Cardiomyopathies are primary diseases of the cardiac muscle and include DCM, which is common in Doberman Pinschers, Great Danes, and Irish Wolfhounds, and ARVC, which is common in Boxers. Both conditions are devastating with regard to the development of debilitating clinical disease, but cardiomyopathy is particularly troublesome with regard to detection of early stages of disease. Detection of early stages of the disease is important for each specific dog as well as for all dogs of a particular breed (because of the desire to perpetuate breeding programs that result in healthy dogs).

The development of cardiomyopathy is characterized by 3 phases of disease.1,2 The first phase includes dogs with a genetic predisposition to the disease without any detectable morphologic or electrocardiographic signs. Detection of dogs in this phase necessitates discovery of the underlying cause of the disease. For example, if DCM or ARVC is caused by a specific genetic defect, identification of the mutant gene or genes responsible and subsequent genomic testing would identify affected dogs. Although cardiomyopathy is believed to be a heritable condition, to our knowledge, the exact cause is unknown. The second phase includes dogs with detectable abnormalities on ECG or echocardiography that do not have overt clinical signs. This phase is often referred to as occult disease. The third phase includes dogs with ECG and echocardiographic abnormalities as well as clinical signs such as heart failure, activity intolerance, inappetence, or syncope. Detection of dogs in the third phase is readily accomplished by use of routine physical examination and diagnostic testing.

Because of the advanced nature of the third phase of the disease, clinical outcome is poor, and many of these dogs have already served as breeding stock. Therefore, detection of disease during the occult phase is desirable in that therapeutic interventions could be initiated sooner, which could ostensibly lead to a better outcome.

Currently, diagnosis of occult cardiomyopathy is achieved by use of a combination of ECG and echocardiographic examinations. Relatively well-defined criteria for occult disease have been established for Doberman Pinschers, Irish Wolfhounds, and Boxers. These

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**Objective**—To evaluate the use of measuring plasma concentrations of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and cardiac troponin-I (cTnI) to detect dogs with occult dilated cardiomyopathy (DCM).

**Animals**—118 client-owned dogs.

**Procedures**—Dogs were prospectively examined by use of ECG; echocardiography; and evaluation of concentrations of ANP, BNP, and cTnI. Occult DCM was diagnosed by evaluation of echocardiographic left ventricular dimensions and detection of ventricular arrhythmias on ECG. Sensitivity and specificity of assays for measurement of plasma concentrations of ANP, BNP, and cTnI to detect dogs with occult DCM were determined.

**Results**—Occult DCM was diagnosed in 21 dogs. A concentration of > 6.21 pg/mL for BNP had a sensitivity of 95.2% and specificity of 61.9% for identifying dogs with occult DCM. In contrast, concentrations of ANP and cTnI had relatively low predictive values.

**Conclusions and Clinical Relevance**—Blood-based screening for occult DCM in dogs can be accomplished by use of a BNP assay. Additional studies should be performed to optimize this method of screening dogs to detect occult DCM. (Am J Vet Res 2007;68:42–47)

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**ABBREVIATIONS**

- **DCM**: Dilated cardiomyopathy
- **ARVC**: Arrhythmogenic right ventricular cardiomyopathy
- **ANP**: Atrial natriuretic peptide
- **BNP**: B-type natriuretic peptide
- **cTnI**: Cardiac troponin-I
- **LVIDd**: Left ventricular internal dimension at end of diastole
- **LVIDs**: Left ventricular internal dimension at end of systole

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criteria primarily depend on evidence of ventricular or atrial arrhythmias (detected by use of serial ECGs or 24-hour ambulatory Holter monitoring) and echocardiographic evaluation of left ventricular dimensions and function.1,3-7 Although these criteria represent the current criterion-referenced standard, they have several drawbacks, including availability (ie, need for experienced echocardiographers), the need for specialized equipment (ie, ultrasonography machines or Holter monitors), high interobserver variability (particularly relevant to the measurement of ventricular function), and financial cost (which may be substantial for owners with large numbers of dogs).

