DEGUM, ÖGUM, SGUM and FMF Germany Recommendations for the Implementation of First-Trimester Screening, Detailed Ultrasound, Cell-Free DNA Screening and Diagnostic Procedures

Empfehlungen der DEGUM, der ÖGUM, der SGUM und der FMF Deutschland zum Einsatz von Ersttrimester-Screening, früher Fehlbildungsdagnostik, Screening an zellfreier DNA (NIPT) und diagnostischen Funktionen

ABSTRACT
First-trimester screening between 11 + 0 and 13 + 6 weeks with qualified prenatal counseling, detailed ultrasound, biochemical markers and maternal factors has become the basis for decisions about further examinations. It detects numerous structural and genetic anomalies. The inclusion of uterine artery Doppler and PlGF screens for preeclampsia and fetal growth restriction. Low-dose aspirin significantly reduces the prevalence of severe preterm eclampsia. Cut-off values define groups of high, intermediate and low probability. Prenatal counseling uses detection and false-positive rates to work out the individual need profile and the corresponding decision: no further diagnosis/screening – cell-free DNA screening – diagnostic procedure and genetic analysis. In pre-test counseling it must be recognized that the prevalence of trisomy 21, 18 or 13 is low in younger women, as in submicroscopic anomalies in every maternal age. Even with high specificities, the positive predictive values of screening tests for rare anomalies are low. In the general population trisomies and sex chromosome aneuploidies account for approximately 70 % of anomalies recognizable by conventional genetic analysis. Screen positive results of cfDNA tests have to be proven by diagnostic procedure and genetic diagnosis. In cases of inconclusive results a higher rate of genetic anomalies is detected. Procedure-related fetal loss rates after chorionic biopsy and amniocentesis performed by experts are lower than 1 to 2 in 1000. Counseling should include the possible detection of submicroscopic anomalies by comparative genomic hybridization (array-CGH). At present, existing studies about screening for microdeletions and duplications do not provide reliable
Introduction

In 2012, one year after market introduction in the USA, the first screening test for trisomies 21, 18, and 13 and the gonosomes using cell-free DNA from maternal blood (cfDNA) was introduced in Germany. The development of simpler and significantly more cost-effective test procedures and intensive marketing resulted in increased use. Recommendations for using cfDNA tests were published in 2015 in the European Journal of Ultrasound [1, 2]. The cfDNA in maternal blood is largely from the mother. Only a significantly smaller portion is from the placenta. For the purpose of clarity, the term cfDNA is thus exclusively used here instead of the terms cell-free fetal DNA (cffDNA) and cell-free placental DNA (cfpDNA).

cfDNA screening, often also called NIPT (noninvasive prenatal testing), is a screening method that always requires clarification via diagnostic procedure in the case of abnormal findings. Combined first-trimester screening, which can be combined with early diagnosis of anomalies and preeclampsia screening ([Table 1] and thus goes far beyond trisomy 21 screening has been long established and is widely used as a screening method [3–5]. Approximately two-thirds of cfDNA tests in Germany are now performed between 11 and 13 gestational weeks, usually after first-trimester screening, even if cfDNA screening starting at 10 weeks as first-line screening is being discussed.

The spectrum of the existing first-trimester screening methods and the useful application of cfDNA tests are discussed in the following. In particular, the elements of screening and the clarification of abnormal findings are taken into consideration.

Elements of screening 11 + 0 to 13 + 6 weeks

Counseling prior to prenatal screening

The law on genetic testing in humans (Genetic Diagnostics Act) [6] and the subsequent guidelines regulate the handling of genetic analyses and prenatal risk clarification on the basis of aneuploidy screening in first-trimester screening. The consequently established Commission on Genetic Testing (GEKO) at the Robert-Koch Institute creates guidelines relating to the generally accepted state of knowledge and technology.

With respect to the Law on Patients’ Rights from 2013 [7], the restriction to physicians in § 7 and informed consent discussion in § 9 of the Genetic Diagnostics Act are pivotal: Prior to obtaining informed consent, the responsible physician must inform the affected person of the nature, significance, and consequences of the genetic testing. After the informed consent discussion, the affected person is to be given appropriate time to think before making a decision about informed consent.

GEKO defined the classification of cfDNA and the corresponding counseling qualifications: In contrast to prenatal risk assessment, tests of circulating placental DNA from the mother’s blood are classified as prenatal genetic analyses for determining genetic properties. As a result, the necessary qualifications, which can be acquired in 72 continuing education units and the corresponding qualification measure [8], are valid for the requirements regarding competence in genetic counseling within the scope of each medical subspecialty.

The scope of counseling with respect to the various prenatal diagnostic testing options has not yet been fully defined. The guidelines of the Federal Joint Committee regarding physician care in
data to calculate sensitivities, false-positive rates and positive predictive values.

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pregnancy and after birth (maternity guidelines) define the early detection of high-risk pregnancies and births as a primary goal of prenatal care. In addition to other medical history factors of high-risk pregnancies, a maternal age of less than 18 years or more than 35 years is specified in section B of the guidelines. First-trimester screening and cfDNA screening are not mentioned in the guidelines. In 2016, the Federal Joint Committee initiated an investigation regarding the introduction of cfDNA screening and commissioned the IQWiG to create an information brochure about prenatal genetic testing options (g-ba.de 2/16/2017).

In a statement regarding the analysis of fetal DNA from maternal blood dated 11/12/2012, the German Society of Human Genetics stated that due to the unnecessary consideration of the risks of diagnostic procedures versus the probability of disease/health problems of the fetus, cfDNA analysis should be made available to every pregnant woman.

When providing counseling regarding primary early screening options without a detailed fetal scan, it must be taken into consideration that only trisomies 13, 18, and 21 show a significant risk for fetal anomalies, cfDNA analysis should be made available to every pregnant woman.

Early diagnosis of anomalies

Early differentiated ultrasound diagnosis at 11+0–13+6 weeks including detailed anatomical evaluation of the fetus, measurement of the fetal nuchal translucency, analysis of the fetal and maternal hemodynamics, and testing of various biochemical parameters in the maternal serum helps to determine the further course of prenatal care. While detailed ultrasound examinations were limited to the second and third trimesters for a long time, the first trimester has become increasingly important for diagnosis since the 1990s. As a result, first-trimester screening now plays a central role in decisions regarding further diagnostic and therapeutic measures.

