Reproducibility of manual measurement of intima-media thickness at distal common carotid artery under a strict measurement protocol by carotid ultrasound in 242 subjects

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Purpose

Background

-Carotid atherosclerosis represents systemic atherosclerosis.

-Carotid ultrasound estimates cardiovascular risk detecting atheroma plaques and measuring intima-media thickness (IMT) of the common carotid artery (CCA).

-Minimal differences in measurements of IMT change classifications to distant quartiles, and being included in the highest and the lowest quartiles depended on IMT differences of 0.3 mm.

-Carotid ultrasound is often performed by sonographers, within and outside of Radiology departments, with different levels of training and experience, in addition to different approaches (manual or semi-automated). The reliability of these approaches is many times simply assumed. The reported variability of IMT measurements changes among studies, and, even more relevant, the statistical assessment has been very diverse, most frequently focused on correlation of measurements rather than agreements. As a result, correlation has been systematically reported much more than agreement. Manual measurements at subjectively selected positions might present significant variability. Therefore, assessing reproducibility is mandatory before cardiovascular risk can be reliably estimated.

-We developed a previous project (PRO1) to assess the intra and inter-observer agreement of these manual measurements between operators with different levels of experience, in our population, reproducing a common clinical scenario. 21 patients underwent two carotid ultrasound examinations on different days. Each operator successively took three distal carotid common artery (CCA) longitudinal images and, after visually deciding what the thicker IMT point was, the IMT was manually measured. Each operator freely decided the angle of incidence and the zoom level for each measurement. The intra and interobserver agreement for IMT range was between good and excellent on left side, but only between fair and good on right side, and generally with wide confidence intervals.

Purpose

Our purpose in this second project (PRO2) is to assess the reproducibility of intima-media thickness (IMT) manual measurements at distal common carotid artery (CCA) under a strict protocol and to determine whether reproducibility improves with respect to the simple protocol developed in PRO1.
Methods and materials

Patients

Subjects recruited for this study were part of a different project at the Internal Department of our Institution. Four groups of patients were examined: healthy people, anti-phospholipid syndrome (APS) diagnosed patients, patients with positive anti-phospholipid antibodies but without criteria for APS, fundamentally thrombosis (AB), and finally patients neither APS nor AB with previous arterial or venous thrombosis at different territories. Subjects were appointed for an ultrasound examination by two operators at the same appointment, with the exception of 3 patients appointed in two different days for the first examination of each operator. A subgroup of healthy subjects from that initial sample received a second appointment and both operators performed a second ultrasound examination.

The study was approved by the Institutional Ethics Committee. Before the ultrasounds, all subjects were interviewed and informed on the characteristics and objectives of the study and signed the written informed consent before beginning the procedure.

Ultrasound and operators

All patients were examined with the same ultrasound scanner and linear probe, an S2000 Siemens, 9L4 MHz (Siemens Healthcare), between March 3rd and July 29th, 2014. Each patient was individually and independently examined by two operators, one with 7 (Operator 1-Op1-) and another with 26 (Operator 2-Op2-) years of experience with general and Doppler ultrasound. Op1 was also a participant of PRO1 for data collection.

Protocol for data collection

Both operators explored each patient in two different days, but not before 15 days from the first exam had passed, and not exceeding 125 days between both exams. They were always blinded to the results of the other operator, and, when performing the second examination, also to those of the previous exams. To perform the ultrasounds, all operators followed the recommendations of the American Society of Echography. Accordingly, the patient stayed in supine position, the neck slightly hyperextended and rotated 45° to the opposite side to probe (figure 1). During the scan, the operator could slightly adjust the neck position to optimize images. A standard probe depth of 4 cm was used; however, increased depth could be exceptionally modified in some patients with larger necks or deeper vessels. The focus and the gain could also be modified. Taking the sternocleidomastoid (SCM) muscle as a reference, 3 longitudinal images from 1 cm distal of each CCA were taken from the probe angulation over anterior, lateral, and posterior SCM edges, respectively, obtaining 3 different imaging planes. The obtained
measurements were called IMT individual anterior, lateral and posterior (IMTindiv-ant, IMTindiv-lat, IMTindiv-post), respectively (figure 1).

