American Heart
Association®

Learn and Live℠

**Individual Measurement and Significance of Carotid Intima, Media, and
Intima-Media Thickness by B-Mode Ultrasonographic Image Processing**

Jang-Ho Bae, Wuon-Shik Kim, Charanjit S. Rihal and Amir Lerman

The online version of this article, along with updated information and services, is
located on the World Wide Web at:

http://atvb.ahajournals.org

Individual Measurement and Significance of Carotid Intima, Media, and Intima–Media Thickness by B-Mode Ultrasonographic Image Processing

Jang-Ho Bae, Wuon-Shik Kim, Charanjit S. Rihal, Amir Lerman

Objective—We assessed the clinical significance of intima (IT), media (MT), and intima–media (IMT) thickness of the common carotid artery using B-mode ultrasonographic image processing.

Methods and Results—One hundred seventy consecutive patients underwent common carotid artery scanning using high-resolution ultrasonography. A total of 150 patients could be analyzed off-line using ultrasonographic image processing, devised for individual measurement of IT, MT, and IMT. By univariate analysis, IT (range, 0.27 to 0.41 mm) was associated with age, whereas MT (range, 0.27 to 0.74 mm) and IMT (range, 0.49 to 1.12 mm) were associated with age, fibrinogen, and creatinine. Among atherosclerosis risk factors, hypertension was associated with thickness of all 3 layers, whereas smoking was associated with IT only. By multivariate analysis, IT was associated with age, hypertension, and smoking, whereas MT and IMT were associated with age, hypertension, and blood urea nitrogen level.

Conclusions—Carotid IT is associated with smoking, whereas age and hypertension are associated with thickness of all 3 arterial layers. Our results suggest a differential response of the vasculature to systemic risk factors. (*Arterioscler Thromb Vasc Biol*. 2006;26:000-000.)

Key Words: carotid arteries ∎ atherosclerosis ∎ risk factors ∎ arterial intima ∎ arterial media

Carotid artery intima–media thickness (IMT), which is the sum of the intima (IT) and media (MT) thickness, is independently associated with atherosclerotic risk factors and adverse cardiovascular events.[1–4] Therefore, it has been used as an important atherosclerosis surrogate in clinical practice and in many clinical studies since Pignoli et al introduced the direct measurement of carotid IMT with ultrasound.[5]

Abnormal neointima formation is the main pathophysiological consequence of obliterative vascular disease, although atherosclerotic changes may also include smooth muscle proliferation and inflammatory processes in the media and adventitia.[6,7] Therefore, we hypothesized that the intima and media have different clinical responses and roles to cardiovascular risk factor exposure and the development and pathophysiology of atherosclerosis.

We aimed to evaluate the response of the intimal and medial layers of the common carotid artery (CCA) to atherosclerosis risk factors, in terms of thickness of the layers, as assessed by high-resolution ultrasonography with an automated image-processing algorithm.

Methods

Study Population

One hundred seventy consecutive patients referred for ischemic heart disease screening were studied. Patients were included in this study if they provided informed consent and did not meet any of the following exclusion criteria: history of neck irradiation, carotid arterial surgery, previous dissection of the aorta or carotid artery, or cervical trauma. Twenty of the 170 patients (11.8%) enrolled were excluded from analysis because of poor delineation of the borders between the intima and media layers. This study was approved by the ethics committee of Konyang University Hospital and conducted in accordance with the Declaration of Helsinki. Patient laboratory data collected within 2 weeks of enrollment included a lipid profile and measurement of blood glucose, homocysteine, and fibrinogen levels.

