Left atrial function and size are associated with prognosis in patients with left ventricular dysfunction.\textsuperscript{1,2} The information provided by PVF patterns can be used to predict atrial performance as well as left ventricular performance.\textsuperscript{3,4} The PVF velocity recordings, which consist of forward flows (S wave [reservoir function] and D wave [conduit function]) and retrograde flows (AR wave [booster function]), have been considered in clinical settings.\textsuperscript{3–8} The PVF velocities are influenced by hemodynamic pressure gradients between the pulmonary vein and left atrium, which has been measured by use of transesophageal echocardiography in sedated dogs.\textsuperscript{7} In another study,\textsuperscript{1} investigators reported that the difference in the duration of pulmonary venous and mitral flow during atrial contraction is related to the relationship between velocities of pulmonary venous flow and plasma concentrations of atrial natriuretic peptide in healthy dogs

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**Objective**—To investigate the relationship between velocities of pulmonary venous flow (PVF) and plasma concentrations of atrial natriuretic peptide (ANP) in healthy dogs.

**Animals**—7 healthy Beagles.

**Procedures**—Dogs were anesthetized, intubated, and positioned in left lateral recumbency. Lactated Ringer’s solution was infused (200 mL/kg/h) for 60 minutes via a cephalic vein. Transmitral flow and PVF velocities were measured echocardiographically by use of the apical 4-chamber view. Pulmonary capillary wedge pressure (PCWP) and ANP concentrations were determined.

**Results**—IV infusion significantly increased heart rate and PCWP. Similarly, the ANP concentration significantly increased from baseline (before infusion of lactated Ringer’s solution) values. Transmitral flow velocities were significantly increased, although the ratio of velocity of the flow during early ventricular diastole (E wave) to velocity of the atrial flow (A wave; E:A ratio) was unchanged. Regarding the PVF velocities, forward flow during ventricular systole (S wave) and retrograde flow during atrial contraction were significantly increased, whereas velocity of the forward flow during ventricular diastole (D wave) was unchanged. Ratio of the velocity of the S wave to velocity of the D wave was increased significantly, and this ratio was significantly correlated with PCWP or ANP concentration. However, the E:A ratio was not correlated with PCWP or ANP concentration.

**Conclusions and Clinical Relevance**—PVF velocities were strongly correlated with PCWP and plasma ANP concentration in clinically normal dogs. Therefore, PVF velocities may serve as a sensitive indicator and provide additional information for monitoring acute preloading conditions and estimating atrial filling abnormalities in dogs. (Am J Vet Res 2008;69:465–470)
dilated cardiomyopathy. Furthermore, studies have revealed that the serum ANP concentration can be used to predict the severity and prognosis in patients with left ventricular dysfunction. It has been reported that the amino acid sequence of ANP in dogs and humans is similar and that serum ANP concentrations are closely correlated with left atrial pressure. However, the relationship between PVF velocity and ANP concentration as a marker of acute atrial filling in dogs is unknown. The study reported here was conducted to evaluate PVF velocity patterns in relation to ANP concentrations as a measure of atrial compliance.

Materials and Methods

Animals—Seven healthy 1- to 2-year-old male Beagles, each of which weighed 8 to 12 kg, were used in the study. Dogs were housed separately in cages and fed commercial dry food; dogs had ad libitum access to water. The study was conducted in accordance with guidelines for institutional laboratory animal care and use for the School of Veterinary Medicine at Kitasato University, Japan.

Anesthesia and preparatory procedures—Dogs were sedated by administration of butorphanol tartrate (0.2 mg/kg, IV) and atropine (0.025 mg/kg, SC); anesthesia was induced by administration of propofol (6.0 mg/kg, IV), and dogs were intratracheally intubated. Anesthesia was maintained by administration of a mixture of 2.0% isoflurane and oxygen. The respiratory rate was monitored and maintained between 35 and 45 mm Hg, and heart rate was monitored by use of an ECG.

Dogs were positioned in left lateral recumbency. Fluoroscopic guidance was used for insertion of a 6-F Swan-Ganz catheter through the right jugular vein into the pulmonary artery. With the balloon inflated, the catheter was advanced until it was wedged in a pulmonary capillary, which was confirmed fluoroscopically and by detection of characteristic pressure waveforms. The catheter in the pulmonary artery was connected to a strain-gauge manometer for PCWP measurements. The PCWP was determined visually at the end of expiration. After these procedures were completed, a stabilization period of 20 to 30 minutes was used to establish a stable baseline condition for echocardiographic measurements and measurement of the PCWP.

