The Effect of Aging on Venous Valves

Kirsten van Langevelde, Alexandr Šrámek, Frits R. Rosendaal

Objective—Age is the strongest risk factor for venous thrombosis. Vessel wall changes such as thickening of venous valves may be one of the contributing mechanisms. We determined thickness and function of venous valves in the popliteal vein with ultrasonography in 77 healthy individuals.

Methods and Results—The study included 6 age groups ranging from 20 to 80 years old. Thickness of the valves was compared between age groups. Valve closure time was assessed as an indicator for valve function. In 69 of 77 participants, valve parameters could be measured. We found an increasing thickness of the valves with age, with a mean thickness of 0.35 mm (range, 0.25 to 0.44 mm) in the group of 20 to 30 years and 0.59 mm (range, 0.30 to 1.21 mm) in the group of 71 to 80 years. The increase in valve thickness per year (linear regression coefficient) was 0.004 mm (95% CI, 0 to 0.009). Valve function was not directly associated with age. Valve thickness, however, was inversely associated with valve function.

Conclusion—Our results show that deep venous valves change with age, with thicker valves in older individuals. The increase of valve thickness with age may be part of the explanation for the age gradient seen in the incidence of venous thrombosis. (Arterioscler Thromb Vasc Biol. 2010;30:2075-2080.)

Key Words: aging ■ ultrasonic diagnosis ■ venous thrombosis ■ venous valves

Deep-vein thrombosis (DVT) of the lower extremities is a disease with an annual incidence of 1 to 2 per 1000.1,2 Risk factors for venous thrombosis can be divided into acquired and genetic risk factors. Among genetic risk factors are deficiencies of antithrombin, protein C, and protein S, which give a high risk for DVT but have low societal impact because of their low prevalence (<0.1%).3 Risk factors such as the prothrombin 20210A and Factor V Leiden mutations are highly prevalent (3% to 8%) and are of intermediate strength.4,5 Surgery and use of oral contraceptives are examples of highly prevalent acquired risk factors.

Aging is the strongest risk factor for venous thrombosis. In people under the age of 40, the incidence is less than 1 per 10 000.6 However, the incidence of venous thrombosis increases to 1 per 100 per year in elderly over the age of 75.7 It is not clear why age is such an important risk factor. Explanations such as a decrease in mobility, an increase in prevalence of diseases with a high thrombotic risk (ie, malignancies, hip fractures), reduced venous compliance in the calf, and damaged venous valves have been suggested.8–10

Venous valves function to ensure that a proper inflow of blood reaches the heart during various cardiovascular adjustments. They can be regarded as flow modifiers that act and react constantly.11,12 Venous valves are bicuspid and are positioned in a valve sinus, which is a local widening of the venous wall. The area between a valve leaflet and the vessel wall is called the valve pocket and is regarded as the place where thrombi originate. Low shear stress areas and stasis of blood flow predispose to thrombus formation. In the deepest part of the valve pockets, fluid circulates with very low velocities, creating a low shear field, thus allowing red cells to aggregate. Stagnation of blood leads to hypoxia, which subsequently causes endothelial damage. In case of nonpulsatile flow, a canine study showed that a thrombus was formed on a valve cusp after only 2 hours.13

Age-related changes of the venous wall and valves have been described in renal veins.14 Muscle fibers in the vessel wall atrophy with increasing age, whereas elastic fiber bundles hypertrophy. With respect to the valves, a gradual thickening with age was seen as a result of an increased number of collagen fiber bundles.14 A recent study showed that the valve sinus plays an important role in maintaining a thromboresistant state. This is achieved by upregulation of anticoagulant proteins such as the endothelial protein C receptor (EPCR) and thrombomodulin in the valvular sinus endothelium as opposed to the vein luminal endothelium. Von Willebrand factor, a procoagulant protein, is downregulated in the valve sinus. It may well be that individual or age-related differences in the thromboresistance profile of the valvular sinus endothelium could modulate thrombosis risk.15

DVT can lead to the postthrombotic syndrome via insufficient venous valves.16,17 About 25% of valvular incompetence in patients with chronic venous disease can be ex-
plained as postthrombotic.18 A thrombus damages the valves mechanically, which results in reflux of venous blood. Whether this mechanism works bidirectional is not yet clear. Aging may lead to damaged valves, ie, thicker and less flexible valves. A decrease in valvular function leads to reflux, resulting in stasis and (possibly) DVT formation.19,20

In this study, we investigated methods to image venous valves in vivo and set out to assess whether aging leads to measurable changes in the thickness and function of valves in the popliteal vein.

