Purpose: We aimed to explore the velocity waveform characteristics of the retinal artery associated with age and the cardio-ankle vascular index (CAVI) as a conventional arterial stiffness marker by applying the Doppler optical coherence tomography (DOCT) flowmeter.

Methods: In this cross-sectional study, DOCT flowmeter imaging was performed in 66 participants aged 21 to 83 years (17 men, 49 women) with no history of eye diseases and no systemic diseases, except for hypertension. Retinal blood velocity waveform was analyzed where several parameters in time (upstroke time, T1, T2, T3, and T4) and area under the waveform (area elevation, area declination, A1, A2, A3, and A4) were extracted. Systolic blood pressure–adjusted Pearson’s coefficients were calculated to determine the correlations of each parameter with age or CAVI.

Results: Corrected upstroke time (UTc) was the waveform parameter most positively correlated with age ($r = 0.497, P < 0.001$). Area declination was the waveform parameter most negatively correlated with age ($r = -0.682, P < 0.001$) and CAVI ($r = -0.601, P < 0.001$).

Conclusions: We extracted the waveform parameters associated with the risks of arterial stiffening. The velocity waveform analysis of the retinal artery with DOCT flowmeter potentially could become a new method for arterial stiffness identification.

Translational Relevance: DOCT flowmeter could evaluate arterial stiffening in a different way from the conventional method of measuring arterial stiffening using pressure waveform. Because the DOCT flowmeter can easily, quickly, and noninvasively provide a retinal blood velocity waveform, this system could be useful as a routine medical examination for arterial stiffening.

Introduction

Arterial stiffening is an independent predictor of cardiovascular diseases and all-cause mortality.1,2 Arterial stiffening is characterized by an increase in the rigidity of the artery wall and is associated with age.3 This age-related arterial stiffening promotes an increase in systolic blood pressure (SBP),4,5 resulting in left ventricular dysfunction and various circulatory disorders.6 In recent years, arterial stiffness has been evaluated using the pulse wave velocity (PWV)7,8 and cardio-ankle vascular index (CAVI),9 which are noninvasively and widely used for routine medical examinations in Japan. PWV and CAVI are calculated by the velocity at which pressure waves move down the vessel.
However, these methods sometimes provide inaccurate values. The PWV fluctuates with the patient’s age and blood pressure because a reflected pulse wave returns to the aorta earlier in elderly people than in young people. CAVI is underestimated in patients with a severe arteriosclerotic femoral artery (ankle-brachial index <0.9) and is reported to be affected by the patient’s body mass index. Moreover, these methods require patients in a supine position and take 5 to 10 minutes to measure. Therefore, there is a need for an additional vascular parameter acquired by different measuring methods.

On top of these systemic impairments, aging and hypertension have long been known to narrow the diameter of the retinal artery, which predicts an incident stroke. Changes in the small artery are partly regulated by blood flow. In practice, several studies have reported that some retinal blood flow parameters may be associated with systemic arterial stiffness. However, few studies have examined an association of the velocity waveform characteristics with age-related vascular changes, most likely because it is challenging to acquire the accurate blood velocity of small vessels. Laser Doppler velocimetry (LDV) has been the only commercially available instrument to measure the absolute value of retinal blood velocity. However, LDV requires a well-trained examiner and takes time to accurately measure the blood velocity, which makes it difficult to use LDV in clinical practice.

Doppler optical coherence tomography (DOCT) was recently demonstrated as a new technology for acquiring the retinal blood velocity. We recently developed a fully automated DOCT flowmeter, which easily, quickly, and noninvasively provides the retinal velocity waveform with both good quality and repeatability. In this study, we measured the arterial–retinal blood velocity waveform in a routine arteriosclerosis checkup.

