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Clinical Experience with Noninvasive Prenatal Testing in Twin Pregnancy Samples at a Single Center in Germany

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Abstract

In this study we wanted to determine the performance of a paired-end sequencing-based noninvasive prenatal testing (NIPT) assay in the detection of common fetal trisomies in twin pregnancy samples. Samples from patients with a twin pregnancy were collected from at least 10 weeks of gestation and analyzed at a single prenatal center in Germany. Results of Anomaly Detected (i.e., high risk) or No Anomaly Detected (i.e., low risk) for trisomy 21, trisomy 18, or trisomy 13 were reported. Follow-up confirmatory outcomes were requested for all cases. A total of 1,658 patients with twin pregnancies submitted samples during the study period; only two of these samples failed resulting in a low failure rate of 0.12%. Of the remaining 1,656 cases, there were 1,625 (98.1%) low-risk and 31 (1.9%) high-risk NIPT samples in our cohort. Of these, follow-up information was available for 301 (18.5%) of the low-risk samples and 19 (61.3%) of the high-risk samples. All of the low-risk cases with follow-up were determined to be true negatives giving an estimated negative predictive value of 100%. Seventeen of the 19 high-risk samples with follow-up were true positives, resulting in an overall positive predictive value of 89.5%. Sensitivities of $>$ 99.9% were noted for both trisomy 21 and trisomy 18, with high specificities of $\geq$ 99.7% observed for all three trisomies. In conclusion, our study showed strong performance of the NIPT assay in the detection of common fetal trisomies in twin pregnancy samples, with high sensitivities, specificities, and positive predictive values observed based on known clinical outcomes along with a low failure rate.

Keywords

- noninvasive prenatal testing
- twin pregnancy
- trisomy
- sensitivity
- specificity
- positive predictive value

Introduction

Since the clinical availability of noninvasive prenatal testing (NIPT) over a decade ago, there has been a vast amount of published data demonstrating the strong performance of NIPT in the detection of a wide range of fetal anomalies including common trisomies,[1–5] sex chromosomal aneuploidies,[6,7] and rarer genome-wide fetal anomalies such as rare autosomal aneuploidies and copy number variants.[8–10] In addition, a large number of professional medical societies are

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supportive of the use of NIPT for prenatal screening.[11–16] In Germany, NIPT has been available since 2012 and is currently used in 50 to 75% of pregnancies.[17] The use of NIPT in Germany is regulated by several professional societies including the German Society of Human Genetics, the German Society for Ultrasound in Medicine (DEGUM), and Fetal Medicine Foundation (FMF) Germany.[18,19] Since 2022, NIPT has been covered by insurance companies in Germany under certain conditions.[17]

While it is well accepted that NIPT has improved performance over traditional prenatal screening in singleton pregnancies, fewer publications have focused on the performance of NIPT in twin pregnancies. A systematic review of traditional prenatal screening for trisomy 21 in twin pregnancies using combined nuchal translucency and first trimester serum screening found a pooled sensitivity of 89.3% and pooled specificity of 94.6%.[20] Prenatal screening by NIPT offers the possibility of a more accurate screening option for these patients. A recent meta-analysis by Judah et al[21] noted a pooled weighted detection rate of 99.0% and false-positive rate of 0.02% for trisomy 21 in twin pregnancies. The use of NIPT in twin pregnancies is supported by multiple professional societies with some restrictions.[13–16,22]

We previously reported on the performance of a paired-end sequencing-based NIPT assay (VeriSeq NIPT Solution v2) for common fetal trisomies in a cohort of cases that included both singleton and twin pregnancy samples[23,24]; results for monosomy X were also provided in these previous studies but for singleton samples only. Here, we focus on the performance of this NIPT assay in a larger cohort of twin pregnancy samples at our laboratory, one of the largest NIPT laboratories in Germany. Results were provided for trisomy 21, trisomy 18, and trisomy 13, with outcome information obtained for a subset of both the high-risk and low-risk NIPT cases.

