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Media and Intima Thickness and Texture Analysis of the Common Carotid Artery

C.P. Loizou, M. Pantzaris, and C.S. Pattichis

Abstract The intima–media thickness (IMT) of the common carotid artery (CCA) is widely used as an early indicator for the development of cardiovascular disease (CVD). It was proposed but not thoroughly investigated that the media layer (ML) thickness (MLT), its composition, and texture may be indicative of cardiovascular risk and for differentiating between patients with high and low risk. In this study, we investigate an automated snakes segmentation method for segmenting the ML and the intima layer (IL) and measurement of the MLT and the intima layer thickness (ILT) in ultrasound images of the CCA. We furthermore investigate the application of texture analysis of the ML of the CCA and how texture is affected by age and gender. The snakes segmentation method was used, and was evaluated on 100 longitudinal ultrasound images acquired from asymptomatic subjects, against manual segmentation performed by a neurovascular expert. The mean $\pm$ standard deviation (sd) for the first and second sets of manual and the automated IMT, MLT, and ILT measurements were 0.71 ± 0.17 mm, 0.72 ± 0.17 mm, 0.67 ± 0.12 mm, 0.25 ± 0.12 mm, 0.27 ± 0.14 , and 0.25 ± 0.11 mm; and 0.43 ± 0.10 mm, 0.44 ± 0.13 mm, and 0.42 ± 0.10 mm, respectively. There was overall no significant difference between the manual and the automated IMC, ML, and IL segmentation measurements. Therefore, the automated segmentation method proposed in this study may be used successfully in the measurement of the MLT and ILT complementing the manual measurements. MLT was also shown to increase with age (for both the manual and the automated measurements). Following the

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segmentation of the three structures, we also investigated the application of texture analysis of the ML of the CCA and how texture is affected by age and gender. The 100 images were separated into three different age groups, namely below 50, between 50 and 60, and above 60 years old. Furthermore, the images were separated according to gender. A total of 61 different texture features were extracted from the intima layer (IL), the ML, and the intima-media complex (IMC). We have found that male patients tended to have larger media layer thickness (MLT) values as compared to the MLT of female patients of the same age. We have also found significant differences among texture features extracted from the IL, ML, and IMC from different age groups. Furthermore, for some texture features, we found that they follow trends that correlate with a patient's age. For example, the gray-scale median GSM of the ML falls linearly with increasing MLT and with increasing age. Our findings suggest that ultrasound image texture analysis of the media layer has potential as an assessment biomarker for the risk of stroke.

Keywords Snakes • Ultrasound imaging • Media layer • Texture analysis
• Common carotid artery

1 Introduction

Cardiovascular disease (CVD – coronary artery disease, cerebrovascular disease, and peripheral artery disease) is the third leading cause of death and adult disability in the industrial world after heart attack and cancer (as reported by the World Health Organization). According to [1], 80 million American adults have one or more types of CVD of whom about half are estimated to be of age 65 or older. Of all the deaths caused by CVD among adults aged 20 and older, an estimated 13 millions are attributed to coronary heart disease and to stroke, with atherosclerosis as the underlying cause. A recent study by the World Health Organization estimates that by 2015, 20 million people will die from cerebrovascular disease (mainly from heart attack and stroke).

Atherosclerosis causes thickening of the artery walls and the intima-media thickness (IMT) (see Fig. 1a) is used as a validated measure for the assessment of atherosclerosis [2]. Specifically, an increased IMT is correlated with an augmented risk of brain infarction or cardiac attack [3]. Moreover, the presence of carotid plaques has been correlated not only to CVD but also to degenerative pathologies such as vascular dementia and Alzheimer's disease [4]. Hence the assessment of carotid wall status is also essential for early identification of risk conditions in asymptomatic patients. Traditionally, the IMT is measured by manual delineation of the intima and the adventitia layers [2] (see Fig. 1a, interface I5 and I7). Manual tracing of the lumen diameter (see Fig. 1a, band Z4) and the IMT by human experts requires substantial experience; it is time consuming, and varies according to the training, experience, and the subjective judgment of the experts. The manual measurements suffer, therefore, from considerable inter- and intra-observer variability [2–10]. It should be noted that parts of this work were also published in [11, 12].

Fig. 1 (a) Illustration of the intima-media complex (IMC, bands Z5 and Z6) of the far wall of the common carotid artery and the automatic IMC segmentation [10]. The media layer thickness (MLT) is defined as the distance between the intima-media and the media-adventitia interface (band Z6), (b) automated ML detection, (c) automated extracted IMC band, (d) automated extracted ML, (e) manual IMC delineation, (f) manual ML delineation, (g) manual extracted IMC, and (h) manual extracted ML. Source [12], © IEEE 2009

In the last 20 years, several automated techniques for the segmentation and measurement of the IMT from longitudinal ultrasound images of the common carotid artery (CCA) have been developed [9–14]. However, there are no studies published in the literature reporting both the manual and the automated segmentation and

measurement of the media layer (ML) and the intima layer (IL) of the CCA in ultrasound imaging. There are only three studies in the literature where manual measurements of the MLT were reported [8, 15, 16]. Earlier research showed that the media layer thickness (MLT) in peripheral arteries does not change significantly with age and that it ranges from 125 to 350 μm [8]. In [15], manual measurements of the thickness of the CCA IMT and MLT were carried out by an expert on 100 subjects aged 70 years old. In this study [15], it was shown that subjects with CVD (coronary heart disease, myocardial infarction, or stroke) had a significantly thinner ML and a thicker IL than healthy subjects. Furthermore, in [16], the IMT, MLT, and ILT of 90 healthy subjects (aged between 10 and 90 years) were manually measured at their radial and anterior tibial arteries. It was shown that age was strongly related with IMT, MLT, and ILT for both peripheral arteries.

The objective of this study was to develop and evaluate a snakes segmentation system enabling the automated segmentation and measurement of the ML and IL in ultrasound images of the CCA and investigate their variability with age groups. We also propose to study changes in textural characteristics that can be associated with disease progression for different age groups. Here, we note that for fully developed plaques in the CCA, texture features derived from statistical, model based, and Fourier based methods have been used to characterize and classify carotid atheromatous plaques from B-mode ultrasound images [11, 30]. We, furthermore, investigated whether textural characteristics extracted from the IL, the ML, and the IMC of the CCA, segmented manually by an expert and automatically by a snakes segmentation system [10, 12], can be associated with the increase in age gender or MLT. Ultimately, texture feature characteristics that vary with age, gender, or MLT might be used to assess the risk of stroke.

The ML (see Fig. 1a, band Z6) is ultrasonographically characterized by an echolucent region, predominantly composed of smooth muscle cells of the media band of the arterial wall and probably by the extracellular matrix of the intima band, which cannot be distinguished from the smooth muscle cell with ultrasound [17]. Due to the acoustic impedance mismatches, the arterial wall bands (intima-media-adventitia) can produce typical bright-dark-bright patterns on ultrasound images [2]. It is, furthermore, proposed but not thoroughly investigated, that not only the IMT but rather the ML (its composition and thickness) may be used for evaluating the risk of a patient to develop a stroke and accounts in general for the risk of the CVD by differentiating between patients with high and low risk.

The IL is a thin layer, the thickness of which increases with age, from a single cell layer at birth to 250 μm at the age of 40 for nondiseased individuals [5]. Further, adaptive physiological thickening of the IL occurs at places where the wall tension is increased, such as arterial bifurcations and on the ML of the artery and may be either eccentric or diffuse [5]. Furthermore, the intima-media complex (IMC) (see also Fig. 1a) becomes more difficult to detect as the age of patients increases, due to the presence of acoustic holes (echo-dropouts), in the IL [6]. The intimal band (see band Z5 in Fig. 1a) may appear as a thin low-contrast structure and, therefore, it is difficult to reliably draw boundaries because smoothing can move the structure edges or make them disappear [2, 7].