### Methods

#### Study Design and Population

A population-based cross-sectional study was performed at Okoppe town hall and Ororon Eye Clinic, a community-based clinic in Hokkaido prefecture located in northern Japan. We recruited community-dwelling adults who had undergone an annual health checkup at Okoppe town and Rumoi city between January 2020 and November 2020. Because previous similar studies reported a correlation coefficient between retinal blood flow parameters and age-related markers of greater than 0.4, we determined that a study with 80% power at the 5% level would require 47 participants to produce a statistically significant correlation. We enrolled 66 participants aged 21 to 83 years (17 men, 49 women) with no history of eye diseases except for mild cataract and no cardiovascular or other systemic diseases except for hypertension, as determined by a preliminary medical interview and ophthalmologic examination (slit-lamp biomicroscopy and fundus photography).

We conducted all methods and experimental protocols according to the guidelines of the institutional review board of Asahikawa Medical University (Asahikawa Medical University Ethics Committee [AMUIEC]) and the tenets of the Declaration of Helsinki. AMUIEC approved all specific protocols of the study (approval No. 19013). All participants provided written informed consent for study participation after they were provided with a complete explanation of the study design and protocol. All of the personal information was encrypted and made anonymous.

#### Retinal Blood Velocity Measurements by the DOCT

We measured the arterial–retinal blood velocity using the DOCT flowmeter. This system consists of a modified version of the commercially available spectral domain optical coherence tomography (OCT) (3D OCT-1 Maestro; Topcon Corporation, Tokyo, Japan). This system included automated alignment software, which automatically focuses, optimizes, and captures the blood vessel. The detailed protocol has been previously published. Briefly, we first captured a color fundus photo centered between the macula and the optic disk and then selected a region of interest of the retinal vessel (Fig. 1A). Flow velocity $v$ from the Doppler shift was incurred by the moving blood cells. $v = \frac{\lambda_0 \Delta \Phi}{4 \pi n t \cos \theta}$ was derived, where $\lambda_0$ is the center wavelength, $\Delta \Phi$ is the phase differences of the adjacent A-line, $t$ is the time interval between the adjacent A-line, $n$ is the refractive index of the blood, and $\theta$ is the Doppler angle between the flow vector and the incident probe beam. To calculate the Doppler angle, we captured a pair of OCT images 200 µm apart across the blood vessels (Fig. 1B). To increase the signal-to-noise ratio, nine OCT images for each scan location were registered and averaged. The vessel center...
Arterial Stiffness and Retinal Blood Flow Waveform

Figure 1. Example of retinal blood flow measurement in a temporal artery. (A) Color fundus image and scanning location (arrow) of DOCT imaging. (B) Two alternative scans captured 200 μm apart across the blood vessel to estimate the Doppler angle using triangular calculation. (C) OCT image. (D) Phase image with color coding. (E) Acquired blood velocity waveform.

Definition of Blood Velocity Waveform Parameters and Waveform Normalization

Figure 2 shows the definitions of waveform parameters derived from a blood velocity waveform. We focused on the upstroke time (UT) in a velocity waveform, as it was previously reported to be associated with arterial stiffness.\(^\text{18,19}\) UT was defined as the transit time from \(V_{\text{min}}\) to \(V_{\text{max}}\) (Fig. 2A). To correct for the effect of heart rate, we calculated the corrected upstroke time (UTc) using the RR interval on the electrocardiogram according to Bazett's formula,\(^\text{28}\) 

\[
UTc = \frac{UT}{\sqrt{RR}}.
\]

The blood velocity waveform was normalized in both velocity (0 to 1) and time (0 to 100) (Fig. 2B). To evaluate the most contributable time sections to arterial stiffening, we divided a cardiac cycle time into four sections (T1, T2, T3, and T4) by a velocity of 0.5 (Fig. 2B). We also calculated the area under the velocity waveform on the time sections (area elevation, area declination, A1, A2, A3, and A4), as microvascular velocity is a vital component of wall share stress that maintains the vascular homeostasis in arteriosclerosis.\(^\text{29,30}\) The area under the velocity waveform was computed as follows:

\[
\text{Area } (t_0 \text{ to } t_1) = \int_{t_0}^{t_1} v(t) \, dt.
\]

The resistance index (RI) in the retinal artery was defined as follows:

\[
RI = \frac{V_{\text{max}} - V_{\text{min}}}{V_{\text{max}}},
\]

which represents the compliance and resistance of the peripheral vessels.