Materials and Methods

Study Cohort

The study cohort consisted of samples from twin pregnancies that underwent NIPT between December 2017 and November 2022. All samples were from a general German and Austrian pregnancy population; subsets of these cases have been published previously.[23,24] Samples had to be at least 10 weeks of gestation for inclusion in the study. Samples were excluded if there was a known vanishing twin or a high-order multiple pregnancy. All study participants provided informed consent for their data to be used for appropriate quality control and improvement of the NIPT assays; all data were deidentified prior to study enrollment. The amedes lab observes the provisions of the German Federal Data Protection Act.

As noted in our previous studies,[23,24] indications for NIPT included advanced maternal age ($\geq$ 35 years), a positive screening test result (serum marker screening or ultrasound), other medical reasons, and patient anxiety. Other medical reasons included previous pregnancy complications such as miscarriage or an affected pregnancy; a genetic aberration in the family; known diseases including diabetes, epilepsy, and carcinoma; medications such as chemotherapy; or consanguinity.

Sample Processing and Analysis

NIPT analysis was carried out using the VeriSeq NIPT Solution v2 assay (Illumina Inc.) as previously outlined.[23,24] This NIPT test is offered under the name "fetalis" by the amedes lab group in Germany and Austria. This assay consists of an integrated platform that uses polymerase chain reaction-free paired-end whole-genome sequencing for the detection of fetal anomalies.[8] The assay uses synthesis by synthesis chemistry, with the use of paired-end sequencing allowing twice as much data to be produced in the same time and effort as single-read sequencing, thereby resulting in improved efficiency.[25] The use of whole-genome sequencing allows for comprehensive screening across the entire fetal genome unlike other targeted NIPT methods, such as a single-nucleotide polymorphism analysis, microarray analysis, or rolling circle amplification, where only certain regions of select chromosomes are analyzed.

There are three main steps to the VeriSeq NIPT v2 assay, namely, sample preparation (plasma isolation, deoxyribonucleic acid extraction, and library preparation), sequencing, and data analysis and report generation. Results are reported for common trisomies (trisomy 21, 18, and 13), as well as fetal sex and sex chromosome aneuploidies in the basic mode (in multifetal pregnancies, only the presence or absence of the Y chromosome is reported); for this study, results were reported for the common trisomies only. Analysis was carried out using VeriSeq NIPT Assay Software v2, where each sample was called as either Anomaly Detected (i.e., high risk) or No Anomaly Detected (i.e., low risk). A fetal fraction estimate was also provided for each analyzed sample. As noted previously,[23,24] the assay software uses a dynamic threshold metric (individualized Fetal Fraction Aneuploidy Confidence Test; iFACT) which takes into account both the fetal fraction estimate and the sequencing coverage for each sample; samples that do not meet this threshold are reported as ouality control failures. A *t*-statistics value is also provided by the assay which can help differentiate between low-risk and high-risk samples.

Collection of Clinical Outcomes

Follow-up is attempted for every case with an NIPT result using a follow-up sheet that is sent to the patient's gynecologist. This form is used to provide information on whether the child was born healthy or whether abnormalities were found in the child. Based on our experience, these feedback forms are often not completed and returned to us in cases of inconspicuous pregnancies. If, after a certain period of time, we have not received any feedback then we try to ask for the clinical follow-up results by phone. However, given the increasing number of NIPT cases that our laboratory now processes, there is not always the capacity available to carry out these additional inquiries. In our experience, discrepancies between a prenatal result and the birth report are typically reported back immediately. We therefore assume that in the frequent cases of nonreporting, the NIPT result corresponds to an inconspicuous birth.

Clinical outcomes were determined by invasive diagnostic techniques (chorionic villus sampling or amniocentesis with

cytogenetic analysis), cytogenetic analysis of products of conception or placenta, postmortem examinations including autopsy or macroscopic assessment of the abortion, postnatal cytogenetic analysis, ultrasound, and newborn physical examination. Cases that had a high-risk result by NIPT (Anomaly Detected) were considered confirmed if they were validated by either invasive prenatal diagnostics or if an abnormality was observed on ultrasound that was consistent with the high-risk NIPT result. Cases that received a low-risk result by NIPT (No Anomaly Detected) were considered confirmed if a healthy newborn lacking the physical features or phenotypes associated with any of the common trisomies was reported by the attending physician.

