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MATERNAL-FETAL MEDICINE

Down's syndrome screening at 11–14 weeks' gestation using prenasal thickness and nasal bone length

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Abstract

Purpose To perform a multicenter prospective study of ultrasound prenasal thickness (PT), and nasal bone length (NBL) measurement at 11–14 weeks' gestation.

Methods Ultrasound PT and NBL determination was performed in 504 normal fetuses and 17 fetuses with Down's syndrome (DS). Measurements were made from mid-sagittal 2D images acquired using a standardized technique during nuchal translucency (NT) examination. PT and NBL values were expressed in multiples of the gestation-specific normal median (MoM) and as the PT/NBL ratio. Information on PT and NBL MoMs was also combined using logistic regression. Results were classified as positive according to whether they were greater than the normal 95th centile for PT, PT/NBL and the DS risk from logistic regression equation or below the 5th centile for NBL.

Results The median value in DS cases and unaffected controls were: PT 1.26 and 0.996 MoM; and NBL 0.596 and 0.993 MoM. The proportion of DS fetuses with positive results was 41% for PT, 65% for NBL, and 82% for both the PT/NBL ratio and DS risk from the logistic regression equation. PT/NBL levels did not vary according to gestational age.

Conclusion The PT/NBL ratio is a valuable first trimester DS screening marker that can be easily determined concomitant with the NT measurement.

Keywords First trimester · Prenasal thickness · Nasal bone length · Down's syndrome · Screening

Introduction

First trimester screening (FTS) for Down's syndrome (DS) at 11–14 weeks' gestation is generally based on ultrasound nuchal translucency (NT) measurements and biochemical serum markers. Performance can be improved by the use of additional ultrasound markers including those in the facial profile determined in the same plane as NT such as nasal bone (NB) absence and the fronto-maxillary facial angle [1–3].

Another ultrasound marker in the facial profile is prenasal thickness (PT), which reflects poor skin elasticity in DS fetuses and could be explained by an over-expression of type IV collagen in the basement membrane of the prenasal area which was recently demonstrated by immune-histochemistry studies [4].

Several second and third trimester studies have investigated PT alone or in combination with nasal bone length (NBL), using the PT/NBL ratio or Gaussian risk calculation [5–11]. The PT is increased in affected fetuses [5, 6, 8, 12] and combinations of PT and NBL show even higher proportions with high levels [8–12].

In recent years, there has been a change in routine DS screening practice from the second to the first trimester. It has not only better performance and provides earlier reassurance, but also at this time, termination of pregnancy, if

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required, is safer, more acceptable to religious tenets, and less traumatic.

However, only three studies have reported on the association between DS and increased PT as early as the first trimester [13–15]. In one study, nine high-risk DS pregnancies were scanned and all had levels above the normal 95th centile [13]. The second study was our retrospective examination of stored images from 44 DS cases and 39% had PT above the normal 95th centile [14]. NBL was also measured and 61% had levels below the 5th centile but 89% had PT/NBL ratio greater than the normal 95th centile. The third study was also based on stored images of 63 DS cases and showed an increased PT in 33% and a PT/NBL ratio above the 97.5 centile in 38% of the DS cases [15].

The current study aims to determine if similar first trimester performance can be achieved when PT and NBL are measured prospectively.

Methods

This was a multicenter prospective observational study conducted between March 2014 and July 2016 on women having FTS either at the antenatal sonographic unit (center 1 and 3, Germany and Switzerland) or in the private facilities of the participating authors (center 2, Israel). An ultrasound NT scan was carried out by experienced certified specialists according to the guidelines of the Fetal Medicine Foundation (FMF) London. The scan crown-rump length (CRL) between 45 and 84 mm included assessment of fetal viability and fetal anomalies.

For the purpose of the current study, the NT scan was extended to include PT and NBL measurements which were not used clinically to assess DS risk. The additional measurements were made without knowledge of the gestational age-specific reference intervals based on the normal population. Ethical approval for the study was obtained by the Ethics committee of Central- and Northwest-Switzerland (EKNZ) No. 2016-01414 (center 3) as well as in the two other participating centers (center 1: No. 053/17, center 2: No. 136/16). According to local regulations, informed consent was not obtained and not required for this anonymized data analysis. Patients were not included if they explicitly refused further use of their anonymized data.

