Quantitative Assessment of Aortic Elasticity With Aging Using Velocity-Vector Imaging and Its Histologic Correlation

Sung-Ai Kim, Kyung Hye Lee, Ho-Yeon Won, Sungha Park, Ji Hyung Chung, Yangsoo Jang, Jong-Won Ha

Objective—Velocity-vector imaging (VVI) represents a valuable new method for noninvasive quantification of vascular properties associated with aging. The purpose of this study was to assess the correlations between VVI parameters and histological changes with aging.

Approach and Results—Fourteen mongrel dogs were classified as either young (n=7; age, 1–2 years; female; weighing 22–29 kg) or senescent (n=7; age, 8–12 years; female; weighing 36–45 kg). The short-axis image of the descending thoracic aorta was obtained for VVI analysis with transesophageal echocardiography. The location of the image was identified using fluoroscopic guidance, and the aortic tissue was extracted. After dividing the aortic wall into 6 segments, both regional and segmental tissue collagen and elastin contents were quantified and correlated with the aortic elastic properties. In the regional analysis, the M-mode–derived aortic dimensions and elastic moduli except for intima-media thickness were not significantly different between the groups, whereas the VVI-derived aortic area and fractional area changes showed more dilated and stiffer aorta in senescent dogs. Also, fractional area change was significantly correlated with the tissue collagen content unlike the M-mode–derived elastic moduli. In the segmental analysis, the radial velocity, circumferential strain, and strain rates of VVI were more reduced in senescent dogs than young dogs, and the radial velocity and circumferential strain showed independent associations with the collagen content of the corresponding aortic wall.

Conclusions—VVI was a feasible method for direct quantification of aortic elastic properties with a significant histological correlation. (Arterioscler Thromb Vasc Biol. 2013;33:1306-1312.)

Key Words: aging ■ aorta ■ collagen ■ elasticity ■ velocity-vector imaging

Aging is a physiological process associated with an increase in cardiovascular morbidity and mortality even in the absence of known cardiovascular risk factors.1 The main feature of arterial aging is the thickening, dilation, and stiffening of the artery,2,3 which is described as senile arteriosclerosis.4 The repeated cycles of distension and elastic recoil of the arterial wall accelerate the fragmentation and depletion of elastin, leading to a substantial increase in the inner diameter and the deposition of collagen with increasing stiffness.5 As a result, the stiffening of the proximal aorta and early wave reflection give rise to the development of isolated systolic hypertension, left ventricular hypertrophy, and heart failure with a basis on a ventricular-vascular coupling mechanism; all of these factors lead to an increase of cardiovascular mortality with aging.5,6

Thus, the assessment of mechanical properties of the artery in humans using noninvasive techniques is of growing importance. For quantification of aortic elastic properties, there are many noninvasive measures, including pulse wave velocity and echocardiographic techniques presented as distensibility, strain, and pressure-strain elastic moduli.7–11 However, these methods represent a global estimation of arterial elasticity and, therefore, have limitations because arterial changes usually begin as regional changes. With the availability of more advanced ultrasound techniques, a novel automated speckle-tracking method using velocity-vector imaging (VVI) software has facilitated the assessment for angle-independent and instantaneous quantification of arterial elastic properties by providing the 2-dimensional–derived tissue radial velocity (RV), circumferential strain, and strain rate in both regional and segmental aspects.12–15

We previously showed that arterial assessment using VVI represents a new method for quantifying vascular alteration not only in the clinical conditions of vasculitis12,13 but also in other disorders associated with aging.14 Despite these investigations, further histological validation will facilitate the clinical application of VVI. Therefore, the purpose of this study was to compare the parameters of VVI between young and senescent dogs and to assess the correlations between the VVI parameters and histological changes.
Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Baseline Characteristics

The young and senescent dogs differed significantly in body weight (24±2 versus 40±2 kg; \( P < 0.001 \)) and both systolic (85±11 versus 120±15 mm Hg; \( P = 0.018 \)) and diastolic (55±11 versus 90±10 mm Hg; \( P = 0.006 \)) blood pressures. The heart rates between the groups were not different (94±12 bpm versus 101±18 mm Hg; \( P = 0.371 \)). The arterial elastic properties using multiple measures and the tissue contents of the aortic wall according to age group are summarized in Table 1.

