Amyloidosis In the Bracco Italiano: Ongoing Research and My Experiences

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In this article, I hope to answer some of the most common questions we have about inherited renal amyloidosis in the Bracco Italiano. I will include information from previous and current research investigating amyloidosis, as well as my own experiences as a veterinarian and breeder of these wonderful dogs.

My foundation bitch died of renal amyloidosis. However, the diagnosis was not made until archived kidney tissue was analyzed six years after her death. By that time, her offspring had been bred and I had second and third-generation Bracchi with amyloidosis. Through my dogs that I've bred and owned, and through networking with owners and breeders across the world, I've been involved in countless cases of amyloidosis in the Bracco over the last 10 years. I am currently involved in ongoing research into this disease at the University of Florida College of Veterinary Medicine. My goal is to provide information (and hope) for families faced with this terrible disease in their beloved dogs.

Any experiences that are anecdotal are noted as such and should be taken with a grain of salt as they are not supported by scientific research.

Delaney - who lived for 16 months after diagnosis with amyloidosis.

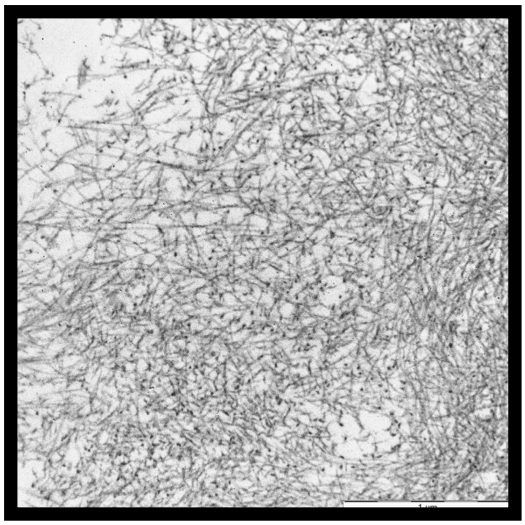
• What is Amyloidosis?

Amyloidosis encompasses a wide variety of diseases that can affect any tissue in the body. Amyloid is an insoluble, waxy aggregate of proteinaceous fibrils (protein chains). The kidney is the most common site of amyloid deposition in the dog. There are many different types of amyloid proteins, but they all are structurally similar and exhibit similar properties in the body and when evaluated under a microscope.

In health, acute inflammation in the body stimulates amyloid protein production by the liver. Circulating amyloid (called Serum Amyloid A, or SAA) exists as microscopic proteins in the blood stream. If there is excessive or chronic production of SAA, it can sometimes lead to these proteins binding together to form chains or fibrils.

Due to the high blood flow and small blood vessels in the kidneys, they are a common place for amyloid fibril deposition. This build up of protein cannot be cleared by the body's normal clean-up mechanisms, and eventually it will begin to affect the kidney's normal function.

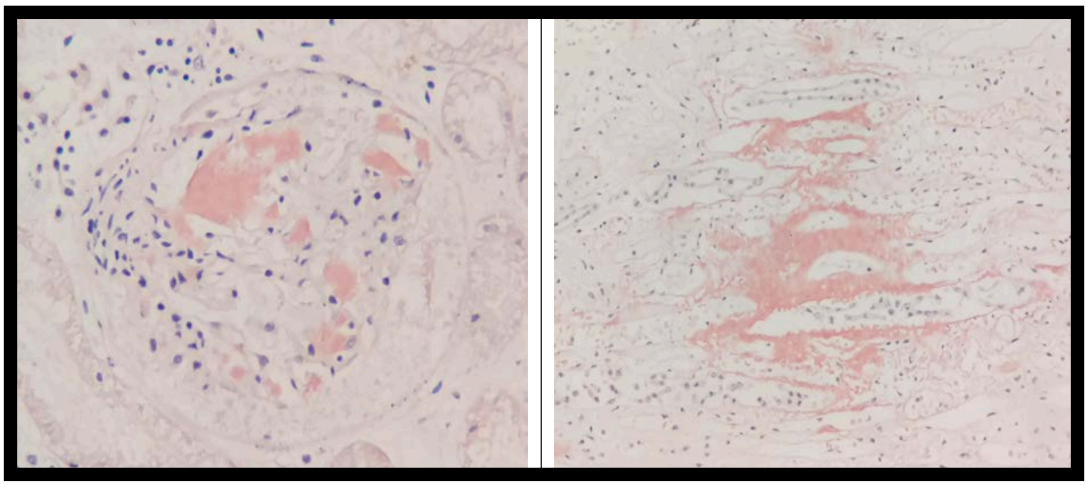
Most commonly in the Bracco Italiano, the amyloid deposition occurs in the section of the kidney called the glomerulus. This section is responsible for making sure that blood proteins stay in the bloodstream and are not spilled into the urine. Thus, when there is damage to the glomerulus, you can see protein loss in the urine (proteinuria).



