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ORIGINAL ARTICLE **ÖZGÜN ARAŞTIRMA**

ULTRASONOGRAPHIC MEASUREMENT OF COMMON CAROTID ARTERY INTIMA-MEDIA THICKNESS: DIRECT COMPARISON OF MANUAL AND AUTOMATED EDGE DETECTION READINGS

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ABSTRACT

INTRODUCTION: It is aimed to compare ultrasonographic intima media thickness (IMT) manual measurement and reading with automatic edge detection algorithms.
METHODS: Manually measured IMT (IMT-manual) and algorithm-measured IMT indices [IMT-maximum (IMT-max), IMT-mean (IMT-avg), IMT-minimum (IMT-min) and IMT-standard deviation (IMT-SD)] were compared in terms of repeatability and variability in recordings and measurements made by 2 different sonographers 3 days apart in 20 healthy controls. The ability of IMT indices to classify vascular risk factors was tested in 606 cases.
RESULTS: : Coefficient of Variation (CV) of IMT indices for "Repeatability" was in the range of 20-30% (acceptable level), except for IMT-SD. The concordance correlation coefficient (ρc) was below 0.9 (Suboptimal) for all IMT indices. Coefficient of Repeatability (CR) for each IMT parameter was over 100 microns. The CV for inter-operator aggrement was 19%-22% for IMT-max and IMT-min, while it was 22.6%-24.8% for IMT-manual. For all IMT methods, ρc was suboptimal (95% confidence intervals [CIs] ranged from 0.745 to 0.863). The mean CR was greater than 100 microns for each IMT index (199.9 for IMT-max, 132.2 for IMT mean, 168.7 for IMT-min, and 151.9 IMT-manual, in microns). The 95% CI lower limit of ROC-AUC of IMT indices was not above 0.6 for any risk factor category. All IMT indices generally tend to increase as the number of risk factors increases (Kendal tau, between 0.07-0.24).
DISCUSSION AND CONCLUSION: Manual IMT measurement techniques and automatic edge detection IMT algorithms have comparable repeatability, reproducibility, and potential for classifying vascular risk factors.
Keywords: Intima, media, thickness, reproducibilty, repeatability, individual variation, ultrasound.

ANA KAROTİS ARTER İNTİMA-MEDİA KALINLIĞININ ULTRASONOGRAFİK ÖLÇÜMÜ: MANUEL VE OTOMATİK KENAR ALGILAMA YÖNTEMLERİNİN KARŞILAŞTIRILMASI

ÖZ

GİRİŞ ve AMAÇ: Elle yapılan ultrasonografik intima media kalınlığı (IMT) ölçüm ve okumasının prototip bir otomatik kenar belirlenimi algoritması ile yapılan ölçümlerle karşılaştırılması amaçlanmıştır.
YÖNTEM ve GEREÇLER: Elle ölçülen IMT (IMT-manual) ile algoritma ile ölçülen IMT indisleri [IMT-maksimum (IMT-maks), IMT-ortalama (IMT-ort), IMT-minimum (IMT-min) ve IMT-standart deviasyon (IMT-SD)] 20 sağlıklı kontrolde 3 gün ara ile 2 farklı nörosonoloğun yaptığı ölçümlerde tekrarlanabilirlik ve değişkenlik açısından karşılaştırıldı. 606 olguda ise IMT indisleri vasküler risk faktörlerini tasnif edebilme kapasitesi açısından test edildi.

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BULGULAR: "Tekrarlanabilirlik" için IMT indekslerinin varyasyon Katsayısı (CV), IMT-SD hariç %20-30 aralığında (kabul edilebilir seviye) bulundu. Konkordans korelasyon katsayısı (ρc) tüm IMT indisleri için 0,9'un (Suboptimal) altındaydı. Her IMT parametresi için tekrarlanabilirlik Katsayısı (CR) 100 mikronun üzerindeydi. Operatörler arası uyum için CV, IMT-max ve IMT-min için %19-%22 arasındayken, IMT-manuel için %22,6-%24,8 idi. Tüm IMT yöntemleri için ρc sub-optimaldi (%95 güven aralıkları [GA] 0,745 ile 0,863 arasındaydı). Ortalama CR, her IMT indeksi için 100 mikrondan büyüktü (IMT-maks için 199,9, IMT ortalaması 133,2, IMT-min 168,7 ve IMT-manuel 151,9, mikron cinsinden). IMT indekslerinin ROC-AUC %95 GA alt sınırı hiçbir risk faktörü kategorisi için 0,6'nın üzerinde değildi. Tüm IMT indeksleri genellikle risk faktörlerinin sayısı arttıkça artma eğilimi göstermiştir (Kendal tau, 0,07-0,24 arası).
TARTIŞMA ve SONUÇ: Manuel ve otomatik kenar algılama algoritmalarının IMT ölçümlerinin tekrarlanabilirlik, yeniden üretilebilirlik ve vascular risk faktörlerini sınıflandırma potansiyeli benzerdir.
Anahtar Sözcükler: İntima, medya, kalınlık, tekrarlanabilirlik, tekrar üretilebilirlik, bireysel varyasyon, ultrason.

