EXPERIMENTAL STUDIES

Doppler Echocardiographic Quantitation of Cross-Sectional Area Under Various Hemodynamic Conditions: An Experimental Validation In a Canine Model of Supravalvular Aortic Stenosis

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The hemodynamic effects on cross-sectional area calculated with the continuity equation were assessed in canine experiments. In 13 open chest dogs, 46 supravalvular aortic stenoses were created by aortic root banding. The cross-sectional area of the stenosis was calculated by Doppler echocardiography with application of the continuity equation before and after the following hemodynamic interventions: protocol 1, atrial pacing at 90, 120, 150 and 180 beats/min after sinus node crush; protocol 2, preload reduction by mild and severe clamping of the inferior vena cava; and protocol 3, afterload augmentation by mild and severe clamping of the descending aorta. In each observation, a dimension of the stenosis was directly measured by two-dimensional echocardiography, and the cross-sectional area was determined as a reference standard.

As a result of the hemodynamic interventions, significant changes were observed in stroke volume and pressure gradient (protocol 1), in cardiac output, stroke volume and pressure gradient (protocol 2) and in heart rate, cardiac output and pressure gradient (protocol 3). Despite these changes in hemodynamic variables, the Doppler-derived cross-sectional area showed no significant change for a given stenosis. In addition, areas calculated with the continuity equation (x) agreed well with those determined by two-dimensional echocardiography (y) (r = 0.96, p < 0.001, y = 0.97x + 0.02, SEE = ±0.06 cm²).

Thus, it is concluded that Doppler echocardiography with application of the continuity equation accurately predicts the stenotic cross-sectional area over a wide range of hemodynamic conditions in supravalvular aortic stenosis.

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In cardiac valve stenosis, the cross-sectional area of the stenosis is an important indicator of the severity of stenosis. Direct visualization of the mitral orifice by two-dimensional echocardiography can be used to determine mitral orifice area in patients with mitral stenosis (1), but this method has several limitations when applied to aortic valve stenosis (2). Recently, Doppler ultrasound quantitation of the stenotic aortic orifice area has been attempted with use of the Gorlin formula (3,4) or the continuity equation (5–7). Good agreement has been demonstrated between areas determined by the Doppler ultrasound methods and those by the conventional Gorlin catheter method in patients at rest. However, significant questions have been raised concerning the accuracy of the Gorlin formula (8,9), particularly when the flow is extremely high or low. Under a high flow condition achieved during exercise, Bache et al. (10) found a questionable increase in orifice area determined by the Gorlin formula in patients with valvular aortic stenosis. In contrast, Segal et al. (9) and Cannon et al. (11) documented experimentally that the Gorlin formula underestimates cross-sectional area in low flow states. Thus, cross-sectional area determined with the Gorlin formula is dependent on hemodynamic conditions; however, it is still unclear whether cross-sectional area determined with the continuity equation is also affected by hemodynamic challenge. The purpose of this study was to assess the effects of hemodynamic variables on the calculation of stenotic area with use of the continuity equation in a canine model of supravalvular aortic stenosis.
Methods

Animal preparation (Fig. 1). Thirteen mongrel dogs weighing 11 to 22 kg (mean 16) were anesthetized with intravenous sodium pentobarbital (25 mg/kg body weight) and were mechanically ventilated with room air by a volume-controlled respirator. The chest was opened through a median sternotomy, and the heart was suspended in a pericardial cradle. A 7F pigtail catheter was inserted into the left ventricle through the left atrial appendage. Another 7F pigtail catheter was inserted into the mid portion of the ascending aorta through the right carotid artery. These catheters were filled with saline solution and coupled to their respective Gould Statham P23ID transducers. After careful calibration and balancing, aortic and left ventricular pressures were recorded simultaneously with the lead II electrocardiogram (ECG). The aorta and main pulmonary artery were carefully separated at the proximal portion of the vessels, and the aorta was banded with an umbilical cord immediately above the sinuses of Valsalva. This cord could be easily tightened circumferentially to produce a supravalvular aortic stenosis. A total of 46 stenoses of various sizes (2 to 5 stenoses for each animal) were created in the 13 dogs. Cardiac output was serially measured by an electromagnetic flow meter (Nihon Kohden MVF-2100), with a probe attached to the main pulmonary artery.

The experiments performed conformed to the Position of the American Heart Association of Research Animal Use adopted November 11, 1984.