The standard plans for early diagnosis of fetal anomalies have been defined in the recommendations and guidelines of the Fetal Medicine Foundation (FMF), International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) and the German Society of Ultrasound in Medicine and Biology (DEGUM) [3, 9, 10].

Anatomical evaluation of the fetus makes it possible to rule out or diagnose a series of anomalies: Syngelaki et al. [11] assigned anomalies at 11+0–13+6 weeks in a population of 45 191 pregnancies to three categories according to their detectability (Table 2).

The detection rate of ultrasound at 11–14 weeks in relation to severe anomalies is 44% according to this study. In a German study including 6879 pregnancies, the detection rate for detailed ultrasound examination at an expert center was 83.7% [12]. The rate of severe anomalies was 1% (27/2788) in the case of an NT <2.5 mm (2788/3094 – 90.1%) and 19.3% (59/306) for an NT of >2.5 mm. A follow-up study by the same group (n = 6,879) showed a prevalence of severe anomalies including chromosomal anomalies of 3.2% (220/6858), with 50.5% (111/220) having an NT <95th percentile and 49.5% (109/220) having an NT >95th percentile [13]. In a meta-analysis of 19 studies including 78,000 pregnant women (prevalence of anomalies 1.2%), the detection rate was 51% [14]. The authors indicated that even 40% of severe anomalies were detected early and that the combination of transabdominal and transvaginal ultrasound allowed a significantly higher detection rate (62% versus 51%).

Evaluation of the 4th ventricle, also referred to as intracranial transparency (IT), and examination of the brain stem can result in early detection of open spina bifida in the first-trimester examination [15, 16]. In a meta-analysis including more than 21,000 fetuses, a sensitivity of 53.5% and a specificity of 99.7% were calculated [17].

The measurement of the fetal nuchal translucency (NT) is highly important not only for aneuploidy screening but also for the early

### Table 1 Nomenclature of the screening tests in the 1st trimester.

<table>
<thead>
<tr>
<th>examination</th>
<th>ultrasound parameters</th>
<th>serum parameters</th>
<th>objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>first-trimester screening</td>
<td>NT</td>
<td></td>
<td>initial anomaly screening aneuploidy screening</td>
</tr>
<tr>
<td>combined first-trimester screening</td>
<td>NT</td>
<td>free β-HCG, PAPP-A</td>
<td></td>
</tr>
<tr>
<td>combined first-trimester screening with markers</td>
<td>NT, NB, DV, TRI</td>
<td>free β-HCG, PAPP-A</td>
<td>primary or secondary clarification of the first-trimester screening finding</td>
</tr>
<tr>
<td>contingent screening</td>
<td>expanded screening depending on the finding of combined first-trimester screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>early diagnosis of anomalies</td>
<td></td>
<td>published quality requirements: DEGUM [10], ISUOG [9], FMF [3]</td>
<td></td>
</tr>
</tbody>
</table>


The term contingent screening is increasingly used to refer to the use of cfDNA screening after prior risk classification based on combined first-trimester screening.
diagnosis of anomalies. In combination with the anatomical evaluation of the fetus, the NT can indicate a number of possible diseases, such as chromosomal and non-chromosomal syndromes, as well as structural anomalies [18–22]. By combining detailed evaluation of the fetus with measurement of the NT and secondary criteria for the detection of trisomies 18 and 13, Wagner et al. achieved a detection rate of 95%, which is similar to that of cfDNA [23].

 Fetuses with heart defects can also have a thickened NT [11,24] often in combination with tricuspid regurgitation and increased pulsatility in the ductus venosus [25,26]. Therefore, a sensitivity of 57.6% for severe heart defects is indicated for the combination of NT measurement and the ductus venosus (one of the two parameters >95th percentile) [27]. However, measurements of the ductus venosus and tricuspid regurgitation with a normal NT have only low detection rates. The combination of an NT >95th percentile with an abnormal ductus venosus and/or tricuspid regurgitation can increase the detection rate for severe heart defects to >50% [28]. This marker screening for severe heart defects is increasingly being replaced by the integration of the four-chamber view and the three-vessel view into the detailed first-trimester examination [29,30].

 In the case of monochorionic twins, the probability of a twin-to-twin transfusion sequence (TTTS) is increased in the case of highly varied measured values for nuchal translucency. In a meta-analysis of 13 studies including 1991 pregnancies, discrepant NT measurements and pathological measurements of the ductus venosus showed a sensitivity of 52.8% and 50%, respectively, for the later development of FFTS [31]. Even in the case of a normal finding, follow-up examinations every two weeks are indicated in monochorionic twins after 14–16 weeks to be able to diagnose symptoms of FFTS or twin-anemia polycythemia sequence (TAPS) in a timely manner [32].

 The probability of live birth of a healthy child can also be estimated based on the NT measurement. Therefore, the probability is 97% for an NT <95th percentile. It decreases in the case of a thickened NT and is only 15% in the case of an NT ≥6.5 mm [33].

 The measurements of fetal nuchal translucency and the secondary criteria nasal bone, ductus venosus and tricuspid regurgitation are the only ultrasound examinations subject to standardized quality control in the form of annual reviews by the Fetal Medicine Foundation London and the Fetal Medicine Foundation Germany. In Germany this quality check was included in the implementation regulations of the RKI [34,35].

cfDNA testing should only be offered after or in connection with professional ultrasound examination [1,10,36]. The significance of early organ examination was shown by a prospective randomized study in which 1400 pregnant women with a normal finding after an expert examination between 11 and 13 weeks underwent either cfDNA screening or combined first-trimester screening according to the FMF algorithm. The false-positive rates for trisomy 21 were 0% for cfDNA screening and 2.5% for combined first-trimester screening [5]. The limitations of this study are the restriction to risk calculation only for trisomy 21 and structural anatomical anomalies and the lack of biochemical parameters that can be useful when screening for other chromosomal anomalies and preeclampsia.

 A lack of early organ examination and the use of primary cfDNA screening can result in structural or genetic anomalies only being detected later.