Carotid Artery Scanning

The CCA was studied with high-resolution ultrasonography (Hewlett-Packard Sonos 5500) with a broadband (11 to 3 L) linear array transducer (Figure 1). Carotid arterial scanning was performed by a certified, blinded sonographer in a dark, air-conditioned room. The far wall of the right CCA was scanned longitudinally while the patient was in the supine position with head extended. To optimize the image quality, the depth control was fixed at 4 cm. The transducer frequency was set to 11 MHz during the entire analysis with an axial resolution of ≈0.2 mm. The gain control was adjusted
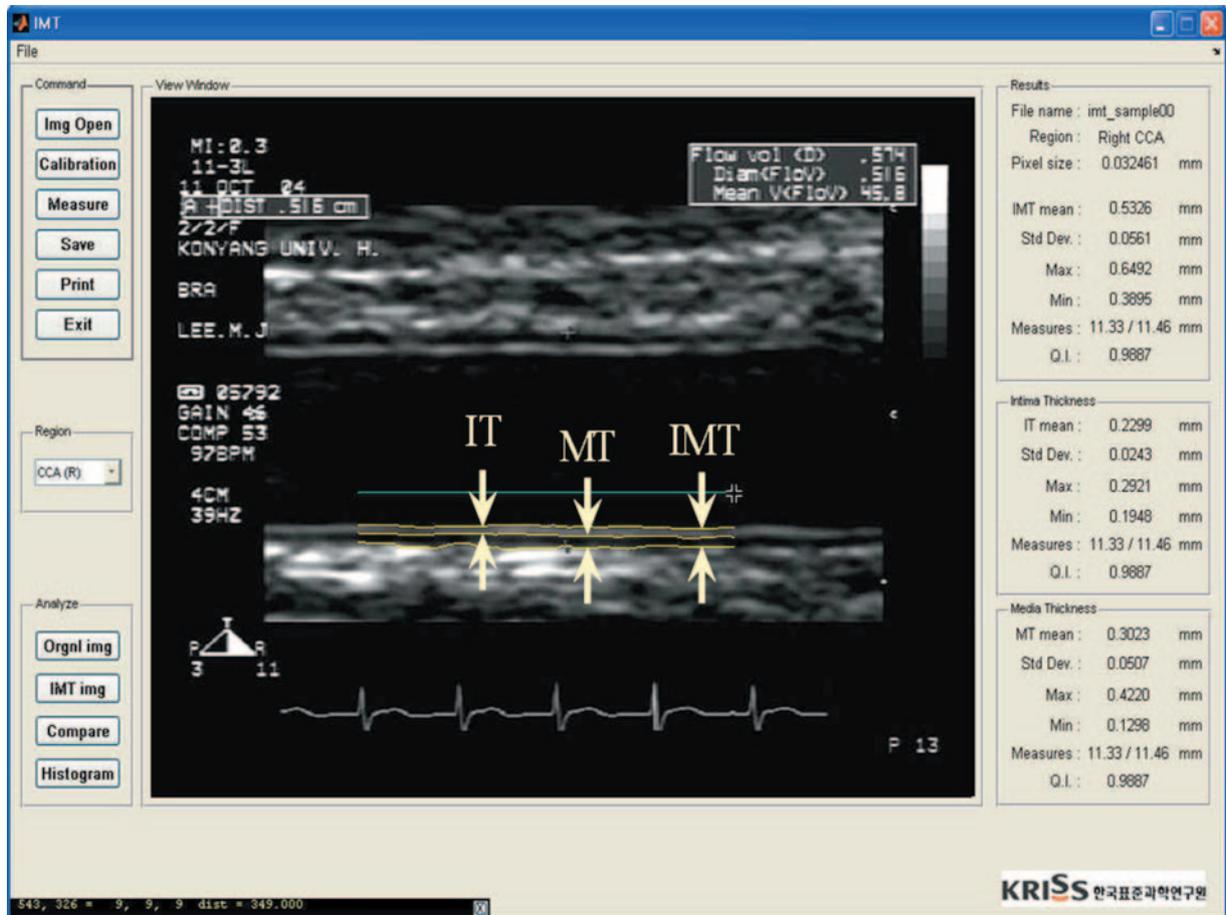
Figure 1. Separate measurements of IT, MT, and IMT using a new method devised in this study. The IT, MT, and IMT were 0.23 mm, 0.30 mm, and 0.53 mm, respectively, in the 11.46-mm segment of CCA at 1 cm proximal to the bulb of this patient.

according to the carotid artery image to obtain a clear delineation of the intima, media, and adventitia layers of the carotid artery far wall. After obtaining a clear carotid artery image, the image was digitally captured for off-line analysis.