Pulse wave velocity, an invasive measure of arterial stiffness in the longitudinal direction, was higher in senescent dogs than in young dogs, with borderline statistical significance. Although intima-media thickness was significantly higher in senescent dogs, other M-mode–derived parameters (such as aortic dimension and pressure-elastic moduli) were not different between the young and senescent dogs. By contrast, VVI–derived parameters showed that a cross-sectional area of the aortic wall was more dilated, and fractional area change (FAC) was significantly reduced in the senescent dogs. In segmental analysis, instantaneous data of aortic deformation derived from VVI, such as RV, circumferential strain, and strain rate, were significantly reduced in the senescent dogs. In addition, tissue quantification from the aortic wall revealed that the elastin content (\( \mu \text{g/mg aorta} \)) was significantly decreased and the collagen content (\( \mu \text{g/mg aorta} \)) was significantly increased in the aortic wall of senescent dogs compared with those of young dogs. Histological sections showed that blue-stained collagen fibers were higher in density in the aortic wall of senescent dogs than they were in young dogs (Figure 1).

Relationships Between Aortic Elastic Indices and Tissue Content

Table 2 shows the correlations between indices of aortic elastic properties and tissue contents of collagen and elastin per subject or per segment. Pulse wave velocity and M-mode–derived pressure-elastic moduli had no significant relationship with the contents of collagen and elastin, whereas VVI–derived FAC showed a significant inverse correlation with the collagen content of the aortic wall.

In the segmental analysis, the RV, circumferential strain, and strain rate of VVI were significantly correlated with the collagen content of the corresponding segment of the aortic wall. However, the mean or segmental content of elastin showed no significant correlations with any aortic indices of elastic properties (Figure 2).

Independent Associations of VVI Parameters With Tissue Collagen Content

When the association between the RV, circumferential strain, strain rate of VVI, and tissue collagen content was assessed (after adjustment for covariates of age group, weight, heart

### Table 1. Comparison of Aortic Elasticity and Tissue Content According to the Age Groups

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Senescent</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per subject</td>
<td>n=7</td>
<td>n=7</td>
<td></td>
</tr>
<tr>
<td>Pulse wave velocity, cm/s</td>
<td>4.34±1.27</td>
<td>5.78±1.03</td>
<td>0.055</td>
</tr>
<tr>
<td>M-mode parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-systolic dimension, mm</td>
<td>11.6±1.7</td>
<td>13.1±2.2</td>
<td>0.276</td>
</tr>
<tr>
<td>End-diastolic dimension, mm</td>
<td>10.5±1.3</td>
<td>12.0±2.2</td>
<td>0.179</td>
</tr>
<tr>
<td>Intima-media thickness, mm</td>
<td>0.31±0.10</td>
<td>0.44±0.07</td>
<td>0.016</td>
</tr>
<tr>
<td>( \beta )-stiffness index</td>
<td>4.23±1.34</td>
<td>4.32±1.74</td>
<td>0.848</td>
</tr>
<tr>
<td>Distensibility coefficient, kPa(^{-1})×10(^{-2})</td>
<td>21.3±20.7</td>
<td>13.6±11.0</td>
<td>0.655</td>
</tr>
<tr>
<td>Young’s elastic modulus, kPa/mm</td>
<td>132±77</td>
<td>141±67</td>
<td>0.482</td>
</tr>
<tr>
<td>VVI parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta, maximal area, cm(^2)</td>
<td>1.16±0.21</td>
<td>1.78±0.20</td>
<td>0.002</td>
</tr>
<tr>
<td>Aorta, minimal area, cm(^2)</td>
<td>1.01±0.18</td>
<td>1.60±0.17</td>
<td>0.002</td>
</tr>
<tr>
<td>Fractional area change, %</td>
<td>13.7±2.2</td>
<td>10.1±1.3</td>
<td>0.006</td>
</tr>
<tr>
<td>Per segment</td>
<td>n=42</td>
<td>n=42</td>
<td></td>
</tr>
<tr>
<td>VVI parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radial velocity, cm/s</td>
<td>0.52±0.24</td>
<td>0.39±0.19</td>
<td>0.014</td>
</tr>
<tr>
<td>Circumferential strain, %</td>
<td>3.82±3.20</td>
<td>2.35±1.85</td>
<td>0.012</td>
</tr>
<tr>
<td>Circumferential strain rate, 1/s</td>
<td>0.88±0.65</td>
<td>0.55±0.37</td>
<td>0.006</td>
</tr>
<tr>
<td>Tissue content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen, ( \mu \text{g/mg aorta} )</td>
<td>1.57±0.93</td>
<td>3.62±2.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Elastin, ( \mu \text{g/mg aorta} )</td>
<td>153.7±72.9</td>
<td>102.0±40.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

VVI indicates velocity-vector imaging.
rate, systolic and diastolic blood pressure, aortic area, and intima-media thickness), the RV and circumferential strain had independent associations with the collagen contents of the corresponding segment of the aortic wall (Table 3).