INTRODUCTION

Ultrasonographically - measured common carotid artery (CCA) intima-media thickness (IMT) is a commonly used surrogate marker for cardiovascular risk prediction (1,2). Since IMT values are submillimetric, its measurement is error-prone, and requires considerable experience, standard technology and meticulous strategy (3). In addition to many parameters such as age, gender, region of interest in the carotid artery, the IMT measurement protocols used are also critical in the variability of IMT values (4-7). In this regard, it can be said that the consensus criteria for IMT methodology are not sufficiently inclusive (8). For example, the standards of sonographer training, standards in off-line reading and softwares used along with quality control criteria were not clarified therein. One of the unclear issues is whether the use of manual IMT measurement and the new automatic edge detection software for off-line reading is equivalent (3,5,9,10). Although manual reading is still the most widely used method in the literature, many, but not all, experts believe that edge-detection algorithms are superior (11-14). This topic has been the subject of very few, contrary to general expectations, studies in the literatüre (5,9-12,15). We herein re-visit the reproducibility of manual IMT measurement in comparison with a standard example of automatic edge detection software in various settings.

METHODS

Patients population and studied parameters: This study was carried out with the data obtained from the ultrasonography protocols part of the recently completed two seperate studies approved by the Hacettepe University Non-Interventional Ethics Committee (Date: 24.07.2013, No: GO 13/243-13 and Date: 04.05.2021, No: 2021/10-37). The consent of the subjects was taken within the scope of the original studies including ultrasonography protocols. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Bilateral IMT recordings were available for off-line analysis in 337 individuals in the first study and 269 in the second study. The correlation between IMT parameters obtained by manual/edge detection software reading and atherosclerosis risk and demographic factors was examined in whole population.

In 20 healthy persons (80 common carotid arteries, 16 female, mean age: 37±10, smoker in 5, hypertension in 4, DM in 1, all were health - care professionals), IMT was measured on both sides and recorded by two sonographers (one experienced and the other novice but very-well trained) with 3 days interval. Then, off-line measurements were performed with the manual (free-hand) method (see below) and an edge-detection IMT software (GE Auto-IMT® program).

Ultrasound: All study scans were performed by a high-resolution B-mode ultrasonography (Logiq® P6 ultrasound system, GE, Milwaukee, WI, USA) with 7-11 MHz linear transducer. Both common carotid arteries (CCA) were imaged from the patient's (usually) right side with the patients lying in the supine position, with their heads slightly turned to the opposite side of the insonation. First, the transducer was moved caudally from the clavicle in a transverse plane to determine the location of the carotid bulb and the presence of atheromatous plaques as defined according to the latest Mannheim consensus report.[8] Then, at the determined position, the probe was rotated to the longitudinal axis. The area where the CCA near and far wall double line view is clearly visible, exactly parallel and (almost) flat, and visually has the largest diameter was carefully determined by vertical and circumferential orientation. Insonation was passed through the internal jugular window by approaching it laterally and/or vertically. Thus, it

was ensured that off-center tangent sections were not taken. ;Then, the screen was frozen and recorded. The area of interest was located at least 5 mm beyond the bulb and kept at least 1 cm long. Simultaneous electrocardiogram monitoring was performed in the majority of cases, and the area of interest was frozen in an automatic cine-loop detection. The depth of focus was generally in the middle of the CCA lumen, the frame rate was above 15 Hz, and the grayscale was optimized for best visualization. Gain settings were freely adjusted to completely separate the double lines on the two walls without creating an intraluminal shadow. For the gain setting, the near and far CCA wall is ensured to have the same brightness, and the gain was reduced if the automatic line overflows within the lumen in edge detection.

IMT measurement: All measurements were made from records stored in the ultrasound machine. Measurements were performed in areas free of atherosclerotic plaques and care was taken to leave at least 5 mm gap between the atherosclerotic plaque (if present) and the area where IMT measurement was made. IMTs were measured using GE company's automatic edge detection program (AutoIMT®) or directly by free hand. In AutoIMT, measurement was made from a standard area of 1 cm or 250 points in length. The measured parameters are “maximum IMT (IMT-max)”, “average IMT (IMT-mean)”, “minimum IMT (IMT-min)” and “IMT standard deviation (IMT-SD)”. Manual IMT was determined as the average of measurements taken from at least 3 points after optimal magnification (usually maximum) was made from the same area. Since manual IMT measurement (IMT-manual) is the measurement of the thickness from the endoluminal edge of the intima to the outer edge of the media, it is expected to correspond to the IMT-max value with the edge detection software (Figure 1).