Earlier research [8] showed that the media layer thickness (MLT) does not change significantly with age ($125\mu\text{m} < \text{MLT} < 350\mu\text{m}$). In a recent study by our group, the median (IQR) of intima layer thickness (ILT), MLT, and IMT were computed from 100 ultrasound images of 42 female and 58 male asymptomatic subjects aged between 26 and 95 years old, with a mean age of 54 years to be as follows: 0.43 mm (0.12), 0.23 mm (0.18), and 0.66 mm (0.18), respectively [12].

In [33], a method has been presented for quantifying the reflectivity of the ML of the distal CCA. It was shown that the GSM of the IM layer is the earliest change representing atherosclerotic disease in the arterial wall that can currently be imaged in vivo. This may be the first marker of atherosclerosis and may precede the development of a significant increase in IMT. This would enable earlier identification of high-risk individuals based on the analysis of the CCA artery wall textural characteristics. In [34], the early structural changes of the CCA in familial hypercholesterolemia were investigated. It was shown that textural characteristics extracted from the IMC were significantly different between patients with and without hypercholesterolemia. In [35], the authors reported on the properties of the GSM of the IMC from a random sample of 1,016 subjects aged exactly 70. They found that the GSM of the IMC of the CCA is closely related to the echogenicity in overt carotid plaques.

While there are several earlier studies suggesting that the instability of the carotid atheromatous plaques can be characterized from B-mode ultrasound images [6,30], we have not found any other studies reported in the literature where the ML textural characteristics have been shown to be associated with the risk of stroke. While in [6,30], the echogenicity in atherosclerotic carotid plaques was evaluated through the gray-scale median (GSM), there are very few attempts made to characterize the IL, the ML, and the intima-media complex (IMC) with a similar gray-scale image intensity analysis. It is evident from the visual inspections of the IMC in the CCA that a great variation in echogenicity does exist. However, the usefulness of this information has not yet been studied.

In [30], the morphology of atherosclerotic carotid plaque was investigated based on the textural characteristic extracted from 230 ultrasound images of carotid plaque, where it was shown that it is possible to identify a group of patients, symptomatic or asymptomatic, at risk of stroke based on these texture features. It was further documented in [36], that carotid endarterectomy in asymptomatic individuals with stenosis greater than 70% reduces the risk of stroke from 2% per year to 1% per year. In another study [37], the relationship of the IMT in the CCA and atherosclerosis was investigated on 182 symptomatic patients (mean age 67 years). It was shown that the IMT was correlated to age, male gender, ischemic heart disease, and presence of plaque or stenosis in any of the carotid bifurcations. In a recent study [38], where the alterations of the CCA with age in ultrasound images were investigated, it was shown that the diastolic and systolic lumen diameters are increasing with age. This reduces wall stress as the elasticity of the wall decreases with age.

2 Materials and Methods

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2.1 Recording of Ultrasound Images

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A total of 100 B-mode longitudinal ultrasound images of the CCA used for the IMC, ML, and IL segmentations were recorded using the ATL HDI-3000 ultrasound scanner (Advanced Technology Laboratories, Seattle, USA), with a linear probe (L74), with a recording frequency of 7 MHz, a velocity of 1,550 m/s and 1 cycle per pulse, which resulted to a wavelength (spatial pulse length) of 0.22 mm and an axial system resolution of 0.11 mm. The technical characteristics of the ultrasound scanner (multielement ultrasound scan head, operating frequency, acoustic aperture, and transmission focal range) have already been published in [10]. Digital images were resized using the bicubic method to a standard pixel density of 16.66 pixels per mm with a resolution of 0.06 mm. This was carried out due to the small variations in the number of pixels per mm of image depth (i.e., for deeply situated carotid arteries, image depth was increased and, therefore, digital image spatial resolution would have decreased) and in order to maintain uniformity in the digital image spatial resolution [19]. The images were logarithmically compressed and were recorded digitally on a magneto-optical drive at size of 768×576 pixels with 256 gray levels. The images were recorded at the Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, from 42 female and 58 male asymptomatic subjects aged between 26 and 95 years old, with a mean age of 54 years. The images were separated into three different age groups depending on age, namely, below 50, between 50 and 60, and above 60 years old, with 27, 36, and 37 subjects in each group, respectively, and also separated into 58 male and 42 female subjects. These subjects had not developed clinical symptoms, such as a stroke or a transient ischemic attack (TIA).

2.2 Image Normalization

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Brightness adjustments of ultrasound images were carried out in this study based on the method introduced in [20] and also used in [10]. It was shown that this method improves image compatibility by reducing the variability introduced by different gain settings, different operators, different equipment, and facilitates ultrasound tissue comparability. Algebraic (linear) scaling of the image was performed by linearly adjusting the image so that the median gray level value of the blood was 0–5, and the median gray level of the adventitia (artery wall) was 180–190 [20]. The scale of the gray level of the images ranged from 0 to 255. Thus the brightness of all pixels in the image was readjusted according to the linear scale defined by selecting the two reference regions. It is noted that a key point to maintaining a high reproducibility was to ensure that the ultrasound beam was at right angles to the adventitia, adventitia was visible adjacent to the plaque, and that for image normalization a standard sample consisting of the half of the width of the brightest area of adventitia was obtained.

2.3 Manual Measurements

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A neurovascular expert delineated manually (using the mouse) the IMC [10], the ML, and the IL on 100 longitudinal ultrasound images of the CCA after image normalization. The IMC was measured by selecting 20–40 consecutive points for the intima (see Fig. 1a, interface I5) and the adventitia (see Fig. 1a, interface I7) layers, and the ML (see Fig. 1a, band Z6) by selecting 10–20 consecutive points for the media (see Fig. 1, interface I6) and the adventitia layers at the far wall of the CCA. The IL (see Fig. 1a, band Z5) was then derived by subtracting the ML from the IMC. The manual delineations were performed using a system implemented in MATLAB[®] from our group. The measurements were performed between 1 cm and 2 cm proximal to the bifurcation of the CCA on the far wall [10] over a distance of 1.5 cm starting at a point 0.5 cm and ending at a point 2.0 cm proximal to the carotid bifurcation. The bifurcation of the CCA was used as a guide and all measurements were made from that region. The IMT, the MLT, and the ILT were then calculated as the average of all the measurements. The measuring points and delineations were saved for comparison with the snakes segmentation method. Two sets of measurements were carried out by the neurovascular expert with a 6-month interval. All sets of manual segmentation measurements were performed by the expert in a blinded manner, both with respect to identifying the subject and to the image delineation.

2.4 IMC, ML, and IL Snakes Segmentation

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A total of 100 ultrasound images of the CCA were segmented using the automated segmentation system presented in [12], to identify IMC, ML, and IL. Segmentation was carried out after image normalization using the automated snakes segmentation system proposed and evaluated on ultrasound images of the CCA in [10], which is based on the Williams and Shah [21]. Initially the IMC was segmented by a snake segmentation system as proposed in [21], where the boundaries I5 (lumen–intima interface) and I7 (media–adventitia interface) were extracted. Details about the implementation of the algorithm can be found in [10].

The upper side of the ML (see Fig. 1, Z6) was estimated by deforming the lumen–intima interface (boundary I5) by 0.36 mm (6 pixels) downwards and then deformed by the snakes segmentation algorithm proposed in [10] in order to fit to the media boundary (see Fig. 1a, interface I6). This displacement of 0.36 mm is based on the observation that the manual mean IMT (IMT_{mean}) is 0.71 mm (12 pixels), and lies between 0.54 mm (minimum or 9 pixels) and 0.88 mm (maximum or 15 pixels) [10]. Therefore, the displacement of the contour, in order to estimate the media should be in average 0.36 mm (6 pixels times 0.06 mm) downwards, which is half of the size of the IMT (the distance between I5 and I7, where I7 is the media–adventitia interface).