**Combined first-trimester screening (combined test)**

The algorithms of first-trimester screening as a combined test of maternal age, nuchal translucency, and the serum parameters ßHCG and PAPP-A make it possible to calculate the probability of the most common trisomies 21, 13, and 18 [37]. The risk algorithms of the Fetal Medicine Foundation (FMF) London and the FMF Germany are used in many countries and also allow the inclusion of the indicated parameters with corresponding certification. Combined first-trimester screening has become established as a very good, cost-effective examination that can be performed by most gynecologists. The detection rates at centers are 90% with a false-positive rate of 3–5% [38]. 2–4% of pregnancies with trisomy 21 are identified in the low-risk group with an first-trimester screening risk of 1:1000 or lower [37]. Approximately 85% of normal pregnancies have an first-trimester screening risk in this range. In the high-risk group, the spectrum of possible diseases is not limited to chromosomal abnormalities that can be detected by cfDNA screening [4,18].

The cut-off values for the intermediate-risk group are controversial. They are characterized by the desire for an optimal combination of high detection rates both for trisomies and other genetic anomalies and low false-positive rates. The higher the cut-off value for the high-risk group, the lower the percentage of pregnancies in which diagnostic procedures are recommended. Every increase in detection rate is associated with an increase in the rate of positive findings. They are thus subject to considerations
regarding health economics as well as to the individual decision of each pregnant woman. Expectant mothers should make a decision only after receiving comprehensive counseling covering the spectrum of anomalies to be detected and the probability of their detection as a function of the cut-off values and an explanation of the safety of diagnostic procedures in expert hands.

In first-trimester screening, the positive predictive values are low but the method has very high negative predictive values. Therefore, based on the latest study data of the FMF London for combined first-trimester screening at a cut-off of 1:100, a sensitivity of 92% and a specificity of 95.4% in relation to trisomy 21, the positive predictive value was 7.34% and the negative predictive value was 99.97%. Similar values apply for trisomies 13 and 18 [39].

Screening using cell-free DNA

Quality parameters

In the initial years prior to and shortly after market introduction, the majority of studies regarding the sensitivity and specificity of cfDNA screening were performed in high-risk populations [40 – 46]. Results from routine populations are now available [47 – 52].

The small total number and high prevalence in some study populations makes evaluation in meta-analyses useful. The meta-analysis published by Gil in 2017 [53] including 35 studies yielded detection rates of 99.7%, 97.9%, and 99.0%, respectively, for trisomy 21, 18, and 13 and 95.8% for monosomy X with false-positive rates of 0.04% for trisomies 21, 18, and 13 and 0.14% for monosomy X (Table 3). Iwarsson et al. achieved similar results [52].

In contrast to earlier studies [54], the meta-analysis by Gil in 2017 used a different statistical approach, i.e., bivariate analysis, as already used in the meta-analysis by Taylor-Phillips [55] and the dependence of the sensitivity-specificity pairs on different cut-off values in the individual studies was taken into consideration. The data pooled from 41 studies were used in a high-risk population and a normal population (Table 4). Detection rates of 95.9% for trisomy 21 (prevalence of trisomy 21 of 1:230), 86.5% and 77.5% for trisomy 18 and 13 (prevalence 1:1000 and 1:2000, respectively) were determined in a normal population. Numerous studies also include a disproportionate number of tests from later gestational weeks.

The positive and negative predictive values of a screening method play an important role in counseling and decision making prior to screening. It must be taken into consideration that the prevalence of the anomaly in question has a significant effect on the positive prediction, even in the case of a high detection rate and high specificity of a test [56]. Even in the case of complete detection of all cases and a very low false-positive rate, the majority of screened cases will receive a “false” finding as soon as the prevalence is lower than the rate of false-positive findings [57]. This must be taken into particular consideration when counseling young pregnant women with a correspondingly low prevalence of trisomies 21, 18, and 13.

Discrepant findings are usually due to the fact that the majority of cell-free DNA fragments are from the mother and only a small portion is from the placenta. cfDNA can therefore provide information regarding placental mosaics and maternal mosaics and chromosomal anomalies. A vanishing twin can also be the reason for a false-positive finding when the cfDNA examination is performed close to the miscarriage event. Therefore, a positive finding must be confirmed by a diagnostic procedure [58].

None of the currently offered testing methods, both the random methods that detect DNA fragments of all chromosomes and the targeted tests that focus on individual chromosomes, differentiates between maternal and placental DNA. The studies published to date have not been able to show any advantages of the different approach of SNP-based methods for differentiating between maternal, placental, and, if available, paternal DNA in relation to detection rates and false-positive rates or the screening spectrum for genetic anomalies.

The percentage of test failures even after repeated examination is specified as 0.5 – 6.4% [59 – 61] (Table 3). A low percentage of placental DNA (“fetal fraction”), which is positively correlated with gestational age and the biochemical parameters PAPP-A and PIgf and negatively correlated with maternal body weight and age and reproductive measures, is often the cause [62 – 64]. Treatment of pregnant women with heparin also often results in a reduced amount of placental DNA [65]. In the group of test failures, a significantly increased rate of fetuses with trisomy 13, trisomy 18 or a triploidy but not trisomy 21 can be observed [47, 62] so that an early detailed fetal scan and if necessary a diagnostic procedure are indicated in these cases. The test failures are not included in most studies. If the failure rate from the first blood sample is taken into consideration, the modeled detection rates for trisomy 21 are in the range of 93 – 97% [66]. Test failures due to a fetal fraction of less than 4% have poor test performance even in the case of a successful second analysis. The fetal fraction of every analysis and the total rate of analyses without a result should be provided by every lab as a quality criterion. Obese pregnant women must be informed of a test failure rate of up to 10% even in the second trimester [64]. Improvement in diagnostic reliability can be expected as a result of a greater sequencing depth and new sequencing techniques such as “paired-end sequencing” [67].