Statistics: All values were given as “mean ± standard deviation”, “median (inter-quartile range [IQR] or 95% confidence interval-95%CI, appropriately)”, or “percent” according to their suitability. Distribution normality was tested by Shapiro-Wilks and Kolmogorov Smirnov tests. Students’ *t* or paired *t*, Wilcoxon’s signs test and analysis of variance were used for evaluaton of numerical data. Pearsons chi-square and Kruskal-Wallis tests were used for non-parametric data.

“Intra-subject variability”, or “Repeatability” was calculated from two measurements made on the same person with 3 days intervals. Because bilateral recordings were made by two different sonographers at two times, IMT-repeatability was calculated for 80 artery (CCA) pairs. The averages of these two measurements were compared with the paired-t test. Passing-Bablok regression analysis was used to determine the difference between the recordings. "Systematic differences" (Intercept A and 95%CI) and "Proportional differences" (Slope B and 95%CI) values were reported. If "CI% for A" contains the value "zero" and "95%CI for B" contains the value "one", the differences calculated are not statistically significant. Linear model validity was examined with the “Cusum test”. This test was supported by “Bland-Altman (BA) plotting”. In addition to presenting BA graphs, the Coefficient of Repeatability (CR) was calculated and reported. This is in microns and indicates the maximum difference that is likely to occur between repeated measures. The aggrement between repeated measurements was determined by "concordance correlation coefficient analysis". In addition to the

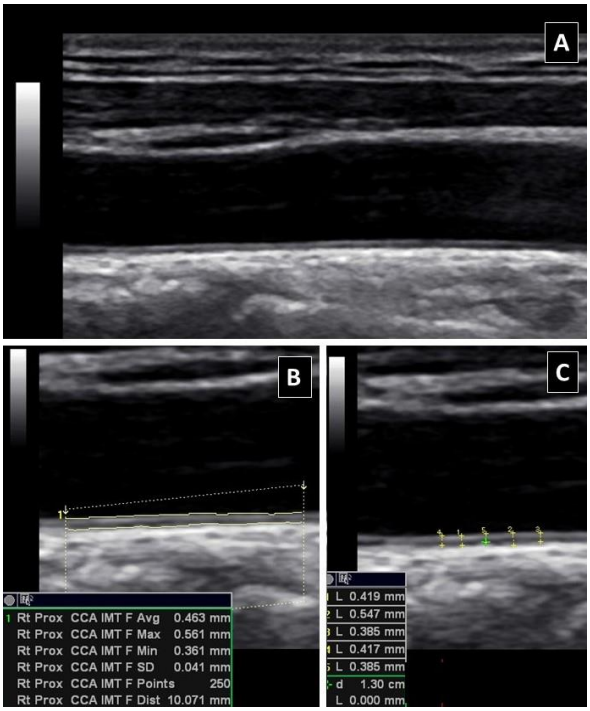


Figure 1. A: Common carotid artery longitudinal B-mode view [Transducer frequency 10 MHz, Gain 60, Dynamic range 72, Frame rate 85 Hz]; **B:** Auto-IMT: IMT indices were measured in a 250 point-1 cm long ROI from the posterior CCA wall. (3x focus); **C:** Manual IMT measurement: Average of 3 values excluding the highest and lowest values of 5 manual measurements.

Concordance Correlation Coefficient (ρc), Pearson correlation coefficient (as a measure of precision) and "bias correction factor" (as a measure of accuracy) were reported. Agreement is considered “poor” if the Concordance Correlation Coefficient is below 0.9.

“Inter-operator variability” was calculated to determine the agreement between operators (one (MAT) with more than 25 year-experienced vascular neurosonologist and the second (EY) is younger but well trained neurologist). Since an operator performed bilateral recordings twice on one patient, 80 CCA samples were used. Ultrasound and offline reading were performed by the same sonographer. Statistical methods detailed above were used for comparison. For the concordance correlation coefficient, "<0.90" is considered "Poor", "0.90 - 0.95" is considered "Moderate", "0.95 - 0.99" is considered "Substantial" and ">0.99" is considered "Almost perfect". For the coefficient of variation, values below 10% were categorized as "Good", between 10-20% as "Good", between 20-30% as "Acceptable", and values higher than 50% were categorized as "Suboptimal".

In the second part of the study, IMT indices were determined according to the presence and number of vascular risk factors. A total of 606 people were included for this section. The study population included 501 people without any including neurological complaints, 32 with a history of stroke, 20 with Parkinson's disease, 38 with a diagnosis of dementia, 11 with a diagnosis of multiple sclerosis, 1 with a history of Parkinson's and stroke, 1 with a history of Parkinson's and dementia, 1 with a diagnosis of dementia and stroke and another 1 with diagnosis of dementia, Parkinson's and stroke history. None of the subjects experienced acute symptoms. Individual consent was obtained for ultrasound study. The "being under treatment" criterion was used for the diagnosis of hypertension, diabetes mellitus and hyperlipidemia. "Obesity" was diagnosed if the body mass index was 30 kg/m^2 and above. It is the criterion currently used to diagnose smoking. IMTmax, IMTmean, IMTmin and IMTmanual “mean” and “standard deviation” values, ROC (Receiver operating characteristic) analysis and AUC (Area under the ROC Curve) mean value (with standard error and 95%CI range) are given for those with and without risk factor/disease. AUC values are interpreted as follows: 0.5-0.6 (unsatisfactory), 0.6-0.7 (satisfactory), 0.7-0.8 (good), 0.8-0.9 (very good), > 0.9 (excellent). Discrimination performance (ROC AUC) of IMT indices was compared with Z test.