PT was measured as described in our previous studies [5, 16, 17]. Briefly, the image of the fetal profile was taken in a strict mid-sagittal plane of the fetal head, identifying the NB, lips, maxilla and mandible. The image was magnified so that two-thirds of the screen still included the head and neck. Similar to our previous studies [18], an anechoic cartilaginous area between the midline of the frontal bone and the NB was located and identified as the nasal bridge. PT was measured from the anterior lower edge of the frontal bone to the shortest point in the facial skin anteriorly (Fig. 1), which differs slightly from the original technique reported by Maymon et al. [5, 16, 17]. The former was preferred because it provides better-defined landmarks, especially in cases in which the NB is absent [5].

NBL was measured in the same image as PT as described previously [2, 19]. The angle of insonation was 90° and two echogenic lines were seen in the mid-sagittal image of the head. One showing the skin of the nose and another one below presenting the NB. The transducer was slightly tilted during the exam to ensure that the nasal bone was identified as more echogenic than the cartilage line and separate from the overlying skin.

Scans were performed with either a 2–5-MHz curvilinear transabdominal transducer or a 5–9-MHz transvaginal probe of ultrasound machines from different companies, and using electronic calipers. Settings were adjusted to ensure that the maximum probe frequency was used. The views and measurements were stored in an electronic database (center 1 and 2) or printed as a thermal hard copy for the record (center 3). Measurements were conducted without a time limitation and only satisfactory images that met these stringent criteria, which were defined at the outset of this study, were included for data processing (Fig. 1).

Cases were included if when the full outcome was known or when there was at least one follow-up at 20–22 gestational weeks. The outcome of pregnancy was obtained from birth records or, when delivery was elsewhere, outcome was obtained from the referring gynecologist or from the mother.

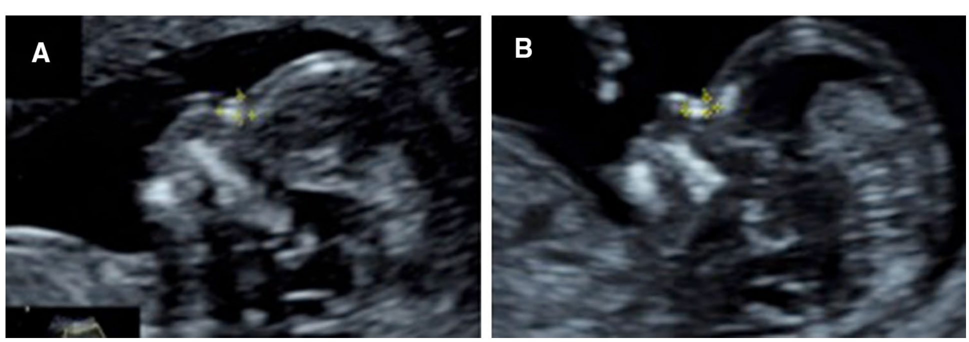

Fig. 1 Two-dimensional ultrasound images demonstrating the measurements of the prenasal thickness (1; PT) and nasal bone length (2; NBL) in a fetus with DS (**a**) and in a normal fetus (**b**). For PT measurement, the mid-sagittal plane was used and the shortest distance between the lowest part of the frontal bone (anterior line) and the skin was measured

Similar to our previous studies [5, 20], exclusion criteria were fetal malformations, chromosomal abnormalities other than DS or various maternal medical complications of pregnancy. Fetuses with a difference of 10 days or more between gestational age by last menstrual period and sonographic biometry were also excluded [5, 20].

PT and NBL values were expressed as multiples of the normal gestation-specific median (MoMs). Medians were derived from the unaffected pregnancies, which were grouped according to CRL: < 55; 55–9; 60–4; 65–9; 70–4; 75–9; and ≥ 80 mm. Regression analysis was performed on the median value against median CRL weighted for the number of pregnancies in the group. Linear, log-linear, quadratic and log-quadratic equations were considered.

Information on the two markers was combined using the PT/NBL ratio and using logistic regression analysis. For this purpose, ten fetuses with absent nasal bone were assigned the value 1.0 mm, the lowest observed measurement among the unaffected pregnancies. Logistic regression was carried out after log10 transformation of the MoMs.