In order to achieve standardization in extracting the thickness of IMC, ML, and IL, segments with similar dimensions were divided based on the following procedure. A region of interest of 9.6 mm (160 pixels) in length, located 1–2 cm proximal to the bifurcation of the CCA on the far wall, was extracted. This was done by estimating the center of the IMC area and then selecting 4.8 mm (80 pixels) left and 4.8 mm (80 pixels) right of the center of the segmented IMC. The selection of the same ML area in length from each image is important in order to be able to make comparable measurements between images and patient groups.

The novelty of the proposed methodology lies in the algorithmic integrated approach that facilitates the automated segmentation and measurements of IL and ML.

2.5 Texture Analysis

In order to estimate textural characteristics extracted from the IL, ML, and the IMC, a total of 61 different texture features were extracted both from the manual and the automated segmented regions of interest where only the most significant are presented. The following texture feature set algorithms were used: (i) statistical features [11,12]: (a) mean, (b) variance, (c) median value, (d) skewness, (e) kurtosis, (f) energy, and (g) entropy. (ii) Spatial gray level dependence matrices (SGLDM) as proposed by Haralick et al. [39]: (a) angular second moment, (b) contrast, (c) correlation, (d) sum of squares variance, (e) inverse difference moment, (f) sum average, (g) sum variance, (h) sum entropy, (i) entropy, (j) difference variance, (k) difference entropy, and (l) information measures of correlation. For a chosen distance d (in this work $d = 1$ was used) and for angles $\theta = 00, 45, 90, \text{ and } 135$, we computed four values for each of the above texture measures. (iii) Gray level difference statistics (GLDS) [40]: (a) homogeneity, (b) contrast, (c) energy, (d) entropy, and (e) mean. The above features were calculated for displacements $\delta = (0, 1), (1, 1), (1, 0), (1, -1)$, where $\delta \equiv (\Delta x, \Delta y)$, and their mean values were taken. (iv) Neighborhood gray tone difference matrix (NGTDM) [41]: (a) coarseness, (b) contrast, (c) busyness, (d) complexity, and (e) strength. (v) Statistical feature matrix (SFM) [42]: (a) coarseness, (b) contrast, (c) periodicity, and (d) roughness. (vi) Laws texture energy measures (LTEM) [42]: LL-texture energy from LL-kernel, EE-texture energy from EE-kernel, SS-texture energy from SS-kernel, LE-average texture energy from LE- and EL-kernels, ES-average texture energy from ES- and SE-kernels, and LS-average texture energy from LS- and SL-kernels. (vii) Fractal dimension texture analysis (FDTA) [42]: The Hurst coefficients for dimensions four, three, and two were computed. (viii) Fourier power spectrum (FPS) [42]: (a) radial sum and (b) angular sum.

2.6 Statistical Analysis

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We computed the mean and median values for the IMT (IMT_{mean} , IMT_{median}), the MLT (MLT_{mean} , MLT_{median}), and the ILT (ILT_{mean} , ILT_{median}), the inter-observer error for the IMT, MLT, and the ILT ($se = \sigma \sqrt{2}$) [9] as well as the IMT ratio [15] ($IMT_{ratio} = MLT/ILT$). We also calculated the coefficient of variation, CV%, for the IMT, MLT, and ILT, respectively, which describes the difference as a percentage of the pooled mean values, where for the media $CV\%_{media} = (se_{media} \times 100)/MLT_{media}$ [9]. The above measurements were also computed for the three different age groups, namely, below 50, between 50 and 60, and above 60 years old and for the male and female subjects. Additionally, in order to assess the intra-observer variability for the neurovascular expert, the manual MLT measurements were repeated 6 months after the first set of measurements.

The Wilcoxon rank sum test, which calculates the difference between the sum of the ranks of two dependent samples, was also used in order to identify if a significant difference (S) or not (NS) exists at $p < 0.05$, between the manual and the snakes segmentation measurements, of IMC, ML, IL, and for the IMT_{ratio} for all 100 image, and between the manual and the automated segmentation measurements IMC, ML, and IL for the three different age groups (below 50, between 50 and 60, and above 60 years old). Furthermore, the Mann–Whitney rank sum test was also applied in order to identify differences between male and female subjects. Also the correlation coefficient, ρ , between the manual and the automated IMT, MLT, and ILT measurements, was investigated, which reflects the extent of a linear relationship between two data sets [22].

In order to investigate how the automated media snakes segmentation method differs from the manual delineation results, we have investigated the regression lines for the mean values of the MLT vs. age. Regression analysis was also used in order to demonstrate the relationship between the IMT, MLT, and ILT mean manual measurements and age of the subjects. Bland–Altman plots [23], with 95% confidence intervals, were also used to further evaluate the agreement between the manual and the automated media segmentation measurements. The results of this analysis are, however, not presented in this study as are the expansion of the studies are published in [10–12]. Furthermore, box plots for the manual and the automated IMT, MLT, and ILT mean measurements were plotted, as well as for the manual IMT, MLT, and ILT measurements for the three different age groups (below 50, between 50 and 60, and above 60 years old).

3 Results

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Figure 1 illustrates an original normalized ultrasound image of the carotid artery with the automated segmentation of the IMC and ML in (a) and (b), the extracted automated IMC and ML bands in (c) and (d), the manual delineations of the IMC and ML in (e) and (f), and the extracted manual IMC and ML bands in (g) and (h),

respectively. It should be noted that the size of the IMC and ML areas presented in Fig. 1c, d, g, h is larger than its original size (enlarged to 300×20 pixels in order to be better visualized) and does not represent the original truth size (see also Sect. 2.4).

The manual (first and second set of measurements) and the automated mean and median measurements for all 100 images, and for the three different age groups, of the IMC, ML, and IL, are presented in Table 1, as well as the inter-observer error, se, the coefficient of variation, CV%, and the IMT_{ratio} . Table 1 shows that the mean $\pm$ sd for the manual first set and the automated IMT measurements at time zero were 0.71 ± 0.17 mm and 0.67 ± 0.12 mm, for the manual first set and the automated MLT measurements were 0.25 ± 0.12 mm and 0.25 ± 0.11 mm, and for the manual first set and the automated ILT measurements were 0.43 ± 0.10 mm and 0.42 ± 0.13 mm, respectively. The inter-observer errors, se, for the manual first set and the automated IMT segmentation measurements were 0.11 and 0.08, whereas for the MLT and ILT measurements were 0.09 and 0.08, and 0.07 and 0.07, respectively. The coefficients of variation, CV%, for the manual first set and the automated IMT measurements were 17.3% and 12.6%, whereas for the manual first set and the automated MLT and ILT segmentation measurements, were 34% and 12.3%, and 16.7% and 11.7%, respectively. The IMT_{ratio} , for the first set of manual measurements for all 100 images and for the three different age groups (below 50, between 50 and 60, and above 60 years old) were 0.5834 ± 0.323 mm, 0.4314 ± 0.242 mm, 0.634 ± 0.273 mm, 0.6867 ± 0.273 mm, respectively. Similar values, but slightly higher, were observed for the second set of manual measurements carried out at month 6, for the IMT, MLT, and ILT (see Table 1). For the IMT_{ratio} using the Wilcoxon rank sum test, there was no significance difference between the automated and the second set of manual measurements ($p = 0.698$); however, there was a significance difference between the automated and the first set of manual measurements ($p = 0.031$) and between the first and the second set of manual measurements ($p = 0.011$).