<table>
<thead>
<tr>
<th>aneuploidy</th>
<th>DR %</th>
<th>FPR %</th>
<th>FF %</th>
<th>NR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>99.7</td>
<td>0.04</td>
<td>10.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>97.9</td>
<td>0.04</td>
<td>8.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>99.0</td>
<td>0.04</td>
<td>7.0</td>
<td>6.3</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>95.8</td>
<td>0.14</td>
<td>10.0</td>
<td>4.1</td>
</tr>
<tr>
<td>SCA</td>
<td>100.0</td>
<td>0.04</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

In the case of twin pregnancies, cfDNA screening is more complex than in singleton pregnancies since the fetuses are either monozygote and thus genetically identical or dizygote in which case it is highly likely that only one fetus would be affected in the case of an aneuploidy. The fetal fraction is usually sufficient in monozygote twins due to the identical genetic properties of the two fetuses (median 10.1%) and is comparable with singleton pregnancies, while the fetal fraction is lower in dizygote twins (median: 7.7%) [68]. In a current meta-analysis [53], five studies on twin pregnancies were examined [68–72] (overview in Table 5). In 24 pregnancies with trisomy 21 and 1100 pregnancies with euploid fetuses, a DR of 100% (95% CI 95.2–100%) and an FPR of 0% (95% CI 0–0.003%) were described. Moreover, 14 cases of trisomy 18 were in the population with 13 being correctly detected and 1 case of trisomy 13 being incorrectly detected as euploid. In 4.87% of the women in this study, the first blood sample did not yield a result. Similar results were achieved by another prospective study in which a result could not be obtained in 5.6% of twin pregnancies after the first blood draw and in 50% after the second blood draw while these values were 1.7% and 32.1% in the compared population of singleton pregnancies [73]. Moreover, this study was able to show that the rate of test failure in twin pregnancies increases with an increasing body-mass index (BMI) and is higher after in-vitro fertilization (IVF) than after natural conception.

In the case of a vanishing twin, cfDNA testing should not be performed since in many cases an aneuploidy probably caused the miscarriage of the fetus resulting in false-positive findings even after a number of weeks [74]. cfDNA is currently not commercially available for higher-order multiple pregnancies. A primary diagnostic procedure should be considered also in women with twin pregnancies after IVF and a high BMI since the failure rates seem to be particularly high here [73].

Screening for trisomy 21 using cfDNA from maternal blood in twin pregnancies has a comparably high detection rate with an equally low FPR rate as in singleton pregnancies. Reliable data regarding the performance of the screening method for trisomy 18 and 13 is currently not available.

### Procedure following findings of ultrasound and first-trimester screening

#### Fetal anomalies

If isolated or complex fetal anomalies are detected on ultrasound, the analysis of cfDNA is insufficient and contraindicated due to the large range of underlying genetic findings. Trisomy 21, 18 or 13 is the cause in only approximately 60% of fetuses [75, 76]. In addition to cytogenetically detectable aneuploidies, structural chromosomal anomalies not detectable with cfDNA are found in 7–8% of cases with a normal karyogram [77, 78]. Therefore, a diagn-
nastic procedure (CVS or amniocentesis) for microscopic karyotyping and if necessary chromosomal microarray analysis for detecting submicroscopic chromosomal anomalies (microdeletions and microduplications) should be performed [77, 79]. Internationally, quick karyotyping (e.g. MLPA or QF-PCR with respect to the common autosomal trisomies 21, 18, and 13 and the gonosomal aneuploidies) followed by a chromosomal microarray analysis is preferred for anomalies for time and cost reasons and conventional cytogenetic karyotyping is not performed [80]. This is currently not the standard in Germany. In the case of a combination of anomalies, Next Generation Sequencing technologies (NGS) such as whole exome sequencing (WES) or whole genome sequencing (WGS) can be used as the next step [81, 82]. These technologies are currently still limited to studies [83].

The above-described procedure is also valid when previously performed cfDNA testing yielded an abnormal result [84].

### High-risk group in combined first-trimester screening

In the high-risk group which is defined above cut-off values of 1:10 to 1:100, the spectrum of possible diseases is not limited to the chromosomal anomalies detectable by cfDNA testing [18, 36]. A diagnostic procedure must be offered to diagnose the possible diseases. Averaging all age groups, trisomies 13, 18, and 21 make up approximately 70 % of all chromosomal anomalies that can be detected by cytogenetic analysis [85, 86]. In the case of abnormal first-trimester screening, other chromosomal anomalies of varying clinical relevance were seen in up to 30 % of cases. Alamillo et al. [86] were able to show in over 23 000 pregnancies that this was the case in 29.9 % of all abnormal karyograms, with 42 % being most common in abnormal first-trimester screening for trisomies 13 and 18. The Danish Fetal Medicine Study Group and the Danish Clinical Genetics Study Group [87] were able to show on the basis of a central country-wide register including approximately 193 000 pregnancies in Denmark (89 % of all pregnant women in the report period) that 23.4 % of all relevant pathological karyograms were not trisomies 13, 18, or 21. The rate of pathological findings increases with the thickness of the nuchal translucency: 10.4 % for an NT thickness between the 95th and 99th percentile and 34.8 % for an NT > 99th percentile. One study including 11 315 pregnancies showed a rate of chromosomal anomalies of 7.1 % (17 % not trisomy 21, 18, or 13) for an NT between the 95th percentile and 3.4 mm. At a size of greater than 3.5 mm to 11.5 mm, the percentage of pathological karyograms increased from 20 % to 70 % [88]. In 1063 cases with an increased NT between the 95th percentile and 3.4 mm [89], pathological karyograms were present in 10 % of cases (68 of 611 fetuses), while they were present in 42 % of cases with an NT greater than 3.4 mm (▶ Table 6).

The increase in the cut-off value between the high-risk group and the intermediate-risk group results in a reduction in the detection rate.

particularly in the case of triploidy and unusual trisomies, the NT values are closer to the normal distribution while they are moderately elevated in unbalanced translocations [90]. In one study, the prevalence of submicroscopic chromosomal anomalies in the group of fetuses with a nuchal translucency > 3.5 mm was not higher than in fetuses without anomalies detectable on ultrasound [91].

### Table 6 Rate of chromosomal anomalies depending on first-trimester screening finding and NT measurement. (Publications without inclusion of chromosomal microarrays).