Receiver operating characteristic (ROC) curves were constructed for NT alone and for the combination of NT + PT/NBL. The area under the curve (AUC) was calculated and compared with the DeLong's test. Differences between the distribution of values in unaffected and DS pregnancies were assessed non-parametrically using the Wilcoxon Rank Sum test. The proportion of values, exceeding the 95th centile of unaffected pregnancies or for NBL, below the 5th centile, was compared using Chi-square test.

Results

Five hundred and four normal fetuses and 17 fetuses with DS were included in the study. The median maternal age was 34 years (range 17–50 years) in unaffected pregnancies and 37 years (31–46 years) in cases with DS ($P<0.005$). The median gestational age was 12 + 3 weeks (10 + 5 to 13 + 6 weeks) for unaffected fetuses and 13 + 0 weeks (10 + 5 to 13 + 5 weeks) for cases with DS ($P=0.13$). The mean CRL was 65 (61–71) mm in unaffected pregnancies and 67 (62–72) mm in fetuses with DS ($P=0.74$).

Among unaffected pregnancies, PT values were best fitted by a log-linear regression with the median given by the formula: $10x$ where $x = -0.20780 + 0.00530 \times \text{CRL}$. For NBL, a log-linear regression was also best with median formula: $10x$ where $x = -0.00125 + 0.00531 \times \text{CRL}$. When weighted regression analysis was performed on the PT/NBL, there was no statistically significant association with CRL. Logistic regression analysis yielded the formula for DS risk ex/(1 + ex), where $x = -5.1472 + 14.4559 \times \log10 \text{ PT (MoM)} - 14.0604 \times \log10 \text{ NBL (MoM)}$, which was highly statistically significant ($P<0.0001$).

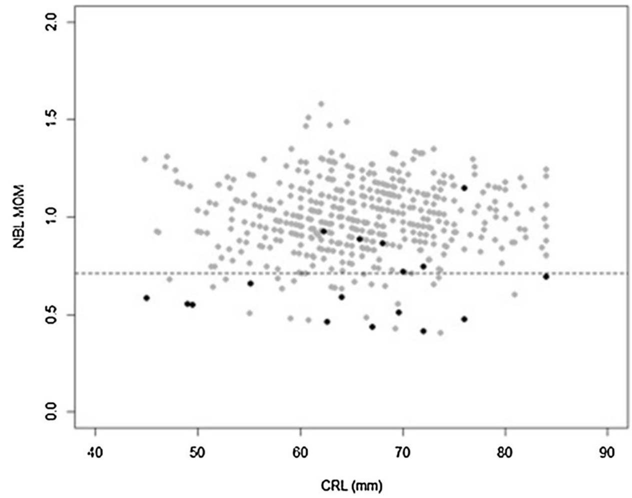

Fig. 2 Distribution of nasal bone length (NBL) in MoM according to crown-rump length [CRL (mm)]. Gray open dots represent normal cases ($n=504$) and dark dots represent the cases with DS ($n=17$). The straight line represents the 5th centile

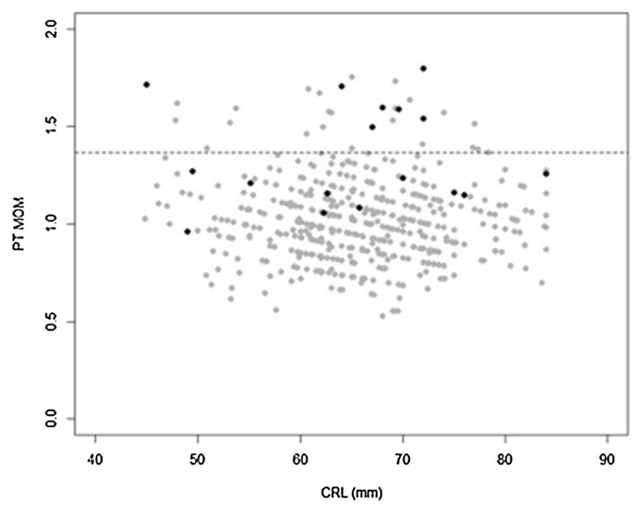

Fig. 3 Distribution of prenasal thickness (PT) in MoM according to crown-rump length [CRL (mm)]. Gray open dots represent normal cases ($n=504$) and dark dots represent the cases with DS ($n=17$). The straight lines represent the 95th centile

Figures 2, 3, and 4 show the individual PT, NBL MoMs and the PT/NBL ratios in DS cases and unaffected pregnancies according to CRL as well as the cutoff centiles. The medians and cutoffs are shown in Table 1 together with the logistic regression medians and cutoff. All the difference in distribution between unaffected and DS pregnancies were highly statistically significant ($P<0.0001$).

