

First Trimester Screening – Current Status and Future Prospects After Introduction of Non-invasive Prenatal Testing (NIPT) at a Tertiary Referral Center

Ersttrimesterscreening: aktueller Stand und Zukunftsperspektiven nach Einführung der nichtinvasiven Pränataldiagnostik (NIPT) in einem Krankenhaus der Tertiärversorgung



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Key words

NIPT, cell-free DNA screening, first trimester screening, prenatal counseling

Schlüsselwörter

NIPT, zellfreier fetaler DNA-Test, Ersttrimesterscreening, pränatale Beratung

received 9.5.2022

accepted after revision 24.7.2022

published online 6.9.2022

Bibliography

Geburtsh Frauenheilk 2022; 82: 1068–1073

DOI 10.1055/a-1787-8803

ISSN 0016-5751

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Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

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ABSTRACT

Objective To investigate the uptake of different components of first trimester screening (FTS) and the impact on invasive diagnostic testing (IPT) since the introduction of non-invasive prenatal testing (NIPT) at a level III center.

Methods Retrospective data analysis was conducted for singleton pregnancies that presented for FTS between 01/2019–12/2019 (group 1, n = 990). Patients were categorized into three risk groups: low risk for trisomy 21 (< 1:1000), intermediate risk (1:101–1:1000) and high risk (≥ 1:100). Uptake of NIPT and IPT was analyzed for each of the risk groups. Results were compared to a previous cohort from 2012/2013 (immediately after the introduction of NIPT, group 2, n = 1178).

Results Group 1 showed a significant increase in the use of NIPT as part of FTS (29.5% vs. 3.7% for group 2, p = 0.001) in all three risk groups. Overall IPT rates were lower in group 1 (8.6%) vs. group 2 (11.3%, p = 0.038), mainly due to a significant reduction of IPT in the intermediate risk group. IPT rates in the high-risk group remained stable over time.

Conclusion Appropriate clinical implementation of NIPT is still currently a challenge for prenatal medicine experts. Our data suggest that widespread uptake of NIPT is becoming more common these days; however, a contingent approach might prevent redundant uptake.

ZUSAMMENFASSUNG

Zielsetzung Ziel dieser Studie war es, die Inanspruchnahme von verschiedenen Komponenten des Ersttrimesterscreenings zu untersuchen sowie die Auswirkungen auf die Inanspruchnahme von invasiven pränatalen Untersuchungen (IPT) seit der Einführung der nichtinvasiven Pränataldiagnostik (NIPT) in einem Krankenhaus der Tertiärversorgung zu prüfen.

Methoden Es wurde eine retrospektive Datenanalyse von Einlingsschwangerschaften vorgenommen, die im Zeitraum von 01/2019–12/2019 für ein Ersttrimesterscreening vorstellig waren (Gruppe 1, n = 990). Die Patientinnen wurden in 3 Risikogruppen eingeteilt: niedriges Risiko für Trisomie 21

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(< 1:1000), mittleres Risiko (1:101–1:1000) und hohes Risiko ($\geq 1:100$). Die Inanspruchnahme von NIPT und IPT wurde für jede der Risikogruppen analysiert. Die Ergebnisse wurden mit denen eines früheren Patientinnenkollektivs aus den Jahren 2012/2013 (gleich nach der Einführung von NIPT, Gruppe 2, n = 1178) verglichen.

Ergebnisse In Gruppe 1 hat sich die Inanspruchnahme von NIPT als Teil des Ersttrimesterscreenings in allen 3 Risikogruppen signifikant erhöht (29,5% vs. 3,7% für Gruppe 2, p = 0,001). Die Inanspruchnahme von IPT in Gruppe 1 war niedriger (8,6%) verglichen mit Gruppe 2 (11,3%, p = 0,038), was

hauptsächlich auf die signifikante Reduktion von IPT in der mittleren Risikogruppe zurückzuführen war. Die Raten für IPT in der Hochrisikogruppe blieben im zeitlichen Verlauf relativ konstant.