Volume overload—Preload was increased by IV infusion of lactated Ringer’s solution (200 mL/kg/h) for 60 minutes via a cephalic vein, which was a modification of the rate reported in another study. During the IV infusion, the influence of volume overloading on several hemodynamic variables was monitored, and the PCWP was approximately 10 mm Hg higher than the baseline value. Echocardiographic examination was performed at 10-minute intervals. Following these procedures, dogs were administered furosemide (2 mg/kg, IV) and allowed to recover from anesthesia.

Echocardiography—Transthoracic echocardiography was performed by use of an ultrasonographic unit with a 12-MHz probe. Echocardiographic measurements were performed at the end of the expiratory phase. The TMF and PVF recordings were obtained by use of pulsed-wave Doppler echocardiography, respectively. Data were stored digitally and subsequently analyzed by a single investigator. Echocardiograms were analyzed by use of the commercial analysis software package supplied with the system. The mean value of 3 cardiac cycles was calculated.

Doppler inflow across the mitral valve was measured by use of the left apical 4-chamber view; the sample volume was positioned at the tip of the mitral valve leaflets. The TMF velocity patterns were traced along the instantaneous highest velocity spectra to determine the peak velocity of the E wave and A wave, and the E:A ratio was calculated.

The PVF velocities were measured for the pulmonary vein of the right caudal lobe by use of the left apical long-axis 4-chamber view. Filter settings were kept to a minimum so that a low-frequency flow was visible. After optimal adjustment of the color gain, Doppler color flow mapping was used to detect pulmonary veins and to align the ultrasound beam with the visible portion of the extraparenchymal pulmonary veins (angle < 20°). The PVF appeared as a red signal along the distal portion of the left atrial border, which indicated flow toward the transducer. The orifice of the appropriate pulmonary vein was visible at the bottom of the red signals. The sample volume was placed appropriately 5 to 10 mm distal to the entrance of the pulmonary vein, which had an axial dimension of 1 to 2 mm. Sweep speed was set at 100 mm/s. The PVF velocity patterns were traced along the instantaneous highest velocity spectra to determine the peak velocity of the S wave, D wave, and AR wave. The S:D ratio was then calculated.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>102±11</td>
<td>103±13</td>
<td>109±13</td>
<td>117±11*</td>
<td>117±9*</td>
<td>119±11*</td>
<td>118±10*</td>
</tr>
<tr>
<td>Hct(%)</td>
<td>35±2</td>
<td>28±3</td>
<td>25±3</td>
<td>25±3*</td>
<td>23±2*</td>
<td>23±2*</td>
<td>23±2*</td>
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<tr>
<td>PCWP (mm Hg)</td>
<td>6±4</td>
<td>8±6</td>
<td>9±5*</td>
<td>10±6*</td>
<td>11±7*</td>
<td>13±8*</td>
<td>11±7*</td>
</tr>
<tr>
<td>Systolic</td>
<td>2±3*</td>
<td>8±6*</td>
<td>10±6*</td>
<td>11±7*</td>
<td>11±7*</td>
<td>11±7*</td>
<td>12±8*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>19±4</td>
<td>42±151</td>
<td>68±324</td>
<td>77±38*</td>
<td>90±38*</td>
<td>98±32*</td>
<td>105±24*</td>
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<tr>
<td>ANP (pg/mL)</td>
<td>5±1*</td>
<td>7±1*</td>
<td>8±1*</td>
<td>9±1*</td>
<td>11±1*</td>
<td>12±1*</td>
<td>12±1*</td>
</tr>
</tbody>
</table>

* Within a variable, value differs significantly from the baseline value (P < 0.001; 1P < 0.05; 1P = 0.01).
Measurement of ANP concentrations—Blood samples were collected from a cephalic vein at baseline (ie, before infusion of lactated Ringer's solution) and at 10-minute intervals thereafter. Blood samples were collected into tubes that contained EDTA; tubes were then centrifuged at 1,500 × g for 10 minutes at 4°C, and plasma was harvested. Plasma ANP concentrations were determined by a radioimmunoassay developed for measurement of human α-ANP. The ANP concentrations were corrected for the Hct%, compared with the baseline value, by use of the following equation:

\[ \text{Corrected ANP concentration} = \frac{\text{measured ANP value}}{\text{baseline Hct%}} \times \frac{\text{measured Hct%}}{\text{baseline Hct%}} \]

