

# Prenatal Invasive Fetal Karyotyping in the Era of First Trimester Screening and Noninvasive Prenatal Testing (NIPT)

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## Abstract

**Objective:** To validate indications for invasive fetal karyotyping in the era of first trimester screening and non invasive prenatal testing.

**Materials and Methods:** We conducted single centre retrospective study between 2009 and 2014. Patients were referred for either first trimester screening or second/ third trimester ultrasound screening. We included all women with invasive testing such as chorionicvilli sampling (CVS) or amniocentesis (AC). The indications for invasive testings were related to suspicious first trimester screening results and pathologic ultrasound findings. Fetal karyotypes and pregnancy outcomes were documented.

**Results:** A total of 724 patients had an AC (69%) or CVS (31%), either. We detected 123 pathologic karyotypes (17%), i.e. 46 trisomy 21 cases, 24 trisomy 18 cases and four trisomy 13 cases. 601 fetuses had normal karyotypes (83 %). 63 pathologic karyotypes were diagnosed by AC (52%) and 60 by CVS (48%). The main indication for CVS was a suspicious first trimester screening (44%), whereas AC indications were maternal age, suspicious ultrasound findings or genetic indications (e.g. family history).

From 189 (27%) normal fetal karyotypes, 115 (60%) had an invasive testing due to suspicious biochemistry results. 36 fetuses (19%) had nuchal translucency (NT) measurements above 2.5 mm and 38 patients (21%) had a combined risk elevation. 60 of these cases had cut-off risks for invasive procedures by combined testing of less than 1:50.

**Conclusion:** Fetal karyotyping is reasonable in high risk situations such as suspicious ultrasound findings in any time of pregnancies. Suspicious biochemistry results from first trimester screening alone may be followed by non-invasive testings (NIPT), especially in high risk pregnancies after ART.

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## Introduction

The screening for fetal aneuploidies in pregnancies started about 60 years ago. Until the beginning of the 1990s advanced maternal age was the main indication for invasive fetal karyotyping. The description of an elevated nuchal translucency in combination with maternal serum markers by Nicolaides and co-workers made it possible to offer a non-invasive screening test to all pregnant women assessing their individual risk of having a fetus with an aneuploidy [1]. Nowadays, the decision for an invasive prenatal diagnostic testing is frequently based on these results. First trimester screening including ultrasonographic measurement of the nuchal translucency (NT) with combined analysis of pregnancy-associated plasma protein (PAPP-A) and free  $\beta$ -chorionic gonadotropine (free  $\beta$ -HCG) has become a widely used screening method to identify chromosomal aberrations in fetuses [1]. Furthermore, an increased NT can be associated with other abnormal findings such as heart defects and numerous genetic syndromes [2], but it may as well go along with a normal fetal outcome [3].

On surplus, the screening for the most frequent aberrant numeric karyotypes such as trisomy 21, 13 and 18 comprises ultrasound with respect to fetal malformations such as cardiac defects, abdominal wall defects, growth restriction or absent nasal bone, for example [4].

Since the beginnings of invasive prenatal testing by amniocentesis or chorionic villi sampling, the major problem was the number of procedure related fetal losses. Many studies proof that the risk of an abortion after CVS or AC is dependent on the operator. It may range from as low as 0.19% up to 1.53% based on results from various studies [5]. Many of these studies were conducted decades ago and probably may not provide an accurate estimate of the current procedure-related risks. Very recently, Akolekar and co-workers performed a systematic review and metaanalysis on this issue and found the procedure related risks of miscarriage much lower

than previously reported [6]. Nonetheless the number of invasive procedures has declined over the last two decades due to the very good results from first trimester screening in the hands of experts and due to the implementation of non-invasive prenatal testing for, at least, trisomy 21, 18 and 13.

In our retrospective single centre study we tried to evaluate the different indications for invasive fetal karyotyping. We also checked the reliability of indications for invasive testing with respect to the results from first trimester screening.

## Methods and Material

This is a retrospective analysis of all pregnancies referred to our single major tertiary care centre of prenatal diagnostics for first trimester screening between 2009 and 2014. Screening was performed according to the guidelines of the Fetal Medicine Foundation (FMF) London [7]. All patient data were documented in the electronic database of our hospital, PIA Fetal Database (TM) (GE, USA).