Reproducibility
The reliability of VVI variables was excellent with high intra-class correlation coefficients (intraobserver, 0.91, 0.94, and 0.91; interobserver, 0.94, 0.94, and 0.92 for RV, circumferential strain, and strain rate, respectively). A Bland–Altman plot showed good intra- and interobserver agreements for RV, circumferential strain, and strain rate (Figure 3).

Discussion
In this study, we investigated the biomechanical and histological changes of the aortic wall that occur with aging. To the best of our knowledge, this study is the first to assess the direct relationships between in vivo aortic elastic properties and tissue contents, and it demonstrated the importance of VVI as a valuable means for assessing aortic elastic properties with a significant histological correlation.

VVI for Assessment of Aortic Elastic Properties
In earlier experimental models designed to determine the in vivo elastic properties of the aorta, instantaneous pressure and diameter signals were converted into stress and strain using a thick-walled cylindrical tube model. Considering the nonlinearity of the stress–strain relationship and the anisotropy of the wall, the individual elastic modulus of structural constituents (elastin, collagen, and smooth muscle) was introduced for better description of elastic behavior of aortic wall in conscious dogs.

Because of the invasive nature of these experiments, noninvasive measures (such as ultrasonic delineation) have been used to assess aortic elastic properties in clinical applications. In this study, VVI enabled automated aortic wall border detection from transesophageal echocardiography images and could quantify the instantaneous deformation in both circumferential and radial directions.

Table 2. Correlation Between Indices of Aortic Elastic Properties and Tissue Contents of Collagen and Elastin

<table>
<thead>
<tr>
<th></th>
<th>Collagen</th>
<th></th>
<th>Elastin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>PValue</td>
<td>r</td>
<td>PValue</td>
</tr>
<tr>
<td>Per subject</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse wave velocity</td>
<td>0.412</td>
<td>0.143</td>
<td>−0.079</td>
<td>0.788</td>
</tr>
<tr>
<td>β-stiffness index</td>
<td>0.349</td>
<td>0.221</td>
<td>−0.064</td>
<td>0.829</td>
</tr>
<tr>
<td>Distensibility</td>
<td>−0.165</td>
<td>0.573</td>
<td>0.174</td>
<td>0.553</td>
</tr>
<tr>
<td>Young elastic modulus</td>
<td>0.253</td>
<td>0.383</td>
<td>0.112</td>
<td>0.703</td>
</tr>
<tr>
<td>Fractional area change</td>
<td>−0.715</td>
<td>0.004</td>
<td>0.153</td>
<td>0.601</td>
</tr>
<tr>
<td>Per segment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radial velocity</td>
<td>−0.387</td>
<td>&lt;0.001</td>
<td>0.140</td>
<td>0.203</td>
</tr>
<tr>
<td>Circumferential strain</td>
<td>−0.292</td>
<td>0.007</td>
<td>0.136</td>
<td>0.218</td>
</tr>
<tr>
<td>Circumferential strain rate</td>
<td>−0.259</td>
<td>0.017</td>
<td>0.158</td>
<td>0.151</td>
</tr>
</tbody>
</table>

Table 3. Regression Analyses Depicting the Independent Association of Velocity-Vector Imaging Parameters With the Collagen Content of the Corresponding Segment of the Aortic Wall

<table>
<thead>
<tr>
<th>Parameters*</th>
<th>β-Coefficient</th>
<th>t</th>
<th>PValue</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radial velocity</td>
<td>−2.58</td>
<td>−3.49</td>
<td>0.001</td>
<td>0.53</td>
</tr>
<tr>
<td>Circumferential strain</td>
<td>−0.14</td>
<td>−2.20</td>
<td>0.03</td>
<td>0.48</td>
</tr>
<tr>
<td>Circumferential strain rate</td>
<td>−0.46</td>
<td>−1.47</td>
<td>0.14</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*Each parameter was adjusted for covariates of age group, weight, systolic and diastolic blood pressure, heart rate, aortic area, and intima-media thickness.
The total stress generated by the wall to resist stretching is commonly attributed to the combined effects of pure elastic, viscous, and inertial behavior.\textsuperscript{19} Peterson et al\textsuperscript{20} stated that inertial force is negligible in physiological conditions, indicating that the behavior of arteries was mainly viscoelastic. However, wall viscosity affected by vascular smooth muscle activation was insignificant in this study because vascular smooth muscle is not significantly activated in the control state or in anesthetized animals.\textsuperscript{21} Thus, VVI-derived parameters in this study merely indicated the purely passive elastic behavior of the aortic wall.