Experimental protocol. Three experimental protocols were designed:

Protocol 1 examined the effect of heart rate on the determination of stenotic cross-sectional area in three dogs. The sinoatrial node was temporarily crushed with a Pean clamp and the heart was paced at 90, 120, 150 and 180 beats/min by atrial pacing. A total of 13 stenoses were created. In three experimental series, because the spontaneous rate after sinoatrial node crush exceeded 90 beats/min, the pacing rate began at 120 beats/min. In another series, the highest pacing rate (180 beats/min) could not be used because of frequent ventricular premature complexes.

Protocol 2 examined the effect of preload reduction on area determination in five dogs. The preload reduction was performed by clamping the inferior vena cava in two degrees of closure: mild, to the extent that the cardiac output was reduced by approximately 25% of the baseline value, and severe, a reduction of 75%. A total of 13 stenoses were created. In eight experimental series, both mild and severe preload reductions were achieved. In the remaining five series, only the effect of mild preload reduction was examined, because the severe preload reduction resulted in significant fluctuation of the hemodynamic state during data acquisition.

Protocol 3 examined the effect of afterload augmentation performed by clamping the thoracic descending aorta in five dogs. A total of 20 stenoses were created. In each experimental series, the descending aorta was clamped in two degrees of closure: mild, to the extent that the ascending aortic pressure increased by 20 to 30 mm Hg, and severe, an increase of >50 mm Hg.

Ultrasound measurements (Fig. 2). The ultrasound apparatus used in this study was a Toshiba Sonolayer SSH 65A. Pulsed and continuous wave Doppler ultrasound signals were analyzed in real time by fast Fourier transform and recorded by a line scan recorder (Toshiba LSR-20B) simultaneously with the ECG at a paper speed of 100 mm/s. A hard copy of the two-dimensional echocardiogram was produced by the same line scan recorder.

The transducer was placed at the apical position and angled medially to depict the long-axis image of the left ventricular outflow tract in a left anterior oblique equivalent view. Flow velocity proximal to the stenosis was measured by pulsed Doppler ultrasound with a 2.5 MHz transducer (Fig. 2A). A sample volume was positioned at approximately the center of the aortic anulus. Although the Doppler beam was directed to be as parallel as possible to the outflow tract, the angle of incidence between the Doppler beam and the axis of the outflow tract ranged from 0° to 33° (mean 11°). Flow velocity of the stenotic jet was measured by continuous wave Doppler ultrasound with a 2.5 MHz transducer coupled to the apical surface of the heart (Fig. 2B). In recording the stenotic jet velocity, the transducer was carefully manipulated to obtain the highest peak velocity, and the Doppler beam was positioned approximately parallel to the flow direction. The diameter of the aortic anulus was mea-
Figure 2. Doppler echocardiographic measurements. A. Flow velocity proximal to the stenosis was measured by pulsed Doppler ultrasound from the apical approach with the sample volume (SV) positioned at the center of the aortic (AO) annulus. The angle of incidence (θ) between the Doppler beam and the center line of the left ventricular (LV) outflow tract was obtained on the mid-systolic-gated two-dimensional echocardiogram. B. Flow velocity in the stenotic jet was measured by continuous wave Doppler ultrasound from the same apical approach. A careful search for the highest peak velocity was made with the aid of the audio signal obtained by recording the stenotic jet velocity. C. Diameter of the aortic annulus (D1) was measured on the mid-systolic-gated two-dimensional echocardiogram of the long-axis image of the left ventricular (LV) outflow tract. D. The minimal diameter of the stenosis (Ds) was measured on the long-axis image of the stenotic portion obtained by two-dimensional echocardiography. LA = left atrium.

The diameter of the aortic annulus was calculated as the distance between the trailing edge and the leading edge of the aortic annulus on the mid-systolic-gated long-axis image of the left ventricular outflow tract (Fig. 2C). In each experiment, the minimal diameter of the stenosis was measured on the long-axis image of the stenotic lesion by two-dimensional echocardiography (Fig. 2D). During ultrasound recording, artificial ventilation was discontinued for 10 to 15 s to avoid the influence of respiration on the hemodynamic variables.