Schlussfolgerung Die angemessene klinische Umsetzung von NIPT stellt zurzeit immer noch eine Herausforderung für Pränatalmediziner dar. Unseren Daten zufolge wird NIPT heute von vielen Schwangeren zunehmend in Anspruch genommen; ein an das Risiko geknüpfter Zugang zu Untersuchungen könnte die redundante Inanspruchnahme von Leistungen verhindern.

Introduction

First trimester screening (FTS) between 11 + 0 and 13 + 6 weeks of gestation has become the basis for decision-making about further diagnostic and therapeutic concepts in early pregnancy [1]. FTS now includes a wide variety of different aspects of prenatal care, such as screening for fetal anomalies, preeclampsia, preterm birth and fetal growth restriction. However, individual risk assessment for chromosomal abnormalities continues to be the essential part of FTS [2, 3, 4, 5, 6].

Combined first trimester screening (cFTS) is based on maternal age, fetal nuchal translucency (NT) and two maternal serum parameters: free β -hCG (human chorionic gonadotrophin) and PAPP-A (pregnancy-associated plasma protein A) [7, 8, 9, 10]. Screening by cFTS results in a detection rate (DR) of approximately 90% for trisomies 21, 18 or 13 at a false-positive rate (FPR) of 5% [11, 12]. The inclusion of additional ultrasound markers, such as hypoplastic or absent nasal bone, tricuspid regurgitation and/or a negative a-wave of the ductus venosus, further improve its screening performance by reducing the FPR to 2.5–3.5% [13, 14].

Clinical implementation of non-invasive prenatal testing by analyzing cell-free fetal DNA from maternal blood has resulted in a remarkable advance in screening for certain fetal aneuploidies [15, 16, 17]. With reported DRs of 99, 96 and 91% for trisomy 21, 18 and 13, respectively, and an overall FPR of 0.35%, NIPT has become widely used in routine clinical practice in recent years [18, 19]. Nevertheless, using NIPT as first-line screening still remains controversial [18]. Limiting FTS exclusively to NIPT screening would result in a loss of substantial additional information including early diagnosis of recognizable fetal structural defects that might raise suspicion of rare autosomal trisomies (RATs), triploidy, and other genetic diseases, and deprive patients of the opportunity of early screening for fetomaternal disorders [18, 19].

In a contingent screening approach, FTS serves as a triage test to determine whether additional NIPT is recommended or not. NIPT should primarily be offered to patients without fetal malformations and an intermediate risk for trisomy 21 [18, 20].

To date, FTS is not part of regular prenatal care in Germany. As for the use of NIPT, there are no binding indications apart from general recommendations by the German Society for Ultrasound in Medicine (Deutsche Gesellschaft für Ultraschall in der Medizin, DEGUM). According to the German Federal Joint Committee (Ge-

meinsamer Bundesausschuss, G-BA), NIPT can be performed at the patients' request without prior risk stratification and regardless of maternal age; however, it must be paid by patients themselves. Since January 2022 a change of the German legal framework has made coverage of the cost of NIPT possible for specific indications [14].

The objective of this study was to explore changes in the uptake of different screening options in the first trimester and to assess the impact on invasive prenatal testing (IPT).

Materials and Methods

This is a single-center retrospective analysis of all pregnancies (n = 1100) that presented for detailed FTS between 01/01/2019 and 31/12/2019 at the University Hospital Bonn, a tertiary referral center for fetal medicine. Cases with multiple gestation (n = 85) and cases lost to follow-up (n = 25) were excluded. Results of the current study (group 1) were compared to a previous cohort of 1178 patients from our institution that had presented directly after the first implementation NIPT between August 2012 and December 2013 (group 2) which were analyzed in the same manner [21].

