ORIGINAL ARTICLE

Nasal bone length: prenasal thickness ratio: a strong 2D ultrasound marker for Down syndrome

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ABSTRACT

Objectives To evaluate the feasibility of incorporating two-dimensional ultrasound measurements of nasal bone length (NBL) and prenasal thickness (PT) into the second-trimester anomaly scan and to determine whether the NBL : PT ratio could help in differentiating euploid and Down syndrome fetuses.

Method Two-dimensional measurements of NBL and PT were obtained from the midsagittal plane of the fetal head at 14–28 weeks of gestation in a Caucasian population at risk for aneuploidy. The screening performances of NBL, PT, and the ratios NBL : PT and PT : NBL were analyzed in euploid ($n = 1330$) and Down syndrome ($n = 33$) fetuses.

Results Nasal bone length and PT alone showed strong correlations with Down syndrome (sensitivity: 76% at 1.88% and 2.35% false positive rate, respectively). However, the NBL : PT ratio showed an even stronger correlation with Down syndrome (false positive rate: 0.9%, sensitivity: 97%). The mean NBL : PT ratio showed a gradual increase from 1.48 to 1.79 (a 21.2% increase) between 14 and 28 weeks of gestation.

Conclusion Two-dimensional ultrasound measurements of NBL and PT, particularly the NBL : PT ratio, are highly sensitive markers for Down syndrome fetuses. © 2014 The Authors. *Prenatal Diagnosis* published by John Wiley & Sons, Ltd.

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INTRODUCTION

Differences in nasal bone length (NBL), determined by ultrasound, have been suggested to differentiate second-trimester euploid and Down syndrome fetuses.[1–6] From analyses of the facial profile, a thickening of the prenasal soft tissue [prenasal thickness (PT)] is also apparent in the vast majority of second-trimester fetuses with Down syndrome. There is evidence that the combination of NBL and PT measurements as a ratio improves the detection of fetal Down syndrome by ultrasound.[6–10]

Despite the multitude of ultrasound soft markers for Down syndrome fetuses – such as increased nuchal fold thickness, cystic hygroma, cardiac anomalies, echogenic intracardiac foci, nasal bone hypoplasia, ventriculomegaly, widened iliac crest angle, short femur/humerus PT, duodenal atresia, echogenic bowel, pyelectasis-hydronephrosis, sandal gap sign, choroid plexus cyst, and midphalanx hypoplasia of the fifth finger (Appendix 1) – there are no very sensitive ultrasound markers in the second trimester that can be used either alone or in combination.[11–13] Furthermore, these markers may not be present in all affected fetuses, and such as all soft markers, they can also be detected in euploid cases.[11]

Preliminary observations using 2D ultrasound measurements of NBL and PT at our tertiary referral center suggested the potential for the second-trimester identification of euploid and Down syndrome fetuses in a mixed-risk population.[14] Therefore, we proposed that both markers and their ratios should be incorporated into the second-trimester fetal anomaly scan for a reliable, cheap, and efficient screening of Down syndrome. The current prospective study examined the clinical value of 2D ultrasound measurements of NBL, PT, and their ratios for differentiating euploid and Down syndrome fetuses in the second trimester (in an at-risk population).

METHODS

Women were referred for genetic counseling and second-trimester anomaly scans to our regional prenatal genetics center because of advanced maternal age (≥35 years); positive screening results; intermediate risk of combined, triple, or integrated tests; and the presence of one or more aneuploidy soft markers in previous ultrasound examinations. Women were recruited for second-trimester assessment and measurement of the NBL and PT values between January 2008 and April 2013. Informed consent was obtained from the mothers before examination at the MEDISONO Fetal and Adult Health Research Center or at the Department of Obstetrics and Gynecology in Szeged, Hungary.