The change trend of IMT values with the increase in the number of risk factors (from zero to five) was examined with the Kruskal-Wallis test (with Post-hoc Dunn test) and Kendall-tau rank correlation analysis was used to determine the change trend. Kendall tau cutoff values are accepted as “weak” for >0.06, “moderate” for >0.26, “strong” for >0.49, and “very strong” for >0.71. Some risk factors were not clarified in 7 cases, and 599 cases were included in the last section.

All statistical calculations and analyses were performed with IBM SPSS Statistics Ver. 22.0 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY). A p-value of <0.05 was accepted to indicate statistical significance.

RESULTS

Intra-subject variability (Repeatability): Intra-subject variability or reproducibility values were obtained by comparing two IMT parameter measurements made 3 days apart on the same subjects. Although there was no statistically significant difference between repeated IMT measurements, measurement variability and dispersion were high for all. Coefficient of Variation values were in the 20-30% band, that is, at an "acceptable" level, except for IMT-SD, which was higher. "Coefficient of Variation" was numerically lowest for IMT-max (20.93% in the first measurement, 19.88% in the second measurement), but there was no significant difference between the IMT indices. In repeated recordings, the 100 micron clinical criterion was exceeded in 16.3% of cases for IMT-max, 13.8% for IMT manual, 5% for IMT-average and 21.3% for IMT-min (Table 1). In terms of the IMT repeatability evaluations at the micron level, it was determined that the Concordance Correlation Coefficient was below 0.9 (Poor) for each parameter (Table 2, Figure 2). Neither "Systematic differences" (95% CI for all parameters included zero, Table 2) nor Proportional differences (95% CI for all parameters included one, Table 2) were detected between the measurements. No indication of systematic error was detected in the Bland Altman plotting examination (Figure 3).

Despite these positivity, it was observed that the average "Coefficient of Repeatability" value for each IMT parameter was over 100 microns (Table 2). The 95% CI lower limits of Coefficient of Repeatability for IMT-max and IMT-manual are 122.8 and 109.9, respectively, and the upper limits are 167.7 and 150.2 (Table 2).

Table 1. Intra-subject variability (Repeatability).

	Measurement			Variation Coefficient (%)		Difference	
	First	Second	p	First	Second	Mean ± SD	>100μ (%)
IMT-mean	496 ± 106	498 ± 106	*0.847*	21.25%	21.32%	40 ± 34	5%
IMT-max	648 ± 136	637 ± 127	*0.178*	20.93%	19.88%	56 ± 46	16.3%
IMT-min	364 ± 102	357 ± 100	*0.450*	27.93%	27.95%	64 ± 53	21.3%
IMT-SD	65 ± 22	63 ± 21	*0.427*	34.02%	33.09%	-	-
IMT-manual	503 ± 121	506 ± 119	*0.668*	24.00%	23.47%	52 ± 39	13.8%

Table 2. Comparison of repeatability performances.

	Concordance correlation coefficient	Coefficient of repeatability	Systematic differences	Proportional differences
IMT-max	0.847 (0.772 to 0.898)	141.8 (122.8 to 167.7)	-53.1 (-178.6 to 11.4)	1.09 (0.98 to 1.29)
IMT-mean	0.877 (0.815 to 0.919)	102.1 (88.5 to 120.8)	-42.6 (-117.2 to 24.2)	1.08 (0.95 to 1.22)
IMT-min	0.652 (0.506 to 0.762)	162.8 (140.9 to 192.6)	-10.8 (-145.8 to 32.0)	1.05 (0.91 to 1.41)
IMT-manual	0.852 (0.779 to 0.903)	126.9 (109.9 to 150.2)	-10.9 (-79.5 to 48.9)	1.02 (0.89 to 1.16)

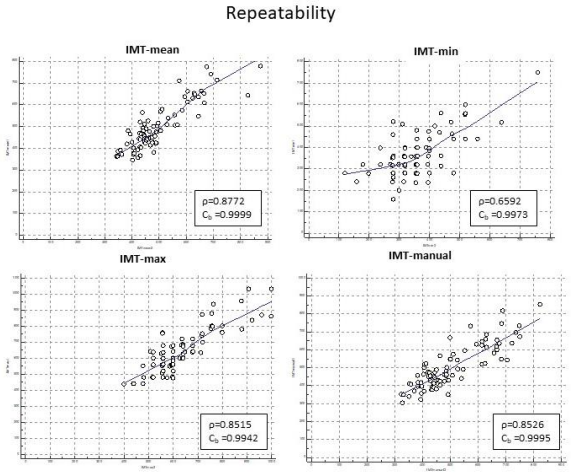


Figure 2. Concordance correlation coefficient analysis diagrams for repeatability: ρ (Pearson correlation coefficient, a measure of "precision") and C_b (Bias correction factor, as a measure of accuracy) were reported.