The natriuretic peptides, ANP and BNP, and the cardiac marker, cTnI, represent blood-based substances that are associated with cardiac structure, function, and injury. Atrial natriuretic peptide and BNP are vasodilatory peptides that are released by myocardial tissue primarily in response to increased stress on the myocardial wall. Cardiac troponin-I is part of the filamentous structure of the cardiac sarcomere and is released as a result of necrosis or injury of myocytes. In humans and dogs, BNP is elaborated relatively early during the course of disease and in proportion to severity of the condition.8-10

In humans, the natriuretic peptides discriminate between cardiac and noncardiac causes of dyspnea and can be used to stratify severity of disease; guide medical treatment; predict outcome; and of particular interest to the study reported here, detect patients with occult left ventricular dysfunction.11,12 Cardiac troponin-I is a marker of myocyte injury, and as such, the blood concentration of cTnI is increased in patients with a wide array of cardiac diseases, including DCM, ARVC, degenerative mitral valve disease, subaortic stenosis, pericardial disease, and infection caused by Trypanosoma cruzi.13-15

During development of cardiac disease, ANP, BNP, and cTnI are all elaborated, presumably along a continuum concurrent with disease severity. Therefore, they may be found in patients with early stages of nonclinical disease. Blood-based testing to detect cardiomyopathy is attractive because of its minimally invasive nature, ease of sample collection, potential for widespread availability, quantitative nature, and theoretic cost efficiency, compared with these factors for other current diagnostic methods. The purpose of the study reported here was to prospectively determine the ability for assay of plasma ANP, BNP, and cTnI concentrations to detect occult cardiomyopathy in a high-risk population of overtly healthy dogs.

Materials and Methods

Sample population—Dogs that were examined for cardiomyopathy screening by personnel in the Cardiology Service of the University of Illinois Veterinary Teaching Hospital were recruited for inclusion in the study. Local and regional breeders were also contacted and encouraged to allow participation of their dogs. Dogs were eligible for inclusion when they were overtly healthy with no clinical signs referable to heart disease (ie, syncope, dyspnea, or activity intolerance). The study protocol was approved by the University of Illinois Institutional Animal Care and Use Committee. Owners provided informed written consent prior to participation of their dogs in the study.

Conventional diagnostic testing—Dogs were subjected to ECG by use of a routine 10-lead ECG.a In addition, a lead-II rhythm strip was recorded for a minimum of 4 minutes for each dog. Standard 2-dimensional, M-mode, and Doppler echocardiographic examinationsb were performed in the dogs. Sedatives were not administered prior to these examinations.

In addition to the standard ECG examination, ECG monitoring was also performed during the ultrasonographic examination by use of the inherent ECG system of the ultrasound machine, and any ventricular arrhythmias were identified. On the basis of results of the ECG and echocardiographic examination, dogs with occult DCM were defined as those that fulfilled at least 1 of the following criteria: 1 or more ventricular premature beats during performance of the 10-lead ECG or lead II rhythm strip or detected during echocardiographic examination; Doberman Pinschers with an LVIDd > 46 mm (> 49 mm in dogs that weighed > 37 kg), as measured by use of the right parasternal, short-axis, 2-dimensional image; Doberman Pinschers with an LVIDS > 38 mm, as measured by use of the right parasternal, short-axis, 2-dimensional image; c or left ventricular fractional shortening < 18% in non-Doberman Pinschers. Left atrial and aortic root dimensions were obtained from M-mode images.

Blood-based assays—Blood samples for ANP and BNP assay were collected in 5-ml chilled tubes that contained EDTA and 0.2 mL of aprotinin. Samples were centrifuged at 5°C at 1,100 × g for 15 minutes. Samples for cTnI assay were collected in sodium heparin tubes and centrifuged at 25°C. Plasma for all 3 assays was transferred to 1.5-ml plastic cryotubes and stored at –80°C until analyzed. All samples were analyzed in batches.

Plasma concentrations for N-terminal pro-ANPd and carboxyterminal BNPwere determined by use of radioimmunoassay kits validated by our laboratory personnel. For statistical analysis, ANP and BNP results that were less than the detection limit of the respective assay were considered to have a value of 0.03 nmol/L and 2 pg/mL, respectively. Plasma cTnI concentration was determined by use of a human assay,1 which has been validated for use in assay of canine cTnI concentrations. For statistical analysis, cTnI results that were less than the detection limit of the assay were considered to have a value of 0.01 ng/mL.