Both sides were examined. The distal CCA was displayed in a longitudinal and perfectly horizontal plane in which, when possible, we saw the carotid bifurcation (figure 2a) and the "double-line" in both the near and far walls of the CCA, corresponding to the blood-intima and intima-media interfaces, respectively, indicating a true perpendicular scanning plane (figure 2a and 2b). Whether seeing the "double line" was impossible, the condition of seeing the distal CCA in all its longitudinal extension for measurement of IMT was maintained. The IMT was manually measured at diastole, where plaques were absent, and recorded in each image. Operators considered the IMT as the maximum distance between internal intima edge and internal adventitia edge (figure 2).

**Fig. 1**: Head position and probe orientations for carotid ultrasound scanning, right-side example. IMTindiv: intima-media thickness individual measurement; ant: anterior; lat: lateral; post: posterior; E1: examination 1; E2: examination 2; CCA: common carotid ultrasound; SCM: sternocleidomastoid muscle.

**References**: RADIOLOGÍA, HOSPITAL MORALES MESEGUER, HOSPITAL MORALES MESEGUER - Murcia/ES
Fig. 2: Optimal images to obtain manual measurements at distal common carotid artery. Both intima-media (empty arrowhead) and media-adventitia (arrowhead) show up as echogenic interfaces at ultrasound, respectively ("double line"). IMT was the maximum distance between internal intimal edge and internal adventitial edge in the distal centimeter of posterior wall of common carotid artery (calipers).

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Operators were instructed to avoid looking at the quantitative value in the screen. The protocol for the second ultrasound examination was the same as for the first examination, but CCA IMTindiv-lat measurement was also obtained with zoom by both operators. For this, each operator zoomed the IMTindiv-lat image and measured again the IMT immediately after obtaining IMTindiv-lat measurement without zoom. Both operators performed the same level of zoom, 2x (figure 3).
Fig. 3: The intima-media thickness individual measurement (IMTindiv) was obtained over lateral edge of SCM muscle (a) and was repeated employing zoom (b) on second exploration on both sides.

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After the exam was over, Op1 archived the three measurements and the zoomed image measurements from the second examination in a Microsoft Office Excel database. The three IMTindiv measurements, the mean of the three IMTindiv measurements (IMTmean), and the thickest of the three IMTindiv measurements (IMTmax) were the variables used for analysis.

Statistical analysis

Variables in Excel tables were exported to SPSS version 20.0 for Windows (IBM, Armonk, NY) and to MedCalc for Windows, versión 12.5 (MedCalc Software, Ostend, Belgium). Variables were presented as mean ± standard deviation. We assessed the intra and interobserver agreement for IMT between the two operators. All agreement analysis was made with the Intraclass Correlation Coefficient (ICC) with 95% confidence intervals (95%CI). Agreement levels of ICC were classified as poor (<0.20), fair (0.21-0.40),
moderate (0.41-0.60), good (0.61-0.80) and excellent (>0.80). Bland-Altman analysis was used to further determine the agreement between measurements obtained by each operator by calculating the bias (mean difference) and 95% limits of agreement (1.96 standard deviations of the difference).

Agreement compared to PRO1

Intra- and interobserver agreement for IMTmean and IMTmax of both examinations of PRO2, according to CCI, were compared with intra- and interobserver agreement for IMTmean and IMTmax of both examinations of PRO1.
**Fig. 1:** Head position and probe orientations for carotid ultrasound scanning, right-side example. IMTindiv: intima-media thickness individual measurement; ant: anterior; lat: lateral; post: posterior; E1: examination 1; E2: examination 2; CCA: common carotid ultrasound; SCM: sternocleidomastoid muscle.

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**Fig. 3:** The intima-media thickness individual measurement (IMTindiv) was obtained over lateral edge of SCM muscle (a) and was repeated employing zoom (b) on second exploration on both sides.
Results

Epidemiologic data of our samples are shown in Figure 4.

Fig. 4: Epidemiologic characteristics of the sample. APS: anti-phospholipid syndrome diagnosed patients; AB: patients with positive anti-phospholipid antibodies but without criteria for APS; E1: examination 1; E2: examination 2.