**Methods**

Ten or more healthy volunteers were enrolled into each of 6 different age groups, ranging from 20 to 80 years of age. Participation was restricted to people without a history of DVT or pulmonary embolism. In addition, previous surgical intervention involving deep or superficial veins in the legs was an exclusion criterion. Participants were selected among acquaintances and colleagues and by advertisement in the monthly newsletter of the Leiden University Medical Center (Leiden, The Netherlands) monthly newspaper. Approval for this study was obtained from the Medical Ethics Committee of Leiden University Medical Center. All participants provided written informed consent according to the Declaration of Helsinki.

Ultrasounds were performed using an adjustable examination table, set to an inclination of 45° anti-Trendelenburg. The incline was used to achieve optimal filling of the venous system. The valves of the popliteal vein were imaged with a 9-MHz linear probe using B-mode ultrasonography (Toshiba Xario Ultrasound Imaging System). In each leg, the transducer was first placed transversely in the popliteal fossa. The popliteal vein was compressed to verify the presence of reversed flow (when present) following valve closure was achieved by applying standardized calf inflatable cuff (Hokanson rapid cuff inflator, Bellevue, Wash). Time measurements were performed in a subgroup of participants. Twelve participants were imaged in each leg of the ultrasound examination. The same procedure as during the first visit was followed. A Bland-Altman plot was made to visualize the differences in valve thickness between the first and second visit (on the y axis). With the within-group standard deviation of 15%, and (difference in population means between the youngest and oldest age groups) of 20%, 10 people per group should be sufficient to detect a difference of 20% or larger in a simple group-by-group comparison. Taking the graded increase in age into account in a linear regression model will yield substantially larger power to detect graded age-dependent changes.

The relationship between age and valve thickness was studied with linear regression analysis. In this study, the regression coefficient (the slope of the regression line) indicates the decrease or increase in valve thickness per year of aging. Multivariable regression analysis was performed to adjust for sex and side of measurement (ie, left or right leg).

Reproducibility of valve thickness and valve function measurements was performed in a subgroup of participants. Twelve participants from different age groups underwent a second ultrasound examination. The same procedure as during the first visit was followed. A Bland-Altman plot was made to visualize the differences in valve thickness between the first and second visit (on the y axis) versus the mean valve thickness of 2 measurements (x axis). With the reproducibility method described by Bland and Altman, the 95% limits of agreement can be calculated for repeated measurements. 95% of differences are expected to be less than 2 SD from the mean difference.24 In this case, the same measurement method (ultrasound) was used by the same observer during 2 different visits. Therefore, the 95% limits of agreement can also be referred to as the repeatability coefficient, which is the difference that will be exceeded by only 5% of pairs of measurements on the same subject.25

**Results**

A total of 77 healthy volunteers participated in the study. In 73 out of 77 participants (95%), 1 or more valves were identified during the ultrasound examination. However, measurable valves according to the measurement criteria were present in 69 out of 77 participants (90%). The Table shows the characteristics of participants with at least 1 measurable valve.

Of all participants, 44% were men, and the oldest age group contained more men than the other age groups. Overall,
a similar proportion of valves was measured in the right (51%) and left (49%) legs of participants. Figure 2 shows an ultrasound image of a valve leaflet measured with Vesselmass. Mean valve thickness was 0.47 mm (range, 0.25 to 2.12 mm). The valves in the right leg (mean, 0.50; range, 0.26 to 2.12 mm) were slightly thicker than in the left leg (mean, 0.44; range, 0.25 to 1.11 mm), with a 95% CI of the difference from −0.09 to 0.21. Mean valve thickness was 0.53 mm (range, 0.25 to 2.12 mm) in men and 0.43 (0.28 to 1.30 mm) in women. Mean valve thickness by age group with a 95% confidence interval is shown in Figure 3.

Linear regression analysis was performed for valve thickness with age as a continuous variable. The regression coefficient was 0.004 (95% CI, 0 to 0.009), indicating an increase of 0.004 mm thickness per year of aging. Figure 4 shows a scatter plot of valve thickness and age with the regression line. In addition, valve thickness is presented on a log scale. The logarithmic transformation gives a better distribution of the data, because points are spread more uniformly in the graph so that outliers are less pronounced.

When the side of measurement (ie, left or right leg) and sex were included in the analysis as covariables, the regression coefficient did not change (0.004; 95% CI, −0.001 to 0.008).