Study Protocol

Before the examination, the participants rested for 10 minutes in a quiet room maintained at 25°C. We then measured the intraocular pressure (IOP) using a noncontact tonometer (Topcon Corporation) and SBP and diastolic blood pressure (DBP) using automated oscillometry. We calculated the mean arterial pressure (MAP) and ocular perfusion pressure (OPP) as follows:

\[
\text{MAP} = \text{SBP} + \frac{1}{3} (\text{SBP} - \text{DBP}); \quad \text{OPP} = \frac{2}{3} \text{MAP} - \text{IOP}.
\]

CAVI was recorded by VaSera VS-1500AE (Fukuda denshi, Tokyo, Japan). An experienced examiner conducted the CAVI measurement, attaching the cuffs bilaterally to the upper arms and ankles of the participants, monitoring the electrocardiogram and heart sound, and checking the records of the CAVI.\(^\text{9}\) The mean value of the right and left CAVI was entered into the analysis. After these experiments, one eye from each participant was imaged using the DOCT flowmeter without mydriasis. We selected a temporal artery with relatively straight segments that were located adequately far from the bifurcations and 1-disk diameter away from the center of the optic disc.\(^\text{25-27}\)
Figure 2. Definitions of blood flow parameters from a blood velocity waveform. (A) Original waveform. Determination of maximum (Vmax) and minimum (Vmin) blood flow velocity. Determination of “upstroke time (UT)” as the transit time from Vmin to Vmax, “area elevation” as the area under the normalized waveform on UT, “area total” as an area on one cardiac cycle, and “area declination” as a subtracted area: area total minus area elevation. (B) Normalized waveform in both the velocity (0 to 1) and time (0 to 100). Determination of T1 as the normalized time from 0 to 0.5 in speeding up velocity, T2 as the time from 0.5 to 1, T3 as the time from 1 to 0.5 in slowing down velocity, and T4 as the time from 0.5 to 0. Determination of A1 as an area under the waveform on T1, A2 as an area on T2 above velocity 0.5, A3 as an area on T3 above velocity 0.5, and A4 as an area on T4.

Statistical Analysis

All data are shown as the mean ± standard deviation. Kruskal–Wallis test was performed to detect age-dependent changes in participant demographics. SBP-adjusted Pearson’s coefficients were calculated between waveform parameters and age or CAVI. In accordance with the stepwise procedure, multivariate linear regression analysis for UTc and area declination was performed. Pearson’s χ² test was performed to compare the frequency of a dicrotic notch among age groups. We considered a P value of <0.05 to indicate a statistically significant difference. All data were analyzed using STATA (StataIC 16; StataCorp LLC, College Station, TX, USA).

Results

Participant Characteristics

Table 1 shows the participants’ baseline characteristics. A total of 17 male and 49 female volunteers were included and equally classified into the following three age groups: young adults, aged 21 to 44 years; middle adults, aged 45 to 65 years; and old adults,
Arterial Stiffness and Retinal Blood Flow Waveform

Table 2. SBP-Adjusted Pearson’s Coefficients Between Retinal Blood Velocity Waveform Parameters and Age or CAVI