Statistical analysis—Data analysis was performed by use of personal computer–based statistical and graphing software. Differences in sex and age between the affected and clinically normal populations were determined by use of χ² and unpaired Student t tests. Results of blood-based tests were logarithmically transformed, and mean ANP, BNP, and cTnI concentrations were compared between affected and clinically normal populations by use of unpaired t tests. Data are reported as the mean ± SEM unless indicated otherwise. Receiver-operating characteristic curves were generated to assess the specificity and sensitivity of ANP, BNP, and cTnI assays for detecting occult disease. For all tests, a value of P < 0.05 was considered significant.
Results

In the study, 118 dogs were examined. Occult DCM was diagnosed in 21 dogs. Occult DCM was diagnosed in 20 dogs on the basis of ECG (n = 11 dogs) or echocardiographic (9) criteria. In addition, 1 Great Dane with left ventricular enlargement (LVIDd, 6.4 cm; LVIDs, 5.0 cm) and a decreased fractional shortening of 21% on initial examination was retrospectively placed into the occult DCM category because of development of biventricular heart failure and atrial fibrillation 3 months after the initial examination. The remaining 97 dogs were deemed healthy by virtue of results on ECG and echocardiographic examination. Thus, the prevalence of occult DCM in our study population was 21 of 118 (17.8%). Sex, age, breed distribution, and echocardiographic measurements for the 2 groups were summarized (Table 1).

Technical complications resulted in the loss of 9 cTnI samples (3 from dogs with occult DCM and 6 from clinically normal dogs); therefore, cTnI-based analysis was performed on only 109 dogs. Dogs with occult DCM had significantly greater plasma concentrations of ANP, BNP, and cTnI, compared with concentrations in clinically normal dogs (Table 2). Analysis of receiver-operating characteristic plots revealed that the area under the curve was highest for BNP, followed by ANP and cTnI. Area under the curve was highest for BNP, followed by ANP and cTnI. In addition, age was not correlated with concentrations in dogs with occult DCM for ANP (r = 0.11; P = 0.29), or cTnI (r = 0.090; P = 0.72). Similarly, age was not correlated with concentrations in dogs with occult DCM for ANP (r = 0.33; P = 0.14), BNP (r = 0.39; P = 0.078), or cTnI (r = 0.033; P = 0.90).

Discussion

Analysis of results of the study reported here revealed that plasma BNP, cTnI, and ANP concentrations are significantly increased in dogs with ECG and echocardiographic evidence of occult cardiomyopathy. This suggests that there is cardiac production and release of these substances relatively early in the course of myocardial disease in dogs; however, of the 3 components evaluated, only BNP assay had adequate sensitivity and specificity to be of diagnostic use. Blood-based testing is unique insofar as its dependency on biochemical variables to assess cardiac function, as opposed to conventional morphologic, ECG, and hemodynamic indices, such as heart size, arrhythmia, or fractional shortening. Over the past 20 years, the concept of myocardial disease has moved from one of altered hemodynamics to one of abnormal neurohormonal activity. In humans, the long-term therapeutic value of neurohormonal drugs, such as angiotensin-converting enzyme inhibitors and β-adrenergic receptor blockers, compared with that for hemodynamic agents, such as digoxin, underscores the importance of heightened neurohormonal activity in myocardial disease. It is widely accepted that neurohormonal derangements are a central driving force in the evolution of cardiac disease. In humans, measurement of plasma ANP and BNP concentrations can help clinicians distinguish cardiac from noncardiac causes of dyspnea, stratify disease severity, provide prognosis, guide medical treatment, and prospectively evaluate at-risk populations for asymptomatic disease. It was reported in a study that measurement of plasma ANP and BNP concentrations was useful in predicting development of heart disease in a population consisting of 3,346 offspring of the original members of the Framingham Heart Study. People with increased concentrations of natriuretic peptides (men with a BNP concentration > 20 pg/mL or an ANP concentration > 0.485 nmol/L and women with a BNP concentration > 23.3 pg/mL or an ANP concentration > 0.599 nmol/L) had a significantly higher risk for heart failure, major cardiovascular events, atrial fibrillation, and death during the subsequent 5-year period. These values were all well below the threshold for diagnosis of clinically apparent heart disease (BNP > 100 pg/mL and indicate that results within the reference range still have clinical meaning. Additionally, that study revealed that the risk for adverse outcome was correlated with the magnitude of change in BNP concentra-