FTS was offered between 11 + 0 and 13 + 6 weeks of gestation and included qualified prenatal counseling followed by a detailed fetal ultrasound examination. Fetal anatomy was evaluated by either abdominal or transvaginal ultrasound and included assessment of nuchal translucency (NT) thickness, nasal bone, tricuspid valve and ductus venosus blood flow in all cases. Gestational age was determined either based on the last menstrual period or, if the discrepancy was more than 7 days, was corrected based on crown-rump length (CRL) measurement. Additional maternal serum biochemistry (Roche Elecsys free β hCG and PAPP-A assay, Roche Diagnostics, Basel, Switzerland) was optional. If indicated or demanded by the parents, invasive and non-invasive prenatal testing was performed subsequently.

FTS was conducted by certified specialists according to the guidelines of the Fetal Medicine Foundation (FMF), London. Individual risk for aneuploidies was calculated on the basis of maternal age together with sonographic findings and, if available, serum biochemistry, using Viewpoint software (v. 5.6, GE Healthcare GmbH, Solingen, Germany).

► **Table 1** Mean maternal age of patients of the two study groups and distribution to risk groups after FTS: high risk $\geq 1:100$, intermediate risk $1:1000-1:101$, low risk for aneuploidy $< 1:1000$.

	Group 1 (1/2019–12/2019) n = 990		Group 2 (08/2012–12/2013) n = 1178		p
	n (%)	Mean maternal age (years)	n (%)	Mean maternal age (years)	
Risk group					
High	111 (11.2)	35.0	182 (15.5)	33.9	0.001
Intermediate	222 (22.4)	37.5	180 (15.3)	37.3	
Low	657 (66.4)	32.0	816 (69.3)	33.3	

Abbreviations: FTS = first trimester screening

► **Table 2** Differences in prenatal testing according to risk category, comparing 2019 (1) with 2012/2013 (2).

	Group	n	No further testing (%)	p	IPT (%)	P	NIPT (%)	p
Overall N = 2168	1	990	626 (63.2)	0.001	85 (8.6)	0.038	292 (29.5)	0.001
	2	1178	1001 (85.0)		133 (11.3)		44 (3.7)	

Abbreviations (in alphabetical order): IPT = invasive prenatal testing; NIPT = non-invasive prenatal testing

Patients were categorized into three risk groups for trisomy 21: high risk $\geq 1:100$, intermediate risk $1:101-1:1000$ and low risk $< 1:1000$. The presence of fetal structural defects, multiple soft markers (absent or hypoplastic nasal bone, negative a-wave of the DV, tricuspid valve regurgitation) or NT > 95 th percentile alone were considered high risk, regardless of the final risk calculation. All patients had post-screening counseling based on their individual results. Uptake of NIPT and IPT was investigated for each of the risk groups.

Data analysis

Statistical analysis was performed using SPSS (v23.0, IBM, Armonk, NY, US). Outcomes were quantified as means for continuous variables and percentages for categorical variables. Fisher's exact test and Chi-squared (χ^2) test were applied for comparisons. A p-value of < 0.05 was considered significant. The Ethics Committee of the University of Bonn does not request formal approval for anonymized retrospective analysis of clinical data.

Results

Description of the study population

A total of 2168 pregnancies were included in this study. 990 patients were seen between 01/2019 and 12/2019 (group 1) and were compared to 1178 patients that had been seen from 08/2012 to 12/2013 (group 2). Comparison of baseline characteristics in terms of maternal age revealed no statistically significant differ-

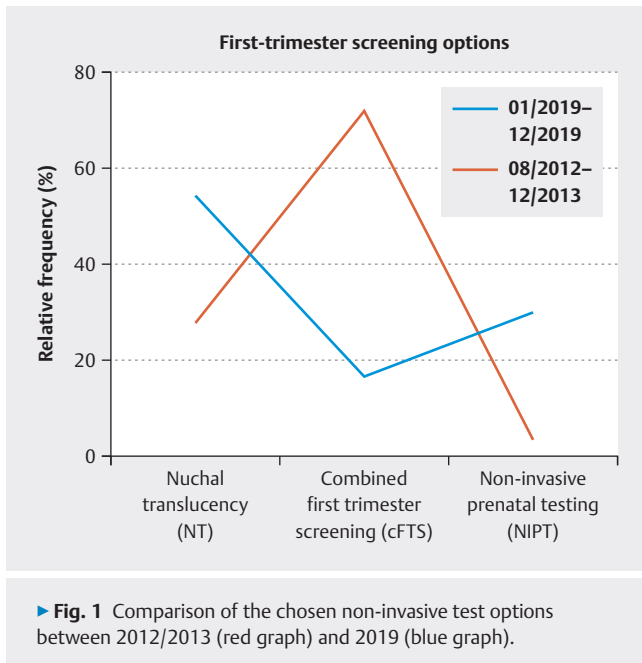
ences. There was a difference in risk group assignment between the two study periods, especially in the intermediate risk group (2012/13: 15.3% vs. 2019: 22.4%, $p = 0.001$) (► **Table 1**).