All ultrasound examinations were performed by the same experienced sonographer in prenatal diagnostics using a transabdominal probe (4-8 MHz Voluson E8; GE Medical Systems) according to the guidelines of the Fetal Medicine Foundation (FMF)

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London[7]. Data were documented in the PIA Fetal Database.

The inclusion criteria: All patients who had an invasive fetal karyotyping over a period of six years from January 1, 2009 to December 31, 2014 were included. As there were no failures in fetal karyotyping in any case, we did not have to define any exclusion criteria.

After informed consent of the patients invasive prenatal diagnosis was performed by either chorionic villi sampling (CVS) or amniocentesis (AC) by one single expert in prenatal diagnostics. All invasive procedures were ultrasound guided and performed by free-handed needle insertion.

Whenever malformations or chromosomal aberrations were diagnosed, human genetic counselling was offered to the patients.

Approval by the local Ethical Committee was given.

Results

Between 2009 and 2014 a total of 724 patients with 713 singleton pregnancies, 10 twin and one triplet pregnancy were examined. Mean maternal age was 35,8 years, 261 patients were primigravidae.

Indications for invasive testing were suspicious first trimester screenings with either increased nuchal translucency, elevated risk for trisomy 13, 18 or 21 by biochemistry results from PAPP-A and free  $\beta$ -HCG or a combined elevated risk in 265 cases (36%). 113 pregnancies had suspicious ultrasound findings in the first or second trimester (15%). 91 patients were referred to our hospital for invasive testing due to a family history with chromosomal or genetic aberrations (12%). Maternal age as the only indication for invasive testing was documented in 256 cases (34%), the mean maternal age in this group was 39,2 years. 23 patients (3%) had a combination of various indications, e.g. personal request or maternal infections such as cytomegaly (Figure 1).

Invasive testing was achieved by amniocentesis (AC) in 500 cases (69%) and chorionic villi sampling (CVS) in 224 cases (31%). (Table 1)

In total, we detected 123 pathologic karyotypes (17%) and 601 normal karyotypes (83%).

Indication of prenatal invasive procedures for fetal karyotyping	AC (n=500)	CVS (n=224)	Total (n=724)
Suspicious first trimester screening	120	145	265
Increased nuchal translucency	19	36	55
Suspicious biochemistry	79	46	125
Combined elevated risk	22	63	85
Maternal age	223	33	256
Suspicious genetic history	50	41	91
Suspicious ultrasound findings	95	18	113
Other indications (request, infections, etc.)	23	0	23
* Total number of indications larger than total population due to double indication			

Table 1: Indications of prenatal invasive procedures for fetal karyotyping according to AC and CVS.

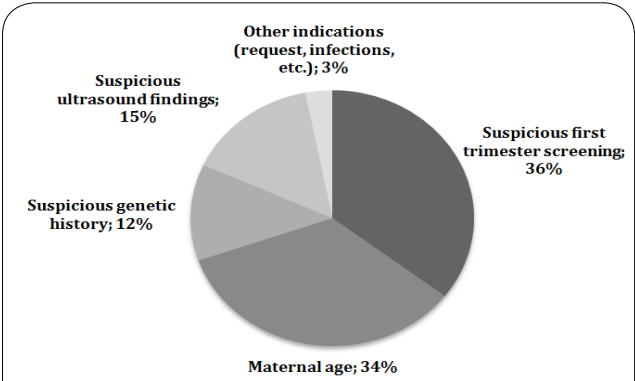


Figure 1: Indications for invasive procedures.

Pathologic karyotypes according to indication for invasive testing

Most frequently, as, expected, we diagnosed 46 cases (37%) of trisomy 21, 24 cases (19%) of trisomy 18 and four cases (3%) of trisomy 13. 15% of the karyotypes were triploidies (7), 45,X0 (4), 47,XXY (5), 47,XXX (2) and 47,XY+4 (1). Furthermore we diagnosed eight cases (7%) with translocations and ten cases (8%) with mosaic status. 11 cases (9%) were diagnosed as microdeletions or duplications (Figure 2).