Table 2 presents the results of the Mann–Whitney rank sum test performed between the male and the female subjects for all three segmentation measurements (IMT, MLT, and ILT) and the IMT_{ratio} . There was no significant difference for the MLT between the male and the female subjects for the first set and automated measurements. Significant differences between the male and the female subjects were only found for the second set of manual measurements for the IMT ($p = 0.01$) and ILT ($p = 0.013$). There was also no significant difference for the IMT_{ratio} between the male and the female subjects.

The Wilcoxon rank sum test between the manual and the automated IMT, MLT, ILT, and IMT_{ratio} measurements was also performed between the three different age groups and it is shown in Table 3. The Wilcoxon rank sum test and the correlation coefficient (with 95% confidence) results performed between the manual and the automated measurements for the IMT, MLT, ILT, and IMT_{ratio} showed that there were no significant differences between these sets of measurements.

The automated MLT values (MLT_A) vs. age using regression analysis gave an equation of $MLT_A = 0.029 + 0.0041 \times AGE$, where the standard error for intercept and slope were 0.0661 and 0.001, respectively. The mean square error due to regression was 0.1509, the residual mean square was 0.013 resulting in an

Table 1 Manual and automated IMC, ML, and IL segmentation measurements in mm for all subjects and for the age groups below 50, between 50 and 60, and above 60 years old

Set of measurements	Thickness (mm)	Mean $\pm$ SD (mm)	Median (IQR)	se	CV (%)	IMT ratio	t10.1
First set of manual measurements at time 0 months	IMT	0.71 $\pm$ 0.17	0.66 (0.18)	0.11	17.3	0.583 $\pm$ 0.323	t10.2
	IMT_50	0.59 $\pm$ 0.13	0.57 (0.13)	0.09	14.9	0.431 $\pm$ 0.242	t10.3
	IMT_60	0.74 $\pm$ 0.18	0.73 (0.23)	0.12	16.8	0.634 $\pm$ 0.273	t10.4
	IMT_g60	0.82 $\pm$ 0.15	0.81 (0.2)	0.10	12.6	0.687 $\pm$ 0.273	t10.5
	MLT	0.25 $\pm$ 0.12	0.23 (0.17)	0.09	34		t10.6
	MLT_50	0.17 $\pm$ 0.09	0.13 (0.15)	0.07	38		t10.7
	MLT_60	0.26 $\pm$ 0.08	0.26 (0.08)	0.06	23.8		t10.8
	MLT_g60	0.31 $\pm$ 0.12	0.33 (0.15)	0.1	12.6		t10.9
	ILT	0.43 $\pm$ 0.10	0.43(0.10)	0.07	16.7		t10.10
	ILT_50	0.41 $\pm$ 0.05	0.39 (0.09)	0.04	10.0		t10.11
	ILT_60	0.43 $\pm$ 0.12	0.42 (0.10)	0.08	19.2		t10.12
	ILT_g60	0.46 $\pm$ 0.08	0.44 (0.06)	0.05	11.4		t10.13
	IMT	0.72 $\pm$ 0.17	0.68 (0.19)	0.12	17.5	0.613 $\pm$ 0.307	t10.14
Second set of manual measurements at time 6 months	IMT_50	0.7 $\pm$ 0.2	0.67 (0.26)	0.14	20.2	0.551 $\pm$ 0.297	t10.15
	IMT_60	0.66 $\pm$ 0.13	0.63 (0.15)	0.09	14.2	0.652 $\pm$ 0.312	t10.16
	IMT_g60	0.78 $\pm$ 0.15	0.73 (0.17)	0.11	14.0	0.632 $\pm$ 0.225	t10.17
	MLT	0.27 $\pm$ 0.14	0.25 (0.09)	0.07	9.8		t10.18
	MLT_50	0.24 $\pm$ 0.12	0.26 (0.21)	0.08	33.5		t10.19
	MLT_60	0.25 $\pm$ 0.08	0.23 (0.11)	0.11	23.2		t10.20
	MLT_g60	0.29 $\pm$ 0.12	0.26 (0.13)	0.07	22.4		t10.21
	ILT	0.44 $\pm$ 0.13	0.44 (0.10)	0.10	21.3		t10.22
	ILT_50	0.45 $\pm$ 0.14	0.44 (0.10)	0.09	21.1		t10.23
	ILT_60	0.41 $\pm$ 0.11	0.40 (0.11)	0.10	18.4		t10.24
	ILT_g60	0.48 $\pm$ 0.11	0.46 (0.08)	0.08	16.1		t10.25
						(continued)	

Table 1 (continued)

Set of measurements	Thickness (mm)	Mean $\pm$ SD (mm)	Median (IQR)	se	CV (%)	IMT _{ratio}	t11.1
Automated segmentation measurements	IMT	0.67 $\pm$ 0.12	0.66 (0.12)	0.08	12.6	0.597 $\pm$ 0.286	t11.2
	IMT_50	0.61 $\pm$ 0.11	0.59 (0.09)	0.08	12.6	0.461 $\pm$ 0.261	t11.3
	IMT_60	0.68 $\pm$ 0.10	0.69 (0.12)	0.07	10.3	0.618 $\pm$ 0.236	t11.4
	IMT_g60	0.74 $\pm$ 0.13	0.73 (0.11)	0.1	12.9	0.760 $\pm$ 0.320	t11.5
	MLT	0.25 $\pm$ 0.11	0.25 (0.10)	0.08	12.3		t11.6
	MLT_50	0.19 $\pm$ 0.11	0.17 (0.09)	0.08	14.0		t11.7
	MLT_60	0.26 $\pm$ 0.10	0.26 (0.12)	0.12	27.1		t11.8
	MLT_g60	0.32 $\pm$ 0.13	0.11 (0.11)	0.10	29.7		t11.9
	ILT	0.42 $\pm$ 0.10	0.42(0.90)	0.07	11.7		t11.10
	ILT_50	0.25 $\pm$ 0.12	0.42(0.90)	0.07	11.7		t11.11
	ILT_60	0.25 $\pm$ 0.12	0.42(0.90)	0.07	11.7		t11.12
	ILT_g60	0.25 $\pm$ 0.12	0.42(0.90)	0.07	11.7		t11.13

IMC intima-media complex, IMT intima-media thickness for all 100 measurements; IMT_50, IMT_60, and IMT_g60: IMT values for the age groups below 50, between 50 and 60, and above 60, respectively; MLT: media layer thickness for all 100 measurements; MLT_50, MLT_60, and MLT_g60: MLT values for the age groups below 50, between 50 and 60, and above 60, respectively; ILT: intima layer thickness for all 100 measurements; ILT_50, ILT_60, and ILT_g60: ILT values for the age groups below 50, between 50 and 60, and above 60, respectively; sd: standard deviation; IQR: inter-quartile range; se: inter-observer error; CV%: coefficient of variation; IMT_{ratio} = MLT/ILT. Source [12], © IEEE 2009

Table 2 Comparison between sex groups (male = M and female = F) for the IMC, IM, IL, and IMT_{ratio} using the Mann–Whitney rank sum test

	First set manual of measurements				Second set of manual measurements				Automated measurements				t12.1
	IMT	MLT	ILT	IMT _{ratio}	IMT	MLT	ILT	IMT _{ratio}	IMT	MLT	ILT	IMT _{ratio}	t12.3
Sex	NS	NS	NS	NS	S	NS	S	NS	NS	NS	NS	NS	t12.4
(M/F)	(0.8)	(0.193)	(0.971)	(0.137)	(0.01)	(0.128)	(0.013)	(0.964)	(0.362)	(0.332)	(0.96)	(0.362)	t12.5

