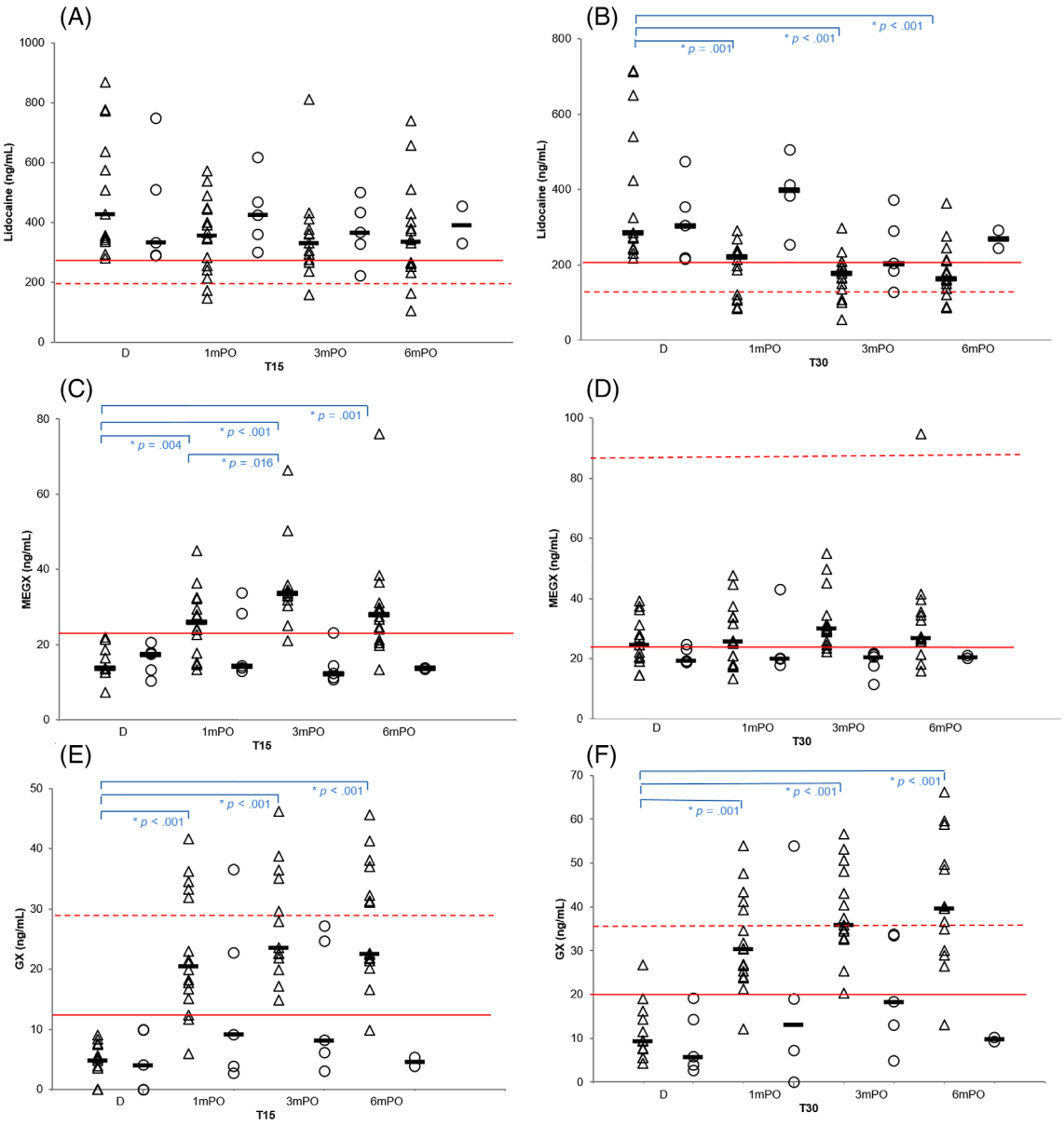

FIGURE 1 Concentrations of different metabolites at different sampling points (T15: 15 minutes after administration of lidocaine; T30: 30 minutes after administration of lidocaine) and at different time points (D: diagnosis; 1mPO: 1 month postoperatively; 3mPO: 3 months postoperatively; 6mPO: 6 months postoperatively) in dogs with extrahepatic portosystemic shunts (EHPSS). A, Lidocaine concentrations at T15; B, lidocaine concentrations at T30; C, monoethylglycylxylidide (MEGX) concentrations at T15; D, MEGX concentrations at T30; E, glycylxylidide (GX) concentration at T15; F, GX concentration at T30. Triangles are dogs with closed EHPSS; circles are dogs with persistent shunting. The red line is the cutoff value for optimal combined sensitivity and specificity. The dotted red line represents the optimal cutoff value with 100% sensitivity