The following criteria determined enrollment into the euploid group : singleton viable pregnancy, 14–28 weeks of gestation, a lack of maternal disease (such as hypertension, toxemia, renal disease, and diabetes mellitus), normal fetal growth, normal amniotic fluid volume, diagnosis of a normal fetal anatomy,

and newborns without chromosomal or structural abnormalities between the fifth and 95th percentile birth weight at delivery.

The study included 1330 euploid and 33 Down syndrome fetuses. The protocol was approved by the ethics committee of the University of Szeged. A routine second-trimester anomaly scan in weeks 18–23 and a detailed examination of the fetal anatomy within 14–17 and 23–28 weeks of gestation were performed using a high-resolution 2D transabdominal ultrasound scanner (Voluson E8 Expert, GE Healthcare, Milwaukee, WI, USA). The facial profile was assessed at the beginning of the scanning sessions to avoid effects of fetal movements that could alter the fetal position. Three image acquisitions were obtained during one scan session, and the best one was used for analysis. If it was not successful, then the patient came back for another scanning session 30–40 min later. The sonographer was blind to the fetal karyotype, and each ultrasound examination was performed before the chromosomal study.

Nasal bone length[1,10] and PT[9] measurements can be obtained on the same image if the face of the transducer was positioned parallel to the nasal bone. The insonation angle should be close to 45°. The following image settings were used: low gain, medium dynamic contrast, and maximum magnification so that the fetal head occupied the entire screen. Images were adjusted to ensure correct midsagittal plane and sharp margins of the skin and the nasal bone. The diencephalon, nasal bone, lips, maxilla, and mandible were used as reference points for the correct measurements of NBL and PT in the midsagittal plane.[3,9] Briefly, PT was measured as the shortest distance from the lower margin of the frontal bone to the outer surface of the overlying skin. The margins of the nasal bone are the proximal and the distal ends of the white ossification line. The NBL and PT were measured using the same view (Figure 1A and B).

Maternal data and sonographic findings were recorded in a database (Astraia Software GmbH, Münich, Germany). The ultrasound imaging data were stored in the local Digital Imaging and Communications in Medicine (DICOM) format via Astraia. Values of NBL and PT were exported to Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed using SigmaPlot (Systat Software Inc., San Jose, CA, USA). Scatter plots of NBL and PT with linear polynomial regression lines and percentile curves (third and 97th) were created. Similarly, scatter plots of NBL : PT and PT : NBL ratios with linear polynomial regression lines and percentile curves (fifth and 95th) were produced. Comparisons between euploid and Down syndrome measurements for NBL, PT [in millimeters (mm) and in multiple of medians (MoMs)], and their ratios (NBL : PT and PT : NBL) were performed using the Mann–Whitney U independent samples test. NBL, PT, and PT : NB and NB : PT ratio correlations were analyzed. No analysis of correlation was performed between any other markers.

RESULTS

The total number of the screened patients was 1470. The mean maternal age in euploid and Down syndrome cases was 30.6 years (16.6–47.1 years) and 31.5 years (21.1–42.3 years). The mean gestational age was 19.6 weeks (14.0–28.9 weeks) for euploid and 20.3 weeks (15.0–25.6 weeks) for Down syndrome cases.

Those excluded were the following: fetal structural abnormalities (24), multiple pregnancy (35), maternal conditions listed in the method (41), and chromosomal abnormalities, such as Turner syndrome ($n=1$), trisomy 18 ($n=4$), and trisomy 13 ($n=2$) (Appendix 2).

Ultrasound markers found in the Down syndrome group were increased nuchal fold thickness ($n=10$), cystic hygroma ($n=2$), cardiac defects ($n=9$), echogenic intracardiac focus ($n=4$), mild ventriculomegaly ($n=4$), short femur ($n=3$), duodenal atresia ($n=1$), hyperechogenic bowel ($n=3$), pyelectasis-hydronephrosis ($n=3$), choroid plexus cyst ($n=4$), sandal gap sign ($n=3$), and midphalanx hypoplasia of the fifth finger ($n=4$) (Appendix 3).