Table 1 also shows the proportion of DS pregnancies with positive results using these cutoffs. The proportion was higher for NBL than for PT (65% compared with 41%)

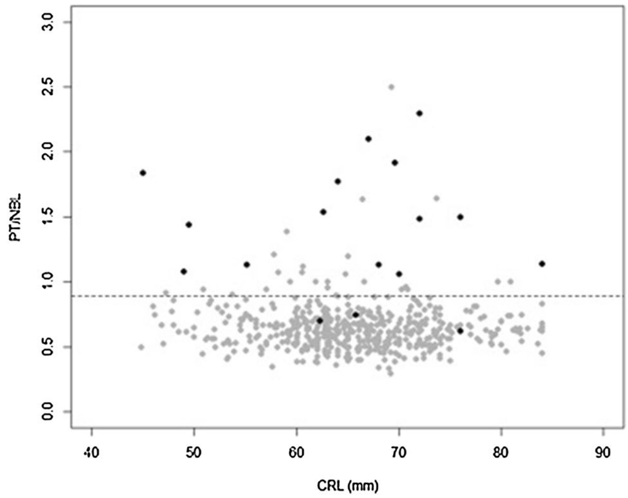

Fig. 4 Distribution of prenasal thickness (PT) to nasal bone length (NBL) in MoM according to crown-rump length [CRL (mm)]. Gray open dots represent normal cases ($n=504$) and dark dots represent the cases with DS ($n=17$). The straight lines represent the 95th centile

and highest for the two methods of combining PT and NBL (both 82%) (Fig. 4).

There were differences between the three centers according to PT. The median values were 0.96, 0.98 and 1.10 MoM ($P<0.0001$) but there were no between-center differences according to NBL. The nasal bone was absent in four (0.8%) normal fetuses and in six (35%) cases with DS ($P<0.0001$).

The Roc analysis for screening performance for Down's syndrome by NT alone showed an area under the curve (AUC) of 0.96 and was compared with the screening performance of NT + PT/NBL which showed an AUC of 0.98. The difference was not statistically significant ($P=0.39$) (Fig. 5).The corresponding sensitivities for fixed false-positive rates of 1, 3 and 5% for NT alone and NT + PT/NBL are shown in Table 2.

Discussion

This study shows the value of PT in combination with NBL for the detection of DS as early as the first trimester of pregnancy. The multicenter study with prospective measurements confirms the results from our previous first trimester single-center retrospective analysis of captured images [21]. PT was a less sensitive marker than NBL but when the two were combined using either the PT/NBL ratio or DS risk from the logistic regression equation sensitivity was higher than either alone.

In our previous and current studies 39% and 42% of DS cases, respectively, had PT above the normal 95th centile. Two other studies investigated PT alone at 11–14 gestational weeks. While the PT was above the normal 95th centile in all 9 cases of DS in one study, 13 pregnancies were selected for investigation if they had other sonographic abnormalities. If PT was associated with such abnormalities, the results will have been biased. The other study used the higher 97.5 centile as cutoff and 33% of the cases had raised PT [15] which is consistent with our two studies using a 95th centile cutoff.

The proportion of DS cases with PT/NBL ratio above the normal 95th centile in our previous and current studies was 89% and 82%, respectively. The other first trimester study reporting combined PT and NBL results used a higher 97.5 centile cutoff and found that only 38% of DS cases were positive. However, in addition to a higher cutoff this study excluded more than half of the fetuses which had an absent NB while our studies included such cases by setting NBL to 1 mm, which is the lowest value observed in normal fetuses.

Although PT and NBL levels in normal pregnancies are known to increase with gestational age, the PT/NBL ratio remains stable in the second trimester [8, 10, 22] and the first trimester as confirmed in the current study.