Image Processing for Separate Measurement of Intima and Media Layers

Carotid IMT is defined as the distance between the luminal border of the intima and the outer border of the media using high-resolution ultrasonography. The far wall images of the CCA usually show 2 parallel echogenic lines separated by a hypoechoic space (Figure 1). Intima and adventitia thickness is considered the thickness from leading edge to far edge of the first and second echogenic lines, respectively, and the media layer is the distance between the 2 brightest echoes.[5,8] The measurement of carotid IMT is becoming more precise by semiautomatic measurement rather than manual measurement.[9]

To measure IT, MT, and IMT effectively, a new method of CCA image processing was devised in this study (Figure 2). First, the CCA image was loaded, and the size per pixel was determined with electronic caliper, which was previously calibrated at 4-cm depth in axial direction of a multipurpose phantom (Model 539, ATS Laboratories). Next, a region of interest (ROI) for each CCA image was selected at least at 1 cm length of the CCA and at 1 cm proximal to the carotid bulb. The ROI was manually moved to more proximal or distal to avoid any plaque in this region, if present (n=7, 4.7%). The quality of the ROI image was evaluated, and the noise was removed with a filtering algorithm.[10] The 3 layers (intima, media, and adventitia) were identified after acquiring the edge images using the Canny edge-detection algorithm.[11] However, if the border between

each layer was not clear enough to be identified with the Canny algorithm, we differentiated each layer by its autocorrelation based on statistical signal processing.[12] To measure the thickness of each layer, the number of pixels corresponding to the thickness of each layer was calculated. Finally, the IT, MT, and IMT were determined by multiplying the calibration factor (millimeters per pixel) by the number of pixels for each of the 3 layers (Figure 1). The mean value of IT, MT, and IMT of CCA was obtained for this study in only images showing a quality index (portion of analyzable segment from at least 1-cm segment of ROI) of ≥0.6.

Validation of IT, MT, and IMT

The carotid artery imaging technique was already certified by Canevas Co after submission of the carotid artery imaging.[13] To estimate the reliability of this method of determining thickness of the layers, the relationship between the IMT value determined by this method and that determined by commercial software (M'ATH Version 2.01; Metris Inc, Argenteuil, France) was evaluated for all 150 CCA images.

The reproducibility and repeatability of the new method for IT, MT, and IMT measurement, including image processing, was validated as follows. One examiner measured IT, MT, and IMT of 15 randomly selected images from 150 CCA images 2 times, each on a different day. Then, another examiner, who was unaware of the clinical information, measured IT, MT, and IMT again. The reproducibility of IT, MT, and IMT measurements was evaluated by comparing the results from the 2 examiners (interobserver) using coefficient of variation (CV) and SD divided by mean, whereas the repeatability was estimated by comparing the 2 results from the first examiner (intraobserver) using CV. The reproducibility and repeat-
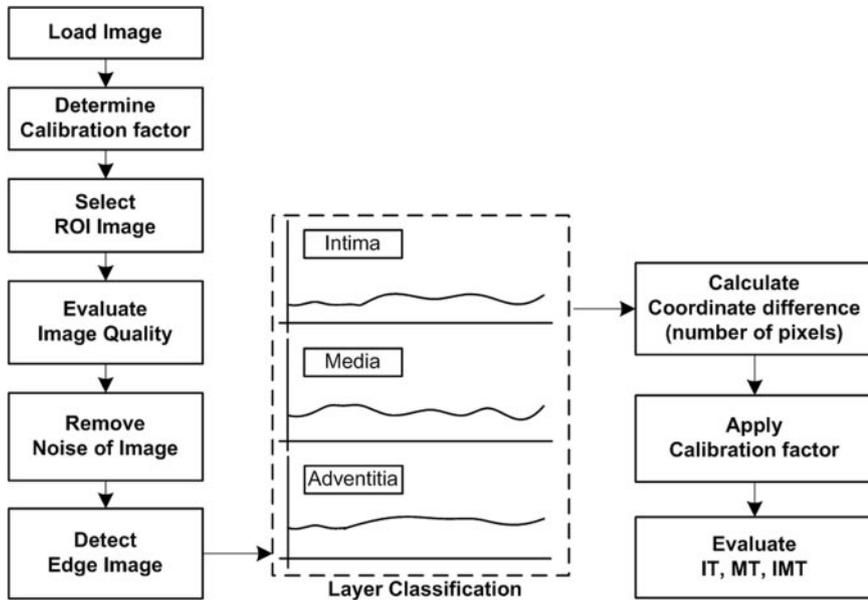
Figure 2. Flow diagram of image processing for separate measurement of the intima, media, and intima–media layers.

ability of IMT measurement with commercial software (M'ATH, Metris Inc) were also estimated with the same method as above.