To verify whether thicker valves were indeed present more often in older individuals than in younger individuals, we performed a dichotomous subanalysis comparing the valves thicker than 0.5 mm in young versus old participants. The young participants group contained the first 3 age categories (20- to 50-year-olds), whereas the older participants group contained the older 3 age categories (51- to 80-year-olds). A normogram showed that valves of 0.5 mm or thicker formed a distinct group. Therefore, we calculated the odds ratio for the presence of thicker valves in older versus younger individuals using 0.5 mm as a cutoff point. We found a crude odds ratio of 5.2 (95% CI, 1.0 to 25.8), indicating that older individuals in our study had a 5-fold increased risk of having valves thicker than 0.5 mm versus younger individuals. After adjustment for sex and the side of the measured valve in a logistic regression model, we found an odds ratio very similar to the crude estimate (odds ratio, 5.0; 95% CI, 0.99 to 25.3).

VCT as a marker of valve function was measured in 66 of 69 (96%) participants and ranged from 0 to 0.127 s. Three participants had no VCT measured because of unavailability of the inflatable cuff. No reflux (defined as time >500 ms of reversed flow through the valve after closure) was present in the study population. Valve function did not decrease with age in this study (regression coefficient, 0.0; 95% CI, 0.0 to 0.001). However, we found that thicker valves were associated with a decrease in valvular function (regression coefficient, 0.036; 95% CI, 0.014 to 0.058). The results suggest that thicker valves have a longer closure time than thinner valves.

To study the reproducibility of valve thickness and VCT measurements, individuals in each age group were asked for a second ultrasound visit. Apart from age group 2 (3 participants) and age group 4 (1 participant), 2 individuals per age group were included.

The mean of the difference between 2 measurements (thickness at visit 1 minus thickness at visit 2) was 0.09 mm.

Table. Characteristics of Participants (N=69) With at Least 1 Measurable Valve Present

<table>
<thead>
<tr>
<th>Age Group (Years)</th>
<th>Participants (N) per Age Group</th>
<th>Sex (% Male per Age Group)</th>
<th>Valve Included in the Analysis (% Right Leg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 to 30</td>
<td>10</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>31 to 40</td>
<td>11</td>
<td>27</td>
<td>36</td>
</tr>
<tr>
<td>41 to 50</td>
<td>10</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>51 to 60</td>
<td>10</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>61 to 70</td>
<td>16</td>
<td>38</td>
<td>56</td>
</tr>
<tr>
<td>71 to 80</td>
<td>12</td>
<td>67</td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>44</td>
<td>51</td>
</tr>
</tbody>
</table>

![Figure 2. Valve leaflet proximal to the ultrasound probe measured in VesselMass. The origin of the valve is marked and subsequently, 2 larger circles with fixed diameters are created by VesselMass. Valve thickness is measured between these 2 circles over a distance of 3 mm.](image-url)

![Figure 3. Barchart with mean valve thickness (mm) by age group. Error bars represent the upper limits of the 95% confidence interval.](image-url)
The 95% limits of agreement were 0.26 to 0.44. Reproducibility of valve thickness measurements was visualized by a Bland-Altman plot (Figure 5A). When we calculate the repeatability coefficient, we expect 95% of all differences to be less than 0.35 around the mean. In our study, 11 of 12 (92%) data points were between the 95% limits of agreement. Figure 5A shows 1 apparent outlier, with an absolute difference between the first and second measurement of 0.62. When we assessed reproducibility without this outlier, the mean difference halved to 0.043 mm (SD 0.064). The 95% limits of agreement were −0.09 to 0.17. The concomitant repeatability coefficient is 0.13. Thus, the outlier had a major influence on repeatability in our sample of 12 participants. The graph below (Figure 5B) shows the corresponding Bland-Altman plot.

VCT measurement was repeated in the same 12 participants. In 1 participant, however, no VCT data were available from the first visit (because the inflatable cuff was unavailable). The mean of the differences between the 2 measurements was 0.015 (SD 0.05), with 95% limits of agreement of −0.08 to 0.11. The repeatability coefficient for VCT is 0.09.

**Discussion**

The results of this pilot study show that in vivo visualization of venous valves and measurement of valve thickness by ultrasound is feasible, and they suggest that aging results in thicker valves and that valve thickness affects valvular function. Valve thickness and function measurements were performed in an examination with a duration of 30 minutes per participant.