IMT intima-media thickness, MLT media layer thickness, ILT intima layer thickness, IMT_{ratio}: IMT ratio = MLT/ILT. The *p* value is also shown in parentheses (S = significant difference at *p* < 0.05, NS = nonsignificant difference at *p* > 0.05) Source [12], © IEEE 2009

Table 3 Comparison between the IMC, ML, IL, and IMT_{ratio} for the manual and the automated segmentation measurements based on the Wilcoxon rank sum test for the three different age groups, below 50 (< 50), between 50 and 60 (50–60), and above 60 (> 60) years old

		Second set of manual measurements												Automated Measurements																	
		IMT				MLT				ILT				IMT _{ratio}				IMT				MLT				ILT				IMT _{ratio}	
		<50	50–60	>60		<50	50–60	>60		<50	50–60	>60		<50	50–60	>60		<50	50–60	>60		<50	50–60	>60		<50	50–60	>60		t13.2	
First set of manual measurements	IMT	<50	S	NS	S													S	S	S											t13.3
		50–60	NS	S	S													S	S	S											t13.5
		>60	S	S	NS													S	S	S											t13.6
	MLT	<50				S	S	S														NS	NS	S							t13.7
		50–60				NS	NS	NS														S	NS	S							t13.8
		>60				NS	S	NS														S	NS	NS							t13.9
	ILT	<50								S	NS	S													NS	NS					t13.10
		50–60								NS	NS	S													NS	NS					t13.11
		>60								NS	S	NS													NS	NS		S	S		t13.12
	IMT _{ratio}	<50													S	S	S										NS	S	S		t13.13
	50–60													NS	NS	NS										S	NS	S		t13.14	
	>60													NS	S	NS															t13.15
	<50																	S	S	S											t13.16
	50–60																	NS	NS	NS						S	NS	S		t13.17	
	>60																	S	NS	NS							S	NS	NS		t13.18

Second set of manual measurements	IMT	<50					t14.1
		50–60					t14.2
		>60	S	NS	NS		t14.3
	MLT	<50					t14.4
		50–60					t14.5
		>60					t14.6
	ILT	<50					t14.7
		50–60					t14.8
		>60					t14.9
	IMT _{ratio}	<50					t14.10
		50–60					t14.11
		>60					t14.12

IMT intima-media thickness, MLT media layer thickness, ILT intima layer thickness, IMT_{ratio} = MLT/ILT; S = significant difference at $p < 0.05$; NS = nonsignificant difference at $p > 0.05$. Source [12], © IEEE 2009

IQR: 0.1257, 0.254, 0.218/0.1577, 0.872, 0.1792/0.0949, 0.1013, 0.0573

Fig. 2 Box plots for the manual measurements of the IMT, MLT, and ILT for the age groups below 50 (50), between 50 and 60 (60), and above 60 years (g60) old (i.e., IMT_50, ILT_60, and MLT_g60 represent the values below the age of 50 for IMC, ML, and IL, respectively). Source [12], © IEEE 2009

F-ratio of 11.6 with a corresponding significance level of $p = 0.001$. This supports the assumption of a linear relationship between age and MLT. The correlation coefficient was $\rho = 0.33$. It was shown that the MLT at the age of 55 is 0.25 mm and that the 95% confidence interval limits for the MLT are both ± 0.24 mm.

The Bland–Altman plot between the first set manual measurements and the snakes segmentation MLT measurements showed that the difference between the manual and the snakes segmentation measurements was -0.01 mm with limits of agreement lying between 0.16 mm and -0.18 mm (note that the pixel resolution was 0.08 mm). The Bland–Altman plot between the second set manual measurements and the snakes segmentation MLT measurements showed that the difference between the manual and the snakes segmentation measurements was 0.01 mm with limits of agreement lying between 0.24 mm and -0.23 mm. The Bland–Altman plot between the first and second set of manual measurements showed that the difference between the first and the second set of the manual measurements was -0.02 mm with limits of agreement lying between 0.16 mm and -0.19 mm. There was also a negative bias, as estimated by the mean difference, which showed that on an average the snakes segmentation algorithm underestimates the area relative to normal delineation.

Figure 2 presents box plots of the IMT, MLT, and ILT, for the three different age groups, extracted from the manual IMC, ML, and IL segmentations for all 100 images of the CCA for the three different age groups (below 50, between 50 and 60, and above 60 years).

Fig. 3 Box plots of the gray-scale median (GSM) texture feature extracted from IL, ML, and IMC. Here we are using automated segmentations. In our description we let IQR denote the inter-quartile distance. From left to right, for the first three plots we have: L50IL for the IL for patients younger than 50 (IQR = 22), 50 to 60 IL for the IL for patients from 50 to 60 years old (IQR = 18.4), and G60IL for the IL for patients who are older than 60 years old (IQR = 15.4). For the next three plots (middle), we have the same age groups for the ML and IQR values of 16, 19.5, and 23.7, respectively. The last three plots (leftmost) show the GSM values for the IMC with IQR values 13.6, 25.2, and 16.9, respectively. In each plot we display the median, lower, and upper quartiles and confidence interval around the median of the GSM. Straight lines connect the nearest observations with 1.5 of the inter-quartile range (IQR) of the lower and upper quartiles. Unfilled rectangles indicate possible outliers with values beyond the ends of the $1.5 \times \text{IQR}$. Source [12], © IEEE 2009

Figure 3 presents box plots of the GSM texture features for the three different age groups. These measurements were extracted using the automated IL/ML/IMC segmentations.

Table 4 presents the median and inter-quartile range (IQR) of selected texture features extracted from the automated IL, ML, and IMC segmentations of the 100 ultrasound images investigated. From Table 4, we can see that most of the texture features extracted from the ML exhibit lower median and IQR values when compared with those extracted from the IL and IMC (e.g.: mean, GSM, STD, contrast, ASM, and coarseness).

In Table 5, we use the Wilcoxon rank sum test to compare selected texture features extracted from automated and manual segmentations. We can see similar results for both manual and automated segmentations. However, we note that there are differences. To avoid issues associated with segmentation differences, we are only interested in textural features that give significant differences for both manual

Table 4 Texture features (median (IQR)) for the IL, ML, and IMC using automated segmentations

	IL	ML	IMC	
Mean	35 (19.3)	21 (25)	33 (22)	t15.1
GSM	35 (18.5)	28 (18)	30 (21.3)	t15.2
STD	16 (6.6)	14 (7)	16 (5.7)	t15.3
Contrast	52 (75)	28 (32)	61 (57)	t15.4
DV	16 (27.8)	81 (56)	32 (32)	t15.5
Complexity	1,704 (3,175)	6,041 (6,762)	4,166 (5,888)	t15.6
ASM	0.09 (0.04)	0.002 (0.003)	0.003 (0.002)	t15.7
Coarseness	20 (14.5)	13 (11)	24 (11.3)	t15.8
SS-TEL	38 (33)	78 (53)	56 (38)	t15.9
Entropy	5.7 (1.15)	6 (1.2)	6.6 (0.7)	t15.10
Roughness	2.46(0.187)	2.2 (0.100)	2.238 (0.079)	t15.11
Periodicity	0.8 (0.07)	0.9 (0.06)	0.8 (0.2)	t15.12
				t15.13

IQR inter-quartile range, *GSM* gray-scale median, *STD* standard deviation, *DV* difference variance, *ASM* angular second moment, *SS-TEL* SS-texture energy laws. Source [11], © CMIG 2009

Table 5 Wilcoxon rank sum comparison tests performed on texture features (first column) extracted from the IL, ML, and IMC using manual (M) and automated (A) segmentations. In boldface, we have the texture features identified by the automated segmentation method that also exhibits significant differences for manual segmentation