<table>
<thead>
<tr>
<th>Waveform Parameter</th>
<th>Association With Age</th>
<th>Association With CAVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>UT</td>
<td>0.427</td>
<td>0.290</td>
</tr>
<tr>
<td>UTc</td>
<td>0.497</td>
<td>0.368</td>
</tr>
<tr>
<td>Area total</td>
<td>−0.363</td>
<td>−0.358</td>
</tr>
<tr>
<td>Area elevation</td>
<td>0.404</td>
<td>0.318</td>
</tr>
<tr>
<td>Area declination</td>
<td>−0.682</td>
<td>−0.601</td>
</tr>
<tr>
<td>RI</td>
<td>0.471</td>
<td>0.522</td>
</tr>
<tr>
<td>T1</td>
<td>0.393</td>
<td>0.286</td>
</tr>
<tr>
<td>T2</td>
<td>0.403</td>
<td>0.327</td>
</tr>
<tr>
<td>T3</td>
<td>−0.579</td>
<td>−0.502</td>
</tr>
<tr>
<td>T4</td>
<td>0.350</td>
<td>0.321</td>
</tr>
<tr>
<td>A1</td>
<td>0.329</td>
<td>0.222</td>
</tr>
<tr>
<td>A2</td>
<td>0.334</td>
<td>0.260</td>
</tr>
<tr>
<td>A3</td>
<td>−0.397</td>
<td>−0.362</td>
</tr>
<tr>
<td>A4</td>
<td>−0.209</td>
<td>−0.190</td>
</tr>
</tbody>
</table>

P < 0.05 was considered to indicate statistical significance (bold), where r was SBP-adjusted Pearson’s coefficient.

Table 3. Multivariate Linear Regression Analysis for UTc or Area Declination as Dependent Variable

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>UTc (× 100)</th>
<th>Area Declination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.148</td>
<td>−0.161</td>
</tr>
<tr>
<td>CAVI</td>
<td>0.451</td>
<td>−0.125</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>0.064</td>
<td>−0.048</td>
</tr>
<tr>
<td>Gender, female</td>
<td>1.678</td>
<td>−0.846</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.148</td>
<td>0.050 to 0.246</td>
<td>0.004</td>
</tr>
<tr>
<td>CAVI</td>
<td>0.451</td>
<td>−0.942 to 1.844</td>
<td>0.520</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>0.064</td>
<td>0.001 to 0.128</td>
<td>0.045</td>
</tr>
<tr>
<td>Gender, female</td>
<td>1.678</td>
<td>−0.477 to 3.832</td>
<td>0.125</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>−0.161</td>
<td>−0.239 to −0.083</td>
<td>0.001</td>
</tr>
<tr>
<td>CAVI</td>
<td>−1.258</td>
<td>−2.365 to −0.153</td>
<td>0.026</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>−0.048</td>
<td>−0.098 to 0.002</td>
<td>0.060</td>
</tr>
<tr>
<td>Gender, female</td>
<td>−0.846</td>
<td>−2.557 to 0.865</td>
<td>0.327</td>
</tr>
</tbody>
</table>

Abbreviations: CAVI, cardio-ankle vascular index; SBP, systolic blood pressure; UTc, corrected upstroke time; 95% CI, 95% confidence interval.

Table 3 shows the multivariate linear regression analysis for UTc or area declination as the dependent variable, where age, CAVI, SBP, and gender were independent variables because age, CAVI, and SBP were associated with the waveform parameters in the univariate analysis (Supplementary Table S1), and gender had been reported to influence retinal blood circulation. Age and SBP were independently associated with UTc. Age and CAVI were independently associated with area declination.

In this study, we identified the velocity waveform characteristics of the retinal artery associated with age and CAVI. UTc was prolonged with aging. Area declination had an inverse association with age and CAVI. The waveform of older adults tended not to have a dicrotic notch. To our knowledge, this is the first study

Discussion

In this study, we identified the velocity waveform characteristics of the retinal artery associated with age and CAVI. UTc was prolonged with aging. Area declination had an inverse association with age and CAVI. The waveform of older adults tended not to have a dicrotic notch. To our knowledge, this is the first study

-aged 66 to 83 years. CAVI and SBP were increased with aging, but DBP and IOP were not influenced much by age. Vessel diameter and retinal blood flow were not influenced by age.

Correlations of Blood Waveform Parameters with Age and CAVI

Table 2 shows the association between waveform parameters and age or CAVI, adjusting for SBP because of the age-dependent changes in the participants’ SBP (Table 1). All parameters derived from speeding up the velocity profile (UTc, T1, T2, A1, A2, area elevation) had positive associations with age and CAVI. In particular, UTc was the strongest positively correlated parameter with age (r = 0.497, P < 0.001) and CAVI (r = 0.368, P = 0.003). In slowing down the velocity profile, area declination was the most negatively correlated parameter with age (r = −0.682, P < 0.001) and CAVI (r = −0.601, P < 0.001).