All invasive tests were amniocenteses, either because maternal age (≥35 years) (18 cases), a positive combined test (≥1 : 250) (12 cases), and second-trimester ultrasound soft markers (three cases).

The three consecutive NBL and PT measurements lasted 3 to 6 min and were completed during the first, the second, and the third attempts in 77%, 19%, and 4% of the cases, respectively.

There was a statistically significant difference ($p<0.0001$) in the NBL : PT ratio between the euploid and Down syndrome groups. Both the NBL and PT alone were found to be strong markers (sensitivity of 76% for both markers) for Down syndrome (Figure 2A and B).

A linear increase was observed in the mean NBL, the mean PT, and the mean NBL : PT ratio according to increasing gestational age between the 14th and 28th weeks. The mean NBL : PT ratio showed a gradual increase from 1.48 to 1.79 between the 14th and 28th weeks of gestation (a 21.2% increase) (Table 1). A total of 14 out of the 1330 euploid pregnancies and 32 out of the 33 Down syndrome cases were under the fifth percentile, with 97% sensitivity, 0.9% false positive rate, and 99% specificity (Table 2.)

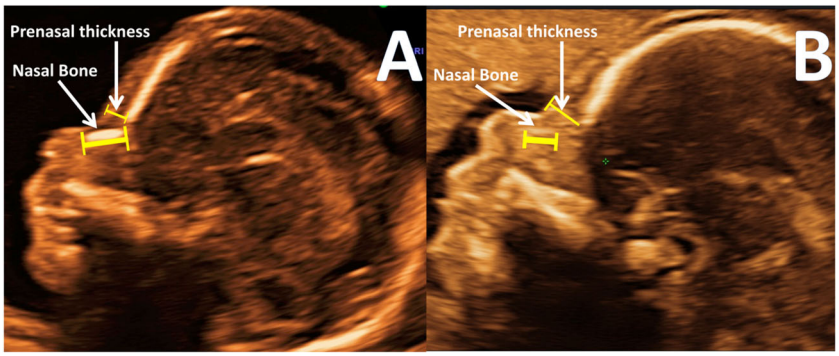

Figure 1 Examples of nasal bone length and prenasal thickness measurements obtained in euploid (A) and Down syndrome (B) fetuses

Evaluating the performance of the ratios, there were 32 true positive and one false negative Down syndrome cases identified. However, using the NBL : PT ratio, the false positive rate was 50% of those using the PT : NBL ratio (Figure 3A and B). The positive and negative cases with the calculated sensitivity, specificity, and false negative rate, using NBL, PT, the NBL : PT ratio, and the PT : NBL ratio for screening Down syndrome are summarized in Table 2.

No correlation has been found between PT and NBL with Spearman Rank Order Correlation test (SROC = 0.830 at $p < 0.05$) supporting that both markers are independent variables. The PT (PT mean: 2.0–5.8 mm) has lower values than the NBL (NBL mean: 3.0–10.0 mm), and PT (axPT average = 1.066) and NBL (axNBL average = 1.084) elevation are also different during the second trimester. Their ratios have different reference ranges because of the inverted counterparts, and the reference range of the NBL : PT ratio is wider than that of the PT : NBL ratio.

DISCUSSION

This 2D ultrasound study demonstrates that NBL and PT measurements can easily be incorporated into routine second-trimester anatomy scans. We confirmed in a potentially high risk Caucasian population that both NBL and PT alone are strong markers of Down syndrome, with both having a sensitivity of 76%. The combination of these two markers as a ratio increased the detection rate to 97% with a 0.9% false positive rate.

Furthermore, the NBL : PT ratio performs slightly better than its inverse counterpart. This is new that the NBL : PT ratio is a better marker than the PT : NBL ratio for detecting Down syndrome fetuses, primarily because it produced less false positive cases, and it can be used in cases where the nasal bone is absent. Moreover, the NBL : PT ratio can easily be calculated during the scan. If the NBL : PT ratio is less than the fifth percentile, a search for other aneuploidy soft markers and invasive fetal karyotyping should be considered.