	Manual (M)			Automated (A)			
	IL-ML	IL-IMC	ML-IMC	IL-ML	IL-IMC	ML-IMC	
Mean	S (0.01)	NS (0.5)	S (0.001)	S (0.02)	NS (0.81)	S (0.004)	t16.1
GSM	NS (0.39)	NS (0.62)	S (0.001)	NS (0.3)	NS (0.45)	S (0.04)	t16.2
Stand. Dev.	S (0.01)	S (0.001)	S (0.001)	S (0.001)	NS (0.1)	S (0.001)	t16.3
Contrast	S (0.001)	S (0.001)	S (0.001)	S (0.001)	NS (0.23)	S (0.001)	t16.4
Diff. Var.	S (0.001)	S (0.001)	S (0.01)	S (0.001)	S (0.007)	NS (0.09)	t16.5
Complexity	S (0.001)	S (0.001)	S (0.04)	S (0.001)	S (0.001)	NS (0.09)	t16.6
ASM	NS (0.3)	S (0.001)	NS (0.13)	S (0.004)	S (0.001)	S (0.001)	t16.7
Coarseness	S (0.001)	S (0.021)	S (0.001)	S (0.001)	S (0.005)	S (0.001)	t16.8
SS-TEL	S (0.009)	S (0.009)	NS (0.13)	S (0.001)	S (0.02)	NS (0.18)	t16.9
Entropy	S (0.017)	S (0.001)	NS (0.85)	S (0.001)	S (0.001)	S (0.008)	t16.10
Periodicity	S (0.001)	NS (0.92)	S (0.001)	S (0.001)	NS (0.07)	S (0.02)	t16.11
							t16.12
							t16.13
							t16.14

IL intima layer, *ML* media layer, *IMC* intima-media complex. The *p* value is shown in parentheses (S = significantly different at $p < 0.05$, NS = nonsignificantly different at $p \geq 0.05$). Source [11], © CMIG 2009

and automated segmentations. For better visualization, we put these features in bold face (see Table 5). Between the IL and the ML, we have significant differences in 10 out of 11 features. Between the IL and the IMC, we have six texture features with significant differences. Similarly, we have eight texture features that exhibit significant differences between the IL and the ML in Table 5. We refer to Table 6 for a summary of the texture feature values.

Table 6 Texture characteristics of IL vs. ML based on the texture feature values given in Table 1

Corresponding features from Table 1	IL	ML	
Mean, GSM	Brighter	Darker	t17.1
Contrast, ASM	Higher contrast	Less contrast	t17.2
Complexity, entropy	Low complexity	High complexity	t17.3
Coarseness	More coarse, i.e., large areas with small gray tone variations	Less coarse, i.e., less local uniformity in intensity	t17.4
Roughness	Slightly rougher	Smoother	t17.5
Periodicity	Less periodical, more heterogeneous	More periodical, more homogeneous	t17.6

Source [11], © CMIG 2009

Table 7 presents the Mann–Whitney rank sum test performed on selected texture features extracted from the automated IL, ML, and IMC segmentations for the three different age groups. It is shown that some of the texture features (GSM, STD, DV, complexity, coarseness, and SS-TEL) exhibit significantly different values between different age groups.

4 Discussion

In this study, both manual and automated IMT, MLT, and ILT measurements are reported for 100 longitudinal ultrasound images of the CCA and their variation with age and sex. The importance of the CCA ML for the evaluation of the risk of CVD was outlined in [2] and shown in [15], that the MLT was thinner for this group of subjects. Also it was shown in [16] that the MLT was strongly related with age for the radial and anterior tibial arteries. It was also found (see Table 6) that the ML is less dark, has less contrast, is more periodic, and is less coarse compared to IL. We have found a decrease of ML GSM with increasing age, as well as decrease with thickness (MLT), that can be attributed to the fact that, at the initial stages of atherosclerosis disease, there is an increased concentration of lipids and hyperplasia of muscle fibers in the ML, which produces hypoechoic (echolucent) structures. It was also shown that the GSM of male patients is brighter than that of the female patients.

Table 1 showed that the manual IMT measurements are larger and have a larger range of values, whereas the first set of manual and the automated MLT measurements are almost the same, while the second set of manual measurements were higher. Similar IMT findings were also reported in [9, 10, 14]. The manual $IMT_{mean} \pm SD$ measurements reported in this study (0.71 ± 0.17 mm and 0.72 ± 0.17 mm for manual first and second sets, respectively) were larger than the snakes segmented measurements (0.67 ± 0.12 mm), and this finding was also reported in other studies. More specifically, the manual vs. the snakes segmented IMT measurements reported

Table 7 Statistical comparisons between texture features extracted from the IL, ML, and IMC for different age groups. Here we use automated (first row) and manual (second row) segmentations. Statistical comparisons were based on the Mann–Whitney rank sum test for the three different age groups. Automated and manual IL, ML, and IMC segmentation measurements in mm (last row)

		IL			ML			IMC			
		<50	50–60	>60	<50	50–60	>60	<50	50–60	>60	
Automated and manual segmentation measurements	GSM	<50	NS (0.3)	NS (0.3)		S (0.01)	S (0.003)		NS (0.08)	S (0.03)	t18.2
		50–60	NS (0.23)	NS (0.23)		S (0.02)	S (0.03)		NS (0.43)	S (0.03)	t18.3
				NS (0.22)			NS (0.25)			NS (0.22)	t18.4
STD	<50		NS (0.36)	NS (0.11)			S (0.01)			NS (0.26)	t18.5
		50–60	NS (0.33)	S (0.03)		NS (0.55)	S (0.04)		NS (0.65)	S (0.03)	t18.6
				NS (0.11)		NS (0.09)	S (0.004)		NS (0.23)	S (0.04)	t18.7
Contrast	<50		NS (0.92)	NS (0.3)			NS (0.08)			NS (0.06)	t18.8
		50–60		S (0.001)		NS (0.7)	NS (0.07)			NS (0.3)	t18.9
				NS (0.56)		NS (0.26)	NS (0.76)		NS (0.3)	NS (0.25)	t18.10
DV	<50			NS (0.55)			NS (0.55)			NS (0.06)	t18.11
		50–60		NS (0.3)			NS (0.22)			S (0.22)	t18.12
				NS (0.33)			S (0.01)			S (0.5)	t18.13
Complexity	<50		NS (0.97)	NS (0.8)		NS (0.18)	S (0.01)		NS (0.55)	S (0.04)	t18.14
		50–60		NS (0.78)		NS (0.19)	NS (0.63)		S (0.03)	S (0.02)	t18.15
				NS (0.67)			NS (0.44)			NS (0.17)	t18.16
				NS (0.07)			NS (0.56)			NS (0.7)	t18.17
				S (0.0001)		NS (0.7)	S (0.005)		NS (0.55)	NS (0.33)	t18.18
				NS (0.13)			NS (0.09)		NS (0.1)	NS (0.33)	t18.19
				NS (0.05)			NS (0.35)		NS (0.42)	NS (0.42)	t18.20
				NS (0.07)			NS (0.87)		NS (0.65)	NS (0.65)	t18.21