Statistics

Statistical data analysis was carried out using Microsoft Excel 2016. Binomial 95% confidence intervals were calculated for sensitivity and specificity estimates. Where applicable, a Student's *t*-test was used to determine statistical significance, with a p-value < 0.05 considered significant.

Results

A total of 102,101 pregnancy samples were analyzed using VeriSeq NIPT Solution v2 assay over the study period, of which 1,658 (1.6%) were from twin pregnancies. Two of the twin pregnancy samples failed to give a result upon NIPT analysis, giving a low failure rate of 0.12% (2/1,658). Of the remaining 1,656 cases, 1,625 (98.1%) were found to be low risk and 31 (1.9%) were high risk for presence of a common fetal trisomy following NIPT (►**Fig. 1**). Demographics for the low-risk, high-risk, and no-results cohorts are provided in ►**Table 1**. When we compared the low-risk and high-risk cohorts, we found that there was a significant difference for both maternal age ($p < 0.001$) and body mass index (BMI) ($p < 0.0001$).

Overall, there were 20 (1.2%) trisomy 21, 8 (0.5%) trisomy 18, and 3 (0.2%) trisomy 13 high-risk calls in our study cohort; demographics for each of these smaller high-risk cohorts are shown in ►**Table 2**. When we compared each of these three groups against the low-risk cohort, we found that there was a significant difference between the trisomy 21 cohort and the low-risk cohort for both maternal age ($p < 0.001$) and BMI ($p < 0.05$). BMI was also significantly different between the trisomy 18 cohort and the low-risk cohort ($p < 0.01$). Follow-up information was available for 320 (19.3%) of the twin pregnancy cases in our cohort, including 301 (18.5%) of the 1,625 low-risk cases and 19 (61.3%) of the 31 high-risk cases (►**Table 3**). All low-risk cases with follow-up (301/301) were determined to be true negatives, giving an estimated negative predictive value (NPV) of 100%. Seventeen (89.5%) of the 19 high-risk cases with follow-up were true positives; there was one false-positive for trisomy 18 and one false-positive for trisomy 13. Overall, for presence of common trisomies in twin pregnancies, we found a sensitivity of > 99.9%, a specificity of 99.3%, and a