Screening options

Overall, a comparison of the chosen screening options during both time periods showed a significant increase of FTS by ultrasound as the sole screening option (► **Fig. 1**). Of those who sought additional screening, biochemistry was less frequently used (overall group 1: $n = 182/990$ vs. group 2: $n = 1634/2271$, 18.4% vs. 72.0%) and NIPT was chosen more often in the patient cohort of 2019 (overall group 1: $n = 292/990$ vs. group 2; $n = 44/1178$, 29.5% vs. 3.7%). Overall group analysis of the decisions by patients with regard to their risk group about further testing showed a significant decrease in those opting for "no further testing" (85.0% vs. 63.2%, $p = 0.001$), but also a strong decrease in IPT (11.3% vs. 8.6%, $p = 0.038$; ► **Table 2**, ► **Fig. 2**), which was mainly caused by a reduction of IPT in the intermediate risk group (7.2% vs. 11.1%, $p = 0.001$). Rates of IPT in the high-risk and low-risk groups remained almost stable over time (53.3% vs. 47.7% and 2.0% vs. 2.4%) (► **Fig. 2**).

NIPT

NIPT uptake increased significantly by almost eight times over time (3.7% to 29.5%, $p = 0.001$) (► **Table 2**), with the majority of patients in group 1 being assigned either to the low (49.8%) or intermediate risk group (35.2%). In contrast, immediately after the



implementation of NIPT in 2012, most patients who opted for NIPT belonged to either the high or intermediate risk group (27.3% and 43.2%).

Of all cases with NIPT in group 1, 10 (n = 10/292, 3.4%) showed abnormal results: n = 4 true positive for trisomy 21, n = 3 true positive for trisomy 18, n = 1 true positive for trisomy 21 and monosomy X (fetal karyotype subsequently obtained by IPT: mos45,X[2]/46,X,+21 [5]/47,XX,+21 [2]), and n = 2 false positive for trisomy 21. No false negative NIPT results were reported.