Two second trimester studies have compared the specificity of the PT/NBL ratio with a bi-variate Gaussian method of combing the two markers in MoMs which estimates DS risk [5, 9]. This is the only first trimester study which has compared PT/NBL with a DS risk method, in this case logistic

Table 1 PT and NBL results in unaffected and DS pregnancies

Marker[a]	Unaffected pregnancies (504)			Down's syndrome (17)	
	Median	Cutoff[b]	Positive[c]	Median	Positive[c]
PT (MoM)	0.996	1.365	26 (5.2%)	1.26	7 (41.2%)
NBL (MoM)	0.993	0.721	26 (5.2%)	0.596	11 (64.7%)
PT/NBL	0.625	0.889	25 (5.0%)	1.44	14 (82.4%)
Logistic regression (*p*)	0.00609	0.0510	25 (5.0%)	0.508	14 (82.4%)

MoM multiple of the normal gestation-specific median

[a]When nasal bone is absent, NBL = 1 mm, the lowest observed value in the unaffected pregnancies

[b]The normal 95th centile or for NBL the 5th centile

[c]Exceeding the cutoff

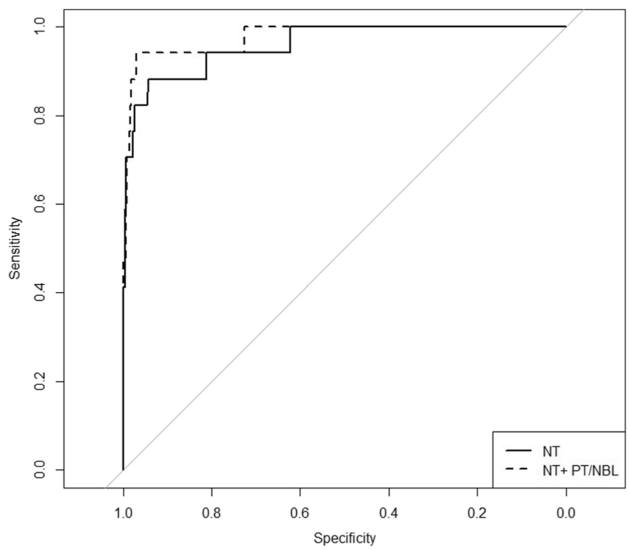

Fig. 5 Receiver operating characteristics (ROC) analysis for the detection of DS in the study population by nuchal translucency (NT, black line, AUC 0.96) and NT + prenasal thickness (PT) to nasal bone length (NBL) ratio (dotted line, AUC 0.98, $P = 0.39$)

Table 2 Sensitivities for the detection of DS with NT alone and NT + PT/NBL at fixed false positive rates of 1, 3, and 5% according to the Receiver operating characteristics (ROC) analysis

	Sensitivity % (95% CI)	
	NT	NT + PT/NBL
False-positive rate		
1%	70.6 (41.1–88.2)	70.6 (35.2–94.1)
3%	82.4 (54.8–100.0)	94.1 (70.6–100.0)
5%	83.2 (64.7–100.0)	94.1 (82.3–100.0)

Data is shown as percentages and 95% confidence intervals

regression, and no difference was found. A secondary analysis of our previous retrospective study also showed the same result (unpublished data analysis). Therefore, it seems that either option can be chosen when investigating these two markers alone. However, when combining PT and NBL with NT or first trimester serum marker levels multi-variate Gaussian or logistic regression analysis would be required.

In the current study, the median NT value in the DS cases was 2.34 MoM and 88% (15/17) had values above the normal 95th centile (1.42 MoM). When the logistic regression was extended to include NT, the proportion of DS cases with high risk increased from 82 to 94%. This analysis shows that the three markers together perform better than each alone or PT/NBL. However, the higher than expected proportion of cases with raised NT may indicate patient selection bias so that in an unselected series the incremental increase in detection may be less than the observed 12%.

Maternal serum PAPP-A and free β-hCG levels were available for 349 unaffected pregnancies but only for 4 DS cases so that a direct assessment of NT and serum markers with the addition of PT and NBL was not possible. Instead an indirect evaluation was made using multi-variate Gaussian risk modeling based on published parameters for the Combined test at 12 weeks' gestation [23], those for PT and NBL from the current series and a standardized maternal age distribution [23]. Among the unaffected pregnancies, there was no statistically significant correlation between the serum markers and PT or NBL, so no correlation was also assumed for the cases. The model predicted DS detection rates for a 5% false-positive rate were: PT and NBL 93%; NT alone 75%; all three ultrasound markers 95%; the Combined test 85% and all markers 97%.