Amyloid Fibrils in Bracco Kidney Tissue - from IVRPS

Another section of the kidney that can be affected are the tubules. These are generally responsible for concentrating urine. When there is tubular damage, you can see dilute urine and increased kidney blood values (blood urea nitrogen, creatinine, or SDMA). The tubules can be directly damaged by amyloid deposition or indirectly from the stress to them caused by ongoing glomerular damage.

While we primarily see amyloidosis as a kidney problem (because that's where the symptoms are usually seen), it often reflects a systemic disease. In the Shar Pei (and possibly the Bracco Italiano), there is an underlying auto-inflammatory condition that precedes amyloidosis. The trigger is unknown, but there is an increased level of inflammation in the body which predisposes to the amyloid production as described above. This is different that an auto-immune disease, because the immune system is not attacking the body's own cells and tissues. In a way, amyloidosis could be considered a very messy and destructive byproduct of chronic inflammation.



Amyloid Deposition (stained a peach color with Congo Red) in a Bracco Kidney - from IVRPS

• What do we know about amyloidosis in the Bracco Italiano?

Amyloidosis is most often diagnosed in young to middle aged Bracchi Italiani. It has been diagnosed in dogs as young as 13 months of age, and as old as 10 years. Most commonly, it is diagnosed around 5 years of age. The range in age may be due to variability in how the disease is inherited or how it progresses. Protein loss in the urine, improperly concentrated urine, or increased kidney values on bloodwork in a young to middle-aged Bracco would be concerning for hereditary kidney disease.

We have not seen any increased risk by sex or color pattern (orange-white and brown-white appear equally affected). It has been diagnosed in many different breeding lines from many countries. We know that it is passed from generation to generation and runs in family lines, although we have not (yet) identified a genetic marker or determined how it is inherited. Due to the complexity of the underlying disease and the small gene pool of the Bracco Italiano, I do not feel that simple pedigree analysis will be adequate to evaluate inheritance.

The presenting symptoms are highly variable. The most common presenting symptom is loss of appetite. Some dogs have increased thirst/urination and weight loss but not all. Seemingly unrelated symptoms (such as joint pain, coughing, and eyelid swelling) have been seen in dogs diagnosed with amyloidosis. This proves the importance of a thorough diagnostic investigation, including urinalysis, for any sick Bracco Italiano.

Most Bracchi Italiani with amyloidosis are proteinuric (have protein loss in the urine). The proteinuria can be very severe. Some complications seen with severe proteinuria include decreased blood protein levels (low albumin, which can lead to edema of the limbs and face, and fluid build up in the abdomen or chest) and an increased risk of abnormal blood clots (thromboembolism, which can affect the lungs, brain, heart, or kidney and result in respiratory distress or sudden death). A couple of dogs have had joint inflammation associated with their disease, similar to Shar Pei Fever (swollen hock syndrome).

Many dogs have a decreased blood albumin level. This could be related to underlying inflammation and/or protein loss in the urine. Some dogs may have increased white blood cell counts or anemia. Abdominal ultrasound is often largely unremarkable, although the kidneys may show a mild loss of their normal layering (called decreased corticomedullary distinction).