Statistical Analysis

All data were analyzed using SPSS (version 12.0; SPSS Inc, Chicago, Ill) statistical software. Values are expressed as mean±SD. Clinical predictors of IT, MT, and IMT were assessed with Pearson correlation coefficient analysis. Correlation of thickness of each layer according to the presence of risk factors was assessed with the independent *t* test. Multivariate linear regression analysis was performed to determine the independent predictors of IT, MT, and IMT. Statistical significance was inferred at $P<0.05$.

Results

Patient Characteristics

Clinical characteristics of the 150 study patients are shown in Table 1. The mean age of the study population was 57 ± 14 years, and 76 (50.7%) were men. Most patients had an atherosclerotic disease, such as coronary artery disease (n=69, 46%) and ischemic stroke (n=2, 1.3%), or atherosclerotic risk factors (n=55, 36.7%), such as hypertension, diabetes mellitus, hypercholesterolemia, and smoking. Twenty-four patients (16%) had comorbid conditions such as bone fractures or malignancy.

Reliability, Reproducibility, and Repeatability of the New Method for IT, MT, and IMT Measurement

The axial calibration factor for CCA images was determined as 0.088 ± 0.002 mm [mm/pixel] by using the electronic caliper. To evaluate the reliability of the new method for IMT measurement, the relationship between the new method and M'ATH in the IMT measurements for 150 CCA images were compared. The IMT ranged from 0.49 to 1.12 mm (mean 0.74 ± 0.12 mm) with the new method and from 0.45 to 1.07 mm (mean 0.71 ± 0.13 mm) with M'ATH software. The correlation between IMT values calculated by the 2 independent methods was excellent ($r=0.980$, $P<0.001$).

The CVs of interobserver measurements were calculated in percentages for each set of duplicate new method and

M'ATH measurements to evaluate the reproducibility for measurement of each layer thickness. The mean CVs of the new method were 0.16% for IMT and 0.21% for both IT and MT. The mean CV of M'ATH was 0.21% for IMT. Eighty-

TABLE 1. Patient Clinical Characteristics

Variable	Value*	No. of Patients
Age, y	57±14	150
Men	76 (50.7)	150
Body mass index, kg/m²	24.5±3.3	135
Hypercholesterolemia	42 (30.9)	136
Hypertension	77 (51.3)	150
Diabetes mellitus	35 (23.3)	150
Smoking, current	29 (19.5)	149
Systolic blood pressure, mm Hg	128±21	145
Diastolic blood pressure, mm Hg	78±14	145
Diagnosis		150
Atherosclerotic disease	71 (47.3)	
Atherosclerotic risk factors	55 (36.7)	
Other comorbid conditions	24 (16.0)	
LV ejection fraction, %	65±11	127
Total cholesterol, mg/dL	186±41	136
Triglycerides, mg/dL	159±92	116
HDL cholesterol, mg/dL	44.5±12.8	112
LDL cholesterol, mg/dL	110±33	113
Fasting blood glucose, mg/dL	133±61	141
Homocysteine, mg/dL	11.7±6.8	75
Fibrinogen, mg/dL	3.2±0.7	61
Hs-CRP, mg/dL	0.21±0.25	89
Blood urea nitrogen, mg/dL	22±18	137
Creatinine, mg/dL	2.1±3.1	137
Uric acid, mg/dL	5.4±1.8	126

HDL indicates high-density lipoprotein; Hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LV, left ventricular. Other comorbid conditions include femur fractures or malignancy. *Values are mean±SD or no. of patients (%).