lidocaine, MEGX, nor GX was detected in any of the samples at diagnosis, so possibly lidocaine was inadvertently not administered to this dog. This dog was not included in the Friedman 2-way analysis to assess the concentrations of different metabolites over time. In 2 dogs, the sample T15 was not collected (both at 3 months postoperatively in dogs with closed EHPSS) and in 2 other dogs the sample T30 was not collected (1 at 1 month postoperatively in a dog with persistent shunting and the other at 6 months postoperatively in a dog with closed EHPSS), due to dogs not being cooperative or because of the presence of a hematoma at the jugular veins secondary to multiple venepunctures. Two dogs with a closed EHPSS feed was not withheld at the 6-month follow-up visit.

4 | DISCUSSION

Based on the results obtained in the current study, the lidocaine/MEGX test is a promising test to distinguish between dogs with a

TABLE 1 Sensitivity and specificity of the optimal cutoff value combining sensitivity and specificity (cutoff 1) and the cutoff value with 100% sensitivity and the highest specificity possible (cutoff 2) to differentiate dogs with closed extrahepatic portosystemic shunts from dogs with persistent shunting

	Cutoff 1	Cutoff 2
Lidocaine		
T15 (AUROC 0.67 (90% CI: 0.55-0.79)		
Concentration (ng/mL)	277.25	191.54
Sensitivity (%)	96.2	100.0
95% CI	(78.4-99.8)	(84.0-100.0)
Specificity (%)	37.9	10.3
95% CI	(21.3-57.6)	(2.7-28.5)
T30 (AUROC 0.88 (90% CI: 0.80-0.95)		
Concentration (ng/mL)	212.55	124.07
Sensitivity (%)	88.5	100.0
95% CI	(68.7-97.0)	(74.0-100.0)
Specificity (%)	80.0	20.0
95% CI	(60.9-91.6)	(8.4-39.1)
MEGX		
T15 (AUROC 0.92 (90% CI: 0.84-0.99)		
Concentration (ng/mL)	23.7	NA
Sensitivity (%)	96.2	NA
95% CI	(78.4-99.8)	NA
Specificity (%)	82.8	NA
95% CI	(63.5-93.5)	NA
T30 (AUROC 0.76 (90% CI: 0.65-0.87)		
Concentration (ng/mL)	25.11	87.26
Sensitivity (%)	73.1	100.0
95% CI	(51.9-87.6)	(84.0-100.0)
Specificity (%)	80.0	3.3
95% CI	(60.9-91.6)	(0.2-19.1)
GX		
T15 (AUROC .93 (90% CI: 0.87-0.99)		
Concentration (ng/mL)	12.4	27.7
Sensitivity (%)	88.5	100.0
95% CI	(68.7-97.0)	(84.0-100.0)
Specificity (%)	96.6	44.8
95% CI	(80.4-99.8)	(27.0-64.0)
T30 (AUROC 0.96 (90% CI: 0.92-0.99)		
Concentration (ng/mL)	19.8	34.1
Sensitivity (%)	84.6	100.0
95% CI	(64.3-95.0)	(84.0-100.0)
Specificity (%)	96.7	70.0
95% CI	(80.9-99.8)	(50.4-84.6)

Abbreviations: AUROC, area under de curve of receiver operating characteristics; CI, confidence interval; GX, glycylxylidide; MEGX, monoethylglycylxylidide; NA: not applicable; T15, blood sample 15 minutes after lidocaine injection; T30, blood sample 30 minutes after lidocaine injection.

closed EHPSS and those with persistent shunting after surgical attenuation. The most promising metabolite to determine EHPSS closure after surgery is MEGX determined 15 minutes after lidocaine administration.