In euploid fetuses, NBL, PT, and the NBL : PT ratio showed a linear increase with advancing gestational age. However, our data do not support previous observations[7,15] that the ratio is constant throughout the second trimester because the increase is more accelerated in NBL than in PT, and their ratio seems to be dependent on the gestational age (Table 1).[15]

The correlation between nasal bone hypoplasia, absent nasal bone, and the correct measurement of NBL in Down syndrome fetuses between 15 and 22 weeks of gestation was published in 2002.[1–3] The importance of increased PT in second-trimester screening for Down syndrome was first reported by Maymon *et al.* in 2005, and this technique has a sensitivity of 70%.[7] They combined PT and NBL measurements, yielding a 27% higher screening detection rate than NBL alone (43%). Three subsequent studies confirmed the association.[8,15,16]

De Jong-Pleij *et al.*, in a retrospective study, first reported that the PT : NBL ratio is a strong marker for Down syndrome. In their analysis of 3D volumes of 106 euploid and 30 Down syndrome cases (20 cases on 3D volumes and ten cases on 2D volumes), the detection rate was 100% with a 5% false positive rate.[16]

Genetic sonography can substantially increase detection rates for combined and quadruple tests, with a more modest increase in sequential protocols.[17] Combining PT and biochemical markers yields an 85% detection rate with a 5% false positive rate. When nuchal fold thickness is added to PT, NBL, and serum markers, the sensitivity increases to 93%.[18] When PT, NBL, and their ratios, all in MoMs, are combined with the lengths of the second and third digits, a 76% detection rate is achieved with a 6.7% false positive rate using a 1-in-200 risk cutoff.[19] The combination of quadruple tests with the measurements of nuchal fold thickness and long bones can yield 90% sensitivity at a 3.1% false positive rate.[20]

Two-dimensional measurements of NBL[21] and PT are feasible in the first trimester[22]; therefore, the markers examined in that study could also be beneficial for earlier Down syndrome detection.

Using a marker similar to PT (e.g., frontonasal fold thickness), one 2D study showed that the ratio of frontonasal fold thickness to NBL in a Latin American low-risk population (1922 pregnancies with four cases of Down syndrome) can easily be obtained during the second-trimester anatomy scan.[15]

This study presents novel evidence that the NBL : PT ratio is a better marker than the PT : NBL ratio for detecting Down syndrome fetuses. Our data indicate that the NBL : PT ratio is

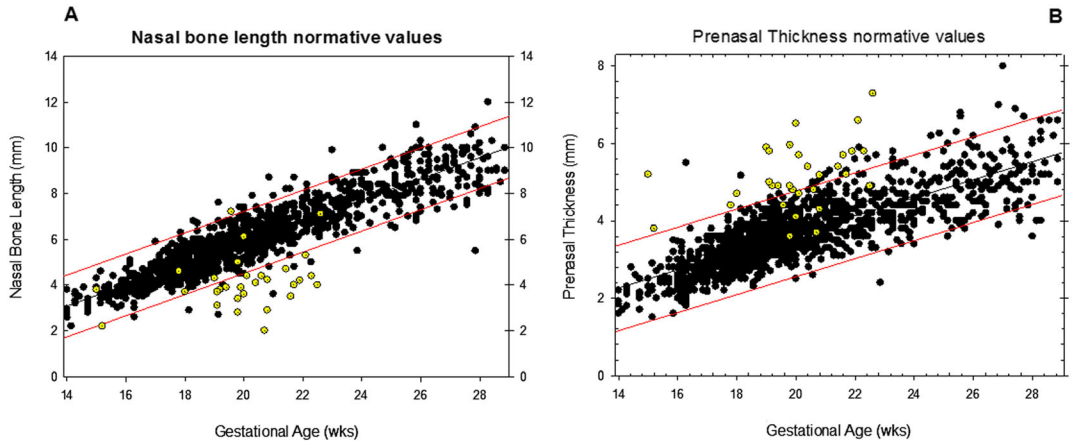

Figure 2 (A) Gestational-age-dependent nasal bone length values in 1330 euploid (black filled circles) and 33 Down syndrome (black open circles) fetuses. Approximately 76% of cases with Down syndrome fell under the third percentile. (B) Gestational-age-dependent prenasal thickness values in 1330 euploid (black filled circles) and 33 Down syndrome (black open circles) fetuses. Approximately 76% of cases with Down syndrome were above the 95th percentile