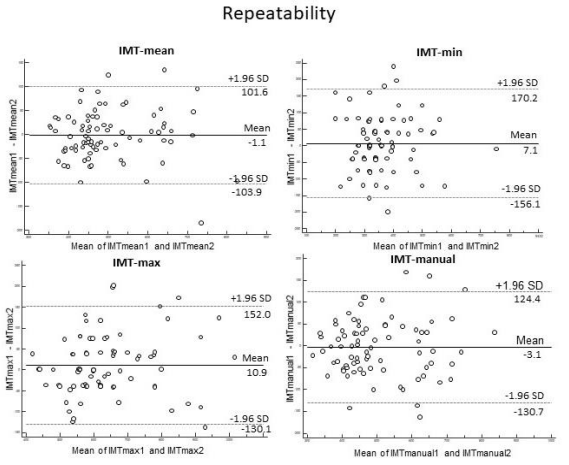


Figure 3. Blandt-Altman Plot for Reproducibility. Mean±1.96xSD values are reported.

Inter-operator aggrement: There was no statistical difference between the means of IMT indices obtained and readed by the two study neurosonologists (Table 3). Similarly, mean differences did not differ between operators. However, the "Coefficient of Variation" was high (albeit at an "acceptable" level) for both sonographers (Table 4). The "Coefficient of Variation" for IMT-max and IMT-min was measured to be numerically lower than in the IMT manual. However, the Coefficient of Variation for former two was between 19%-22%. The mean coefficient of variation measurements for the IMT-manual was 22.6% and 24.8%, indicating that the distribution of averages was quite wide (Figure 4). The percentage of measurement differences exceeding 100 microns (0.1 mm), which is the most commonly (though not fully consensus) accepted "clinical cut - off value", occurred in 15% of cases for IMT-mean, in 26.3% for IMT-max, in 17.5% for IMT-min, and in 21.3% for IMT-manual, and no statistically significant difference was found between these frequencies. In further evaluation of the agreement between operators,

the concordance correlation coefficient was found to be suboptimal (95% CIs were between 0.745 and 0.863) for all IMT methods and parameters. The average Coefficient of Repeatability is greater than 100 microns for each IMT index (199.9 for IMT-max, IMT mean 133.2, IMT-min 168.7 and IMT-manual 151.9, in μ). No statistical difference was detected between these values. Although it remained at a marginal level in Bland Altman plotting and Passing Bablok tests, it was determined that there was a systematic (111.7 micron) and proportional (95% upper limit of CIs of the ratio was 0.99) difference for IMT-max (Figure 5). For IMT-manual, the proportional difference reached borderline significance (95% upper limit of CIs of the ratio was 1.0).

Table 3. Inter-operator agrement.

	Measurement			Variation coefficient (%)		Difference	
	Sonographer-1	Sonographer-2	P	Sonographer-1	Sonographer-2	Mean±SD	>100μ (%)
IMT-mean	496 ± 103	498 ± 108	*0.731*	20.83%	21.72%	51 ± 45	15%
IMT-max	642 ± 122	642 ± 140	*0.970*	19.00%	21.78%	75 ± 70	26.3%
IMT-min	357 ± 109	365 ± 92	*0.429*	30.48%	25.26%	64 ± 58	17.5%
IMT-SD	65 ± 21	63 ± 22	*0.498*	32.89%	34.33%	-	
IMT-manual	508 ± 126	502 ± 113	*0.468*	24.81%	22.57%	62 ± 46	21.3%

Table 4. Comparison of performances in terms of inter-operator agreement.

	Concordance correlation coefficient	Coefficient of repeatability	Systematic differences*	Proportional differences**
IMT-max	0.694 (0.564 to 0.791)	199.9 (173.2 to 236.6)	111.7 (5.8 to 220.5)	0.83 (0.66 to 0.99)
IMT-mean	0.791 (0.693 to 0.863)	133.2 (115.3 to 157.6)	58.8 (-15.1 to 120.4)	0.88 (0.74 to 1.04)
IMT-min	0.632 (0.484 to 0.745)	168.7 (146.1 to 199.6)	-15.1 (-173.2 to 39.8)	1.03 (0.85 to 1.46)
IMT-manual	0.788 (0.691 to 0.858)	151.9 (131.6 to 179.7)	72.1 (-4.5 to 129.9)	0.84 (0.72 to 1.00)