Currently, several liver function tests are available; however, a simple, rapid, cost-effective liver test that accurately determines shunt closure postoperatively is lacking. Based on the data of this study, taking 1 blood sample 15 minutes after administration of lidocaine gives adequate information with a sensitivity of 96.2% and specificity of 82.8%. This makes it a much faster test than the serum bile acid stimulation test which takes 2 hours to complete. In addition, the latter test is a sensitive test to diagnose PSS; yet, reported sensitivity and specificity to determine shunt closure 3 to 6 months postoperatively is 85% and 74% respectively,[11] which appears inferior to the sensitivity obtained by MEGX measurement 15 minutes after lidocaine administration. Other liver function tests commonly used in the postoperative phase are measurement of blood ammonia concentration after feed withholding and ammonia tolerance test; the former having a sensitivity of 19% to 44% and specificity of 100%,[11,12] the latter a sensitivity of 89%, and a specificity of 85%.[12] Furthermore, care should be taken as the ammonia tolerance test can trigger hepatic encephalopathy in dogs with persistent shunting.[29]

Data from this study show that by determining MEGX 15 minutes after intravenous administration of lidocaine, more than 80% of the dogs with persistent shunting were correctly identified, and only 4% of dogs with closed EHPSS had false positive results postoperatively. Hence, the lidocaine/MEGX test is currently the most sensitive blood test available to diagnose successful EHPSS closure 3 months after surgery. At an individual level, the increase in MEGX and GX concentrations already reached statistical significance as early as 1 month after gradual attenuation of EHPSS in dogs with closed EHPSS (while no evolution was observed in dogs with persistent shunting). However, statistical differences between the median concentrations of metabolites in dogs with closed EHPSS and those with persistent shunting were only detectable 3 months postoperatively. Although it has been suggested that ameroid constrictors close much earlier than 3 months postoperatively,[30,31] it can be speculated that it takes longer than 1 month for cytochrome P450 in the liver to function optimally. Based on the results of this study, the lidocaine/MEGX test seems a promising adjuvant test in dogs that have normal ammonia concentrations after surgical attenuation. In dogs with increased ammonia concentrations it is advised to perform medical imaging to differentiate between dogs with a persistent EHPSS and those with MAPSS.[11] In dogs with ammonia concentrations within normal limits and high MEGX concentrations at T15, the EHPSS is likely closed, and therefore additional medical imaging to determine shunt closure might not be of added value. Lidocaine has a high hepatic extraction ratio, which is a measure for the perfusion of the liver. Metabolism of lidocaine into MEGX and GX, on the contrary, gives information about the hepatic metabolism. In an experimental study in dogs that underwent ligation of 1 of the portal branches, dogs in which a portocaval anastomosis was created and dogs in which both 1 of the portal branches was ligated and a portocaval anastomosis was created, the lidocaine/

MEGX test was performed. [24] Findings of this study suggest that MEGX reflects the function of mitochondria in the hepatic cells.[24] In dogs with a congenital EHPSS, lidocaine clearance significantly increased after surgical attenuation in dogs with closed EHPSS, whereas no significant change in lidocaine concentrations over time could be detected in dogs with persistent shunting. Parallel findings were observed for MEGX and GX, indicating that not only the liver perfusion but also the hepatic metabolism improved significantly in dogs with closed EHPSS.

The lidocaine/MEGX test is currently used in human medicine and determination can be performed in several laboratories over the world. It is, however, important to keep in mind that the LOQ of the method to analyze dog plasma is required to be much lower compared to human plasma samples. Reference values of MEGX in healthy dogs 15 minutes after lidocaine administration are 34 to 79 ng/mL, which are about half of the reference values in human medicine, who receive the same dose of lidocaine.[18,21] This is most likely due to a slower capacity for MEGX formation in dogs compared to people.[21]

In order to perform a reliable lidocaine/MEGX test, lidocaine needs to be strictly administered intravenously, as extravasation might result in falsely decreased values. As a consequence, intravenous injection via a peripheral intravenous catheter is preferred over injection through a needle. More research is needed to determine how much influence it would have if blood samples are not taken exactly 15 minutes after lidocaine administration. Furthermore, it would be interesting to investigate the added value of GX in dogs that have MEGX concentrations that are close to the cutoff value and to determine the ideal timing after surgery to perform the lidocaine/MEGX test.