63 (52%) pathologic karyotypes were diagnosed by an AC, and 60 (48%) pathologic karyotypes were diagnosed by a CVS. The main indication for CVS was a suspicious first trimester screening in 55 cases (44%), whereas AC had a higher percentage of maternal age, suspicious ultrasound findings or genetic indications. Altogether 21 patients (17%) with a suspicious first trimester screening did not opt for immediate CVS, but decided to have an AC in the second trimester. Eight of these patients had their first trimester screening in other institutions and were referred to our centre for invasive testing after 13+6 weeks of gestation.

The 46 cases of trisomy 21 were diagnosed by CVS (27) in 59% and AC (19) in 41% of the cases. All of them had a suspicious first trimester screening or additional abnormal ultrasound findings such as AV channel (3 cases), fetal hydrops (9 cases), short femur, distended jugular lateral sacs, polyhydramnios, duodenal atresia, hypoplastic nasal bone, tricuspid insufficiency or hydronephrosis.

The 24 cases of trisomy 18 were diagnosed by CVS (17) in 70% and AC (7) in 30%. All of them had some typical ultrasound findings such as a fetal omphalocele or heart defects.

Trisomy 13 (4) and Turner syndrome (4) were diagnosed by CVS in three cases and AC in one case, respectively. All of them had the typical ultrasound findings such as holoprosencephaly, heart defects and fetal hydrops.

We diagnosed seven cases of triploidy by AC (5) and CVS (2), showing severe fetal retardation and oligohydramnios.

With respect to risk calculations in the first trimester screening for trisomy 21, 18 and 13 we obtained the following results: from 60 patients 57 cases (95%) had an adjusted risk of less than 1:50 and three cases (5%) of more than 1:100. From these three cases two trisomy 21 fetuses had an NT of 2.6 mm. One trisomy 18 fetus had an NT of 2.0 mm with unsuspicious biochemistry but revealed definite other ultrasound markers such as an omphalocele and a ventricular septal defect.

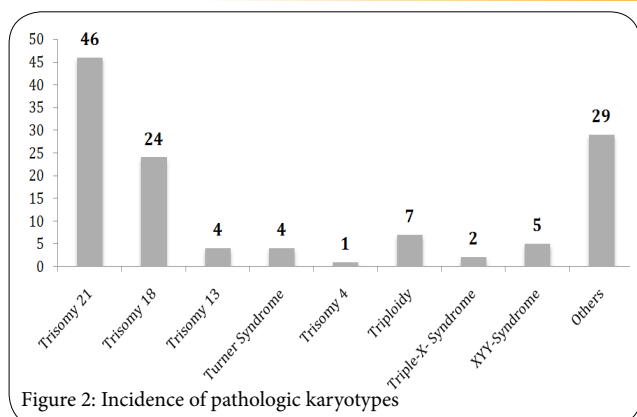


Figure 2: Incidence of pathologic karyotypes

### Normal karyotypes according to indication for invasive testing

189 patients (26% of the overall population) with a normal fetal karyotype had an invasive procedure following a suspicious first trimester screening, 99 of those patients had an AC and 90 patients had a CVS. 115 of these cases had suspicious biochemistry results, 36 patients had an NT above 2.5 mm and 38 patients had a combined risk elevation. Of these patients 60 women had an adjusted risk of less than 1:50.

79 patients opted for invasive testing due to pathologic ultrasound findings during first and second trimester screening, 20 patients had various other indications such as fear of fetal chromosomal aberrations in general. 242 of 256 patients (94%) with maternal age as the only indication for invasive testing had normal fetal karyotypes. 77 patients had a family history with genetic diseases (Table 3).

Indication of prenatal invasive procedures with normal fetal karyotype	AC (n=437)	CVS (n=164)	Total (n=601)
Suspicious first trimester screening	99	90	189
Increased nuchal translucency	11	25	36
Suspicious biochemistry	74	41	115
Combined elevated risk	14	24	38
Maternal age	209	33	242
Suspicious genetic history	41	36	77
Suspicious ultrasound findings	74	5	79
Other indications (request, infections, etc.)	20	0	20
* Total number of indications larger than total population due to double indication			

Table 3: Indication of prenatal invasive procedures with a normal fetal karyotype.

To all of our knowledge we had no report on a missed diagnosis of trisomy 21, 18 or 13 after a first trimester screening. Overall reported fetal loss after invasive testing was less than 0.5%, which was one CVS and no AC. There were two reported leakages after AC followed by normal term births.