<table>
<thead>
<tr>
<th>author</th>
<th>criterion</th>
<th>n</th>
<th>pathological karyotype (%)</th>
<th>percentage of all pathol. karyotypes (%)</th>
<th>trisomies and SCAs (%)</th>
<th>other anomalies (%)</th>
<th>percentage of all other anomalies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kagan 2006</td>
<td>NT &gt; 95th perc.</td>
<td>11 315</td>
<td>2168 (19.2)</td>
<td>100</td>
<td>2014 (92.9)</td>
<td>154 (7.1)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>NT ≥ 3.5 mm</td>
<td>4206</td>
<td>1661 (39.4)</td>
<td>76.6</td>
<td>1557 (93.7)</td>
<td>104 (6.3)</td>
<td>67.5</td>
</tr>
<tr>
<td>Åyräs 2013</td>
<td>NT &gt; 95th perc.</td>
<td>1063</td>
<td>224 (21.5)</td>
<td>100</td>
<td>206 (91.9)</td>
<td>18 (8.0)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>NT ≥ 3.5 mm</td>
<td>384</td>
<td>159 (41.4)</td>
<td>71.0</td>
<td>145 (91.2)</td>
<td>14 (8.8)</td>
<td>77.8</td>
</tr>
<tr>
<td>Petersen 2014</td>
<td>NT &lt; 95th perc.</td>
<td>209 257</td>
<td>682 (0.33)</td>
<td>53.4</td>
<td>429 (62.9)</td>
<td>253 (37.1)</td>
<td>84.9</td>
</tr>
<tr>
<td></td>
<td>NT ≥ 95th perc.</td>
<td>5966</td>
<td>596 (10.0)</td>
<td>46.6</td>
<td>551 (92.4)</td>
<td>45 (7.6)</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>NT ≥ 99th perc.</td>
<td>1362</td>
<td>422 (31.0)</td>
<td>33.0</td>
<td>391 (92.6)</td>
<td>31 (7.3)</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>Comb. first-trimester screening risk ≤ 1:300</td>
<td>185 620</td>
<td>352 (0.19)</td>
<td>31.4</td>
<td>174 (49.4)</td>
<td>178 (50.6)</td>
<td>67.9</td>
</tr>
<tr>
<td></td>
<td>&gt; 1:300</td>
<td>8018</td>
<td>770 (9.6)</td>
<td>68.6</td>
<td>686 (89.1)</td>
<td>84 (10.9)</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td>&gt; 1:100</td>
<td>4002</td>
<td>667 (16.7)</td>
<td>59.4</td>
<td>603 (90.4)</td>
<td>64 (9.6)</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>&gt; 1:10</td>
<td>734</td>
<td>378 (51.5)</td>
<td>33.7</td>
<td>365 (96.5)</td>
<td>13 (3.5)</td>
<td>5.0</td>
</tr>
</tbody>
</table>

NT: nuchal translucency, SCA: sex chromosome anomaly. Special features of the studies: Kagan: Population only NT > 95th percentile; only karyograms, no array-CGH; no data regarding the number of fetuses with anomalies; Åyräs: Population only NT > 95th percentile; only karyograms, no array-CGH; 74 with anomalies; Petersen: no data regarding the number of fetuses with anomalies; no classification according to karyogram and array-CGH.
The prevalence of submicroscopic chromosomal structural anomalies that can only be detected via array-CGH (pathological CNVs) in populations with an abnormal NT is the subject of various studies using different NT cut-off values: Lund et al. found pathological CNVs in 132 fetuses with NT values > 3.5 mm in 12.8 % of cases [92]. Maya et al. [93] used absolute NT values and found pathological CNVs in 0.9 % of cases for NT values < 3.0 mm with normal cytogenetics, in 1.8 % for NT values between 3.0 and 3.4 mm, and in 3.6 % of cases for values > 3.4 mm (Table 7).

Tørring et al. [94] showed that PAPP-A is reduced to 0.2 – 0.5 MoM (median 0.34 MoM) in the group of uncommon trisomies while the NT values were only slightly elevated. f-ßHCG and PAPP-A were usually significantly reduced, i.e., 0.2 MoM and 0.15 MoM, respectively, in triploidies [95].

The Danish Fetal Medicine Study Group showed that in the case of an indication for diagnostic procedure with a risk for trisomy 21 of > 1:300 and for trisomies 13 and 18 of > 1:150 diagnostic procedure was offered to approximately 5 % of pregnant women and a detection rate of > 90–95 % for chromosomal abnormalities was achieved [95]. Another study in a population with a lower prevalence [39] showed that 75.1 % of chromosomal abnormalities were detected in the case of an first-trimester screening risk of > 1:10 in this subgroup (1.4 % of examined pregnancies). In total, 5.3 % of pregnant women had a cut-off value of > 1:100. In this group, 88.6 % of anomalies that can be detected by conventional cytogenetics were found (Table 8).

To limit access to diagnostic procedures and genetic diagnosis to high-risk groups with NT values ≥ 3.5 mm or risks of ≥ 1:10 in...
first-trimester screening does not seem justified given the risk of miscarriage of 0.2% for chorionic villus sampling and 0.1% for amniocentesis [96, 97] with the goal of maximum detection rates. Individual counseling of pregnant women in the case of abnormal findings in first-trimester screening is of central importance.

Intermediate-risk group and low-risk group in first-trimester screening

The established spectrum of diseases that can be detected by the cfDNA screening method is currently still limited to trisomies 21, 18, 13 and gonosomal anomalies. From today’s standpoint, the use of NIPT analysis can be useful in normal fetuses and in the case of an intermediate risk according to first-trimester screening, which is between the cut-off values for the low-risk group and the high-risk group. In this population, additional ultrasound markers, such as the nasal bone, ductus venosus and tricuspid regurgitation, have been examined to date. A combination model including first-trimester screening with a broad spectrum of detectable diseases followed by cfDNA analysis for a certain population can combine established and new screening methods in a useful way [98].

If the use of NIPT analysis is limited to a population with a first-trimester screening risk between 1:10 and 1:1000, the secondary test method would be used in approximately 20% of cases. 28% of pregnancies with trisomy 21 are in this risk group [36]. An upper cut-off value of 1:100 would reduce the intermediate-risk group to 16% and increase the high-risk population to 5%. The rate of false-positive findings would increase from 0.8% to 4.6%, the rates of detected trisomies 21, 18, and 13 from 86% to 93%, and the rate of other detected aneuploidies from 44% to 65% [39].