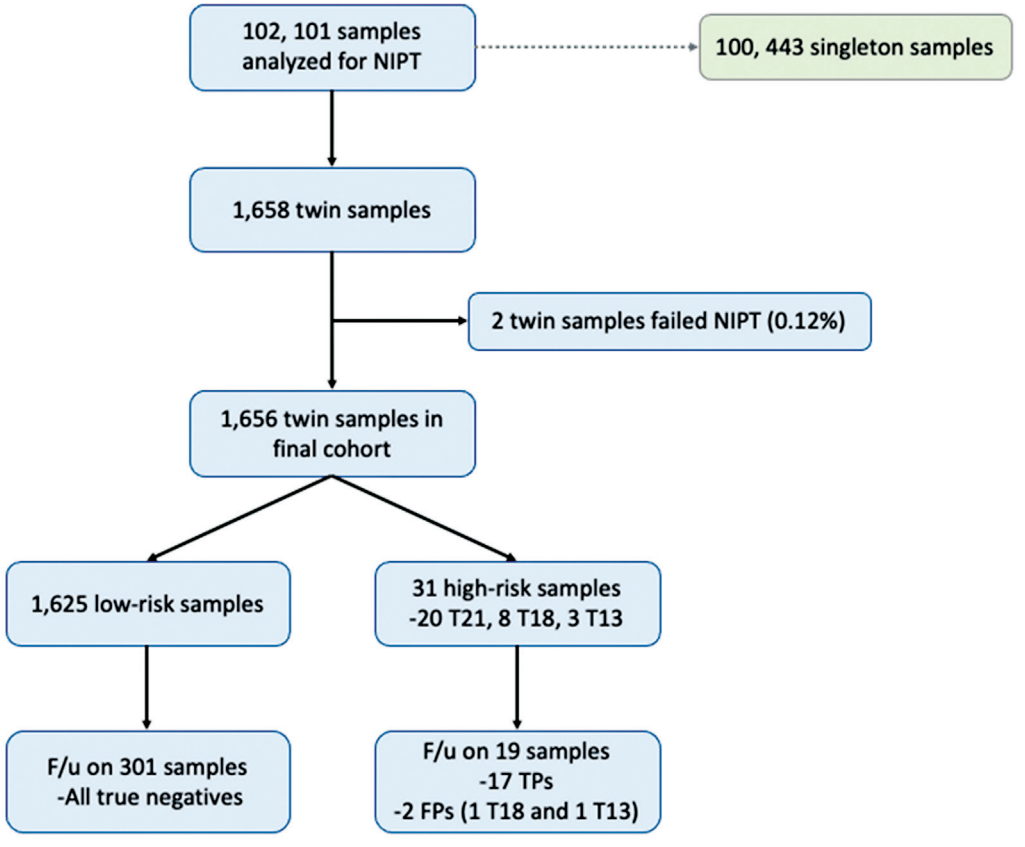

Fig. 1 Study flowchart. FP, false positive; F/u, follow-up; T, trisomy; TP true positive.

Table 1 Demographics of the low-risk, high-risk, and no-results by NIPT cohorts

Variable	Low-risk cohort ($n = 1,625$)	High-risk cohort ($n = 31$)	No-results cases ($n = 2$)	*p*-Value[a]
Mean maternal age, y	33.84 ± 0.11	36.39 ± 0.64	30.00 ± 1.45	0.00038
Mean BMI	25.83 ± 0.16	23.30 ± 0.43	19.15 ± 3.04	0.00008
Mean gestational age, wk	12.73 ± 0.05	13.00 ± 0.56	13.50 ± 1.52	0.61817
Mean fetal fraction, %	12.56 ± 0.12	13.13 ± 0.71	—	0.38964

Abbreviations: BMI, body mass index; NIPT, noninvasive prenatal testing.
[a]Statistical significance was calculated between the low-risk and high-risk cohorts.

Table 2 Demographics of patients with a high-risk NIPT call for trisomy 21, trisomy 18, or trisomy 13

Variable	Trisomy 21 ($n = 20$)	Trisomy 18 ($n = 8$)	Trisomy 13 ($n = 3$)
Mean maternal age, y	36.40 ± 0.54	36.63 ± 2.15	35.67 ± 1.20
Mean BMI	23.79 ± 0.56	22.56 ± 0.68	22.03 ± 1.32
Mean gestational age, wk	13.54 ± 0.85	12.13 ± 0.47	11.76 ± 0.67
Mean fetal fraction, %	13.15 ± 0.73	12.25 ± 1.71	15.33 ± 1.76

Abbreviations: BMI, body mass index; NIPT, noninvasive prenatal testing.

Table 3 Performance metrics for twin pregnancy cases with a high-risk NIPT result for a common trisomy

Performance metric	All high-risk cases	Trisomy 21 cases	Trisomy 18 cases	Trisomy 13 cases
Number of cases	31	20	8	3
Cases with follow-up, *n* (%)	19 (61.3)	12 (60.0)	6 (75.0)	1 (33.3)
True positives	17	12	5	0
False positives	2	0	1	1
Sensitivity, %	> 99.9	> 99.9	> 99.9	N/a
Specificity, %	99.3	> 99.9	99.7	99.7
PPV, %	89.5	100	83.3	0.0
Theoretical PPV range, %	54.8–93.5	60.0–100	62.5–87.5	0.0–66.7

Abbreviations: N/a, not applicable; NIPT, noninvasive prenatal testing; PPV, positive predictive value.

positive predictive value (PPV) of 89.5% (potential PPV range of 54.8–93.5%). When we looked at performance of the assay for each of the trisomies, we noted high specificities (≥ 99.7%) for all three trisomies, with a sensitivity of > 99.9% for both trisomy 21 and trisomy 18. In addition, our study had a PPV of 100% for trisomy 21 (potential range of 60.0–100%) and 83.3% for trisomy 18 (potential range of 62.5–87.5%) as shown in ►**Table 3**.