Data analysis. At least six clearly recorded beats, which were not always consecutive, were measured and averaged for quantitative analysis. The time-velocity integral of the flow proximal to the stenosis (TV1) was defined as the area under the peak temporal velocity envelope of the Doppler shift frequency pattern, corrected for the angle of incidence of the Doppler beam. The time-velocity integral of the stenotic jet flow (TV2) was obtained in the same way but without angle correction. These areas were digitized by a digital planimeter (Planix 7). Cross-sectional area proximal to the stenosis (A1) was calculated with use of the mid-systolic diameter of the aortic annulus, assuming circular shape. According to the theoretic consideration described previously (5), the cross-sectional area of the stenosis (A2) was determined by the formula A2 = A1 x TV1 / TV2. The cross-sectional area of the stenosis was also determined with use of the diameter of the stenotic portion (Ds), correctly measured on the two-dimensional echocardiogram: Area = \( \pi(D_s/2)^2 \).

Reliability and reproducibility of the measurements. We validated the reliability of the cross-sectional area calculated...
from the measurement of the diameter of the stenosis. In 27 stenoses, we carefully visualized the short-axis image by two-dimensional echocardiography and digitized the cross-sectional area. Good agreement was found between areas directly digitized by a planimeter (y) and those calculated from the diameter, assuming a circular shape (x) (y = 1.04x + 0.13, r = 0.97, p < 0.001, SEE = ±0.03 cm²).

To test the reliability and reproducibility of the measurements, one observer on two occasions (for intraobserver variability) determined the cross-sectional area of the stenosis with the continuity equation and also with the stenotic diameter from the two-dimensional echocardiographic image in 10 randomly selected sets of ultrasound recordings. Another observer independently performed the area determinations for the same 10 sets of recordings (for interobserver variability). The observers did not know each other’s results. The mean differences between the observations were 0.01 ± 0.01 cm² (intraobserver) and 0.02 ± 0.01 cm² (interobserver) for cross-sectional areas determined with use of the continuity equation and 0.03 ± 0.02 cm² (intraobserver) and 0.03 ± 0.04 cm² (interobserver) for those determined by two-dimensional echocardiography. The mean absolute differences between the observations expressed as a percent of the first observer’s first observation were 3.8% (intraobserver) and 4.0% (interobserver) for areas determined with the continuity equation and 10.2% (intraobserver) and 10.5% (interobserver) for those determined by two-dimensional echocardiography.

Statistical analysis. All values are expressed as mean values ± SD. The significance of difference in the variables obtained for each condition studied was assessed by analysis of variance and paired t tests. When the number of the observations differed among conditions, only paired data were used for statistical analysis. Statistically significant probability values were accepted at p < 0.05. Correlation between the areas determined with use of the continuity equation and those determined by two-dimensional echocardiography was performed by simple linear regression analysis.

Results

Hemodynamic conditions and cross-sectional areas during interventions. Overall results of the effects of the changes in heart rate, preload and afterload on the hemodynamic variables and the cross-sectional area determined with the continuity equation are summarized in Table 1. Individual data on hemodynamic indexes and stenotic cross-sectional area obtained in protocols 1, 2 and 3 are plotted in Figures 3, 4 and 5, respectively. In each condition achieved by the hemodynamic interventions, the cross-sectional area of the stenosis determined by two-dimensional echocardiography showed no significant change for a given stenosis.

Effects of changes in heart rate (Fig. 3). Cardiac output showed no difference among the four pacing rates. Stroke volume was significantly reduced as pacing rate increased (p < 0.005). The instantaneous maximal pressure gradient significantly decreased at a heart rate of 150 and 180 beats/min compared with that at a slower pacing rate (p < 0.05 for a heart rate of 150 versus 90 and 120 beats/min, and for a heart rate of 180 versus 150 beats/min, p < 0.01 for a heart rate of 180 versus 90 and 120 beats/min). Cross-sectional
Effects of preload reduction (Fig. 4). Heart rate was not significantly affected by preload reduction. Cardiac output ($p < 0.005$) and stroke volume ($p < 0.01$ for baseline versus mild preload reduction; $p < 0.001$ for baseline versus severe preload reduction and for mild versus severe reduction), as well as the instantaneous maximal pressure gradient ($p < 0.001$), were significantly decreased as preload was reduced. The stenotic cross-sectional area determined with use of the continuity equation showed no significant change for the same preparation.