This is the first study describing in vivo measurement of valve thickness in deep veins in combination with the assessment of valvular function. We developed a technique to quantify valve thickness in the popliteal vein. This is the largest study in which the effect of aging on valve thickness is quantified. Other data were based on autopsy studies...
including smaller numbers of valves and a narrower age range. \textsuperscript{14,26}

Valves in our study were found to increase in thickness with age, although the increase was modest and not consistent over all age groups. The results of other studies support our findings. Histology studies also found increasing thickness of valves with age in renal, \textsuperscript{14} great saphenous, \textsuperscript{23} and femoral veins. \textsuperscript{26} Thickening of the valve cusps with age was described to be due to increased collagen deposition and a thickened lamina elastica. The latter leads to elasticity loss of the valves. \textsuperscript{26} In a study comparing venous compliance in the calf between young (median age, 22 years) and older volunteers (median age, 64 years), a 45\% reduction in compliance was found in older versus younger participants. \textsuperscript{8} As the efficiency of the calf muscle pump decreases with age, \textsuperscript{28} venous flow can become further impaired. This decline can result in stasis and possibly even reflux. Venous valves are supported by the calf muscles in their movement of blood against gravity. These results indicate that an increase in collagen-to-elastin ratio as well as venous wall thickening are important changes in the aging process of veins. \textsuperscript{8} Similar changes can be expected to be seen in the venous valves, as they are part of the venous wall.

In contrast to other studies, we did not find VCT (ie, the time of reversed flow after valve closure) to be increased with age. \textsuperscript{29} It is debatable whether VCT is a good marker for valve function and whether a subtle decline with age is detectable by this technique. None of the participants in our study fulfilled the criteria for reflux and therefore primary venous insufficiency. An explanation for our findings is that because of our exclusion criteria (ie, a history of DVT or surgery of the lower extremity veins), we have selected a population with a remarkable quality of veins and well-functioning valves. Regarding venous insufficiency, participants were therefore expected to be healthier than patients from other studies. In addition, other data\textsuperscript{29} concerned superficial veins, and we studied only the popliteal vein in 1 location. Having reflux of the superficial veins does not necessarily mean that coexisting reflux of the deep veins is present. \textsuperscript{30} Moreover, reflux originates most often in the superficial system and extends slowly toward the deep veins. \textsuperscript{31,32} Thus, the participants in our study might have had reflux in other segments of the deep veins or in the superficial veins.

In a clinical setting, other methods such as the reflux volume index, are more discriminating in the assessment of severity of valvular insufficiency and in the serial follow-up of patients with chronic venous dysfunction. \textsuperscript{33}

We assessed reproducibility of valve thickness and VCT measurements between 2 separate visits. Our results show that for valve thickness reproducibility was moderate, as 1 in 12 measurements was distinctly out of the 95\% limits of agreement area. When the outlier was not included in the analysis, the repeatability coefficient improved substantially from 0.35 to 0.13. We found that VCT measurements were very reproducible. It should be noted, however, that VCT measurements had a narrow range, and none of the participants had more than 500 ms of reversed flow. Reproducibility may be overestimated because of the small range, and it is not inconceivable that reproducibility decreases with wider ranges. To study this, future studies should also include individuals with less favorable functioning venous valves.

The use of ultrasound has a number of advantages, such as high resolution, dynamic imaging, and noninvasiveness of the examination. A dynamic modality is inevitable for imaging of venous valves because of the quick and irregular nature of valve movements, led by blood flow and muscle contractions. However, ultrasound has drawbacks because it is highly operator dependent. In our study, because of the fine structure of the venous valves, not all of the images obtained contained a measurable valve. In addition, only the valve leaflet proximal to the ultrasound probe was sharply visualized and measurable. Although our study is the largest study done so far on venous valves, groups remained small, and a higher number of participants would have given more information.

In conclusion, the results of our study show that aging results in a modest increase in venous valve thickness and suggest that the thickening contributes to a decline in valve function. These age-related changes may partly explain why aging is such an important risk factor for venous thrombosis. More research is needed to confirm these findings. Future studies should include participants with and without complaints of venous insufficiency to improve generalizability of the association between aging and valve thickness.

Acknowledgments
We acknowledge R.J. van der Geest, designer of the program Vesselmass; F.M. Helmerhorst for inviting participants of the Oranje Sociëteit Warmond, founded September 7, 1945; and all of the volunteers who participated in the study.

Sources of Funding
Dr van Langevelde was supported by the Leducq Foundation, Paris, France, for the development of Transatlantic Networks of Excellence in Cardiovascular Research (Grant 04 CVD 02).

Disclosures
None.

References


The Effect of Aging on Venous Valves
Kirsten van Langevelde, Alexandr Sránek and Frits R. Rosendaal

Arterioscler Thromb Vasc Biol. 2010;30:2075-2080; originally published online July 29, 2010;
doi: 10.1161/ATVBAHA.110.209049

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/30/10/2075

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the
Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for
which permission is being requested is located, click Request Permissions in the middle column of the Web
page under Services. Further information about this process is available in the Permissions and Rights
Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online
at:
http://atvb.ahajournals.org//subscriptions/