Table 1 The mean, the third, and the 97th percentiles of nasal bone length and prenasal thickness (PT) and the mean, the fifth, and the 95th percentiles of the ratios of nasal bone length to PT and PT to nasal bone length of euploid fetuses between 14 and 28 weeks of gestation

	Nasal bone length (mm)			Prenasal thickness (mm)			NBL-to-PT ratio			PT-to-NBL ratio		
Gestational age (weeks)	3rd percentile	Mean	97th percentile	3rd percentile	Mean	97th percentile	5th percentile	Mean	95th percentile	5th percentile	Mean	95th percentile
14	1.867	3.088	4.310	1.265	2.265	3.266	1.023	1.476	1.928	0.486	0.686	0.886
15	2.324	3.545	4.766	1.495	2.496	3.496	1.046	1.498	1.950	0.478	0.678	0.877
16	2.781	4.002	5.223	1.726	2.726	3.726	1.068	1.520	1.972	0.470	0.669	0.869
17	3.239	4.459	5.679	1.957	2.956	3.956	1.090	1.542	1.994	0.461	0.661	0.861
18	3.723	4.943	6.163	2.201	3.200	4.199	1.113	1.565	2.017	0.453	0.652	0.852
19	4.180	5.399	6.619	2.431	3.430	4.429	1.136	1.587	2.039	0.444	0.644	0.844
20	4.636	5.856	7.076	2.661	3.660	4.660	1.158	1.609	2.061	0.436	0.636	0.835
21	5.093	6.313	7.533	2.891	3.891	4.890	1.180	1.632	2.083	0.428	0.628	0.827
22	5.550	6.770	7.990	3.122	4.121	5.120	1.202	1.654	2.105	0.420	0.619	0.819
23	6.033	7.254	8.474	3.365	4.365	5.364	1.225	1.677	2.129	0.411	0.611	0.810
24	6.490	7.711	8.931	3.595	4.595	5.595	1.247	1.699	2.151	0.403	0.602	0.802
25	6.946	8.167	9.388	3.825	4.825	5.825	1.269	1.721	2.173	0.394	0.594	0.794
26	7.403	8.624	9.846	4.055	5.055	6.056	1.291	1.743	2.196	0.386	0.586	0.786
27	7.886	9.108	10.330	4.298	5.299	6.300	1.314	1.767	2.219	0.377	0.577	0.777
28	8.342	9.565	10.788	4.528	5.529	6.531	1.336	1.789	2.242	0.369	0.569	0.769
Correlation coefficient (r) (95% CI) $p = <0.0001$	0.916 (−1.000–0.923)			0.815 (−1.000–0.829)			0.285 (−1.000–0.326)			−0.244 (−0.286–1.000)		
Total increase in percent	346.87%	209.70%	150.29%	258.00%	144.08%	99.96%	30.54%	21.20%	16.25%	−24.10%	−17.05%	−13.18%

Table 2 Statistical characteristics of the performance of the screening for Down syndrome using NBL, PT, their multiple of medians and their ratios

	NBL	PT	NBL + PT	NBL MoMs	PT MoMs	NBL : PT	PT : NBL
Sensitivity (%)	75.75	75.75	87.88	69.70	69.70	96.97	96.97
Specificity (%)	98.12	97.65	97.14	98.34	96.84	99.10	98.42
False positive rate (%)	1.88	2.35	2.86	1.65	3.16	0.90	1.58
False negative rate (%)	24.24	24.24	12.12	30.30	30.30	3.03	3.03

NBL, nasal bone length; PT, prenasal thickness; MoMs, multiple of medians.