As stated above, most Bracchi have amyloid deposition in the glomerulus of the kidney. The tubules are usually also affected, either directly by amyloid deposition (less common) or secondary inflammation (more common). Many dogs have chronic kidney disease changes when their kidney tissue is evaluated under a microscope. Deposition of amyloid in other body tissues is possible, and it is unknown what portion of Bracchi have systemic amyloidosis.

In ongoing research, roughly 13% of apparently healthy Bracchi Italiani had evidence of kidney disease on a single routine screening performed at a national breed event in the USA. The normal prevalence of kidney disease in a population of dogs is closer to 0.4%.

The survival time for amyloidosis is poor once the dog becomes symptomatic. In our initial results, we found that the median survival time after diagnosis (half of the dogs lived longer, half did not survive as long) was 75 days. More severely affected dogs lived only days or weeks after diagnosis. If intervention is achieved before they show symptoms, survival times may be significantly improved with treatment. There is no cure for amyloidosis, and it is considered ultimately life limiting for most dogs diagnosed with the disease.

I know of several dogs who are presumed to have amyloidosis that were diagnosed prior to showing symptoms and are now months to years into treatment. We suspect that the deposition of amyloid may occur over years in some cases. It is impossible to know if these dogs would have a more slowly progressive disease regardless of intervention - but it gives us hope that routine screening can provide a window for therapy to extend quality life for some dogs with this disease.

In my anecdotal experience, the disease takes several forms. Some dogs have a more severe form that is quickly fatal. These dogs are often younger (1-3 years of age) and have severe protein loss in the urine, but their kidney values on bloodwork are likely to be normal. There are more slowly progressive forms, which may lead to both protein loss and increased kidney values on bloodwork. It is possible to also have dogs who have increased kidney blood values but are not proteinuric, but this appears less common.

Also, in my personal experience with the breed, it seems that there are a number of puppies who experience episodes of fever, increased thirst and urination (which can be profound), and increased white blood cell counts. This seems to happen most commonly around 6-10 months of age. In many puppies, full diagnostics do not reveal a cause of these changes and the symptoms resolve within a couple of months. I suspect this is another manifestation of the underlying auto-inflammatory condition, but we cannot say for sure without additional research. I suspect these puppies may grow up to be at higher risk of developing amyloidosis as adults (and some may begin amyloid deposition at a young age and be fatally affected by 1-3 years), but it is still unclear.

• How can you test for amyloidosis?

Definitive diagnosis of amyloidosis requires evaluation kidney tissue under a microscope. The most common test is called Congo Red staining. The Congo Red will turn amyloid protein a peach or pink color (and bright green under polarized light). The amyloid fibrils can actually be visualized using electron microscopy (this is a more advanced test and is not frequently performed).

If a kidney biopsy is performed, I recommend evaluation by the International Renal Veterinary Pathology Service associated with the Ohio State University. This can be done as a diagnostic test in a sick dog, or post-mortem to confirm diagnosis. Kidney biopsies are performed under general anesthesia surgically or using an ultrasound-guided needle. If the biopsy is performed by an experienced veterinarian the risk of complications (bleeding and worsening of kidney disease) is very low. Your dog may need to be hospitalized overnight for monitoring after biopsy or they may be able to go home the same day. Activity restriction is generally recommended for 2 weeks after the procedure (no running, jumping, or rough play) as the skin and kidney heal.

Standard diagnostic tests that your veterinarian may perform to evaluate for kidney disease would include blood and urine testing. Bloodwork (including creatinine, blood urea nitrogen, albumin levels, and possibly SDMA) is critical but only part of the picture. Any creatinine level above 1.4mg/dL is concerning for kidney disease if the urine is not highly concentrated. The difficult part about blood testing is that the creatinine does not increase until approximately 75% of kidney function is lost. The SDMA is slightly more sensitive and can detect a roughly 40% loss of kidney function, but it is not foolproof. Due to the risk of false results, further investigation is necessary if an abnormal SDMA is found. It is a very good adjunctive test but should not be used alone.