<table>
<thead>
<tr>
<th>GENDER</th>
<th>E1</th>
<th>E2</th>
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<tr>
<td>Male</td>
<td>121 (54.1 %)</td>
<td>18 (50.0 %)</td>
</tr>
<tr>
<td>Female</td>
<td>111 (45.9 %)</td>
<td>18 (50.0 %)</td>
</tr>
<tr>
<td>Total</td>
<td>242 (100 %)</td>
<td>36 (100 %)</td>
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<th>AGE</th>
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<th>E2</th>
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<tr>
<td>Valid</td>
<td>242</td>
<td>36</td>
</tr>
<tr>
<td>Mean</td>
<td>51.50</td>
<td>44.47</td>
</tr>
<tr>
<td>Median</td>
<td>50.00</td>
<td>45.00</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>13.013</td>
<td>10.668</td>
</tr>
<tr>
<td>Minimum</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Maximum</td>
<td>83</td>
<td>68</td>
</tr>
</tbody>
</table>

All subjects upon second examination were healthy. About 50% were men in both examinations. The age mean was 51.5 and 44.5 for first and second ultrasounds respectively, but the age range was wide, between 22 and 83, and between 22 and 68 years old in the first and second examinations respectively. The first ultrasonographic assessment and measurement of carotid IMT was performed on the 242 subjects. Two of the 3 patients who exceptionally received a second appointment for undergoing the first examination of each operator did not attend, so their measurements were lost. The second ultrasound assessment and measurement of carotid IMT were performed on all
36 subjects. One right CCA IMTindiv-post from Op2 was lost, because the image file failed and couldn't be found later in the in our local Picture Archiving and Communication System (PACS). Final data collection is shown in Fig. 5 on page 21.

**Agreement analysis**

For both operators, the intraobserver agreement by ICC was moderate to excellent on right and left CCA for all IMTindiv (ICC 0.524-0.807). Intraobserver agreement for zoomed and non-zoomed IMTindiv-lat measurements was excellent for both operators and both sides (ICC > 0.840). Intraobserver agreement revealed a narrow Bland-Altman limits of agreement for all IMTindiv on both sides (the widest limit ranged from 0.42 to -0.37 mm for left IMTindiv-post Op1) and for zoomed measurements (the widest limit ranged from 0.24 to -0.21 for left zoomed Op1 measurement) (figures 6, 7, 8 and 9).

IMTmean intraobserver agreement by ICC was excellent on right and left CCAs for both operators, except a good agreement on left CCA for Op1 (ICC 0.665) (figure 6). IMTmax intraobserver agreement by ICC was good for Op1 and excellent for Op2, on both sides respectively (figure 6). For both operators and both sides, IMTmax intraobserver agreement was always worse than IMTmean, with a wider 95% CI for ICC (figure 6) and wider limits of agreement on Bland-Altman analysis (figures 6, 10 and 11).
Fig. 6: Intraobserver agreement for intima-media thickness individual (IMTindiv), intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) measurements. Superior chart: Intraclass Correlation Coefficient, with 95% confidence intervals (CI). Inferior chart: Bland-Altman analysis, with mean difference and 95% limits of agreements.

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Interobserver agreement was good or excellent for all IMTindiv measurements on both sides on the first (ICC from 0.742 to 0.835) and the second (ICC from 0.605 to 0.784) examinations according to ICC. Interobserver agreement for zoomed IMTindiv-lat measurements was moderate or good for both operators on both sides in the second examination (ICC 0.605-0.699), but worse than non-zoomed IMTindiv-lat measurements (figures 12 and 13). Bland-Altman limits of agreement were narrow for all IMTindiv on both sides (the widest ranged from 0.44 to -0.39 mm for left IMTindiv-lat in the second examinations) and for zoomed measurements (the widest ranged from 0.45 to -0.41 for the left zoomed measurement) (figures 14-17).

IMTmean interobserver agreement was excellent for both operators on both sides and for both examinations (figures 12 and 13). IMTmax interobserver agreement ranged from moderate to excellent and, as for intraobserver agreement, it was worse than IMTmean.
(figures 12 and 13) for both operators on both sides and for both examinations, and with wider limits of agreement on Bland-Altman analysis (figures 12, 13, 18 and 19).

Fig. 12: Interobserver agreement for intima-media thickness individual (IMTindiv), intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) in the first examination. Superior chart: Intraclass Correlation Coefficient, with 95% confidence intervals (CI). Inferior chart: Bland-Altman analysis, with mean difference and 95% limits of agreements.