TABLE 2. Reproducibility Data Summary* for the New Method Versus M'ATH

	New Method			M'ATH
	IMT	IT	MT	IMT
CV (%)				
0≤0.05	2 (13)	2 (13)	5 (33)	2 (13)
0.05≤0.1	4 (27)	1 (7)	2 (13)	1 (7)
0.1≤0.15	3 (20)	4 (27)	1 (7)	5 (33)
0.15≤0.2	2 (13)	1 (7)	2 (13)	1 (7)
0.2≤0.25	1 (7)	2 (13)	0	0
0.25≤0.3	1 (7)	1 (7)	2 (13)	2 (13)
≥0.3	2 (13)	4 (27)	3 (20)	4 (27)
Mean, %	0.16	0.21	0.21	0.21
SD, %	0.14	0.15	0.26	0.14
Range, %	0.0–0.46	0.0–0.51	0.0–0.98	0.0–0.51

n=15. *Values describe the no. of CCA images corresponding to the ranges of CV (%), and the related percentages are described in the parentheses.

seven percent of the CVs from the duplicate new method IMT measurements were <0.3%. Of the CVs for duplicate M'ATH IMT measurements, 73% were <0.3%. Eighty percent of the CVs form the duplicate new method MT measurements were <0.3%. Of the CVs for duplicate new method IT measurements, 73% were <0.3%. Any of the CVs from the duplicate new method IT, MT, or IMT measurements were <1% (Table 2). The repeatability was evaluated by calculating the mean CV of intraobserver measurements with 2 different measurements for each of 15 CCA images and resulted in <0.3% for IT, MT, and IMT using the new method and for IMT using M'ATH software.

Correlations With Carotid Wall Thickness

The IT ranged from 0.27 to 0.41 mm (mean 0.32 ± 0.03 mm) and was associated with age ($r=0.379$, $P<0.001$), whereas the MT ranged from 0.27 to 0.74 mm (mean 0.42 ± 0.09 mm) and was associated with age ($r=0.452$, $P<0.001$) and fibrinogen ($r=-0.286$, $P=0.03$) and creatinine ($r=-0.269$, $P=0.001$) levels. Carotid IMT ranged from 0.49 to 1.12 mm (mean 0.74 ± 0.12 mm) and was associated with age ($r=0.496$, $P<0.001$) and creatinine value ($r=-0.251$, $P=0.003$). The mean carotid IT, MT, and IMT were not associated with body mass index, left ventricular ejection fraction, systolic and diastolic blood pressure, total cholesterol, triglyceride, high- and low-density lipoprotein choles-

TABLE 3. Comparison of Layer Thickness According to Atherosclerosis Risk Factors

	Value*		
Risk factor	IT	MT	IMT
Hypertension			
+	0.39±0.03	0.45±0.10	0.77±0.13
−	0.31±0.03‡	0.40±0.07‡	0.71±0.11‡
Smoking			
+	0.33±0.04	0.42±0.08	0.75±0.13
−	0.32±0.03†	0.42±0.09	0.74±0.12
Diabetes mellitus			
+	0.33±0.03	0.43±0.09	0.75±0.12
−	0.32±0.03	0.42±0.09	0.74±0.13
Hypercholesterolemia			
+	0.32±0.04	0.44±0.11	0.75±0.14
−	0.32±0.03	0.41±0.08	0.73±0.11

*Values are mean±SD thickness (millimeters) of each layer for patients with (+) or without (−) each risk factor for atherosclerosis. †$P< 0.05$, ‡$P< 0.005$ compared with patients with risk factor.

terol, fasting blood glucose, homocysteine, high-sensitivity C-reactive protein, fibrinogen, blood urea nitrogen, and uric acid levels.

Thickness of all 3 layers was significantly higher in patients with hypertension than in those without hypertension ($P<0.005$) (Table 3). Among smokers, only the intima layer was significantly thicker compared with that in nonsmokers ($P<0.05$). The presence of other risk factors such as diabetes mellitus and hypercholesterolemia were not significantly correlated with thickness of each layer of the carotid wall (Table 3).

Multivariate Analysis of Predictors of Carotid Wall Thickness

Multivariate predictors of thickness of each layer of the carotid wall are shown in Table 4. Age and hypertension were independently associated with increased thickness of all 3 layers. Smoking was independently associated with only IT. Blood urea nitrogen level was independently associated with MT. Blood urea nitrogen level was also an independent predictor of IMT of the CCA.