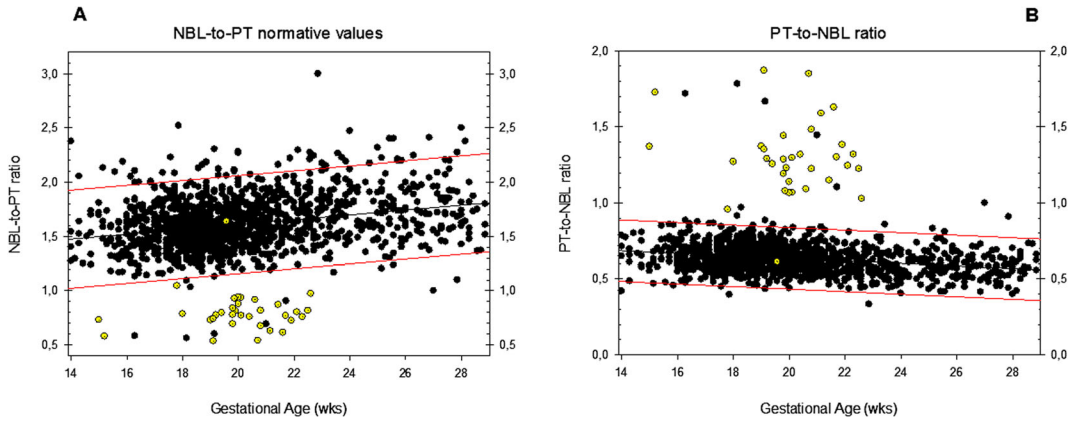

Figure 3 (A) Scatterplot of the ratio of nasal bone length to prenasal thickness in 1330 euploid (black filled circles) and 33 Down syndrome (black open circles) fetuses. All fetuses, except one, with Down syndrome fell under the fifth percentile. (B) Scatterplot of the ratio of prenasal thickness to nasal bone length in 1330 euploid (black filled circles) and 33 Down syndrome (black open circles) fetuses. All fetuses, except one, with Down syndrome were above the 95th percentile

superior to currently used investigated ultrasound markers alone or in combination with each other or even in combination with maternal biochemistry. A limitation of our study is that it was performed on a mixed-risk Caucasian population. However, a point in favor of this study is that it allowed us to test the performance of these markers on a relatively large group of fetuses with Down syndrome. This study focused on a Caucasian population, and further studies are needed to evaluate the sensitivity of the ratios across different ethnic populations.

CONCLUSION

In conclusion, 2D ultrasound measurements of NBL and PT can easily be performed within the second-trimester anomaly scan, and their ratios appear to be highly sensitive and specific markers for euploid and Down syndrome fetuses. The 2D measurements of these markers and their ratios can be incorporated into the second trimester anatomy scan.

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WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Preliminary 2D and retrospective 3D studies show that the ratio of prenasal thickness to nasal bone length (PT : NBL) is a strong marker of second-trimester Down syndrome fetuses.

WHAT DOES THIS STUDY ADD?

- The ratio of nasal bone length to prenasal thickness (NBL : PT) had performed better than its inverse counterpart for the screening of trisomy 21.
- In euploid fetuses, the PT : NBL and the NBL: PT ratios showed gradual increases over time.
- Two-dimensional ultrasound measurements of NBL and PT were successfully incorporated into the routine second-trimester anomaly scan.