### Discussion

Prenatal diagnosis has experienced some tremendous shifts in counselling women with respect to the risks of fetal chromosomal

aberrations. While in the last century maternal age was the main indication for invasive testing by AC or CVS in early pregnancies, nowadays women are recommended to have a first trimester screening including NT measurement and detailed ultrasound evaluation of the fetus. Furthermore, early fetal echocardiography and neurosonography may improve the diagnostic results [8, 9]. Moreover, it is the intention to counsel patients with respect to possible pregnancy complications such as preeclampsia, intrauterine growth restriction (IUGR) or maternal diabetes mellitus [10].

Certainly, another major problem in prenatal counselling nowadays is the rising number of pregnancies after ART treatment. Naturally, many women in this population have an advanced age so that their personal risk for a pregnancy with chromosomal aberrations is elevated. Also naturally, the fear for fetal loss with or without invasive procedures is immense. Therefore, these patients are in the need of an extremely careful prenatal counselling recommending an ultrasound examination including a correct risk calculation for chromosomal aberrations or further pregnancy complications.

In our study, first trimester screening had a good prognostic reliability for invasive testing. From 60 cases with trisomy 21, 18 and 13, as much as 95% had an adjusted risk of more than 1:50. This is in good congruence with or even better than the standards suggested by the FMF London [11].

Furthermore, adding surplus markers from fetal echocardiography such as pathologic Doppler in the ductus venosus or tricuspid regurgitation helped to identify severe cases of Turner's syndrome in our cohort. In 2009 Kagan and co-workers as well as Maiz and co-workers published their data proving the importance of extended ultrasound examinations of the fetal heart [12, 13].

From 123 pathologic karyotypes we identified 38 chromosomal aberrations (31%), which are not supposed to be detected by first trimester screening such as sex chromosomal aberrations, translocations or micro deletions. The main indication for invasive testing in these cases was a suspicious ultrasound screening in either the first or second trimester. Translocations and chromosomal mosaicism in association with severe abnormal ultrasound findings have a major impact on counselling parents prenatally with respect to the fetal outcome.

On the other hand, certain sex chromosomal aberrations such as triple X syndrome or Klinefelter syndrome, in our opinion, require very distinct counselling with respect to induced abortions [14].

35% of our patients had an invasive prenatal testing due to their maternal age alone. These numbers have constantly declined over the six-year period of this study. Furthermore, like in previous other studies, we found constantly declining numbers of AC in relation to CVS. This is in accordance with a study by Tabor and co-workers. They found a constantly decreasing number of AC over a period of eleven years, whereas CVS procedures according to suspicious first trimester screening results were rising [15]. The results of our study support the fact that major indications for an AC may be pathologic ultrasound findings in the second trimester screening or several genetic family risks that can be detected in amniocytes rather than chorionoc villi due to a better resolution of the karyogram [16].

Nonetheless women attitude toward prenatal diagnosis depends on various implications. Although the numbers of invasive prenatal testing are constantly declining, there are patients who opt for a primary fetal karyotyping. Definitely, the major argument of these

patients is the fear of a life with a severely impaired child. Counselling these parents must, at least, include all aspects and options of legal termination of pregnancy [17].

Results from non-invasive cfDNA testing (NIPT) are promising, as far as trisomy 21, 18 and 13 or sex chromosomal aberrations are concerned. Results with respect to the detection of microdeletions are promising, as well [14, 18].

Nevertheless, there is a worldwide consensus that, for the moment being, pathologic results from cfDNA must be confirmed by invasive testing.

Once again our results show that the defined pathologic ultrasound findings such as heart defects, abdominal wall defects, absent nasal bone or growth restriction in the first or second trimester, have a good correlation with aberrant fetal karyotypes and should be followed by an invasive testing, if this is an option for the parents. In contrast, suspicious biochemistry analysis without abnormal ultrasound findings may be an indication for a cfDNA prenatal testing.

## Conclusion

We could show once again that combined first trimester screening is a reliable method for aneuploidy screening in the hands of experts. Maternal age alone should, no further, be an indication for invasive prenatal testing. A distinct family history remains one indication for molecular genetic testing as well as suspicious ultrasound findings at any time of pregnancy [19].

Furthermore, our own number of fetal losses with less than 0.5 % suggests that invasive prenatal procedures are a rather safe method in the hand of an experienced operator.

## Competing Interests

The authors declare that they have no competing interests.

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