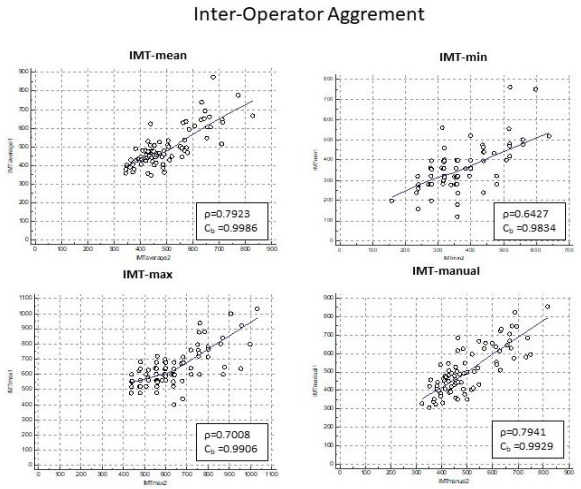


Figure 4. Concordance correlation coefficient analysis diagrams for Inter-operator variability: ρ (Pearson correlation coefficient, a measure of "precision") and C_b (Bias correction factor, as a measure of accuracy) were reported.

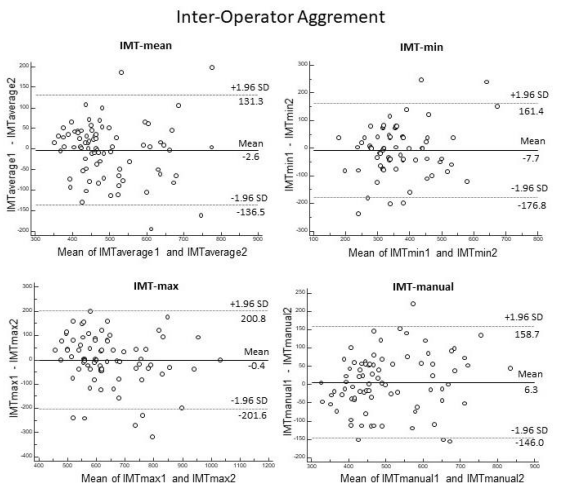


Figure 5. Blandt-Altman Plot for Inter-operator variability: Mean±1.96xStandard deviation values are reported.

Correlaton with atherosclerosis risk factor: In this section, IMT values of 606 cases were analyzed. The average age of the cases is 64.5±9.9 years and the female rate is 56%. Hypertension was detected in 49.7%, diabetes mellitus in 22.6%, hyperlipidemia in 36.5%, coronary artery disease in 21.3%, smoking in 38.9% and obesity in 42.4% of the cases. The number of risk factors was "0" in 10.9% of cases, "1" in 29.5% of cases, "2" of 29% of cases, "3" of 17.5% of cases, "4" of 10.8% of cases and "5" of 1.8% of cases (total cases were 599).

The ROC-AUC 95% CI lower limit was not above 0.6 for any risk factor. The ROC-AUC mean values of all IMT indexes for hypertension were above 0.6 ("Satisfactory") and no statistical difference was detected between them. While IMTmax, IMTmean and IMTmanual AUC values for diabetes mellitus were in the 0.6-0.7 range, it

remained as 0.591 (“non-satifactory”) for IMTmin. There was no statistical difference between IMT Indices. The mean AUC-ROC values for dyslipidemia, smoking, and obesity were below 0.6, and no significant difference was detected between the indices (Table 5).

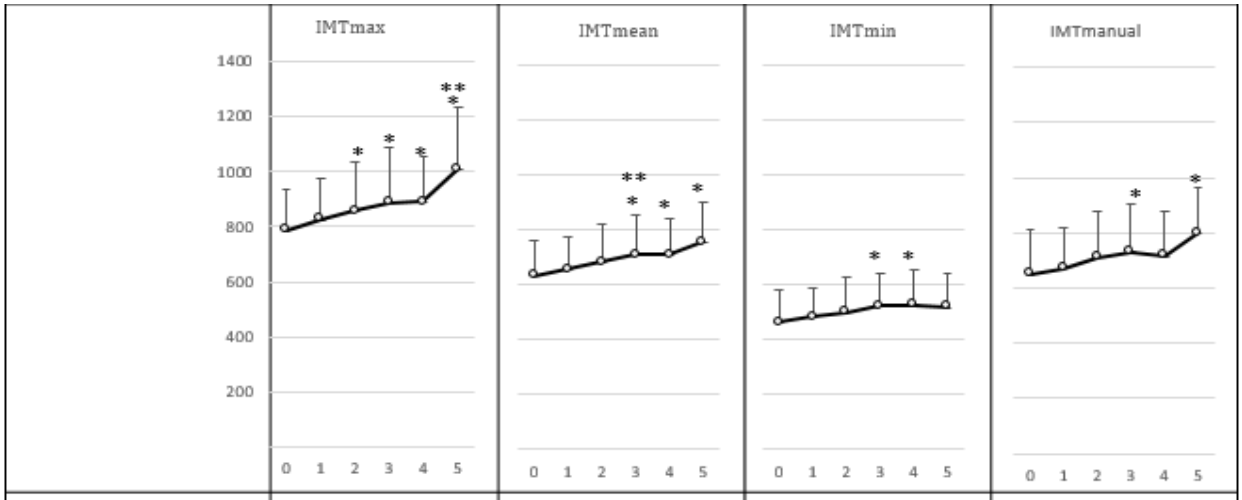
All IMT indices generally tend to increase as the number of risk factors increases (Figure 6). In the Kendal test performed for the size of these increasing trends, the proportional correlation (Kendal tau values) is between 0.07-0.24 for all indices, that is, in the "weak" correlation range.