Discussion

In our study, we found that the VeriSeq NIPT Solution v2 assay showed strong performance in the detection of common fetal trisomies in twin pregnancy samples, with high sensitivities, specificities, and PPVs observed based on known clinical outcomes. In addition, our study had a very low failure rate of 0.12%. Some previous studies with NIPT in twin pregnancies have shown higher failure rates, with the most recent Position Statement from the International Society for Prenatal Diagnosis[22] noting that initial failure rates for NIPT in twin pregnancies across 10 different studies ranged from 1.6 to 13.2%, with a median of 3.6%. A recent multicenter study by van Riel et al[26] found that initial failure rates ranged from 0 to 11.7% among the different genetic centers, which could be reduced to an overall rate of 1.2% after resampling.

As noted earlier, first trimester combined screening in twin pregnancies has been shown to have a relatively low detection rate of 89.3% and a high false-positive rate of 5.4%.[20] As patients with high-risk screening results are typically counseled to undergo confirmatory diagnostic testing, a high false-positive rate can lead to unnecessary invasive diagnostic procedures which may have a higher risk of loss in twin pregnancies.[27] As our study has shown, NIPT offers patients with twin pregnancies a more accurate approach to screen for fetal trisomies, in particular for trisomy

21. Based on known clinical outcomes, we observed both a sensitivity and specificity of > 99.9% for trisomy 21. A meta-analysis by Liao et al[28] also found that NIPT has both high sensitivity and specificity for trisomy 21 screening in twin pregnancies. In addition, our study had a high overall PPV of 89.5%, with a PPV of 100% for trisomy 21 and 83.3% for trisomy 18. This is similar to that observed in a recent study which found that a single-nucleotide polymorphism-based NIPT approach in twin pregnancies gave a PPV of 88.7% for trisomy 21 and 72.7% for trisomy 18.[29] For patients with twin gestations, it is very important that they receive appropriate counseling prior to prenatal screening and that they are made aware of the limitations of each of the different types of prenatal screening options and also the need for diagnostic confirmation of any detected aneuploidy.

One of the strengths of our study was that it involved a large number of twin samples from a general pregnancy population, allowing us to provide evidence on the performance of the NIPT assay in that patient population. Another strength is that the study was carried out at a single prenatal center, which is one of the largest labs in Germany, where we have over 10 years of experience with NIPT. A limitation of our study was that follow-up was not available for all samples in our cohort, particularly for the low-risk cases. The limited follow-up data on the low-risk cases prevented us from providing a true calculation of the NPV. Based on the 18.5% of these cases that did have clinical outcomes available, we estimated the NPV to be 100%. It is possible that there were some false-negative cases in our cohort that we were not made aware of, although discrepancies between a NIPT result and the birth report are typically reported back to our laboratory immediately. We were, however, able to obtain follow-up on a majority (61.3%) of the high-risk samples. Future steps will include obtaining diagnostic testing outcomes or birth outcomes on all study samples.

Another limitation of our study was the lack of zygosity data for our twin samples. Overall, about one-third of twin pregnancies are monozygotic and two-thirds are dizygotic.[30] NIPT is more complex in dizygotic pregnancies as the two fetuses have different genotypes but typically only one fetus (if any) will have an aneuploidy.[31] In addition, although the overall fetal fraction is higher in twin pregnancies, the individual contribution per fetus is lower and each fetus can contribute different amounts of fetal fraction.[31–35] This can lead to a decreased performance of NIPT in dizygotic pregnancies. A recent study by Kantor et al noted that the PPVs of NIPT for common trisomies were lower in dizygotic compared to monozygotic pregnancies.[29] As monozygotic twins have the same genotype except in rare cases, and with the higher overall fetal fraction observed in twin pregnancies, the performance of NIPT in monozygotic pregnancies will be at least equivalent to that observed in singleton pregnancies.[31,34] Therefore, the higher the proportion of monozygotic twins in a twin cohort, the closer the accuracy of the assay will be to that of singletons.