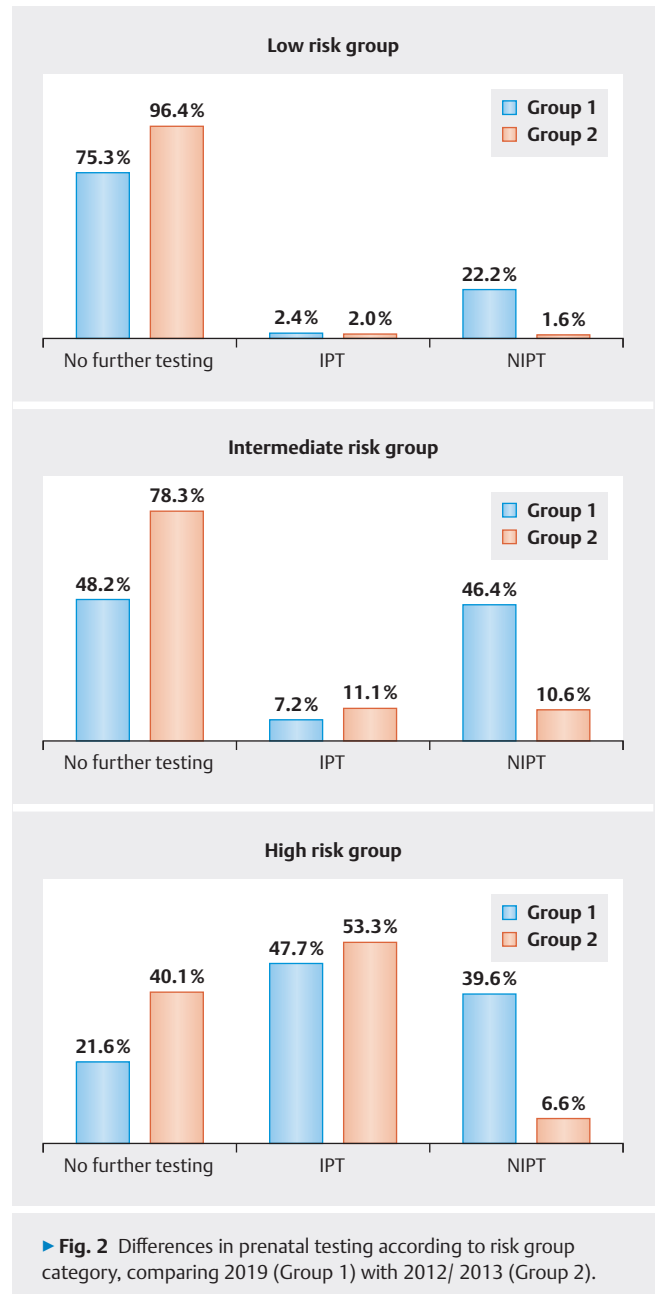
All but one of the cases that had true positive NIPT results for aneuploidy showed an abnormal first trimester ultrasound. Thus, only one case with true positive NIPT for trisomy 21 later on had a completely normal FTS. In contrast, the two patients with false positive NIPT results both showed inconspicuous FTS.

IPT

Of all patients that underwent IPT in group 1, 43.5% (n = 37/85) had abnormal results. 75.7% (n = 28/37) had aneuploidy, such as trisomy 21, 18 or 13, while the rest (24.3%, n = 9/37) was diagnosed with other chromosomal abnormalities. These results are comparable to findings for group 2. There were no cases of miscarriage or other complications after IPT in both groups.

Discussion

Implementation of NIPT with its significantly improved screening performance for trisomy 21, together with the continued decrease in NIPT cost and the expansion of its diagnostic possibilities in the recent years, has been a challenge for all advocates of classic FTS [14]. After considering the respective advantages and disadvantages of different screening methods, international societies have so far been unable to decide on a consensus with regards to an algorithm for the use of NIPT in early pregnancy. Counseling, recommendations and handling therefore remain a challenge (at least in Germany), especially as cost-effectiveness and politics are



increasingly important, in addition to social and ethical issues [14, 19].

This is the first study to evaluate changes in the uptake of NIPT over a period of almost 10 years in a single center in Germany. Our study shows that in our center, first trimester screening for chromosomal abnormalities has changed dramatically since NIPT was introduced. While combined FTS, consisting of a detailed fetal ultrasound examination together with maternal serum biochemistry, was considered the gold standard in the past, less than 1/5 of patients opt for this “classical” cFTS nowadays. At our center, NIPT uptake has almost increased by eight times until its first introduction and the overall use of IPT has significantly decreased, mainly due to increased uptake of NIPT in the intermediate and low-risk group. A continuous reduction of its costs between the two study periods, together with a decrease in patients opting for additional

serum biochemistry (that might lead to a further decrease of their calculated risk for aneuploidy) might in part explain these changes.

Overall distribution of the different risk groups (high, intermediate and low risk) for aneuploidies showed significant changes between 2013 and today. An increase in the intermediate risk group (2012/13: 15.3% vs. 2019: 22.4%, $p = 0.001$) could be particularly observed and might, at least in part, be explained by a lower utilization of additional serum biochemistry with the possibility of further risk reduction in group 1, as mentioned above. Because it is a tertiary referral center, the proportion of patients belonging to the high-risk group (11.2%) is considerably higher compared to other studies [22, 23], and this should be kept in mind.