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Fig. 13: Interobserver agreement for intima-media thickness individual (IMTindiv), intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) in the second examination. Superior chart: Intraclass Correlation Coefficient, with 95% confidence intervals (CI). Inferior chart: Bland-Altman analysis, with mean difference and 95% limits of agreements.

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Agreement compared to PRO1

IMTmean and IMTmax intraobserver agreement of PRO2 was better on the right CCA compared to PRO1. However, agreement on left CCA was sometimes even worse in PRO2 compared to PRO1 (figure 20).
Fig. 20: Comparision of intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) intraobserver agreement of the project 1 (PRO1) and the project 2. Intraclass Correlation Coefficient (ICC), with 95% confidence intervals (CI).

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Regarding the interobserver agreement, the IMTmean and IMTmax of PRO2 was always better on the right CCA compared to PRO1 (figure 21-24). However, although left IMTmean and IMTmax agreement was good or excellent according to ICC in PRO2, it stayed very similar and sometimes even worse than in PRO1 (figures 21 and 23).
Fig. 21: Comparision of intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) interobserver agreement of the first examination of the project 1 (PRO1) and the first examination of the project 2. Intraclass Correlation Coefficient (ICC), with 95% confidence intervals (CI).

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**Fig. 22:** Comparison of intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) interobserver agreement of the second examination of the project 1 (PRO1) and the first examination of the project 2. Intraclass Correlation Coefficient (ICC), with 95% confidence intervals (CI).

**References:** RADIOLOGÍA, HOSPITAL MORALES MESEGUIER, HOSPITAL MORALES MESEGUIER - Murcia/ES
**Fig. 23:** Comparision of intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) interobserver agreement of the first examination of the project 1 (PRO1) and the second examination of the project 2. Intraclass Correlation Coefficient (ICC), with 95% confidence intervals (CI).

**References:** RADIOLOGÍA, HOSPITAL MORALES MESEGUER, HOSPITAL MORALES MESEGUER - Murcia/ES
Fig. 24: Comparison of intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) interobserver agreement of the second examination of the project 1 (PRO1) and the second examination of the project 2. Intraclass Correlation Coefficient (ICC) with 95% confidence intervals (CI).

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Fig. 5: Characteristic of intima-media thickness (IMT) at distal centimeter of common carotid artery (CCA) (superior table) and data collection of both examinations (inferior table, data in absolute number of measurements). E1: examination 1; E2: examination 2.

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Fig. 6: Intraobserver agreement for intima-media thickness individual (IMTindiv), intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) measurements. Superior chart: Intraclass Correlation Coefficient, with 95% confidence intervals (CI). Inferior chart: Bland-Altman analysis, with mean difference and 95% limits of agreements.

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**Fig. 7:** Intraobserver agreement for right intima-media thickness individual measurements (IMTindiv). Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2. E1: examination 1; E2: examination 2.

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Fig. 8: Intraobserver agreement for left intima-media thickness individual measurements (IMTindiv). Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2. E1: examination 1; E2: examination 2.

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**Fig. 9:** Intraobserver agreement for right and left zoomed and non-zoomed intima-media thickness individual (IMTindiv) lateral measurements in second examination. Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2. E2: examination 2.

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**Fig. 10:** Intraobserver agreement for right and left intima-media thickness mean measurements (IMTmean). Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2. E1: examination 1; E2: examination 2.

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**Fig. 11:** Intraobserver agreement for right and left intima-media thickness maximum measurements (IMTmax). Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2. E1: examination 1; E2: examination 2.

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Fig. 12: Interobserver agreement for intima-media thickness individual (IMTindiv), intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) in the first examination. Superior chart: Intraclass Correlation Coefficient, with 95% confidence intervals (CI). Inferior chart: Bland-Altman analysis, with mean difference and 95% limits of agreements.

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Fig. 14: Interobserver agreement for right intima-media thickness individual measurements (IMTindiv) in the first examination. Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2.

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**Fig. 15:** Interobserver agreement for left intima-media thickness individual measurements (IMTindiv) in the first examination. Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2.

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Fig. 16: Interobserver agreement for right intima-media thickness individual measurements (IMTindiv) and for right zoomed IMTindiv lateral measurement in the second examination. Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2. E2: examination 2.