Table 3 shows the multivariate linear regression analysis for UTc or area declination as the dependent variable, where age, CAVI, SBP, and gender were independent variables because age, CAVI, and SBP were associated with the waveform parameters in the univariate analysis (Supplementary Table S1), and gender had been reported to influence retinal blood circulation. Age and SBP were independently associated with UTc. Age and CAVI were independently associated with area declination.

Figure 3 presents examples of the arterial–retinal blood velocity waveform in young adults with low CAVI (Figs. 3A, 3B) and old adults with high CAVI (Figs. 3C, 3D). The time interval from the nadir to peak of the velocity was longer in older adults than in young adults, which was likely reflected in the high levels of UTc, T1, T2, A1, A2, and area elevation. T3, A3, and area declination were smaller in older adults than in young adults. The waveforms of the young adults tended to have a dicrotic notch (Figs. 3A, 3B) and an increased velocity in early diastole (Figs. 3A, 3B, arrow). Pearson’s χ² test showed the frequency of a dicrotic notch was significantly increased in young adults (young adults, 12/17; middle adults, 11/21; older adults, 4/28; P < 0.001).
Figure 3. Representative velocity of the retinal artery in young adults with low CAVI (A, B) and older adults with high CAVI (C, D). A dicrotic notch (circle) and increased velocity in early diastole (arrow) found in young adults.

to analyze the velocity waveform and demonstrate the association of waveform parameters with aging and arterial stiffening through the application of the DOCT flowmeter. Before the invention of the DOCT, LDV had been the only commercially available instrument to measure the absolute value of retinal blood velocity.21,22

However, LDV has some disadvantages. First, it requires a strict focus on the target vessel and maintenance of strict eye fixation for measurement, which makes it difficult to use LDV in clinical practices. Second, the retinal blood flow should be considered a parabolic flow to acquire the blood flow velocity. The acquired velocity waveform comprises only maximum blood velocity at the vessel point. DOCT has been developed as a new technology to provide an average velocity at the vessel by obtaining the velocity profile across the whole vessel. We selected a temporal artery with relatively straight segments, checking the artery with parabolic flow. In this study, we were able to analyze the waveform using the DOCT, as it easily, quickly, and noninvasively provided actual velocity waveform.

Several studies have suggested that stiffening of the large artery provokes structural and functional retinal vascular abnormalities.13,14,18–20 Possible mechanisms of the microvascular alternations include pressure-induced remodeling31,32 and flow-induced remodeling.15–17 When the aortic wall stiffens with aging, the aorta loses a cushioning function, which maintains steady blood flow and microvascular pressure. This loss of cushioning function transmits an excessive harmful pressure into the microvascular system.33 To normalize the excessive pressure, the small vessels vasoconstrict, thicken, and eventually reduce an organ perfusion.31,32 In the retinal arteries, aging and hypertension have been reported to narrow the diameter of the retinal artery13,14 and provoke a reduction in retinal flow.20 This vascular remodeling might be responsible for the increase in retinal RI of elderly participants in this study. Dervenis et al.34 reported an association between lifestyles (age, gender, body height, body weight, smoking, alcohol consumption, and diet) and retinal vessel diameters. In this study, only one temporal artery was measured, and its diameter did not correlate with age or CAVI. There were no associations between waveform parameters and vessel diameter in the univariate analysis (Supplementary Table S1). We believe that vessel diameter had little effect on our results, as the velocity waveform was acquired without...
calculation of vessel diameter. However, participants’ lifestyles should have been included in our analysis, as the lifestyles might influence the retinal blood velocity. On the other hand, one possible mechanism for the microvascular alternations includes flow-induced remodeling.\(^{31,32}\) In previous animal experiments, a chronic flow reduction caused a structural narrowing of the small arteries, whereas a chronic flow elevation caused a structural widening.\(^{15–17}\) Because flow changes may precede structural changes, we can observe a flow change related to arterial stiffening before the vessel structure changes. An analysis of retinal flow could possibly detect early changes in age-related vascular flow before structural remodeling occurs.