Since some Bracchi have normal blood values and abnormal protein loss in the urine - urinalysis is a requirement and should be performed concurrently with blood testing. This allows your vet to evaluate for proteinuria and determine if an increase in creatinine is due to kidney disease (ie: the creatinine is increased and the urine is not maximally concentrated) or due to dehydration (ie: the creatinine is increased and the urine is appropriately concentrated).

To measure urine concentration, your vet will analyze a urine specific gravity (measurements from 1.000, which is like water, to 1.050, which is highly concentrated). This compares the concentration of the urine to the concentration of blood (a specific gravity of 1.008-1.012, or “isosthenuric”). Anything more dilute or more concentrated than the blood tells you that the kidneys are doing their job of maintaining normal hydration. If the urine concentration is from 1.008-1.012 then it is concerning that the kidneys may not be working appropriately. Any dog with an increased creatinine should have a urine specific gravity greater than 1.025 — a value more dilute than that is concerning for kidney disfunction. A dog must lose approximately 66% of it’s kidney function before they lose the ability to concentrate their urine, so even this is a late change.

In addition to urine concentration, your vet will evaluate your dog’s urine for abnormal compounds, such as glucose (sugar), ketones, blood, and protein. Protein loss is most commonly seen in Bracchi with amyloidosis. A bed-side test called a dipstick is available and gives a broad sense of protein loss (on a scale of 0-3+). The dipstick can be unreliable, however, and I always recommend a urine protein:creatinine ratio (UPC). A UPC provides a quantitative measurement of protein loss. Any UPC greater than 0.4 is cause for concern. Some Bracchi with amyloidosis may have a severely increased UPC of 10-15 or even higher (the highest I have seen was 30). Controlling proteinuria is an important part of treatment, and the UPC provides an accurate way to track changes in protein loss.

A large amount of blood (ie: if the urine is visibly discolored red or pink) may falsely increase the UPC, but a small amount of blood should not significantly change the UPC.

Most urine testing is done with urine collected that day at the vet’s office. This is a good starting point, but for long term management it may be necessary to collect first-morning urine from three days in the row to evaluate the UPC. This is called a “pooled” sample. Equal volumes of urine are collected from three days in a row (ideally the first urination in the

morning) and stored in the refrigerator. You can take these three samples to your vet who will combine equal volumes of each into a single “pooled” sample for testing. This is good because proteinuria (and thus the UPC) can vary from day to day and a pooled sample gives you a better overall view of the amount of protein lost.

Measuring blood pressure and urine culture (to look for bacterial infection in the urinary tract) are indicated in any dog with evidence of kidney disease. High blood pressure and urinary tract infections can both be the cause of kidney disease and also a consequence of kidney disease.

I recommend following the American College of Veterinary Internal Medicine guidelines (“Consensus Recommendations for the Diagnostic Investigation of Dogs with Suspected Glomerular Disease”) and the International Renal Interest Society diagnostic and staging guidelines for kidney disease (www.iris-kidney.com).

• Screening for amyloidosis

I recommend routine kidney health screenings for all Bracco Italiano dogs starting at 1 year of age. This should include bloodwork (creatinine, blood urea nitrogen, albumin, +/- SDMA) and urinalysis (including a urine protein:creatinine ratio/UPC) every year. For dogs with a family history (grandparent, parent, sibling, or parent’s siblings) of kidney disease, I recommend testing every 6 months.

I recommend that breeding dogs be screened as above within 6 months of breeding. Unfortunately, with the limitations of our current diagnostics, there is no predictive value in these tests. They do not guarantee that the parents will not go on to develop kidney disease, and they do not guarantee the puppies will be at a lower risk of kidney disease. These tests can only tell us that the dog tested does not have advanced kidney disease at the time of testing. Even kidney biopsies prior to breeding may have false-negative results (miss the amyloidosis) and cannot guarantee the dog won’t develop amyloidosis later in life. However, I firmly believe all breeding dogs should be tested.