The biomechanical properties of the major arteries are largely dependent on the qualitative or quantitative changes of scleroproteins, such as collagen and elastin; these are the major structural constituents that determine the passive mechanical properties of the aortic wall.\textsuperscript{22} Elastin confers elasticity, whereas collagen, which is $\approx 100\times$ stiffer than elastin, provides rigidity.\textsuperscript{23} In a stress–strain relationship at low pressure, the resistance to stretch is the result of elastin alone, whereas at high pressure, the resistance is caused by collagen. This trend indicates the presence of increasing collagen recruitment and mechanical function transitioning to the stiffer at high strain region.\textsuperscript{24,25} In this study, the maximal values of the segmental RV, circumferential strain, and strain rate were significantly correlated with the collagen content of the corresponding segment, whereas the elastin content was not. This finding is consistent with those of previous studies showing that biomechanical properties in vitro at maximal strain above the breakpoint may reflect the state of the collagen within the vessel,\textsuperscript{26} and that maximal stiffness is positively correlated with collagen-associated fluorescence in the rat aorta.\textsuperscript{27} Further, after adjusting for the confounding variables affecting the quantitative change of collagen, the RV and circumferential strain showed independent associations with the tissue collagen content of the aortic wall.

**Biomechanical and Histological Alterations of the Aortic Wall With Aging**

The dominant feature of arterial aging is the thickening, dilatation, and stiffening of the arterial wall accompanying the biochemical alterations.\textsuperscript{2,3} To maintain basal levels of tensile stress (Laplace law), progressive thickening of the vessel wall occurs as a result of the proliferation and migration of vascular smooth muscle responding to the mechanical stretch created by synthesis of collagen.\textsuperscript{23,28,29} In addition, the fatiguing effects of tensile stress by repeated pulsation produce fracture and breakdown of elastic fibers, which lead to a substantial increase in the inner diameter of the aortic wall.\textsuperscript{30,31} In the present study, the aortic walls of senescent dogs were more thickened and dilated than those of young dogs. These morphological changes as increased vessel area in senescent dogs were identified by speckle tracking the instantaneous vessel wall motion using the VVI method, whereas M-mode–derived dimensions except for intima-media thickness could not differentiate these structural alterations between the groups.

For the functional properties of vascular aging, FAC from VVI was significantly reduced in the senescent dogs, whereas M-mode–derived elastic moduli (including $\beta$-stiffness, distensibility and Young pressure-strain elastic modulus) showed no significant difference between the groups. Also, in the segmental analysis, instantaneous data of aortic deformation with VVI as RV, circumferential strain, and strain rate were significantly reduced in senescent dogs compared with those of young dogs. These findings suggest the limitations of M-mode as a 1-dimensional approach and the superiority of
2-dimensional approach by speckle tracking that enables the instantaneous vessel wall motion for a correct estimation of the structural and functional changes in entire circumference of vessel wall.

Biochemical alteration of arterial aging is the fragmentation and depletion of elastin and deposition of collagen undergoing nonenzymatic glycation and oxidation of free amino groups to form advanced glycation end products. When we quantified the tissue contents within the aortic wall, the collagen content was increased, and the elastin content was decreased with aging; these findings are in line with the results of previous works, although conflicting results also have been reported regarding the changes of the absolute amount of scleroprotein with aging. This discrepancy may be related to the differences in methodology, the source of tissue, species differences (including rats, dogs, and nonhuman primates), and data units between the studies where collagen values were expressed per square millimeter or per milligram dry weight of the aortic tissue.

Figure 3. Bland–Altman plots for intra- and interobserver agreements of velocity-vector imaging parameters. RV indicates radial velocity; and SR, strain rate.