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APPENDIX 1. Ultrasound soft markers for Down syndrome fetuses in the second trimester

Nuchal fold thickness (NF)
Cystic hygroma
Cardiac anomalies
Echogenic intracardiac foci/golf ball sign
Nasal bone hypoplasia (NBL)
Increased prenasal thickness (PT)
Widened iliac crest angle
Short femur
Short humerus
Ventriculomegaly
Duodenal atresia
Pyelectasis-hydronephrosis
Echogenic bowel
Sandal gap sign
Choroid plexus cyst
Midphalanx hypoplasia of the fifth finger

APPENDIX 2. Excluded euploid pregnancies

	Number of cases (*N*)
Multiple viable pregnancy	35
MATERNAL disease	18
Abnormal amniotic fluid volume	10
Fetal structural abnormalities	24
Chromosomal or structural abnormalities	7
Abnormal birth weight at delivery(<5th and >95th)	41
Fetal loss/death in second and third trimester	3

APPENDIX 3. Trisomy 21 cases scan results

	Number of cases (*N*)
Increased nuchal fold thickness	10
Cardiac defects	9
Echogenic intracardiac focus	4
Mild ventriculomegaly	4
Choroid plexus cyst	4
Midphalanx hypoplasia of the fifth finger	4
Hyperechogenic bowel	3
Pyelectasis-hydronephrosis	3
Short femur	3
Sandal gap sign	3
Cystic hygroma	2
Duodenal atresia	1

APPENDIX 4. Interobserver and intraobserver variability

Table A. Interobserver and intraobserver variability of nasal bone length (NBL) and prenasal thickness (PT) in absolute (mm) and relative (%) values at 95% limits of agreement (LoA) and their confidence intervals (CI)

Intraobserver ($n = 1.330$)						
Measurement	Mean relative difference	95% CI	95% Lower LoA	95% CI	95% Upper LoA	95% CI
NBL	0.59%	0.398% to 0.786%	−6.48%	0.398% to 0.786%	7.66%	7.330% to 7.993%
PT	0.97%	0.787% to 1.163%	−5.86%	−6.185% to −5.543%	7.81%	7.493% to 8.134%
Measurement	Mean difference	95% CI	95% Lower LoA	95% CI	95% Lower LoA	95% CI
NBL (mm)	0.028	0.0164 to 0.0403	−0.406	−0.4268 to −0.3860	0.463	0.4427 to 0.4835
PT (mm)	0.039	0.0320 to 0.0464	−0.223	−0.2351 to −0.2105	0.301	0.2889 to 0.3135
Interobserver ($n = 102$)						
Measurement	Mean relative difference	95% CI	95% Lower LoA	95% CI	95% Upper LoA	95% CI
NBL	−0.14%	−0.769% to 0.494%	−6.47%	−7.551% to −5.386%	6.19%	5.112% to 7.276%
PT	−0.11%	−0.649% to 0.436%	−5.55%	−6.477% to −4.617%	5.33%	4.404% to 6.264%
Measurement	Mean difference	95% CI	95% Lower LoA	95% CI	95% Upper LoA	95% CI
NBL (mm)	−0.010	−0.067 to 0.047	−0.442	−0.540 to −0.344	0.421	0.324 to 0.519
PT (mm)	−0.004	−0.030 to 0.023	−0.204	−0.249 to −0.159	0.197	0.151 to 0.242

Intraobserver and interobserver variability

These are preliminary data for intraobserver and interobserver variability; a Bland–Altman analysis[23] was used to describe intraobserver and interobserver variability.

Methodology: Two images were saved: one with calipers and one without calipers. To assess interobserver variability, the measurements of these two markers were repeated after the scanning by another operator, who remeasured the markers as previously described.The intraobserver variability analysis was performed on the three images, which were taken during the scan session.

Results: The limits of agreement (LOA: 95% CI) were −6.48% to 7.66% and −5.86% to 7.81% for NBL and PT, respectively. The respective interobserver variability 95% limits of agreement were −6.47% to 6.19% and −5.55% to 5.44% (Appendix Table A). There is a very low and not significant difference that has been confirmed.

Conclusion: There is a need to have further studies on the measurement education and on the intraobserver and interobserver variability of PT and NBL, and have it published as a separate article.