Statistical analysis—Data are reported as mean ± SD. Values after changes in preload conditions were compared with baseline values by use of a 1-factor repeated-measures ANOVA. A 2-way ANOVA was used to compare changes between the E:A ratio and the S:D ratio. Significance of the differences between mean values at baseline and for each condition was tested by use of the Tukey multiple comparison test. Regression analysis was performed to determine the correlation between plasma ANP concentration and mean PCWP. A significant correlation (r = 0.68; P < 0.001) was detected between the concentration of ANP and mean PCWP.
Table 2—Mean ± SD values for hemodynamic and Doppler echocardiographic data before (baseline; time 0) and during volume overloading in 7 healthy dogs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>E wave (cm/s)</td>
<td>50.0</td>
<td>62.0</td>
<td>70.0</td>
<td>70.0</td>
<td>74.0</td>
<td>79.0</td>
<td>72.0</td>
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<tr>
<td>A wave (cm/s)</td>
<td>27.0</td>
<td>35.0</td>
<td>38.0</td>
<td>40.0</td>
<td>41.0</td>
<td>44.0</td>
<td>43.0</td>
</tr>
<tr>
<td>E:A ratio</td>
<td>1.9</td>
<td>0.6</td>
<td>1.8</td>
<td>1.3</td>
<td>1.8</td>
<td>0.2</td>
<td>1.7</td>
</tr>
<tr>
<td>S wave (cm/s)</td>
<td>12.3</td>
<td>22.5</td>
<td>30.0</td>
<td>36.7</td>
<td>41.0</td>
<td>45.0</td>
<td>42.0</td>
</tr>
<tr>
<td>D wave (cm/s)</td>
<td>34.1</td>
<td>30.1</td>
<td>29.0</td>
<td>31.1</td>
<td>31.0</td>
<td>30.0</td>
<td>29.0</td>
</tr>
<tr>
<td>S:D ratio</td>
<td>0.4</td>
<td>0.2</td>
<td>0.8</td>
<td>1.1</td>
<td>1.3</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>AR wave (cm/s)</td>
<td>14.0</td>
<td>15.0</td>
<td>21.0</td>
<td>21.0</td>
<td>23.0</td>
<td>23.0</td>
<td>25.0</td>
</tr>
</tbody>
</table>

See Table 1 for key.

Results

Hemodynamic variables that markedly changed as a result of volume overloading were summarized (Table 1). Heart rate and PCWP increased significantly (P < 0.001) from baseline to the endpoint. Similarly, plasma ANP concentration increased significantly (P < 0.001) from baseline to the endpoint. Furthermore, the ANP concentration was positively correlated (r = 0.68; P < 0.001) with PCWP (Figure 1).

Representative TMF and PVF recordings were obtained during acute volume overloading (Figure 2). Echocardiographic measurements were calculated (Table 2). The TMF and PVF velocities were increased significantly by acute volume overloading. Although the E and A waves were increased significantly (P < 0.001) from the baseline values, the E:A ratio remained unchanged. However, transesophageal Doppler echocardiography was used to record the PVF pattern, which revealed the S, D, and AR waves as PVFs. The S and AR waves were increased significantly (P < 0.001) from baseline to the endpoint, whereas the D wave was unchanged. As a result, S:D ratios increased significantly (P < 0.001) from baseline to the endpoint. In addition, values for the S:D ratios were increased significantly (P < 0.001), compared to values for the E:A ratios (Figure 3).

The mean PCWP and E:A ratios were not correlated (r = −0.06; Figure 4). However, mean PCWP and S:D ratios were significantly correlated (r = 0.69; P < 0.001). Similarly, ANP concentration and E:A ratios were not correlated (r = −0.09; Figure 5).

Figure 3—Comparison of the E:A ratio (white squares) and S:D ratio (black circles) in 7 healthy dogs before (baseline; time 0) and during IV infusion of lactated Ringer’s solution (200 mL/kg/h) for 60 minutes to induce volume overload. Values for the E:A ratio. *: †Within a ratio, value differs significantly (* P = 0.01; †P < 0.001) from the baseline value.