In the current study, there were differences between the three centers according to PT. The median values were 0.96, 0.98 and 1.10 MoM. Whilst this was highly statistically significant (Kruskal–Wallis test $P < 0.0001$), it will not have materially altered the results since there were no DS cases in the center with the high median. There were no significant between-center differences according to NBL. There were difference between the centers according to PT. It will not have materially altered the results since there were no DS cases in the center with the high median. There were no significant between-center differences according to NBL. Our previous retrospective study showed that intraclass correlation between observers was high, but higher for NBL then for PT and the data stemmed from one center [14]. However, it is known that center-specific differences exist for example for NT measurement even after extensive formal training which is due to the variability of the placement of the calipers rather than to the scanning technique itself [24]. Repeated assessment of images and feedback to the examiner are important for achieving a consensus [25]. The fact the examiner of center three was trained in center one for PT measurement while center two, with the higher median, trained independently leads us to the assumption the caliper placement could be a possible cause. Means to overcome such differences are training, accreditation and ongoing audit procedures like for NT measurements. Meanwhile, equations for centers or even for operators could be used [26].

Both first and second trimester studies now show that combining PT and NBL has a high sensitivity and when used with other tests that examine the facial profile no additional images are required; the recommended approach is to capture the best NB measurement and place the calipers on it for PT measurement (Fig. 1). This will not increase substantially the time needed for an examination [5]. Moreover, unlike first trimester NT [21] and second trimester nuchal skin-fold [27], which are measured differently, or other sonographic markers, which are detected during the mid-gestation "genetic sonogram" [27–31], PT and NBL are measured exactly the same during the first and second trimester of pregnancy. In the era of non-invasive prenatal testing (NIPT), the importance of first trimester screening for aneuploidies by ultrasound has changed. While sensitivities of more than 95% can be achieved with combined, ultrasound-based multimarker screening at a false-positive rate of 2.5% [32], the false-positive rate of NIPT is much lower and lies below 0.1% at a sensitivity of 99% for trisomy 21, 13, and 18 [33]. Therefore, it is unlikely that this marker will change the trend in current first trimester screening. We believe, however, that the PT/NBL ratio can be of benefit in immediate counseling at the time of the ultrasound scan before biochemical screening or NIPT is available. It may also help for triage of patients, towards invasive testing or towards NIPT especially in situations where health insurance coverage for prenatal testing is restricted. Our results suggest that PT can be incorporated as an additional marker at the time of first trimester NT screening and there seems to a trend to improve current ultrasound-based screening algorithms, although no significant difference could be shown in the ROC analysis in this study ($P = 0.39$). This is an easy landmark to identify and measure, while simultaneously doing the NT scan. It increases the valuable information achieved from the same image, while the fetus is lying in the mid-sagittal plane and will not substantially increase the time required for the examination. Both PT and NBL are readily subject to quality assessment by monitoring the median MoM and standard deviation, as is currently done with first trimester NT. The results also favor measuring NBL rather than simply classifying NB absence and presence.

In conclusion, our data support the concept that the prenasal skin edema seen in fetuses with DS is present as early as the first trimester, when PT could be a useful marker. Furthermore, when combined with NBL it can be highly sensitive in DS detection.

Author contributions GMB: project development, data collection, data analysis, and manuscript writing. RM: data collection and manuscript writing/editing. SS: data collection. HC: data analysis and manuscript writing/editing. UG: project development and manuscript editing. AG: project development, data collection, data analysis and manuscript writing/editing.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest. The authors state that they had full control of all primary data and that they agree to allow the Journal to review their data if requested.

Ethical standards Ethical approval for the study was granted by the Ethics Committee of Central- and Northwest-Switzerland (EKNZ, Basel, Switzerland), No. 2016-01414. Approval was additionally obtained the two other participating centers (center 1: No. 053/17, center 2: No. 136/16). According to local regulations, informed consent was not obtained and not required for this anonymized data analysis. Patients were not included if they explicitly refused further use of their anonymized data.

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