Discussion

The present study had several main findings. First, to our knowledge, this is the first study showing the differential

TABLE 4. Multivariate Analysis of Independent Predictors of Intima, Media, and Intima-Media Thickness*

	IT		MT		IMT	
Variable	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Age	0.455 (0.001 to 0.002)	0.001	0.284 (0.000 to 0.004)	0.02	0.351 (0.001 to 0.006)	0.006
Hypertension	−0.371 (−0.038 to −0.007)	0.006	−0.327 (−0.100 to −0.015)	0.009	−0.294 (−0.120 to −0.011)	0.02
Smoking	−0.416 (−0.047 to −0.011)	0.002	0.008 (−0.062 to 0.066)	0.96	−0.113 (−0.113 to 0.056)	0.49
Blood urea nitrogen	0.003 (−0.001 to 0.001)	0.98	0.394 (0.001 to 0.004)	0.003	0.296 (0.000 to 0.004)	0.02
Diabetes mellitus	0.009 (−0.029 to 0.030)	0.97	−0.234 (−0.095 to 0.000)	0.05	−0.282 (−0.179 to 0.035)	0.18

*All of the models included all variables used in univariate analysis as independent variables. Only those variables that remained significant after backward elimination are shown. CI indicates confidence interval.

significance of carotid arterial intima and media thickness using this novel method of calculating the thickness. Second, smoking is associated with the thickness of the intima, whereas age and hypertension are associated with the thickness of all 3 layers. Third, these results suggest that the atherosclerotic process may be different in the context of different cardiovascular risk factors.

Clinical Implications

Our results suggest that each layer of the CCA wall can be affected by specific cardiovascular risk factors in different ways. That is, the early stages of development of atherosclerosis may not be confined to just the intimal layer. In particular, we found that hypertension was associated with thickening of all the 3 layers. Therefore, future clinical studies having specific risk factors such as hypertension or smoking, as in our study, need to focus on specific layers of the vessel wall with this new technique.

In terms of different implications and determinants of noninvasive phenotypes of atherosclerosis, carotid wall morphological phenotypes measured by ultrasound, such as carotid IMT, total plaque area, and carotid stenosis, had different associations with specific atherosclerosis risk factors like our study in some way. For example, traditional atherosclerotic risk factors were more strongly associated with total carotid plaque area than carotid stenosis. Furthermore, carotid IMT was significantly associated with hypertension, whereas total plaque area was associated with smoking and plasma cholesterol.[14,15]

Our study and these previous studies suggest that the different phenotypes of atherosclerosis have different implications and determinants. Therefore, future studies should examine specific phenotypes within a specific context of different cardiovascular risk factors.

Reliability of Measuring an Individual Arterial Wall

The axial calibration factor for CCA images determined by our new method was 0.088 mm [mm/pixel] in 4-cm depth using a multipurpose phantom and Hewlett-Packard Sonos 5500 system. In the same condition of depth and ultrasound, this calibration factor was provided as 0.09 [mm/pixel] by M'ATH software. Moreover, the correlation between IMT values calculated by our new method and by M'ATH software was excellent ($r=0.980$, $P<0.001$). These 2 results support that our new method of IMT measurement is as reliable as M'ATH software. The CV, which is less than 1% for reproducibility and less than 0.3% for repeatability of IT or MT measurement, and the reasonable correlation of IT or MT with the atherosclerosis risk factors support the reliability of our measurement method. Even though the axial resolution was ≈0.2 mm with the system used and the 11-MHz transducer, we measured the IT and MT (range, 0.27 to 0.74 mm) by counting the number of pixels, which had a resolution as small as 0.088 ± 0.002 mm. In addition, we could determine the edge between lumen and intima for IT and the edge between intima and media for MT, respectively, by using not only the Canny edge detection algorithm[11] but also the statistical signal processing method.[12] The IT and MT

determined in our research may slightly differ from the real thickness, possibly because of the lack of histological examination. However, the correlations of IT or MT with the atherosclerosis risk factors are valid, because we tried to obtain the best clear delineation between the first echogenic (intima) and echolucent line (media) of the each carotid arterial wall[5,8] using total gain and time gain compensation control, which allowed us to delineate each layer with a quality index of ≥0.6 in 150 patients of a total of 170 patients. The excellent reproducibility and repeatability of IT, MT, and IMT support the validation of our new measurement. Moreover, the correlations inspect the relative variation of IT or MT depending on the atherosclerosis risk factors.