### Table 1—Mean ± SEM values for characteristics of 21 dogs affected with occult DCM and 97 clinically normal dogs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinically normal</th>
<th>Occult DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>46</td>
<td>10</td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
<td>11</td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doberman Pinscher</td>
<td>73</td>
<td>16</td>
</tr>
<tr>
<td>Great Dane</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Boxer</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Age (y)</td>
<td>4.7 ± 0.3</td>
<td>6.7 ± 0.7*</td>
</tr>
<tr>
<td>LVIDd:Ao</td>
<td>1.51 ± 0.02</td>
<td>1.77 ± 0.041</td>
</tr>
<tr>
<td>LVIDs:Ao</td>
<td>1.13 ± 0.02</td>
<td>1.40 ± 0.061</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>25.6 ± 0.45</td>
<td>21.4 ± 2.0*</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>2.65 ± 0.05</td>
<td>3.00 ± 0.11*</td>
</tr>
<tr>
<td>Ao (mm)</td>
<td>2.79 ± 0.04</td>
<td>2.63 ± 0.10</td>
</tr>
<tr>
<td>LAD:Ao</td>
<td>0.97 ± 0.02</td>
<td>1.15 ± 0.051</td>
</tr>
</tbody>
</table>

*Within a row, value differs significantly (*P = 0.01; †P < 0.001) from the value for clinically normal dogs.

### Table 2—Mean ± SEM plasma concentrations of ANP, BNP, and cTnI in 21 dogs affected with occult DCM and 97 clinically normal dogs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinically normal</th>
<th>Occult DCM</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP (nmol/L)</td>
<td>0.269 ± 0.013</td>
<td>0.346 ± 0.033</td>
<td>0.030</td>
</tr>
<tr>
<td>BNP (pg/mL)</td>
<td>6.51 ± 0.66</td>
<td>14.35 ± 1.60</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>cTnI (ng/mL)</td>
<td>0.06 ± 0.01</td>
<td>0.21 ± 0.10</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Concentrations were considered to differ significantly between groups at P < 0.05. The cTnI results represent samples from only 109 dogs (18 with occult DCM and 91 clinically normal dogs).
trations, such that for each 1 SD increase in plasma BNP concentration, risk for heart failure or death increased 77% and 94%, respectively.

Similar results were found in 2 other studies\textsuperscript{12,20} in asymptomatic but high-risk human populations (ie, the elderly or people with diabetes, hypertension, or hyperlipidemia). Those studies revealed that an increase in plasma BNP concentrations could be used to predict study subjects with underlying systolic dysfunction\textsuperscript{20} or eventual progression to more clinical disease.\textsuperscript{12} Although still controversial in practice,\textsuperscript{21,22} applying BNP testing specifically to a high-risk population (ie, one with higher prevalence) enhances the use of testing by decreasing the number of false-positive results.

Analysis of our results indicated that assay of plasma BNP concentrations was effective in identifying occult DCM in a population of dogs at high risk for disease. In populations with an extremely low incidence of disease, high specificity is desired to avoid numerous false-negative results that require additional and more expensive testing. In populations with a relatively high incidence of disease, high sensitivity is desired to detect all patients that have disease (ie, avoid false-negative results). Investigators in 1 study\textsuperscript{13} reported that BNP testing in humans is maximally effective when the investigated population has a disease prevalence > 1%. Prevalence of cardiomyopathy in the adult Doberman Pinscher, Boxer, and Great Dane populations is well above this threshold and may be as high as 40%.\textsuperscript{1}

In the study reported here, the lower prevalence of occult DCM (17.8%) may have been influenced by the inclusion of relatively young dogs (14 dogs were < 24 months old). We chose to select assay cutoff values that maximized sensitivity so that the number of false-negative results was kept to a minimum. In dogs with occult disease, false-negative results are potentially disastrous because these animals would be included for breeding purposes. Furthermore, they would not receive medical treatment or follow-up care that may help slow progression of the disease. The impact of false-negative results includes financial considerations (ie, monetary cost associated with future hospitalization and medications) as well as more intangible costs associated with psychologic and programmatic damage to the patient, owner, and breeding program.