Diagnostic procedures

In the case of abnormal cfDNA screening results, a diagnostic procedure to verify or falsify the screening finding must be performed [99, 100]. When selecting the diagnostic procedure, it must be taken into consideration that cfDNA originates largely from the trophoblast cells and not from the fetus. As in chorionic villus sampling (CVS), abnormal findings, in particular for trisomy 18, can be based on mosaics about 20% of which represents the fetus and 80% the cytotrophoblast cells [58, 101]. CVS should usually be performed after 11 + 0 weeks for genetic diagnosis. Given a normal fetus in the detailed ultrasound examination, amniocentesis is the method of choice starting at 15 + 0 weeks because the examination is performed using purely fetal cells and the risk of a mosaic is minimized. Prior to the decision to perform prenatal diagnostic testing, every pregnant woman must receive comprehensive information and counseling regarding the information provided by the various genetic lab tests and the possible risks of diagnostic procedures. The indications for offering a diagnostic procedure and further clarification during counseling are:

- Fetal malformations [76]
- Early growth restriction [23, 102]
- Nuchal translucency > 95th percentile

The finding of an increased nuchal translucency thickness is often seen during initial screening between the 11th and 13th gestational week and should be an indication for expanding screening to include additional anatomical and biochemical parameters or further diagnostic testing by experts [23, 80, 87, 88].

- Increased risk according to first-trimester screening

The present studies used various cut-off values. Every increase in the cut-off value lowers the detection rates both for numeric and structural chromosomal anomalies as well as for pathological CNVs that are not detected by cfDNA. The resulting positive rates depend on the quality of first-trimester screening and the parameters that are used. At a cut-off value of 1:100 for all trisomies, diagnostic procedures were offered to between 2.1% and 4.6% [39, 87, 103] of all pregnant women. Lowering the cut-off value to 1:300 yielded positive rates of 4.1% [87] and 10.4% [39]. The rate of detected anomalies other than trisomies and aneuploidies of the gonosomes would increase from 24% to 32% at a lower cut-off value [87] and that of pathological CNVs from 14% to 25% [80]. Syngelaki [103] indicates that most retrospective studies do not detect more than half of these “other” anomalies so that their detection rates are overestimated.

- Abnormal biochemical findings

PAPP-A < 0.2 MoM or fßHCG < 0.2 or > 5 MoM [80, 87, 94]

- Abnormal cfDNA screening findings [75, 104]

- Wishes of the pregnant woman

The desire to rule out genetic anomalies in fetuses is expressed even without preceding aneuploidy screening. From a medical standpoint, it must be taken into consideration that the preventative care guidelines still specify a maternal age of 35 or older as a risk factor.

The following genetic lab tests can be performed using the acquired cells:

- Conventional microscopic karyotyping (G-band technique with a resolution of 7–10 million bases)
- Fluorescence in situ hybridization (FISH)
- Quantitative real-time polymerase chain reaction (qPCR)
- Molecular genetic examination of the submicroscopic structure of the chromosomes via comparative genomic hybridization (array-CGH with a significantly higher resolution of 25,000 – 100,000 bases)
- Individual gene analyses

In relation to all pregnancies, the incidence of chromosomal anomalies is 0.44% [85]. In the case of an abnormal ultrasound finding, the rate of abnormal karyograms from chorionic villi and amniotic cells is 2% with 1.8% being clinically relevant. 72.7% of pathological karyograms are trisomies 13, 18, 21 and anomalies of the sex chromosomes. Other anomalies are found in 27.3% of cases [105]. The majority of the over 2100 structural chromosomal anomalies (90%) can only be detected via chromosomal microarrays (array-CGH) with a resolution of up to 25–100 Kb [106]. The clinical significance of pathological structural changes can be described in more than 99% of cases [75]. Microdeletions and (more rarely) microduplications (pathological “copy number variations” (CNVs)) are found in 2.5% of all pregnancies, in
approximately 1% of fetuses with normal ultrasound scans, and slightly more frequently in isolated abnormal serum biochemistry [77, 107].

In abnormal fetuses (malformation and/or IUGR), pathological karyograms are found in 14–30% of cases [108, 109]. The rate in the case of NT values > 95th percentile is similar (22–38%) [89, 91, 110]. In the case of a normal karyogram and abnormal ultrasound findings, an array-CGH must be offered. Pathological CNVs are seen in 6–10% of cases [77, 78, 111]. In fetuses with multiple, particularly dysmorphism-related, symptoms, a targeted search for monogenic diseases possibly on the basis of relevant databases must be performed. In the case of dorsonuchal edema and malformations, over 100 genetic syndromes with single gene mutations such as Noonan syndrome are known [112]. In total, more than 5000 dysmorphic syndromes are described and particularly pronounced entities such as skeletal dysplasia can be effectively visualized on ultrasound [113, 114]. Molecular genetic diagnostic testing can be performed with Sanger sequencing or NGS-based panels from any fetal material.

The counseling of pregnant women with respect to the risk of miscarriage due to the diagnostic procedure should be based on current large studies that have shown that the miscarriage rate at expert centers is 1:1000 for amniocentesis and 1:500 for chorionic villus sampling [115–117] or does not differ statistically from the natural miscarriage rate in the particular risk group [96, 97]. A miscarriage rate of 1% from a prospective randomized study published in 1986 [118] no longer reflects current knowledge.

In light of the comprehensive genetic diagnostic testing options, the very low risk associated with diagnostic procedures, the age-independent prevalence of pathological CNVs, the limitations of cfDNA screening and the fact that only approximately 80% of chromosomal aneuploidies are associated with abnormal ultrasound findings, every pregnant woman should be given the option of undergoing a diagnostic procedure and microarray analysis [119, 120].

**Screening for rare aneuploidies, gonosomal aneuploidies, microdeletion syndromes, and monogenic diseases**

**Rare aneuploidies**

While a number of studies regarding the detection of the most common trisomies using cfDNA screening of maternal blood are available, there is minimal data regarding the detection of rarer aneuploidies, deletions and duplications.

Rare trisomies have a prevalence of 0.3–0.8% [121, 122]. They can be caused by uniparental disomy (UPD) in which case the fetus inherited both homologous chromosomes from one parent (e.g., trisomy 6, 7, 14, 15, 16) or a placental mosaic can be present. The latter can be responsible for fetal growth restriction. In 13% of cases, placental mosaics are representative of an actual fetal mosaic [123]. Detection rates for the diagnosis of rare aneuploidies based on cfDNA are not provided due to a lack of follow-up data. The false-positive rates are 0.7% for the total population and the positive predictive value was only 8% [122]. Some authors are calling for the release of the results of rare trisomies due to their clinical significance [124]. The American College of Medical Genetics and Genomics (ACMG) recommends not screening for rare aneuploidies with cfDNA [125].