There are limitations to our study. First, our sample size was relatively small; a larger sample size would provide more conclusive results. To obtain in vivo cross-sectional images of descending thoracic aorta during transesophageal echocardiography, all experiments were performed under general anesthesia, which could produce age-dependent effects on aortic mechanics. All dogs included in this study were female because of the difficulty in finding senescent male dogs, hence our results were limited to the characteristics of aortic elasticity of female dogs with aging. We obtained the image of descending thoracic aorta at which VVI analysis would be performed and then quantified the tissue contents at the same location to correlate the histology. Thus, our results reflect the association between the collagen and elastin composition and elastic property of the descending thoracic aorta. Regarding the variation of elastin:collagen gradient along the course of the aorta, our results might not be applicable to entire aorta. Finally, the blood pressures of senescent dogs were higher than those of young dogs. Because the blood pressure is one of the important determinants of vascular expansion, it may not be possible to discriminate the impact of hypertension on aortic indices independently of the aging process in this study. Wang et al reported that, in similar ages, hypertensive patients showed dilated aortic diameter and decreased ascending aortic wall motion compared with those of healthy subjects. However, in our previous study assessing the elastic properties of carotid artery with VVI in healthy volunteers, we demonstrated that...
the FAC of older subjects (50–59 years) was significantly lower than those of younger subjects (20–29 years; FAC, 10.8% versus 17.8%; 735–740). Despite similar blood pressures. In addition, RV, strain, and strain rate of older subjects were also significantly lower than those of younger subjects (RV, 0.13 versus 0.27 cm/s; strain, 4.69% versus 8.53%; strain rate, 0.35 versus 0.72 s⁻¹; all P values <0.01). These findings suggest that VVI parameters could be an independent marker of vascular aging independent of hypertension.

**Conclusion**

VVI exhibited the unique ability to provide both regional and segmental alterations of aortic elastic properties with aging, and the magnitude of instantaneous aortic deformation computed by VVI analysis showed significant associations with the collagen content of the aortic wall. In clinical applications, VVI would provide better description of the elastic behavior of the aortic wall and could be useful to detect early vascular changes in various clinical situations.

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**Disclosures**

None.

**References**

Significance

Velocity-vector imaging as a novel automated speckle-tracking method has facilitated the angle-independent and instantaneous quantification of arterial elastic properties. This is the first to assess the direct relationships between in vivo aortic elastic properties using velocity-vector imaging and the tissue contents of the aortic wall. We identified that velocity-vector imaging exhibited the unique ability to provide both regional and segmental alterations of aortic elastic properties with aging and showed a significant association with the collagen content of aortic wall. The assessment of arterial elasticity using velocity-vector imaging would provide better understanding of elastic behavior of vessel wall and could be useful to detect early structural and functional vascular changes in various clinical conditions.
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Materials and Methods

Animals
Fourteen mongrel dogs were classified as either young (n=7, mean age: 1 to 2 years, female, weighing 22 to 29 kg) or senescent (n=7, mean age: 8 to 12 years, female, weighing 36 to 45 kg). All animal procedures were performed with the approval of the Institutional Animal Care and Use Committee, and the dogs were handled in accordance with National Institutes of Health Guidelines for the care and use of animals. Animals were housed in rooms maintained at 20-23°C with a 12:12 hour light-dark cycle for seven days as an adaptive period. All dogs were free of apparent cardiovascular disease at the time of the study and had no history of cardiovascular problems.

Anesthesia and hemodynamic monitoring
Premedication was accomplished with atropine (0.05 mg/kg IM). Endotracheal intubation was performed following an intravenous dose of zoletil (5 mg/kg IV) and rompun (0.2 mg/kg), and then mechanical ventilation (Servo 900C, Siemens Elema, Lund, Sweden) was administered with a tidal volume of 10 mL/kg and a FiO2 of 1.0. The respiratory rate was adjusted to maintain an end-tidal CO2 concentration in the range of 4.0% to 5.0%. After intubation, general anesthesia was maintained with inhaled enflurane (1.5 to 2.0%). A 6 F sheath (Cordis Corp, Miami Lakes, FL, US) was placed in the common femoral artery and served as the route of both hemodynamic monitoring and for the passage of 5-Fr measurement catheters (Cordis Corp, Miami Lakes, FL, US) from the descending thoracic aorta (DTA) to the abdominal aorta for pressure monitoring. Electrocardiogram and oxygen saturation were continuously monitored throughout the procedure.

Invasive measurement of pulse wave velocity
Pulse wave velocity (PWV) was measured along the descending thoraco-abdominal aorta using the foot-to-foot velocity method. Briefly, waveforms were obtained using a fluid-filled system (5-Fr right Judkin’s catheter) at the DTA and abdominal aorta. At each site, the pressure waves were simultaneously recorded with the electrocardiography at the speed of 100 mm/s using a polygraph. We defined T1 as the time interval from the starting point of the QRS complex to the foot of the pressure wave in the DTA; T2 was the time interval from the starting point of the QRS complex to the foot of the pressure wave in the abdominal aorta. We measured T1 and T2 from three different QRS complexes and pressure waves and computed a mean value to minimize any errors. The time delay (T) was calculated as T2-T1, and the distance (D) was obtained by the length of the catheter between the two recording sites. PWV was determined using the
The following formula: $PWV = \frac{D \ (m)}{T \ (s)}$. Parameters were calculated beat-to-beat and averaged over periods of 4 s every 30 s for 2 min.