We must make the most of the testing we have available, however we must also understand and be honest about the limitations of that testing. For this reason, it is important that we work towards a DNA marker for screening breeding dogs in the future. Having kidney biopsies performed on affected dogs (which can often be used for DNA extraction) and DNA banking (blood or cheek swabs) on affected and healthy Bracchi are important steps moving forward.

If kidney disease is diagnosed, full diagnostic investigation should be performed to evaluate for other causes of kidney disease besides amyloidosis. These may be more treatable and have a better prognosis. This may include abdominal x-rays and ultrasound, urine culture, blood pressure, infectious disease testing (Leptospirosis, Lyme’s disease, Leishmaniasis, others).

• What is the treatment for amyloidosis?

Unfortunately, we do not currently have a cure for amyloidosis. Treatment is aimed to minimize symptoms and reduce ill effects of the disease.

- IRIS Treatment Guidelines for Chronic Kidney Disease offers a good guideline.
- The Consensus Recommendations for Standard Therapy of Glomerular Disease In Dogs is an excellent resource (Journal of Veterinary Internal Medicine, 2013).

- Treatment for Uremia (Increased Kidney Blood Values) and Dehydration
  - Increased blood toxins from kidney failure can lead to nausea and vomiting, stomach ulcers, loss of appetite, and diarrhea. Anti-nausea medications and medications to make the stomach fluid less acidic may be used.
  - Dogs with kidney disease can easily become dehydrated. They may require supplemental fluids under the skin, or even have a feeding tube placed for water and nutritional support in some cases.
  - Azodyl is a prebiotic supplement that is available to help reduce the severity and effects of uremia. It does not improve survival or change prognosis. In my experience, it helps to reduce the severity of uremia and may be a beneficial adjunctive therapy.
- Treatment for Proteinuria:
  - Ongoing proteinuria can cause damaging systemic effects (blood clots, edema) and can worsen kidney disease. There are multiple medications which can help to minimize proteinuria.
  - ACE Inhibitors are drugs that help reduce protein loss in the urine. They often cause a mild decrease in blood pressure as well. These may not be effective as a single therapy for protein loss due to amyloidosis. Examples include Enalapril and Benazapril.
  - Angiotensin Receptor Blockers (ARBs) are newer medications that reduce protein loss from the kidneys. These are generally much more effective than ACE Inhibitors but are often slightly more expensive and may have a bigger effect on blood pressure. Examples include Telmisartan (my preferred medication to treat proteinuria) and Losartan.
  - Any medication that decreases protein loss in the urine has the potential to harm the kidneys or cause a drop in blood pressure, but most dogs tolerate treatment very well and the benefits far outweigh the risks. For this reason, it is generally recommended that you recheck blood values and blood pressure one week after starting any of these medications.
  - If a dog is proteinuric and has low blood albumin (protein), they are likely at risk for abnormal clot formation. These dogs may benefit from blood thinners (such as low dose Aspirin, Clopidogrel, Rivaroxaban).
- Treatment for High Blood Pressure:
  - Kidney disease can cause high blood pressure, which can in turn worsen kidney disease. ACE Inhibitors and ARBs (see above) can be used to manage high blood pressure. In some cases, additional medications (such as Amlodipine) may be needed.
  - Untreated high blood pressure can lead to blindness, stroke, mental changes, and worsening of kidney disease.
- Treatment of Increased Phosphorus
  - Increased blood phosphorus levels have been showed to have deleterious effects for patients with kidney disease and is linked to shorter survival times.
  - Your vet may prescribe a phosphorus restricted diet or a phosphate-binding medication.
- Anti-Inflammatory Therapy
  - Colchicine is used in humans with amyloidosis to help reduce amyloid deposition. It is unproven in dogs, but it is often recommended in Shar Pei with this disease. Once a dog is in kidney failure, it is unlikely to be beneficial but it may help dogs who are diagnosed earlier in their disease. It can have significant gastrointestinal side effects, but at appropriate doses these are usually minimal. I generally recommend this medication in Bracchi with presumed amyloidosis as it may be beneficial and is unlikely (at appropriate doses) to cause harm. If a dog is having significant side effects from Colchicine, I would stop the drug. Colchicine can be expensive, but generic or compounded forms are available (in the USA GoodRx.Com provides free coupons online).
  - Supplemented Omega-3 fatty acids are beneficial to dogs with kidney disease. Some prescription diets are already supplemented with Omega-3s (in the forms of DHA and EPA, or fish oil). The goal is to increase the ratio of Omega-3s to Omega-6s, so it is important to make sure any fish oil supplement has low Omega-6 levels. Also, be cautious as some fish oil supplements contain Vitamin D, which can be toxic in high doses. All of