Effects of afterload augmentation (Fig. 5). Heart rate was significantly decreased by afterload augmentation ($p < 0.01$ for baseline versus mild afterload augmentation; $p < 0.005$ for baseline versus severe augmentation and for mild versus severe augmentation), as was cardiac output ($p < 0.05$ for baseline versus mild augmentation and for mild versus severe augmentation) and for baseline versus severe augmentation ($p < 0.01$ for baseline versus severe augmentation). The instantaneous maximal pressure gradient was significantly reduced as afterload was increased ($p < 0.005$). Despite these changes in the hemodynamic conditions, the Doppler-derived cross-sectional area of the stenosis showed no significant change.

Accuracy of cross-sectional area (Fig. 6). Regardless of the intervention performed, the stenotic cross-sectional areas determined with the continuity equation showed excellent agreement with those determined by two-dimensional echocardiography ($r = 0.96, p < 0.001$, $y = 0.95x + 0.02, \text{SEE} = \pm 0.06 \text{cm}^2$, where $x$ denotes the cross-sectional area determined by two-dimensional echocardiography and $y$ the area determined with the continuity equation).

Comparison of the Gorlin method with the continuity equation method (Fig. 7). In experimental protocol 1 (heart rate control), the stenotic cross-sectional area determined with the Gorlin formula was compared with the area determined with use of the continuity equation. There was a fair overall correlation between areas determined with the continuity equation and those determined with the Gorlin method ($r = 0.76, p < 0.001, n = 48$). The Gorlin-derived area showed a significantly lower value than the Doppler-derived area.
derived area \( y = 0.58x + 0.06 \), where \( x \) denotes areas determined with the continuity equation method and \( y \) denotes areas determined with the Gorlin formula. This was evident in the dogs whose stroke volume was <10 ml \( (n = 15, y = 0.13x + 0.13) \) and less evident in the dogs whose stroke volume was >20 ml \( (n = 7, y = 0.85x + 0.02) \).

**Discussion**

In this study we assessed the effect of changes in hemodynamic variables on the calculation of the cross-sectional area with use of Doppler echocardiography in an open chest canine model of supravalvular aortic stenosis. The results demonstrate that the present Doppler method enables reliable estimation of stenotic cross-sectional area, independently of a wide range of changes in heart rate, preload and afterload.

**Implications of pressure gradient across a stenosis.** Clinically, the pressure gradient is widely used for the evaluation of the severity of a stenosis, and Doppler echocardiography permits noninvasive estimation of the gradient with satisfactory accuracy by means of the simplified Bernoulli equation (12–15). However, the pressure gradient depends directly on the flow rate. Therefore, though the pressure gradient is an important indicator of the pressure load imposed on the
upstream chamber of the heart, it is not necessarily a measure of the severity of the stenosis, especially in a severe case (16) or in a patient with low cardiac output (17) or additional regurgitation (18).

**Hemodynamic effects on stenotic orifice area.** Previous Doppler echocardiographic studies (5–7) have shown that use of the continuity equation allows a reliable determination of stenotic orifice area in humans at rest. However, few studies have referred to hemodynamic effects on the stenotic orifice area. Cannon et al. (11) demonstrated that the stenotic porcine valve area digitized on videotape showed no significant change over a wide variety of flow volumes in a flow model experiment. Gorlin et al. (19) also documented that the mitral orifice area did not change significantly before or after exercise in patients with mitral stenosis. Recently, Abascal et al. (20) preliminarily documented that stenotic aortic orifice area determined with the continuity equation showed no change after dobutamine infusion. However, Bache et al. (10) demonstrated that aortic orifice area calculated with the standard Gorlin formula showed a slight but statistically significant increase during exercise in isolated aortic stenosis. They did not verify whether the area increased during exercise; rather, they noted that this result might be attributable to a computational artifact involved in the standard Gorlin formula, in which the square root of the mean pressure gradient is used in practice where, in theory, the mean square root of the instantaneous pressure gradient should be used (10). Because the continuity equation method does not require such a mathematic substitution, the accurate determination of stenotic orifice area by this method may be a promising means of obtaining specific information on the severity of a stenotic lesion under a wide variety of hemodynamic conditions.

**Implications of the present experimental design.** In this study we designed a canine model closely resembling supravalvular aortic stenosis. This model is similar in design to that used by Smith et al. (21) to validate the Doppler-derived pressure gradient in aortic stenosis. In a steady flow model experiment we demonstrated that the stenotic orifice area determined with use of the continuity equation agreed well with the actual orifice area regardless of the changes in volumetric flow rate. In a living subject, multiple factors, including preload, afterload, heart rate and contractility, might be complexly intertwined to achieve the equilibrated hemodynamic state. Because such a complex regulation of the hemodynamic condition can hardly be simulated by a flow model experiment, we designed the present animal experiment for further investigation.