Lidocaine is a cheap and widely available analgesic drug that has a wide safety margin.[32] Lidocaine in plasma is partially unbound (pharmacologically active fraction) and partially bound to plasma proteins (reservoir fraction).[15] In healthy humans, 19.9% to 38.8% is unbound, 20% is bound to albumin and the remainder is bound to acute phase protein α-1 acid glycoprotein.[33] Lidocaine has a high hepatic extraction ratio,[18] with other organs, such as the kidneys eliminating only a small fraction.[34]

For the lidocaine/MEGX test only a low dose of lidocaine (1 mg/kg) is needed and no adverse effects are anticipated. Yet, in order to ensure good test results, plasma needs to be separated and sent frozen to the laboratory. Previous studies have shown that hepatic encephalopathy in dogs with PSS is associated with an inflammatory state, in which acute phase proteins, such as C-reactive protein, are increased.[35] It is unclear if α-1 acid glycoprotein, another marker of acute inflammation, is increased in dogs with EHPSS. Similar to the situation in humans where lidocaine is bound to α-1 acid glycoprotein, the amount of α-1 acid glycoprotein (and possibly other acute phase proteins) might also affect the bound fraction of lidocaine in dogs. An experimental study in rats with portocaval shunts, however, failed to detect an increase in α-1 acid glycoprotein 4 weeks after creation of the PSS.[36,37] It is not known so far whether α-1 acid glycoprotein is increased in dogs with EHPSS. As the affinity for the cytochrome P enzymes is high for drugs with a high hepatic extraction ratio, both bound and unbound drugs will be metabolized.[38] Consequently, an increased bound fraction of lidocaine decreases the pharmacologically active fraction, which, for the purpose of the lidocaine/MEGX test would be advantageous. Indeed, although previous studies[22-24] and the present study have shown that the 1 mg/kg body weight dose of lidocaine is safe in dogs with liver dysfunction, this even further decreases the risk of potential toxicity of lidocaine, without affecting the outcome of the lidocaine/MEGX test. At this moment, it is unsure what the expected bound fraction of lidocaine in dogs with EHPSS is and if this would be different in dogs with overt versus mild hepatic encephalopathy.

As the activity of cytochrome P450 enzymes in the liver in humans is known to be dependent on genetic factors, age, sex, nutritional status, and drugs,[15] individual variations in lidocaine metabolism are also to be expected in dogs. It has been described in healthy dogs that females have a higher concentration of MEGX 15 minutes after lidocaine administration than males; although this difference was no longer observed 30 minutes after lidocaine administration. In the same study, MEGX concentration was not influenced by the age of the dogs.[21] In the current study, clear differences were not observed between dogs of different sexes nor ages. Statistical analyses could, however, not be performed due to the small number of dogs included.

Besides the promising results, this study has also several limitations. Although all owners were asked to keep their dog fasted for 12 hours, some dogs were not fasted and, hence, lipemic samples were obtained. Furthermore, as multiple blood samples were taken, some samples were hemolytic. The influence of lipemia and hemolysis was not investigated in this study; however, there were no indications that any of these factors had a clear impact on the results. It also needs to be mentioned that the length of the storage time between different samples varied greatly. As all samples were analyzed in batch, the oldest samples were frozen at −80°C during 3 years. Nevertheless, MEGX is reported to be a stable metabolite as multiple freeze-thaw cycles and storage at −20°C during 8 months did not change the concentration significantly.[21,39] Furthermore, only a relatively small number of dogs was included, especially the number of dogs with persistent shunting was low. Additionally, some dogs were not presented for all follow-up visits and in other dogs, not all blood samples could be collected. Despite this limited cohort of dogs, statistical significance was reached. However, a 90% CI for the AUROC had to be used, in order for the CI interval to be within the normal range, which might imply that the CI is narrower than it actually is.

In conclusion, the lidocaine/MEGX test is an assay that shows promise to differentiate between dogs with closed EHPSS and dogs with persistent shunting after gradual attenuating surgery. It is a safe test that only takes 15 minutes to complete and is easy to perform. More investigations are, however, warranted in a larger cohort of dogs with PSS as well as other liver diseases to consolidate these promising results. Furthermore, the lidocaine/MEGX test should be tested in healthy dogs to establish accurate reference values and cutoff values.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approval: EC 2014/179; Deontological committee: 2015N03.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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