my Bracchi receive supplemental Omega-3 fatty acids, even if they have not been diagnosed with kidney disease.

- Nutrition
  - There is a significant debate about what is the best diet for a dog with kidney disease. It has been shown that phosphorus restriction and supplemented Omega-3 fatty acids are beneficial and improve survival times.
  - The amount of protein that is best for a dog with kidney disease is disputed. I generally recommend once a dog has a persistently increased creatinine, they should be started on a kidney diet that is restricted in protein. For dogs with known kidney disease but normal blood values, some senior or “early stage” kidney diets can provide Omega-3 fatty acids and restricted phosphorus with a more moderate amount of protein. These diets that are only slightly protein restricted may be better to minimize protein malnutrition in chronic kidney disease.
  - For dogs who have protein loss in the urine, but normal creatinine and other blood values, I generally do not restrict their protein intake. This is my personal preference, and some veterinarians may approach treatment differently. For these dogs, I prefer to provide a moderate protein diet with supplemented Omega 3-s as to minimize protein malnutrition. For proteinuric dogs with chronic disease, or cases with a high-normal/increased phosphorus, then a phosphorus restricted diet is beneficial (or even adding a phosphate binder).
  - There are many prescription diets available through your veterinarian, or you can work with a veterinary nutritionist for a home-cooked meal plan.
  - For dogs with severe kidney disease and a decreased appetite, nutrition becomes very important. With end-stage disease, I take the approach that “all nutrition is good nutrition.” If they are not willing to eat their prescription diet, try something else. Some dogs may benefit from feeding tube placement - however in cases of amyloidosis and chronic kidney disease, we must ask ourselves the goal of our treatment. If the dog is otherwise a happy, healthy young Bracco, then a feeding tube is a viable option. However, if your dog is feeling very ill, not eating, and not responding to medical management, then it may be seen as extending suffering when humane euthanasia is more appropriate. Many cases of acute kidney injury (where a partial or complete cure is possible) benefit from feeding tube placement and it is very beneficial. Feeding tubes can be used for water supplementation and medication administration in dogs that are eating on their own. The most common is an esophageal feeding tube, which is easy to place and most dogs adapt very well to having it in place.

Delaney had a feeding tube placed for fluid administration. It did not affect her appetite or ability to eat normally. Her tube was in place for 5 months, during which time she had a normal appetite and energy level, but needed more fluids than could safely be given under the skin.

• What is the prognosis for amyloidosis?

There is no cure for amyloidosis. The prognosis once symptoms develop is poor. In our initial evaluation of Bracchi Italiani with familial kidney disease, the survival time after diagnosis was less than 3 months for most dogs. One study in 2012 found a survival time of only 5 days when they evaluated 91 dogs with renal amyloidosis in Shar Pei and non-Shar Pei dogs.

In my experience, I have seen several dogs diagnosed before they become symptomatic (thanks to routine screening). These dogs can be managed for months or years with medication and diet. We don't know if these individuals might have a more chronic, subtle form of the disease or if they are truly responding to treatment, but I think there is a window for intervention that may give dogs a significant amount of quality time with their families despite a terrible diagnosis.