In our study, UTc was prolonged with an increase in age. This result is consistent with a previous study that found the UT extended in the aged group with arterial stiffening using an LDV.\(^{18,19}\) Several studies have suggested that the UT was a useful tool for detecting cardiovascular diseases using a pulse volume recording with oscillometric cuff technology.\(^{35,36}\) Prior studies using a pulse-tracing technology in the apex cardiogram reported that prolonged UT was associated with an increase in left ventricular wall stress and a decrease in left ventricle function.\(^{37,38}\) This age-induced decline in cardiac function probably led to the prolonged UTc in this study.

In addition, we found that area declination had a negative association with age and CAVI. Area declination comprises two parts: late systolic and diastolic phases. As a diastolic phase mainly occupies the area declination, it is important to consider the diastolic flow. Organ blood flow in diastole depends on the compliance of large elastic arteries, which is explained by the Windkessel theory.\(^{39}\) When blood pressure rises in systole, large elastic arteries distend, and they recoil when the blood pressure falls in diastole. This damping function maintains organ blood flow in diastole, when cardiac ejection stops. Aortic stiffening is known to disrupt the damping function and to decrease the organ blood flow in diastole. This may explain the negative association of area declination with age and CAVI in this study. The waveforms of young adults tended to have a dicrotic notch (Figs. 3A, 3B, circle) and an increased velocity in early diastole (Figs. 3A, 3B, arrow). A dicrotic notch represents the closure of the aortic valve and separates systole from diastole.\(^{40}\) The dicrotic notch is reported to diminish with aging and arterial stiffening using pulse pressure recording.\(^{40,41}\) Those results are consistent with the findings of the current study. On the other hand, several studies have suggested that aortic stiffening has a direct influence on the blood velocity of the carotid artery.\(^{42,43}\) In these studies, aortic stiffening caused an early wave reflection and a diastole reverse flow in the descending aorta, which probably leads to an increase in carotid blood velocity in early diastole. Contrary to these studies, we demonstrated an increased blood velocity in early diastole in young adults but not in older adults with arterial stiffening. We should note the hemodynamic differences by organs. Arm arteries, for instance, have a retrograde flow in diastole.\(^{44,45}\) The mechanism in which the early diastolic increment in retinal blood velocity was found in young adults remains unclear. However, age-related aortic stiffening may directly influence the characteristics of retinal blood velocity waveform. Further studies are needed to evaluate the impact of the waveform characteristics on age-related aortic stiffening.

**Study Limitations**

There were some limitations to our study. First, we included a relatively small number of participants, and the gender distribution was somewhat skewed (i.e., a greater number of women than men). However, there was no difference in the values of almost all of the waveform parameters between men and women (Supplementary Table S2). UTc and area declination were associated with age and CAVI regardless of gender and SBP (Table 3). In addition, we performed the multivariate analysis without taking into account other confounding factors (participants’ axial length, body weight, height, lifestyles, and use of antihypertensive drugs) due to the insufficient number of participants. Further studies with a larger number of participants are needed.

Second, because of the cross-sectional nature of this study, our findings do not suggest a causal relationship between the waveform parameters and age-related vascular stiffness. Therefore, further prospective studies are warranted to determine whether the waveform changes in the retinal artery have predictive power with respect to future cardiovascular events and mortality.

**Conclusion**

We extracted the waveform characteristics associated with the risks of atherosclerosis by applying the DOCT flowmeter. In the current study, we evaluated the correlation between age or CAVI and the waveform characteristic patterns, peaks, and areas as the parameters. Future work should confirm the relationships through multivariate analysis and clustering.
in machine learning as the number of data increases. Because the DOCT flowmeter can easily, quickly, and noninvasively provide a blood velocity waveform of the retinal artery with patients in a sitting position, waveform analysis could be useful as a routine medical examination for arterial stiffening.

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