

PORTAL SYSTEMIC SHUNT COLLECTION PROGRAM

By Dr. Sharon Center

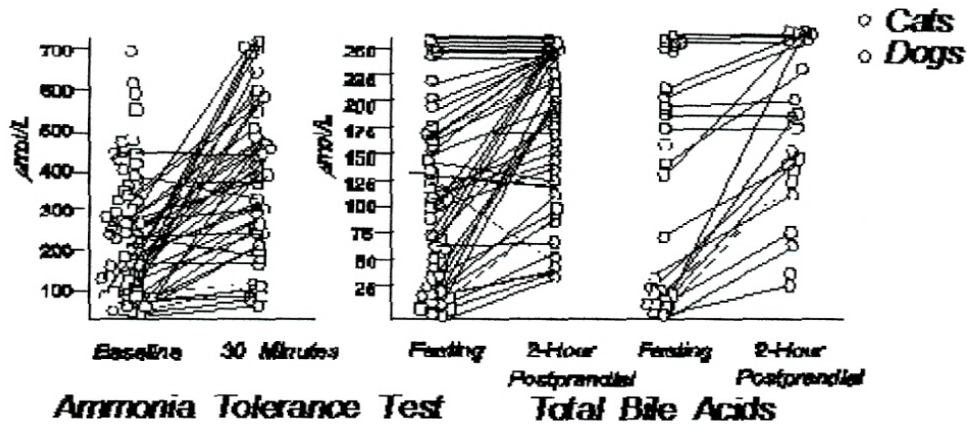
The ongoing genome wide study for the inheritable cause of liver vascular malformations (portosystemic venous anomalies [PSVA] and microvascular dysplasia [MVD]) in Cairn Terriers is progressing. Blood samples have been collected from a large informative pedigree involving more than 150 dogs and the DNA has been extracted. An informative pedigree is one in which there are normal dogs, PSVA dogs, and MVD dogs. Rather than just focusing on dogs with PSVA, the study is including MVD as a manifestation of hepatic vascular malformation. Previously completed studies of expansive pedigrees in other small "terrier" type breeds have repeatedly demonstrated that these two abnormalities appear in the same families of dogs of many different small breeds. In the next paragraph, we describe why this is important in the genotyping study and will later explain why the bile acid test rather than blood ammonia measurements are necessary in this work.

PSVA denotes the presence of an abnormal blood vessel that circuits blood (draining from the intestinal tract) around the liver rather than to the liver. Normally this blood in the Portal Vein is cleansed by the liver removing substances that are "toxic" and adding important metabolites. One of those toxins is ammonia which the liver transforms into urea (eliminated by the kidney). MVD denotes the presence of microscopic vascular malformations in the liver. These lesions difficult to discern from those observed in dogs with PSVA. In fact, these lesions do not disappear after surgical ligation in the majority of dogs with PSVA. The associated MVD explains why serum bile acids (SBA) do not completely normalize in successfully ligated dogs. Dogs with MVD may "shunt" blood around a liver lobe yet have circulation to another liver lobe. This has been proven in MVD dogs by radiographs made after injection of a contrast agent into the portal vein and also recently by MRI imaging. In looking for the genetic cause of PSVA, it is absolutely essential that dogs with MVD be identified to avoid them being labeled as phenotypically normal. The word phenotype denotes the physical appearance of a trait that is used as a marker for the genetic trait in question.

It is important to understand these points to carefully consider steps taken to identify the involved genetic factors as well as the utility of SBA and blood ammonia testing in recognizing affected dogs. The SBA test is very sensitive for detection of abnormal blood flow to the liver

and through the liver. Paired testing with fasting and 2-hour postprandial samples optimizes test performance. Screening with a fasting SBA value is absolutely not recommended to rule out a liver problem. The SBA detects both MVD and PSVA and is the only test that can be used to phenotypically label dogs as affected or not affected for the genotyping project. The blood ammonia test can be used to detect dogs with PSVA if the test is properly conducted (see below) but it will not reliably detect dogs with MVD. The following graph illustrates the comparable utility of these tests in dogs and cats with PSVA.

Figure 1: S. Center, College of Veterinary Medicine, Cornell University



With careful examination of this graph and noting that baseline fasting ammonia values may be normal in dogs and cats with PSVA, a bedside blood ammonia monitoring unit has been widely used in Europe during the last 15 years. With this test a dipstick is saturated with blood and inserted into a measuring device. The release of ammonia from blood allows volatilized ammonia to diffuse into a color indicator pad allowing detection of a color change by the measuring device. These units have not been approved for use in the United States by the FDA and are not currently for sale in the United States. The routine analysis of ammonia in most laboratories involves a chemical reaction reliant on an enzymatic reaction. It is well documented that these measurements are difficult to replicate for a number of reasons. The problems involve the lability of ammonia in blood (you cannot store blood or mail blood for analyses) and environmental contamination (air

contaminated by open urine containers, sweat on hands, cigarette smoke, other factors). It also is known that ammonia challenge testing (administering protein containing food or administering ammonium chloride orally or rectally) also can be problematic. Several authors have published data proving that fasting blood ammonia values can miss dogs with PSVA. While administration of ammonia chloride (orally or rectally) to provoke an ammonia challenge can increase the performance of the blood ammonia test, this may result in toxicity in a PSVA patient and is not recommended. Since the SBA test accomplishes the same level of detection for PSVA (consult figure 1), is safer, the samples can be mailed because the SBA are very stable in blood, this has become the testing standard for most progressive veterinary practices. Most dogs with PSVA have at least one of paired samples (fasting, 2-hour postprandial) demonstrating very high SBA (>150 $\mu\text{mol/L}$) concentrations. When the SBA is coupled with an examination of urine sediment looking for ammonium biurate crystals (a product of high sustained ammonia) this identifies dogs with both high SBA and high blood ammonia concentrations. Urine evaluation for ammonium biurate crystals are not as affected by storage as the blood ammonia test.

A bedside ammonia test has been used to test dogs for PSVA in Europe for some time. Early work reported its ability to detect high ammonia concentrations in the Irish Wolfhound, a breed with an unusually high incidence of PSVA involving a large vessel within the liver (patent ductus venosus). Terriers usually have PSVA that are in the abdomen outside the liver (extrahepatic). However, detection of high ammonia concentration in Irish Wolfhound pups does not always correspond to the presence of a PSVA and seemingly reflects slow closure of an embryologic vessel that bypasses the liver in the fetus. This was initially reported by the group using the ammonia checker device as an inborne error of metabolism in the breed. The blood ammonia test does not identify the dogs with MVD.

Back to the genotyping study: Dr. Center and her collaborators have a post doctoral student working on the project during the summer which will help with the progress of the project. Initial microsatellite screens have been completed in a large pedigree of affected Tibetan spaniels and these findings are helping to select genomic areas of interest for the Cairn Terrier PSVA/MVD genotyping. SNP association testing for PSVA/MVD is underway using blood from a number of different breeds. Initial progress in DNA preparation was delayed by the unfortunate aflatoxin outbreak in the Northeast over the holiday period in which Dr. Center played a key role in guiding patient management.

Several group collections of blood for genotyping and SBA determinations have been completed. The SBA concentrations have been measured and results have been mailed to participants. All results are coded by dog number to maintain anonymity and confidentiality. Samples were evaluated in large batches to provide economical analyses.