Triploidy detection via cfDNA is greatly affected by the usually low placentinal DNA fraction in maternal blood. Therefore, triploidies are usually not detected [126–128] even though the sonographic and biochemical findings are abnormal in first-trimester screening in up to 90% of cases [23]. Due to the low placentinal DNA fraction, triploidies like trisomy 18 and other anomalies are very common (3%) in the group of examinations without a result (no call results) [129]. Following cfDNA without a result, a detailed ultrasound examination possibly with a diagnostic procedure is recommended [127].

**Sex chromosome aneuploidies, early detection of fetal sex**

The most common sex chromosome aneuploidies (SCAs) are monosomy 45, X (Ullrich-Turner syndrome), 47, XXX (Triple-X syndrome), 47, XXY (Klinefelter syndrome) and 47, XYY (Diplo-Y syndrome). The prevalence of SCAs is 0.8–1% with monosomy 45, X being most common (approx. 70%) [122, 130]. The accuracy of cfDNA screening for the determination of normal fetal sex is greater than 99%. The diagnostic significance for SCAs is significantly lower. A combined evaluation of three studies published between 2013 and 2015 yielded a detection rate of 89% for monosomy 45, X, and between 82% and 90% for the other three SCAs [131]. One meta-analysis found a higher detection rate for monosomy 45, X (95.8%) and an FPR of 0.14%. The detection rate in this publication is 100% for other SCAs and the FPR is 0.004% [53]. However, closer analysis of the underlying industry-sponsored publications shows a high rate of “lost to follow-up” cases of up to 70% in some of the studies [130]. The information regarding diagnostic validity is therefore applicable only on a very limited basis. In particular, the positive predictive value (PPV) for SCAs seems low. For monosomy 45, X it is approximately 30% [131]. A newer, also industry-sponsored, study calculates a PPV of 70% for monosomy 45, X [122]. Independent studies show that the PPV for SCAs is lower: between 38% and 50% for monosomy 45, X and between 17% and 50% for 47, XXX, 47, XXY, and 47, XYY [128, 132, 133]. The discordant findings can be due to placental mosaics but also to a corresponding abnormal maternal karyotype. Based on 522 SCA cases, Grati et al. showed that a confined placental mosaic (CPM) was present in 122 cases (23.4%) while a true fetal mosaic (TFM) was seen in 43 cases (8.2%). This relates primarily to fetuses with monosomy 45, X with normal ultrasound findings. The positive predictive value of an abnormal cfDNA analysis is therefore only approximately 53% in this group, while the PPV would be 98.8% in the case of an abnormal ultrasound finding such as fetal nuchal edema or hygroma [134]. Both in the case of a normal finding regarding fetal sex after cfDNA and in a pathological finding, sonographic verification of the fetal sex organs should be performed to rule out developmental disorders [135]. Due to the ethical problems with respect to
providing notification of SCAs, the European and American societies of human genetics currently recommend not providing notification of such findings after cfDNA [136]. The American College of Medical Genetics and Genomics recommends comprehensive counseling regarding the issues prior to cfDNA screening [125].

According to the Genetic Diagnostics Act, notification of the fetal sex may not be provided prior to 14+0 weeks. However, in individual cases, advance determination of the sex is important. Particularly in the case of adrenogenital syndrome (AGS), it is important to determine the sex before seven weeks: Virilization is to be prevented in female fetuses by administering steroids but the side effects and effectiveness are a topic of discussion [137]. The sex can be determined even at this early time with cfDNA analysis. The test systems focus on the detection of SRY or DYS14 [138]. If these cannot be detected, treatment would be initiated. A further use is the determination of the sex in X-chromosome diseases such as Duchenne muscular dystrophy. Also in the case of an unclear sex on ultrasound and the differential diagnoses clitoris hypertrophy vs. hypospadias, the use of cfDNA analysis could become more important.

**Microdeletions/microduplications**

Microdeletions and microduplications (pathological copy number variations (CNVs)) are very small structural anomalies that cannot be detected by conventional microscopic chromosome analysis. They are diagnosed in 1–1.7 % of pregnancies with normal findings and are thus much more common than trisomy 21 in younger pregnant women [139]. Reliable diagnosis of pathological CNVs can only be achieved from fetal samples via array-CGH (see the section “Diagnostic procedures”). However, many of the over 2100 known CNVs are extremely rare [106]. Therefore, the prevalence of the most common microdeletion, i.e., microdeletion 22q11.2 (DiGeorge syndrome), is 1:4000 to 1:1000. Additional microdeletions, such as Cri-du-Chat syndrome (microdeletion 5p15), have rates of significantly less than 1:10000, in some cases less than 1:100 000 [140]. In contrast to the trisomies, the rate of microdeletions is independent of maternal age. For several years, the providers of cfDNA screening tests have been using various techniques to screen for pathological CNVs in addition to the most common trisomies. These changes are difficult to detect with cfDNA due to their size of less than 5–7 megabases (Mb). At present, only CNVs > 3 Mb, probably even only > 6 Mb can be detected by cfDNA [140, 141]. The majority of companies limit their offer to the most common larger microdeletions, such as 22q11.2 (DiGeorge syndrome), 15q (Prader Willi/Angelman syndrome) and 5p15 (Cri-du-Chat syndrome). Therefore, in the best case 0.1–11 % of pathological CNVs are currently detected by cfDNA [120, 140, 142, 143].

The publications of various providers regarding cfDNA screening for microdeletions are based largely on retrospective evaluations of existing serum samples of fetuses with postnatally detected diseases and allow only partial calculation of the true diagnostic value since there are high “lost to follow-up” rates of up to 70 % of cases or no information regarding the populations is provided [122, 144–146]. Therefore, reliable detection rates cannot be calculated from the available data. A retrospective proof of concept study yielded a theoretical detection rate of 74 % for all examined CNVs [147]. Given a false-positive rate for the entire examined population of >1 % and low rates of anomalies, combining the available data yields low positive predictive values between 4 % and 5 % for most pathological CNVs [140]. According to this, the majority of abnormal findings would be false-positive.