Although afterload augmentation (protocol 3) decreased the cardiac output by 6.7% and 13.9% from the basal condition for mild and severe afterload augmentation, respectively, the stroke volume did not change significantly because of the counterbalanced reduction in heart rate. In contrast, when the heart rate was controlled by atrial pacing (protocol 1), the cardiac output remained constant for different pacing rates, and consequently, the maximal 50% reduction in stroke volume could be obtained. Also, preload reduction by partial clamping of the inferior vena cava (protocol 2) resulted in about a 25% reduction in cardiac output, which can be found clinically, for example, in a patient with diminished cardiac performance. However, the low output state (<30% of the control condition) produced by the severe preload reduction might not be seen in living humans. Nevertheless, the calculated stenotic cross-sectional area showed no significant change, a finding in agreement with the directly measured cross-sectional area of the stenosis. When aortic stenosis is associated with mitral stenosis, cardiac output may be decreased because of reduced left ventricular filling volume. Under such conditions, the pressure gradient across the aortic valve may be inadequate to assess the degree of aortic stenosis. Our experimental results indicate that even under such low flow conditions, the present Doppler-derived method provides an accurate value for the stenotic area.

**Previous clinical studies (3–7) have generally used the area determined with use of the Gorlin formula as a reference standard.** However, the Gorlin method has been shown to include the possibility of 20% to 40% error even in the absence of regurgitation; (8) and, more importantly, a stenotic area determined with the Gorlin method underestimates the actual area under critically low flow conditions (9,11). Our results were consistent with previous reports in which area determination by the Gorlin method results in a significant underestimation of the actual area under such critically low flow conditions as those generally seen in our experiment. Thus, it may not be adequate to use the Gorlin method of determining cross-sectional area as a reference standard in our study.

**Limitations of the study.** The present model of supravalvular stenosis may differ in morphologic and hemodynamic behavior from valvular stenosis in clinical patients. First, the phasic change in the cross-sectional area is expected to be smaller in supravalvular than in valvular stenosis, because the opening and closing motion of the valve might be affected by the flexibility of the valve structure, especially when the flow is critically low. Second, because the pressure loss across the hourglass-shaped stenosis is less than that in an orifice stenosis, the relation between the stenotic jet velocity and the proximal velocity in the supravalvular stenosis might be different from that in the valvular stenosis even when both have the same cross-sectional area. Third, irregular adhesion of the leaflets in valvular stenosis might distort the flow direction and velocity profile at the stenosis, which has been encountered as a possible limitation to applying the present Doppler method to valvular aortic stenosis. Thus, the results of the present study might not be directly applicable to valvular stenosis.

The accuracy of the quantitated area was assessed by
comparing it with the area obtained by two-dimensional echocardiography. The cross-sectional area to be used as a reference standard should preferably be determined on the short axis image of the stenosis. However, in the present open chest setting, significant care and time are needed to obtain the exact short-axis image of the stenosis by two-dimensional echocardiography. In 27 preparations of the stenosis, we preliminarily compared the cross-sectional area that was directly digitized on the short-axis image and the area calculated with use of the diameter measured on the long-axis image of the stenosis, assuming a circular shape. As a result, good agreement was found between the areas, and thus, the cross-sectional area of the stenosis was determined with use of the diameter of the stenosis. Two-dimensional echocardiography has a limitation in resolution, but we made every effort to lessen the error in the measurement of diameter by using the optimal condition of a high resolution transducer. As a result, a clear image of the stenosis could be obtained, and the diameter ranging from 4 to 12 mm could be measured without difficulty and with good reproducibility. The results indicate the accuracy of the cross-sectional area of the stenosis obtained with two-dimensional echocardiography, in contrast to that in patients with valvular aortic stenosis (2).

We assessed the hemodynamic effects of heart rate, preload and afterload on area calculation, but we did not verify the effect of contractility. However, because the present area determination is, in principle, based on the conservation of flow volume rate at two different positions in the tract, a change in stroke volume may be the primary factor affecting the area determination.

Conclusions. The present experimental study revealed that in a model of supravalvular aortic stenosis the Doppler echocardiographic applications of the continuity equation accurately predict stenotic cross-sectional area over a wide range of hemodynamic conditions.

References