**Transesophageal echocardiography and velocity-vector imaging analysis**

Following measurement of PWV, transesophageal echocardiography (TEE) was performed using a 5-MHz multiplane TEE probe (Vivid i, GE Healthcare, Milwaukee, WI, US), which was advanced to the lower esophageal position (40 to 60 cm from the upper incisor). The transducer was then positioned to visualize the proximal DTA just distal to the aortic arch. In the transverse plane, digital cine loops and continuous video recordings of short-axis images of the DTA were acquired for off-line analysis. No atheromatous plaque was observed in all animals of both groups.

Blood pressures at the same location were measured simultaneously with the acquisition of TEE images. The precise location of the probe in the DTA at which the short-axis image was obtained was identified using fluoroscopic guidance based on the level of the counterpart rib. The M-mode was also recorded at the same location of the proximal DTA, and the maximal systolic diameter (Ds), minimal diastolic diameter (Dd) and intima-media thickness (IMT) of DTA were measured as previously described.

With the obtained TEE images, off-line analyses were performed using a velocity-vector imaging (VVI) workstation (Syngo®, US Workplace, Siemens, Mountain View, CA, US). Fundamentally, VVI uses a two-dimensional speckle tracking method in which the blood-tissue border is traced manually over one frame of a cine loop and automatically tracked through the cardiac cycle. For vascular analysis of VVI, the fractional area change (FAC) defined as the percent change of the cross-sectional area (CSA) between systole and diastole ($FAC \ (%) = \frac{[\text{largest CSA} - \text{smallest CSA}]}{[\text{largest CSA}] \times 100}$) can be calculated by speckle tracking along the entire circumference of the vessel wall (Figure 1-A). Instantaneous vessel wall deformations were analyzed by dividing the vessel into six segments; the segmental value of tissue radial velocity (RV) and the circumferential strain and strain rate were obtained by tracing the displacement of ultrasonic speckles (Figure 1-B,C).

**Determination of pressure-elastic modulus**

The following indices of aortic elasticity were calculated using the M-mode data as the aortic diameter: ($D_s$, $D_d$) systolic, diastolic and pulse pressures ($P_s$, $P_d$, $PP$, respectively). Stiffness parameter, $\beta$, an index of arterial wall stiffness, was calculated as $\ln \ (P_s/P_d) / [(D_s-D_d) / (D_d)]$. The distensibility coefficient in $10^{-2} \times \text{kPa}^{-1}$ was $[2 \times (D_s-D_d) \times D_d + (D_s-D_d)^2] / PP \times D_d^2$. Young’s elastic modulus in $10^3 \text{kPa/mm}$ was figured as follows: $[(P_s-P_d) / (D_s-D_d)] \times (D_d/\text{IMTd})$. 

\[ (P_s-P_d) / (D_s-D_d)] \times (D_d/\text{IMTd}) \]
Histologic analysis
After a left thoracotomy was performed, the descending thoracic aorta was carefully exposed. The location of the aorta in TEE image was identified using the level of counterpart rib in fluoroscopic guidance (Figure 2) and a total length of 10 cm of each DTA was excised with the lesion in TEE image as a center. The proximal tip and the dorsal aspect (the opposite of the esophagus) of the excised DTA was marked as point of reference to sectionalize the structure into six segments for matching with the corresponding six segments of VVI analysis (Figure 2, 3). Samples taken from the thoracic aorta were preserved in 10% buffered formalin for tissue staining, including hematoxylin and eosin, picric acid sirius red (collagen staining), and orcein (elastin staining).

Elastin
The segments of the thoracic aorta were dissected into six fragments, and their drying weights were recorded using a Sartorius R balance with a precision of 0.01 mg. Tissues were cut into small fragments after re-hydration. For the conversion of insoluble elastin to water-soluble α-elastin, the insoluble small fragments were boiled in 0.25M oxalic acid at 100°C for 60 min. Soluble α-elastin was measured using the Fastin™ elastin assay kit (Biocolor Ltd., Newtownabbey, Northern Ireland, UK) according to the manufacturer’s protocol.