In conclusion, based on the clinical outcomes available at this time, we determined that the VeriSeq NIPT Solution v2 assay allows for screening of fetal chromosomal anomalies in twin pregnancies with a high overall sensitivity, specificity, and PPV, along with a very low failure rate.

Conflict of Interest
None declared.

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References

1 Taneja PA, Snyder HL, de Feo E, et al. Noninvasive prenatal testing in the general obstetric population: clinical performance and counseling considerations in over 85 000 cases. Prenat Diagn 2016;36(03):237–243
2 Dar P, Curnow KJ, Gross SJ, et al. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. Am J Obstet Gynecol 2014;211 (05):527.e1–527.e17
3 McCullough RM, Almasri EA, Guan X, et al. Non-invasive prenatal chromosomal aneuploidy testing–clinical experience: 100,000 clinical samples. PLoS One 2014;9(10):e109173
4 Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2017;50(03): 302–314
5 Iwarsson E, Jacobsson B, Dagerhamn J, Davidson T, Bernabé E, Heibert Arnlind M. Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18 and 13 in a general pregnant population and in a high risk population - a systematic review and meta-analysis. Acta Obstet Gynecol Scand 2017;96(01):7–18
6 Samango-Sprouse C, Banjevic M, Ryan A, et al. SNP-based non-invasive prenatal testing detects sex chromosome aneuploidies with high accuracy. Prenat Diagn 2013;33(07):643–649
7 Mazloom AR, Džakula Ž, Oeth P, et al. Noninvasive prenatal detection of sex chromosomal aneuploidies by sequencing circulating cell-free DNA from maternal plasma. Prenat Diagn 2013;33 (06):591–597
8 Pertile MD, Flowers N, Vavrek D, et al. Performance of a paired-end sequencing-based noninvasive prenatal screening test in the detection of genome-wide fetal chromosomal anomalies. Clin Chem 2021;67(09):1210–1219
9 Soster E, Boomer T, Hicks S, et al. Three years of clinical experience with a genome-wide cfDNA screening test for aneuploidies and copy-number variants. Genet Med 2021;23(07):1349–1355
10 van Prooyen Schuurman L, Sistermans EA, Van Opstal D, et al; Dutch NIPT consortium. Clinical impact of additional findings detected by genome-wide non-invasive prenatal testing: follow-up results of the TRIDENT-2 study. Am J Hum Genet 2022;109 (06):1140–1152
11 Dondorp W, de Wert G, Bombard Y, et al; European Society of Human Genetics. ; American Society of Human Genetics. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. Eur J Hum Genet 2015;23(11):1438–1450
12 Salomon LJ, Alfirevic Z, Audibert F, et al; ISUOG Clinical Standards Committee. ISUOG updated consensus statement on the impact of cfDNA aneuploidy testing on screening policies and prenatal ultrasound practice. Ultrasound Obstet Gynecol 2017;49(06): 815–816
13 Gregg AR, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med 2016;18(10):1056–1065