Several studies have been published comparing different approaches for the reasonable implementation of NIPT in clinical practice. These studies have especially compared first-tier versus contingent screening [20, 24, 25, 26]. Many international societies have favored recommending NIPT for intermediate risk patients [14, 27]. However, in some countries there is still no consistent strategy or guideline, and different cut-offs in the definition of high, intermediate and low risk cause additional difficulties when comparing different studies [23, 28].

To date, German health insurance providers only cover the costs of NIPT for special indications [29]. Consistent with previous findings, our data suggests that implementation of NIPT seems to be more effective with a contingent-screening approach and only subsequent to detailed ultrasound evaluation of the fetus [29, 30, 31]. Almost 90% of patients in the high-risk group had abnormal ultrasound findings and invasive testing rather than NIPT was therefore immediately recommended. Just like Manegold et al., we also made the observation that NIPT is still used predominantly by women with normal FTS for additional reassurance [21]. However, this could lead to unnecessary dilemmas, especially when faced with false positive results. In our study, we observed 10 cases of abnormal NIPT results, 8 of which were subsequently confirmed by IPT. Of these 8 fetuses, all but one showed sonographic abnormalities that would have justified invasive testing right away, but patients chose NIPT instead. On the other hand, the two cases with abnormal NIPT findings subsequently disproved by IPT had a normal fetal sonographic assessment.

Our results again highlight the importance of a detailed first trimester ultrasound examination. Moreover, of all patients in our study that showed abnormal IPT results, 24.3% were diagnosed with chromosomal abnormalities other than trisomy 21, 18 and 13. They would have been missed using a strategy that focuses primarily on NIPT. Rates of undiagnosed congenital abnormalities of up to 34% have been described with a NIPT-only screening approach [32]. This, together with the generally low rates of miscarriages and other complications after IPT, should be kept in mind when counseling patients. Chromosomal microarray analysis (CMA) and whole exome sequencing (WES) have enormously expanded the diagnostic spectrum in fetuses with abnormal ultrasound findings and must therefore be discussed with affected parents [19]. Some authors even suggest using IPT and microarray analysis immediately if the fetus presents with isolated NT of 3.0 mm or above [33].

Rates for additional serum biochemistry decreased significantly between the two study periods. In group 1, 9 patients with normal first trimester ultrasound scan results faced an increased adjusted risk for fetal aneuploidy after serum biochemistry. Seven of them had additional NIPT and two patients had IPT, and results were normal in all cases. Kozłowski et al. immediately recommend CMA if either beta-HCG or PAPP-A levels are below 0.2 MoM or if free beta-hCG exceeds 5.0 MoM [14]. However, in our opinion, it remains to be seen how the uptake of additional serum biochemistry will develop in the future, as it might lead to unnecessary uncertainties for patients, especially if first trimester ultrasound shows no signs of fetal aneuploidy.

Apart from the general limitations of retrospective studies, the main limitations of our study include the fairly high number of high-risk patients which could lead to selection bias. Also, our results might have been influenced by demographic, socio-economic and cultural aspects as well as specific policies of the German healthcare system. In addition, considerably decreased NIPT costs are likely to have contributed significantly to its increased uptake in the recent months and years and therefore must be taken into account.

Conclusion

Appropriate clinical implementation of NIPT still poses a challenge for prenatal medicine experts nowadays. Our data suggests that widespread uptake of NIPT is becoming more common these days; however, a contingent approach might prevent the redundant uptake. In order to obtain truly informed consent during the counseling process, a detailed fetal sonography is, as ever, crucial. The range of additional diagnostic tools must still be discussed with patients individually, based on maternal age, medical (and especially obstetric) history and sonographic findings, to obtain a diagnosis as effectively and quickly as possible and to pave the way for subsequent multidisciplinary patient care.

We suggest that implementation of NIPT in FTS is best achieved with a contingent approach, and its costs should be covered by German healthcare providers accordingly.

Note

No acknowledgements to be mentioned. All authors declare that they received no funding for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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