Collagen
Collagen content was determined by measuring the hydroxyproline levels using a hydroxyproline colorimetric assay kit (Biovision Inc., Milpitas, CA, USA). Aortic segments were dried, rehydrated with dH2O 200 μl per segment and minced with microscissors. To hydrolyze the sample, 12N HCl was added in a pressure-tight, Teflon capped vial at 100°C for 3 hr. Ten microliters of each hydrolyzed sample were evaporated under a vacuum dryer, and the samples were incubated with Chloramine T and dimethylaminobenzaldehyde for 90 min at 60°C. The colorimetric assay was measured with absorbance at 560 nm in a microplate reader.

Statistical analysis
All data are expressed as mean ± standard deviation. For comparisons between the groups, t test or Mann–Whitney U test was used for independent samples according to the distribution. Comparisons between the VVI parameters and tissue contents were assessed by Pearson’s or Spearman’s correlations. Independent associations between VVI parameters and collagen contents after adjusting for baseline covariates were evaluated using multiple linear regression analyses. The intraclass correlation coefficient (ICC) and Bland-Altman analysis with 95% confidence limits were used to calculate intra- and interobserver variability of RV, circumferential
strain and strain rate. The clinical significance of ICC was interpreted as follows: excellent, ICC >0.75; fair-to-good, 0.4 ≤ ICC ≤0.75; and poor, ICC <0.4. A p-value <0.05 was considered to be significant.
References


Velocity-Vector Imaging (VVI): 혈관노화를 평가할 수 있는 새로운 비침습적 영상검사 방법

김광일 교수
분당서울대학교병원 노인병내과

Summary

배경
Velocity-vector imaging (VVI)은 혈관노화에 따른 변화를 비침습적으로 평가하는 데 유용하다. 이 연구는 VVI 지표와 혈관노화에 따른 구조적 변화의 상관관계를 알아보기 위해 수행하였다.

방법 및 결과
7마리의 젊은 개(나이 1-2세, 암컷, 22-29 kg)와 7마리의 늙은 개(나이 8-12세, 암컷, 36-45 kg)를 대상으로 한 실험에서 경식도초음파 검사를 통해 하행 대동맥의 단면영상을 획득하여 VVI 분석을 수행하였다. 투시 유도하에 영상의 위치를 확인하였고 대동맥 조직을 채취하였다. 대동맥벽을 6개의 분획으로 구분한 후 부위별/분획별 콜라겐과 엘라스틴 조직의 함량을 정량분석하고 대동맥의 탄성 정도와 비교하였다. 방사선고도화 영상의 위치를 확인하였고 대동맥 조직을 채취하였다. 대동맥벽을 6개의 분획으로 구분한 후 부위별/분획별 콜라겐과 엘라스틴 조직의 함량을 정량분석하고 대동맥의 탄성 정도와 비교하였다. 부위별 분석에서 M-mode로 측정한 대동맥의 직경과 내막-중막벽 두께를 제외한 탄성계수는 양군 간에 유의한 차이가 없었으나 VVI로 측정한 대동맥 면적과 단면적 변화 정도는 늙은 개에서 보다 확장되었고 경직되어 있었다. M-mode로 얻은 탄성계수

결론
VVI는 대동맥의 구조적 특성과 유의한 상관관계를 보이며 대동맥 탄성 정도를 직접적으로 평가하는 것이 가능한 방법이다.
노화가 진행됨에 따라 동맥의 혈관벽에는 여러 가지 변화가 일어나며 이 중 대표적인 것이 동맥벽 두께의 증가이다. 사후 부검을 통한 연구에서 연령의 증가에 따라 대동맥벽의 두께가 증가되었다는 사실이 보고된 바 있고 이러한 변화는 죽상동맥 경화증의 빈도가 낮은 집단에서도 관찰되었다. 또한, 연령의 증가함에 따라 대동맥이 확장되고 경직도가 증가하는 변화를 보이는 것으로 알려져 있다. 이러한 현상은 심장이 박동할 때마다 대동맥의 탄성섬유가 반복적으로 부하를 받게 되는데, 오랜 시간에 걸쳐 이러한 부하를 받게 되면 -마치 고무줄을 반복적으로 늘려도 줄이려는 작업을 반복하면 결 국에는 고무줄이 끊어지거나 적절한 탄성을 유지하지 못하고 늘어나버리는 것처럼- 대동맥벽의 고무줄 성분과 같은 탄성섬유들이 점차 분절되면서 적절한 탄성을 유지하지 못하고 확장 및 경직성 변화를 보이는 것으로 알려져 있다. 또한, 노화로 인한 대동맥벽의 콜라겐 증가 및 콜라겐 섬유간의 공유교차결합, 탄성섬유의 감소, 혈관 석회화 등의 변화가 생기며 이러한 변화 역시 혈관의 경직성 변화에 기여하는 것으로 알려져 있다.