14 American College of Obstetricians and Gynecologists (ACOG), Society for Maternal-Fetal Medicine (SMFM) Screening for fetal chromosomal abnormalities: ACOG Practice Bulletin Summary, Number 226. Obstet Gynecol 2020;136(04):859–867
15 Benn P, Borrell A, Chiu RW, et al. Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn 2015;35(08):725–734
16 Audibert F, De Bie I, Johnson JA, et al. No. 348-Joint SOGC-CCMG guideline: update on prenatal screening for fetal aneuploidy, fetal anomalies, and adverse pregnancy outcomes. J Obstet Gynaecol Can 2017;39(09):805–817
17 Liehr T, Harutyunyan T, Williams H, Weise A. Non-invasive prenatal testing in Germany. Diagnostics (Basel) 2022;12(11): 2816
18 Kozlowski P, Burkhardt T, Gembruch U, et al. DEGUM, ÖGUM, SGUM and FMF Germany recommendations for the implementation of first-trimester screening, detailed ultrasound, cell-free DNA screening and diagnostic procedures. Ultraschall Med 2019; 40(02):176–193 Empfehlungen der DEGUM, der ÖGUM, der SGUM und der FMF Deutschland zum Einsatz von Ersttrimester-Screening, früher Fehlbildungsdiagnostik, Screening an zellfreier DNA (NIPT) und diagnostischen Punktionen
19 Schmid M, Klaritsch P, Arzt W, et al. Cell-free DNA testing for fetal chromosomal anomalies in clinical practice: Austrian-German-Swiss recommendations for non-invasive prenatal tests (NIPT). Ultraschall Med 2015;36(05):507–510
20 Prats P, Rodríguez I, Comas C, Puerto B. Systematic review of screening for trisomy 21 in twin pregnancies in first trimester combining nuchal translucency and biochemical markers: a meta-analysis. Prenat Diagn 2014;34(11):1077–1083
21 Judah H, Gil MM, Syngelaki A, et al. Cell-free DNA testing of maternal blood in screening for trisomies in twin pregnancy: updated cohort study at 10-14 weeks and meta-analysis. Ultrasound Obstet Gynecol 2021;58(02):178–189
22 Palomaki GE, Chiu RWK, Pertile MD, et al. International Society for Prenatal Diagnosis Position Statement: cell free (cf)DNA screening for Down syndrome in multiple pregnancies. Prenat Diagn 2021;41(10):1222–1232
23 Borth H, Teubert A, Glaubitz R, et al. Analysis of cell-free DNA in a consecutive series of 13,607 routine cases for the detection of fetal chromosomal aneuploidies in a single center in Germany. Arch Gynecol Obstet 2021;303(06):1407–1414
24 Eiben B, Borth H, Kutur N, et al. Clinical experience with noninvasive prenatal testing in Germany: analysis of over 500 high-risk cases for trisomy 21, 18, 13 and monosomy X. Obstet Gynecol Res 2021;5(01):. Doi: 10.15761/OGR.1000157
25 Illumina VeriSeq NIPT Solution v2 Datasheet. Accessed May 11, 2023 at: https://emea.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/veriseq-nipt-solution-data-sheet-1000000032015.pdf
26 van Riel M, Brison N, Baetens M, et al. Performance and diagnostic value of genome-wide noninvasive prenatal testing in multiple gestations. Obstet Gynecol 2021;137(06):1102–1108
27 Hopkins MK, Dugoff L. Screening for aneuploidy in twins. Am J Obstet Gynecol MFM 2022;4(2S):100499
28 Liao H, Liu S, Wang H. Performance of non-invasive prenatal screening for fetal aneuploidy in twin pregnancies: a meta-analysis. Prenat Diagn 2017;37(09):874–882
29 Kantor V, Mo L, DiNonno W, et al. Positive predictive value of a single nucleotide polymorphism (SNP)-based NIPT for aneuploidy in twins: experience from clinical practice. Prenat Diagn 2022; 42(13):1587–1593
30 Audibert F, Gagnon A. No. 262-prenatal screening for and diagnosis of aneuploidy in twin pregnancies. J Obstet Gynaecol Can 2017;39(09):e347–e361
31 Benn P, Rebarber A. Non-invasive prenatal testing in the management of twin pregnancies. Prenat Diagn 2021;41(10):1233–1240
32 Struble CA, Syngelaki A, Oliphant A, Song K, Nicolaides KH. Fetal fraction estimate in twin pregnancies using directed cell-free DNA analysis. Fetal Diagn Ther 2014;35(03):199–203
33 Hedriana H, Martin K, Saltzman D, Billings P, Demko Z, Benn P. Cell-free DNA fetal fraction in twin gestations in single-nucleotide polymorphism-based noninvasive prenatal screening. Prenat Diagn 2020;40(02):179–184
34 Norwitz ER, McNeill G, Kalyan A, et al. Validation of a single-nucleotide polymorphism-based non-invasive prenatal test in twin gestations: determination of zygosity, individual fetal sex, and fetal aneuploidy. J Clin Med 2019;8(07):937
35 Leung TY, Qu JZ, Liao GJ, et al. Noninvasive twin zygosity assessment and aneuploidy detection by maternal plasma DNA sequencing. Prenat Diagn 2013;33(07):675–681