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Fig. 17: Interobserver agreement for left intima-media thickness individual measurements (IMTindiv) and for left zoomed IMTindiv lateral measurement in the second examination. Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2. E2: examination 2.

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**Fig. 18:** Interobserver agreement for right and left intima-media thickness mean (IMTmean) and maximum (IMTmax) measurements in the first examination. Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2.

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Fig. 19: Interobserver agreement for right and left intima-media thickness mean (IMTmean) and maximum (IMTmax) measurements in the second examination. Bland-Altman analysis, with mean difference and 95% limits of agreements. Op1: operator 1; Op2: operator 2. E2: examination 2.

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Fig. 20: Comparison of intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) intraobserver agreement of the project 1 (PRO1) and the project 2. Intraclass Correlation Coefficient (ICC), with 95% confidence intervals (CI).

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**Fig. 21:** Comparision of intima-media thickness mean (IMTmean) and intima-media thickness maximum (IMTmax) interobserver agreement of the first examination of the project 1 (PRO1) and the first examination of the project 2. Intraclass Correlation Coefficient (ICC), with 95% confidence intervals (CI).

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Conclusion

According to our results, to measure CCA IMT under a strict measurement protocol doesn’t always get an acceptable reproducibility, and doesn’t always improve reproducibility with respect to a simple and more usual protocol. Intraobserver agreement obtained under a strict measurement protocol ranges from moderate to excellent and interobserver agreement ranges from good to excellent, except for zoomed measurements, with worse interobserver agreement than non-zoomed ones. Intra and interobserver IMTmean agreement are always better than IMTmax one.

Our main purpose has been to assess the reproducibility of manual ultrasound measurements at distal CCA from different longitudinal image planes obtained under a strict measurement protocol following the American Society of Echography recommendation for IMT measurements. There are few previous papers analyzing the reproducibility of manual and automatized carotid IMT measurements. Some of them have only made a correlation analysis but agreement was not actually assessed. For other researchers, correlation based on coefficients of variation or the difference of means was acceptable or very high, but the interobserver agreement was not studied in the latter. Unlike those studies, we assessed intra and interobserver agreements with the intraclass correlation coefficient and Bland-Altman analysis. On the other hand, some papers assess reproducibility of measurements at different carotid areas such as in the internal carotid artery, the carotid bifurcation or distal CCA and reproducibility of intimal measurement versus intima-media thickness. Our research includes a circumferential scan at distal centimeter of both CCA, getting three measurements from three imaging plane angulations over anterior, lateral and posterior of the SCM muscle, obtaining images from the postero-medial, medial and antero-medial CCA intima-media walls thickness, respectively (figure 1). Moreover, we have compared the agreement of manual measurement of IMT CCA obtained under a simple and common protocol with measurements under a stricter, laborious and consuming time one. Agreement under a strict protocol, although overall acceptable, had an evident variability and didn’t always improve reproducibility with respect to the simple protocol of a usual clinical scenario. Sonographers and cardiologists must be conscious of this point when IMT measurements are considered a sign of cardiovascular risk. Other factors other than technical protocol of manual measurements may influence on the strength of agreement. IMTmean (averaged three IMT measurements in distal centimeter of CCA) is the American Society of Echography’s recommendation for IMT manual quantification and, as our results corroborate, is less variable than IMTmax. The small sample for the second examination (n=36) can be considered a limitation to assess the agreement; however, although global agreement was better in the first examination, with a greater sample (n=243), it didn't improve significantly with respect to a project employing a simple protocol of measure. The use of manual measurement with electronic calipers instead of automatic image analysis could be considered another limitation. However, we don't think our manual approach to IMT assessment is a real limitation if we consider that
this is also the most common acquisition method. Including different types of patients could be considered another limitation, but we don’t think it influences the assessment of reproducibility of IMT measurement.

In conclusion, although agreements of CCA IMT measurements under a strict protocol are acceptable, variability is important, ranging from moderate to excellent. IMTmean measurement is more reproducible than IMTmax one. A strict protocol doesn’t always improve reproducibility with respect to the simpler protocol of a usual clinical scenario. Other factors that influence reproducibility outside technical protocol of measurement should be researched.
References