An independent study examining the cfDNA tests of various providers finds a positive predictive value of 0 % for microdeletions and a high number of test failures (“non-reportables”) for these anomalies (65 %) [148].

A relevant ethical problem is the possible detection of maternal CNVs or maternal tumors based on cfDNA screening for pathological CNVs [121, 129]. Direct diagnosis via array-CGH from chorionic villi or amniotic fluid eliminates this problem because only placental or fetal DNA is analyzed.

The guidelines of multiple societies state that cfDNA screening for pathological CNVs cannot be recommended [125, 136, 149, 150].

**Determination of fetal blood group**

Fetal blood group determination is important particularly in the case of a positive antibody test and rhesus D-negative pregnant women. If the fetus is rhesus D-negative, immunological fetal anemia cannot occur. Chitty et al. showed that the detection rate for rhesus D via cfDNA after 12 weeks is over 99.7 % [151]. Fetal blood group antigens Kell, C, c, E and e can also be determined via cell-free DNA [152]. Based on these results, there is a discussion as to whether the fetal rhesus D factor should be determined in rhesus-negative women and the administration of anti-D should be limited to women with rhesus D-positive fetuses.

**Detection of monogenic diseases**

The spectrum of cfDNA testing was already expanded to include monogenic diseases such as achondroplasia and thanatophoric dysplasia in 2007. In Great Britain, cfDNA detection of these two diseases, Apert syndrome and paternal mutations of cystic fibrosis have already been approved by the NHS. Since the cfDNA test is possible beginning in the 9th gestational week, an advantage could be the very early exclusion of recurrence [138]. The number of potentially detectable diseases far exceeds those named above and primarily includes additional autosomal-dominant diseases, such as tuberous sclerosis, as well as several autosomal-recessive entities, such as autosomal-recessive polycystic kidney disease [153].

**First-trimester screening for maternofetal disease screening**

The use of cell-free DNA from the placenta for the prediction of placenta-based diseases has also been studied [154, 155]. However, no relevant dose change of placental cfDNA in pregnancies that later developed placenta-based pregnancy complications could be found in studies performed at 11°–13° weeks [156–159]. Combination with biochemical markers [160] or uterine
Doppler measurements [161] also did not improve prediction rates.

With the key publication entitled “Turning the pyramid of prenatal care” [3], Nicolaides expanded genetically oriented first-trimester screening to include early screening for maternofetal diseases. Maternofetal diseases are more common than fetal genetic anomalies by a factor of approximately 10 and can generally be prevented. Models for early risk prediction have been developed for the pregnancy complications preeclampsia [162 – 165], fetal growth restriction [166], miscarriage and stillbirth [167], gestational diabetes [168, 169], fetal macrosomy and preterm delivery [170].

The present model shows that it is possible in principle to screen for the main problems of pregnancy already between 11+0 and 13+6 weeks and to develop prediction models for multifactorial diseases on the basis of individual risk factors [171, 172]. However, the performance of early prediction tests in pregnancy has been only moderate to date and validation studies are not available in most cases [173, 174].

Preeclampsia (PE)

For example, successful development has been seen for the early prediction of PE [175] with good test performance [162, 164, 176] and confirmation by external validation in an unselected population [177]. The breakthrough regarding prevention was achieved with the reduction in the incidence of PE via the early administration of low-dose aspirin: in the ASPRE study pregnant women were screened for PE with the FMF algorithm at 11+0 – 13+6 weeks. In the high-risk group (risk > 1:100), the administration of ASS (150 mg/day, beginning at 11 – 14 weeks) reduced the incidence of PE < 37 weeks by 62 % (P = 0.004) and PE < 34 weeks by 82 % [178].

Newer biophysical methods make it possible to determine the pulse wave velocity and the augmentation index for detailed evaluation of the maternal pulse wave. Early prediction of the PE risk in the first trimester is also the focus of scientific interest here [179, 180].

In the case of a previous Cesarean section, early screening between the 11th and 14th weeks for indications of scar defects [181, 182] and primarily for signs of an increased risk of placenta accreta [183] is extremely important for early presentation at a prenatal center. Current studies by Timor-Tritsch show advantages of early detection of scar implantation, as early as 8 – 10 weeks [184] and allow the option of early minimally invasive treatment [185].

First-trimester screening is no longer used only for aneuploidy screening. The expansion of the first-trimester scan to include maternofetal medicine will become increasingly important since the effectiveness of preventative measures will benefit greatly from an early start and thus early risk detection.

Primary cfDNA screening performed as early as possible carries the risk that a normal cfDNA screening finding will result in possible structural anomalies or other genetic anomalies not being detected until 20 weeks or not at all. The updated consensus statement of the ISUOG expresses the concern that primary cfDNA screening in the low-risk population could have a negative effect both on the quality of counseling prior to cfDNA testing and on diagnostic ultrasound imaging in the subsequent weeks [186].

The acceptance of cfDNA screening tests is largely due to the fear of complications from diagnostic procedures [187].

The expansion of screening to include additional anomalies with a largely low prevalence further complicates counseling.

A main problem of current cfDNA tests is the dominance of maternal DNA fragments. All counting methods cannot differentiate between maternal and placental DNA. SNP-based methods are based on a comparison of maternal, fetal, and paternal nucleotide sequences but this basic advantage has not yet been able to be verified.

Methods for isolating individual fetal cells [188, 189] or examining microRNA [190, 191] as well as the isolation of trophoblast cells from cervical smears [187, 192] or from embryonic cells after coelocentesis [193] have been described in small study series. Faster and cheaper sequencing techniques could provide new diagnostic possibilities even in the case of small cell numbers or fragments.

The indispensable and overdue inclusion of chromosomal microarrays and the possibility of whole exome sequencing (WES) [83] in prenatal genetic diagnostic testing and the new data regarding the low complication rates of diagnostic procedures should be reason to reevaluate genetic analyses.

Conflict of Interest

U Gembruch participated 2013 – 2015 in a clinical follow-up study on PraenaTest supported by LifeCodexx.
KO Kagan runs a prospective study on cfDNA supported by Ariosa.
T Schramm is member of Scientific Advisory Board at GE Healthcare Viewpoint.

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