Figure 4—Relationship between the mean PCWP and TMF (A) or between the mean PCWP and PVF (B) measured in 7 healthy dogs. The mean PCWP was significantly correlated (r = 0.69; P < 0.001) with the S:D ratio but not with the E:A ratio (r = −0.06).
Discussion

Concerning the PVF velocity patterns, the S wave corresponds to the period during ventricular systole when the left atrial pressure decreases as a result of atrial relaxation, which allows blood in the pulmonary veins to fill the left atrium. After the mitral valve opens, left atrial pressure decreases as a result of blood flowing from the left atrium into the left ventricle until the pressures equalize, which allows blood in the pulmonary veins to refill the left atrium. Therefore, the systolic and diastolic PVF velocities may reflect abnormalities in left atrial and ventricular compliance and relaxation. However, there are few data on the relationship between abnormality-related changes in atrial function during acute filling and PVF measured by use of transthoracic echocardiography. In the study reported here, we determined that PVF patterns can be evaluated in dogs by use of transthoracic echocardiography and that the abnormality in atrial filling is related to plasma ANP concentration and PVF.

Analysis of our data revealed that volume overload caused an increase in the systolic PVF velocity and a marked increase in the S:D ratio, which was closely correlated with PCWP and plasma ANP concentration. In other studies, investigators reported that systolic PVF velocity increases following volume overloading but that diastolic PVF velocity remains unchanged. In addition, the S:D ratio is strongly correlated with increases in left atrial pressure. Volume overloading increases the systolic PVF volume but not the early diastolic PVF volume in clinically normal dogs, and changing rate of the ratio between the systolic PVF volume and early diastolic PVF volume is significantly correlated with the change in mean left atrial pressure. In addition, ANP is released from the atrium in response to wall stretching, which causes vasodilatation, natriuresis, and inhibition of the renin-angiotensin-aldosterone system. It has also been reported that volume overloading causes an immediate change in the plasma ANP concentration. In addition, the ANP concentration is significantly correlated with PCWP and left atrial pressure in dogs. Analysis of data from the study reported here confirmed that ANP was released from the atrium immediately as a result of acute volume overload and that there was a significant correlation between the PVF pattern and ANP concentration. These results, which are consistent with those in other reports, indicated that the systolic PVF velocity immediately reflected atrial reservoir function and that the S:D ratio and ANP concentration indicated an acute filling abnormality in dogs.

Recently, PVF velocities have been used in conjunction with TMF velocities. It has been reported that PVF patterns have the potential to be used to differentiate pseudonormalization. In that study, investigators classified patients into 4 groups on the basis of TMF patterns (clinically normal, relaxation failure, pseudonormalization, and restrictive). The LVEDP was significantly higher and systolic PVF velocity significantly lower in the pseudonormalization and restrictive groups than in the relaxation-failure and clinically normal groups. Furthermore, the diastolic PVF velocity was significantly lower in the relaxation-failure group than in the other 3 groups. In the study reported here, systolic PVF velocity and TMF patterns were significantly increased with volume overloading, whereas diastolic PVF velocity was unaffected. Consequently, the S:D ratio increased significantly from the baseline value, whereas the E:A ratio was unchanged. Other investigators reported that the E:A ratio is unchanged with volume overloading until the LVEDP becomes moderately increased in clinically normal dogs. However, when the LVEDP exceeds approximately 20 mm Hg, an increase in the E wave and a decrease in the A wave cause a marked increase in the E:A ratio. Therefore, the PVF pattern recorded by use of transthoracic echocardiography may serve as a sensitive indicator of an acute filling abnormality in dogs.

A limitation of the study reported here was that transthoracic echocardiography was used to investigate the response to volume overloading as determined by PVF velocity. Therefore, we cannot exclude the possibility that anesthesia may have modulated PVF velocity.
A complete autonomic block was not used in this study because reflexive autonomic changes may have affected filling variables of the heart. In addition, it has been reported\(^\text{1,23}\) that PVF is affected by age, body weight, and heart rate. Additional chronic heart diseases may lead to other responses; therefore, validation of the PVF velocities in patients with heart disease and in healthy control dogs is required to evaluate the entire range of responses.

Although E:A ratios were not changed by moderate volume unloading, the S:D ratios were significantly increased in healthy dogs. In addition, the S:D ratio was significantly correlated with PCWP or plasma ANP concentration. Analysis of these results suggests that PVF patterns can be used to estimate acute filling abnormalities, which will be useful for predicting atrial filling and atrial stretch.

References