Table 5. Vascular risk groups and IMT indices.

		IMTmax	IMTmean	IMTmin	IMTmanual
Hypertension	+	888.7 ± 178.1	702.8 ± 132.8	518.2 ± 117.6	733.0 ± 162.4
	-	815.1 ± 159.5	647.6 ± 127.5	473.9 ± 116.4	663.1 ± 153.5
	p	<0.001	<0.001	<0.001	<0.001
	AUC±SE	0.629±0.023	0.637±0.023	0.621±0.029	0.632±0.023
	%95CI	0.589 to 0.668	0.597 to 0.676	0.581 to 0.660	0.592 to 0.671
Diabetes	+	914.2 ± 195.5	721.3 ± 141.9	526.9 ± 126.4	746.7 ± 159.8
	-	833.9 ± 161.5	661.7 ± 127.3	487.2 ± 115.3	683.9 ± 159.7
	P	<0.001	<0.001	0.001	<0.001
	AUC±SE	0.620±0.027	0.629±0.027	0.591±0.028	0.620±0.027
	%95CI	0.580 to 0.659	0.589 to 0.668	0.550 to 0.630	0.580 to 0.659
Dyslipidemia	+	858.1 ± 172.1	680.8 ± 134.7	503.0 ± 118.0	701.4 ± 164.3
	-	848.7 ± 173.6	672.1 ± 132.1	492.2 ± 119.5	696.3 ± 160.5
	p	0.521	0.439	0.284	0.705
	AUC±SE	0.525±0.0243	0.526±0.0246	0.531±0.0248	0.512±0.0246
	%95CI	0.484 to 0.565	0.485 to 0.567	0.490 to 0.571	0.471 to 0.553
Smoking	+	868.7 ± 180.6	688.2 ± 140.1	509 ± 123.5	706.2 ± 171.2
	-	841.3 ± 167.8	666.6 ± 128.1	487.4 ± 115.7	692.6 ± 155.8
	p	0.059	0.052	0.033	0.314
	AUC±SE	0.546±0.0242	0.546±0.0244	0.560±0.0242	0.515±0.0244
	%95CI	0.505 to 0.587	0.505 to 0.586	0.520 to 0.601	0.474 to 0.556
Obesity	+	859.6 ± 181.9	677.3 ± 137.5	490.4 ± 119.8	699.5 ± 162.4
	-	846.1 ± 166.9	673.6 ± 130.3	500.3 ± 118.8	697.2 ± 162
	p	0.345	0.735	0.315	0.860
	AUC±SE	0.517±0.0239	0.502±0.0238	0.533±0.0237	0.501±0.0239
	%95CI	0.476 to 0.557	0.461 to 0.543	0.492 to 0.573	0.460 to 0.542

DISCUSSION AND CONCLUSION

The manual (free-hand) "reading" method, as herein described, in IMT measurement was compared with a prototype commercial algorithm that performs automatic edge detection. The results obtained by the two methods are generally compatible with each other in terms of repeatability consisting of intra-subject and intra-operator variability. This emphasizes that the manually read IMT data that have long accumulated in the literature are still valid today. However, it is somewhat difficult to say the same for the change cut-off values, whisch is at the sub-millimeter level, even several microns (2,5,16,17), used for progression and resolution of subcilinical atherosclerosis. In our study, no statistical difference was detected in the averages of two recordings made within a relatively short (3 days) interval on the same person (a method that is often used in the literature but is not sensitive enough). Likewise, no systematic or proportional variability or bias was detected between repeated measurements and readings for any IMT parameter. However, it was determined that the variability of measurements for all IMT indices was high and the distribution range was wide. Concordance correlation coefficients are far from ideal. This coefficient is similar to the intraclass correlation but is more resistant to sample distribution anomalies (18). The concordance correlation coefficients we determined correlated well to the intraclass correlation coefficient limits reported in the literature. However, in some cases identified in some studies, the correlation coefficient exceeded 90%, that is, favorable. But, in these cases it can still be seen that the dispersion is high and the upper tolerance limits are similarly low (7). The repeatability of IMT methods in the same person is only at an acceptable level, and the measurement difference exceeds 100 microns in about 15 (from 5% to 21.3%) percent of the individuals in our study. The frequency of measurement differences exceeding 100 microns