In the case of occult DCM, false-positive results, although reducing the overall financial effectiveness of the assay, prompt additional diagnostic procedures that are not necessarily harmful to the patient; thus, the costs associated with false-positive results are primarily financial rather than medical. False-positive results can be reduced by selecting assays with high specificity and by selecting a population with a high incidence of disease (ie, adult large-breed dogs). In our study, we chose to screen breeds of dogs with a known predisposition for cardiomyopathy. Our results may have been further improved by restricting screening to only dogs that were older than a specified age.

Despite concentrations that were significantly increased in dogs with occult DCM, compared with results for clinically normal dogs, ANP and cTnI assays did not have the requisite sensitivity or specificity to serve as a useful diagnostic test. At a cutoff value that achieved adequate sensitivity, both ANP and cTnI assays resulted in the detection of occult DCM in 21 of 118 dogs that were prospectively screened.

![Figure 1](image-url) - Receiver-operating characteristic curve of the sensitivity and specificity of blood-based assays for ANP (circles), BNP (squares), and cTnI (diamonds) for use in detection of occult DCM. Notice that BNP has the best assay performance as determined by the highest sensitivity and specificity. The dotted line represents an assay with no predictive value.

<table>
<thead>
<tr>
<th>Assay</th>
<th>AUC</th>
<th>P-value*</th>
<th>Cutoff value</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP</td>
<td>0.844</td>
<td>&lt; 0.001</td>
<td>6.210 pg/mL</td>
<td>95.2</td>
<td>61.9</td>
<td>98.4</td>
<td>26.1</td>
</tr>
<tr>
<td>cTnI</td>
<td>0.740</td>
<td>&lt; 0.001</td>
<td>0.030 ng/mL</td>
<td>88.9</td>
<td>41.8</td>
<td>95.0</td>
<td>23.2</td>
</tr>
<tr>
<td>ANP</td>
<td>0.658</td>
<td>0.005</td>
<td>0.244 nmol/L</td>
<td>85.7</td>
<td>47.4</td>
<td>93.9</td>
<td>26.1</td>
</tr>
</tbody>
</table>

*Values were considered significant at \(P < 0.05\).

AUC = Area under the receiver-operating curve. NPV = Negative predictive value. PPV = Positive predictive value.

See Table 2 for remainder of key.
in more false-positive than true-positive results (ie, specificity < 50%). The main stimulus for ANP release is increased atrial pressure; therefore, usefulness of the ANP assay likely increases as disease severity worsens. In dogs with clinical disease, ANP assay is useful in distinguishing cardiac from noncardiac causes of dyspnea.1 Cardiac troponin-I is a marker of myocyte injury and is increased in various cardiac and noncardiac conditions.13,14,24 Analysis of results of the study reported here suggests that cTnI assay has relatively low specificity, compared with that for assay of the natriuretic peptides, and is not useful as a screening tool.

The study reported here has several limitations. It is possible, perhaps even likely, that our clinically normal population contained dogs with occult disease. We used a single echocardiographic examination and in-hospital ECG to diagnose occult DCM and did not perform 24-hour ambulatory Holter monitoring. In addition, we were not able to perform longitudinal follow-up monitoring to confirm our initial diagnosis. Thus, the study results should be interpreted cautiously, and more definitive studies that include additional diagnostic testing and longitudinal follow-up monitoring are required before the diagnostic usefulness of neurohormonal tests can be established.

Another limitation of our study involves the difference in age between the clinically normal and affected groups. It is not entirely surprising that dogs in the affected group were older than nonaffected dogs because the prevalence of cardiomyopathy increases as dogs reach middle age25; however, analysis of our results indicated that age was not correlated with the plasma concentration of ANP, BNP, or cTnI in either study group. In healthy humans, BNP concentrations are relatively increased in young children, decrease after adolescence, and then increase again in older people.26,27 Another study13 of a population of healthy dogs indicated a small but significant age-related increase in cTnI concentrations. To our knowledge, the effect of age on ANP and BNP concentrations in dogs has not been reported and requires further study.

The number of Boxers in our study was relatively low, compared with the number of Doberman Pinschers. In another study,28 investigators found that the plasma BNP concentration in a population of 13 affected Boxers was not increased, compared with the concentration in control dogs. Additional studies targeted toward cardiomyopathy in specific breeds are indicated.

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References

23. Heidenreich PA, Gabens MA, Fonanow GC, et al. Cost-effective-