Previous Studies on Intima and Media Layers of the CCA Wall

Features of atherosclerosis include foam cell infiltrates, fibroplastic intimal thickening, and atheronecrosis.[16] Neointimal formation after long-standing hypertension, atherosclerosis, and mechanical vascular injury is "the pathologic hallmark of obliterative vascular disease," such as primary atherosclerosis, poststent restenosis, and allograft vasculopathy.[17] The neointima develops by a migration, proliferation, and accumulation of vascular smooth muscle cells in the intima.[18] The coronary artery undergoes fibroplastic intimal thickening beginning at age 15 years. MT is correlated with necrosis indirectly through associations with foam cells and fibroplasia.[16,17]

These findings are based on histological examination of autopsy specimens and on animal studies.[16–18] Most studies have focused on formation of the neointima rather than the media layer. However, most studies related to the carotid artery have measured IMT noninvasively, which represents the sum of IT and MT, although there might be a significant difference in level of atherosclerosis between the intima and media. Nevertheless, the CCA IMT is well known to be associated with atherosclerosis risk factors, atherosclerotic disease, and adverse cardiovascular events.[1–3] At present, measurement of carotid IMT is recommended as a tool for primary prevention over various other noninvasive surrogates in the American Heart Association guidelines.[4]

Atherosclerosis Risk Factors and Carotid IT and MT

Similar to the results of others,[1–3] age and hypertension were independent predictors of carotid IMT in our study. Age was a more highly significant independent predictor of IT than MT.

Of interest, only IT was independently associated with smoking. However, detailed smoking history, such as total pack-years and duration of smoking, was not obtained from the patients. This may explain why carotid IMT did not show an association with smoking. In other words, the association between smoking and increased carotid IMT may be a result of the association between smoking and the increased carotid IT. This result is somewhat similar to autopsy findings by Berenson et al.[19] They reported that smoking was associated with an increased percentage of intimal surface involved with

fibrous plaques in the aorta and fatty streaks in the coronary vessels.

Presence of hyperlipidemia was not associated with a significant difference in the thickness of the 3 layers but tendency of increasing thickness of the each layer, although hypercholesterolemia was reported to be associated with an increased carotid IMT in human.[20] This result may be explained by the study population, in which the patients had heterogeneous disease entities such as atherosclerotic disease, atherosclerosis risk factors, or other comorbid conditions. These disorders are already associated with increased carotid arterial wall thickness. In addition, the study population was generally healthy (eg, low lipids and moderate incidence of atherosclerosis risk factors).

Diabetes mellitus was not associated with the increased thickness of the 3 layers in the present study, although it is also well known to be related to increased carotid IMT. This unusual finding might be caused by the same reason as stated above. That is, among our study patients, 56 of 71 patients with atherosclerotic disease did not have diabetes, although 20 of 55 patients with atherosclerosis risk factors had diabetes. In particular, these 2 groups may have had no significant difference in carotid arterial wall thickness.

Study Limitations

This study had 3 limitations. The first was insufficient axial resolution of $\approx$0.2 mm with the system used to measure the carotid IT and MT, which may probably range from 0.27 to 0.74 mm by implementing the image pixel calibration factor of 0.088 [mm/pixel]. Nevertheless, the excellent reproducibility and repeatability of carotid IT, MT, or IMT measurement support the reliability of our measurement method. Moreover, our measurement method is thought to be effective because the correlations of IT or MT with the atherosclerosis risk factors in our study inspect the relative relation between them. The second limitation was that the ideal study population would have been homogeneous. That is, a control group should have been compared with patients with a single risk factor or atherosclerotic disease to separately identify the effect of atherosclerosis risk factors or atherosclerotic disease on each vessel wall measurement, such as carotid IT, MT, and IMT. Despite this limitation, several findings in our study group were significant. The third limitation was that we could not evaluate why some risk factors affect specific vessel layers. This should be studied in the future with histological findings.