0 Risk	788.2 ± 150.7	632.3 ± 125.0	461.7 ± 118.3	649.6 ± 160.1
1 Risk	826.8 ± 149.9	653.1 ± 116.2	480.8 ± 106.2	671.4 ± 144.1
2 Risk	858.7 ± 175.9	679.7 ± 135.8	498.7 ± 127.1	711.9 ± 165.7
3 Risk	887.7 ± 197.6	706.7 ± 147.1	521.3 ± 118.0	731.1 ± 174.5
4 Risk	889.7 ± 164.6	706.9 ± 134.5	524.7 ± 125.1	716.4 ± 161.4
5 Risk	1009.3 ± 223.4	755.1 ± 142.8	517.0 ± 119.4	800.0 ± 162.8
Kruskal-Wallis p*	0,000027	0,000046	0,001797	0,000588
Kendall's Tau	0,151 (0,0870 to 0,206)	0,154 (0,0951 to 0,205)	0,129 (0,0730 to 0,194)	0,134 (0,0762 to 0,191)

Post-hoc Dunn test; *Greater than 0 risk; **: Greater than 1 risk

Figure 6. In the upper bank, the increase of IMT values according to the number of risk factors is shown. The values are given in the table in the lower bank. Kruskal-Wallis test followed by Dunn test was performed post-hoc. **Greater than "0-risk" and **Greater than "1-risk".

has been reported at this level or higher in all studies in the literatüre (12,19). Therefore, clinically significant IMT changes (to meet the resolution or progression criterion) can be considered to be in the range of 150-200 microns (0.15-0.20 mm) for the maximum IMT method. However, it should be underlined that a large population study is required for this normative data. Because the data in the literature is not that solid (5,20,21).

In our study, it was determined that the aggreement of IMT measurements between operators was not at the optimal level between experienced and inexperienced (but well-trained) operators. In fact, no statistical difference was detected between the mean values or differences in repeated measures of the IMT indices obtained and read by the two neurosonologists. But, differences in "repeatability" of measurements of different operators was significant (Mean "coefficient of repeatability" is greater than 100 microns for each IMT index) and aggreement was suboptimal ("Concordance Correlation coefficient" below 0.9 for all IMT indices). Additionally, it was determined that maximum IMT methods (both manual and automatic edge detection algorithms) may have a high rate of systematic and proportional errors (As documented Table 4 and Figure 4 and 5). This observation underlines that the participation of different operators in different time periods in the prospective studies using IMT surrogates may be another cause of error. Importantly, experienced and inexperienced sonographers alike can make inaccurate measurements (22). We did not investigate inter-reader aggreement in our study given the sonographers in our study also read their recording. If these two (recording and reading) processes are performed by different persons, it can be predicted that there may be increased

errors resulting from both measurement and reading.

In the second part of our study, the ability of IMT indices to discriminate vascular risk factors was compared. It was observed that the strength of the IMT indices measured manually with the electronic caliper of the ultrasound device and the strength of the IMT indices obtained with the semi-automatic border detection software were similar. In terms of discrimination of hypertension and diabetes mellitus, manual-IMT, IMT-max and IMT-mean capacity remained only as satisfactory level. However, since the lower limit of the 95% confidence interval of these indices is below 0.6, it should be taken into consideration that none could have a sufficient clinical yield. In addition, none of the IMT indices were observed to be discriminatory for dyslipidemia, smoking and obesity (Table 5). The data in the literature indicating IMT is thicker in the presence of vascular risk factors (as in our study other than dyslipidemia and obesity) are convincing (13,20,23-25). However, there are also studies showing that IMT thickening is not closely related to risk factors (26). The other result of our study is that as the number of vascular risk factors increases, IMT-manual, IMT-maximum and IMT-mean values show an increasing trend approaching the "medium" strength. Again, in this respect, no significant difference was observed between manual and semi-automatic measurements.

In conclusion, manually tracked IMT and semi-automatic edge-detection traced IMT are interchangeable. If IMT indices are used as indicators of vascular risk in an individual approach, threshold limits should be at the level of 150-200 microns, not 100 microns or 0.1 mm (27).

We think that the 10 micron/year progression and regression limit recommendations stated in the literatüre (2,17) are too low compared to measurement variability.

IMT automation can of course make readings faster and more accurate. However, it seems unlikely that IMT automation would ultimately impact the significant variability in acquisition of ultrasound images.

Several case control and cohort studies, such as the multi-ethnic study of atherosclerosis, found no significant difference in cardiovascular outcomes when comparing edge-detected CCA IMT with manual-traced IMT (15,28).

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Ethics

Ethics Committee Approval: The study was approved by Hacettepe University Non-Interventional Ethics Committee (Date: 24.07.2013, No: GO 13/243-13 and Date: 04.05.2021, No: 2021/10-37).
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