Coarseness	<50	NS (0.19)	NS (0.08)	NS (0.56)	S (0.004)	NS (0.4)	NS (0.3)	t19,1
		NS (0.37)	NS (0.3)	NS (0.5)	S (0.01)	NS (0.5)	NS (0.4)	t19,2
	50–60		NS (0.7)	S (0.003)			NS (0.2)	t19,3
			NS (0.6)	S (0.01)			NS (0.08)	t19,4
SS-TEL	<50	NS (0.07)	NS (0.14)	S (0.008)	S (0.0001)	NS (0.83)	NS (0.71)	t19,5
		NS (0.24)	NS (0.22)	S (0.02)	S (0.04)	NS (0.78)	NS (0.25)	t19,6
	50–60		NS (0.06)	NS (0.09)			NS (0.27)	t19,7
			NS (0.2)	NS (0.11)			NS (0.09)	t19,8
Entropy	<50	NS (0.65)	NS (0.9)	NS (0.34)	NS (0.08)	NS (0.83)	S (0.02)	t19,9
		NS (0.59)	NS (0.7)	NS (0.9)	NS (0.3)	S (0.81)	S (0.04)	t19,10
	50–60		NS (0.88)	NS (0.36)			NS (0.27)	t19,11
			NS (0.5)	NS (0.7)			NS (0.4)	t19,12
Thickness (mm)		0.42 ± 0.10	0.42 ± 0.10	0.19 ± 0.11	0.26 ± 0.10	0.32 ± 0.13	0.61 ± 0.11	0.74 ± 0.13
		0.41 ± 0.05	0.43 ± 0.12	0.46 ± 0.08	0.17 ± 0.09	0.26 ± 0.08	0.31 ± 0.12	0.59 ± 0.13
0.74 ± 0.18 0.82 ± 0.15								

IL intima layer, ML media layer, IMC intima-media complex. The *p* value is shown in parentheses (S = significant difference at *p* < 0.05, NS = nonsignificant difference at *p* ≥ 0.05). Source [11], © CMIG 2009

in other studies were (0.93 mm vs. 0.88 mm) [9], (0.72 mm vs. 0.63 mm) [13], (0.8 mm vs. 0.7 mm) [14], and (0.92 mm vs. 0.88 mm) [29]. There is a large variation in the IMT measurements between the different studies because different material and different measuring methodologies were used.

The manual first set MLT, ILT measurements, and IMT_{ratio} in our study were 0.25 ± 0.12 mm, 0.43 ± 0.10 mm, and 0.5834 ± 0.323 mm, respectively (similar values were obtained for the second set of manual measurements and the automated measurements). In [15], the manual IMT, MLT, and ILT measurements carried out for the CCA by an expert using high-resolution ultrasound with a broadband probe and a 25 MHz center frequency, between subjects with and without CVD, were 0.75 ± 0.17 mm vs. 0.72 ± 0.20 mm ($p = 0.4$), 0.45 ± 0.14 mm vs. 0.38 ± 0.16 mm ($p < 0.05$), and 0.17 ± 0.006 mm vs. 0.22 ± 0.06 mm ($p < 0.0001$), respectively. It was shown in [15] that subjects with CVD (coronary heart disease, myocardial infarction, or stroke) had a significantly thicker IL and a thinner ML than healthy subjects. The IMT_{ratio} also differed significantly between subjects with and without a diagnosis of CVD (0.43 ± 0.20 vs. 0.75 ± 0.48 , $p = 0.0002$). The MLT and ILT were manually measured from the radial and tibial arteries in 90 subjects (10 to 90 years old), using a 55 MHz two-dimensional ultrasound transducer [16]. The values reported for the MLT ranged from 0.159 ± 0.039 mm to 0.227 ± 0.038 mm, whereas for the ILT the values ranged from 0.49 ± 0.08 mm to 0.81 ± 0.019 mm, respectively. Finally, in [8], it was found that the median MLT of muscular arteries was 0.6 mm in the absence of a lesion, decreasing to 0.1 mm in the area of maximal atherosclerosis. The in vivo study was performed in 29 patients undergoing coronary or peripheral vascular procedures using intravascular ultrasound with a 30 MHz frequency ultrasound probe.

It is shown from Table 1 that for the MLT measurements, the standard deviation, sd, for both the manual (0.12 and 0.14 for the first and second set of manual measurements, respectively) and the automated (0.11) and the coefficient of variation, CV% (34% and 9.8% for the first and second set of manual measurements, and 12.3% for the automated measurements), are large. This is explained due to the fact that the ML is very small with complex texture and, therefore, very difficult to be estimated correctly either manually or automatically.

Table 1 also shows that the inter-observer errors, se, for the IMT and the MLT, are larger for the manual segmentation measurements (for both the first set and the second set of manual measurements). Also, the results from Table 1 showed that high intra-observer variabilities occur when manual measurements are made. Smaller values for se were obtained for the automated segmentation measurements, indicating a better performance of the automated vs. the manual measurements as documented in [10, 22].

Table 2 showed that no significant differences were found between men and women for the IMT, MLT, ILT, and the IMT_{ratio} , as also shown in [15] and that the IMT_{ratio} did not differ significantly between male and female groups.

The nonsignificant (NS) differences between the manual and the automated segmentation method, estimated by the Wilcoxon rank sum test show that the manual measurements may be replaced by the snakes segmentation measurements

with confidence. It was also shown that the manual measurements of the expert at time 0 vs. time 6 months were NS. The correlation coefficient, ρ , showed that the strongest relationship existed between the MLT manual first set and the automated segmentation measurements (with $\rho = 0.72$), where higher values were reported. The Wilcoxon rank sum test performed in Table 3 showed that the first set of manual and automated IMT measurements between all the age groups are mostly significantly different (S), whereas the manual and automated measurements of the MLT and ILT are mostly nonsignificantly different (NS). Furthermore, the Wilcoxon rank sum test for the IMT_{ratio} showed that there are no clear statistical significance between the different age groups; therefore, this measure may not be used as an indicative value for atherosclerotic disease with increasing age.

Based on the Bland–Altman test a small discrepancy, of around 0.01 mm, was found between the manual and the automated MLT measurements. On this basis the two methods (manual and snakes segmentation) can be considered interchangeable. There are no other studies reported in the literature, where Bland–Altman plots were used for comparing the manual and automated MLT measurements. The Bland–Altman plot was used in [29], where 24 carotid ultrasound images were analyzed by two experts for validating the results of IMT measurements using a newly developed system by comparing them with those obtained using previous methods and showed no evidence of bias between the two methods.

This study shows that there might be a linear relationship between MLT automated measurements and age, where all measurements showed an increase with age. Similar findings were also reported for the IMT in [10]. It was also shown that in this study the values of the mean IMT in a carotid artery vary between 0.54 mm and 0.88 mm, depending on age, and this is also consistent with other studies [2, 6, 14, 28], whereas the values of the MLT vary between 0.13 mm and 0.37 mm. Furthermore, in [16], the IMT, MLT, and ILT of 90 healthy subjects (aged between 10 and 90 years), were manually measured at their radial and anterior tibial artery. It was shown that age was strongly related with IMT, MLT, and ILT of both peripheral arteries.

Similar to [37], we have also found that the IMT is generally larger in men than in women. Furthermore, in agreement with [28, 36], we have also found that the IMT increases linearly with age.

In [43], increased IMT has been found in young adults with childhood-onset chronic kidney disease (CKD). The disease stage at which these patients first develop abnormalities of arterial texture is unknown. It was also shown that in 55 children with stages 2 to 4 CKD, thickening of IMT occurs early in the course of the disease and is most prominently marked in dialyzed patients. Furthermore, morphologic alterations were found in both muscular and elastic type arteries as early as in the second decade of life. The degree of thickening depends on the degree of renal dysfunction. The authors also found that 61% of children with stages 2 to 4 CKD had IMT equal to or exceeding the 95th percentile for age.

It was shown in [33] that the GSM of the IM layer is the earliest change representing atherosclerotic disease in the arterial wall that can currently be imaged in vivo. This may be the first marker of atherosclerosis and may precede the

development of significant increase in IMT. This would enable earlier identification of high-risk individuals based on the analysis of the CCA artery wall textural characteristics.