혈관 노화의 특징적인 현상인 동맥의 내막-중막 두께의 증가, 혈관 내피세포의 기능장애, 대동맥 경직도 증가를 평가하기 위한 침습적 및 비침습적인 방법들이 개발되어 있는데 현재 임상적으로 흔히 사용되는 비침습적인 방법으로 맥파전달속도(PWV)와 반향 맥파에 의해 발생하는 파형 증가에서 구하는 augmentation index (AIx) 등이 있다. 특히 맥파전달속도는 비교적 쉽게 측정할 수 있고, 검사결과의 재현성이 양호한 것으로 알려져 있어 임상적으로 유용하게 이용될 수 있다. 또한 대동맥 맥파전달속도의 증가는 고혈압, 당뇨병, 말기신부전 환자 등에서 주로 관찰되는 현상으로 알려져 있다

**Figure 1. 혈관 경직도의 측정.**

1. 맥파전달속도(Pulse Wave Velocity)
   
   \[ PWV = \frac{\text{Distance}}{\Delta t} \]

2. Augmentation Index (AIx)

   \[ \text{Augmentation index} = \frac{\text{Augmented pressure (A)}}{\text{Pulse pressure (PP)}} \]
자 및 노령 환자에서 심혈관사건의 독립된 예측인자
임이 알려져 있으며 대규모 코호트 연구에서도 대동
맥 맥파전달속도가 다른 전통적인 위험인자에 비하
여 향후 심혈관사건을 예측하는데 있어 더 우수한
지침이 될 수 있음을 보고하기도 하였다.2 이러한 연
구 결과를 바탕으로 유럽고혈압학회/유럽심장학회
의 고혈압 치료지침에서는 경동맥-대퇴동맥 맥파전
달속도(carotid-femoral PWV, cfPWV)를 추천검사
항목에 포함시켰으며, 10 m/sec가 넘으면 무증상 장
기 손상이 있는 것으로 분류하고 있다.3 한편, 최근 바관혈적으로 말초동맥(대개는 요골동맥)
에서 applanation tonometry로 동맥 파형을 얻고,
 이를 transfer function으로 대동맥의 파형을 구하
는 장비가 개발되어 있으며, 이를 이용하여 대동맥압
과 AIx를 구할 수 있다. Carotid AIx가 말기신부전 환
자에서 전체 및 심혈관질환에 의한 사망의 독립적인
위험인자임을 보고한 연구들이 있고, 다른 연구에서
는 요골동맥의 파형을 이용하여 구한 AIx가 관동맥
중재술을 시행 받는 환자군에서 사망, 심근경색증 등
의 심혈관사건에 대한 독립적인 위험인자임을 보고
하고 있다.

그러나 이러한 검사방법은 동맥의 전반적인 변화를
평가하는 것으로 대동맥의 국소적인 노화에 따른 구
성성분의 변화와 이로 인해 압은 혈액학적 변화 및 기
능적 평가를 반영하는 것은 불가능하였다. 본 논문
에서는 국소적인 혈관벽의 노화 정도를 평가하기 위
해 경식도 초음파를 통한 VVI를 활용하였고 그 결과
VVI의 여러 지표가 혈관의 구조적인 변화를 잘 반영
함을 보여주면서 비교적 비침습적으로 혈관의 국
소적 노화를 평가할 수 있는 유용한 방법이 될 수 있
음을 동물모델을 통해 증명하였다. 이러한 방법을 활
용하여 혈관노화의 진행 정도를 비침습적으로 추적
관찰할 수 있을 것으로 생각되며, 또한 혈관노화의
진행을 조절하는 데 효과적일 수 있는 약제 사용 전
후에 지표를 측정함으로써 치료에 따른 반응 정도를

REFERENCES
1. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders
   in cardiovascular disease enterprises: Part I: aging arteries: a "set up"
   of aortic stiffness in elderly individuals: a subgroup of the
   for the management of arterial hypertension: The Task Force for
   the management of arterial hypertension of the European Society
   of Hypertension (ESH) and of the European Society of Cardiology