Conclusion

The importance of this study is that each atherosclerosis risk factor seems to affect the arterial wall in its own way. The effects were seen even in this heterogeneous study population, in which patients may have already had increased arterial wall thickness. Our novel noninvasive method for individual measurement of carotid artery IT, MT, and IMT will be useful in future clinical studies and helpful in many in vivo trials in humans.

Disclosure(s)

None.

References

1. de Groot E, Hovingh GK, Wiegman A, Duriez P, Smit AJ, Fruchart JC, Kastelein JJ. Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. *Circulation.* 2004;109(suppl III):III-33–III-38.
2. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med.* 1999;340:14–22.
3. Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. *J Am Coll Cardiol.* 2003;42:1037–1043.
4. Smith SC Jr, Greenland P, Grundy SM. AHA Conference Proceedings. Prevention conference V: Beyond secondary prevention. Identifying the high-risk patient for primary prevention: executive summary. American Heart Association. *Circulation.* 2000;101:111–116.
5. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation.* 1986;74:1399–1406.
6. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr., Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler Thromb.* 1994;14:840–856.
7. Guyton JR, Klemp KF. Development of the lipid-rich core in human atherosclerosis. *Arterioscler Thromb Vasc Biol.* 1996;16:4–11.
8. Rodriguez-Macias KA, Naessen T, Bergqvist D. Validation of in vivo noninvasive high-frequency ultrasonography of the arterial wall layers. *Ultrasound Med Biol.* 2001;27:751–756.
9. Baldassarre D, Tremoli E, Amato M, Veglia F, Bondioli A, Sirtori CR. Reproducibility validation study comparing analog and digital imaging technologies for the measurement of intima-media thickness. *Stroke.* 2000;31:1104–1110.
10. Wang Z, Bovik AC. A universal image quality index. *IEEE Signal Processing Lett.* 2002;9:81–84.
11. Canny J. A computation approach to edge detection. *IEEE Trans Pattern Anal Mach Intell.* 1986;8:679–898.
12. Kay SM. *Fundamentals of Statistical Signal Processing. Volume I: Estimation Theory.* Englewood Cliffs, NJ: Prentice Hall International Inc; 1993:197–267.
13. Bae JH, Bassenge E, Lim DM, Synn YC, Kim KY, Schwemmer M. Effects of lacidipine on vascular responses in patients with coronary artery disease. *Int J Cardiol.* 2005;101:377–383.
14. Spence JD, Hegele RA. Noninvasive phenotypes of atherosclerosis: similar windows but different views. *Stroke.* 2004;35:649–653.
15. Al-Shali K, House AA, Hanley AJ, Khan HM, Harris SB, Mamakeesick M, Zinman B, Fenster A, Spence JD, Hegele RA. Differences between carotid wall morphological phenotypes measured by ultrasound in one, two and three dimensions. *Atherosclerosis.* 2005;178:319–325.
16. Tracy RE. Medial thickness of coronary arteries as a correlate of atherosclerosis. *Atherosclerosis.* 1998;139:11–19.
17. Vazquez-Padron RI, Lasko D, Li S, Louis L, Pestana IA, Pang M, Liotta C, Fornoni A, Aitouche A, Pham SM. Aging exacerbates neointimal formation, and increases proliferation and reduces susceptibility to apoptosis of vascular smooth muscle cells in mice. *J Vasc Surg.* 2004;40:1199–1207.
18. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature.* 1993;362:801–809.
19. Berenson GS, Srinivasan SR, Bao W, Newman WP III, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults: the Bogalusa Heart Study. *N Engl J Med.* 1998;338:1650–1656.
20. Poli A, Tremoli E, Colombo A, Sirtori M, Pignoli P, Paoletti R. Ultrasonographic measurement of the common carotid artery wall thickness in hypercholesterolemic patients. A new model for the quantitation and follow-up of preclinical atherosclerosis in living human subjects. *Atherosclerosis.* 1988;70:253–261.