In [34], the texture features of 12 subjects (28 ± 2 years) with familial hypercholesterolemia (FCH) and without CVD, before and 3 months after treatment with atorvastatin were investigated, where the entropy and the ASM were extracted from the IMC band. Before treatment of atorvastatin significantly higher entropy (normal: 0.082 ± 0.02 vs. FCH : 0.57 ± 0.12) and lower ASM (normal: 0.01 ± 0.0011 vs. FCH : 0.004 ± 0.0001) values were found when compared to the normal tissue.

The normalization method used in this study was documented to be helpful in the manual contour extraction [25] as well as the snakes segmentation of the IMC [10] and the atherosclerotic carotid plaque segmentation [26] and texture analysis [11]. Moreover, this method increased the classification accuracy of different plaque types as assessed by the experts [27]. Ultrasound image normalization was carried out, prior to segmentation of the IMT in [24], where histogram equalization was performed on carotid artery ultrasound images for increasing the image contrast.

4.1 Study Limitations

It has been demonstrated in [2, 31] that the blood–endothelium (intima) interface and the media–adventitia interface correspond to their anatomical/histological counterparts. This is not the case with the intima–media interface because the width of the white band closer to the lumen is gain dependent and, therefore, variable. Thus the measurement of the MLT represents only an ultrasonic measurement (as also discussed above). It should be noted, however, that part of the ML, shown in ultrasound image as an echolucent band (Fig. 1a, band Z6), contains the hypo echoic muscular arterial tunica media and perhaps hypo echoic part of the arterial tunica intima. It is well known that atherosclerosis causes an increase of the arterial tunica intima thickness. As has been also reported earlier in the literature [13], the histological boundaries between the arterial tunica intima and media (internal elastic lamina) in ultrasound images cannot be accurately delineated. Therefore, we hypothesize that the increase of the MLT with age as reported in our study (see also [10]) may be a misleading finding, since part of the ML, as shown in ultrasound images, may belong to the arterial tunica intima. We recommend that the above hypothesis should be taken into account in future studies aiming in studying the ML texture and morphology. It should be furthermore noted that there are no other studies reported in the literature where ML segmentation and measurements were performed on longitudinal ultrasound images of the CCA.

While IMT is an established measure of the vascular wall disease [2], GSM is less commonly used while other features have not been used before. GSM analysis has previously mainly been performed on plaques and then found to be related to histological features of the plaque, such as the elastin and calcium content, as well as to the size of the lipid-rich necrotic core [44]. However, the histological correlate

to variation in the ML texture features has to be evaluated and the prognosis impact of these new variables has to be investigated. It has also been observed that there is an increase in the granularity in association with atherosclerotic disease [31]. A granular IMC indicates more advanced atherosclerosis, which may precede the development of significant IMT thickening.

A limitation of the proposed method is that the manual and automated ML and IL segmentation measurements were performed in linear segments of the CCA because in the clinical praxis, the experts are delineating the IMC and the ML only in those parts of the vessel where there are no significant artifacts, signal drop outs, and structure irregularities [2, 9, 10, 20, 27, 35].

Another limitation of the proposed method is the presence of acoustic shadowing together with strong speckle noise, which hinders the visual and automatic analysis in ultrasound images [9, 10]. Such images as well as images with extensive echolucency and calcification, where the ML was not well visually recognized, were excluded from the study. Backscattered ultrasound is also angle dependent. During the recording of the images, a standard recording technique was used to adjust the position of the probe so that the ultrasound beam was at right angles to the arterial wall. This improved the IMC visualization. Moreover, the new spatial compound imaging technique might optimize further carotid ultrasound imaging [25, 27]. Due to the small size of the ML and the estimation and positioning of the initial snake contour may sometimes result to segmentation errors. This should be placed as close as possible to the area of interest, otherwise it may be trapped into local minima or false edges and converge to a wrong location.

As it has already been mentioned in Sect. 2.1, the axial resolution of the system used was 0.11 mm, which indicates that structures greater than 0.11 mm can be correctly visualized and measured (see also [32]). In addition, it is noted that the pixel resolution was 0.06 mm. For structures thinner than 0.11 mm, the two echo interfaces (i.e., of ML) cannot be separated and measurements of these structures are not possible. For the dataset investigated in this study, 6% of the measurements were below 0.11 mm; therefore, these measurements may be considered as unreliable. It should be noted, however, that with new ultrasound equipment and new probes, that are available having a recording frequency in the range of 15 MHz, this problem will be highly limited. In another study where the variability of the IMT between experts and between the manual and the automated IMT measurements was investigated [32], it was shown that the reproducibility of the IMT measurements may be further improved if the IMT mean value is computed from multiple ultrasound images (frames) of the carotid, instead of the mean value obtained from only one frame [32]. These can be also investigated in a future study for the case of the MLT and the ILT measurements.

In the present study, in less than 8% of the cases, the positioning of the initial snake contour was not calculated correctly. Furthermore, there were also another 6% of the cases where the ML structure was very small and the two snake contours were trapped together. For these cases the user of the proposed system may run the snakes initialization procedure again in order to estimate the correct initial snake contour, and during the snakes deformation the user may interact and manually correct the contour.

5 Concluding Remarks and Future Work

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It was clearly illustrated in this study that both manual and automated IMT, MLT, and ILT measurements could be carried out successfully. Also, it was shown that there was overall no significant difference between the manual and the automated measurements, between the male and the female subjects, as well as that these increase with age.

The segmented IMC, ML, and IL bands may be used for extracting texture features [30], which might be able to differentiate between subjects with high and low risk of stroke. Furthermore, the change of these textural characteristics with age may give additional information for characterizing subjects in different risk groups. In a recent study [35], it was shown that the gray-scale median of the IMC of the CCA is closely related to the echogenicity in overt carotid plaques. This finding suggests that the gray-scale median and other texture features extracted from the IMC or the ML bands could have prognostic impact for the assessment of cardiovascular risk. However, the IMT_{ratio} was not found to be statistically of significant difference with increasing age.

Our texture analysis showed that: (a) there are significant differences between some texture features extracted from the IL, ML, and IMC (mean, gray-scale median (GSM), standard deviation, contrast, difference variance, and periodicity), (b) some of the texture features can be associated with the increase (difference variance, entropy) or decrease (GSM) of patient's age, (c) the GSM of the ML falls linearly with increasing ML thickness (MLT) and with increasing age, (d) the GSM of male subjects is larger than that of female subjects (see Fig. 5.4), and (e) male and female subjects may be better distinguished using texture features extracted from the IMC.

Risk factors like smoking, blood pressure, inflammation markers, and cholesterol correlate to the traditional carotid IMT [1, 3, 4, 16]. It would have been valuable to relate these risk factors to AM-FM analysis and not only the age as done in this study. Ongoing studies from our group will give more data on this topic.

Future work will investigate whether it is possible to identify a group of patients at risk of atherosclerosis based on their texture features extracted from the IL, the ML, and the IMC of high-resolution ultrasound images of the CCA. It may also be possible to identify and differentiate those individuals into high- and low-risk groups according to their cardiovascular risk before the development of plaques. The proposed methodology may also be applied to a group of people, which already developed plaques in order to study the contribution of the ML texture features to cardiovascular risk. Both groups of patients may benefit by prognosing and managing future cardiovascular events. Another possible future application of the proposed methodology is that it can be used to investigate possible effects of statins or other drugs in texture feature changes of the ML of the CCA.

The methodology presented in this study will be further evaluated on ultrasound images of the CCA collected on a large-scale epidemiological study by our group as well as for the segmentation and measurement of curved segments of the bifurcation bulb of the CCA.

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